Community-based waste management strategies in relation to a targeted Nepalese community

A thesis submitted for the degree of

Doctor of Philosophy

Institute for Sustainability & Innovation/College of Engineering & Science

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by

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Declaration

I, Anusuya Joshi, declare that the PhD thesis entitled **'Community-based waste management strategies in relation to a targeted Nepalese community'** is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, references and footnotes.

This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

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August 2015

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Journal publications, conference proceedings and conference presentations relevant to the scope of this thesis.

Refereed Conference Proceedings (copies provided in the accompanying CD)

1. Joshi, A., Reeve, D., Ngeh, L. N., Guneratne, J. & Orbell, J. D. 2013. Exploring vermifiltration for wastewater treatment at the community level in Nepal. *In*: 11th IWA Conference on Small Water & Wastewater Systems and Sludge Management, October 28-30, 2013, Harbin, China.

2. Joshi, A., Reeve, D., Ngeh, L. N. & Orbell, J. D. 2014. Challenges in the design of a community-based vermifiltration system for wastewater treatment. *In*: 2nd IWA Specialized International Conference on Ecotechnologies for Wastewater Treatment – EcoSTP 2014, June 23-27, 2014, Verona, Italy.

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3. Joshi, A., Reeve, D., Ngeh, L. N. & Orbell, J. D. 2014. A community-based wastewater treatment system to improve water quality in the Bagmati River. *In:* IWA specialized conference on Global challenges: Sustainable wastewater treatment and resource recovery, October 26-30, 2014, Kathmandu, Nepal. (Poster Presentation)

Poster presentations (copies provided in the accompanying CD**)**

1. Joshi, A., Reeve, D., Ngeh, L. N., Guneratne, J. & Orbell, J. D. 2013. Presentation: Adopting an integrated approach to alleviate the environmental impact on the Bagmati River. *In*: Victoria University Postgraduate Association and Graduate Research Centre Poster Exhibition, October 11, 2013, Melbourne, Australia.

2. Joshi, A., Reeve, D., Ngeh, L. N. & Orbell, J. D. 2014. Method development ethanol, volatile fatty acid and lactic acid determinations in Takakura composting fermentation solutions. *In:* Victoria University Postgraduate Association and Graduate Research Centre Poster Exhibition, October 10, 2014, Melbourne, Australia.

Refereed Journal Publications in preparation

Anusuya Joshi, Lawrence N. Ngeh, Darrell Reeve and John D. Orbell, "Towards ZERO waste – an evaluation of an innovative approach to municipal solid waste management in a Nepalese community", to be submitted to *Waste Management*, in preparation.

Anusuya Joshi, Lawrence N. Ngeh, Darrell Reeve and John D. Orbell, "Municipal wastewater management in developing countries – new directions*",* to be submitted to *Critical Reviews in Environmental Science and Technology*, in preparation.

Anusuya Joshi, Lawrence N. Ngeh, Darrell Reeve and John D. Orbell, "A critical study of a centralized wastewater treatment facility in the Kathmandu Valley – an argument for decentralized systems in developing countries", to be submitted to *Journal of Environmental Management*, in preparation.

Anusuya Joshi, Lawrence N. Ngeh, Darrell Reeve, Jeremy Guneratne and John D. Orbell, "A long-term study of factors affecting the efficiency of the treatment of domestic sewage by vermifiltration", to be submitted to *Bioresource Technology,* in preparation.

Anusuya Joshi, Lawrence N. Ngeh, Darrell Reeve and John D. Orbell, "A scientific assessment of the Takakura composting method", to be submitted to *Bioresource Technology*, in preparation.

Abstract

The Bagmati River, which is of great cultural and religious importance to the Nepalese people, is also a major water resource. Its degradation, as a result of urbanization and industrialization, affects water quality and poses a threat to the environment and to human health - as well as resulting in water scarcity. In particular, the discharge of sewage directly into the river without prior treatment contributes significantly to river water pollution, whereas municipal solid waste dumping onto the river bank and development activities are major contributors to the deteriorating situation of the river basin overall. This study addresses this problem from a number of perspectives including an examination of two appropriate community-based technologies that may contribute to the sustainable management of domestic waste and sewage and that will also minimize the impact on the surrounding environment, especially the Bagmati River. Thus an existing composting method, the Takakura Composting Method (TCM), for municipal solid waste management was scientifically investigated with a view to optimizing its current performance. Vermifiltration (VF), which exploits earthworm metabolism to remove contaminants from sewage effluent, was also scientifically investigated in order to develop its potential for treating domestic sewage at the community level. Also investigated is the potential for both of these technologies to be integrated into the community for resource recovery.

In the context of this project, a community based eco-audit provided insight into the waste generation, power and water usage in a target community - namely: Ward Number 20 of Lalitpur Sub-metropolitan City, Lalitpur, Nepal. For this community, most of the households adopt a waste segregation method and already use the Takakura composting method to separate organic waste - in order to prepare compost at the household level. The survey revealed that 65 % of the waste is organic in nature. In terms of wastewater (domestic sewage, a combination of black and grey water) generation, each household generates an average of 200.6 L of wastewater per day, with the approximate average water use being 235.9 L per day; i.e. 85 % of water used is discharged as wastewater. However, none of the households treat wastewater on-site

and the sewage water is collected from households through a system that discharges directly into the Bagmati River without any prior treatment.

A pilot-scale three-layered biological Vermifiltration (VF) system, which consisted of distinct layers of soil, sand and gravel, was designed and constructed at City West Water's sewer mining project site, the Sunshine Golf Course Treatment Plant, Victoria, Australia, where domestic sewage (as influent) could be accessed easily. The VF was assessed for its efficacy for the filtration of domestic sewage, as measured by the quality of the treated effluent. The unit was operated in two different Phases I & II (with soil type 1 & soil type 2 respectively). Water quality parameters such as temperature, pH, conductivity, dissolved oxygen (DO), biological oxygen demand (BOD_5), chemical oxygen demand (COD), turbidity, total suspended solids (TSS), nitrogen content, phosphorous content, levels of heavy metals such as zinc, cadmium, mercury, lead and certain bacteria (*E.Coli*, Faecal coliform) were continuously monitored. The VF performance was found to be significantly effective in removing turbidity, TSS, COD, BOD_5, NO_2-N and NO_3-N; with removal efficiencies of 87 %, 82 %, 41 %, 94 %, 84 % and 92 %, respectively, for Phase I. For Phase II, turbidity, TSS, COD, NO_2-N and NO_3-N removed by 83 %, 86 %, 52 %, 98 % and 93 %, respectively. The physico-chemical and biological profile of the system throughout the operation period demonstrated a significant removal of pollutants - especially suspended solids and organic matter.

A pilot-scale Takakura composting system, analogous to a system that has already been implemented at the community level in developing countries, was constructed and scientific experiments were carried out with a view to optimizing its performance. Scientific research on this system, at this level and to this extent, has not been conducted before. At first, two different types of fermentation solutions (salt-based and sugar-based) were prepared utilizing locally available vegetable/fruit waste and fermented products. The physico-chemical parameters such as temperature, pH, conductivity, total organic carbon, nitrogen and the concentration of volatile fatty acids, lactic acid and ethanol were monitored with a view to optimizing the fermentation solutions (FS) with respect to time and substrate quantity. Then, three different

compositions of seeding inoculates (SIs) were prepared utilizing the native microorganisms isolated in the FSs. Finally, three different TCM matrices (of compost) were prepared utilizing the three different compositions of SIs and the compost quality was assessed by monitoring parameters such as temperature profile, pH, conductivity, carbon-to-nitrogen ratio, available nutrients (nitrogen, phosphorus and potassium), micronutrients and trace metals - over 35 days. The compost maturity test involved four different methods - germination percentage, plant bioassay, C:N ratio and Fourier Transform Infrared (FTIR) spectroscopy. A new index was introduced to assess the health of test plants in terms of the number of leaves and height of the plant, termed the 'Bushiness Index' (BI). These studies revealed that the ideal FS could be obtained with a desired quality by varying time and substrate quantity. With respect to the quality of TCM matrices, the matrix with very high nutrient levels were found to be unfavourable for seed germination and seedling growth, which suggests that a too high a nutrient level in the compost could exhibit phytotoxic characteristics. However, when the same matrix was mixed with garden soil (GS), it was found that it imparts nutrients to the GS, which produced relatively healthier plants. The findings of this study showed that the TCM compost is "tunable" as required, which can be achieved by selecting the appropriate seeding inoculate. The study also supports the claim of the inventor that TCM is an innovative technology, which is simple, fast and easy to adopt at the community and household levels.

Also of relevance to the appropriateness of the above technologies for this community is the current role and performance of the centralized Guheshwori Wastewater Treatment Plant (GWWTP), which is operated by the High Powered Committee for Integrated Development of the Bagmati Civilization (HPCIDBC). Therefore, this has also been investigated in terms of existing data records and constitutes part of the field work for this study. Notably, the river water monitoring at the point of discharge revealed that although the treatment plant itself satisfactorily treats the wastewater for discharge, operational requirements necessitate the mixing of the effluent and untreated influent through a by-pass, resulting in a net contamination of the river. Thus, a consideration of the data from the GWWTP, both existing and collected as part of this project, suggests that centralised conventional treatment systems such as GWWTP are not economically and technologically viable for a developing country like Nepal. This supports the

adoption of alternative approaches such as the community-based Vermifiltration system studied here, as a means of more reliably ameliorating the discharge of wastewater into the river environment. Furthermore, the domestic wastewater effluent could be diverted for irrigation purposes. Similarly, solid waste management at the local level, through a method such as Takakura composting, could divert up to 65 % of organic waste that currently goes to landfill.

Executive Summary

Background

Anthropogenic activities generate waste, which is problematic for the environment and which is generally not considered to be useful to the community. The cost of appropriate technologies associated with waste management has become an emerging challenge worldwide. This is especially the case in developing countries where resources are often limited and economic development issues tend to be given priority, regardless of how improper and inefficient handling of community and industrial waste harms human health and the environment. There is a growing realization, however, that waste generation can be effectively reduced at source and that many waste materials can be recovered and exploited. For the purpose of this project, such waste may be conveniently divided into the categories of solid and liquid wastes. Other categories, such as air pollution, are outside the scope of this thesis but are, of course, also relevant and will be alluded to as appropriate. Solid waste refers to food wastes, paper, cardboard, plastics, textiles, leather, yard wastes, wood, glass, tin cans, aluminium, other metals, ashes, street leaves, special wastes and household hazardous wastes – some of the sources of generation being residential, commercial, institutional, industrial and municipal solid waste (Tchobanoglous et al. 2002). Liquid waste refers to sewage effluent which is the combination of grey and black water from domestic, industrial and commercial sectors (NWQMS 1997).

Within a defined conceptual framework, which is depicted schematically in Scheme 1, this study focuses specifically on certain aspects of solid and liquid waste generated within a small community in the developing country of Nepal. More specifically, this study focuses on contributing to the development a community-based waste management strategy for a Nepalese target community, namely; Ward No. 20 of Lalitpur Sub-metropolitan City (LSMC), with a view to alleviating the environmental impact on the local Bagmati River. A particular emphasis has been placed on the adoption and development of community-based innovative technologies, such as

Takakura Composting and Vermifiltration. In this regard, it is recognised that an efficient, effective and systematic waste management strategy is required in such communities to ensure that waste has no adverse impact on the receiving environment. Such a management strategy, in addition to the technological considerations, takes into account societal, environmental and economic aspects that are acceptable to the communities and that aspires to environmental best practice.

The need for sustainable innovative waste management technologies

Generally, waste management is a complex process, which engages many different technologies and inter-disciplinary expertise. It involves multiple stages including waste generation itself, waste handling and separation, storage and processing at the source, collection, transfer and transport, processing and transformation of waste and the ultimate disposal or reuse. Whilst planning to develop a strategy for waste management in the target community, an Integrated Waste Management (IWM) approach has been adopted. IWM is defined as "*the selection and application of suitable techniques, technologies and management programs to achieve specific waste management objectives and goals*" (Tchobanoglous et al. 2002, p.1.8). In terms of solid waste management, basic management strategies identified by the United States Environment Protection Agency (USEPA) for IWM include source reduction, recycling and composting, combustion (waste-to-energy) and landfill. Many waste treatment and disposal/reuse methods have been researched and adopted in recent years including thermal treatment, incineration, gasification/pyrolysis, open burning, composting and anaerobic digestion.

In terms of sewage effluent management, NWQMS (1997, p.10) has suggested that the strategy should consider the sewerage system as a whole. Such a strategy should also address different aspects of waste minimisation, managing the collection system, managing the treatment system. It should also include efficient process control within the treatment plant and proper sludge handling, effluent reuse and discharge of the remaining effluent to land, coastal waters and inland waters. Again, treatment and disposal/reuse emerge as ultimate management options.

Chapter 1 of this thesis investigates the current status of solid and liquid waste management, in terms of generation, collection, treatment and disposal/reuse, in the Global and the Nepalese contexts. Moreover, current innovative technologies for organic solid waste management and sewage effluent treatment have been reviewed and appropriate candidate technologies identified on the basis of technical simplicity and affordability with respect to the targeted Nepalese community. The requirements for further scientific investigation in order to optimize such technologies have formed a basis for the research that has been carried out for this thesis. Such research has been carried out with a view to establishing a research platform for future development.

A community eco-audit in order to characterise and quantify the environmental factors that affect well-being

With changing life styles and consumer consumption patterns, the use of environmentally damaging materials in environmentally unfriendly ways has affected waste generation patterns in many cities of developing countries (Alam et al. 2008). The target community investigated here is no exception to this, where waste generation is increasing with an increase in population density and extended settlement. Moreover, the lack of public awareness of available solid waste management and collection facilities causes people to dump waste in public places posing a risk to human health and the local environment. Particular problems include solid waste dumping along river banks and near other surface water sources (e.g. ponds) and sewage effluent discharge, particularly into the Bagmati River, without prior treatment. Such factors have led to an increasing demand for energy (electricity) and freshwater. Unfortunately, the government is not able to meet this demand and people are forced to live with such shortages. This study proposes a self-sustained community which manages solid waste and liquid waste at the local level and which, ultimately, does not rely on the government for centralized facilities.

Chapter 2 characterises the existing (representative) target community, Ward No. 20 of Lalitpur Sub-metropolitan City (LSMC), and discusses the current solid waste and sewage effluent management practices. An eco-audit conducted in the community

characterises and quantifies solid waste (Local Government Association Northern Territory 2009) and identifies the stakeholders involved in waste management. Moreover, it provides information on water usage, source of water supply, water storage, on-site wastewater treatment and type of toilet (pour/flush) in residential buildings. Furthermore, it explores the current status of power usage, potential solar power use and rainwater harvesting.

The performance of the Guheshwori Wastewater Treatment Plant (GWWTP) and its impact on river water quality

In many developing countries centralised sewage treatment facilities are in use (Clarkson et al. 2010). However, many of these do not perform to capacity due to a lack of local expertise to ensure their ongoing operation and maintenance (Wagner and Pinheiro 2001). This is certainly the situation in Kathmandu, Nepal. The Guheshwori Wastewater Treatment Plant (GWWTP), which was built with a view to minimizing the direct discharge of sewer into the Bagmati River, is operated by the High Powered Committee for Integrated Development of the Bagmati Civilization (HPCIDBC), and has a service area of 537 hectares, covering a population of 198,000 with a design wastewater flow of 0.190 m^3/s. The design parameters of water quality for influent and effluent are 270 mg/L and 25 mg/L for BOD_5, 1150 mg/L and 250 mg/L for COD; and 216 mg/L and 100 mg/L for TSS, respectively. However, it operates to only 60% efficiency of its capacity due to many operational and maintenance issues (personal communication).

Chapter 3 addresses the current performance of the GWWTP in terms of water quality parameters for inlet and outlet, and identifies the social, economic and environmental factors associated with its operation. Additionally, the Bagmati River water quality has been assessed upstream and downstream of the GWWTP effluent discharge point. Moreover, an assessment of the Bagmati river from upstream Sundarijal (where the river enters the Kathmandu valley) to downstream Chovar (where the river exists the Kathmandu valley) has been carried out and described. These studies allowed an informed comparison to be made between the centralized GWWTP and the potential implementation of decentralized vermifiltration systems.

Vermifiltration as a tool for sewage effluent management and the reuse of recovered effluent

Sewage effluent collected from Nepalese residential and commercial buildings via the centralized sewer system and treatment facility are ultimately discharged into the Bagmati River and a significant proportion of this effluent is directly discharged into the River. Effective community-based decentralised wastewater treatment systems are a potential solution to this problem. People living downstream utilize river water for various purposes such as washing dishes and clothes, bathing and as drinking water for cattle. Thus, to ameliorate the health risk and to protect public health and the environment, a simple technology which treats sewage effluent with a synchronous earthworm-microorganism mechanism was investigated as a potential treatment system. Such a system aims to maximize the reuse of effluent, minimise adverse impacts to land and the contamination of water bodies and to maintain agreed water quality objectives for receiving waters when discharged to surface waters. The community-based treatment system treats and disposes the effluent locally which also enables the community to save costs related to transport and transfer.

Chapter 4 investigates the feasibility of vermifiltration as a potential sewage effluent treatment technology for Nepalese communities. A model vermifiltration pilot plant was designed and set up at a local (Victoria, Australia) water authority's site, i.e. City West Water's, Sunshine Golf Course 'Sewer Mining Site' in order to access a continuous supply of domestic influent. The performance of the vermifiltration unit in terms of the influent and effluent water quality has been scientifically evaluated by tracking various physical, chemical and biological water quality parameters over time. These include temperature, pH, conductivity, DO, BOD_5, COD, turbidity, TSS, nitrogen content, phosphorous content, heavy metals and microorganisms. A particular interest of these experiments was to elucidate the role of the worms themselves. This work is with a view to advancing this technology for implementation in the Nepalese context and, as such, is a 'model' system.

Optimization of an existing Takakura composting method for source reduction of organic solid waste

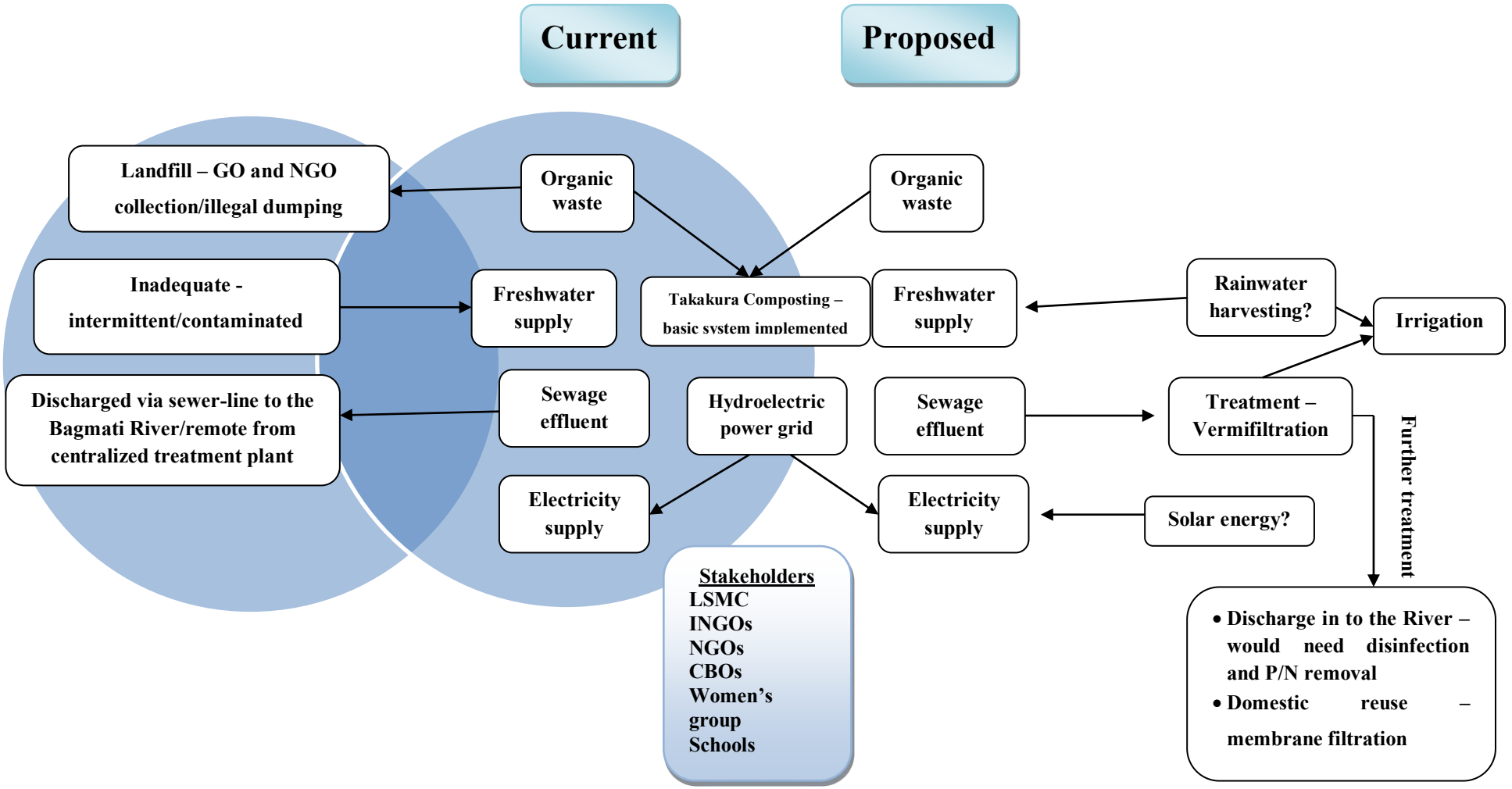
In Nepalese communities traditional composting methods have been practiced from ancient times to manage organic solid waste. Thus, clusters of houses in the community were arranged in such a way (called Saaga) so that a group of households collectively produce compost which can then be shared for use on their farms (fields), although this was far from being a hygienic and environmentally benign practice. Although this practice is now diminishing, local governments still encourage households to manage organic solid waste at the source by the promotion of household composting. Encouragingly, the adoption of the more hygienic and environmentally benign Takakura composting method in the target community is a part of the local government's strategy to reduce organic solid waste at the source. However, this composting method, which has already been adopted in many households in this target community, has not yet been rigorously scientifically investigated.

Thus, Chapter 5 initiates a scientific investigation on the existing Takakura composting system in order to explore the potential of further optimizing this technology. The Takakura method requires fermentation solutions to be simply prepared using waste food and vegetable scraps in order to isolate native microorganisms as inoculate for seeding the compost. A scientific investigation, conducted from 2011 to 2014, has assessed these solutions in terms of ethanol, volatile fatty acids and lactic acid produced with respect to retention time and substrate concentration. It also researches the details of seeding compost preparation, its maturity and use. Finally, it optimizes the compost in relation to the relative proportions of seeding inoculate. Prepared composts have been rigorously assessed by monitoring pH, the C:N ratio, Nitrogen, Phosphorus and Potassium (NPK) values, micronutrients and germination indices with respect to plant trials.

Chapter 6 summarises the overall strategy to develop a model for a community-based waste management system, especially in terms of organic solid waste and sewage effluent, and other environmental factors such as power and freshwater supply as

supporting factors. Further, it provides overall recommendation on the basis of the outcomes of the project.

Scheme 1 – Conceptual Framework for Waste Management



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Abbreviations and Terms

3Rs	Reduce, Reuse and Recycle
5Rs	Refusal, Reduction, Reuse, Recycling and Responsibility
ADB	Asian Development Bank
Aerobic	A condition in which atmospheric or dissolved oxygen is present in an environment.
AFR	African region
Alkalinity	The capacity of water or wastewater to neutralize acids
Ambient Temperature	Temperature of the surroundings
Anaerobic	A condition in which atmospheric or dissolved oxygen is not present in an environment
APHA	American Public Health Association
BKM	Bhaktapur Municipality
BOD_5	Biochemical oxygen demand
C/N	Carbon to nitrogen ratio
$C_6H_{10}O_5$	Cellulose
CA, CB & CC	Compost A, Compost B and Compost C
CBOs	Community based organizations
CBS	Central Bureau of Statistics, Nepal
CH_4	Methane
CO	Carbon monoxide
CO_2	Carbon dioxide
COD	Chemical oxygen demand; oxygen reducing capabilities of wastewater
Conductivity	Electrical conductance per unit distance
CWs	Constructed wetlands

CWW	City West Water, Melbourne, Australia
DEWATs	Decentralized Wastewater Treatment Systems
DO	Dissolved oxygen
DRIFT	Diffuse reflectance infrared fourier transform spectroscopy
EF1	Effluent from first layer of the VF, soil layer
EF2	Effluent from second layer of the VF, sand layer
EF3	Effluent from third layer of the VF, gravel layer; this represents the quality of the final effluent from the VF
EM	Effective microorganism
EPA	Environment Protection Authority
FOG	Fat, oil and grease
FS	Fermentation solution
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
GDP	Gross domestic product, an indicator of the economic health of a country
GON	Government of Nepal
GOs	Government organizations
GP	Germination percentage
GS	Garden soil
GWWTP	Guheshwori wastewater treatment plant
H_2	Hydrogen
H_2O	Water
H_2S	Hydrogen sulphide
HH	Households
HIC	High income countries
HLR	Hydraulic loading rate

HPCIDBC	High Powered Committee for Integrated Development of the Bagmati Civilization
HPLC	High performance liquid chromatography
HRT	Hydraulic retention time
ICP - OES	Inductively coupled plasma optical emission spectrometry
IGES	Institute for Global Environmental Strategies
IN	Influent, domestic sewage wastewater
INGOs	International non-governmental organizations
IWM	Integrated waste management
IWWM	Integrated wastewater management
JICA	Japan International Cooperation Agency
JOCV	Japan Overseas Cooperation Volunteers
KMC	Kathmandu Metropolitan City
LA	Lactic acid
LIC	Low income countries
LSMC	Lalitpur Sub-metropolitan City
m^3/day	Cubic meter per day
MBR	Membrane bio-reactor
MCM	Million cubic meters
MENA	Middle East and North Africa region
Mesophilic	Organisms requiring temperature between 25 – 40 ° C to thrive
mg/L	Milligram per liter; measurement of mass concentration
MJ/Nm^3	Megajoule per normal cubic meter , heat value
MLD	Million litres per day
MRFs	Materials recycling facilities
mS/cm	Millisiemen per centimeter; unit of conductivity

MSW	Municipal solid waste
MSWM	Municipal solid waste management
N_2	Nitrogen
NGOs	Non-governmental organizations
NH_3	Ammonia
NH_3-N	Ammonium nitrogen
NM	Native microorganism
NO_2-N	Nitrite nitrogen
NO_3-N	Nitrate nitrogen
NTU	Nephelometric turbidity unit; unit of turbidity
O_2	Oxygen
OCs	Operating conditions
OECD	Organisation for Economic Co-operation and Development region
pH	Water quality parameter that measures the activity of hydrogen ion
RB	Rice bran
RDF	Refused derived fuel
RECPHEC	Resource Centre for Primary Health Care
RH	Rice husk
SBR	Sequential Batch Reactor, wastewater treatment system
SEM	Scanning electron microscope
SGCTP	Sunshine golf course treatment plant
SI	Seed inoculate
SMC	Sibu Municipality Council
T	Temperature,°C
TCM	Takakura Composting Method

TDS	Total dissolved solids; combined content of all dissolved organic and inorganic material in wastewater
Thermophilic	Organisms requiring temperature between 40 – 80 °C to thrive
THM	Takakura Home Method
TN	Total nitrogen
TOC	Total organic carbon; amount of carbon in an organic compound
TP	Total phosphorus
TSS	Total suspended solids; particles that remain in suspension in wastewater
Turbidity	Cloudiness of water due to suspended solids
USEPA	United States Environment Protection Agency
UASB	Up-flow Anaerobic Sludge Bed wastewater treatment technology
UNEP	United Nations Environment Program
v/v	Volume by volume concentration
VC	Vermicompost
VF	Vermifiltration system
VFA	Volatile fatty acid
WB	Wheat bran
WHO	World Health Organization
Worms	Earthworms
WSSCC	Water Supply and Sanitation Collaborative Council
WTE	Waste-to-energy technology, one of the waste management systems
µS/cm	Microsiemen per centimeter; unit of conductivity

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Sample of the questionnaire on Eco-Audit in Ward No. 20, Lalitpur Sub-metropolitan City.

Filled-up questionnaires (in CD).

Chapter 3: Appendix 3.1 - Figures

Figure 1 The ammonium nitrogen (NH_3-N) test in wastewater (Influent and Effluents). The vial (a) is transparent before the test, the vial (b) is for blank test and the vial (c) with green colour indicates the presence of NH_3-N in samples.

Figure 2 The nitrite nitrogen (NO_2-N) and the nitrate nitrogen (NO_3-N) test in wastewater (Influent and Effluents). The equipment used is DR890 colorimeter.

Chapter 3: Appendix 3.2 - Figure

Figure 1 The temperature, pH, conductivity, DO, turbidity and TSS profile for Influent and Effluent at the GWWTP for October 2013 (this study). The error bars represent the standard error, where n=3.

Chapter 4: Appendix 4.1 - Figures

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Figure 2 The inductively coupled plasma – optical emission spectrophotometer used for the determination of heavy metals, nutrients and macronutrients.

Figure 3 The chemical oxygen demand (COD) test in wastewater (Influent and Effluents).

Figure 4 The total phosphorus (TP) test in wastewater (Influent and Effluents). The equipment used is DR5000 spectrophotometer.

Chapter 4: Appendix 4.2 - Figure

Figure 1 Calibration curves for Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn. Altogether six standards were prepared (0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L). The wave length at which the measurement was taken is indicated in the respected calibration curves.

Chapter 4: Appendix 4.3 - Box plot obtained for Phases I and II
(for normality and outlier tests) – in CD.

Chapter 4: Appendix 4.4 - Box plot obtained for Phases I

(based on HRT - for normality and outlier tests) – in CD.

Chapter 4: Appendix 4.5 - Tables

Table 1 Normality test for Phase I datasets, which contains 22 sampling sets including 7 sets "without worms" and 15 sets "with worms". Here, green colour represents the normal dataset and red colour represents the non-normal dataset. Asterisk (*) represents the datasets with extreme outlier/s.

Table 2 Normality test for Phase I datasets, which contains 22 sampling sets including 11 sets with 13 hours HRT, 6 sets with 19.5 hours HRT and 5 sets with 182 hours HRT. These sets do not consider "without worms" and "with worms". Here, green colour represents the normal dataset and red colour represents the non-normal dataset. Asterisk (*) represents the datasets with extreme outlier/s.

Table 3 Normality test for Phase II datasets, which contains 13 sampling sets including 3 sets "without worms" and 10 sets "with worms". Here, green colour represents the normal dataset and red colour represents the non-normal dataset. Asterisk (*) represents the datasets with extreme outlier/s.

Chapter 4: Appendix 4.6 - Hypothesis outcomes

1. The effect of filter layers

A. Phase I, Without worms

B. Phase I, With worms

C. Phase II, Without worms

D. Phase II, With worms

Chapter 4: Appendix 4.7 - Hypothesis outcomes

2. The effect of worms - Phase I and Phase II

Chapter 4: Appendix 4.8 - Hypothesis outcomes

3. The effect of soil type - Without worms and With worms

Chapter 4: Appendix 4.9 - Hypothesis outcomes

4. The effect of HRT(comparison Effluent 3 among retention time of 13 hours, 19.5 hours and 182 hours)

Chapter 4: Appendix 4.10 - The significance test outcomes

The effect of filter layers
The effect of worms
The effect of soil type
The effect of HRT/HLR

Chapter 5: Appendix 5.1 – setting up of worm farm

Figure 1 Can-O-Worms® TUMBLEWEED Worm Farm set up at Werribee campus.

Chapter 5: Appendix 5.2

Figure 1 GC peak for 10% DL Lactic acid.

Chapter 5: Appendix 5.3

Figure 1 FTIR spectra for (a) Compost A – day 35 and (b) vermicompost (see **Appendix 5.3** in CD for details).

Chapter 5: Appendix 5.4

Figure 1 DRIFT FTIR spectra for (a) Compost A – day 35 and (b) vermicompost (see **Appendix 5.4** in CD for details).

CHAPTER 1: Introduction and Background

1.1 Municipal Solid Waste Management

1.1.1 Background

Municipal Solid Waste (MSW) and Municipal Solid Waste Management (MSWM) have been defined diversely by various authors/authorities. According to Schübeler et al. (1996) MSW is defined as "... *refuse from households, non-hazardous solid waste from industrial, commercial and institutional establishments (including hospitals), market waste, yard waste and street sweepings*", and "*MSWM includes all phases of waste collection, recycling, treatment and disposal."* The main objective of MSWM is to protect environmental and human health, which are vulnerable to poor management of solid waste. MSWM aims to promote the quality of the urban environment by controlling environmental pollution including waste, air, soil and cross media pollution. In addition, MSWM aims to support the efficiency and productivity of the economy by providing required services for waste management and ensuring its efficient use. The employment and income generated by well-planned waste management activities is an ultimate endeavour of MSWM (Schübeler et al. 1996).

An Integrated Waste Management (IWM) approach is usually considered by countries in relation to income levels, whilst planning to develop strategies for waste management. IWM is defined as "... *the selection and application of suitable techniques, technologies and management programs to achieve specific waste management objectives and goals*" (Tchobanoglous and Kreith 2002, p.1.8). In terms of solid waste management, basic management strategies for IWM identified by the United States Environment Protection Agency (USEPA) include source reduction, recycling and composting, combustion (waste-to-energy) and landfill[1]. At first, the strategies evaluate local needs and conditions, and then select the most appropriate management activities that suit the conditions. While planning for

[1] http://www.epa.gov/climatechange/wycd/waste/downloads/overview.pdf

IWM, the institutional, social, financial, economic, technical, and environmental factors are all considered.

Usually, solid waste is considered as the by-product of urban activities due to a resource-incentive and consumer based economic lifestyle. Thus, the management of solid waste has emerged as a serious challenge since more than 50 per cent of the world's population live in cities and it is estimated than the world's population in as it was in 2000, will be the population living in cities by 2050 (Hoornweg and Bhada-Tata 2012). Thus, the volume and composition of the waste is directly linked to the economic development of a country. The World Bank (Hoornweg and Bhada-Tata 2012) estimated that almost 1.3 billion tonnes of MSW are generated globally every year, i.e, 1.2 kg/capita/day. The World Bank reported that 161 countries in the world, with a total urban population of 2,982 million, generated 3,532,255 tonne of MSW per day, which equates to 1.19 kg per capita per day. The projected population for these 161 countries for the year 2025 is 7,648 million of which 4,287 million is urban. Thus, these countries are estimated to generate 6,069,705 tonnes of MSW per day in 2025, which comes to be 1.42 kg per capita per day (Hoornweg and Bhada-Tata 2012). This projection estimates that the urban population in high income countries (HIC) will increase by 43.7 %, increasing total urban MSW generation per day by 71.8 %, in 2025.

1.1.2 Waste generation in high income countries

Most of the HICs are from the Organisation for Economic Co-operation and Development (OECD) region and the Middle East and North Africa (MENA) regions. The World Bank (Hoornweg and Bhada-Tata 2012) reported that 46 HICs with 774 million of urban population generated 1,649,546 tonne of MSW per day, i.e., 2.13 kg urban MSW per capita per day - as presented in **Table 1.1**. The collection of this generated MSW in HICs was quite promising, with a 76 – 100 % MSW collection rate. For example, the total MSW collection rate was found to be 100 % for HIC countries such as Austria and Germany, and 76 % for Ireland. With respect to the disposal of MSW, different countries had taken different strategies, the most popular approach was found to be landfill following recycling. Australia was found to landfill 69.7 % of the total MSW and to recycle the remaining 30.3 %. However, Norway was found to landfill only 26 % MSW whereas 15 % was composted, 34 % recycled and 25 % was managed by waste-to-energy (WTE) technology. **Table 1.1**

projects that the urban population in HICs will increase by 17.8 %, increasing total urban MSW generation per day by 13.9 % by 2025.

Table 1.1 Municipal Solid Waste (MSW) generation in high income countries (HICs) - current status and the projections for 2025. The data presented here are adapted from The World Bank (Hoornweg and Bhada-Tata 2012).

	Current available data			Projected data for 2025		
Country	Total urban population	MSW generation per capita (kg/capita/day)	Total MSW generation (tonnes/day)	Total urban population	MSW generation per capita (kg/capita/day)	Total MSW generation (tonnes/day)
Antigua and Barbuda	24,907	5.5	137	35,000	4.3	151
Australia	16,233,664	2.23	36,164	22,266,000	2.1	46,759
Austria	5,526,033	2.4	13,288	6,204,000	2.15	13,339
Bahamas, The	252,689	3.25	822	346,000	2.9	1,003
Bahrain	574,671	1.1	630	875,000	1.6	1,400
Barbados	92,289	4.75	438	152,000	4	608
Belgium	10,265,273	1.33	13,690	10,511,000	1.8	18,920
Brunei Darussalam	282415	0.87	247	426,000	1.3	554
Canada	21,287,906	2.33	49,616	31,445,000	2.2	69,179
Cyprus	595,707	2.07	1,230	760,000	2.1	1,596
Czech Republic	7,547,813	1.1	8,326	7,575,000	1.65	12,499
Denmark	4,684,754	2.34	10,959	5,027,000	2.15	10,808
Estonia	931,657	1.47	1,367	903,000	1.7	1,535
Finland	3,301,950	2.13	7,030	3,805,000	2	7,991
France	47,192,398	1.92	90,493	53,659,000	2	107,318
Germany	60,530,216	2.1	127,816	61,772,000	2.05	126,633
Greece	6,755,967	2	13,499	7,527,000	2	15,054
Hong Kong, China	6,977,700	1.99	13,890	8,305,000	2	16,610
Hungary	6,717,604	1.92	12,904	7,011,000	2	14,022
Iceland	280,148	1.56	438	314,000	1.7	534
Ireland	2,589,698	3.58	9,260	3,564,000	3	10,692
Israel	5,179,120	2.12	10,959	8,077,000	2.1	16,962
Italy	39,938,760	2.23	89,096	42,205,000	2.05	86,520
Japan	84,330,180	1.71	144,466	86,460,000	1.7	146,982
Korea, South	38,895,504	1.24	48,397	41,783,000	1.4	58,496

Kuwait	2,683,301	5.72	15,342	3,934,000	4	15,736
Luxembourg	390,776	2.31	904	473,000	2.2	1,041
Macao, China	466,162	1.47	685	535,000	1.75	936
Malta	384,809	1.78	685	416,000	2	832
Netherlands	13,197,842	2.12	27,945	14,860,000	2.1	31,206
New Zealand	3,612,147	3.68	13,293	4,229,000	3	12,687
Norway	3,605,500	2.8	10,082	4,187,000	2.3	9,630
Oman	1,629,404	0.7	1,142	2,700,000	1.15	3,105
Portugal	6,162,205	2.21	13,616	7,389,000	2.15	15,886
Qatar	759,577	1.33	1,014	1,066,000	1.7	1,812
Saudi Arabia	15,388,239	1.3	20,000	29,661,000	1.7	50,424
Singapore	4,839,400	1.49	7,205	5,104,000	1.8	9,187
Slovak Republic	3,036,442	1.37	4,164	3,300,000	1.6	5,280
Slovenia	986,862	1.21	1,192	958,000	1.7	1,629
Spain	33,899,073	2.13	72,137	37,584,000	2.1	78,926
Sweden	7,662,130	1.61	12,329	8,525,000	1.85	15,771
Switzerland	5,490,214	2.61	14,329	6,096,000	2.3	14,021
Trinidad and Tobago	144,645	14.4	2,082	291000	10	2,910
United Arab Emirates	2,526,336	1.66	4,192	5,092,000	2	10,184
United Kingdom	54,411,080	1.79	97,342	59,738,000	1.85	110,515
United States	241,972,393	2.58	624,700	305,091,000	2.3	701,709

1.1.3 Waste generation in the low income countries

Most of the lower income countries (LICs) are from the African region (AFR). The World Bank (Hoornweg and Bhada-Tata 2012) reported that 38 LICs with 343 million of urban population generated 204,802 tonnes MSW per day, i.e., 0.6 kg urban MSW per capita per day, as presented in **Table 1.2**. The collection of this generated MSW in the LICs was not efficient, with a 10.6 – 55.0 % MSW collection rate. For example, the total MSW collection rate was found to be 11 % for Haiti, whereas the collection of urban MSW was found to be 94 % for Nepal. With respect to the disposal of MSW, different countries had adopted different strategies, the most popular approach was found to be landfill following dumping.

The African country Uganda was found to landfill 100 % of the total MSW whereas Cambodia was found to dump 100 % of the 75 % urban waste collected. **Table 1.2** projected that the urban population in LICs will increase by 97.1 %, increasing total urban MSW generation per day by 185.3 % by 2025.

Table 1.2 Municipal Solid Waste (MSW) generation by low income countries (LICs) - current status and the projections for 2025. The data presented here are adapted from The World Bank (Hoornweg and Bhada-Tata 2012).

	Current available data			Projected data for 2025		
Country	Total urban population	MSW generation per capita (kg/capita/day)	Total MSW generation (tonnes/day)	Total urban population	MSW generation per capita (kg/capita/day)	Total MSW generation (tonnes/day)
Bangladesh	38,103,596	0.43	16,384	76,957,000	0.75	57,718
Benin	3,147,050	0.54	1,699	7,286,000	0.75	5,465
Burkina Faso	2,549,805	0.51	1,288	6,899,000	0.75	5,174
Burundi	700,922	0.55	384	2,577,000	0.8	2,062
Central African Republic	1,596,934	0.5	795	2,634,000	0.7	1,844
Chad	2,566,839	0.5	1,288	6,566,000	0.7	4,596
Comoros	161,070	2.23	359	405,000	2.1	851
Congo, Dem. Rep.	18,855,716	0.5	9,425	48,980,000	0.75	36,735
Cote d'Ivoire	9,006,597	0.48	4,356	15,677,000	0.7	10,974
Eritrea	878,184	0.5	438	2,368,000	0.7	1,658
Ethiopia	12,566,942	0.3	3,781	30,293,000	0.65	19,690
Gambia	822,588	0.53	438	1,726,000	0.75	1,295
Ghana	11,680,134	0.09	1,000	19,713,000	0.5	9,857
Haiti	3,227,249	1	3,233	7,966,000	1.4	11,152
Kenya	6,615,510	0.3	2,000	16,952,000	0.6	10,171
Lao PDR	1,916,209	0.7	1,342	3,776,000	1.1	4,154
Madagascar	4,653,890	0.8	3,734	11,350,000	1.1	12,485
Malawi	2,288,114	0.5	1,151	6,158,000	0.8	4,926
Mali	3,900,064	0.65	2,534	8,987,000	0.95	8,538
Mauritania	1,197,094	0.5	603	2,203,000	0.8	1,762
Mozambique	7,706,816	0.14	1,052	14,493,000	0.5	7,247
Myanmar	12,847,522	0.44	5,616	24,720,000	0.85	21,012

Nepal	3,464,234	0.12	427	10,550,000	0.7	7,385
Niger	2,162,063	0.49	1,068	5,503,000	0.75	4,127
Nigeria	73,178,110	0.56	40,959	126,634,000	0.8	101,307
Pakistan	60,038,941	0.84	50,438	104,042,000	1.05	109,244
Rwanda	1,573,625	0.52	822	3,831,000	0.85	3,256
Sao Tome and Principe	88,673	0.49	44	155,000	0.9	140
Senegal	4,693,019	0.52	2,438	8,992,000	0.85	7,643
Sierra Leone	2,029,398	0.45	904	3,949,000	0.85	3,357
Solomon Islands	50,992	4.3	219	183,000	4	732
Tajikistan	1,653,091	0.89	1,479	2,774,000	1.2	3,329
Tanzania	9,439,781	0.26	2,425	21,029,000	0.55	11,566
Togo	2,390,840	0.52	1,233	5,352,000	0.85	4,549
Uganda	3,450,140	0.34	1,179	9,713,000	0.65	6,313
Vietnam	24,001,081	1.46	35,068	40,505,000	1.8	72,909
Zambia	4,010,708	0.21	842	6,862,000	0.55	3,774
Zimbabwe	4,478,555	0.53	2,356	7,539,000	0.7	5,277

1.1.4 Characteristics of Municipal Solid Waste

Detailed waste characterization is essential for the sustainability of efficient and effective solid waste management. It also prompts policy makers to adopt appropriate waste management strategies. The characteristics of municipal solid waste (MSW) varies with communities and countries[2] and is based on geographic location, economic conditions, climatic conditions, season, extent of urbanization and many other social factors such as food habits, local activities and cultural traditions (Ogwueleka 2009; Das and Bhattacharyya 2013). Generally, MSW is characterised by the type of waste and its quantity and properties. MSW is mainly comprised of organic waste, paper, plastics, metals and glass. The various types of waste and their sources are presented in **Table 1.3**.

[2]http://www.britannica.com/EBchecked/topic/553362/solid-waste-management#toc72378

Table 1.3 MSW characterization based on the type of waste and its source - adapted from Hoornweg and Bhada-Tata (2012).

Type	Sources
Organic	Food scraps, yard (leaves, grass, brush) waste, wood, process residues.
Paper	Paper scraps, cardboard, newspapers, magazines, bags, boxes, wrapping paper, telephone books, shredded paper, paper beverage cups. Strictly speaking paper is organic but unless it is contaminated by food residue, paper is not classified as organic.
Plastic	Bottles, packaging, containers, bags, lids, cups.
Glass	Bottles, broken glassware, light bulbs, colored glass.
Metal	Cans, foil, tins, non-hazardous aerosol cans, appliances (white goods), railings, bicycles.
Other	Textiles, leather, rubber, multi-laminates, e-waste, appliances, ash, other inert materials.

The composition of MSW generated globally in 2009 is delineated in **Figure 1.1**, which shows that the majority of MSW comprises of organic waste followed by paper, plastic, glass and metal. "Other waste' is that listed in **Table 1.3**.

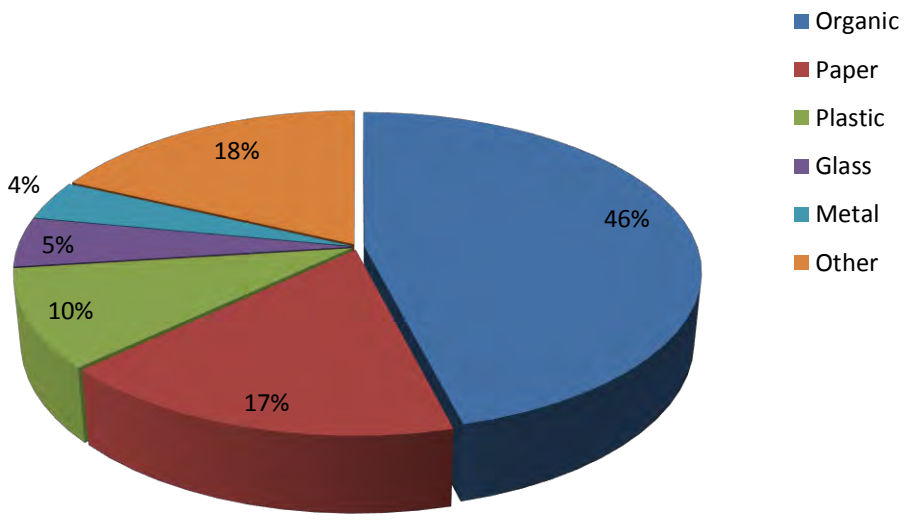

Figure 1.1 The composition of the global municipal solid waste generated in 2009 (Hoornweg and Bhada-Tata 2012).

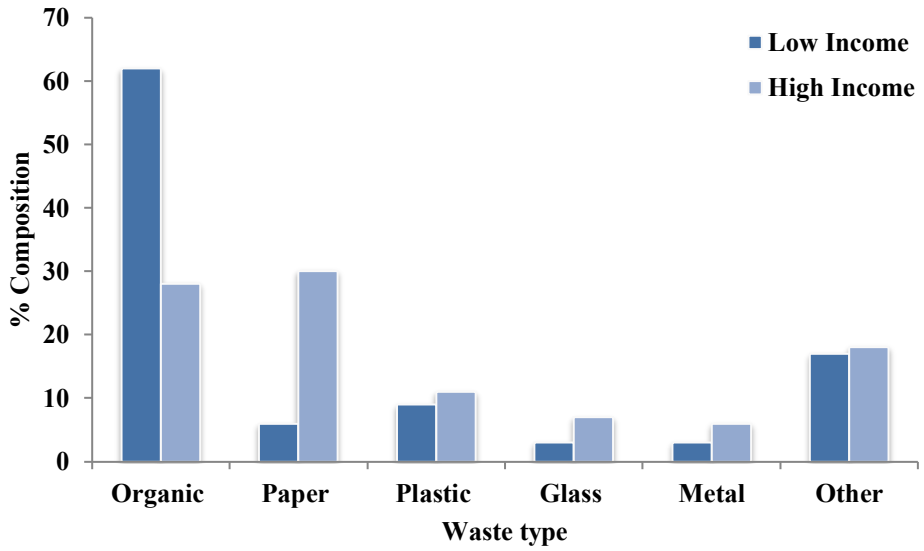

Figure 1.2 A comparison between the compositions of the municipal solid waste generated in high income countries (HICs) and low income countries (LICs) (Hoornweg and Bhada-Tata 2012).

The composition of organic waste is significantly higher in low income countries than in high income countries; 62 % for LIC and only 28 % for HIC, as presented in **Figure 1.2**. However, the composition of inorganic waste such as paper, plastic, metal and glass is higher in HIC than in LIC. This variation in the composition of the MSW could be attributed to the high consumption of packaging materials in HICs.

Chandrappa and Das (2012b) characterized MSW based on a function of the lifestyle and living standards of a region's inhabitants. The MSW generation in relation to the GDP per capita shows that the countries with high GDPs generate higher quantities of waste with higher fractions of non-degradable waste. Conversely, the countries with low GDPs generate higher quantities of waste with higher fractions of degradable organic waste. For example, Japan with a $31,267 GDP per capita (2005 US $) generated 1.47 kg MSW per capita per day, whereas Nepal with a $1,550 GDP per capita (2005 US $) generated only 0.5 kg MSW per capita per day.

The characteristics of MSW affect the overall management of waste such as the storage, collection, transfer and final disposal. For example, Ogwueleka (2009) reported that the density of solid waste in Nigeria ranged from 250 - 370 kg/m^3 which is higher than the solid waste densities found in developed countries. This increases the requirement for high

capacity waste storage and collection facilities and reduces the effectiveness of compaction for waste transfer.

1.1.5 The rule of the "5Rs" for sustainable waste management

The "5Rs" which are refusal, reduction, reuse, recycling and responsibility are the most important factors in sustainable MSWM. In a traditional waste hierarchy, only 3Rs (Reduce, Reuse and Recycle) tend to be discussed. However, even more "Rs" are being proposed to integrate over time, such as Rethink, Repair, Regulate, Research, Redesign[3]. By refusing and reducing products, which are not environmentally sound, prevents the production of such products, hence minimizing the extraction of raw materials[4]. A simple amendment in the consumption pattern such as avoidance of single-used/disposable goods, selection of products with less packaging, and taking one's own bags while shopping, minimizes waste generation. An adoption of recent technologies also helps to reduce waste, for example, use of electronic mail and news promotes paperless communication. The product that cannot be refused or reduced should be reused without modification. For example, the use of empty tins, glass and plastic bottles as storage containers, newspapers as packaging materials, and donating used clothes to charity, all serve to prevent waste generation. Recycling is the processing of waste into the same or different products, such as food waste that can be used as feed stocks for cattle or plastic products that can be melted and remoulded to the same or different product. Again, the product that cannot be further processed can be used to recover resources. For example, anaerobic digestion of food wastes to recover energy, which is discussed further in **Section 1.1.6**. Resource recovery is usually done through source segregation and materials recycling facilities (MRFs) (Pichtel 2005). However, it should be noted that reuse and recycle was never a novel approach in low income developing countries (Chandrappa and Das 2012a). Due to poverty, the waste for someone was often being used as a resource for another so that they could save or earn money. Responsibility for the waste is probably the most essential of the Rs. Every producer and consumer of the product should be responsible for its safe and efficient management after the production and use of the product. 'Product stewardship' is the best approach to make producers and consumers equally responsible. According to the Australian Government, product stewardship "*acknowledges that those*

[3] https://journeytotheplasticocean.wordpress.com/2013/01/30/reduce-reuse-recycle/
[4] http://www.epa.nsw.gov.au/wastestrategy/waste-hierarchy.htm

involved in producing, selling, using and disposing of products have a shared responsibility to ensure that those products or materials are managed in a way that reduces their impact, throughout their lifecycle, on the environment and on human health and safety"[5]. However, despite adopting the 5Rs approach, still there will be a large amount of waste remaining to be disposed of. Arguably, the only way to dispose of such waste in an environmentally sound way is to use correctly-designed and well-operated sanitary landfill sites (Schübeler et al. 1996).

1.1.6 Waste recycling technologies

Despite the fact that MSWM has negative effects, as it promotes disease causing microorganisms, attracts disease vectors, generates foul odours, diminishes the aesthetic value of the environment, occupies space that could be used for other purposes and pollutes the environment, MSWM also has a potential to be recovered as raw material to produce goods, feedstock for composting and to derive fuel (Liu and Liptak 1997). However, the recovery of materials and/or energy is usually dependent on the chemical composition of the solid waste – the individual chemicals as well as the heat value (Samah et al. 2013). The chemical composition is generally represented as the proximate and ultimate composition of MSW. The proximate composition includes ash content, volatile matter and fixed carbon while ultimate composition also includes carbon, hydrogen, nitrogen, chlorine, sulfur and oxygen. Liu and Liptak (1997) have provided the values for these compositions as percentages based on dry MSW (p. 1153). The MSW with a high content of nitrogen and moisture, such as food wastes, are more easily biodegradable than those with low nitrogen and moisture content such as wood and cotton. Thus, the MSW with less nitrogen and moisture content are not suitable for composing. With respect to the energy value, plastic wastes have higher heat value than paper and organic wastes, as delineated in **Table 1.4**.

[5] http://www.environment.gov.au/protection/national-waste-policy/product-stewardship

Table 1.4 Representative heat values of MSW. The values shown here are the higher heating values[6], and in this case the energy required to drive off the moisture formed during combustion is not deducted.

Waste type	Dry-basis heat value (Btu/lb)	As-received heat value (Btu/lb)	Moisture content (%)
Organic	9154	6175	32.5
Food waste	8993	3108	65.4
Yard waste	7731	3565	53.9
Wood	8430	7186	14.8
Textiles	9975	8733	12.4
Paper	7587	5767	24.0
Plastic	16499	14301	13.3
Inorganic	0	0	0.0

Source: Liu and Liptak (1997)

In many developing countries, the waste composition mainly comprises the organic waste, which has high energy value, **Figure 1.2** & **Table 1.4**. The biodegradable part of MSW can be converted to usable products and ultimately to energy in a number of ways, including (1) combustion to produce steam and electricity; (2) pyrolysis to produce a synthetic gas, liquid or solid fuel, and solids; (3) gasification to produce synthetic fuel; (4) biological conversion to produce compost; and (5) biodigestion to generate methane and to produce a stabilized organic humus. The appropriate technologies for solid waste recovery and recycling are discussed below with respect to the relevant physical, chemical and biological transformation processes.

1.1.6.1 Physical transformation

The principal physical transformations that are performed in solid waste management systems are (1) component separation, (2) mechanical volume reduction, and (3) mechanical size reduction. The physical transformation of solid waste is usually carried out for the processing and the recovery of the individual waste components (Tchobanoglous et al. 1993).

[6] The higher heating value (HHV) includes the latent heat of vaporisation of the water created during combustion.

Component separation is a process to transform a heterogeneous waste into a number of homogeneous components. This process recovers the reusable and recyclable materials from MSW, removes contaminants from separated materials to improve the specifications of the separated material and removes hazardous wastes. The most used methods for separation are hand/manual sorting, in which people physically remove materials from the waste stream, magnetic field separation (magnetic materials are separated from non-magnetic materials), and automated sorting where materials are separated based on their individual characteristics.

Mechanical volume reduction, also termed densification, is the process of reducing the initial volume occupied by a waste and increase the density of recovered materials in order to reduce transportation costs and simplify storage. This is usually done by the application of force or pressure. Some of the examples of mechanical volume reduction include, the use of baling for cardboard, paper, plastics, and aluminium cans, and the use of cubing and pelletizing for the production of densified refuse derived fuel (RDF) (Tchobanoglous and Kreith 2002). In most cities in the HICs, the vehicles used for the collection of wastes are equipped with compaction mechanisms so that the quantity of waste collected per trip can be increased.

Mechanical size reduction is a process to reduce the size of waste material in order to obtain a final product that is reasonably uniform and considerably reduced in size in comparison to its original form. Practically, the terms shredding, grinding, and milling are used interchangeably with mechanical size reduction. The waste materials thus reduced in size are more efficient to transport and process. For example, in Nepal recyclable plastic bottles are shredded into small plastic pieces before being exported to China.

1.1.6.2 Chemical transformation

The chemical processes for the transformation of MSW mainly involve (1) combustion (chemical oxidation), (2) pyrolysis and (3) gasification. Chemical transformation of solid waste changes the phase (e.g., solid to liquids, solids to gas, etc.) of materials, in order to reduce the volume and/or to recover the products.

Combustion (Chemical oxidation), traditionally known as incineration, is a chemical process in which an organic material reacts with oxygen to produce heat. The temperature in the combustion furnace, the time of residence of the combustion products at the furnace temperature and turbulence within the furnace, affect the combustion of materials (Tchobanoglous and Kreith 2002). Thus in presence of excess air and under ideal conditions, the combustion of the organic fraction of MSW may be represented as follows:

$$\text{Organic matter} + \text{excess air} \rightarrow N_2 + CO_2 + H_2O + O_2 + \text{ash} + \text{heat}$$

The end products derived from the combustion of MSW include hot combustion gases – composed primarily of nitrogen (N_2), carbon dioxide (CO_2), water (H_2O, fuel gas), oxygen (O_2) and non-combustible residue. USEPA has listed three types of technologies for the combustion of MSW, namely mass burn facilities, modular systems and refuse derived fuel systems[7].

Pyrolysis is a process of a destructive distillation of a solid, carbonaceous, material in the presence of heat and in the absence of stoichiometric oxygen (Tchobanoglous and Kreith 2002). It is an exothermic reaction. An idealized pyrolytic reaction is shown as follows:

$$C_6H_{10}O_5 \xrightarrow{\text{heat}} CH_4 + 2CO + 3H_2O + 3C$$

The three major components resulting from the pyrolysis of the organic portion of MSW are – a gas stream primarily containing hydrogen (H_2), methane (CH_4), carbon monoxide (CO), CO_2, and various other gases, depending on the organic characteristics of the waste material being pyrolyzed; tar and/or an oil stream that is liquid at room temperature and that contains chemicals such as acetic acid, acetone, and methanol; and a char consisting of almost pure carbon plus any inert material that may have entered the process.

Gasification is a process involving partial combustion of carbonaceous materials to generate a combustible fuel gas rich in carbon monoxide, hydrogen, and some saturated hydrocarbons, principally CH_4 - a mixture known as syngas (Klein 2002). The process involves the partial oxidation (combustion) of a solid waste in which insufficient oxygen is provided. Gasification is an exothermic process and some heat is also required to initiate the process.

[7] http://www.epa.gov/wastes/nonhaz/municipal/wte/basic.htm

Generally, the syngas generated from gasification has a net calorific value[8] of 4 - 10 MJ/Nm^3. This gas has many applications such as burning in a boiler to generate steam which may be then used for power generation or industrial heating. Also, it may be used as a fuel in a dedicated gas engine (Arena 2012).

1.1.6.3 Biological transformation

The biological transformation of the organic fraction of MSW may be used to reduce the volume and weight of the material. It involves composting (aerobic and/or anaerobic) technology to produce compost, which is a humus-like material that can be used as a soil conditioner, and anaerobic digestion technology to recover energy. The biological transformation of organic waste is typically carried out by various microorganisms such as bacteria, fungi, yeasts and actinomycetes. These biological processes that may be used for the conversion of the organic fraction of MSW are discussed below.

Composting is a process involving the biological decomposition of the organic solid waste that is controlled by a diverse microbial community, dominated especially by naturally occurring bacteria, actinomycetes, fungi and protozoa (Pichtel 2005), in presence (aerobic) or absence (anaerobic) of oxygen. The composting of MSW involves three steps – preparation, decomposition and product preparation (Chandrappa and Das 2012a). Besides microbial activities, the MSW composting process is affected by many other factors such as the nature of the waste, dissolved organic carbon content, electrical conductivity, the C/N ratio, the moisture content, temperature, pH, aeration and available nutrients (Adhikari et al. 2008; Adhikari et al. 2010; Shen et al. 2015). This is discussed further in **Chapter 5**. Some of the popular composting systems include open systems such as turned piles, turned windrows, static piles using stir blowing, or closed systems such as rotating drums, tanks (Epstein 1996; Michel Jr et al. 1996; Pichtel 2005) and other systems such as Vermicomposting (Sinha et al. 2008a; Sinha et al. 2008d). Composting the organic fraction of MSW under aerobic condition can be represented by the following equation:

$$\text{Organic matter} + O_2 + \text{nutrients} \rightarrow \text{new cells} + \text{resistant organic matter} + CO_2 + H_2O + NH_3 + SO_4^{2-} + \text{heat}$$

[8] http://altenergymag.com/content.php?issue_number=09.06.01&article=zafar

The principal end products in the above reaction are new cells, resistant organic matter, carbon dioxide, water, ammonia, and sulphate. Compost is the resistant organic matter that remains, which contains a high percentage of lignin that is difficult to break down biologically in a relatively short time. Lignin, found most commonly in newsprint, is the organic polymer that holds together the cellulose fibers in trees and certain plants. Thus, composting is the biological recycling of organic waste into fertilizer, which retrieves nutrients from organic wastes.

Anaerobic digestion is a biological process involving the conversion of the biodegradable part of MSW to a gas containing carbon dioxide and methane (CH_4), under anaerobic conditions. This conversion can be represented by the following equation (Pichtel 2005):

$$\text{Organic matter} + H_2O \rightarrow \text{new cells} + \text{organic matter} + CO_2 + CH_4 + NH_3 + H_2S + \text{Heat}$$

The anaerobic digestion of organic solid waste occurs in three stages, namely hydrolysis, acid generation and methanogenesis. The microbial communities, represented by *Bacteroidales, Lactobacillales, Rhodospirillales, Clostridiales and Synergistales,* in the digester, are responsible for the conversion of organic solid waste into soluble organic compounds, such as Volatile Fatty Acids (VFAs) (Wan et al. 2013). These intermediate products further serve as substrates for methanogens, to produce 'biogas'. Temperature is one of the key factors which affect the digestion process; usually the digestion is carried out under mesophilic (Dong et al. 2010) or thermophilic (Beevi et al. 2015) conditions. The gas generation rate in thermophilic conditions is reported to be two to three times higher than in mesophilic conditions (Cecchi et al. 1991; Fernández-Rodríguez et al. 2015).

1.1.6.4 Importance of waste transformations in solid waste management

The above discussed physical, chemical, and biological transformations are used (1) to improve the efficiency of solid waste management operations and systems, (2) to recover reusable and recycled materials, and (3) to recover conversion products and energy. The segregation of biodegradable and non-biodegradable parts of waste from MSW is required for the transformation processes. Thus, chemical and biological transformation involves energy recovery from waste by converting non-recyclable waste materials into useable heat,

electricity, or fuel through a variety of processes. These include combustion, pyrolysis, gasification, anaerobic digestion and recovery of landfill gas. These processes are known as waste-to-energy (WTE) technologies (Pham et al. 2014; Arena et al. 2015).

1.2 Wastewater management

1.2.1 Background

Water and sanitation in developing countries has been emerging as a challenging issue exacerbated by water scarcity. The ecosystems of the earth stand to be affected by a loss of biodiversity and agricultural production with water shortages (Latif et al. 2011). Renewable internal freshwater resources per capita is decreasing worldwide (6945.9 m^3 in 2002, 6307 m^3 in 2010, 6125.7 in 2012 and 6055.1 in 2013) with the exception in few countries including Belarus, Germany and the Russian Federation[9] (The World Bank). In addition, this is likely to promote diseases and malnutrition, increase social instability and conflict and impact economic growth[10] (WSSCC).

The World Health Organization (WHO/UNICEF, 2010) has estimated that 884 million people in the world do not have access to improved drinking water and almost all of that population represents developing countries – Sub-Saharan Africa alone accounting for one third. Based on the estimation of the World Health Organization (WHO), almost 3.2 million people die annually from infectious diseases such as diarrhoea caused by inadequate water availability for good sanitation and by the use of contaminated water (Miller and Spoolman 2007). The United Nations Environment Program (UNEP) reports that out of the total world's freshwater withdrawal, 70 – 90 % is used for agriculture[11]. According to the World Bank (2009), out of the total annual global freshwater withdrawals (3,908.3 billion cubic meters), only 11.7 % is withdrawn for domestic uses while 70.2 % is for agricultural use.

[9] The World Bank http://data.worldbank.org/indicator/ER.H2O.INTR.PC/countries/1W?display=default&http://data.worldbank.org/indicator/ER.H2O.INTR.PC/countries/1W?display=graph 20/04/2015
[10] Water Supply and Sanitation Collaborative Council http://www.wsscc.org/topics/water/water-overview
[11] UNWater http://www.unwater.org/water-cooperation-2013/water-cooperation/facts-and-figures/en/ 24/04/2015

Rapid population growth, a rise in living standards, increasing urbanization and industrialization are some of the reasons behind increasing fresh water demand (Massoud et al. 2009) in developing countries. For instance, in the Arabian Peninsula alone, the domestic water demand is projected to rise to about 10580 MCM in 2025. In Riyad City, Saudi Arabia, the increase in the daily water consumption from 0.219 million m^3/day in 1980 to 1.153 million m^3/day in 1995 and the projection of 3.098 million m^3/day in 2025 illustrates the increasing water demand overtime (Abderrahman 2000).

According to the Global Water Policy Project, most cities in developing countries discharge 80 – 90 % of their untreated sewage directly into rivers, streams, and lakes - whose waters are then used for drinking water, bathing, and washing clothes (Miller and Spoolman 2007). Kivaisi (2001) has presented the example of Lake Victoria in Africa, the second largest lake in the world, which has been greatly affected by anthropogenic activities. Discharge of untreated municipal/domestic wastewater into surface water bodies, which is generally practiced in most developing countries, is a threat to human health and aquatic ecosystems. However, wastewater collection, treatment and its safe discharge is an extra burden to such countries - where development issues take precedent over environmental management. Hence, water resources management for water use in various sectors is one of the primary challenges for developing countries. Though there is a great potential for wastewater recovery and its reuse to address water scarcity, wastewater treatment has been given less priority (Clarkson et al. 2010). Systems with minimum operational costs, simple technology and low energy demand should be considered whilst considering 'environmental performance, economic factors and social sustainability'. In addition, a consideration of the recovery of water, energy and nutrients as resources should also be at the forefront when designing wastewater treatment/management technologies (Fuchs et al. 2011).

Integrated wastewater management (IWWM), as the most widely discussed strategy, has been adopted to address current issues related to water. IWWM considers the life cycle of the wastewater, from its generation to its ultimate reuse and plans for reuse - and involves factor such as wastewater generation and composition, collection, treatment, disposal and recycling. In this management process, sludge treatment should also be included along with wastewater treatment. The sustainability of IWWM depends upon affordability - in terms of capital, operational & maintenance costs, functionality - in terms of locally available technical and

support staff, and reliability - wastewater treatment to produce effluent that is safe for reuse, environmentally sound and considerate of climate-change (Abbassi and Al Baz 2008).

As in developed countries, the increasing scarcity of fresh water, the recovery and safe reuse of treated wastewater for various purposes has also become a particular area of interest in developing countries. Many biological systems for the treatment of municipal/domestic sewage wastewater have been investigated by many researchers - including constructed wetlands (Solano et al. 2004; Kayranli et al. 2010; Wu et al. 2010; Vasudevan et al. 2011), sand filtration (Bahgat et al. 1999; Hamoda et al. 2004; Jenkins et al. 2011), Up-flow Anaerobic Sludge Bed UASB (Sato et al. 2006; Khan et al. 2011), Oxidation Ditch (Chen et al. 2012) and vermifiltration (Sinha et al. 2008b; Xing et al. 2010a; Wang et al. 2011b. Biological wastewater treatment involves the removal of biomass by the conversion of suspended and dissolved solids present in the wastewater - with the help of bacteria (Droste 1997; Tansel 2008). The effluent produced from these systems has the potential to be reused in various sectors including farm irrigation (Fach and Fuchs 2010), in the garden and for toilet flushing.

1.2.2 Water use and wastewater generation in high income countries

Globally, 70 % of total water withdrawal is used in the agricultural sector, 22 % in the industrial sector and only 8 % for domestic use[12]; thus agricultural water withdrawal is the major sector withdrawing significantly more water than any other sector (UNEP 2007). In terms of water use in the HICs, 59 % is used in the industrial sector, 30 % in the agricultural sector and 11 % for domestic use. In most of the HICs, agricultural water withdrawal is higher than municipal water withdrawal; however, it varies from country to country, depending upon its application and is affected by other factors such as rainfall. For example, in Greece, agricultural water withdrawal is 90.4 % while municipal water withdrawal is only 8.9 % of the total water withdrawal (2007 figures), but in Denmark, agricultural water withdrawal is 36 % but municipal water withdrawal is 58.5 % (2009 figures). However, in Germany, industrial water withdrawal is more significant than agricultural and municipal, with only 0.25 % for agriculture and 17.9 % for municipal - the remaining 81.9 % being for industrial purposes (2007 figures). For a country where rainfall is abundant all year round,

[12] http://www.unwater.org/downloads/Water_facts_and_trends.pdf 24/05/2015

such as the United Kingdom, agricultural water withdrawal is < 1 %. Global municipal wastewater production depends on water withdrawal for municipal use. Again, different countries generate differnt amount of municipal wastewater. Mateo-Sagasta et al. (2015) reported that some of the countries, taken together, produce more than half of the global municipal wastewater production - for example, China, India, the United States of America, Indonesia, Brazil, Japan and Russia (where more than 80 % of the global urban population lives) produce more than 167 km^3 of wastewater. With respect to wastewater treatment, the HICs treat approximately 70 % of generated wastewater. In Australia, the use of treated wastewater for irrigation is required due to limited rainfall (Anderson et al. 2008). The data available for water use[13] and wastewater production, collection and treatment[14] in the HICs is given in **Table 1.5**.

[13] Water use in high and low income countries http://www.fao.org/nr/water/aquastat/data/query/results.html
[14]Wastewater production, collection and treatment http://www.fao.org/nr/water/aquastat/data/query/results.html

Table 1.5 Water use and wastewater profiles in high-income countries (10^9 m^3/year). For the data presented here, the respective years are indicated in parentheses.

High income countries	Agricultural water withdrawal (10^9 m^3/year)	Municipal water withdrawal (10^9 m^3/year)	Total water withdrawal (10^9 m^3/year)	Produced municipal wastewater	Collected municipal wastewater	Treated municipal wastewater	Direct use of treated municipal wastewater for irrigation purposes
Antigua and Barbuda	0.0018 *(2012)*	0.0072 *(2012)*	0.0115 *(2012)*	-	-	0.0002 *(1990)*	-
Australia	12.97 *(2013)*	4.259 *(2013)*	19.75 *(2013)*	2.094 *(2008)*	-	2 *(2013)*	0.28 *(2013)*
Austria	0.1 *(2002)*	0.608 *(2008)*	3.657 *(2002)*	1.054 *(2006)*	1.919 *(2010)*	1.899 *(2010)*	-
Bahrain	0.1592 *(2003)*	0.1779 *(2003)*	0.3574 *(2003)*	0.151 *(2011)*	0.101 *(2011)*	0.076 *(2012)*	0.009 *(2008)*
Barbados	0.0548 *(2005)*	0.02 *(2005)*	0.081 *(2005)*	-	-	-	-
Belgium	0.037 *(2007)*	0.728 *(2007)*	6.216 *(2007)*	1.249 *(2002)*	0.1501 *(1999)*	-	-
Brunei Darussalam	0.0053 *(1995)*	0.1515 *(2009)*	0.092 *(1994)*	-	-	-	-
Canada	4.749 *(2010)*	8.99 *(2000)*	42.2 *(1986)*	6.613 *(2009)*	5.819 *(2009)*	3.549 *(2009)*	-
Chile	29.42 *(2006)*	1.267 *(2006)*	35.43 *(2006)*	1.112 *(2011)*	1.067 *(2011)*	0.768 *(2011)*	0.138 *(2008)*
Croatia	0.0086 *(2010)*	0.534 *(2009)*	0.6286 *(2010)*	0.256 *(2011)*	-	0.209 *(2011)*	-
Cyprus	0.159 *(2009)*	0.019 *(2009)*	0.184 *(2009)*	0.0221 *(2005)*	0.023 *(2010)*	0.019 *(2013)*	0.011 *(2010)*
Czech Republic	0.04 *(2009)*	0.709 *(2005)*	1.699 *(2007)*	1.248 *(2009)*	-	-	-
Denmark	0.238 *(2009)*	0.386 *(2009)*	0.66 *(2009)*	0.5 *(2010)*	0.24 *(2010)*	-	-
Equatorial Guinea	0.001 *(2000)*	0.0158 *(2005)*	0.0174 *(2000)*	-	-	-	-
Estonia	0.004 *(2009)*	0.054 *(2005)*	1.796 *(2007)*	0.385 *(2009)*	0.311 *(2009)*	0.19 *(2009)*	-
Finland	0.05 *(2005)*	0.404 *(2005)*	1.634 *(2005)*	-	-	-	-
France	3.143 *(2009)*	5.775 *(2007)*	31.62 *(2007)*	3.79 *(2008)*	3.77 *(2008)*	3.77 *(2008)*	-
Germany	0.081 *(2007)*	5.128 *(2007)*	32.3 *(2007)*	5.287 *(2007)*	5.213 *(2007)*	5.183 *(2007)*	-
Greece	8.458 *(2007)*	0.846 *(2007)*	9.471 *(2007)*	-	0.568(2007)	0.566 *(2007)*	0.069 *(2010)*
Iceland	0.07 *(2005)*	0.081 *(2005)*	0.165 *(2005)*	-	-	-	-
Ireland	0.0033 *(1998)*	0.799 *(2005)*	0.79 *(1979)*	0.783 *(2010)*	0.751 *(2010)*	0.54 *(2010)*	-
Israel	1.016 *(2009)*	0.712 *(2004)*	1.954 *(2004)*	0.5 *(2010)*	0.48 *(2010)*	0.45 *(2007)*	0.279 *(2004)*
Italy	12.89 *(2007)*	9.095 *(2008)*	45.41 *(2000)*	3.926 *(2007)*	-	3.902 *(2007)*	0.087 *(2006)*
Japan	54.62 *(2007)*	17.4 *(2000)*	90.04 *(2001)*	16.93 *(2011)*	12.02 *(2011)*	11.56 *(2011)*	0.0116 *(2009)*

Kuwait	0.4919 *(2002)*	0.4483 *(2005)*	0.9132 *(2002)*	0.292 *(2010)*	-	0.219 *(2012)*	0.109 *(2012)*
Latvia	0.051 *(2007)*	0.16 *(2000)*	0.418 *(2002)*	0.282 *(2009)*	-	0.128 *(2009)*	-
Lithuania	0.079 *(2009)*	0.156 *(2005)*	2.378 *(2007)*	0.262 *(2009)*	-	0.128 *(2009)*	-
Luxembourg	0.0008 *(2010)*	0.043 *(2009)*	0.0602 *(1999)*	0.09 *(2003)*	0.04 *(2008)*	0.04 *(2008)*	-
Malta	0.019 *(2009)*	0.0344 *(2005)*	0.0539 *(2002)*	0.02 *(2009)*	-	0.002 *(1993)*	-
Monaco	0 *(2009)*	0.005 *(2009)*	0.005 *(2009)*	0.008 *(2009)*	-	0.006 *(2009)*	-
Netherlands	0.071 *(2008)*	1.252 *(2008)*	10.61 *(2008)*	1.934 *(2010)*	1.875 *(2010)*	1.875 *(2010)*	-
New Zealand	3.207 *(2010)*	1.02 *(2000)*	4.753 *(2002)*	-	-	0.284 *(1997)*	-
Norway	0.845 *(2006)*	0.833 *(2006)*	2.939 *(2006)*	0.93 *(2010)*	0.883 *(2010)*	-	-
Oman	1.168 *(2003)*	0.134 *(2003)*	1.321 *(2003)*	0.09 *(2000)*	0.045 *(2010)*	0.009 *(2010)*	0.024 *(2004)*
Poland	1.159 *(2009)*	3.667 *(2009)*	11.96 *(2009)*	2.271 *(2011)*	2.089 *(2011)*	1.356 *(2011)*	-
Portugal	6.178 *(2002)*	1.086 *(2005)*	8.463 *(2002)*	0.577 *(2009)*	0.54 *(2009)*	0.27 *(2009)*	-
Puerto Rico	0.0738 *(2005)*	0.9043 *(2005)*	0.995 *(2005)*	-	-	-	-
Qatar	0.262 *(2005)*	0.174 *(2005)*	0.444 *(2005)*	0.274 *(2008)*	-	0.117 *(2012)*	0.078 *(2012)*
Republic of Korea	15.96 *(2003)*	6.62 *(2002)*	25.47 *(2002)*	7.838 *(2011)*	-	6.583 *(2011)*	-
Russian Federation	13.2 *(2001)*	13.4 *(2001)*	66.2 *(2001)*	12.32 *(2011)*	11.33 *(2011)*	-	-
Saint Kitts and Nevis	0.0002 *(2012)*	0.0154 *(2012)*	0.0156 *(2012)*	-	-	-	-
Saudi Arabia	20.83 *(2006)*	2.13 *(2006)*	23.67 *(2006)*	1.546 *(2010)*	1.144 *(2010)*	1.063 *(2010)*	0.535 *(2010)*
Singapore	0.0076 *(1975)*	1.078 *(2005)*	0.19 *(1975)*	0.511 *(2013)*	0.511 *(2013)*	0.511 *(2013)*	-
Slovakia	0.0178 *(2010)*	0.32 *(2007)*	0.688 *(2007)*	0.56 *(2009)*	-	-	-
Slovenia	0.002 *(2009)*	0.165 *(2009)*	0.942 *(2009)*	0.1724 *(2010)*	-	0.126 *(2010)*	-
Spain	19.64 *(2008)*	5.765 *(2008)*	32.46 *(2008)*	3.183 *(2004)*	-	3.16 *(2004)*	-
Sweden	0.107 *(2007)*	0.974 *(2007)*	2.616 *(2007)*	0.671 *(2010)*	-	0.436 *(2010)*	-
Switzerland	0.0684 *(2010)*	1.004 *(2005)*	2.614 *(2000)*	1.409 *(2011)*	1.409 *(2011)*	1.084 *(2011)*	-
Trinidad and Tobago	0.0167 *(2011)*	0.2376 *(2011)*	0.3832 *(2011)*	-	-	0.289 *(2006)*	-
United Arab Emirates	3.312 *(2005)*	0.617 *(2005)*	3.998 *(2005)*	0.5 *(1999)*	-	0.265 *(2001)*	0.14 *(2012)*
United Kingdom	1.111 *(2008)*	7.419 *(2005)*	13.03 *(2007)*	4.089 *(2011)*	4.048 *(2011)*	4.048 *(2011)*	-
United States of America	192.4 *(2005)*	65.44 *(2005)*	478.4 *(2005)*	60.41 *(2008)*	47.24 *(2008)*	40.89 *(2008)*	0.33 *(2004)*
Uruguay	3.17 *(2000)*	0.41 *(2000)*	3.66 *(2000)*	-	-	-	-

1.2.3 Water use and wastewater generation in low income countries

In terms of water use in LICs, 82 % of total water is withdrawn for agricultural use, 10 % for industrial use and only 8 % for domestic use[15]. Agricultural water withdrawal is significantly higher in most LICs than municipal and industrial water withdrawal, with only with few exceptions, see **Table 1.6**. Similar to the HICs the water withdrawal varies from country to country depending upon its application. For example, in Bangladesh, agricultural water withdrawal is 87.8 % whereas municipal water withdrawal is only 10.0 % (2008 figures) but in Sierra Leone, agricultural water withdrawal is 21.5 %, whereas the municipal water withdrawal is 52.3 % (2005 Figures). Needless to say, municipal wastewater generation is dependent on the municipal water withdrawal and usage. However, for LICs, the data that is available on wastewater generation, collection, treatment and use is not adequate. Sato et al. (2013) reported that only ~ 8 % of the wastewater generated in HICs is treated. The use of untreated wastewater for irrigation is a common practice in these countries due to; the unavailability of alternative sources of irrigation, the need to reduce the buying of fertilizers and to save the cost of accessing fresh water (Sato et al. 2013).

Thus, a comparison of the water use and wastewater production, collection and treatment between the HICs and LICs shows, not unexpectedly, that the income (economic) level of the country affects overall trends of water and wastewater management. The HICs, with high industrial activity, withdraw more water for industrial use and the LICs, involved more with the agriculture sector, withdraw more water for agricultural use. Generally, municipal water use is less than agricultural and industrial use in both HICs and LICs. Here the focus is on municipal wastewater production stemming from municipal water use. With respect to the use of wastewater, Sato et al. (2013) estimated that about 1.5 - 6.6 % of the global irrigated area of 301 million hectors is irrigated with wastewater. However, the practice of using treated wastewater is common in HICs and the use of untreated wastewater is common in LICs.

[15] http://www.unwater.org/downloads/Water_facts_and_trends.pdf 24/05/2015

Table 1.6 Water use and wastewater profile in low-income countries (10^9 m^3/year). For the data presented here, the respective years are indicated in parentheses.

Low income countries	Agricultural water withdrawal (10^9 m^3/year)	Municipal water withdrawal (10^9 m^3/year)	Total water withdrawal (10^9 m^3/year)	Produced municipal wastewater
Afghanistan	20 *(1998)*	0.2034 *(2005)*	20.28 *(2000)*	-
Bangladesh	31.5 *(2008)*	3.6 *(2008)*	35.87 *(2008)*	0.725 *(2000)*
Benin	0.059 *(2001)*	0.041 (2001)	0.13 *(2001)*	-
Burkina Faso	0.4207 *(2005)*	0.3756 *(2005)*	0.818 *(2005)*	0.0024 *(2011)*
Burundi	0.222 *(2000)*	0.0431 *(2005)*	0.288 *(2000)*	-
Cambodia	2.053 *(2006)*	0.098 *(2006)*	2.184 *(2006)*	1.184 *(2000)*
Central African Republic	0.0004 *(2005)*	0.0601 *(2005)*	0.0725 *(2005)*	-
Chad	0.6722 *(2005)*	0.1037 *(2005)*	0.8796 *(2005)*	-
Comoros	0.0047 *(1999)*	0.0048 *(1999)*	0.01 *(1999)*	-
Democratic People's Republic of Korea	6.61*(2005)*	0.9028 *(2005)*	8.658 *(2005)*	-
Democratic Republic of the Congo	0.0719 *(2005)*	0.4649 *(2005)*	0.6836 *(2005)*	-
Eritrea	0.55 *(2004)*	0.031 *(2004)*	0.582 *(2004)*	0.018 *(2000)*
Ethiopia	5.204 *(2002)*	0.81 *(2005)*	5.558 *(2002)*	-
Gambia	0.0392 *(1999)*	0.0412 *(2005)*	0.0905 *(2000)*	-
Guinea	0.2929 *(2001)*	0.2248 *(2005)*	0.5533 *(2001)*	-
Guinea-Bissau	0.144 *(2000)*	0.0341 *(2005)*	0.175 *(2000)*	-
Haiti	1.209 *(2009)*	0.258 *(2005)*	1.2 *(2000)*	-
Kenya	2.165 *(2003)*	0.47 *(2003)*	2.735 *(2003)*	-
Liberia	0.0123 *(2000)*	0.0802 *(2005)*	0.1308 *(2000)*	-
Madagascar	16.13 *(2000)*	0.2956 *(2005)*	16.5 *(2000)*	-
Malawi	1.166 *(2005)*	0.1431 *(2005)*	1.357 *(2005)*	-
Mali	5.075 *(2006)*	0.107 *(2006)*	5.186 *(2006)*	0.0117 *(2010)*
Mozambique	0.69 *(2001)*	0.2537 *(2005)*	0.8842 *(2001)*	-
Myanmar	29.57 *(2000)*	-	33.23 *(2000)*	0.016 *(2000)*
Nepal	9.32 *(2006)*	0.1476 *(2005)*	9.497 *(2006)*	-
Niger	0.6565 *(2005)*	0.0617 *(2012)*	0.9836 *(2005)*	0.0047 *(2011)*
Rwanda	0.102 *(2000)*	0.0614 *(2005)*	0.15 *(2000)*	-
Sierra Leone	0.0457 *(2005)*	0.111 *(2005)*	0.2122 *(2005)*	-
Somalia	3.281 *(2003)*	0.015 *(2003)*	3.298 *(2003)*	-
South Sudan	0.24 *(2011)*	0.193 *(2011)*	0.658 *(2011)*	-
Tajikistan	10.44 *(2006)*	0.647 *(2006)*	11.49 *(2006)*	4.7 *(2004)*
Togo	0.076 *(2002)*	0.1407 *(2005)*	0.169 *(2002)*	-
Uganda	0.259 *(2008)*	0.328 *(2008)*	0.637 *(2008)*	0.007 *(2012)*
United Republic of Tanzania	4.632 *(2002)*	0.527 *(2002)*	5.184 *(2002)*	-
Zimbabwe	3.318 *(2002)*	0.589 *(2002)*	4.205 *(2002)*	0.194 *(2012)*

1.2.4 Wastewater Characteristics

Wastewater is water from the community which has been contaminated via various uses and is a mixture of sewage, agricultural drainage, industrial waste effluent and hospital discharge (Tchobanoglous et al. 2003; Latif et al. 2011). Thus raw wastewater contains a wide range of contaminants that includes pathogens such as bacteria, viruses, parasitic protozoans and helminths (Gerardi 2005), organic and inorganic materials, nutrients and oxygen demanding wastes from various sources (Miller and Spoolman 2007). TSS, biodegradable organics, dissolved inorganics, heavy metals, nutrients, pathogens and priority organic pollutants are the most analysed parameters in wastewater - being the major constituents of concern (Crites and Tchobanoglous 1998). The major source of organics in domestic wastewater is human excreta (Droste 1997).

The characterization of any wastewater is vital in planning and designing its collection, treatment, disposal and reuse. Generally, the characterization is done in terms of its physical, chemical and biological properties. The physical properties involve temperature, colour, total solids and odour. The chemical constituents include - organic such as carbohydrates, fat, oil and grease (FOG), pesticides, phenols, proteins, priority pollutants, surfactants and volatile organic compounds; inorganic such as alkalinity, chlorides, heavy metals, nitrogen, pH, phosphorus and sulphur; gases such as hydrogen sulphide, methane and oxygen. The biological constituents include animals, plants and protists such as eubacteria, archaebacteria and viruses (Tchobanoglous et al. 2003).

1.2.5 Centralized vs. decentralized approaches to wastewater management

The centralized approach to wastewater management is based on conventional wastewater collection and treatment systems, and involves disposal/reuse of the treated effluent far from the point of origin; whereas the decentralized approach is based on the collection, treatment and disposal/reuse of wastewater from individual homes and communities at or near the point of origin (Crites and Tchobanoglous 1998). Cook et al. (2009) have reviewed the definition for decentralized wastewater management from different authorities and presented it in simple terms as "*... the systems installed and operated to deliver effluent/wastewater services near to the point of generation in small to medium development areas*". Therefore,

decentralized systems are usually feasible where there is a need to reduce wastewater flow to a centralized treatment system and there is potential for reusing treated wastewater for non-potable purposes. The selection of the correct wastewater management systems, centralized and/or decentralized, depends on many factors and some of the factors to be considered are ecological assessment, hygienic appraisal, analysis of the withdrawal of natural resources and an option for water, energy and nutrients recovery[16] (Orth 2007).

In developing countries, highly centralized approaches are popular for the management of national water resources (Clarkson et al. 2010). However, many wastewater treatment plants in developing countries do not perform to capacity due to a lack of proper knowledge on their operation and maintenance (Wagner and Pinheiro 2001). Centralized wastewater treatment systems are not suitable for developing countries because of the large costs involved in their construction and operation/maintenance (Chen et al. 2009; Massoud et al. 2009). Still, the transformation of a centralized to a decentralized approach is not an easy task and is not possible to achieve in a short period of time. Most centralized systems are conventional and based on high technology and they collect large volumes of wastewater for treatment. In contrast, natural decentralized wastewater treatment systems are popular in small communities due to low costs and high efficiency. In the decentralized management approach, the wastewater is collected, treated and reused or disposed of at a point near to its point of generation. Small wastewater treatment systems are considered to be one approach to decentralized wastewater management. Such systems include pit latrines, composting toilets and septic tanks. Beside these, improved on-site treatment systems used in developing countries include inverted trench systems and aerated treatment units. Moreover, activated sludge treatment, trickling filters, anaerobic or facultative lagoons, anaerobic digestion and constructed wetlands are more advanced treatment systems. Many of these systems have been used in both developed and developing countries (Bogner et al. 2007). Soil based wastewater treatment systems such as constructed wetlands and sand filters and conventional land based treatment systems such as septic tank–soil trench systems, are some of these natural decentralized wastewater treatment systems (Crites and Tchobanoglous 1998). Simple conventional septic tanks are the most popular and the most used decentralized treatment systems worldwide (Clarkson et al. 2010) - especially in developing countries.

[16] http://www2.gtz.de/Dokumente/oe44/ecosan/en-centralised-versus-decentralised-wastewater-systems-2007.pdf

This study has reviewed decentralised biological wastewater treatment systems in terms of the removal efficiency of pollutants such as organic materials and nutrients, **Table 1.7**. Some of these technologies are discussed as follows.

1.2.6 Wastewater treatment technologies

1.2.6.1 Constructed Wetlands

The potential of constructed wetlands (CWs) for wastewater treatment was first experimented in Germany by Seidel in early 1950s (Vymazal et al. 2006). Constructed wetlands (CWs), with subsurface systems, have been in use in Europe, and CWs with free water surface systems have been used in North America and Australia since late 1960s. However, natural wetlands have been used in the United Kingdom for more than a hundred years. Constructed wetlands are biological wastewater treatment systems which are similar to natural wetlands and use natural processes utilizing wetland plants, microorganisms and soil for the treatment of municipal and industrial wastewater (APHA 1998; Crites and Tchobanoglous 1998; Vymazal 2011). Subsequently, many other types of constructed wetlands emerged to treat wastewater from various sources. Vymazal (2011) has listed examples of the use of constructed wetlands for various kinds of wastewater in different countries. Though CWs are used for treating industrial and agricultural wastewaters, landfill leachates and storm runoffs (Kouki et al. 2009), the CWs focused on municipal/domestic wastewater treatment are only discussed here. In Taiwan, CWs have been constructed to reduce river pollution which is caused by the direct discharge of sewerage into rivers without prior treatment. The Kaoping River Rail Bridge Constructed Wetland (KRRBCW) is the largest constructed wetland in Taiwan, which covers 120 hectres with a capacity to receive 17,000 to 19,000 m^3 of wastewater per day. This treatment plant has two systems containing eleven basins and more than twenty wetland plant species such as reed, cattail and bulrush. An investigation carried out by Wu et al. 2010, showed that the CW system removed 97 %, 55 % and 30 % of total coliforms, BOD_5 and nutrients respectively. However, the study revealed that the wetland sediment contained high amounts of metals (Cu, Fe, Zn, Cr and Mn), organic content and nutrients, thus raising concern over the potential release of these pollutants into the wetland system.

Table 1.7 Some decentralized wastewater treatment techniques.

Treatment Techniques	Countries practiced/ experimented	BOD_5 Removal %	COD Removal %	Total Nitrogen	Total Phosphorus	Total suspended solid %	Capacity/ Volume (m^3/day)	Reference
Anaerobic Filter using small lava stones	Mexico	-	38 - 48	-	-	73 - 79	-	Gonzalez-Martinez et al. (2011)
Rotating Biological Contactor	China	97 - 99	90 - 93	89 – 92 *	48	97 - 98	10	Zhang & Tan (2010)
Ceramic Filter (Clay and Rice bran) as a membrane in MBR	Bangladesh	> 99	97 - 99	88 - 98	72 – 96	-	-	Hasan et al.(2011)
Anaerobic Digestion + Aerobic Sand Filtration	Indonesia	-	86	99	-	-	-	Fach and Fuchs (2010)
Up-flow Septic Tank/ Baffled Reactor (USBR)	Egypt	81	84	89	-	-	-	Sabry (2010)
Constructed Wetlands	India Nepal	73 - 90	80	38 - 48	40 – 56	89 - 75	-	Vasudevan et al. (2011) Singh et al. (2009)
Sequential Anaerobic-Aerobic	-	-	85 - 93	-	-	85 - 95	-	Kassab et al. (2010)
Up-flow Anaerobic Sludge Blanket	Israel	-	78#	-	-	-	-	Lew et al. (2009; 2011)
Vermifiltration	China	91 - 98	80 - 90	35 – 51 *	-	90 - 98	-	Xing et al. (2005) Sinha et al. (2008c)

Life cycle assessment carried out by Fuchs et al. (2011), to compare CWs with conventional wastewater treatment such as activated sludge technology, revealed that CWs have comparatively less environmental impact in terms of global warming potential and energy use (Dixon et al. 2003).

Vertical flow constructed wetlands (VFCW) are more efficient than horizontal flow constructed wetlands (HFCW) in terms of denitrification (nitrogen removal). Moreover, VFCWs have less footprint and hence less environmental impact over the entire life cycle in order to meet the same effluent standards compared to HFCW (Fuchs et al. 2011).

Sedimentation, filtration, chemical precipitation and adsorption, microbial interaction and plant uptake are some of the mechanisms that are involved in constructed wetlands for the removal of pollutants such as pathogens, organic and inorganic matter (Kivaisi 2001). The most prevalent problem with CWs is clogging caused by the accumulation of suspended solids and sludge produced by the microorganisms, leading to system failure by reducing oxygen supply (Kouki et al. 2009). The clogging problem in CWs can be solved by reducing loading rates. Moreover, some of the beds can be left to rest so that the organic materials responsible for blockage can degrade hence restoring the hydraulic conductivity (Rousseau et al. 2008). However, pre-treatment of wastewater before discharging into the constructed wetlands can protect it from clogging due to removal of solid particles and this can also reduce the land requirement due to less organic loading (Ayaz et al. 2012). For example, the pilot-scale hybrid wetland built for domestic wastewater treatment at the campus of TIBITAK-MRC in Turkey receives almost 3000 L/day from 30 residents after pre-treatment in anaerobic reactors.

CWs are becoming popular in developing countries because of their simple design, effective contaminant removing capacity, reliability and low operational cost. Despite its popularity and efficiency in removing pollutants, the lack of availability of land in highly urbanised areas (Singh et al. 2009) and the high costs associated with treatment plant construction and maintenance (Rammont and Amin 2010) present impediments to the application of CW systems. In this regard, highly effective filter materials such as lava sand, which has high purification efficiency, can be used to reduce the land requirement. A comparative study on the physical, chemical and mineralogical properties of lava sands, conventional sands and fluviatile sands, show that the zeolite minerals present in lava sand make it more efficient

than other two types of sand. A CW with lava sand as a filter material, constructed at Saarland, Germany, achieved a removal efficiency of 93.2 % and 77.5 % of COD and phosphorous respectively (Bruch et al. 2011). This system had a population equivalent (PE) of 100, was planted with *P. australis* (reed) and had an average loading of 80 mmd^{-1} in dry weather and 120 mm^{-d} in rainy season.

Many developing countries with tropical and subtropical climates have a potential for using CWs due to the remarkable biological activities of the biota in the wetlands of such regions (Kivaisi 2001). Pollutant removal by macrophytes in CWs is defined by their developmental stages. Macrophytes such as reed and cattails grow to their optimum size during the autumn season, beginning their life cycle during the spring. The most common species of macrophytes used in CWs is *Phragmites australis* (Duarte et al. 2010). However, these can be replaced by ornamental plants such as *Zantedeschia aethiopica*, *Strelitzia reginae*, *Anthurium andreanum* and *Agapanthus africanus,* which have economic value (Zurita et al. 2011).

1.2.6.2 Up-flow Anaerobic Sludge Bed

Many studies have been conducted on the application of anaerobic processes in domestic/municipal wastewater treatment. The various technologies include, the anaerobic fluidized membrane bioreactor (Kim et al. 2011), the anaerobic filter using small lava stones as a filter media (González-Martínez et al. 2011), anaerobic digestion reactors (Gallagher and Sharvelle 2009), granular sludge blanket reactors (McAdam et al. 2011), anaerobic baffled reactors ABR (Singh et al. 2009), up-flow septic tank/baffled reactors USBR (Sabry 2010) and the UASB (Sato et al. 2006; Gomec 2010; Lew et al. 2011).

Anaerobic treatment of wastewater is widely used worldwide and is a proven technology - due to its simplicity, reliability, robustness and high efficiency. It produces comparatively less sludge than aerobic processes, thus less sludge handling is required. Moreover, no aeration is required, which reduces cost (Lew et al. 2009). Anaerobic biological degradation of organic materials produce methane which has an energy content of 37 MJ/m^3. This was first observed by scientists in the seventeenth century and was applied to raw wastewater in

the 1950s (Droste 1997). Thus, key features of anaerobic systems such as less land requirement, low cost of operation, less sludge production, low energy consumption and energy production in the form of methane gas, are encouraging factors for the adoption of this system rather than an aerobic system. The comparison made by Lettinga (2008) on the advantages and disadvantages of anaerobic systems over aerobic systems, considering both as a first biological treatment step, shows that the beneficial features of anaerobic systems outweigh those of aerobic systems.

Among the thousands of full-scale anaerobic treatment systems, that treat a wide range of industrial wastewater worldwide, approximately 60 % are based on the up-flow anaerobic sludge bed UASB (Jantsch et al. 2002; Karim and Gupta 2003; Gomec 2010) due to its key features that include, high organic loading rates, short hydraulic retention time and less energy consumption (Tchobanoglous et al. 2003). However, the studies have shown that many full-scale UASB facilities are in operation and many are under construction to treat domestic wastewater (Foresti et al. 2006; van Haandel et al. 2006). An investigation conducted by Latif et al. (2011), on the treatment of seven different types of wastewater by an UASB, indicates that a UASB reactor can be applied successfully to a wide range of wastewaters. However, in an anaerobic reactor, some of the environmental variables such as temperature, pH, mixing, ammonia and sulfide control and nutrient requirements, influence the microorganisms' habitat, which affect the process efficiency of a UASB. Moreover, the sludge bed in a UASB, created by the accumulation of suspended solids and bacterial growth in the bottom of the reactor, affects the efficacy as well. Studies on the application of anaerobic reactors for the treatment of different types of wastewater in variable conditions in order to optimize efficiency are on-going. For example, Lew et al. (2009) studied the effect of low temperature (below 20 °C) on the anaerobic degradation pathway and kinetics of domestic wastewater. Lew et al. (2011) also investigated the efficiency of the anaerobic reactor in temperate climates and found that the COD removal rate reduced to 42 % at 10 °C from 78 % at 28 °C with a hydraulic retention time of 6 h. A reduced performance can be improved by integrating an anaerobic filter AF at the top of the reactor of a UASB or by fixing a settler above a gas-liquid-solid separator GLSS.

Despite its efficiency in removing pollutants, even for a high loading rate, and having many more advantages (Latif et al. 2011), UASB effluent often requires further treatment to meet effluent discharge standards (Henze et al. 2008). Hence, it is usually integrated with post

treatment systems such as polishing ponds, constructed wetlands, duckweed ponds, aerated fixed bed reactors, dissolved air floatation (DAF), a submerged aerated bio-filter (SABF), a trickling filter (TF), a rotating biological contactor (RBC), chemically enhanced primary treatment (CEPT) and a zeolite column, a sequencing batch reactor (SBR), vermifiltration (VF), an activated sludge process, and flash aeration in order to get effluent of required quality.

1.2.6.3 Vermifiltration

Vermifiltration (VF) technology, which is also called lumbrifiltration, was first introduced in 1992 by Prof. José Toha at the University of Chile. A full scale VF sewage treatment plant, known as the TOHA vermifiltration system, has been constructed with a treatment capacity of 1000 persons per day (Sinha and Valani 2011). Since then many studies have been conducted on the optimization and performance on VF technology.

VF is defined as 'a process that separates wastewater solids by allowing wastewater to be gravity-fed over the filtration material' (Wang et al. 2011a). *Eisenia fetida* (Indian tiger worm) is a common species of earthworm chosen for wastewater treatment due to its unique feature of its body performing as a 'biofilter'. Other worm species used in VF are versatile 'waste-eating' earthworm species such as the Red Tiger Worm (*Eisenia andrei*) and the Indian Blue Worm (*Perionyx excavates*) (Sinha et al. 2008a; Li et al. 2012; Wang et al. 2013). These worms act as 'an aerator, grinder, crusher, chemical degrader and a biological stimulator'. They are capable of bio-accumulating metals, including heavy metals such as cadmium, mercury, lead, copper, manganese, calcium, iron and zinc in high concentrations. Sinha et al. (2010a) defines vermiculture technology as 'economically viable, environmentally sustainable and socially acceptable technology. Furthermore, he adds that the technology based on earthworms are 'self-promoted, self-regulated, self-improved and self-enhanced, low or no-energy requiring zero-waste technologies, easy to construct, operate and maintain'.

Various studies have been conducted using earthworms in filter beds as a 'biofilter' both at the laboratory and pilot scale. A VF system in China (Wang et al. 2011b) used cubic stages and a tank, each stage comprised of four layers of filter bed; soil, silver sand, fine detritus and

cobblestones. These researchers argue that their system efficiency is related to the "running time" (residency time), the increasing nitrification ability between the stages and the function of metal (Al, Fe, Ca) oxides. Taylor et al. (2003), in his study on a commercial on-site domestic wastewater treatment system 'Biolytix', observed that earthworms are capable of colonizing the filter bed and that this defines the efficiency of the filtration process. The comparative study conducted by Sinha et al. (2008a), found that filtration using earthworms was more effective in removing contaminants than filtration without earthworms. Vermifiltration technology is like 'killing two birds with a single stone'. On the one hand, it is a safe wastewater management technology and on the other hand, it helps sustainable agriculture by producing compost (Sinha et al. 2008a). The use of earthworms for the management of the organic solid waste by means of 'vermicomposting' was in practice for many years. However, its use in wastewater management is a new approach and represents novel technology.

Some researchers have applied vermicomposting to sludge stabilization. For instance, research conducted at Murdoch University involved the application of vermicomposting for the destabilization of sludge from wastewater treatment plants. The vermicomposting reduces the quantity of sludge to be sent to landfills by using the compost produced as fertilizer. The study has listed the advantages of large-scale vermicomposting for sewage sludge stabilization which makes it a viable option for developing countries (Bajsa et al. 2004).

Previous studies show that VF has many applications for the treatment of a wide range of wastewaters. For example, swine wastewater (Li et al. 2008a), rural sewage (Xing et al. 2010b) and household wastewater (Xing et al. 2010a). Only a few known vermifiltration technology studies have been carried out in Chile, India, China, Zimbabwe and Australia. **Table 1.8** and **1.9** provides an overview on some of the previous research on such VF technology.

Table 1.8 Characteristics of various vermifiltration units studied previously, using different filter media, worm species and operating conditions (OCs) and for a range of wastewaters.

Reference	Type of wastewater	Worm species	No. of worms introduced	Filter material
Kumar et al. (2015)	Synthetic domestic wastewater	*Eisenia fetida*	150 ind, Stocking density of 1000/m^3	River bed material Wood coal Glass balls Mud balls
Li et al. (2012)	Raw sewage	*Eisenia andrei*		Quartz sand Turf Wood chips Fibre
Tomar & Suthar (2011)		*P. sansibaricus*	22-24.5 g/L	Large & small stones Gravel Pebble Plastic net Saw dust Dry leaves Sand
Yang et al. (2011)	Municipal	*Eisenia fetida*	11440 ind/m^2	Quartz sand Zeolite
Fang et al. (2010)	Domestic	*Eisenia fetida*	1.69 kg	Artificial soil Sand Gravel Cobble
Wang et al. (2010)	Rural domestic	*Eisenia fetida*		Converter slag Coal cinder
Xing et al. (2010a)	Domestic	*Eisenia foetida*	21000 ind/m^2	Quartz sands Ceramsite
Lu et al. (2009)	Municipal sewage-sludge	*Eisenia fetida*		Quartz sand
Li et al. (2008b)	Swine wastewater	*Eisenia andrei*		Wood chip Bark Peat Straw Vermicompost
Sinha et al. (2008a)	Sewage	*Eisenia fetida* *Eisenia andrei* *Perionyx excavaus* *Eudrilus euginae* *Lumbricus rubellus*	20000 ind/m^2	Gravel Garden Soil

In China, Wang et al. (2011b) investigated a VF system with four filter media; soil mixed with sawdust in 3:1 ratio by volume, earthworm, sand, detritus and cobblestone. They found that the VF layer, in which soil was mixed with saw dust, was more effective than the other layers due to higher porosity and larger surface area[17]. This finding is supported by Kumar et al. (2015), who argue that filter media with relatively larger surface area help to accumulate

[17] More information on the effect of porosity and surface area is provided in a practice guide by Klobes, P., Meyer, K. & Munro, R. G. 2006. *Porosity and specific surface area measurements for solid materials,* US Department of Commerce, Technology Administration, National Institute of Standards and Technology.http://www.glb.nist.gov/customcf/get_pdf.cfm?pub_id=854263 22/03/2014

biomass and perform more efficiently. Many filter media/beds have been used in VF by various researchers. These include ceramsite (Liu et al. 2009), soil mixed with saw dust (Wang et al. 2011b) and quartz sand (Xing et al. 2010a). **Table 1.8** lists the wide variety of materials used as a packing material/filter media in vermifiltration, such as ceramsite, gravel, stones, cobblestones, pebble, saw dust, quartz soil, zeolite, sand, silver sand, soil, detritus, wood coal, mud balls etc. Based on these studies, it may be concluded that the type of media used can affect the treatment efficiency. However, it is noteworthy to mention that the particle size distribution of the filter media also makes difference as it influences microbial activity and flow rates.

Table 1.9 A comparative study on the performance efficiency of some investigated vermifiltration units. Parameters are reported in % removal.

Filter system	HLR, $m^3/m^2/d$	BOD_5	COD	TDS	TSS	NH_4-N	TP	TN	Reference
Lab-scale vermifilter, VFR	1.5	81	72	56	73	76	-248	-	Kumar et al. (2015)
Lab-scale vermifilter, VFC		75	65	54	61	74	-219	-	
Lab-scale vermifilter, VFG		73	62	50	38	58	-156	-	
Lab-scale vermifilter, VFM		71	60	49	36	54	-165	-	
Four-layered vermifilter	0.93	98	70	95	95	-	-	-	Manyuchi et al. (2013)
Three-stage tower earthworm ecofilter	0.25	-	88	-	-	99	99	90	Fang et al. (2010)
	0.5	-	84	-	-	99	99	84	
Three-stage tower vermifiltration	-	-	81	-	-	98	98	60	Wang et al. (2011b)
	-	> 90	80 - 90	90 - 92	90 - 95	-	-	-	Sinha et al. (2010b)
Pilot-scale vermifilter	-	55 - 66	47 - 65	-	57 - 78	21 - 62	-	8 - 15	Xing et al. (2010b)
	-	-	-	-	-	60	30	50	Li et al. 2008b
	-	90 - 98	80 - 86	-	95 - 98	30 - 60	-	-	Xing et al. (2005)

Note: Here, VFR, VFC, VFG and VFM represent the vermifilter with river bed material, wood coal, glass balls and mud balls respectively

A comparative study conducted by Xing et al. (2011) on suitable filter media suggests ceramsite as preferred because of its low sludge yield and good vermicast sludge stabilization. Moreover, cuticle injury of worms in a ceramsite bed was found to be less than that in a quartz sand bed. Similarly, a laboratory-scale study by Wang et al. (2011b) provides evidence of effective removal of COD by the soil sawdust-earthworm layer, and the authors argue that the four-layer VF layer could be effective for domestic wastewater treatment.

In Australia, Sinha et al. (2008a) conducted a comparative study of the vermifiltration system with and without earthworms in the top layer of filter media. The study found that VF with earthworms was more effective in removing contaminants compared to without earthworms. Taylor et al. (2003) observed that the removal rate of COD and BOD_5 from the influent was more efficient as it passed the vermicompost filter bed, and showed that the filter-depth plays a significant role in declining oxygen demand.

With respect to the effect of hydraulic retention time (HRT) and hydraulic loading rate (HLR) on the treatment efficiency of VF, previous studies illustrate that high HRT and low HLR are favourable to obtaining better treatment efficiency. For instance, the study conducted by Fang et al. (2010) on the effect of HLR on the removal of contaminants from synthetic domestic water showed a variance in nutrient removal efficiency; with improved efficiency with a low HLR, see **Table 1.9**. Similarly, a study on variability in the HRT also resulted in a variation in removal efficiency, as reported by Xing et al. (2010a), who observed that the BOD_5, COD, SS, TN and NH_4-N removal rate decreased with an increase in HLR and a decrease in HRT.

In terms of the performance of VF, worm density and their health is another factor which will affect the treatment efficiency. Xing et al. (2010b) observed less density of adults and clitellated earthworms in their VF unit and found that the density of hatchlings and cocoons was high - which is evidence that worms are capable of breeding and incubating in the filter. This study reported that adult worms contribute more than the younger ones in removing contaminants; hence a decline in adult worms in the filter decreased the efficiency of the system. Thus it is important to maintain optimum conditions in the environment where these worms reside. Hughes et al. (2007) reported that the optimum pH for earthworm survival ranges from 6.2 to 9.7. A detail discussion on the basic environmental requirements or the factors affecting the worms, are provided in Chapter 4, **Section 4.1.2.2**.

Moreover, studies have revealed that this simple and low technology biological process is capable of handling a large variation in wastewater characteristics. A series of studies conducted by Wang et al. (2010; 2011a; 2011b; 2013) observed the various physical, chemical and biological processes in VF related to the adsorption of small particle organisms, colloid organisms, molecules and ions and oxidation and reduction. These are the activities that efficiently remove organic matter from the influent. Similarly, in various VF processes the majority of N removal is due to nitrification followed by denitrification (Sinha et al. 2008b; Wang et al. 2010). Thus, Li et al. (2012) has defined the vermifiltration process as a 'sponge' and Sinha et al. (2008a) described the earthworms' body as a 'biofilter'. The biological wastewater treatment process involves the removal of organic pollutants by ingestion, adsorption through the body wall and a biodegradation process in conjunction with other living organisms – i.e. microbes (Sinha et al. 2008a; Tomar and Suthar 2011).

Various designs and combinations have been used to enhance the treatment efficiency of VF. For instance, Xing et al. (2005) studied VF combined with an up-flow anaerobic sludge blanket (UASB) - by setting up a pilot plant at the Shanghai Quyang Wastewater Plant for more than one year. The combination of UASB with VF produces fertilizer (soil conditioner) as a sludge, which only needs to be removed from the system, every six months. This system might be suitable for developing countries because it fulfils sustainable wastewater management criteria. Tomar and Suthar (2011) successfully investigated a combined VF and constructed wetland system at the pilot scale.

Considering the simplicity and efficiency of the technology, many researchers argue that this decentralized biological wastewater treatment technology is economic and suitable for developing countries. Xing et al. (2010a) suggests that VF is suitable for the rural community of China to treat wastewater on-site. The researchers argue that the earthworm bio-filter saves almost 48.72 % in cost compared to conventional activated sludge (Xing et al. 2010a). Another investigation carried out by a group of researchers in China (Wang et al. 2011a) claims that VF is the most economical technology for treating domestic wastewater compared to other proposed solutions such as constructed wetland, soil infiltration and vegetation based wastewater treatment. Bajsa et al. (2004) has listed the benefits of this technology as pollution free, odourless, low cost, with no requirement for the transportation of raw sludge and producing valuable end product instead sludge.

The potential of reuse of the treated wastewater for various applications is another issue that has been investigated. Liu et al. (2009) claims that the effluent from ceramsite vermifiltration is suitable to reuse for flushing toilets, floor washing and garden/crop irrigation. A major concern for the reuse of such wastewater seems to be of nutrients and pathogens. This is discussed further in Chapter 4, **Section 4.3.8**.

1.2.6.4 Sand filtration

Many researchers (Bahgat et al. 1999; Hamoda et al. 2004; Massoud et al. 2009; Zheng et al. 2009; Fach and Fuchs 2010; Bruch et al. 2011) have considered sand filtration as a suitable technology for wastewater treatment in developing countries because it is simple, cheap and needs very little technical knowledge of operation/maintenance - and produces high quality effluent if designed properly. The mechanisms for a sand filter to clarify wastewater are filtration, chemical sorption and assimilation (Lesikar 1999). The initial 20 cm surface layer of the filter is biologically active; therefore major organic breakdown occurs in this layer. However, chemical sorption occurs all over the filter bed. Fach and Fuchs (2010) reported that a laboratory based vertical flow sand filter which used 2/8 mm round gravel and 0/2 mm lava sand was highly efficient in removing 99 % of ammonium and 86 % of COD at a hydraulic loading rate of 75 l/(m^2*day) and 140 g COD/(m^2*day), without any clogging. González-Martínez et al. (2011) used lava stones as a biological filter for municipal wastewater treatment. An anaerobic pilot filter consisting of a 19 cm tube was built vertically and was fed from the bottom with the municipal wastewater with respect to two different hydraulic retention times (HRT) and with two different organic loading rates. Chen et al. (2009) advocated a multi-soil-layering system as a novel technology for wastewater treatment with high efficiency. The authors argue that "the high purification capacity of soil arises from many of its environment-related features, including developed pore systems, co-existence of aerobic-anaerobic organisms and hydrophilic-hydrophobic conditions, as well as habitat for various kinds of microorganisms" (p. 255). Sand size and residence time are the major factors which affect the efficacy of the filter, hence fine sand and long residence time are recommended for efficient removal of bacteria, viruses and turbidity (Jenkins et al. 2011). Sand particles used in the filter should be of the same size otherwise small particles may fill the spaces between the large particles and the system may clog (Lesikar 1999). Sand filtration is a simple technique that provides a habitat for natural bacteria to survive aerobically. Use of

a biosand filtration system (BFS) for drinking water purification has been in common usage for many years (Baig et al. 2011; Jenkins et al. 2011).

1.2.6.5 Ceramic filtration

Membrane filtration is popular in developed countries due to its high organic pollutant and nutrient removal efficiency. However, filter units with organic polymer or inorganic ceramics are not viable in developing countries due to associated high cost. Hence, some researchers have experimented on low-cost ceramic membranes made with local materials such as clay and rice bran (Hasan et al. 2011; Shafiquzzaman et al. 2011), mineral and fly ash (Jedidi et al. 2009; Jedidi et al. 2011), Moroccan natural clay and phosphate (Palacio et al. 2009) and Tunisian natural illite clay (Khemakhem et al. 2007). For example, Hasan et al. (2011) suggested the use, in developing countries, of a ceramic filter made with 80 % clay soil and 20 % rice bran (fired at 900 °C) in a Membrane Bioreactor (MBR), due to the associated low-cost. Their experiments showed a high removal efficiency of BOD_5, COD, N, P and TOC.

1.2.6.6 Novel technologies

Tsuzuki et al. (2010) argues that the introduction of 'soft interventions' in household activities help to reduce wastewater pollutant discharge. For example, if a paper filter is used in kitchens, BOD_5, COD, TN and TP will be removed by 7 %, 7 %, 21 % and 4 %, respectively. Moreover, the fat, oil and grease (FOG) discharge can be reduced simply by prohibiting the dumping of cooking oil and grease into the kitchen sink or wiping the utensils with a paper towel before washing them.

The on-site zero-water discharge system proposed by Wu et al. (2011) is another novel technology for treating wastewater biologically. The system consists of four major stages: anaerobic tank, aerobic bioreactor, activated soil filter and waste-collecting well. The system was applied to treating wastewater from Panlong village of Kunming City, China - with a population of 15,000. The study demonstrated that the system with a hydraulic loading of 350 $m^{3/}$day has 86 %, 87 %, 80 % and 71 % removal efficiency of COD, SS, TP and TN, respectively. The researchers recommend the application of the system, especially in

developing countries, considering its effectiveness in removing pollutants due to its low cost and environmentally friendly features.

A technology designed and developed by the ZAO ECOS Company in Russia, based on a combined system of physicochemical and biological treatment for "weak" wastewater, demonstrated that effluent from the wastewater treatment plant was suitable to be used in fish-breeding reservoirs due to highly efficient nitrogen removal, involving nitrification, denitrification and anammox processes (Nozhevnikova et al. 2012).

Many companies in developed countries have introduced innovative technologies for the recovery of municipal wastewater on-site, with a range of capacities and treatment standards (e.g. Australian-based companies include - Aqua-nova, BioSeptic, Fuji Clean and Supertreat) and United States-based companies include - Orenco and Siemens). Many of these offer combined biological technologies and claim that their technologies are energy efficient, have low footprints and provide high quality effluent. One of the largest European companies in waste and wastewater recovery, Veolia Water[18], manages, operates and maintains thirty-four wastewater treatment plants across Australia and New Zealand, with a treatment capacity varying from 0.1 MLD to 259 MLD. BIOSEP is their most popular technology, being compact in design and with a unique combination of biological treatments using activated sludge and immersed membrane filtration (Veolia).

The Rhizopur® process, developed by Suez Environment, is a combination of three technologies; attached growth treatment such as a trickling filter or a rotating biological contractor, infiltration percolation in a vertical flow constructed wetland and mineralization in a vertical flow constructed wetland - such as using reed beds. Aguilera Soriano et al. (2011), put forward such technology as being suitable for small communities because of its small footprint. In this regard, carbon removal and nitrification in a trickling filter and the use of reed beds reduces the size of the constructed wetland. Moreover, the naturally occurring aeration in the trickling filter and in the reed beds reduces energy requirements by limiting the need for pumping - hence reducing the operating costs. It is reported that the process has high removal efficiencies for BOD_5, COD and TSS, which are more than 90 %, 80 % and 90 % respectively.

[18] An official website: http://www.veolia.com.au/about-veolia/veolia-water

Wormsmart[19] is a single tank biological waste treatment system which "works with nature" and does not use mechanical instruments such as an aerator or blower during the treatment process. The system uses earthworms as a 'biofilter' which break down solid faeces, toilet paper and other organic matter by vermiculture technology.

Ozzi Kleen[20] is sewage and wastewater treatment technology produced by Suncoast Wastewater Management. This has been operating in Australia since 1983 and has more than 14,000 household sewerage treatment systems in operation. Ozzi Kleen wastewater treatment system doesn't use a septic process and only use 'a unique cyclic fully aerobic sewage treatment process'. This environmentally friendly system produces nutrient-rich water which can be reused in the garden. Their more recently developed advanced model has a nutrient removal function which produces water that is suitable for reuse for environmentally restricted applications, such as highly sensitive catchment areas or small-sized blocks.

FujiClean[21] is another biological wastewater treatment system that treats domestic wastewater in an eco-friendly manner and produces recycled water that is safe to reuse in the garden. The system was developed in Japan in the early 1960's and more than 1.6 million units of the domestic wastewater treatment systems have been installed worldwide. The company claims that the system is reliable, efficient, safe, easy to install and maintain, and with a low operational cost. Moreover, the system is odourless and quiet. Recently, the system has been recognized for its high level of nitrogen removal. FujiClean produces two models: the CE-1500 EX and the CRX-1500 that treat wastewater to different levels.

The Living Machine®[22] developed by biologist John Todd is an ecological approach to purifying sewage. The purification process begins with sewage flowing into a series of large open tanks in a solar greenhouse. The first set of tanks contain algae and microorganisms that decompose organic waste in the presence of sunlight – with nutrients being taken up by plants such as water hyacinths, cattails and bulrushes. The water then passes through an artificial marsh of sand, gravel, and bulrush plants that filter out the algae and remaining organic wastes. Some plants absorb toxic metals (e.g. Pb, Hg) and remove pathogens by a

[19] An official website: http://www.wormsmart.com.au/
[20] An official website: http://www.ozzikleen.com/
[21] An official website: http://www.fujiclean.com.au/
[22] An official website: http://www.livingmachines.com/Home.aspx

natural antibiotic process. The effluent from the marsh passes into an aquarium tank which contains snails and zooplankton, which consume the microorganisms. Finally, the effluent is passed to another artificial marsh after ten days of holding in the aquarium. Ultraviolet light or ozone is used for disinfection of the effluent if drinking quality water is required. This system applies three of the four scientific sustainability principles: a) the use of solar energy – a form of renewable energy, b) the use of a natural process for the removal and recycling of nutrients and other organic materials & c) the reliance on a diversity of organisms and natural processes.

1.3 Solid waste and wastewater management in the Nepalese context

In Nepal, solid waste and wastewater, generated in significant amounts in the context of unplanned urbanization, are not being managed adequately due to a high demand on municipal services. Such inadequate and inefficient practices for the management of solid waste and wastewater creates serious health and environmental hazards, especially to those who live along the Bagmati River - as the collected solid waste and wastewater is usually dumped and discharged in and around the river (Dangi et al. 2009; Dangi et al. 2011). The population growth rate, as delineated in **Figure 1.3**, can be considered as indicative of the proportional increase in the solid waste and wastewater generation, that poses a major challenge for municipalities to collect, recycle, treat and dispose of waste.

However, solid waste management prior to the 1980s was quite simple and was relatively uncomplicated due to a smaller population, less industrial activity and an abundance of land (Pokhrel and Viraraghavan 2005). Conflict among government authorities with respect to allocation of responsibility for solid waste management and issues related to the consequences of sanitary landfill sites on the well-being of local residents undermined efficient waste management.

With respect to solid waste generation, data is available only in terms of 58 municipalities in the country, and indicates that an average household (HH) size of 5.4 generates an average HH waste of 0.79 kg/day, which accounts for a total HH waste generation of 769.6 tons/day and a total MSW generation of 1435.05 tons/day for all municipalities combined (ADB

2013). WaterAid[23] reported that less than half of the waste generated in the country gets collected and most of the collected waste is dumped in an unsustainable manner. However, some municipalities such as Kathmandu, Lalitpur and Bhaktapur have introduced relatively more efficient and effective waste management systems.

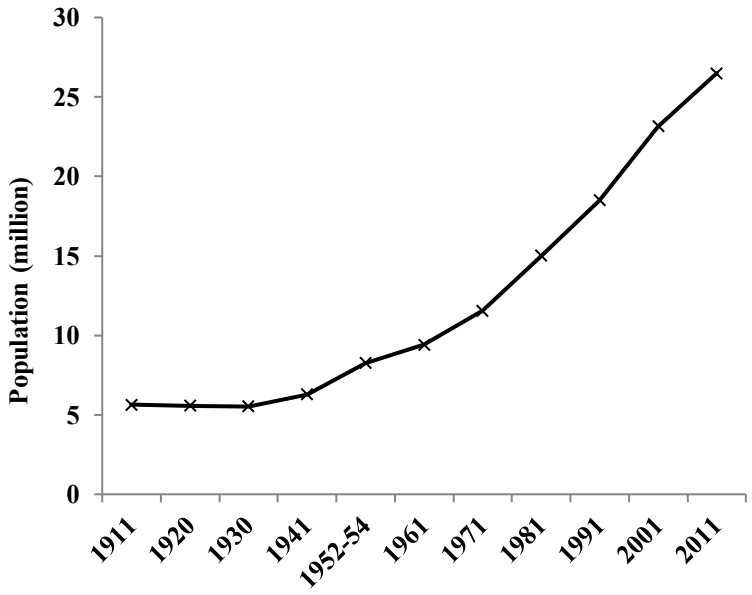

Figure 1.3 Population size of Nepal, over a 100 year period. (Source: Environment Statistics of Nepal, CBS 2013).

With respect to the total volume of wastewater production in Nepal, reliable data is not available. Thus an estimate of total wastewater generation is based on the average consumption of water per capita per day, which is 75 liters per capita per day in the urban areas and 40 liters per capita per day in the rural areas. It is then assumed that 85 % of this ends up as a domestic wastewater (UNEP 2001); thus wastewater production in the country is estimated to be 296 MLD (Nyachhyon 2006). With respect to water use, the total annual water withdrawal is 9787 million m^3, which is 359 m^3 per inhabitant, out of which 98 % goes for irrigation and only 2 % is for municipalities[24]. Total wastewater generation recorded for the five municipalities in the Kathmandu Valley is 99,622 MLD and the total collection is 49,811 MLD, which is only 50 % of the total wastewater generated (Rana et al. 2007).

[23] www.wateraid.org/~/media/.../solid-waste-management-nepal.pdf
[24] http://www.fao.org/nr/water/aquastat/countries_regions/asia_southeast/table32.pdf

1.4 Adopting the most appropriate technology

The selection of a sustainable "Best Available Technology" which is affordable and well suited for a small community is a challenging mission. Massoud et al. (2009) argues that "economically affordable, environmentally sustainable and socially acceptable" technologies are the most practical for developing countries. Moreover, the technology should involve the community from the planning phase to the design and construction phase. The above review on the waste management shows that developing countries require sustainable technologies that are less technical, affordable and that fit into local conditions. Thus, this study has identified "Vermifiltration" technology for potential sewage wastewater treatment. It has been proposed by a number of researchers that this technology is innovative, simple, less technically demanding and cost effective, as discussed in more detail in **Section 1.2.6.3**. With respect to solid waste management, this study is limited to organic waste management, and has also considered complimentary "Takakura Composting" technology - which has already been introduced in the targeted community and accepted by the community, this is further discussed in **Chapter 2 and Chapter 5**.

1.5 Research aims

For a representative Nepalese community, Ward Number 20 of Lalitpur Sub-metropolitan City, Lalitpur, Nepal, in the vicinity of the Bagmati River, this project was aimed at identifying the most appropriate technologies for the sustainable management of the community's domestic waste and sewerage; that also minimizes the impact on the surrounding environment, especially the River. A particular emphasis was placed on cost effectiveness and innovation and technologies that have the potential to contribute to the local economy and which engage and involve the community.

1.6 Scope of the study

LUZZA Nepal, a non-governmental organization, was developing Ward No. 20 of the Lalitpur Sub-metropolitan City (LSMC) as a model community with a 'Zero Waste

Approach'. The ward comprises of 14 Toles (a cluster of households). This project was initiated in terms of solid waste management at the local level with a unique model of waste segregation, collection and recovery. For example, the organization has introduced the Takakura composting technology to manage organic solid waste. Moreover, the organization was piloting bio-methanation technology in association with organic solid waste management - with the aim of energy recovery. Thus, this project adopted Ward No. 20 as the target community and performed a comprehensive waste audit to characterize and quantify the waste of this community.

For the target community, an investigation was carried out to assess the domestic sewage wastewater quality that discharges effluent to the Bagmati River. An attempt was made to access power and water consumption data for the community from the relevant authorities and departments. To complement this information, such data was also estimated from the community's power and water meters. This was carried out in full consultation with the social leaders of the community, which includes executives of the Tole development committees and women's groups.

Water quality monitoring at the point of effluent discharge and of the river water upstream and downstream from the discharge point was performed. Water quality parameters such as temperature, turbidity, pH, DO, COD, nitrogen content, phosphorous content, TSS, heavy metals and certain bacteria (*E Coli*, *Fecal coliform*) was assessed. Although the analysis equipment and methods of the High Powered Committee for Integrated Development of the Bagmati Civilization (HPCIDBC) laboratory in Nepal was used for the determination of these parameters, the standard methods (APHA 1998) was followed in Australia and Australian guidelines were used for water quality monitoring/reporting and effluent management.

The technologies for solid waste management and sewage wastewater treatment which are in current practices in Nepal and other developing countries were explored, and investigated for their efficacy. In addition, information on the different technologies available worldwide to address these issues was collected, assessed, and a database was prepared. A number of technologies such as those relating to localized sewage treatment (Vermifiltration) and composting (Takakura Home Method - THM) were selected and assessed in Australia for their optimization and applicability in Nepal.

However, this study does not consider the details of implementing the selected technologies, which were assessed in Australia, into the target community due to time and budget constraints. Therefore this was not considered to be part of the scope of this thesis. The limitations with respect to the different components of this project have been discussed separately in the individual chapters.

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CHAPTER 2: Community-based waste management strategies in a targeted community

2.1 Introduction

2.1.1 Background

In Nepal, as in other developing countries, Municipal Solid Waste Management (MSWM) is a major challenge - amongst many other priorities. The current practice of waste management in the Kathmandu valley has invited many problems, which are a threat to the environment and human health. Continuous confusion associated with landfill and dumping sites has resulted in Valley Street and open public places becoming free dumping areas, making the valley people vulnerable to communicable diseases. Indeed, municipal solid waste (MSW) is a major factor contributing to the deterioration of the Bagmati River basin.

MSW is defined as waste from households, commercial and institutional establishments, parks and gardens, street sweeping as well as treated hospital waste. Of the total MSW, household waste contributes 50 - 75 %. The characteristics of the waste generated is usually affected by various factors, including physical factors such as altitude, temperature, rainfall and humidity; and socio-economic factors such as population, economic status and consumption patterns (ADB 2013). For example, **Figure 2.1** depicts the waste generation (tons/year) with respect to the population in the Kathmandu Metropolitan City (KMC) area over the period from 1986 to 2003. The plot clearly shows that the waste generation increases in proportion to the increase in population (Alam et al. 2008).

A major component of the waste generated is generally organic waste followed by plastics and paper/paper products. Other waste such as glass, metals, textiles, inert materials, rubber and leather are found only in small quantities. **Table 2.1** presents the % composition of the daily solid waste generated with respect to the type of waste in three major cities of the Kathmandu valley; namely the Kathmandu Metropolitan City (KMC), Lalitpur Sub-metropolitan City (LSMC) and Bhaktapur Municipality (BKM). In all the three cities, the

organic waste composition was significant, ranging from 60 – 75 % of the total waste generated. Although organic waste generation fluctuated over time, it was still a major component of the total solid waste generated.

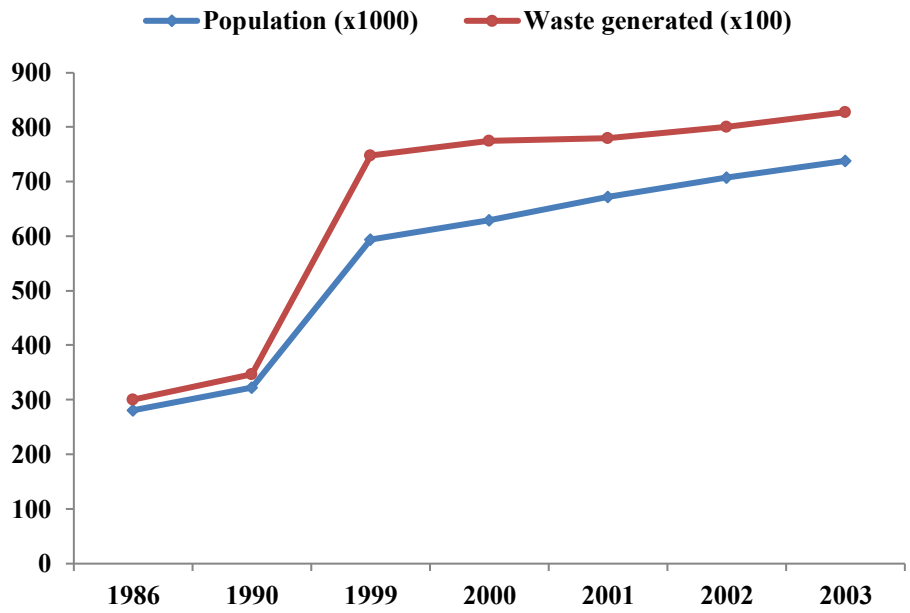

Figure 2.1 Waste generation (tonnes/year) in the Kathmandu Metropolitan City with respect to population from 1986 to 2003.

Various studies conducted on the characteristics of the municipal solid waste under consideration show variations in the data. A study conducted by Nippon Koei Co. Ltd. and Yachiyo Engineering Co. Ltd. (2005) found that almost 71 % of the MSW comprises of organic waste while the rest includes paper, plastic and other inorganic waste (Nippon Koei Co. Ltd. and Yachiyo Engineering Co. Ltd. 2005; Dangi et al. 2011). Domestic food waste is a major proportion of organic waste which is manageable locally (LUZZA Nepal 2010) with waste minimization approaches such as composting - and energy recovery using anaerobic biogas production (Joshi 2008; Gomec 2010). In developing countries, local government usually fails to implement sustainable MSWM due to lack of proper technology and financial barriers (Okot-Okumu and Nyenje 2011; Sanneh et al. 2011). However, in the developed world, urban planning integrates MSWM as an important environmental factor (Larsen et al. 2010; Zhang et al. 2010). A comparative study (Zhang et al. 2010) provides an overview of MSWM and recycling potential in developed and developing countries.

Table 2.1 Daily solid waste generation in the Kathmandu Metropolitan City, Lalitpur Sub-metropolitan City and Bhaktapur Municipality with respect to the type of waste (% of average collection).

Type of waste	**Kathmandu Metropolitan City**					
	2001	***2005***	***2006***	***2009***	***2011***	***2012***
Organic waste	69.0	70.9	69.0	63.2	63.2	63.2
Paper	9.0	8.5	9.0	9.0	9.0	9.0
Plastic	9.0	9.2	9.0	10.8	10.8	10.8
Glass	3.0	2.5	3.0	5.4	5.4	5.4
Metals	1.0	0.9	1.0	0.4	0.4	0.4
	Lalitpur Sub-metropolitan City					
	2004	***2005***	***2006***	***2009***	***2011***	***2012***
Organic waste	67.5	67.5	67.5	60.6	71.6	67.5
Paper	8.8	-	8.8	13.2	9.4	8.8
Plastic	11.4	15.4	11.4	10.0	12.1	11.4
Glass	1.6	-	1.3	2.8	1.7	0.9
Metals	0.9	-	0.9	1.7	-	-
	Bhaktapur Municipality					
	2003	***2005***	***2006***	***2009***	***2011***	***2012***
Organic waste	70.2	75.0	75.0	71.0	70.7	70.7
Paper	2.4	-	3.3	2.8	3.5	3.5
Plastic	3.2	6.4	3.4	6.5	7.0	7.0
Glass	1.3	-	1.5	2.1	0.4	0.4
Metals	0.1	-	0.3	0.4	2.3	-

Source: (CBS 2013a)

Based on a study conducted by Nippon Koei Co. Ltd. and the Yachiyo Engineering Co. Ltd. (2005), in 2004 in the Kathmandu valley, KMC generates a higher volume of waste than LSMC and BKM, **Table 2.2**. With respect to waste collection, only 69 – 81 % of the total waste generated is collected by the municipality. The rest of the uncollected waste was left to the local residents to manage, most of which was dumped on the riverside or onto open spaces. The data shows that the LSMC is comparatively less efficient in waste collection than KMC and BKM.

Table 2.2 Waste generation and collection in municipalities.

	Generation	Collection	% collection	Projected generation in 2015
KMC	308.4	250	81	547.9
LSMC	75.1	52	69	135.4
BKM	25.5	19	75	46.2

Source: (Nippon Koei Co. Ltd. and Yachiyo Engineering Co. Ltd. 2005)

Out of 58 municipalities in the country, only a few are provided with sanitary landfill facilities and many are lacking proper landfill sites and well-planned MSWM strategies. Thus, most of municipalities dump collected solid waste onto open spaces, riversides or roadsides - as depicted in **Figure 2.2**. It is regrettable to observe that such wastes are being dumped without any kind of processing, although a significant part of the total budget of the municipality is spent on environmental management[25]. The costs associated with the management of solid waste in the LSMC for the period 2006/07 to 2012/13 is shown in **Figure 2.3**. This figure shows that the waste disposal cost has increased significantly over a six year period. Moreover, most of the MSWM budget is spent only on the collection of waste with its disposal getting only little attention[26]. The World Bank (Hoornweg and Bhada-Tata 2012) also reported that collection cost represents 80 – 90 % of the MSWM budget in low income countries.

Thus, to understand the trend of waste generation and its management, a community was chosen where a project has already been initiated in terms of solid waste management at the local level with a unique model of waste segregation, collection and recovery. A local non-governmental organization, LUZZA Nepal, initiated the "Towards ZERO Waste" program in Ward No. 20 of Lalitpur Sub-metropolitan City in December 2008, with a view to develop it as a model community.

[25] 23.32% of the total budget of the municipality is for the environmental management, which comes second after the infrastructure development (23.99 % of the total budget).
http://www.kathmandu.gov.np/pdf/51summaryof%20exp&revenue.pdf
[26] Personal communication, Environment division LSMC

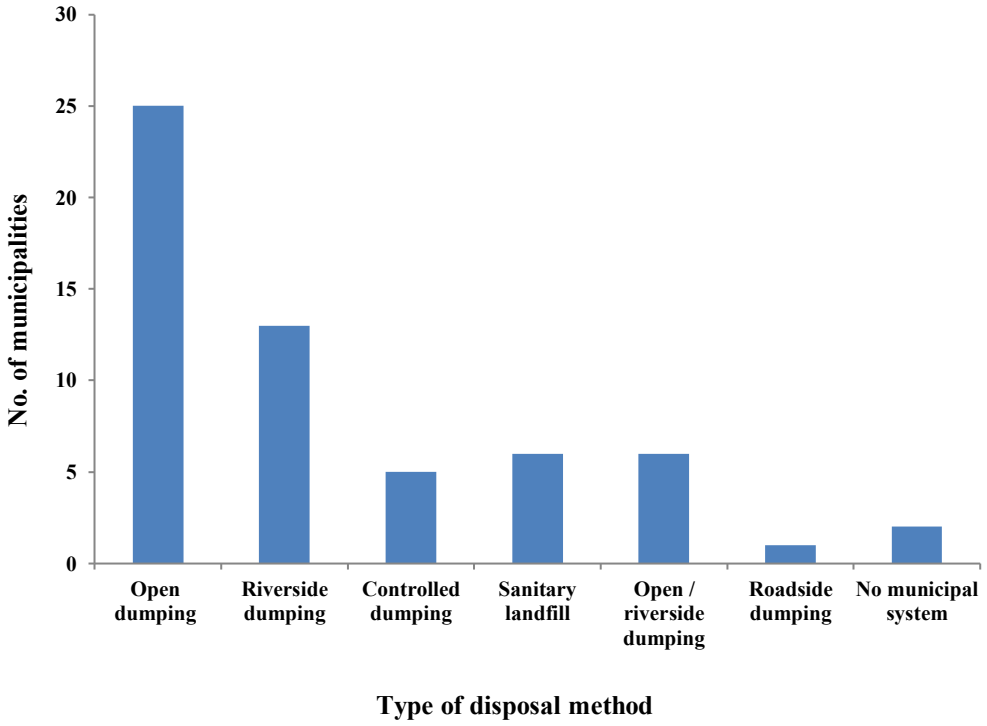

Figure 2.2 Solid waste disposal methods in municipalities (58 municipalities have been considered as of 2013) Source: (ADB 2013)

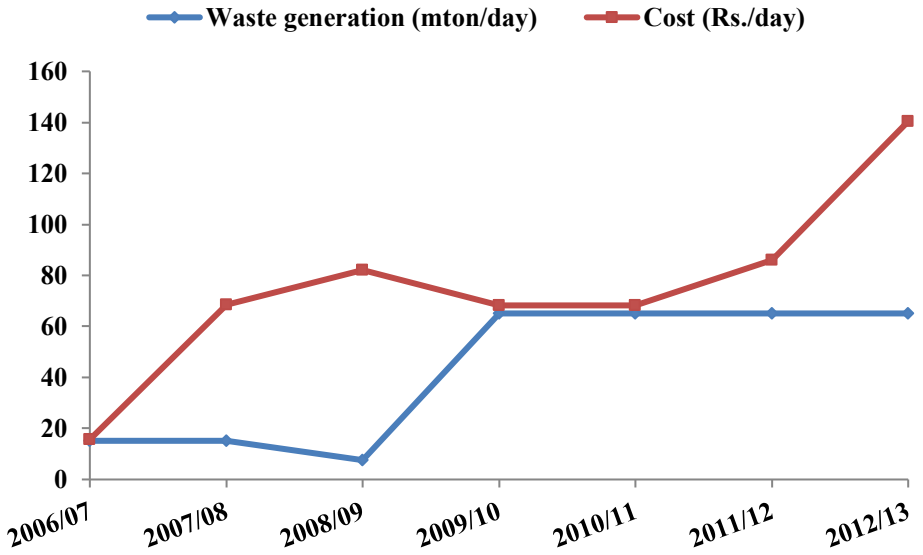

Figure 2.3 Waste generated in LSMC and the associated cost for the waste management, from 2006 to 2013. Note: The unit for the cost associated with the management of waste generated is on 1000 Rs. Per day. Source: (CBS 2013a, b)

2.1.2 The target community

Lalitpur Sub-metropolitan City (LSMC) is one of the municipalities in the Lalitpur district. It is geographically located in the hilly area of the central development region of the country. LSMC is administratively divided into 22 wards[27], and the size of a ward ranges from 2.43 km^2 to 0.09 km^2. The target community for this study is one of the 22 wards of the LSMC, namely Ward No. 20, which is 0.20 km^2 in area and comprises of 14 Toles (clusters of households). An overview on the distribution of population and households (HH) in the ward is presented in **Table 2.3**. The total population of the ward is 7721, as per the 2011 census (CBS 2012b), which counts for 3.5 % of the LSMC population and 1.6 % of the Lalitpur district population. The total population constitutes of 3958 males and 3763 females, residing in 1978 households (HHs) with an average HH size of 3.9 and a male to female ratio 1.05.

Table 2.3 An overview on the demographic information on the country, district, municipality and ward level.

Observed parameters	Nepal[28](2012a)	Lalitpur district[29](2014)	LSMC[30](2012b)	Ward No. 20[5] (2014)
Total population	26494504	468132	220802	7721
Male	12849041	238082	113781	3958
Female	13645463	230050	107021	3763
Sex ratio	0.94	1.03	1.06	1.05
No. of household	5427302	109797	54581	1978
Average household size	4.88	4.26	4.06	3.9
Area (km^2)	147181	385	15.43	0.20
Population density /km^2	180.01	1216.0	14574.4	38605

Source: CBS census 2011

The total solid waste generation from LSMC is approximately 90 tons per day although only 70 tons per day is collected.

[27]Information from an official website of LSMC http://lalitpur.org.np/e_cityataglance_statistics.php 04/04/2015
[28] Census Info Nepal 2011 http://www.dataforall.org/dashboard/nepalcensus/ 05/04/2015
[29] CBS 2014 http://cbs.gov.np/wp-content/uploads/2014/03/Volume05Part01.pdf 05/04/2015
[30] CBS 2012 http://cbs.gov.np/wp-content/uploads/2014/04/25Lalitpur_WardLevel.pdf 05/04/2015
http://www.dataforall.org/dashboard/nepalcensus/ 05/04/2015

2.1.3 The baseline study

A baseline study was conducted in November 2008, at Ward No. 20 of LSMC, to explore the current waste management practices in the target community. This study was carried out in line with the local non-governmental organization, LUZZA Nepal, which aims to design and introduce innovative waste management programs into target communities - taking a 'ZERO Waste Approach'. Therefore, this study also assessed each HH's willingness to participate in the ZERO waste programs, their willingness to pay for improved service levels and HH's commitment to the sustainability of the program.

Figure 2.4 The community people being interviewed by the volunteers. (LUZZA Nepal)

A simple household survey technique, Willingness to Pay (WTP) methodology, was applied to get the desired information. The WTP is a method in which a member of household of the community is asked a series of structured questions designed to determine whether they are willing to pay for time, service, and fees for a good service. The random sampling methodology was applied and included HHs randomly chosen from all 14 Toles of the Ward, so that it could include a diverse population and represent a community. Considering the total HH as 650[31], almost 20 per cent of the total household i.e., 134 HHs were involved in the study. The collected data was analyzed using EXCEL.

[31]The approximation was made based on the total HH number of 638 mentioned in the 2006 voter's list. This information was obtained from the office of the Ward No. 20, LSMC.

The study showed that 1035 people were residing in 134 HHs, with an average HH size of 7.7. The total population constituted of 53.4 % male and 46.6 % female, with a 1.1 male-to-female sex ratio. The data presented in **Table 2.4** shows that the community was found to generate 330.4 g organic waste/HH/day (data derived by combining leftover foods and vegetable remnants). The study does not show the volume of the total waste generated. Thus, the volume of the total waste generated was derived based on the observation of this study that organic waste constitutes 65 % of the total. Therefore, the volume of the total waste calculated was 507.7 g waste/HH/day, which accounts for 65.9 g of waste/capita/day. Based on this data, the community was found to generate a total volume of 330 kg waste/day.

Table 2.4 An overview on the type of waste generation in the target community based on the baseline survey conducted in November 2008 by LUZZA Nepal.

Waste Type	Sample	HH	Community
Leftover foods, *g/day*	*11870*	88.6	57590
Vegetable remains, *g/day*	*32400*	241.8	157170
Papers/Newspapers, */ day*	*299*	2.2	1450
Plastic Bags, */ day*	*387*	2.9	1877
Old Clothes, */ year*	*577*	4.3	2799
Furniture/ Accessories, */ year*	*14*	0.1	68
Batteries, */ year*	*98*	0.7	475
Bottles (glass/plastic), */ year*	*1826*	13.6	8857

The study also revealed that 42 % of HHs practice waste segregation, by separating organic and inorganic waste into different bins. However, they were not able to dispose of the waste separately due to the lack of a separate collection system for the segregated waste. Out of the segregated waste, 86 % of HHs composted biodegradable organic waste by the traditional method and some fed it to animals/birds. With respect to the mode of the management of waste, 14 % of HHs was found to burn the waste, 60 % were found to throw it into the garbage and 5 % sold it to scavengers[32].

In terms of the management of the sewerage generated from the community, 88 % of HHs had a sewer pipe connected to the city's sewerage system, whilst 6 % did not have a sewer

[32] Scavenger is defined as 'a person who searches through and collects items from discarded materials, or, a street cleaner.' http://dictionary.reference.com/browse/scavenger

pipe connected. The HHs without a sewer pipe had a septic tank in their backyard and only a few of them were interested in being connected to the sewerage system. With respect to a rainwater collection/harvesting system, only 10.4 % are practicing this through a traditional collection system, i.e. collecting rain water from rooftops in tubs and drums. Not scientific techniques had been implemented. However, 31 % of HHs expressed their interest to install any type of rain water collection/harvesting system in the future, considering the water supply shortage.

Finally, assessing the commitment from the community to participate actively in ZERO Waste programs was a main objective of the baseline study, as only significant community participation can make any program a success. In this regard, it was encouraging to find that 70.12 % of HHs expressed their willingness to participate. They showed their commitment to give time, service and also to pay fees. 29.85 per cent of HHs were not interested in participating in the program.

2.1.4 ZERO waste strategy in the community and its outcome

Considering the interest of a sizeable majority of the community to participate in the ZERO waste programs, LUZZA Nepal initiated the "Towards ZERO Waste" program in Ward No. 20 in December 2008, with a view to develop it as a model community. Government organizations (GOs) such as the Environment Section of LSMC, the Ward Office of LSMC, the Solid Waste Management and Resource Mobilization Center (currently known as the Solid Waste Management Technical Support Center), and INGO, the Japan International Cooperation Agency (JICA) supported the program, whereas the NGO, the Resource Centre for Primary Health Care (RECPHEC) funded it. Moreover, women's groups of the community and volunteers (interns at LUZZA Nepal) also contributed in a bid to reach out to the community - this was crucial to make the program successful. This innovative approach to waste management was the first of its kind, and is summarized in **Figure 2.5**.

First of all, a consultation was done with all the stakeholders (GOs, NGOs, INGOs and women's groups) as mentioned above. Then, a baseline study was conducted with a view to knowing the willingness of the community to collaborate and to assess waste characteristics and other relevant information. The program was initiated in the community with the

'orientation program'. Thus the members of the women's group and the volunteers were oriented with respect to the strategy to be taken and were then mobilized within the community. They visited each HH in the ward and made them aware of the consequences of poor waste management practices. At the same time, they were encouraged to understand the waste hierarchy so that the quantity of waste generation that goes to landfill could be reduced. Then, they were provided with two types of waste bins – green with a lid for organic waste and red/blue without a lid for inorganic waste.

The HHs were encouraged to compost the organic waste at the household level. Those HHs not able to do so, were encouraged to give the organic waste to the waste collector. The door-to-door waste collector was mobilized in the community to collect separated waste once a week for inorganic waste and on alternative days for organic waste. Those HHs interested in composting were trained in the Takakura Home Method (THM) and they were provided with compost baskets. A detailed discussion on the THM is presented in Chapter 5, **Section 5.1**. The collected organic waste from the community was planned to be composted using two techniques – the Takakura community composting method and a bio-methanation process. With respect to inorganic waste, the collected waste was sent/sold to the LSMC's recycle centre. For the effective implementation of the THM, each HH practicing this composting was visited by a volunteer, to monitor the on-going progress, and provide technical assistance if needed.

Beside the HHs, the educational institutes (schools and colleges) in the Ward were involved in the awareness program. Most of the schools and colleges in the Ward were familiarized with the components of the 'ZERO waste strategy'. The intention in involving these institutes in the program was based on the theory that children are able to motivate their parents in efficient waste management. These institutes were provided with educational materials (pamphlets, posters) and big bins to encourage students to segregate waste.

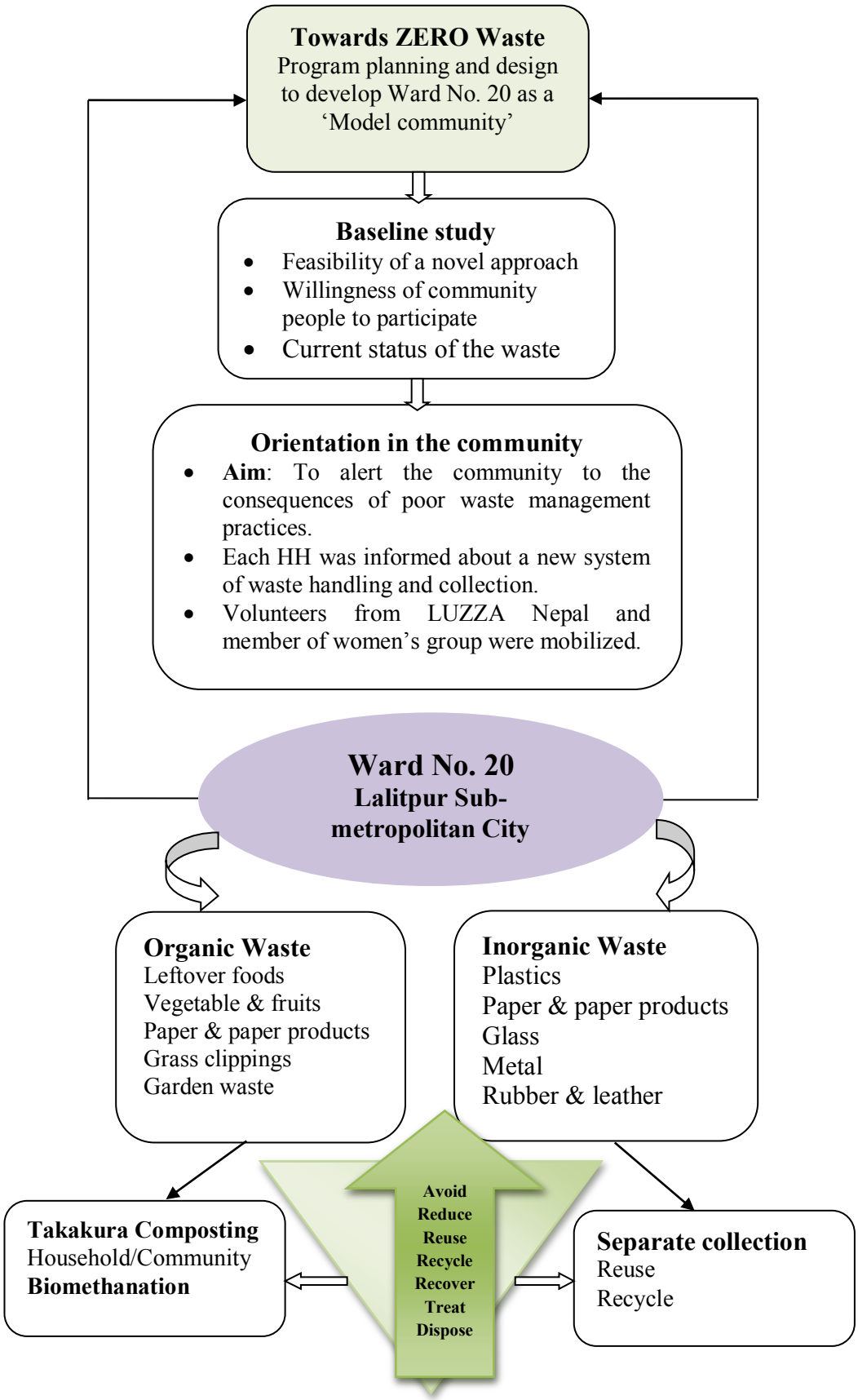

Figure 2.5 The waste management approach taken for the "Towards ZERO Waste Program" at Ward No. 20 of LSMC.

One of the major activities in this strategy was to reduce the use of plastic bags (for shopping) by encouraging people to use cotton/paper bags as an alternative. Thus, they were motivated to carry reusable bags from home. To accomplish this goal, each HH was provided with cotton bags and participating supermarkets were supplied with cotton/paper bags at a subsidized rate. Moreover, students in the schools were encouraged to avoid bringing lunch in plastic bags and once a school could achieve a plastic free environment, it was declared as the "Plastic Bag Free Zone".

2.1.5 Research objectives

The main objective of this Chapter was to conduct an eco-audit via a survey of a targeted Nepalese community so that the information on waste characteristics in terms of waste generation and management, and other relevant household (HH) facilities, could be obtained. This survey was conducted within the framework of the "Towards ZERO waste" program and was aimed at providing a basis for identifying viable community-based technologies for the management of the waste generated.

2.1.6 Limitations of the study

This part of the research is a background study for the adaptation of the best available waste management technologies into Nepalese communities. This occupies only 10 % (approximately) of the study. Thus, due to time constraints and a limited budget, the researcher could only spend limited time in the field in Kathmandu, Nepal. The major project was located in Melbourne, Australia. Therefore, the study was limited to the HHs in the target community, which was participating in LUZZA Nepal's "Towards ZERO Waste" program. Moreover, the researcher collected only one sampling data set and those who were involved in the previous baseline study collected other datasets.

(a) Orientation for Women's group & volunteers

(b) Awareness rally in the community

(c) Composting training on Takakura Home Method

(d) Participants of the training

(e) Door-to-door collection of waste

(f) Monitoring of household composting.

Figure 2.6 Various activities undertaken in the community during ZERO waste programs. (LUZZA Nepal)

2.2 Materials and Methods

2.2.1 The sampling for the study

This survey was conducted in Ward No. 20 of the Lalitpur Sub-metropolitan City (LSMC), with a sample size of 63 households - that were participating in the "Towards ZERO Waste" program[33]. This represents approximately 10 % of the total households in the community. The survey was conducted from October to December 2013 and the data were collected twice a month from each household. A list of the HHs, who participated in the first set of data collection, was prepared and the same HHs were sampled for the rest of the datasets.

2.2.2 The community survey

The community survey was conducted in October, November and December 2013. Each participating household (HH) was assessed twice a month, so that 6 sets of data could be collected in a 3 month duration. The first set of data was conducted under the direct supervision of the principal researcher and also involved researchers who participated in the previous baseline study conducted in 2008. The remaining survey was conducted by researchers who were previously trained by the principal researcher.

For this survey, a household was defined as a number of people using the same kitchen rather than the number of houses, as one house could be shared by many families. Each HH was provided with two different kinds of waste bins; a green bin with lids for organic waste and a red/blue bin for recyclable waste, as part of the "Towards ZERO Waste" program. During the survey, each HH was instructed to keep the waste generated in a given day (24 hours) for investigation. Thus, the researchers gravimetrically assessed the previously segregated waste (in the two categories) by wet weight, separating the waste provided by the HHs into nine different categories; namely organic waste, plastics, paper and paper products, metals, glass, textiles, rubber and leather, dirt and hazardous waste .

[33] Based on the 2011 census, the number of total household was 1978, however based on the voter's list, 2006, it was only 638. Thus, this study considered only of those HHs that participated in the "Towards ZERO Waste" program.

The "head" of each household, or the person responsible for waste management (often female), was selected to respond to the questionnaire which seeks information on waste segregation, waste disposal, and water usage/on-site wastewater treatment. Objective questions and relevant measured data were included in the questionnaire - that is provided in **Appendix 2.1**. A survey of this kind is expected to have minimal error.

2.2.3 Data analysis and presentation

All the filled questionnaires were collected and posted from Lalitpur, Nepal, to the author in Melbourne, Australia. The data was tabulated and analysed in EXCEL in order to present it in tabular and graphical form. In terms of demographic information, only one survey was taken from each HH and the results are presented in **Table 2.3**. The information on waste volume was collected twice a month for three consecutive months. The data presented in **Figure 2.8 (b)** represents the % composition for an average of six datasets from each HH collected over three months, which is again averaged for all 63 HHs sampled. The information on power consumption was gathered utilizing the meter reading in each HH and the readings were collected once a month. Thus, the calculated power usage is the average of three datasets, which is again averaged for 63 HHs. In terms of the information on daily water usage, this is based on approximate values provided by the respondents. The water meter readings were not reliable since the HHs use water supply sources other than tap water. The remaining information in the questionnaire was asked only once to the respondent and presented here accordingly.

2.3 Results and Discussion

2.3.1 Demographic information

The study revealed a total population of 322 residents in 63 HHs, which represent 4.2 % of the population of the ward, with an average HH size of 5.1. The ward itself represents 3.5 % of the total population of the LSMC (220802), **Table 2.3**. The total population consisted of 50.7 % male and 49.3 % female, with a male-to-female sex ratio of 1.03. Moreover, the total

population consisted of 80.7 % adults and 19.3 % children. An overview of these demographics is summarized in **Table 2.5**.

Table 2.5 An overview (%) on the demographic observation in the target community.

Total HHs	Total Population	Adult%	Child %	Female %	Male %	Sex ratio	HH size	Population density/km^2
63	322	80.7	19.3	49.3	50.7	1.03	5.1	38605

In this study, it is interesting to note that the HH size decreased from 7.1 in a baseline study to 5.1. However, it is higher than the HH size reported in the 2011 census (3.9). This variation in HH size might be attributed to various factors such as variations in HH sampling protocols, sample size and/or temporal errors. One significant reason might also be due to the change in family structure; the traditional practice to live in extended families is declining and people are becoming more prone to live as "nuclear families".

2.3.2 Waste characterization

The waste characteristics have been grouped into 9 categories; namely organic waste, plastics, paper and paper products, metals, glass, rubber and leather, textiles, dirt and hazardous waste. Observations made on the waste characterization are discussed below.

Figure 2.7 The waste was separated into categories and weighed as shown. A survey was conducted in this community to characterize the waste generated each day.

2.3.2.1 Waste volume

The data presented in **Table 2.6** shows that the community was found to generate a total of 499.6 g of waste per HH per day. A major volume of the total waste generated by the community consists of organic waste, as depicted in **Figure 2.8 (b).** The waste generation based on the waste type may be shown in decreasing order as organic waste > plastics > paper and paper products > glass > metals > dirt > textiles. Rubber and leather waste and hazardous materials were not found in the HH waste investigated in this study. Thus, the total waste generation per capita per day was found to be 98.0 g, which consists of 63.7 g of organic waste, 12.7 g plastics, 10.4 g paper and paper products, 6.0 g glass, 3.3 g metals, 1.4 g dirt and 0.4 g textiles. Thus, considering the total HH number of 1978 and 499.6 g waste per HH per day, this ward is estimated to generate 0.99 tonne (988.21 kg) of waste per day.

Table 2.6 A breakdown on the composition of the community household waste; values presented are secondary data from ADB (2013) for Nepal[#] and LSMC, and primary data from this study for Ward No. 20. Columns 4 & 5 correspond to each other (with data presented in % composition and volume in g, respectively).

Type of waste	Nepal[#] (%)	LSMC (%)	Ward* (%)	Volume of waste** (g)
Organic waste	66	77.94	65.05	325.00
Plastics	12	9.81	13.01	65.00
Paper & paper products	9	5.23	10.65	53.20
Metals	2	0.66	3.34	16.67
Glass	3	1.99	6.16	30.76
Rubber and leather	1	0.75	0.00	0.00
Textiles	2	0.74	0.40	2.00
Dirt	5	2.86	1.40	7.00
Hazardous wastes	0	0	0	0

[#] 58 municipalities[34]
*the target community - Ward No. 20
**volume of waste generated in the Ward No. 20, in g/HH/day.

[34] The number of municipalities in the country has reached 130, after the Nepalese government announced an additional 72 municipalities in 2014. http://www.ekantipur.com/2014/05/08/top-story/govt-declares-72-new-municipalities-with-list/389310.html

Gratifyingly, the composition of the waste generated in the target community resembles the observation carried out for 58 municipalities, by the ADB (2013), **Figure 2.8 (a)** & **(b).** The major components of the waste generated are quite similar, except for the rubber and leather waste which was not found in this study. But for 58 municipalities, it comprises of 1 % of the total volume.

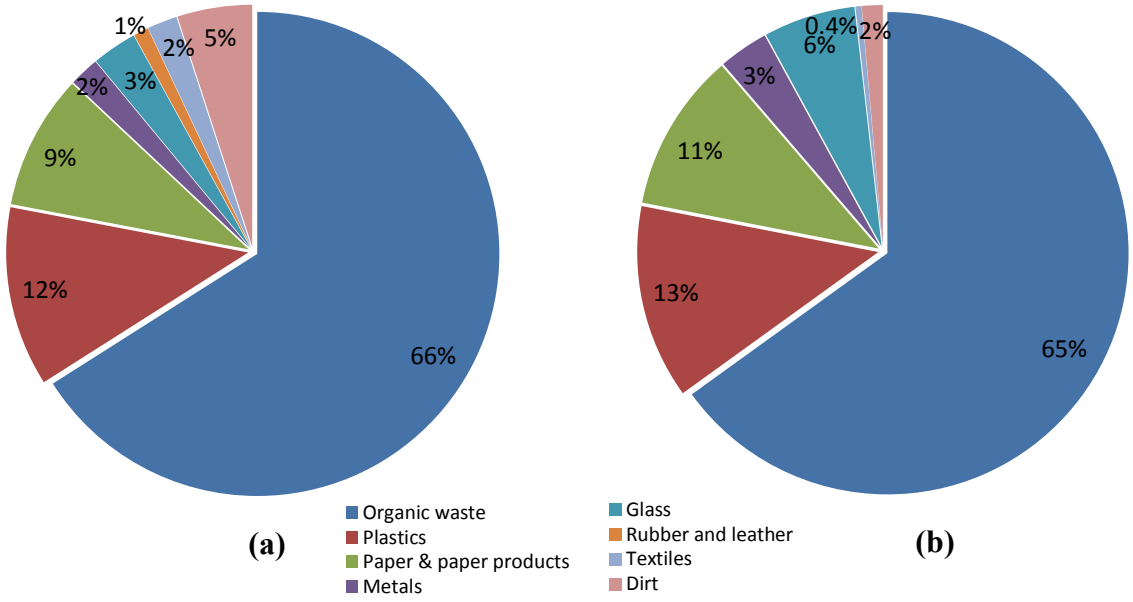

Figure 2.8 Waste generation in (a) Municipalities - average of 58 municipalities (b) Ward No. 20 – in this survey.

In addition, in 2012 the Central Bureau of Statistics (2013a) reported the same pattern of waste composition in the total waste generated in KMC, LSMC and BKM, **Figure 2.9**. A comparative study on the composition of the major waste types among these municipalities, shows that LSMC generated less volume of organic waste than KMC but a higher volume than BKM. In terms of paper/paper products and plastics, BKM generated comparatively less waste than LSMC and KMC. This variation in waste composition in different municipalities might be attributed to the life style of the people living in these municipalities. The people living in BKM have less HHs (only 17639) than in KMC (254,292) and LSMC (5481) and live more traditionally than those in KMC and LSMC. Moreover, the commercial activities are less in BKM than in KMC and LSMC, thus producing less volume of paper and plastics.

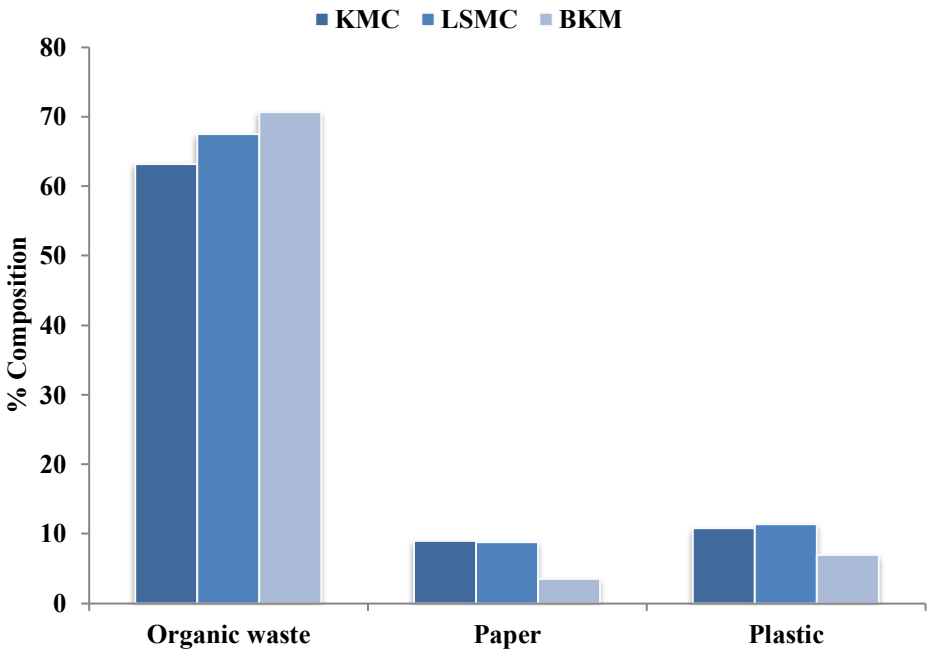

Figure 2.9 The % composition of daily solid waste generated for the major components, in KMC, LSMC and BKM in year 2012 (CBS 2013a).

2.3.2.2 Waste segregation

It was encouraging to find in this community survey that 100 % of the HHs were segregating organic and inorganic waste. As mentioned above in **Section 2.1.4**, organic waste was separated and stored in a green bin and inorganic waste, that can be recycled, was placed in a blue/red bin. Note that a study conducted by Alam et al. (2008) reported that 89 % households in KMC were willing to separate organic waste from others.

It is unfortunate that, although the people in the community are willing to store waste based on its type, there is no such collection system provided by the government. However, the local government has taken an initiative for the management of the separated waste by the establishment of the Community Mobilization Unit (CMU). This unit provides training to the community on the techniques of recycling inorganic waste at the local level. Also, separated waste such as milk pouch (packet) and PET bottles can be provided to the CMU.

2.3.2.3 Current practices of waste management

Traditional practices of the solid waste management in the old urban settlements of the Kathmandu valley can be considered to be quite sophisticated. With a low population and limited industrial activities, the majority of the waste composition is organic in nature and is mostly food waste. Thus, as a traditional practice, people used to dump the organic waste in an open space or courtyard, located between houses with a common entrance, generally called 'Saaga'. When this waste matures, the HHs used the waste as fertilizer for agricultural land (Pokhrel and Viraraghavan 2005). Thus, residents were responsible for the management of their own waste. In early 1950s, the government gave the responsibility for waste management to the municipalities, considering people's health and sanitation, which gradually eliminated the traditional practice. With the establishment of the sanitary landfill in 1986, the practice of collecting waste from communities and dumping it in landfill started. Thus, the practice of local management of waste diminished with the introduction of modern systems.

Figure 2.10 (a) Waste collected from roadside dumping (b) Residents dumping waste onto the collection vehicle. The truck from the LSMC collects waste from the community every morning/every alternative day. (Photos taken by Dr. Lawrence Ngeh)

At present, MSW management is mainly based on this collection and dumping system. The collection of waste from the communities is mainly done by municipalities. However, in some communities, municipalities have mobilized NGOs and private sectors to collect waste. In LSMC, the number of environmentally related NGOs affiliated with the Social Welfare Council has reached 66. Despite the efforts of municipalities, NGOs and the private sector,

the collection efficiency remains unsatisfactory. For example, KMC, LSMC and BKM generated 308.4, 75.1 and 25.5 tons of waste per day respectively, in 2004, whilst only 250, 52 and 19 tons of waste per day were actually collected (Nippon Koei Co. Ltd. and Yachiyo Engineering Co. Ltd. 2005). The rest of the waste was either dumped in open space or along the river.

This study shows that the community practices waste segregation and the majority of HHs compost organic waste and sell recyclable materials such as newspaper to "scavengers" for recycling. In addition, they sell milk pouches and plastic bottles to the CMU of LSMC. The rest of the waste is dumped in the municipality's waste collection system or collected by private companies using a door-to-door collection system with a fee. (The HHs have to pay a fee to the private sector). In this community, a waste collection truck from LSMC arrives at the collection point, usually a few stops in each Tole, signalling its arrival by whistling. Then, HH residents have to dispose of their waste onto the truck, **Figure 2.10**. The waste is collected as mixed waste; there is no system to collect segregated waste separately. Thus, 93.7 % of HHs were found to dump their waste into the municipality's collection trucks, 6.3 % give it to private sector and only 3.2 % were found to dump the waste in open spaces/roadside.

2.3.2.4 Composting

As mentioned above, 100 % of HHs sampled in this study was successful in separating organic waste from the rest of the waste. The separated organic waste was found to be composted by 98.4 % of the HHs. The Takakura Home Method was used by 95.2 % of the population and 3.2 % used the bin composting method.

The CMU of LSMC is promoting the bin composting method in LSMC. However due to some economic and technical constrains, it was not as popular as THM. THM was an easy, simple and fast composting system and it was popular among women's groups. Most of the respondents said that they use the compost in their home garden and some also use it in agricultural fields.

Figure 2.11 The THM compost monitoring during the community survey. The HHs practicing THM were requested to show their compost bin, and the researcher monitored it for the optimum condition such as moisture and the physical appearance of the compost. (Photo taken by Dr. Lawrence Ngeh)

2.3.3 Wastewater characterization

The volume of the wastewater generation in the community is influenced by various factors such as the availability of water for domestic and commercial use, the lifestyle of people living in the community and the types of commercial sector, e.g., hotels, restaurants, offices, schools, and shops. In the urban area of the Kathmandu valley, the potential domestic wastewater generation was estimated to be 124 million litres per day (MLD) in 2000, and only 38% of it was collected through the sewerage system (Tchobanoglous et al. 2003). After the commencement of the Melamchi Water Supply Project, the water supply in the Kathmandu valley is assumed to increase, thus the current volume of wastewater will definitely increase (Rana et al. 2007). To obtain an overview of the wastewater generation in the target community, the following factors were investigated and presented below.

2.3.3.1 Water supply and usage in the community

The major source of drinking water in the Nepalese community is tap water through household pipeline connection. Other sources are tubewells/boreholes, wells/kuwa (covered and uncovered), traditional water spouts, river water etc, **Figure 2.12**.

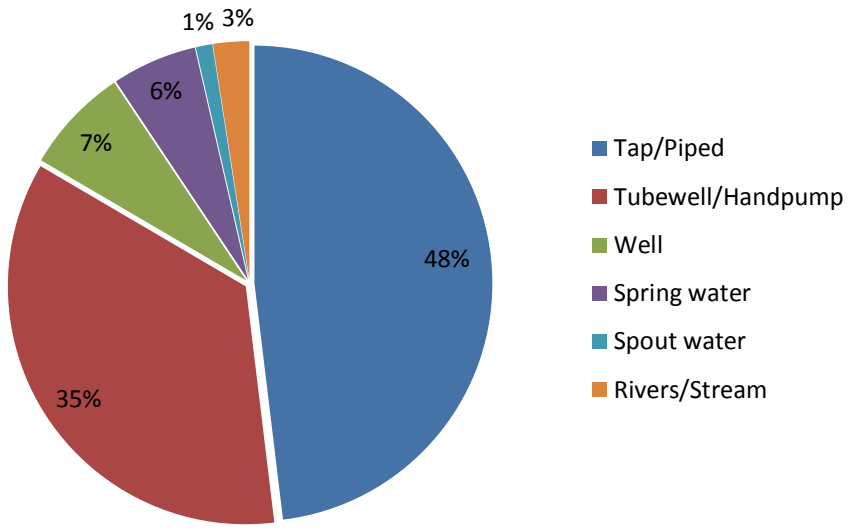

Figure 2.12 Sources of drinking water supply in Nepal. Source: CBS CensusInfo Nepal 2011[35]

Though the CBS (2012c) reported that the Nepalese community has improved drinking water supply, with 83 % of the total population having access to safe drinking water, the CBS (2011) reported that in the urban area of the Kathmandu valley, 68 % of HHs rated the drinking water supply facility as 'worst'. In the urban areas, most of the HHs are dependent on the government's water supply system i.e., tap/piped water. However, in the rural areas people have access to drinking water from other sources such as wells/kuwa and the river. For example, in the Kathmandu district, 70 % of HHs had a pipeline connected, of which 35 % was in a rural area and 86 % was in an urban area (CBS 2005).

Like many communities, the drinking water supply in the Kathmandu valley is not adequate or satisfactory; tap water is supplied only for a few hours in 3 to 5 days. Thus, people have to rely on other sources of water. **Table 2.7** presents a comparative study of the drinking water supply in three districts in the Kathmandu valley, namely Kathmandu, Lalitpur and Bhaktapur, in 2001 and 2011. The table shows that the tap water supply significantly decreased over this time in Kathmandu and Lalitpur, whereas the water supply from other sources such as tubewells, wells, spouts and rivers increased. However, in Bhaktapur the piped water supply was found to improve, so that the dependency on wells and tubewells for water supply decreased.

[35] Source of water supply http://www.dataforall.org/dashboard/nepalcensus/ 05/04/2015

Table 2.7 An overview (%) on access to drinking water by households, comparison between 2001 and 2011.

District	Tap		Well		Tubewell		Spout		River		Others	
	2001	*2011*	*2001*	*2011*	*2001*	*2011*	*2001*	*2011*	*2001*	*2011*	*2001*	*2011*
Kathmandu	84.1	62.0	6.3	8.0	5.7	8.0	2.6	4.4	0.1	0.3	1.3	17.3
Lalitpur	83.1	68.6	9.8	11.6	1.2	1.0	4.5	4.3	0.2	0.2	1.3	14.4
Bhaktapur	74.6	77.9	11.7	9.7	7.2	3.8	4.9	4.9	0.1	0.1	1.5	3.6

Source: (Rana et al. 2007; CBS 2012a)

This study also showed that the majority of the HHs in the target community rely on tap water and that they were not satisfied with the government's water supply system. Due to the inadequacy in the supply system, they have to rely on other sources such as tubewells and wells. Some HHs have to obtain water from tankers. In this case, they are paying for water to both the government and the private sector.

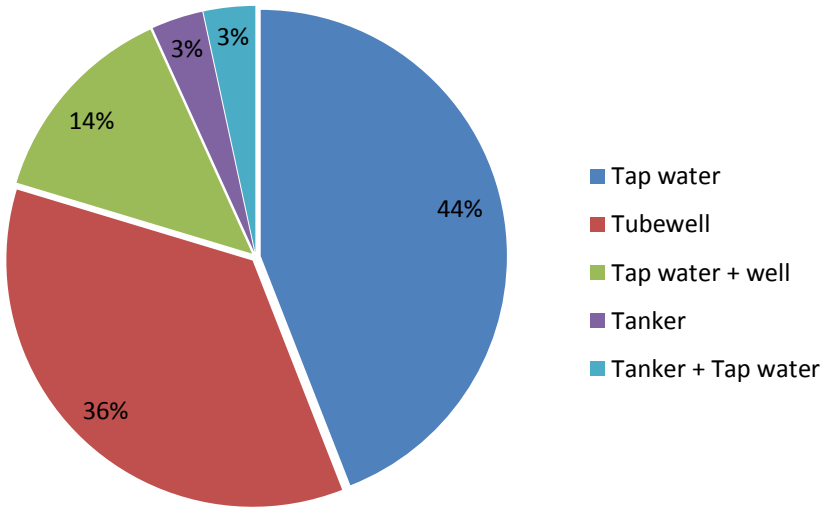

Figure 2.13 Source of water supply in Ward No. 20, as observed in this study.

Figure 2.13 shows that 44 % of HHs in Ward No. 20 rely on tap water, 36 % on tubewells (borehole), 14 % on wells and tap water, 3 % on tankers and 3 % on both tankers and tap water. Due to the unreliable water supply, a common practice has been developed to build underground water tanks or keep water tanks on roof tops, **Figure 2.14**, which diminish the aesthetic environment of the community.

Figure 2.14 The black PVC tank and silver colored aluminum tank on the roof top. A yellow arrow shows a well in a courtyard of the community. (Photo taken by Dr. Lawrence Ngeh)

This survey showed that the HHs had underground water tanks with an average capacity of 4623 L, ranging from 800 – 22000 L. With respect to roof top water tanks, the average capacity was found to be 897 L, ranging from 100 – 4000 L.

On average, the water use in the community was found to be 235.9 L per day per HH. Each HH was estimated to discharge 200.6 L of wastewater in the sewerage system per day, based on the estimate of 85 % of water used being discharged as wastewater (Tchobanoglous and Burton 1991; Liu and Liptak 1997). Thus, the community (ward) is estimated to discharge 396.8 m^3 of wastewater per day, into the sewerage system.

2.3.3.2 Households connected to sewer line

According to the CBS (2012c), only 44 % of the total population of the country has toilet facilities. However, the situation is improving with increasing awareness of both health and sanitation. CensusInfo Nepal 2011 reported that the HHs without toilets was 59.5 % in 2001, which reduced to 38.2 % in 2011. However, some households in the Kathmandu valley still do not have toilet facilities. **Table 2.8** shows that in the urbanized districts of the valley, still there are no toilet facilities in 1.2, 4.3 and 3.0 % if HHs in Kathmandu, Lalitpur and Bhaktapur, respectively.

Table 2.8 An overview of the toilet facilities in HHs. Data presented here are the % of the total HHs in the respective district.

District	Without toilet	Flush toilet (Public sewerage)	Flush toilet (Septic tank)	Ordinary toilet
Kathmandu	1.2	68.9	19.7	9.5
Lalitpur	4.3	44.8	38.8	11.1
Bhaktapur	3.0	48.2	38.1	10.1

With the inception of modern facilities in the community and/or changes in life style, the popularity of flush toilets is increasing, with increasing numbers of HHs installing flush toilets. According to CensusInfo 2011, the HHs with flush toilets was only 15.6 % in 2001, which increased to 41.7 % in 2011. Thus, HHs with ordinary toilets decreased in 2011, only 19.5 % HHs had ordinary toilets, which was 23.7 % in 2001. The increasing trend of using flush toilets in HHs also increased the amount of sewage discharged in the sewer line. The CBS (2013a) reported that the LSMC was connected with a total urban sewerage system of 65.9 km in 2011. Thus, the sewerage service of 1 km covers an urban population of 3388.

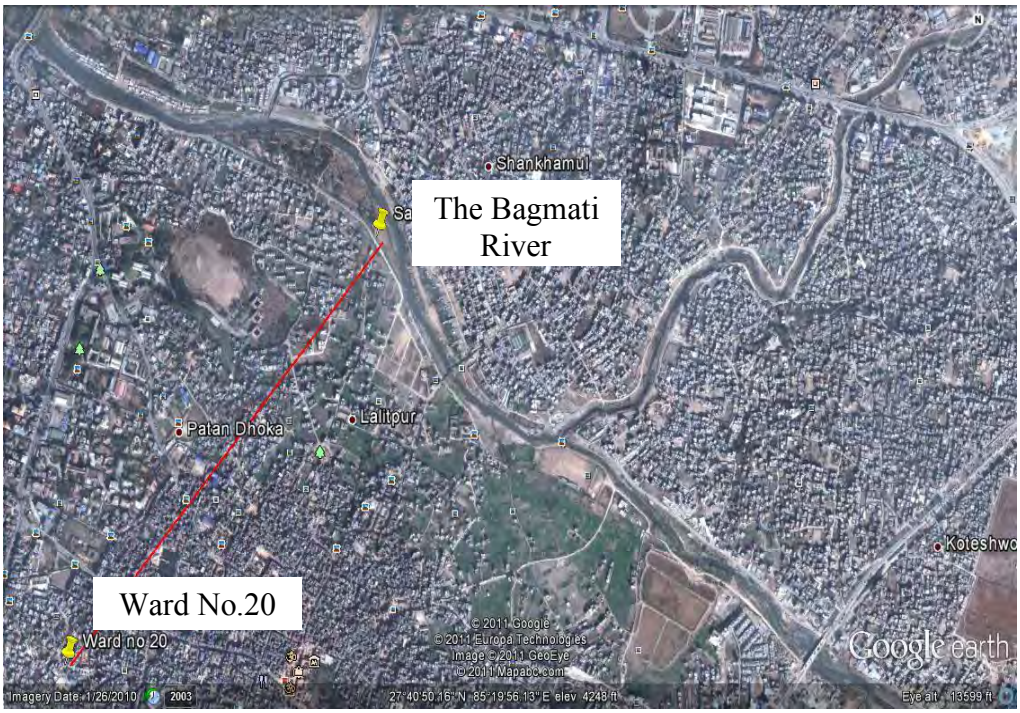

Figure 2.15 The Bagmati River and the Ward No. 20. The sewage collected from the Ward is discharged to the Bagmati River with a central sewerage system.

In the target community, 100 % of the HHs were connected to the sewer line, via a central sewerage system, which is directly discharged to the Bagmati River, without any prior treatment, **Figure 2.15**. The survey showed that HHs in this community have not adopted any on-site wastewater treatment system and only 17.5 % of HHs practice rainwater/stormwater collection using a traditional method (roof top water collection). Thus, the volume of sewage increases as there are no separate systems available for the rainwater/stormwater collection.

2.3.4 Power usage

As depicted in **Figure 2.16**, in Nepal, the residential sector consumes approximately 90 % of the total energy. Only 10 % is utilized by other sectors such as commercial, industrial, transport and agriculture sectors. It is encouraging to observe the decrease in energy consumption in 2010/11 after a gradual increase in energy consumption from 2001/02 to 2009/10.

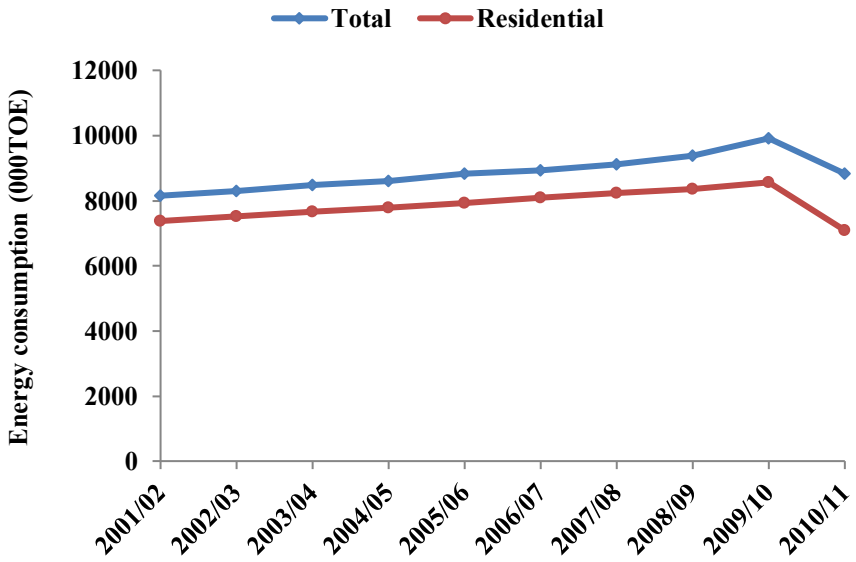

Figure 2.16 Total energy consumption in the country from 2001/02 to 2009/10 and the energy consumption by the residential sector in the same period. Source: (CBS 2013a)

The type of fuel used for the energy production and consumption by different sectors is presented in **Table 2.9**, which shows that most of the energy consumption is based on traditional fuels. For example in 2012/13, 79.9 % of energy consumption was based on the energy produced by the traditional sector, 18.5 % was based on the commercial sector while only 1.7 % was based on renewable energy.

Table 2.9 An overview on the energy consumption by sector and type of fuel. The % expressed for the individual sector is for 2012/13. (CBS 2013a)

Traditional, 79.9 %	Commercial, 18.5 %	Renewable, 1.7 %
Fuel wood	Coal, Electricity	Bio-gas
Agriculture residue	LPG, Kerosene	Micro-hydro
Animal dung	Gasoline	solar
	High speed diesel	
	Light diesel oil	
	Fuel oil	
	Air turbine fuel	
	Other petroleum	

The current study shows that most of the HHs are dependent on electricity for energy, with an electricity usage of 108 units (kw) per HH per month. For cooking purposes, most used kerosene and LPG along with electrical energy. 11.1 % of HHs were found to be using solar power, which is limited to hot water systems only and not for cooking and other applications. According to the 2011 census data, the electricity consumption per HH per month is 234 units[36], which is more than double as found in this study in 2013. Electricity outages for long periods (8 - 12 hours a day) might be one of the reasons for less energy consumption in this survey.

2.4 Conclusions and suggested further research

- The total population of 322 reside in 63 HHs, which represent 4.2 % of the population in the Ward, with an average HH size of 5.1. The total population consisted of 50.7 % male and 49.3 % female, with a male-to-female sex ratio of 1.03.

- The community was found to generate 499.6 g of solid waste per HH per day. The total waste generated by the community mainly consisted of organic waste, being 65.05 % of the total volume. This was followed by plastics, paper and paper products, glass, metals, dirt and textiles, which constituted 13.01 %, 10.65 %, 3.34 %, 6.16 %, 0.40 % and 1.40%, respectively.

[36] http://www.lalitpur.org.np/e_cityataglance_statistics.php

- Rubber and leather were not found in the household waste during the community survey. Also, hazardous waste was not found.

- With respect to waste segregation based on the type of waste, 100 % of the sampled HHs were found to separate organic waste from other waste. Recyclable materials such as milk pouches, plastics, paper and bottles were kept separately.

- The separated organic waste was found to be composted by 98.4 % of the HHs. The Takakura Home Method was used by 95.2 % of HHs and 3.2 % used the bin composting method. The reusable or recyclable materials were sold to the community mobilization unit (CMU) or to scavengers.

- With regard to the waste which could not be composted or sold to scavengers or given to the CMU, 93.7 % of HHs were found to dump its waste in the municipality's collection truck, 6.3 % were collected by the private sector and only 3.2 % was found to be dumped in open spaces/roadside.

- A major source of drinking water supply in the community was found to be tap water through pipelines - provided by the government. Thus, in Ward No. 20, 44 % of HHs were found to rely on tap water, 36 % on tubewells (borehole), 14 % on wells and tap water, 3 % on tankers and 3 % on both tankers and tap water.

- Since the government's water supply service was not reliable, a common practice is to build underground water tanks or keep water tanks on roof tops, for water storage.

- This survey showed that the average capacity of the underground water tank was 4623 L, ranging from 800 - 22000 L and the average capacity of a rooftop water tank was 897 L, ranging from 100 - 4000 L.

- Water usage in the community was found to be 235.9 L per day per HH on average. Thus, each HH was estimated to discharge 200.6 L of wastewater into the sewerage system per day. 100 % of HHs are connected to the sewer line, and the community discharges 396.79m^3 of wastewater in the central sewerage system per day.

- With respect to power usage, most of the HHs were found to be dependent on electricity; each HH was found to use 108 units per month. However, due to power shortages, the electricity supply was not adequate, with 8 - 12 hours of load shedding per day.

Thus, this study shows that the "Towards ZERO Waste" program has, to some extent, addressed waste management problems and the associated challenges associated at the community level. This will definitely minimize adverse impacts on the environment and human health caused by poor MSWM. The management of waste generated at the local level, will help to make MSWM, independent of the ongoing conflicts associated with landfill and dumping sites. Such conflicts had turned Valley Street and open public places into free dumping sites, which made the valley people more vulnerable to health risks such as disease.

Moreover, the management of > 65 % of the waste generated in the community at the local level will divert > 65 % waste that would otherwise sent to landfill. This shows that waste can be utilized as a resource in itself.

Hence, novel and innovative, best available technologies for waste management, including solid and liquid wastes, should be adopted urgently. The first priority should be given to source reduction, and public awareness programs should be conducted to encourage communities to adopt the Waste Hierarchy approach. The Takakura Home Method of composting, being simple, easy and fast, should be extended in the existing community, and promoted in other communities as well.

2.5 References

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CHAPTER 3: The significance of the Bagmati River monitoring program and the performance of the Guheshwori Wastewater Treatment Plant

3.1 Introduction

3.1.1 The Bagmati River

Nepal is a landlocked South Asian country that shares its border with China in its north and with India in its east, west and south. The country is 147,181 km^2 in area and has a population of 31 million (UN 2012). The context of this study is the Kathmandu valley, which is located between latitudes 27º 32' 13" and 27º 49' 10" north and 85º 11' 31" and 85º 31'38" east, with an average temperature ranging from 10 to 24 ºC and an annual rainfall of 1400 mm/year (GON 2009; Pant and Dongol 2009). The rate of population growth in the Kathmandu valley is high and the population is projected to be about 34 million by 2016. It is believed that the civilization of the valley originated along the (holy) Bagmati River that runs through the heart of the city of Kathmandu. The river is 35 km long and originates from Bagdwar at Shivapuri in the north of the Kathmandu valley and leaves the valley via a narrow gorge at Chovar, in the south of the valley. The river has a total catchment area of about 157 km^2. The flow in the river is affected by the seasonal variation; its average flow is 15.6 m^3/sec and the low flow is 0.15 m^3/sec in April (Dixit and Gyawali 2011). Many important religious and cultural temples/shrines, as well as popular heritage sites, are situated along the banks of the river. Fed by springs and monsoon rains, the river is a major water resource and its ongoing degradation, as a result of urbanization and industrialization, is seriously affecting the water quality. This poses a threat to the environment and human health as well as leading to water scarcity. Since few settlements are located in the proximity of the source, the upstream water quality is still acceptable. However, as the river flows downstream, it is very badly affected by anthropogenic activities such as direct sewage discharge, solid waste dumping, river bank encroachment for road construction, squatter settlements and sand mining.

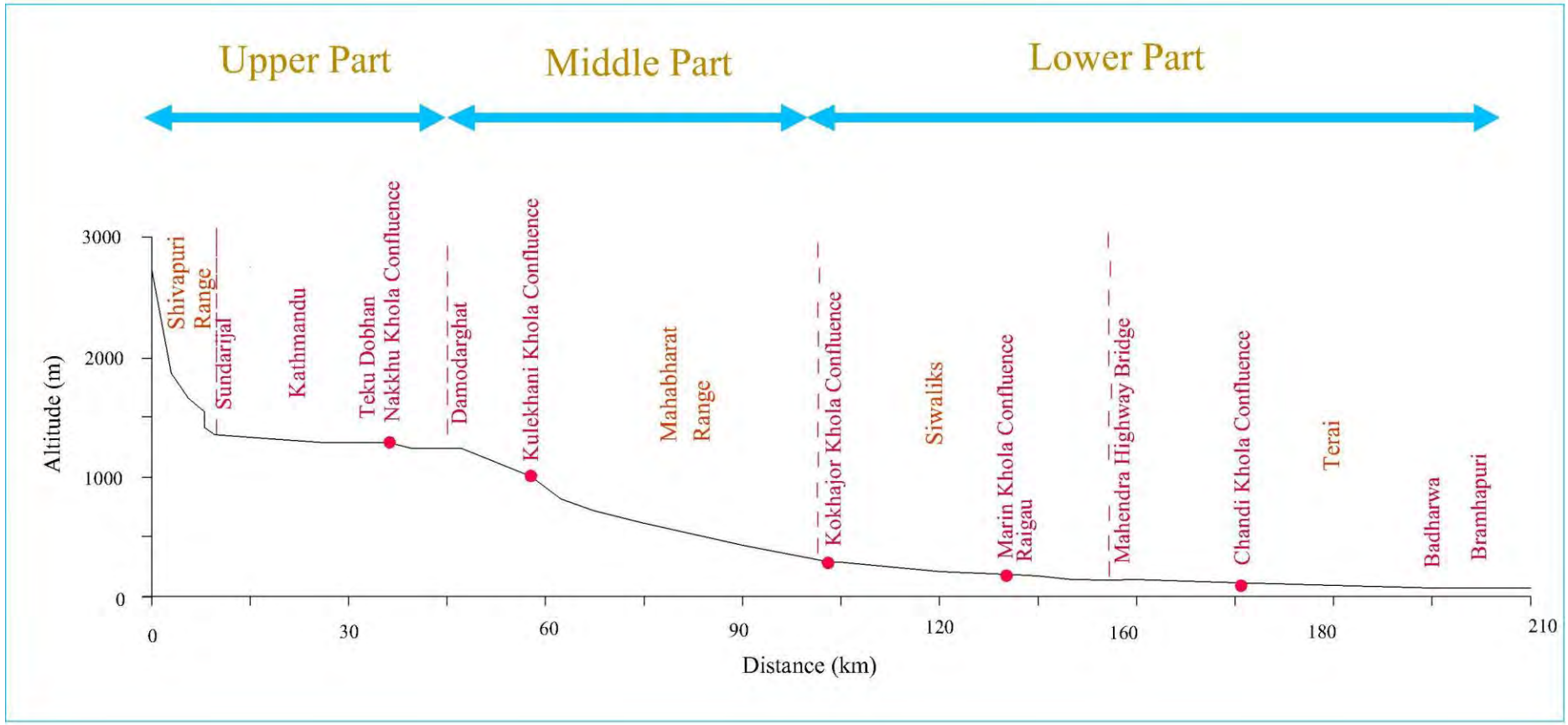


Figure 3.1 The Bagmati River Basin, divided into sub-basins, namely-Upper Bagmati, Middle Bagmati and Lower Bagmati, based on morphology and land use. The basin in Nepal covers about 3638 km^2 (Koirala et al. 2013).

The river may be divided into three different parts based on morphology and land use – i.e. the upper part, middle part and lower part, **Figure 3.1**. The upper part covers the Shivapuri range and the Kathmandu valley, the middle part covers the Mahabharat range and the lower part covers the Siwaliks and Terai regions. Pradhan (2005) has taken the Saprobic approach[37] and has divided the river into four standard water quality classes – Class I for non-polluted, Class II for moderately-polluted, Class III for heavily polluted and Class IV for extremely polluted.

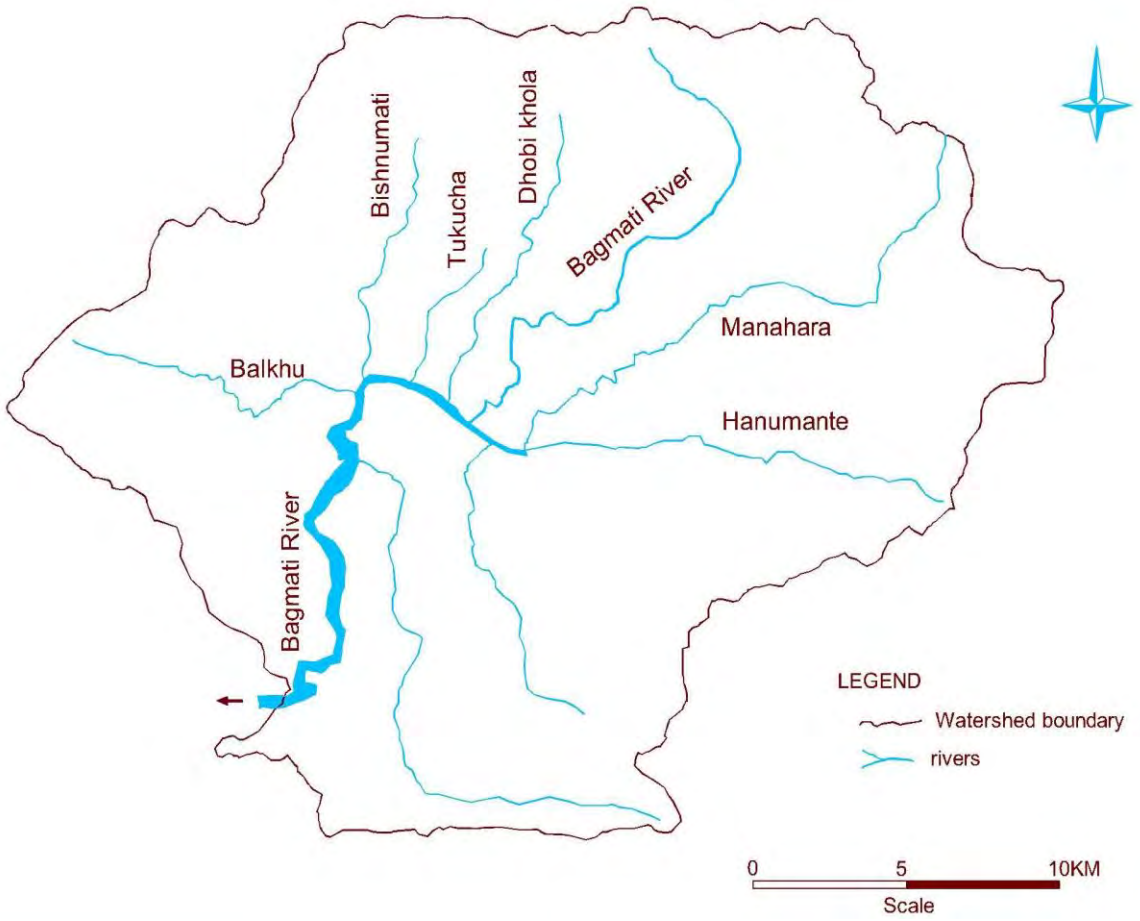


Figure 3.2 The Bagmati River with its six major tributaries. Adapted from (Kanel et al. 2007)

The Bagmati River receives flow from six major tributaries: Manahara khola[38], Hanumante khola, Dhobi khola, Tukucha khola, Bishumati khola and Nakkhu khola, **Figure 3.2**. The three major settlements in the Kathmandu valley – Kathmandu, Lalitpur and Bhaktapur, are located on the banks of the Bagmati River and its tributaries.

[37]The "Saprobic approach" describes the relationship between riverine ecology and river water quality. - GON2009, p.17
[38] 'Khola' is 'River' in a Nepalese language.

3.1.2 The Tributaries of the Bagmati River

Manahara is the longest tributary of the Bagmati River, which originates from Manichaur danda[39] in the north-east and flows towards south-east (GON 2009). The river is 23.4 km long and has a total catchment area of 285.35 km^2, which mainly covers the agricultural land. Similar to the Bagmati River, the river water quality degrades as it flows downstream. Hanumante, Salinadi, Godavari khola, Kodku khola and Ghatte khola are the major tributaries of Manahara.

Bishumati khola originates from Bishnudwar at Shivapuri, north of the Kathmandu valley, which flows to the south. The river is 17.3 km long and has a total catchment area of 109.3 km^2. Many important cultural and religious sites are situated along the bank of the river. This river is also heavily affected by the direct discharge of wastewater, solid waste dumping and direct withdrawal of water by individual households. Chharchhare, Ludi, Sangla, Mahadev, Samakhushi, Bhachakhushi and Mananmati rivers are the major tributaries of Bishnumati.

Dhobi khola (Rudramati) originates from the Shivapuri danda and flows towards the city, which confluences with the Bagmati River at Buddhanagar. The river is 18.2 km long and has a total catchment area of 31.2 km^2. The upstream of the river mostly covers agricultural land and still has good water quality. As the river flows towards the city, it becomes impacted by human activities such as sewage discharge, solid waste disposal, sand mining etc. Its major tributaries are Khahare khola and Chahkhuncha khola.

Tukucha khola (Ichhumati) originates from Maharajgunj, in the Kathmandu valley, and confluences with the Bagmati River at Thapathali. The river is 6.4 km long and has a total catchment area of 8.94 km^2. The entire riverbank is heavily impacted by people for residential buildings, road construction and by squatters. Similar to other rivers, this river is also influenced by sewage discharge and solid waste dumping. This river does not have any tributary.

Nakkhu khola originates from Bhardeu and confluences with the Bagmati River near the Chovar gorge. The river is 17.6 km long and has a total catchment area of 51.44 km^2. From

[39]'Danda' is 'Hill' in a Nepalese language.

the upstream of the river the water is diverted for irrigation. As the river approaches downstream, it receives sewage waste from households as well as industrial waste. Nallu and Lele khola are the major tributaries of this river.

Hanumante khola originates from Mahadev danda, at the eastern part of the Kathmandu valley. The river is 23.5 km long and covers the highly urbanized areas of Bhaktapur and Thimi. The river bank is used as a dumping site for the solid waste generated from the Bhaktapur area. Untreated sewage from Bhaktapur is also discharged directly in this river.

The rivers have been categorized into five zones on the basis of the water quality in the river segment and population density. Zone 1 is a natural conservation core zone, Zone 2 is a rural zone, Zone 3 is a peri-urban zone, Zone 4 is an urban zone and Zone 5 is a downstream zone. The natural conservation core zone covers the Shivapuri National Park, where the origin of the Bagmati River and its tributaries are located. The river is not polluted yet as the population density is still very low. Therefore, the river quality of Zone 1 has been classified as Class I. The rural zone covers the border area of Zone 1 which is mostly the agriculture land. The Bagmati River and its tributaries flow from this zone. The population density is higher than in Zone 1 and the river water is moderately-polluted. Thus the river water has been classified as Class II. The peri-urban zone covers the peri-urban area of the Kathmandu valley. Zone 3 has higher population density than Zone 2 and includes many urbanizing villages. The river water quality is heavily polluted in this zone and it has been classified as Class III. The urban zone is the most highly urbanized area of the Kathmandu valley. It consists of the five municipalities, namely Kathmandu, Lalitpur, Bhaktapur, Madhyapur Thimi and Kirtipur. Zone 4 is highly populated and the water quality in the river is extremely polluted. Thus, the river has been classified as Class IV. The downstream zone covers Sundarighat to Katuwal daha, from where the Bagmati River leaves the Kathmandu valley. Zone 5 has comparatively less population density than Zone 4 and consists of agricultural land. However, the river water quality in this zone is extremely polluted due to upstream discharge and this zone is classified as Class IV.

3.1.3 The river ecosystem and wastewater

The river ecosystem has been adversely affected as a consequence of the decrease in water discharge into the river, degradation of water quality, degradation of catchment quality, the narrowing and deepening of the waterway and the depletion of aquatic biodiversity (GON 2009). The diversion of a huge amount of water from upstream - for drinking and irrigation, has significantly decreased the water discharge in the river. Consequently, the river flow has decreased and the habitat for aquatic life has been destroyed. An alternative source of water supply, such as the Melamchi water supply project, could reduce pressure on the Bagmati River and increase the discharge (Khadka and Khanal 2008). The unhealthy practices of open defecation, poor sanitation, household solid waste disposal onto the river banks, runoff from agricultural land containing chemical fertilizers and pesticides are a few of the causes of river water degradation in the upstream part of the river, especially in Zones 1 and 2. Additional pollution in Zone 3 comes from wastewater discharge from industries, such as poultry, piggeries, saw mills, paper mills, dying industries, textiles etc. Municipal solid waste dumping onto the riverbank and sewage discharge into the river without any treatment are major contributors to the degraded water quality in Zones 4 and 5. In addition, waste from hospitals, industries and commercial institutes such as super markets are also contributing to the degradation of river water.

Land encroachment for human settlement and the conversion of forest land into agricultural land, as a consequence of unplanned and rapid urbanization, are degrading the river catchment quality. It is vital to maintain the catchment quality; otherwise it will reduce the base flow of the river, which is required to maintain a steady flow downstream. The mobilization of the community forest user groups to retain the forest in the catchment area is one important pathway to preservation.

Sand extraction from the river bed, encroachment of the river bank for road construction and settlements and unplanned construction of gabion[40] structures along the river bank are some of the reasons for the narrowing and deepening of the waterway. As a consequence of these actions, the flow velocity of the water increases, which can damage infrastructure in the

[40] Gabions are free-draining walls that are constructed by filling large galvanized steel baskets with rock. http://www.gabionwallsystems.com/

proximity of the river. Moreover, the ground water table might become lower due to the discharge of groundwater into the river to balance the reduced water level. The restoration of the river bed and awareness among local residents can mitigate this issue.

A healthy river system can be demonstrated by the presence of aquatic flora and fauna such as fish, amphibians, reptiles, micro and macro invertebrates and birds. Due to a highly polluted river system, many of these species are becoming endangered or even extinct, especially in the urban core. Consequently, there is an increasing imbalance in the river ecosystem. No freshwater fish species, that are visible in rural areas, can be seen as the river approaches the urban area.

3.1.4 Culture and heritage

Many cultural and religious sites are located along the banks of the river, as well as popular national heritage sites. Some of the important cultural and heritage sites are Gokarna, Pashupati, Sankhamul, Teku dovan etc. The river is closely associated with the traditional rituals of the Nepalese people. Cremation is performed on the banks of the holy river; with people in the Kathmandu valley preferring to be cremated along the river bank in proximity to the Pashupati dham. Keane (2013) reported that an average of thirty seven cremations a day was performed in this area. Such rituals have an adverse impact on the river environment and river water quality, since cremation remains and material from other rituals are dumped straight into the river. A diminishing of cultural and religious values has lessened the traditional norm of respect for the river. Consequently, people have also started to dump solid waste and to discharge sewage into the river. Indeed, the river water has now been polluted to the level that “holy baths” should be avoided. Thus, it is vital to restore the river in order to protect its cultural and religious significance, **Figure 3.3**.

Figure 3.3 Pashupati dham - a famous cultural and heritage site along the Bagmati River (Keane 2013).

3.1.5 Riverside land use and socio-economic condition

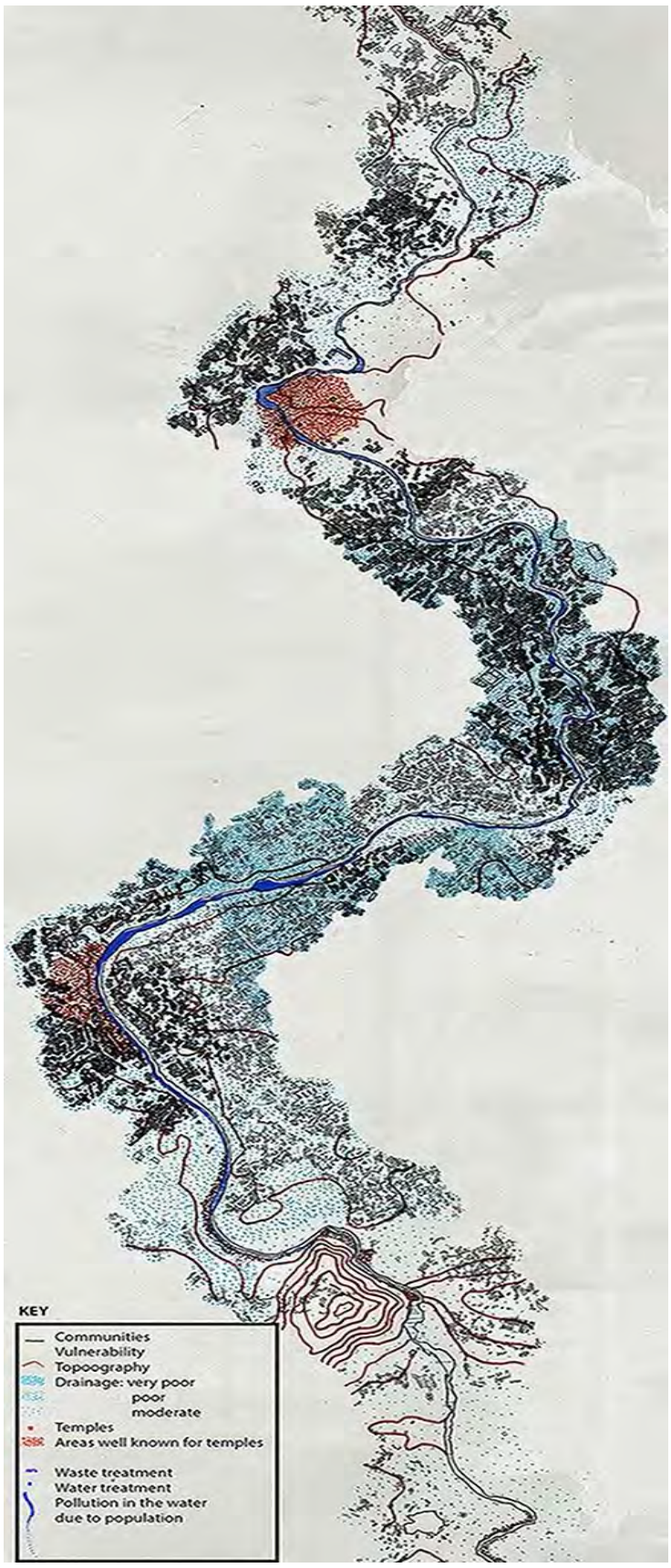
Riparian vegetation along the river bank keeps the river environment healthy and retains its aesthetic value. Moreover, it is important for the balance of the river's ecosystem. The upstream part of the river, which lies in the natural conservation zone, has good riparian vegetation. However, as the river flows downstream, it runs through agricultural land. Once it reaches urban areas, the riverside land is significantly impacted by road construction, squatter settlements, private residences, commercial complexes, schools and hospitals. Such unplanned riverside land use has diminished the aesthetic value of the river, which is still maintained in the National park area and where temples/shrines/monuments are located.

3.1.6 A review of the water quality monitoring of the Bagmati River

Many studies (Wolfe 2000; Green 2003; Pradhan B 2005; Kannel et al. 2006; Bhatt and McDowell 2007; Kannel et al. 2007a; Kannel et al. 2007b; Bhatt and Gardner 2009; Kanel et al. 2011; Pandey et al. 2011) have been conducted on the Bagmati River Basin to assess the water quality of and the effect of pollution on human and aquatic life and water availability. These studies have found that sand extraction, land encroachment for different purposes, municipal solid waste dumping on the river bank and development activities are major

contributors to the deteriorating situation of the river basin, whereas the discharge of sewage directly into the river without prior treatment is responsible for a significant part of the river water pollution (GON 2009; Kanel et al. 2011). The Bagmati Action Plan (Pradhan B 2005; GON 2009) has been developed by the Government of Nepal to conserve and restore the river. Thus the river has been classified into five zones based on the water quality in each river segment and the population density of that area. The water quality of the urban zone has been identified as the most polluted, i.e. class IV. The Central Bureau of Statistics provides some information on water quality standards and guidelines for various uses - with a list of policies, acts and rules that govern the river environment (CBS 2008).

The river is home to a wide range of aquatic life and is a valuable source of water for drinking and irrigation (Horan 1990; Haslam 1992). Many factors such as seasonal variation, surface runoffs, biotic and abiotic activities contribute pollutants to the river water (Vega et al. 1998). However, anthropogenic activity is one of the major reasons for river pollution and degradation. The river is the ultimate site for the disposal of waste, solid or/and liquid, especially for a landlocked country like Nepal. Ideally, wastewater should be allowed to discharge in the river only after proper treatment - but this is currently not the case. There are many environmental and health impacts, both for humans and animals, who comes into direct contact with water that receives untreated or insufficiently treated municipal wastewater (Akpor and Muchie 2011). In this regard, some studies recommend the need to develop appropriate technologies that will treat wastewater in an 'environmental, societal and economic sustainable' way (Muga and Mihelcic 2008). Moreover, a municipal wastewater treatment flow-sheet has been proposed which combines physical and chemical processes with biological processes to recover energy, water and nutrients (Sutton et al. 2011). Keane (2013), for example, has proposed a simple and cost effective technology for the treatment of the wastewater, having put forward a plan and a design of wastewater treatment technology that is based on a sand filtration system - in order to minimize the impact of untreated wastewater discharge into the Bagmati River.



The mapping of the Bagmati River as presented by Keane (2013), includes: a description of the drainage – indicated by blue dots (dense for very poor to light for moderate), communities vulnerable to diseases – indicated by bolded black lined boxes, temples – indicated by red squares and most popular temples – indicated by red dots. The dark blue lines indicate the extent of the river pollution due to population density. This figure illustrates that the river water quality is comparatively worse where the drainage system is very poor and, as a consequence, the communities are more vulnerable to disease. Moreover, the degree of river water pollution is higher around the temple areas, which might be due to the more populous religious and traditional rituals that are performed in the vicinity of the temples and the river itself. Notably, Keane observed the interaction of the Nepalese people with the river to be ideally "a practice of both purity and cleanliness that was rooted in a search to be pure upon entry into the next life" (Keane 2013).

Figure 3.4 Mapping of the Bagmati River (Keane 2013).

The components of wastewater and their receiving water bodies should be analysed to adopt an effective integrated water management strategy. BOD_5, COD, DO, TDS, TSS, conductivity, pH, temperature, nutrients (nitrogen, phosphorus) and heavy metals are the major water quality parameters that are frequently tested (Horan 1990). High values of most of these parameters (except DO) are considered to be indicative of high levels of water pollution, although a high value of DO is an indicative of better water quality.

Table 3.1 presents a brief overview of some of these water quality parameters as observed at different stations along the Bagmati River at different time periods. The data from Paudel (1998) is from the study carried out by the Water and Energy Commission Secretariat/Nepal Environmental and Scientific Services in 1997. Kannel et al. (2007a) have recorded the averaged data collected during the period 1999 to 2003 in pre-monsoon, monsoon, post-monsoon and winter seasons. Regmi (2013) has recorded data from a study conducted at the Sundarighat sampling station. All these studies show that the river water quality deteriorates as it flows downstream. For instance, in **Table 3.1**, Paudel (1998) reported that the COD was found to be 274 mg/L at Pahsupati dham which increased up to 367 mg/L at Chovar. Kannel et al. (2007a) observed 5.4 mg/L BOD_5 at Gokarna, which increased up to 57 mg/L at Sundarighat. Bhatt and McDowell (2007) also reported a similar finding and concluded that water quality along the Bagmati River is not acceptable for any purpose and the whole ecosystem has been affected due to the pollution. They observed the influence of human and geochemical processes on changes in river chemistry throughout the Kathmandu valley, in terms of nutrients, organic matter and the major cations and anions.

With an increasing scarcity of fresh water, the recovery and safe reuse of treated wastewater for various purposes has become a particular area of interest. In this regard, many companies in developed countries have introduced innovative technologies for the recovery of municipal wastewater on-site with a range of capacities and treatment standards (e.g. such Australian based companies include -Aqua-nova, BioSeptic, Fuji Clean and Supertreat) and in the United States such companies include – Orenco and Siemens). Many of them offer combined biological/chemical technologies and claim their technologies to be energy efficient, to have a low footprint and to provide high quality effluent.

Table 3.1 Available data on the water quality of the Bagmati River at different sampling stations.

Bagmati River monitoring stations	Gokarna		Pashupati dham		Sankhamul		Sundarighat			Chovar
Observed Parameter	*Paudel (1998)*	*Kannel et al.(2007a)*	*Paudel(1998)*	*Kannel et al.(2007a)*	*Paudel(1998)*	*Kannel et al. (2007a)*	*Paudel(1998)*	*Kannel et al.(2007a)*	*Regmi(2013)*	*Paudel(1998)*
Temperature (°C)	-	19.24	-	20.99	-	20.76	-	19.66	-	-
pH	7.6	7.4	6.5	7.2	7.1	7.25	7.1	7.4	7.2	7.1
Conductivity (µS/cm)	70	51.8	360	185.8	410	304.9	740	435.5	-	720
Turbidity (NTU)	-	-	-	-	-	-	-	-	183	-
TDS (mg/L)	56	30	288	94	-	151	592	218	-	576
TSS (mg/L)	-	102	-	203	328	513	-	244	115	-
DO (mg/L)	6.7	7.7	<0.5	3.98	<0.5	3.26	<0.5	2.24	0.9	<0.5
COD (mg/L)	22	17	274	60	90	67	378	93	320	367
BOD_5 (mg/L)	-	5.4	-	41	-	48	-	57	296	-
NH_3-N (mg/L)	0.16	1.7	16.8	7.5	18.6	10	43	19	110	38.8
NO_2-N (mg/L)	-	0.08	-	0.28	-	0.27	-	0.47	-	-
NO_3-N (mg/L)	-	1.55	-	2.23	-	2.01	-	3.91	18	-
TP (mg/L)	-	0.37	-	0.84	-	2.4	-	3	-	-

One of the largest European companies in waste and wastewater recovery, Veolia Water, manages, operates and maintains thirty-four wastewater treatment plants across Australia and New Zealand with the treatment capacity varying from 0.1 MLD to 259 MLD. BIOSEP™ is their most popular technology, being compact in design and with a unique combination of biological treatment (activated sludge) and immersed membrane filtration. This technology is environmentally safe and provides high quality treated effluent[41].

However, many such technologically advanced wastewater treatment plants in developing countries do not perform to capacity due to a lack of proper knowledge on their operation and a lack of maintenance (Wagner and Pinheiro 2001). For example, both the wastewater treatment plant, which is under the ownership and direct supervision of the High Powered Committee for Integrated Development of the Bagmati Civilization (HPCIDBC) and which is based on activated sludge technology (with the capacity to treat 16.4 MLD), and the treatment plant located at Gokarna, which is based on Reed Bed technology, are experiencing these problems (HPCIDBC, personal communication). Bright-Davies and Jachnow (2013) have described some pilot projects around the Kathmandu valley that implement Decentralized Wastewater Treatment Systems (DEWATs)[42] in these communities to reduce or eliminate open defecation. As a result, these communities achieved improved sanitation and environmental hygiene. Considering the success of these pilot-projects and the shortcomings of the centralized treatment systems in the Kathmandu valley, Bright-Davies and Jachnow (2013) argue that DEWATs may be an alternative approach. The United Nations Environment Programme (2001) has also recommended the introduction of wastewater treatment plants at the local community level. According to USEPA[43], "*adequately managed decentralized wastewater systems are a cost-effective and long-term option for meeting public health and water quality goals, particularly in less densely populated areas.*" In Nepal, constructed wetlands with reed bed technology is popular as DEWATS, and is installed in many hospitals and schools (Jha and Bajracharya 2014).

In addition, rainwater harvesting is being considered as an alternative approach for addressing fresh water scarcity (Alam et al. 2011; Farreny et al. 2011). However, rainwater storage over long periods of time in a monsoonal climate such as Nepal requires special

[41]http://technomaps.veoliawatertechnologies.com/biosep/en/municipality.htm
[42]A detail discussion on DEWATs is done in Chapter 1, Section 1.2.5.
[43]http://water.epa.gov/infrastructure/septic/index.cfm

consideration. In addition, the diversion of rainwater reduces stormwater runoff to the river and reduces the quantity of wastewater to be treated.

Urban planning criteria for the assessment of the sustainability of a city are closely related to water management issues (Novotny et al. 2010). With a view to minimizing the environmental impact on the river and for an informed consideration of integrated wastewater management and for the appropriate design of wastewater utilities, information technology planning and decision making systems such as the Geographic Information System (GIS) (WEF 2011) and SANEX© (Loetscher 1999; Loetscher and Keller 2002) are useful tools. The Water Environment Federation (2011) states "a GIS can become a key tool to support utility decision making and planning if the GIS is well integrated with other data sources. … a GIS can be a powerful tool for communicating with the public" (p. 39). For example, researchers have discussed mathematical models for conventional pollutant evaluation such as QUAL2K and QUAL2Kw (Kanel et al. 2007).

The restorations of the Thames River in Britain (Doxat 1977; Wood and Ager 1982; Kinniburgh and Barnett 2010)) and the Cheong Gye Cheon River (Shin and Lee 2006) in Korea provide evidence that a polluted river is capable of regaining its original form if a holistic approach is taken and planned efforts are made by the government with community participation.

A review of the known literature shows that water quality monitoring of the Bagmati River to date has been limited to the river body itself and has not yet been carried out for the discharge point sources. Moreover, the studies have left out important water quality parameters in municipal wastewater such as heavy metals which have the potential to impact on the environment and health (Akpor and Muchie 2011). Thus, this study aims to address these gaps in the previous studies.

3.1.7 Wastewater treatment systems in the Kathmandu valley

In the Kathmandu valley, many small decentralized wastewater treatment systems, based on different technologies, have been constructed. On the other hand, only a few centralized wastewater treatment systems are in place. However, many of the latter are not functional at

all and only few of them are partially functional. A summary of these centralized wastewater treatment plants is provided in **Table 3.2**. A detailed discussion has been carried out for the Guheshwori wastewater treatment plant (GWWTP) since it forms part of this study.

Table 3.2 Current centralized wastewater treatment systems in the Kathmandu valley.

Capacity, MLD	Location	Treatment system	Current status
16.4	Guheshwori, Kathmandu	Oxidation ditch,	Partially operating
15.4	Dhodighat, Lalitpur	Aerobic. Facultative ponds, Anaerobic.	Not operating
2.4	Sallaghari, Bhaktapur	Aerated lagoon,	Partially operating
1.1	Kodku, Kathmandu	Aerobic. Facultative ponds, Anaerobic	Partially operating
0.4	Hanumanghat, Bhaktapur	Oxidation ditch	Not operating
0.05	Sunga community Bhaktapur Wastewater Treatment Plant.	Constructed wetland.	Operating

(Shukla et al. 2012; Regmi 2013; Jha and Bajracharya 2014)

3.1.7.1 An assessment of the Guheshwori Wastewater Treatment Plant

Previous studies (Pokhrel and Ha 2001; Pradhan B 2005; Kannel et al. 2006; Bhatt and McDowell 2007; Kannel et al. 2007a; Kannel et al. 2007b; Kanel et al. 2011) on the water quality of the Bagmati River have reported that the river water quality deteriorates as it flows downstream to more populated areas. Upstream (rural areas), human sewage and fertilizer are found as the major contaminants, whereas downstream (urban areas) municipal sewage and solid waste are the major contaminants. The need to treat municipal wastewater before discharge on to the river prompted the construction of the GWWTP, designed in 1996 and started its operation in 2001 (Green 2003), see **Figure 3.5** and the schematic diagram depicted in **Figure 3.6**.

This is located on the banks of the Bagmati River and has the capacity to treat 16.4 MLD of wastewater collected from surrounding areas-from Gokarna to Tilganga (Regmi 2013). The treatment plant covers 5 hectares and it is designed for a population of approximately 198,000. This treatment plant is based on activated sludge technology, consisting of an oxidation ditch with aerators where microbes decompose organic matter. Due to the complexity of such treatment processes, conventional centralized wastewater treatment systems such as the GWWTP involve high operational and maintenance costs and require highly skilled operators.

Table 3.3 The GWWTP design parameters. (WEPA Nepal Dialogue site visit report, 2010); note: Mixed Liquor Suspended Solids (MLSS); Food to Microbe ratio (F/M).

The GWWTP design parameters	
Service area	5.37 km^2
Service population (1996)	58,000
Projected population (2021)	198,000
Wastewater produced	80 L/capita/day
WWTP footprint	51 m^2
Energy consumption	2.3 KW-hr/kg BOD_5
Annual operating costs	$167,000 US
Design flow	0.19 m^3/s (4.3 MGD)
MLSS	3,500 mg/L
F/M	0.34

Table 3.4 The GWWTP design performance.

Parameter (mg/L)	Influent	Effluent	% Removal
BOD_5	270	25	91
COD	1150	250	78
TSS	216	100	54
TKN	48	30	38
NH_3-N	41.7	22.1	47
TP	6.7	3.2	52

An assessment of the performance of the GWWTP since its establishment was not possible due to the unavailability of the water quality monitoring data. Thus it is not clear whether such water quality monitoring was conducted or not. Only limited data was available and the

assessment presented in this thesis was necessarily based on any secondary data that was available and the primary data obtained in this study on an extended field trip[44].

Figure 3.5 The Guheshwori Wastewater Treatment Plant showing the oxidation ditch and the surrounding catchment area. Pictures were taken by the researcher on 24/10/2013 during the field trip to Nepal. (Photos take by Anusuya Joshi)

Table 3.5 presents the data recorded for the month of April in 2002, 2003, 2012 and 2013. Despite the fluctuation in the values for some parameters such as TSS and COD, for influent, it is clear that the influent strength is getting "stronger" over time. The higher the concentration of organic matter in the influent, the stronger the "strength" (Mara 2013). For instance, the BOD_5 recorded in 2002 was 376 mg/L, which increased to 538 mg/L in 2013. Moreover, DO being one of the important parameters that reflects the quality of water, was found to be 1.2 mg/L in 2002, reducing to 0.3 mg/L in 2013.

The performance efficiency of the GWWTP has been evaluated in terms of the removal efficiency of pollutants as demonstrated by the values of TSS, COD, BOD_5 and NH_3-N, and the % increase in DO, as presented in **Figure 3.7**. The TSS in the influent was 295 mg/L in 2002, which increased to 648 mg/L in 2013. The TSS removal efficiency was low in 2012 (59 % only) compared to 2002 (81 %).

[44]The PhD student and the Associate Supervisor travelled to Kathmandu, Nepal from Melbourne, Australia, to investigate the performance of the GWWTP.

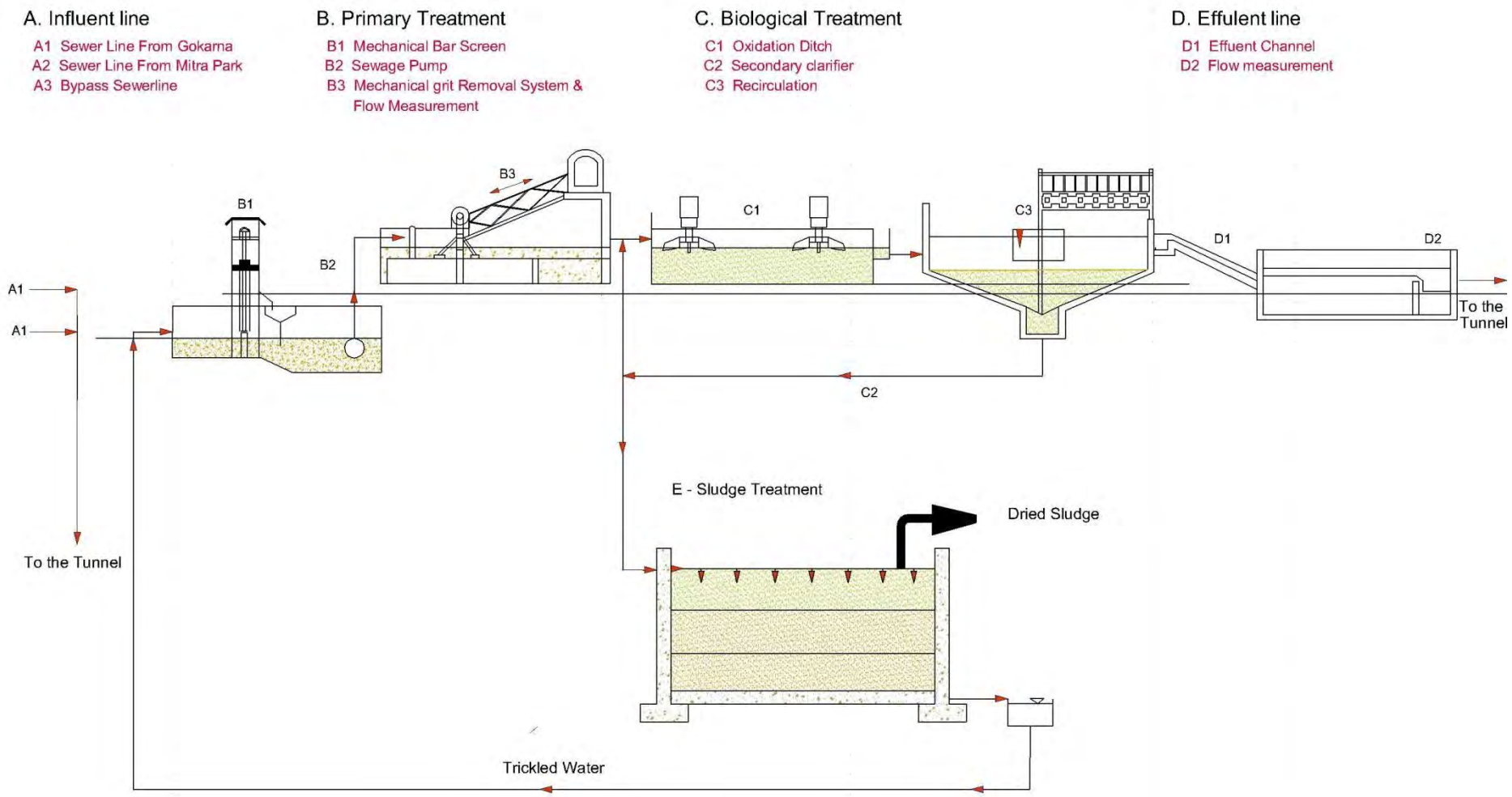


Figure 3.6 Schematic diagram of the GWWTP.

With respect to COD, the removal efficiency was significantly less in 2013 (43 % only) than in 2003 (87 %). However, the removal efficiency of the BOD_5 seems to be better than the other parameters (84 % in 2002 and 79 % in 2013). The NH_3-N in the influent was found to be more than double in 2013 (140 mg/L) in comparison to 2002 (53 mg/L).

However, the GWWTP has only low NH_3-N removal efficiency, 17 % in 2002 and only 7 % in 2013. In terms of DO, it increased by 320 % (0.5 to 2.1 mg/L) in 2012 and by 273 % in 2013 (0.3 to 1.1 mg/L). The influent DO was significantly less in 2012 than in previous years.

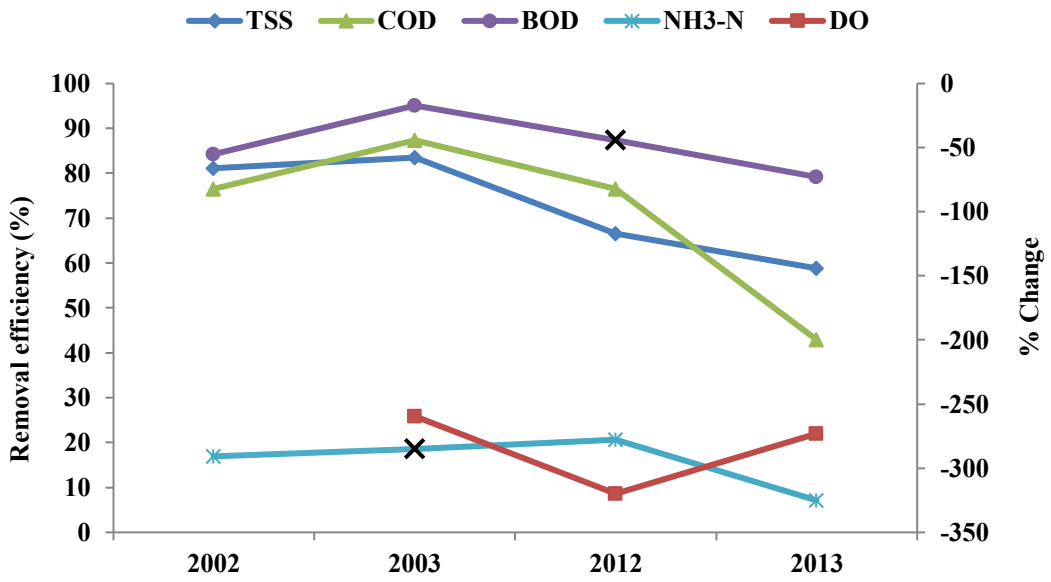


Figure 3.7 A temporal comparative study for the month of April in the years indicated, with respect to the performance of the GWWTP - showing the % removal efficiencies for TSS, COD, BOD_5 and NH_3-N and the % DO change. The data points represented by × are "dummy" values since the actual data was not available and are included to indicate the trend. The data is drawn from Table 3.5.

The data for pH, total organism (TO) count and the faecal coliform (FC) count was available only for the year 2013. In terms of pH, the slightly acidic influent became alkaline after the treatment process (6.9 to 7.4). In terms of the biological observation, the removal efficiency was found to be 93 % for the TO count whereas it was only 24 % for the FC count.

Thus, this overview clearly depicts that the treatment efficiency of the GWWTP decreased over-time. In addition, the comparison between the current % removal, **Table 3.5**, and the design parameters and design performance of the GWWTP, **Tables 3.3** & **3.4**, reflects that

the current treatment efficiency for COD, BOD_5 and NH_3-N is significantly less. However, the current removal efficiency for TSS is slightly higher than the design parameter. There may be many reasons behind this reduction of treatment efficiency; one might be maintenance issues. Moreover, this could be related to the operation of the aerators in the system. It is worth mentioning here that the aerators could not be operated at full phase due to the electrical supply shortages.

Considering the technical and financial issues in the operation and maintenance of the GWWTP, with current technology, Regmi (2013) has recommended making a few amendments in the current system. A comparison of the current system with the suggested system, Sequential Batch Reactor (SBR), is presented in **Table 3.6**.

The SBR is a kind of an activated sludge treatment technology, known as a fill-and-draw activated sludge treatment system. However it can be operated under non-steady state condition, unlikely a traditional activated sludge technology. The operating mechanism of SBR constitutes of six cycles, namely anoxic fill, aerated fill, react, settle, decant and idle[45] (Vigneswaran et al. 2008). This system has smaller footprints as the treatment process is carried out in a single basin, which only requires less land. The flexibility in the operating system allows the treatment cycle to undergo anaerobic, anoxic and aerobic processes to enhance the organic matter removal and nutrition reduction in a single tank.

[45] http://www.eolss.net/sample-chapters/c07/e6-144-11.pdf

Table 3.5 Performance of the GWWTP based on the parameters listed below, for the month of April in 2002, 2003, 2012 and 2013, as reported in Regmi 2013 (secondary data). The value reported in parenthesis for 2013 is the primary data for this study.

Observed Parameters	2002		% Change	2003		% Change	2012		% Change	2013		% Change
	Influent	*Effluent*		*Influent*	*Effluent*		*Influent*	*Effluent*		*Influent*	*Effluent*	
pH	-	-		-	-		-	-		6.9 (6.5)	7.4 (7.2)	-8 (-12)
TSS (mg/L)	295	56	81	422	70	83	314	105	67	648 (547)	267 (91)	59 (83)
DO (mg/L)	-	-		1.2	4.3	-260	0.5	2.1	-320	0.3 (0.4)	1.1 (3.4)	-273 (-750)
COD (mg/L)	744	175	77	1069	135	87	1356	319	76	672	384	43
BOD_5 (mg/L)	376	60	84	437	22	95	-	-		538	112	79
NH_3-N (mg/L)	53	44	17	-	-		58	46	21	140 (78)	130 (64)	7 (18)
TO count* (cfu/100 mL)	-	-		-	-		-	-		3.00E+08	2.00E+07	93
FC count (cfu/100 mL)**	-	-		-	-		-	-		1.53E+04	1.17E+04	24

Note: Data provided in the parenthesis, for 2013, is the primary data from this study. The rest is the secondary data.
*TO count – Total organism count
**FC count – Faecal coliform count

Table 3.6 Comparison of the GWWTP – proposed Sequential Batch Reactor with the existing activated sludge technology (Regmi 2013).

Parameters	GWWTP (current)	GWWTP (proposed)
Influent flow rate, m^3/day	16416	16416
Treatment type	Continuous	Batch
Time required for treatment, hours	24	5/batch
BOD_5 removal rate, %	79.18	97.4
COD removal rate, %	42.85	84
TSS removal rate, %	58.84	93.6
Fecal coliform removal rate, %	23.52	96
No. of blowers for aeration	6	2
Time for aeration, hours/day	24	10 (2 hours/batch)
Required energy for blowers, HP	60	20
Electrical energy usage, unit/kg BOD_5	2.3	0.77
Ratio of wastewater treated to amount of land, m^3 wastewater/m^2 land/day	0.003	0.004

Moreover, the existing system can be modified to the SBR, utilizing the basins in the current system (Regmi 2013). The current power shortage will not affect the performance of the proposed SBR system as it is a huge drawback in influencing the performance efficiency of the GWWTP.

To reduce adverse impacts of the effluent discharge into the river and to maintain the health of the river environment, the Government of Nepal has provided some guidelines. The guidelines on the tolerance limits for effluents discharged into inland surface water and the Nepal water quality guidelines for the protection of aquatic ecosystem, for some of the parameters under consideration, are listed in **Table 3.7**.

Table 3.7 Nepalese Standards for River Water Quality.

	Tolerance limits for effluents discharged into inland surface water [46]*	Nepal water quality guidelines for the protection of aquatic ecosystem **
Temperature	Shall not exceed 40 °C	"Water temperature should not be allowed to vary from the background[47] average daily water temperature considered to be normal for that specific site and time of day, by > 2 °C or by 10% whichever estimate is the more conservative".
pH	5.5 - 9.0	"pH values could not be allowed to vary from the range of the background pH values for a specific site and time of day, by > 0.5 of a pH unit, or by > 5% and should be assessed by whichever estimate is more conservative."
DO		80-120 (% saturation)
TSS	50 mg/L	"Any increase in TSS concentrations must be limited to < 10 % of the background TSS concentrations at a specific site and time."
TDS		"TDS concentrations should not be changed by > 15% from the normal cycle of the water body under unimpacted conditions at any time of the year."
COD	250 mg/L	No entry
BOD_5	50 mg/L	No entry
NH_3-N /TN	50 mg/L	"Inorganic nitrogen concentrations should not be changed by more than 15% from that of the water body under local unimpacted condition at any time of the year; The trophic status of the water body should not increase above its present level, though a decrease in trophic status is permissible (see effect):The amplitude and frequency of natural cycles in inorganic nitrogen concentrations should not be changed."
Fe		"The iron concentration should not be allowed to vary by more than 10% of the background dissolved iron concentration for a particular site or case, at specific time."
Zn	5 mg/L	<2 µg/L
Cu	3 mg/L	<1.4 µg/L for very hard water
Mn		<180 µg/L

* Source: Nepal Gazette , 2058/01/17 (30 April 2001) and 2060/ 03/09 (23 June 2003),In (CBS 2008), ** Source: Department of Irrigation: Ground Water Project (Nepal Gazette (Number 10, BS, 2065-03-02), In (ADB 2013).

[46]Definition of Inland surface water in http://legal-dictionary.thefreedictionary.com/Inland+Waters 12/03/2015

[47]In this Table the reference to "background" values does not indicate where such values are documented or may be found. This, perhaps, suggests the need for such a database to be readily accessible.

3.1.8 The Bagmati Action Plan (2009-2014)[48]

The Bagmati Action Plan (2009 -2014) was introduced in 2009 by the Government of Nepal and the National Trust for Nature Conservation (NTNC) with a view to conserve and restore the Bagmati River and its tributaries. The action plan has identified the following "interactions" of the river – i.e. what the river is used for (GON 2009, p.5).

i. Major source of municipal, industrial and irrigation water for the Kathmandu valley and for cultural and religious practices.
ii. Disposal of water-borne effluents and deposition of solid waste along the banks.
iii. Extraction of sand.
iv. Space for public infrastructure, e.g. roads and water tanks.
v. Preferred zone for squatters and other encroachments.

Realizing that current practices of river usage have seriously affected the river environment and have caused acute water scarcity, the action plan aims to carry out the following activities, based on the requirement of the individual Zone (GON 2009, pp. 56-67).

1. Undertake various measures to protect and enhance water resources and increase water discharge into the river.
2. Undertake various measures to conserve the catchment area and aquatic biodiversity.
3. To maintain and enhance the river water quality.
4. To renovate and conserve the cultural and heritage sites.
5. To promote tourism.
6. To prevent discharge of wastewater and solid waste into river.
7. Promote sustainable agricultural practices.
8. Regulate urban growth and industrial activities.
9. Control and relocate squatter settlements from the river banks.
10. To improve river water quantity and quality through the proper management of water and wastewater.
11. To improve the self-purification processes of the river.

[48]Provided in CD.

3.1.9 Research objectives

One of the main objectives of this PhD study is to research the adoption of the best available decentralized wastewater treatment technology with a view to minimizing adverse impacts on the water quality of the Bagmati River, **Section 1.5**. Thus, to place this in context, this research also aims to assess the Bagmati River water quality and the performance of the centralized wastewater treatment system, the GWWTP. More specifically, the research described within this chapter has been directed towards:

- Reviewing the current wastewater treatment systems that are available in the Kathmandu valley.
- Assessing, via previous data and via an experimental field trip to Nepal, the water quality of the Bagmati River, from upstream (Sundarijal – where the river enters the Kathmandu valley) to downstream (Chovar – where river leaves the Kathmandu valley).
- Analysing this data in order to assess the current impact of the GWWTP on the Bagmati River and to provide a framework for the consideration of alternative or adjunct biological wastewater treatment systems such as Vermifiltration.

3.1.10 Limitations of the study

This part of the research program was intended to provide a background study for considering the adoption of the best available waste management technologies in Nepalese communities. As such, this part of the project constitutes approximately 10 % of the research program. Due to the time constraints and a defined budget, the researcher could only spend a limited amount of time in the field (Kathmandu, Nepal), given that the major part of the project was located in Melbourne, Australia. Moreover, the laboratory at the High Powered Committee for Integrated Development of the Bagmati Civilization (HPCIDBC), where the author was granted access, had limited resources. Much of the equipment and reagents for the water and wastewater analysis had to be taken over to Nepal from Melbourne. Therefore, only the portable items and reagents which could be transported without any difficulty were taken over to enable appropriate analyses to be conducted by the researcher herself. Consequently,

only those parameters which are commonly used to assess the water and wastewater quality were chosen - as listed in **Section 3.2.4, Table 3.7**.

3.2 Materials and Methods

Current practice for the management of wastewater (sewage) generated by the communities and the research design for this part of the study is presented in the schematic shown in **Figure 3.8**. The figure shows that a part of the wastewater generated by the communities is discharged directly into the river and a part of it is sent to the GWWTP for treatment. At the GWWTP, wastewater is passed to the treatment unit only at the times when the plant is fully operational (i.e. when there is a full electrical power supply). At other times the wastewater is discharged through a by-pass channel. Both the treated effluent wastewater and the by-passed influent, are combined for discharge into the Bagmati River, at Tilganga as shown by the broken line in **Figure 3.8**.

The research design for the water quality monitoring of the Bagmati River and the performance of the GWWTP is discussed as follows:

3.2.1 The Bagmati River water quality monitoring plan

As part of this project, an on-site study was conducted on a 25 km stretch of the Bagmati River from Sundarijal (upstream), where river enters the Kathmandu valley, up to Chovar (downstream) - where the river leaves the valley, **Figure 3.9**. The water monitoring was carried out at seven different stations: Sundarijal, Gokarna, Pashupati dham, Sankhamul, Thapathali, Sundarighat and Chovar. In Pashupati dham, the water samples were collected from Aaryeghat and Tilganga, which are approximately 100 m apart, in the vicinity of where the wastewater from the GWWTP is discharged. All the samples collected were analyzed in triplicate for temperature (T), pH, dissolved oxygen (DO) and conductivity (Cond.). Due to time constraints, only samples from Pashupati dham were tested for the additional parameters - nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), ammonia- nitrogen (NH_3-N), and heavy metal concentrations such as zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn), **Figure 3.8**.

The locations for the river water monitoring stations have been chosen based on the previous studies conducted by Kannel et al. (2006; 2007a) and Bhatt and McDowell (2007). Thus the segments of the water bodies, where the water samples were taken, were shallow and well-mixed. Surface water sampling was performed by immersing a sample bottle by hand just below the surface, at a depth of 0.25 – 0.5 m (EPA 2011). The protocol, stated in the Australian Guidelines for Water Quality Monitoring and Reporting (ANZECC/ARMCANZ 2000) as well as the Environmental Water Quality Guidelines for Victorian Riverine Estuaries (EPA 2011), was followed. All occupational health and safety procedures were observed. This included the researchers being accompanied by a local resident/guide throughout the sampling program.

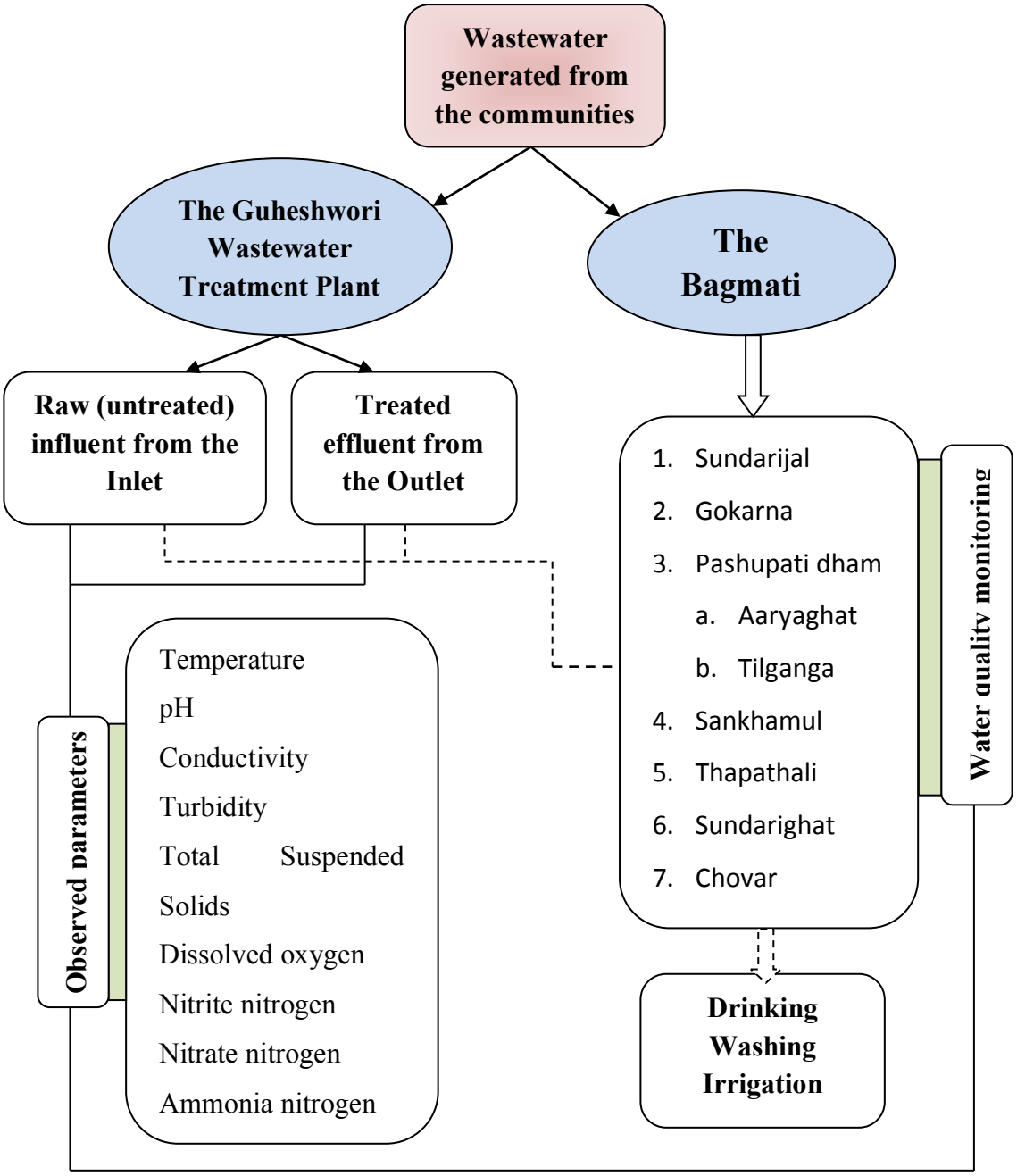


Figure 3.8 The current process and the research design for the monitoring of the Bagmati River water quality and the efficiency of the GWWTP.

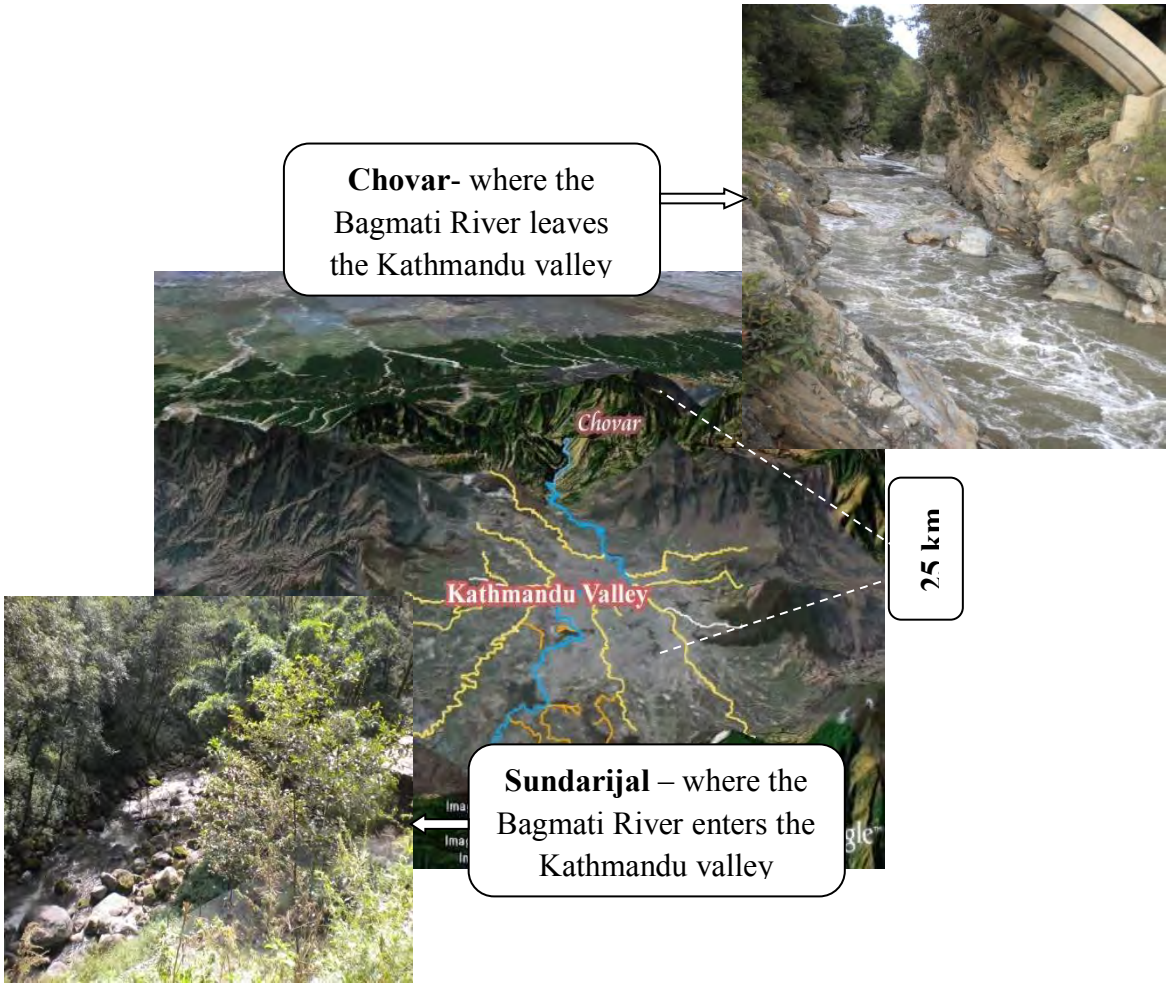


Figure 3.9 The river stretch in the Kathmandu valley, from Sundarijal (upstream) to Chovar (downstream). Photos taken by the author, Anusuya Joshi.

To clarify the protocol a "case study" has been provided as follows:

Case Study: Water sampling and analysis in Kathmandu, Nepal

In order to collect the primary data relating to the Bagmati River water quality and the efficiency of the Guheshwori Wastewater Treatment Plant (GWWTP), the PhD student (Ms Anusuya Joshi) and the Associate Supervisor (Dr. Lawrence Ngeh), travelled to Kathmandu, Nepal, from Melbourne, Australia.

Figure A On-site measurement of the river water. **Figure B** The HPCIDBC laboratory.

Bagmati River monitoring

The river water monitoring program for the selected 25 km stretch of the Bagmati River was conducted on Saturday October 19, 2013, at seven stations: Sundarijal, Gokarna, Pashupati dham, Sankhamul, Thapathali, Sundarighat and Chovar. Thus, the researchers started early in the morning, travelled 23 km from Lalitpur to Sundarijal, by a Jeep (as most of the road was rough), to where the first sampling station was located. This location is in a hilly area with dense vegetation and can only be accessed by foot. The researchers carried the instrument (multipurpose HACH meter with different probes), plastic jars (to analyze water), notepad and pens. For safety and security, they were accompanied by at least one local resident at all times. The water quality parameters such as temperature, pH, conductivity and DO were monitored on-site and recorded, **Figure A**. Otherwise, the sample was collected, stored and transferred to the HPCIDBC laboratory, **Figure B**, for further analysis. The same procedure was repeated for all the sampling stations. Though it was accessible in some places, the river bank was found to be full of solid wastes or human faeces (due to open defecation). Whilst monitoring river water quality at these sampling stations, the surrounding river environment was also observed. The monitoring program was concluded at Chovar, downstream of the Bagmati River.

3.2.2 The Guheshwori Wastewater Treatment Plant

To evaluate the efficiency of the GWWTP, an investigation on the influent and effluent wastewater quality was conducted on-site from October 21-25, 2013. This constitutes *"primary data"* that will be combined and compared to existing *"secondary data"*. The influent samples were collected in triplicate from the influent tank which is located just after the bar rack (screen) and before the flow pass to the grit chamber, **Figure 3.6**. Similarly, the effluent samples were collected in triplicate from the outlet of the treatment plant. To collect grab samples, a bucket and rope was used and the samples were transferred into bottles. Triplicate samples were collected few minutes apart. The collected samples were transported to the HPCIDBC laboratory, which is located on the premises of the GWWTP. Thus, since it did not take a long time for transportation, all samples were analyzed on the same day for the listed parameters and using the methods given in **Table 3.8**.

3.2.3 Analytical methods

For the Bagmati river water quality monitoring, the river water samples were collected at the sites mentioned in **Section 3.2.1**, **Figure 3.8**. Thus collected water samples were analyzed for the parameters listed in **Table 3.8**, based on standard methods (APHA 1998) and/or the Hach methods (Hach Company 1997 - 2009). The parameters such as temperature, pH and DO were measured on-site.

From each sampling point, three different samples were collected and all the samples were analyzed in triplicate. Finally, the average of each sampling point was used for data analysis. Only limited parameters were chosen for the analysis due to the limitations as described in **Section 3.1.10**. Again, this constitutes the "primary data" that will be combined and compared to existing "secondary data".

Table 3.8 Test parameters and methods used for analysis.

Observed Parameter	Standard method (APHA)	Equipment/Method
Temperature (°C)	2550	Hach HQ40d
pH	2310/2320	Hach HQ40d
Conductivity (mS/cm)	2510	Hach HQ40d
Turbidity (NTU)	2130	Hach DR890 / 8237
Total suspended solids (mg/L)	2540 D	Hach DR890 / 8006
Dissolved oxygen (mg/L)	4500-O	Hach LDO Probe/ 10360
Nitrite nitrogen (mg/L)	4500-NO_2	Hach DR890 / 8153
Nitrate nitrogen (mg/L)	4500-NO_3	Hach DR890 / 8039
Ammonia nitrogen (mg/L)	4500-NH_3	Hach DR890 / 8155
Zinc (mg/L)	3000	Hach DR890 / 8009
Copper (mg/L)	3000	Hach DR890 / 8506
Iron (mg/L)	3000	Hach DR890 / 8008
Manganese (mg/L)	3000	Hach DR890 / 8034

3.2.3.1 Physico-chemical measurements

The pH, conductivity and DO of the influent and the effluents were determined using a multipurpose Hach HQ40d portable meter with a gel-filled pH electrode, a conductivity probe and a DO probe respectively. The temperature of the sample was recorded with the same equipment at the time of the pH measurement. The equipment was calibrated prior to the measurement of the each parameter according to the manufacturer's specifications. These parameters were measured directly by immersing the electrode into the respective samples.

Turbidity was measured using a DR 890 spectrophotometer according to the procedure described in the Hach method 8237. The vial was filled with a 10 mL sample, vortexed and subsequently measured by placing the vial in the spectrophotometer.

Total Suspended Solid (TSS) was measured by a DR 890 spectrophotometer with the procedure described in the Hach method 8006. The vial was filled with a 25 mL sample, vortexed and measured by placing the vial in the spectrophotometer.

3.2.3.2 Nutrients

3.2.3.2.1 Ammonia nitrogen

The Ammonia Nitrogen (NH_3-N) was determined via a standard method, namely 4500- NH_3 (APHA, 1998) or HACH method 8155 (salicylate method) using a Hach DR890 colorimeter. A sample cell was filled with 10 mL of deionized water. Another sample cell was filled with a 10 mL sample. The contents of one ammonia salicylate powder pillow (Catalog No. 26531-99) were added to each sample cell, mixed and allowed to react for 3 minutes. Then, the contents of one ammonia cyanurate powder pillow (Catalog No. 26532-99) were added to each sample cell, mixed and allowed to react for 15 minutes. The sample cell filled with deionized water was used as a blank prior to the measurement of the samples. Here, ammonia compounds combine with chlorine to form monochloramine, which reacts with salicylate to form 5-aminosalicylate. In the presence of a sodium nitroprusside catalyst, the 5-aminosalicylate oxidizes to form a blue coloured compound. The blue color combines with the yellow color from excess reagent present to give a green-colored solution, see **Appendix 3.1**. The absorbance was measured at 655 nm.

3.2.3.2.2 Nitrite nitrogen

Nitrite Nitrogen (NO_2-N) was determined using a standard method 4500- NO_2 (APHA 1998) or a HACH method 8153 (ferrous sulfate method), using a Hach DR890 colorimeter. A sample cell was filled with 10 mL of a pre-filtered sample and the content of one NitriVer 2 Nitrite reagent powder pillow (Catalog No. 21075-69) was added. The content of the sample cell was mixed thoroughly for 10 minutes before measurement. 10 mL of the sample itself was used as a blank. Here, ferrous sulphate in an acidic medium reduces nitrite to nitrous oxide and the ferrous ions react with the nitrous oxide to give a greenish-brown complex, see **Appendix 3.1**.

3.2.3.2.3 Nitrate nitrogen

Nitrate Nitrogen (NO_3-N) was determined using a standard method (APHA 1998) or HACH method 8039 (cadmium reduction method) using a Hach DR890 colorimeter. One NitraVer 5 Nitrate reagent powder pillow (Catalog No. 21061-69) was added to the sample cell filled with 10 mL of the filtered sample, shaken vigorously and left standing for 5 minutes.10 mL of the sample itself was used as a blank. Here, cadmium metal reduces nitrate present in the sample to nitrite. Then, the nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt, which couples with gentisic acid to form an amber-coloured product.

3.2.3.3 Heavy metals

3.2.3.3.1 Copper

Copper (Cu) was determined by the standard method 3000 (APHA 1998) or the HACH method 8506 (Bicinchoninate Method), using a Hach DR890 colorimeter. A sample cell was filled with a 10 mL sample and the reading was taken as a blank (by pressing zero). Then, the contents of one CuVer 1 Copper Reagent Powder Pillow (Catalog No. 21058-69) were mixed into the sample cell and left undisturbed to react for 2 minutes. The reading was recorded as mg/L of Cu. Here, the Cu present in the sample reacts with a salt of bicinchoninic acid to form a purple coloured complex.

3.2.3.3.2 Iron

Iron (Fe) was determined using the standard method 3000 (APHA 1998) or the HACH method 8008 (FerroVer Method) using a Hach DR890 colorimeter. A sample cell was filled with a 10 mL sample and the reading was taken as a blank (by pressing zero). Then, the contents of one FerroVer Iron Reagent Powder Pillow (Catalog No. 21057-69) were mixed in the sample cell and left undisturbed to react for 3 minutes. The reading was recorded as mg/L of Fe. Here, FerroVer Iron Reagent reacts with all soluble iron and most in soluble forms of

iron in the sample and produces soluble ferrous iron. This reacts with 1,10-phenanthroline indicator in the reagent and forms an orange coloured complex.

3.2.3.3.3 Manganese

Manganese (Mn) was determined using the standard method 3000 (APHA 1998) or the HACH method 8034 (Periodate Oxidation Method) using a Hach DR890 colorimeter. A sample cell was filled with a 10 mL sample and the reading was taken as a blank (by pressing zero). Then, the contents of one Buffer Powder Pillow (Catalog No. 21076-69) was added into the sample cell and mixed until it dissolved. Again, the contents of one Sodium Periodate Powder Pillow (Catalog No. 21077-69) was mixed in the sample cell and left undisturbed to react for 2 minutes. The reading was then recorded as mg/L of Mn. Here, after buffering the sample with citrate, the manganese present in the sample was oxidized to purple permanganate by sodium periodate. The intensity of the resulting purple colour is directly proportional to the manganese concentration.

3.2.3.3.4 Zinc

Zinc (Zn) was determined using the standard method 3000 (APHA 1998) or the HACH method 8009 (Zincon Method) using Hach DR890 colorimeter. A sample cell was filled with 20 mL of digested sample. Then, the contents of one ZincoVer 5 Reagent Powder Pillow (Catalog No. 21066-69) was added in the sample cell and mixed until it dissolved. 10 ml of the orange solution obtained after the mixing was then transferred to another sample cell, which was used as a blank. 0.5 mL of Cyclohexanone (Catalog No. 14033-32) was added to the orange solution in the first sample cell and shaken vigorously for 30 seconds. Then, it was left undisturbed to for 3 minutes. The reading was recorded as mg/L Zn. Here, Zinc and other metals contained in the sample complex with cyanide. When cyclohexanone is added to the sample, it selectively releases zinc. Zinc reacts with 2-carboxy-2'-hydroxy-5'-sulfoforamazyl benzene ("zincon") indicator and forms a blue colour, the intensity of which is proportional to the zinc concentration.

3.3 Results and Discussion

3.3.1 Bagmati River water quality

As alluded earlier in **Section 3.2.1**, the river water quality for the seven sampling stations – namely; Sundarijal, Gokarna, Pashupati dham (Aaryeghat and Tilganga), Sankhamul, Thapathali, Sundarighat and Chovar, and the observed surrounding river environment including land uses, are discussed below.

Table 3.9 Observed values from the Bagmati River water monitoring at 8 different sampling sites.

Observed Parameter	Sundarijal	Gokarna	Aaryeghat	Tilganga	Sankhamul	Thapathali	Sundarighat	Chovar
Temperature (°C)	18.3	23.1	21.1	23.1	19.9	22.8	23.8	22.3
pH	-	6.20	6.45	6.78	7.35	7.00	7.14	7.20
Conductivity (µS/cm)	23.89	53.48	87.72	431.61	249.33	316.00	418.33	388.67
Turbidity (NTU)	-	20	29	241	59	188	35	70
TSS (mg/L)	-	19	26	225	51	186	27	46
DO (mg/L)	8.56	7.33	7.43	3.11	6.63	1.44	0.84	6.39

The results presented in **Table 3.9** represent the mean values for triplicate samples for all the observed parameters, on the day of river monitoring - except for Pashupati dham, for which the river water sampling was conducted over three consecutive days. The high values of Standard Error (SE) that were obtained for some parameters, as presented below for individual sampling stations in histograms, reflect the wide range of measurements on different sampling dates. For example, the NH_3-N value in Tilganga was recorded as 6 mg/L on 20/10/2013, 14 mg/L on 21/10/2013 and 44 mg/L on 23/10/2013.

3.3.1.1 Sundarijal

This sampling station is located in zone 1 and zone 2, the natural conservation zone and the rural zone respectively. These are in the upper part of the river which is not yet highly impacted by the human activities. However, the river water, which flows from its origin to

this sampling station, passes through two villages, where the sanitation is considered to be poor due to open defecation and run-off from agricultural land. Otherwise, it is unaffected by any other source of contamination and the water is clear in appearance.

Figure 3.10 Sundarijal sampling stations (a) Upstream and (b) Downstream. (Photos taken by the author, Anusuya Joshi)

In Sundarijal, the water sample was taken at three different sites – Site A is upstream of the river situated in Ward No. 8, Site B is midstream and situated in Ward No. 1 and Site C is downstream and located in Ward No. 2, **Figure 3.10.** Site A is covered with riparian vegetation, unaffected by any local human activities. Site B is surrounded by agricultural land and Site C is surrounded by light vegetation and huge rocks. These sites seem to be affected by human activities, though not found to be contaminated with sewage. Nearby the Site A, people were harvesting corps and along Site B, people were washing cloths and utensils.

Figure 3.11 depicts the temperature, conductivity and dissolved oxygen recorded for the river water at Sites A, B and C. The temperature at these three sites ranged from 17.4 °C to 19.7°C, from upstream to downstream, with a lower temperature evident for the more highly vegetated areas A and B. River water temperature is a crucial factor that affects the habitat of aquatic life and also influences water quality[49]. Higher temperature tends to increase toxicity and reduce the DO in the water. In terms of the conductivity, it may be seen to increase

[49]http://www.fondriest.com/environmental-measurements/parameters/water-quality/water-temperature/#watertemp1

gradually as the river flows downstream, with the value ranging from 20.1 µS/cm to 29.2 µS/cm, such values are considered to represent low conductivity. At all three sites, the observed DO values (> 8 mg/L) indicate that the water is well-oxygenated and suitable for aquatic life. This DO value is consistent with the DO value of 8 mg/L recorded by HPCIDBC on October 2012, for Sundarijal (ADB 2013).

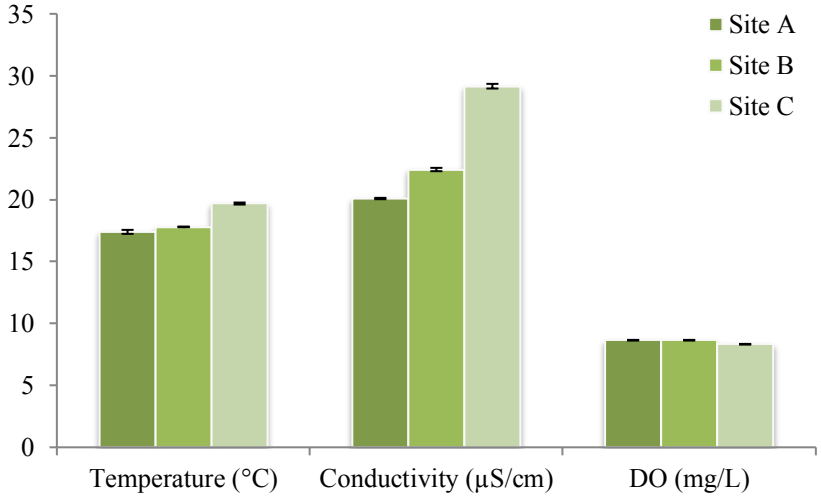


Figure 3.11 The temperature, conductivity and dissolved oxygen observed at the three different sites at the Sundarijal sampling station. The error bars represent the standard error (SE), where n = 3.

3.3.1.2 Gokarna

Figure 3.12 Gokarna sampling station, (a) Sampling site and (b) Upstream to the sampling site. (Photos taken by the author, Anusuya Joshi)

When the river flows to Gokarna from Sundarijal, it passes through the rural zone and the land is agricultural (ADB 2013), **Figure 3.12.** The sampling station is located in the proximity of the Gokarneshwor temple, where many cultural and traditional rituals are performed - including cremation on the bank of the river. The area is surrounded by an empty open field with only light vegetation. Up to this area, minimal human activity was observed and the water appeared clear.

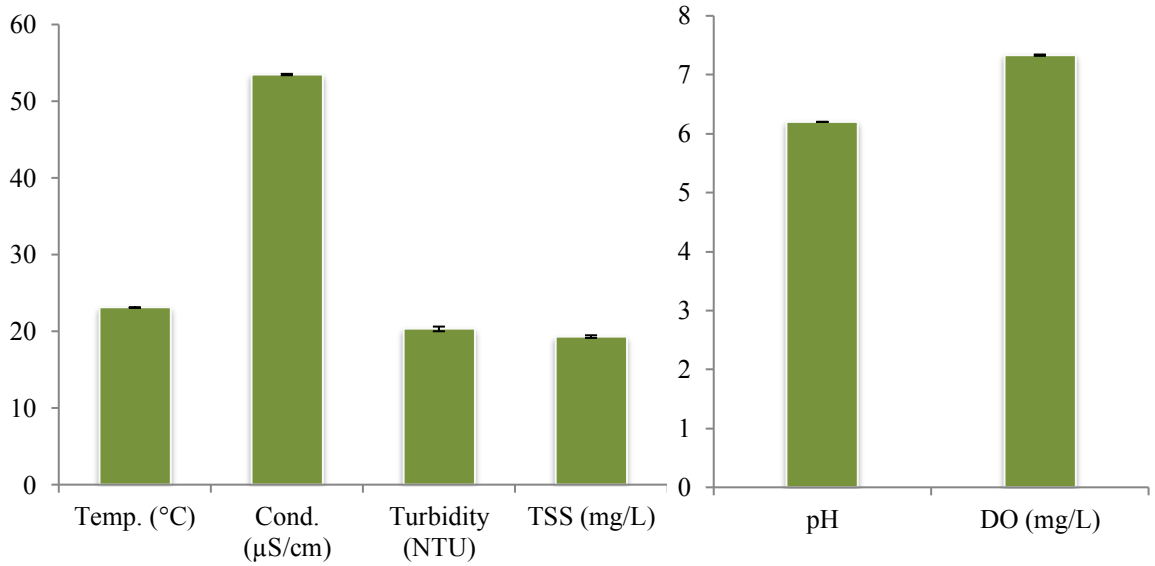


Figure 3.13 The temperature, conductivity, turbidity, TSS, pH and DO observed at the Gokarna sampling station. The error bars represent the standard error (SE), where n = 3.

Figure 3.13 depicts the temperature, conductivity, turbidity, TSS, pH and DO observed at the Gokarna sampling station. The temperature recorded was 23.1 °C, which was higher than at Sundarijal and which might be due to less vegetation in the surrounding area. Also, the sunlight started to fall on the water. With respect to the ambient temperature for Kathmandu on the day of sampling i.e., October 19, 2013, the high temperature recorded was 24°C and the low temperature recorded was 11°C[50]. The conductivity was found to be 53.5 µS/cm which is also comparatively higher than for Sundarijal. The pH was found to be acidic with a value of 6.2. Runoff from the agricultural land is likely to contribute to relatively increased conductivity and lower pH. The DO in the river water was recorded as 7.3 mg/L, less than upstream, but still considered good and suitable for aquatic life. The turbidity and TSS was found to be 20 NTU and 19 mg/L. The DO and conductivity values in this study reflect the secondary data values of 2007, **Table 3.1**.

[50] http://www.worldweatheronline.com/Kathmandu-weather-history/NP.aspx 04/04/2015

3.3.1.3 Pashupati dham

The third sampling station for river water monitoring was at Pashupati dham, which is located in the peri-urban zone (zone 3). The water sample was collected upstream (Aaryeghat) and downstream (Tilganga), in order to assess the effect of pipeline discharge from the GWWTP on the water quality at Pashupati dham, **Figure 3.14.**

Figure 3.14 Pashupati dham sampling station (a) Aaryeghat (Upstream) and (b) Tilganga (Downstream), Yellow arrow showing discharge pipeline. (Photos taken by Anusuya Joshi)

This station is located at the proximity of the Pashupati Nath temple, where many cultural and religious rituals are performed. Most of the remains of the rituals and cremation ceremonies are dumped into the river. Aaryeghat is not affected by sewer wastewater whereas it has been introduced in Tilganga through a large pipeline drain from the GWWTP discharge (as indicted above).

Figure 3.15 depicts the temperature, pH, DO, conductivity, turbidity and TSS observed at the Aaryeghat (upstream of the river) and Tilganga (downstream of the river) - the two sampling sites of the Pashupati dham sampling station. The temperature downstream (23.1 °C) was found to be significantly higher than the temperature upstream (21.1 °C). The increased temperature may be due to the discharge from the GWWTP. In terms of pH, it was found to be acidic at both sites; 6.5 and 6.8 at Aaryeghat and Tilganga respectively. Just after the upstream sampling site, the remaining burnt wood ash from cremation ceremonies is washed into the river, which is likely be a reason for a slightly higher pH at downstream.

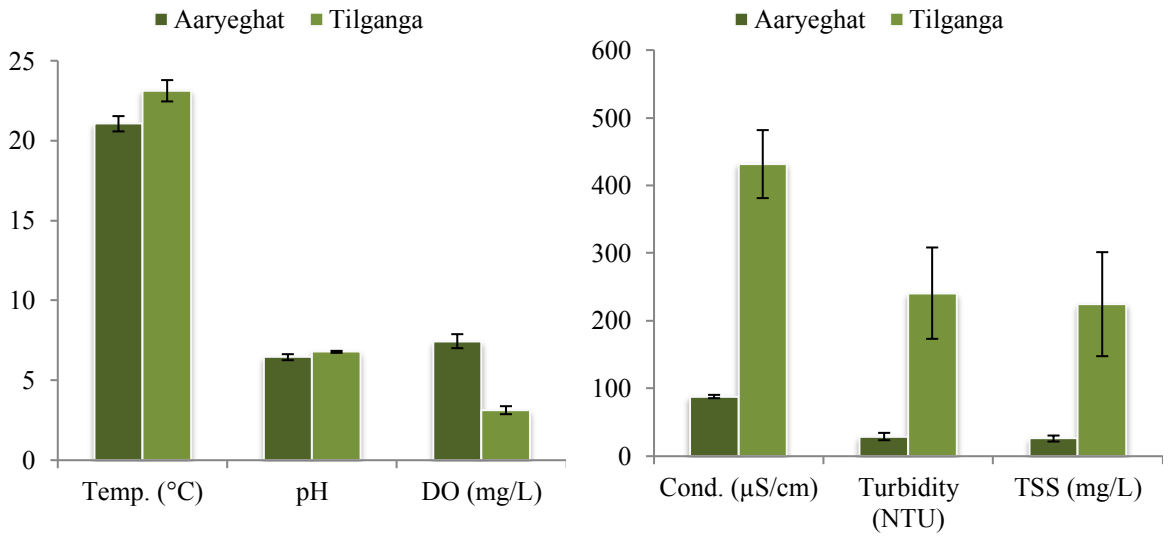


Figure 3.15 The temperature, pH, DO, conductivity, turbidity and TSS observed at the Pashupati dham sampling stations. The error bars represent the standard error (SE), where n = 3.

A significantly higher DO was recorded at Aaryeghat than in Tilganga; 7.4 mg/L for former and 3.1 mg/L for later. Moreover, the conductivity, turbidity and TSS were found to be significantly higher for Tilganga than for Aaryeghat. The conductivity, turbidity and TSS recorded were 87.7µS/cm, 29 NTU and 26 mg/L, respectively at Aayeghat and 431.6 µS/cm, 241 NTU and 225 mg/L, respectively at Tilganga. This indicates that the discharge from the GWWTP is responsible for the lower DO and the increase in the conductivity, turbidity and TSS in the river water down to Tilganga. In addition, solid waste (mostly organic waste) and cattle dung were observed to be dumped along the river bank on the pathway to Tilganga. The values obtained for the Tilganaga sampling stations are consistent with Kannel et al. (2007a), **Table 3.1**.

Figure 3.16 depicts the NH_3-N, NO_2-N, NO_3-N, Fe, Zn and Cu concentrations observed at the Aaryeghat and Tilganga sampling sites. The NH_3-N concentration in the river water increased from 10.2 mg/L to 21.2 mg/L, the NO_2-N concentration increased from 1.4 mg/L to 2.3 mg/L and NO_3-N concentration increased from 1.1 mg/L to 3.0 mg/L, on going from upstream to downstream. Bhatt and McDowell (2007) reported that NH_3-N contributed almost all of the nitrogen in the observed dissolved nitrogen, and NO_3-N concentration was found to be negligible. This is consistent with these results although the NO_2-N and NO_3-N concentrations cannot be described as negligible.

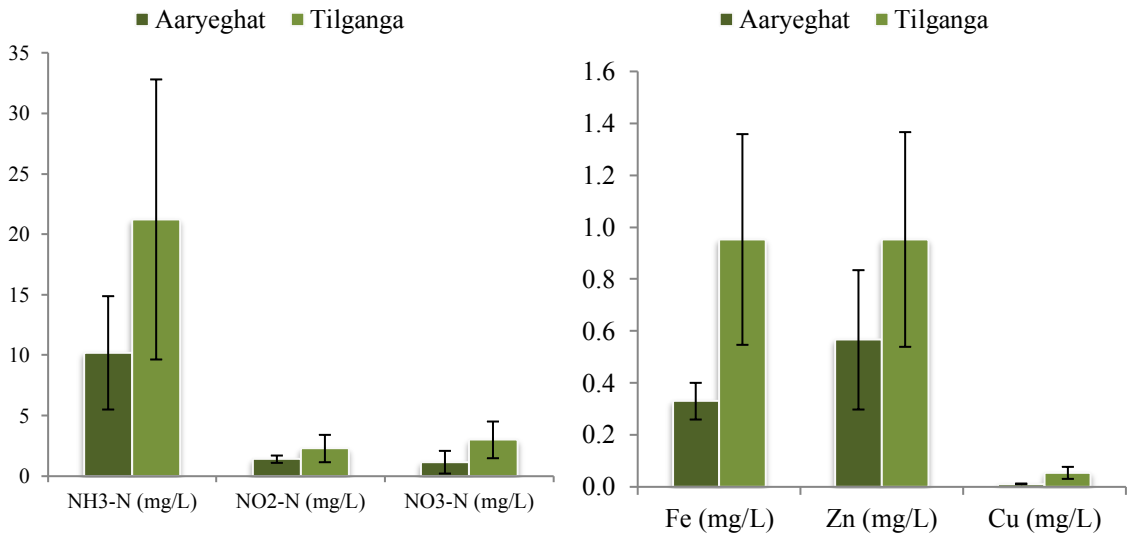


Figure 3.16 The NH_3-N, NO_2-N, NO_3-N, Fe, Zn and Cu concentrations observed at the Pashupati dham sampling station. Error bars represent the standard error (SE), where n = 3.

However, this effect might be due to a rapid denitrification and limited nitrification with low oxygen availability. In terms of the heavy metals, the concentrations were found to be significantly higher in the Tilganga sample than in Aaryeghat. Generally, the presence of heavy metals is not expected in surface water, it oxidizes in air and precipitates as insoluble hydroxides, sulfides, sulfates or carbonates (Commonwealth of Australia 2005). The Fe concentration increased from 0.33 mg/L to 0.95 mg/L, Zn increased from 0.57 mg/L to 0.95mg/L and Cu increased from 0.01 mg/L to 0.05 mg/L, on going from upstream to downstream. However, Mn was not detected in the river water at all. This comparison between the river water quality, upstream and downstream from the GWWTP discharge, shows that, even though treated, the discharge diminishes the river water quality.

3.3.1.4 Sankhamul

The Sankhamul sampling station is in urban zone, which is moderately impacted by anthropogenic activities. This station is in the vicinity of the cremation site for the residents of Lalitpur and is also a religious site for the performance of daily rituals, **Figure 3.17**. Many squatter settlements and residential buildings are situated along the bank of the river and their associated discharges are connected directly into the river. Moreover, solid waste can be seen dumped at many locations along the river as well as open defecation. The waste from the

offering from rituals such as plastic bags/bottles can also be seen floating on the water and the water itself is turbid and filthy.

Figure 3.17 Sankhamul sampling station (a) Upstream river segment and (b) Downstream river segment. (Photos taken by the author, Anusuya Joshi)

Figure 3.18 gives the temperature, pH, DO, conductivity, turbidity and TSS observed at the Sankhamul sampling station monitored at three sampling sites - A, B and C. Site A is upstream, where the surrounding environment is open field with light vegetation. Site B is midstream, where most of the rituals are performed. Temples, shrines and cremation sites are located on one side of the river bank and squatter settlements on the other side. Site C is downstream where newly constructed residential buildings are situated.

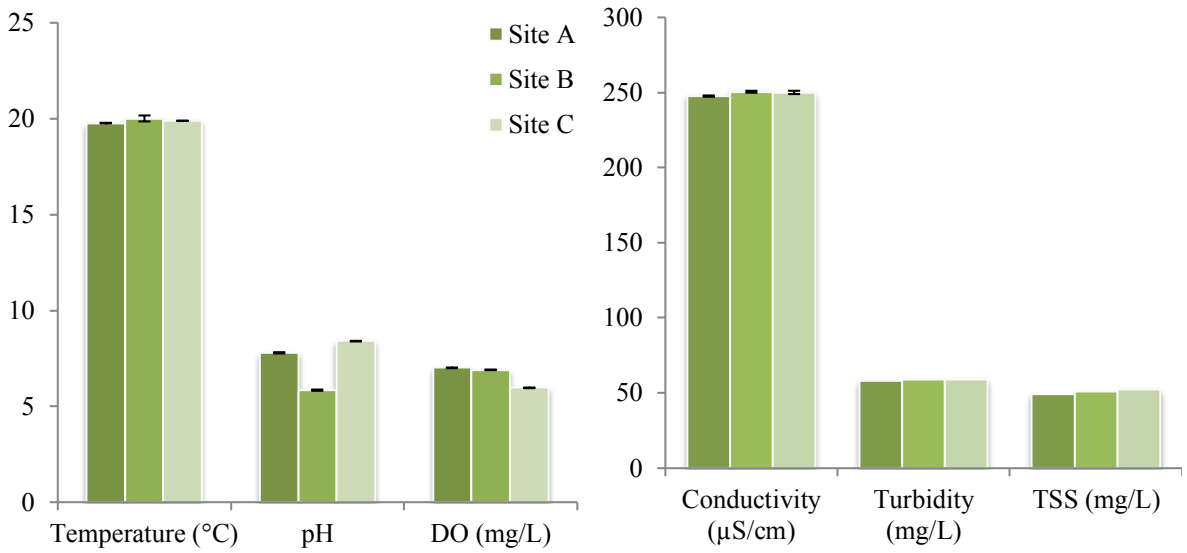


Figure 3.18 The temperature, pH, DO, Conductivity, turbidity and TSS observed at the Sankhamul sampling station. The error bars represent the standard error (SE), where n = 3.

The temperature was found not to vary significantly for these three sites - thus 19.8 °C, 20 °C and 19.9 °C were recorded for Sites A, B & C respectively. In terms of pH, Site B was acidic (5.9) whereas Sites A and C were basic (7.8 and 8.4 respectively). The DO level in the river water could be considered as good for Sites A, B and C (7.0 mg/L, 6.9 mg/L and 6.0 mg/L, respectively). The relatively lower DO at Site C of the river is likely to be due to the input into the river downstream of Site B. Not much difference was found in conductivity, turbidity and TSS values for Sites A, B and C. The conductivity recorded was 247.7 µS/cm, 250.3 µS/cm and 250.0 µS/cm for Sites A, B and C, respectively. The turbidity recorded was 58 mg/L for Site A and 59 mg/L for Sites B and C. The TSS was found to be 49 mg/L, 51 mg/L and 52 mg/L respectively. This indicates that the water quality along the river in the Sankhamul station did not change significantly from upstream to downstream. Though the sampling site is moderately affected by human activities, it does not seem to be affecting the river water quality significantly. This observation might be attributed to a high flow of river during the sampling period. The rain was recorded for Kathmandu with the highest rainfall of 11.8 mm at 5.45 am in the previous day[51] and the rainfall of 0.3 mm at 2.45 am in the morning of the sampling day[52].

[51] Weather information for Kathmandu on 15/10/2013 http://www.worldweatheronline.com/Kathmandu-weather-history/NP.aspx 04/04/2015
[52] Weather information for Kathmandu on 16/10/2013 http://www.worldweatheronline.com/Kathmandu-weather-history/NP.aspx 04/04/2015

3.3.1.5 Thapathali

Figure 3.19 Thapathali River water sampling station. (Photo taken by the author, Anusuya Joshi)

This sampling station is located in the urban zone, which is highly affected by human activities. This location is surrounded by residential buildings, hospitals, squatter settlements and commercial buildings such as a supermarket, **Figure 3.19**. Although being in the city centre, human faeces and solid waste was found to be dumped at many places along the banks of the river.

A bridge connecting the two districts Kathmandu and Lalitpur passes over this sampling station. During the dry season/summer, this part of the river is more like a drain and gives off an unpleasant (rotten egg) smell. This creates a health threat to the local residents as well as to passers-by.

Figure 3.20 presents the temperature, conductivity, turbidity, TSS, pH and DO observed at the Thapathali sampling station.

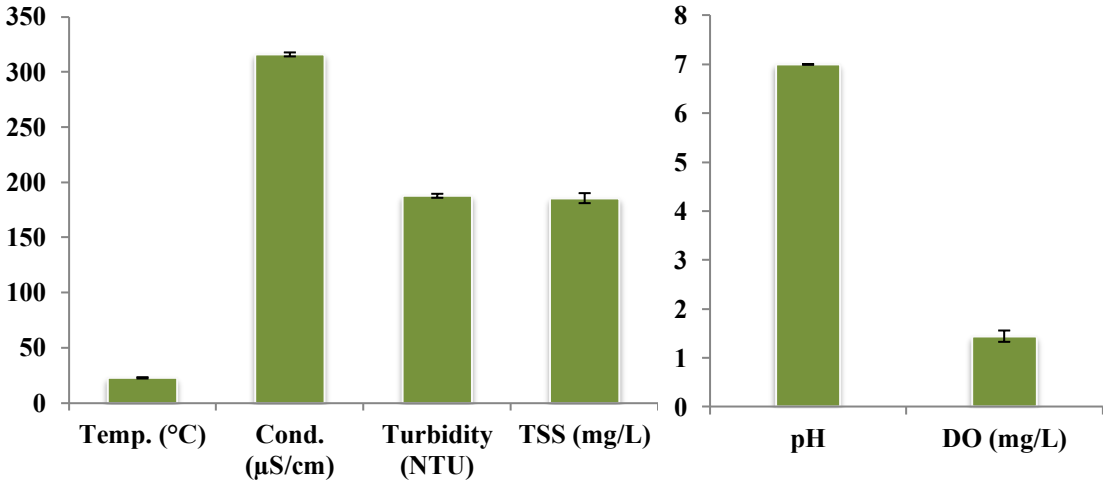


Figure 3.20 The temperature, conductivity, turbidity, TSS, pH and DO observed at the Thapathali sampling station. The error bars represent the standard error, where n = 3.

The temperature recorded was 22.8 °C with pH 7 and DO 1.4 mg/L. The low DO level is consistent with the rotten egg smell (hydrogen sulphide) in this area and is an indication of contamination due to decomposing organic matter that reduces the dissolved oxygen level (Commonwealth of Australia 2005). It is interesting to note a neutral pH value despite the low DO value that is reduced dramatically upon flowing from Sankhamul to Thapathali. The conductivity, turbidity and TSS were found to be significantly higher than in Sankhamul. The recorded conductivity, turbidity and TSS were 316 µS/cm, 188 NTU and 186 mg/L, respectively. This result indicates that the river water quality is deteriorating as it flows downstream.

3.3.1.6 Sundarighat

This sampling station is located in the urban zone, and is expected to be the most impacted amongst all the stations, **Figure 3.21**. Downstream to Thapathali, the river bank is effectively being used as a solid waste transfer station and dumping site by both the municipality as well as private organizations. In addition, the river bank has been encroached upon for road and park construction.

Figure 3.21 Sundarighat river water sampling station. (Photo taken by the author, Anusuya Joshi)

Figure 3.22 depicts the temperature, conductivity, turbidity, TSS, pH and DO observed at the Sundarighat sampling station.

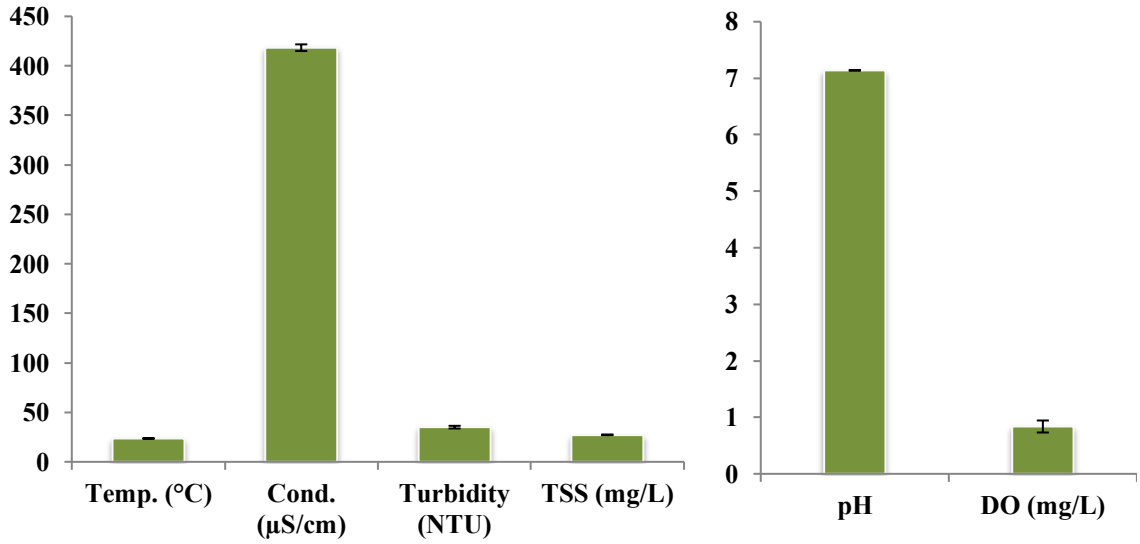


Figure 3.22 The temperature, conductivity, turbidity, TSS, pH and DO observed at the Sundarighat sampling station. The error bars represent the standard error, where n = 3.

The temperature, pH and DO were found to be 23.8 °C, 7.1 and 0.8 mg/L, respectively. The low value of DO indicates that this stretch of the river water is not suitable to support any

aquatic life. Part of river that passes through the city area is considered as being “biologically dead”. The low turbidity (35 NTU), TSS (27 mg/L) and the high conductivity (418.3 µS/cm) is likely to be due to the low water flow which allows the suspended particles to settle.

3.3.1.7 Chovar

Figure 3.23 Chovar river water sampling station. (Photo taken by the author, Anusuya Joshi)

The final sampling station was Chovar, which is located in the downstream zone, **Figure 3.23**. Before reaching this section, the river passes through a narrow gorge. The surrounding area is covered with moderate vegetation and a popular temple is situated on the bank. At the time of the sampling, the author and the Associate Supervisor observed the water being used for washing utensils, clothes and cattle. The remains of offerings from the temple and other solid waste were found to be dumped on the river bank.

Figure 3.24 depicts the temperature, conductivity, turbidity, TSS, pH and DO observed at the Chovar sampling station. The temperature, pH and DO were found to be 22.3 °C, 7.2 and 6.4mg/L, respectively. The higher DO might be due to the aeration caused by the rapid water flow through the gorge. The conductivity, turbidity and TSS were found to be 388.7 µS/cm, 70 NTU and 46 mg/L, respectively. The water quality seems to be better here, which could be attributed to a self-purification process as the river flows downstream without any further input of pollutants.

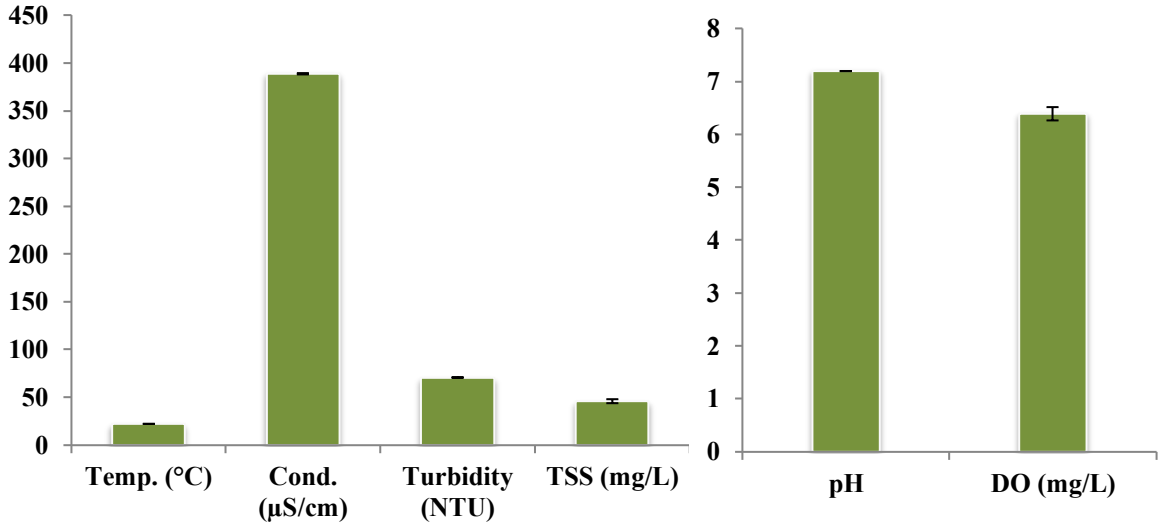


Figure 3.24 The temperature, conductivity, turbidity, TSS, pH and DO observed at the Chovar sampling station. The error bars represent the standard error, where n = 3.

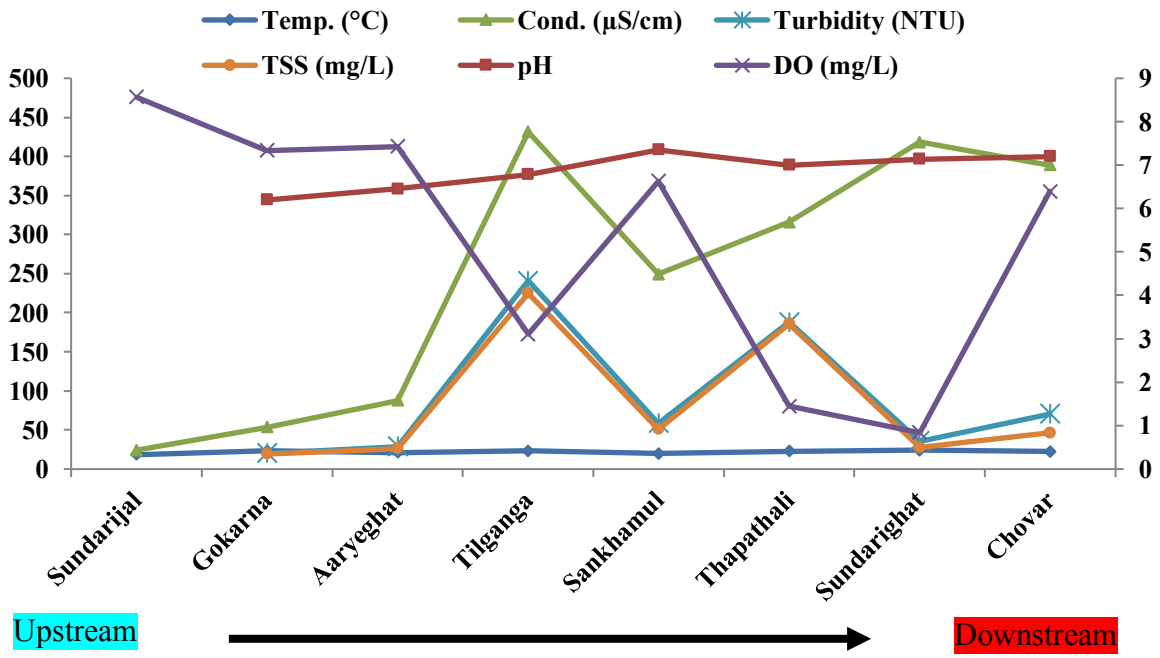


Figure 3.25 The temperature, conductivity, turbidity, TSS, pH and DO observed throughout the upper part of the Bagmati River, in this study. For Sundarijal and Sankhamul, the data points represent the average values for three sampling sites. For Pasupati dham, two sampling sites are presented in this plot.

The trends in the above data, as depicted in **Figures 3.11 to 3.24** for the individual sampling stations, upon moving from upstream to downstream is shown in **Figure 3.25.** This provides

an overall profile of the upper part of the Bagmati River in relation to the data obtained at the seven sampling stations. **Figure 3.26** provides a profile of the upper part of the Bagmati River in relation to the data obtained at the three sampling stations, based on previous studies (see **Table 3.1**).

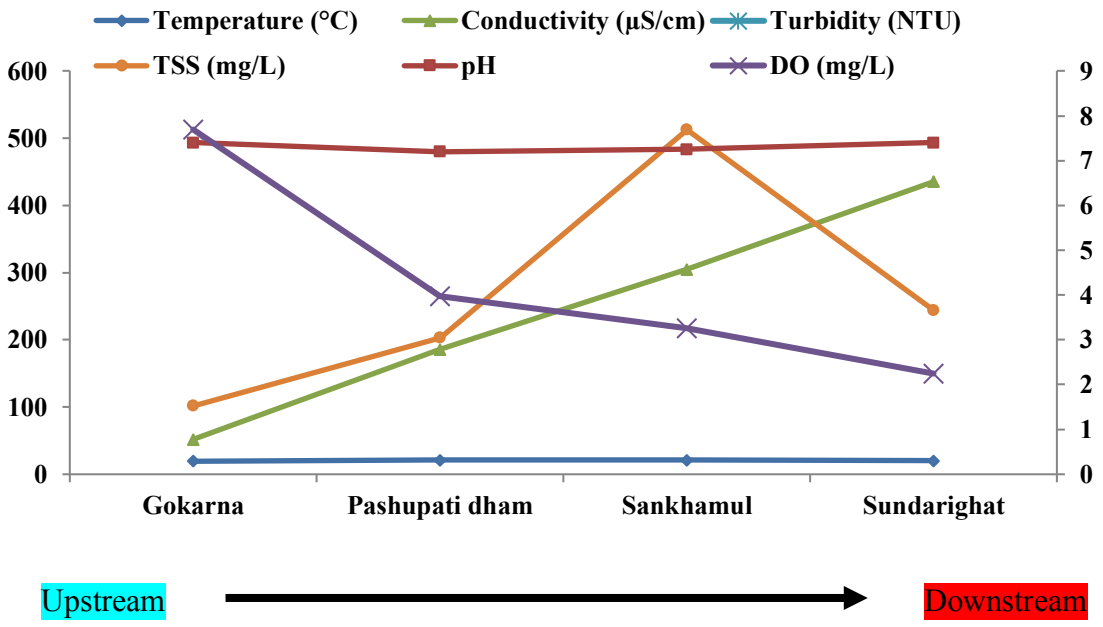


Figure 3.26 The temperature, conductivity, turbidity, TSS, pH and DO observed throughout the upper part of the Bagmati River, in previous studies (see, **Table 3.1**).

From the above current data, it can be seen that the Bagmati River water quality still deteriorates as it flows downstream to the more populated urban area. This finding is consistent with previous studies on the river water quality (Pradhan B 2005; Bhatt and McDowell 2007; Kannel et al. 2007a; Kannel et al. 2007b). The river water quality monitoring conducted by the High Powered Committee for Integrated Development of the Bagmati Civilization (HPCIDBC) also reported that river water quality deteriorated as it moved downstream[53]. In this regard, the desirable values reported by the HPCIDBC are 20 - 50 NTU for turbidity, 5 - 7 mg/L for DO and 0 - 30 mg/L for BOD_5. In terms of DO, the ADB (2013) also reports that the desired DO in the river water to be > 5 mg/L and that a DO < 2 mg/L is considered detrimental to most aquatic life. For our observations, the desired DO was recorded in the upstream segment up to Sankhamul (except Tilganaga, which may be attributed to the mixing of effluent from the GWWTP) and downstream in Chovar. In

[53] River water quality tested at several sampling stations at the upper part of the Bagmati River on various sampling dates are provided. http://bagmati.gov.np/bagmati-water-quality-test-report.php

Thapathali and Sundarighat, it was found to be detrimental at < 2 mg/L. With respect to the tolerance limits in relation to the Nepalese Standards for River Water Quality, **Table 3.7**, the temperature and pH are in the required range, i.e.< 40 °C and from 5.5 to 9, respectively, for all sampling stations. However, the TSS values were significantly higher for Tilganga and Thapathali. This observation suggests that the river segment, from Thapathali to Sundarighat, is the most highly impacted and polluted segment of the Bagmati River.

3.3.2 Performance of the Guheshwori Wastewater Treatment Plant

The wastewater treatment efficiency of the GWWTP was evaluated by comparing the relative Influent and Effluent water quality with respect to the water quality parameters shown in **Table 3.10**. The *"Primary data"* was collected for the month of October 2013 on the field trip to Nepal that was an integral part of this study. The *"Secondary data"* is the existing data from the months January to August 2013 that was obtained from the HPCIDBC. It is possible that variation in sampling times, analytical methods and instrumentation might affect the observed measurements.

3.3.2.1 Influent and Effluent Characteristics

The Influent (IN) is the sewage wastewater received from the surrounding catchment area of the GWWTP. It has been characterized based on the parameters listed in **Table 3.10**. The primary data for October 2013 from this study and the secondary data from January to August 2013 obtained from HPCIDBC were averaged to obtain the presented values. A wide range for some of the observed parameters such as COD, TSS, TDS and turbidity is probably due to the variation in water use by customers. This could also be related to water availability. In summer, the water supply in the Kathmandu valley is limited, forcing people to use less water to flush the toilet. This practice would lead to an increase in the concentration of pollutants in the Influent over this period. Indeed, Ellingson (2010) reported that the water crisis in the Kathmandu valley is one of the reasons for wastewater being more highly concentrated with pollutants compared to Western countries. In this regard the influents from Kathmandu and Melbourne have been compared in **Section 4.2.4.9**.

Table 3.10 Influent (IN) and Effluent (EF) characteristics at the GWWTP. The values are derived from combining the data collected in this study (*primary* data) and the data obtained from the HPCIDBC for the year 2013 (*secondary* data).

Observed parameter (Unit)	Mean		Median		Standard error		Standard deviation		Range		Minimum		Maximum	
	Influent	*Effluent*	*Influent*	*Effluent*	*Influent*	*Effluent*	*Influent*	*Effluent*	*Influent*	*Effluent*	*Influent*	*Effluent*	*Influent*	*Effluent*
Temp. (°C)	21.7	22.3	23.6	24.2	0.3	0.4	3.5	4.3	13.3	16.9	12.2	10.1	25.5	27
pH	6.75	7.37	6.7	7.35	0.01	0.01	0.15	0.16	1.03	0.80	6.40	7.00	7.43	7.80
DO (mg/L)	0.01	3.31	0.00	3.20	0.01	0.07	0.08	0.82	0.65	3.71	0.00	1.20	0.65	4.91
TSS (mg/L)	338	97	350	100	8	3	78	26	411	112	178	40	589	152
Turbidity (NTU)	441	113	450	118	9	4	90	42	499	315	252	40	751	355
TDS (mg/L)	443	289	480	290	13	9	87	60	280	224	280	180	560	404
COD (mg/L)	1031	232	1141	288	42	13	288	90	854	255	542	85	1396	340
Chloride (mg/L)	113.5	93	121	88	4.6	4	31.6	28	94	92	68	54	162	146
Total alkalinity (mg/L)	235	210	243	190	7	7	47	50	152	165	158	150	310	315
NH_3-N (mg/L)	78	64	77	58	12	10	24	20	48	45	55	48	103	93
NO_2-N (mg/L)	1.7	1.2	2.1	1	0.6	0.4	1.2	0.7	2.6	1.6	0.0	0.7	2.6	2.2
NO_3-N (mg/L)	0.9	0.6	0.7	0.1	0.4	0.5	0.9	0.9	2.0	2.0	0.0	0.03	2.0	2.0
Fe (mg/L)	1.5	0.83	1.3	0.85	0.2	0.03	0.4	0.07	0.9	0.16	1.1	0.74	2.0	0.90
Zn (mg/L)	0.31	0.14	0.17	0.01	0.21	0.13	0.41	0.26	0.90	0.53	0.00	0.00	0.90	0.53
Cu (mg/L)	0.03	0.01	0.03	0.01	0.02	0.01	0.04	0.01	0.07	0.03	0	0.00	0.07	0.03

3.3.2.2 A temporal comparison between influent and effluent water quality parameters for 2013

3.3.2.2.1 Temperature

Figure 3.27 depicts the time averaged temperature for Influent and Effluent at the GWWTP, from January 2013 to August 2013 and for October 2013.

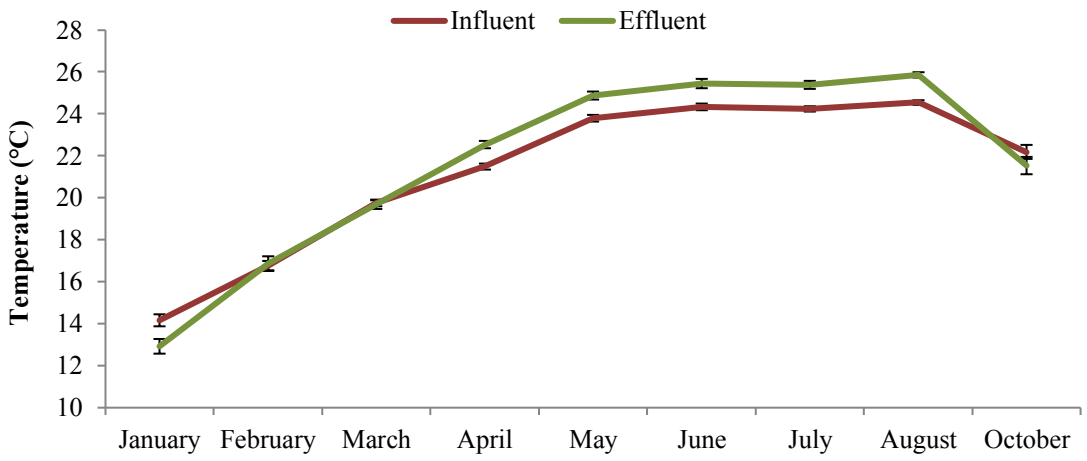


Figure 3.27 The time averaged Temperature for Influent and Effluent at the GWWTP for 2013. The error bars represent the standard error, where, n = 12 for January; n = 13 for February; n = 14 for March & April; n = 16 for May & July; n = 21 for June; n = 19 for August; n = 4 for October.

The temperature of the Influent and the Effluent increased gradually from January to August, and starts to decrease in October. The Influent and Effluent temperature trends are seen to correspond. The recorded temperature profile of the Influent and Effluent throughout the observation period is due to the effect of ambient temperature[54]. In October, the Influent temperature ranged from 21.4 °C to 22.7 °C whereas the Effluent temperature ranged from 20.7 °C to 22.5 °C. Thus the Effluent temperature was found to be slightly less than the Influent at this time of the year.

[54] http://www.nepal.climatemps.com/ presents the maximum and minimum temperature profiles throughout the year.

3.3.2.2.2 The pH Profile

Figure 3.28 depicts the time averaged pH for Influent and Effluent at the GWWTP, from January 2013 to August 2013 and for October 2013.

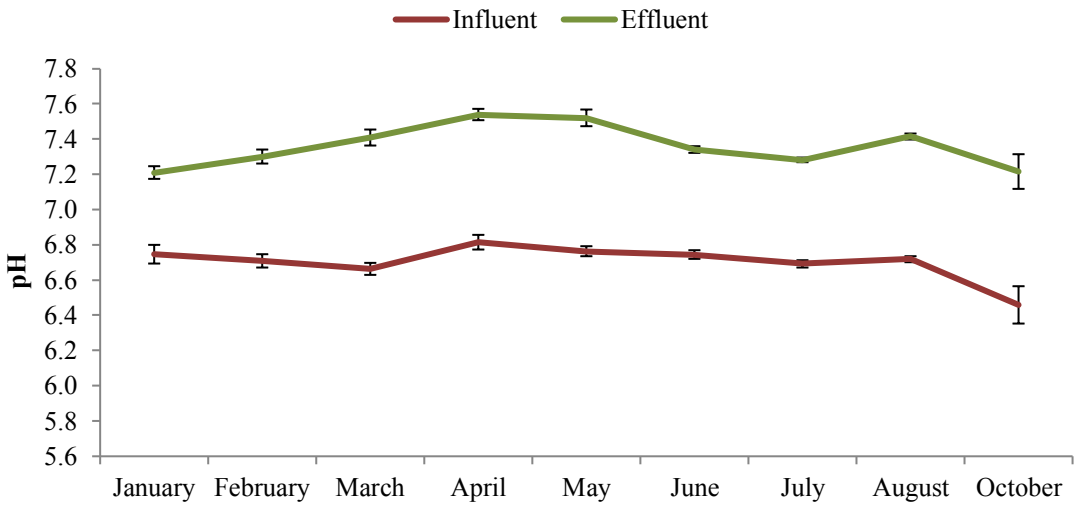


Figure 3.28 The time averaged pH for Influent and Effluent at the GWWTP for 2013. The error bars represent the standard error, where, n = 11 for January; n = 13 for February & April; n = 14 for March; n = 16 for May & July; n = 21 for June; n = 19 for August; n = 4 for October.

The pH of the Influent ranged from 6.5 to 6.8 whereas the pH of the Effluent ranged from 7.2 to 7.5. This pH profile shows that the Influent was found to be acidic throughout the year and attained a slightly basic pH after treatment. Our measurements in October 2013 were found to be consistent with this trend with Influent pH being acidic and attaining a basic pH after the treatment process. The average Influent pH of 6.5 increased to 7.2 in the Effluent.

3.3.2.2.3 Conductivity

Figure 3.29 depicts the time averaged conductivity for Influent and Effluent at the GWWTP, from February 2013 to August 2013 and for October 2013.

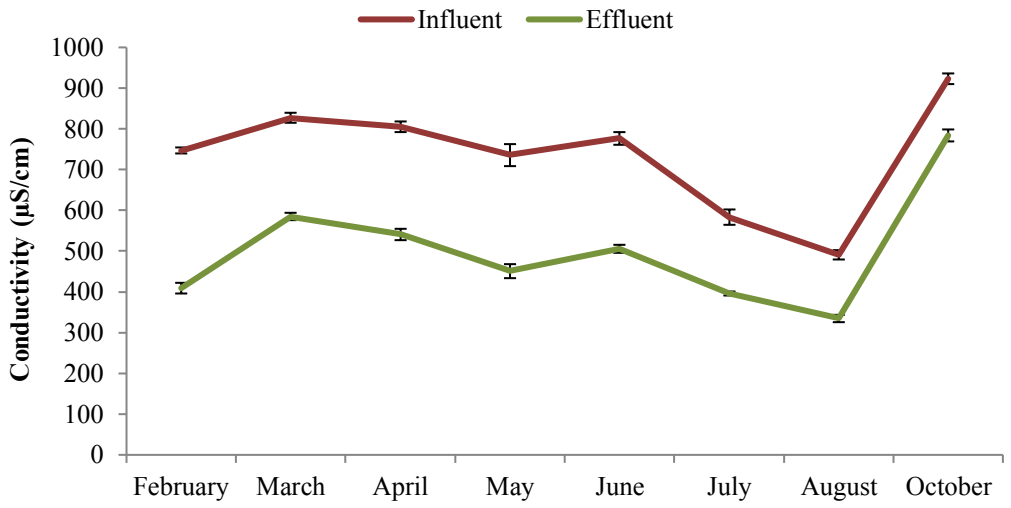


Figure 3.29 The time averaged conductivity for Influent and Effluent at the GWWTP for 2013. The error bars represent the standard error, where, n = 6 for February, March, April & June; n = 7 for May; n = 8 for July & August; n = 4 for October.

Here, the data available on TDS from the HPCIDBC has been converted into conductivity using the conversion relation, TDS = EC ÷ 1.5625[55]. The conductivity of the Influent ranged from 491.4 - 923.4 µS/cm whereas the conductivity of the Effluent ranged from 334.6 - 784 µS/cm. The highest TDS removal achieved was 45 % in February, the average removal was 35 %. The TDS of the Influent ranged from 315 - 530 mg/L whereas the TDS of the Effluent ranged from 214 - 374 mg/L. The highest TDS removal achieved was 45 % in February, the average removal was 35 %. For October 2013, the Influent conductivity ranged from 895 - 950 µS/cm whereas the Effluent conductivity ranged from 745 - 817 µS/cm. The average conductivity changed by 15 % in the Effluent after the treatment.

3.3.2.2.4 Dissolved Oxygen

In a wastewater treatment process, an increase in the DO concentration is expected in the effluent. As discussed earlier, the ultimate discharge of effluent is into the river and it is desirable that the DO is high enough (> 5 mg/L) so as not to affect the aquatic life. Indeed, dissolved oxygen is considered to be the most important parameter for the assessment of river water quality (ADB 2013).

[55]TDS = EC ÷ 1.5625, where TDS is total dissolved solids and EC is electrical conductivity (which refers to conductivity) http://www.gympcentss.eq.edu.au/classwork/cs2_7/pdf/Week4_s/4_WaterQualStand.pdf

Figure 3.30 depicts the time averaged DO for Influent and Effluent at the GWWTP, from January 2013 to August 2013 and October 2013.

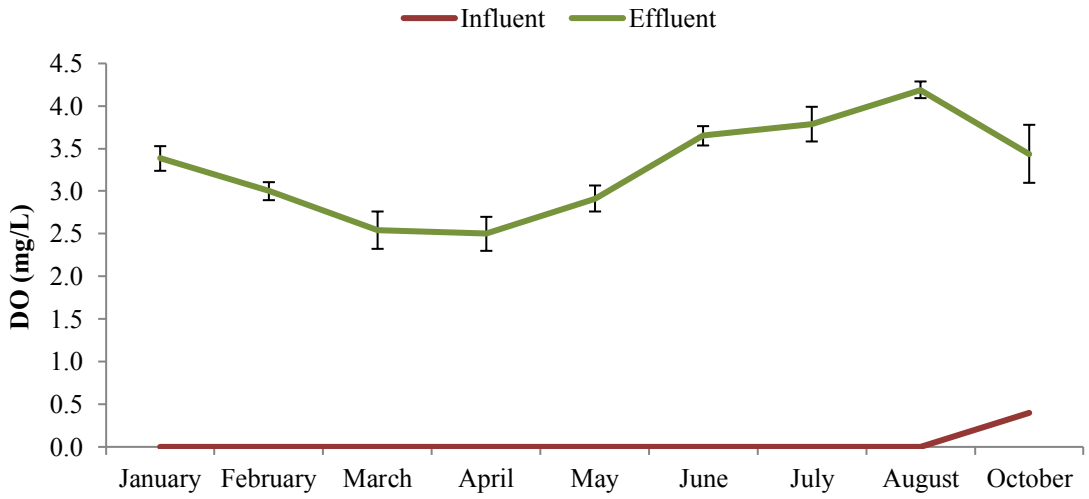


Figure 3.30 The time averaged DO for Influent and Effluent at the GWWTP for 2013. The error bars represent the standard error, where, n = 12 for January; n = 13 for February & April; n = 14 for March; n = 16 for May & July; n = 20 for June; n = 19 for August; n = 4 for October.

The initial DO of the sewage wastewater was considered to be 0 mg/L, in a study conducted by the HPCIDBC. After treatment, the Effluent DO increased significantly and ranged from 2.5 - 4.2 mg/L. The variation in the increase of DO might be due to a disparity in the functioning of the aeration units. In October 2013, the DO in the Influent ranged from 0.2 - 0.7 mg/L whereas it ranged from 2.5 - 4.0 mg/L in the Effluent. The findings of this study are consistent with the findings of the HPCIDBC.

3.3.2.2.5 Turbidity

Figure 3.31 depicts the time averaged turbidity for Influent and Effluent at the GWWTP, from February 2013 to June 2013, August and October 2013. The turbidity in the Influent ranged from 326 - 670 NTU and from 55 - 139 in the Effluent. The turbidity removal efficiency was found to be highest in August, at 83 % - with the average removal rate being 75 %. In October, the Influent turbidity ranged from 596 - 751 NTU, which is comparatively higher than that recorded by the HPCIDBC for previous months. The higher turbidity value on this month might be due to the sampling on the consecutive days after a few rainy days. A high level of turbidity may result from soil erosion within catchments after rain

(Commonwealth of Australia 2005). However, the Effluent ranged from 102 - 149 NTU, resembling Effluent values for the previous months. The average turbidity removal in the Effluent was found to be 82 %.

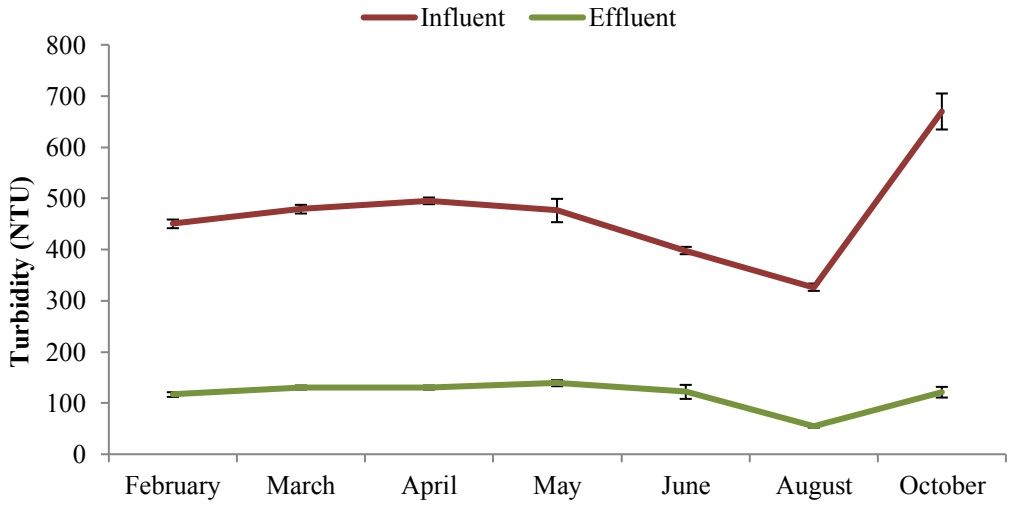


Figure 3.31 The time averaged turbidity for Influent and Effluent at the GWWTP for 2013. The error bars represent the standard error, where, n = 13 for February; n = 14 for March & April; n = 15 for May; n = 16 for June; n = 19 for August; n = 4 for October.

3.3.2.2.6 Total Suspended Solids

Figure 3.32 depicts the time averaged TSS for Influent and Effluent at the GWWTP, from January 2013 to August 2013 and October 2013.

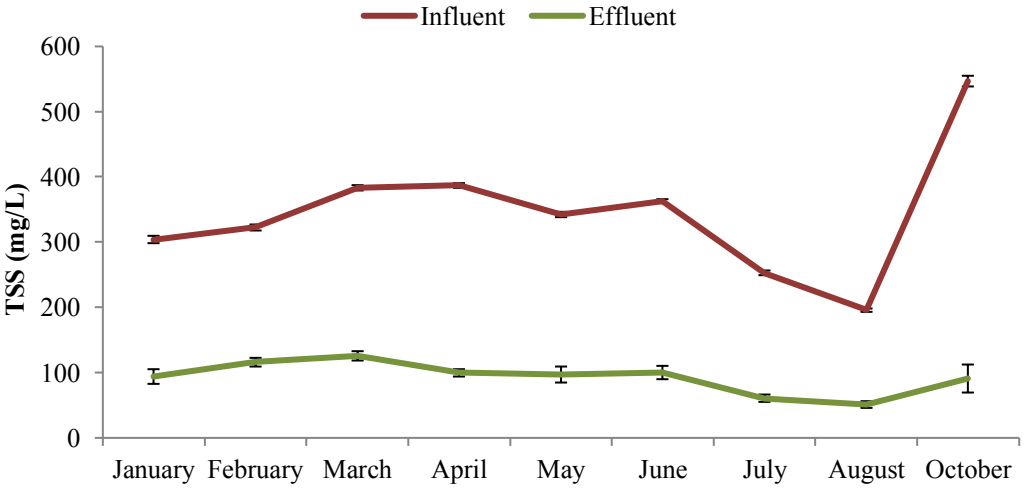


Figure 3.32 The time averaged TSS for Influent and Effluent at the GWWTP for 2013. The error bars represent the standard error, where, n = 9 for January; n = 13 for February; n = 14 for March & April; n = 15 for May; n = 7 for June; n = 19 for July &August; n = 4 for October.

A large variation in the TSS value was observed for the Influent throughout the year, ranging from 196 - 547 mg/L. Interestingly, the Effluent value ranged from 51 - 126 mg/L, being less variable than for the Influent. The comparatively higher value observed for the October Influent could be due to the rain just before the sampling day. The highest TSS removal was found to be in October (by 83 %), the average TSS removal was found to be 72 %.In October, the Influent TSS ranged from 492 - 589 mg/L, whereas it ranged from 75 - 113 mg/L in the Effluent. The average TSS removal was found to be 83 %.

A graphical representation of the *primary* data collected for the temperature, pH, conductivity, DO, turbidity and TSS, on 21 - 24 October, 2013, is provided in **Appendix 3.2**.

3.3.2.2.7 Ammonium, nitrite and nitrate nitrogen

High nutrients may boost a toxic algal bloom which may reduce dissolved oxygen in the receiving water (EPA Victoria 2009). **Figure 3.33** depicts the NH_3-N, NO_2-N and NO_3-N profile for Influent and Effluent at the GWWTP in October 2013.

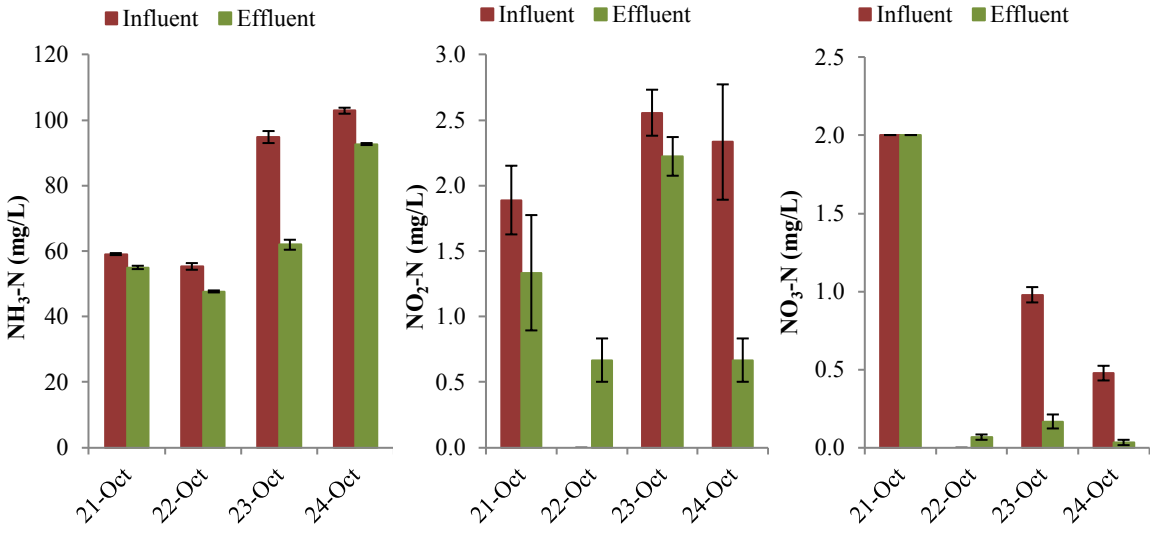


Figure 3.33 The NH_3-N, NO_2-N and NO_3-N profile for Influent and Effluent at the GWWTP for October 2013. The error bars represent the standard error, where n = 3.

The NH_3-N in the Influent ranged from 55 - 103 mg/L whereas it ranged from 48 - 93 mg/L in the Effluent. The average NH_3-N in the Effluent was significantly reduced by 18 %. The NO_2-N in the Influent ranged from 0 - 2.6 mg/L whereas it ranged from 0.78 - 2.2 mg/L in

the Effluent. The average NO_2-N in the Effluent was significantly reduced by 28 %.The NO_3-N in the Influent ranged from 0 - 2.0 mg/L whereas it ranged from 0.03 - 2.0 mg/L in the Effluent. The average reduction of NO_3-N in the Effluent was found to be 34 %, after treatment.

3.3.2.2.8 Heavy metals

The presence of the heavy metals, Cu, Fe, Mn and Zn, in wastewater (as soluble ions) may be indicated by characteristic properties such as colour, bitter taste and blue stains (e.g. corroded copper pipes) (Commonwealth of Australia 2005). These metals are toxic to the aquatic environment if elevated, **Table 3.7**. Heavy metal input in the wastewater seems unlikely as there are not many industries in the vicinity of the treatment plant. However, fertilizers and some metals used in plumbing and pipes may be the potential source of heavy metals in the influent. Of the observed heavy metals, namely - Cu, Fe, Mn and Zn, Mn was not detected at all. **Figure 3.34** presents the Cu, Fe and Zn profiles for the IN and EF at the GWWTP for October 2013.

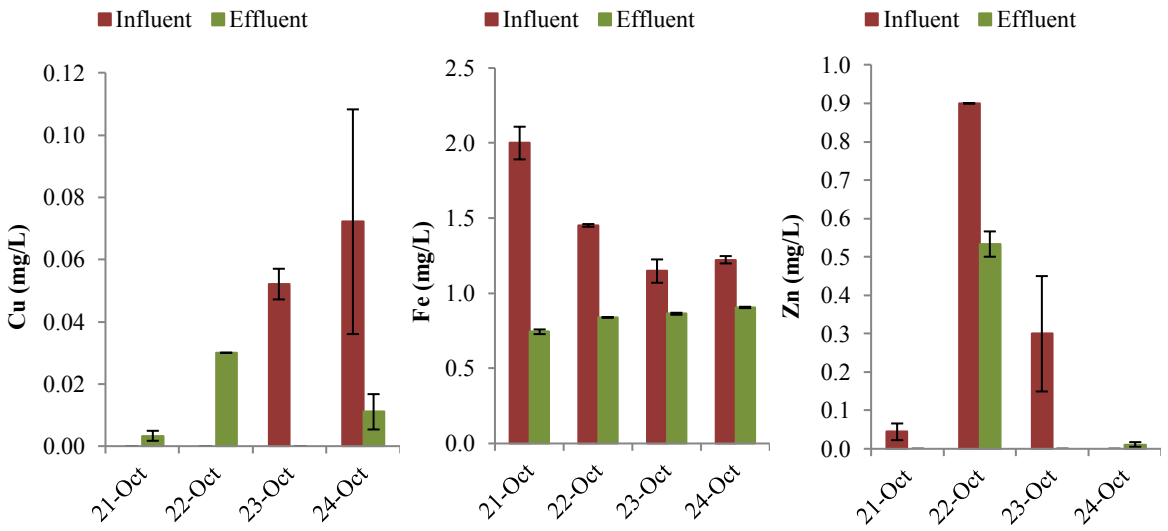


Figure 3.34 The Cu, Fe and Zn profile for Influent and Effluent at the GWWTP for October 2013. The error bars represent the standard error, where n = 3.

The Cu concentration in the IN was found to be low; the highest value observed being 0.07 mg/L. In the EF, it was also found to be low, with the highest value 0.01 mg/L. It is interesting to observe that on the first two sampling occasions, no Cu was detected in the IN, but it was recorded in the EF. This might be attributed to the corrosion of pipes and fittings

from the treatment process. On average, the Cu in the EF was found to be reduced by 64 %. The Fe concentration in the IN ranged from 1.15 - 2.0 mg/L whereas it ranged from 0.74 - 0.91 mg/L in the EF. The Fe concentration in the EF was found to be reduced by 42 %. The Zn concentration in the IN ranged from 0.04 - 0.9 mg/L whereas it ranged from 0 - 0.5 mg/L in the EF. The Zn concentration in the EF was found to be reduced by 56 %. These values for Cu, Fe and Zn in the EF are found to be in the tolerance range according to the limit/guidelines, **Table 3.7**. Here, the significant reduction of the observed heavy metals suggests that the GWWTP is quite efficient in heavy metal removal. The reduction in the concentration may be attributed to the precipitation of the metals in insoluble form such as metal hydroxides, sulfides/sulfates and carbonates (Armenante 1997)[56].

3.3.2.2.9 Chemical Oxygen Demand

Figure 3.35 presents the time averaged COD for Influent and Effluent at the GWWTP from January 2013 to August 2013.

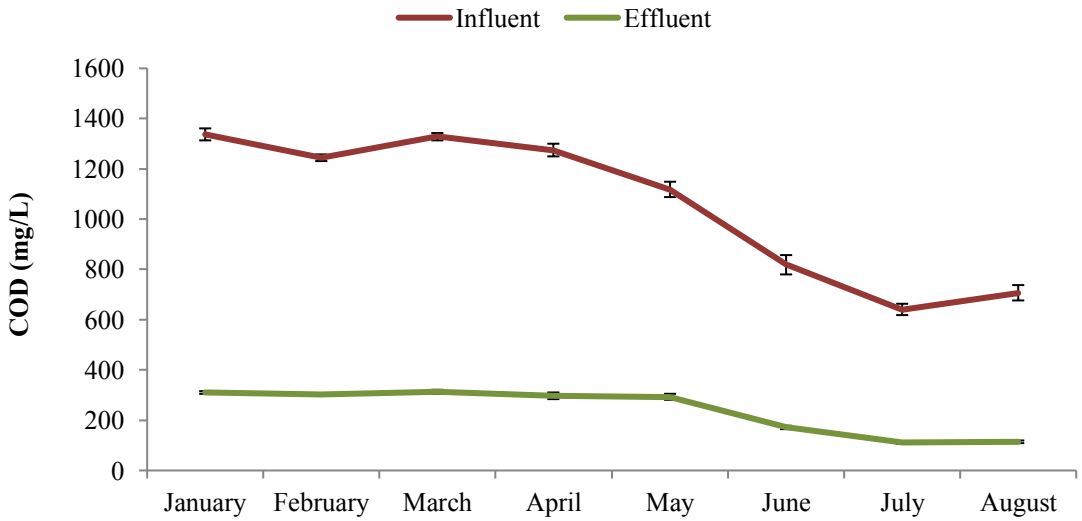


Figure 3.35 The time averaged COD for Influent and Effluent at the GWWTP for 2013. The error bars represent the standard error, where, n = 5 for January & June; n = 6 for February, March & May; n = 7 for July & August

[56]Armenante 1997 http://cpe.njit.edu/dlnotes/che685/cls06-2.pdf

The IN COD ranged from 640 - 1337 mg/L whereas it ranged from 113 - 313 mg/L in the EF. The highest reduction of COD achieved was 84 % in August, with an average reduction of 78%, **Table 3.11**. Thus the treatment system is quite effective in the removal of COD.

3.3.2.2.10 Chloride

Though chloride is one of the essential factors required for aquatic life, an elevated level of chloride in the receiving water could have an adverse impact on the ecosystem. It may affect aquatic life by altering reproduction rates, increasing species mortality rates or by changing the entire local ecosystem. The main source of chloride in wastewater is the use of water softeners by households. Other contributors could be agricultural waste containing biological waste, solid waste, hazardous waste, and used oil, which might contain chloride (Fontenot and Lee 2013).

Figure 3.36 presents the time averaged chloride for Influent and Effluent at the GWWTP from January 2013 to August 2013.

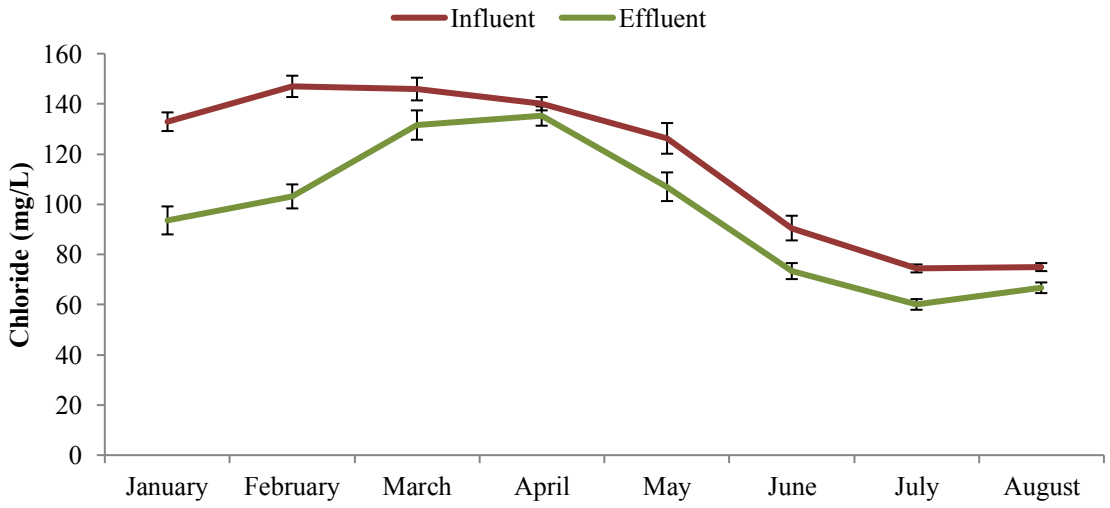


Figure 3.36 The time averaged chloride for Influent and Effluent at the GWWTP for 2013. The error bars represent the standard error, where, n = 6 for January, February, March, May &June; n = 4 for April; n = 7 for July & August

The IN chloride ranged from 74 - 147 mg/L, whereas it ranged from 60 - 135 mg/L in the EF. The highest reduction achieved was 30 % in February, the average reduction was found to be 17 %. The concentration of chloride recorded in the GWWTP Influent/Effluent was quite low

compared to the one recorded for Morris's sewage treatment pond[57], which is above 700 mg/L (2012 data), and quite a bit less than the Minnesota Pollution Control Agency (MPCA) standard at 230 mg/L. Thus, it is unlikely any adverse impact on the receiving water (the Bagmati River).

3.3.2.2.11 Total Alkalinity

A measure of the total alkalinity of water indicates its capacity to neutralize or buffer acids[58]. The abundance of carbonates, bicarbonates and other ions in wastewater determine the total alkalinity[59]; usually, detergents and soaps are the major source. This buffering effect plays a vital role in the protection of aquatic life as it protects against dramatic changes in the pH. Generally, most of the natural water has a total alkalinity in the range of 10 to 500 mg/L. No limits on alkalinity have been noted as it is not considered to be a water pollutant factor. **Figure 3.37** presents the time averaged total alkalinity (TA) for Influent and Effluent at the GWWTP from January 2013 to August 2013

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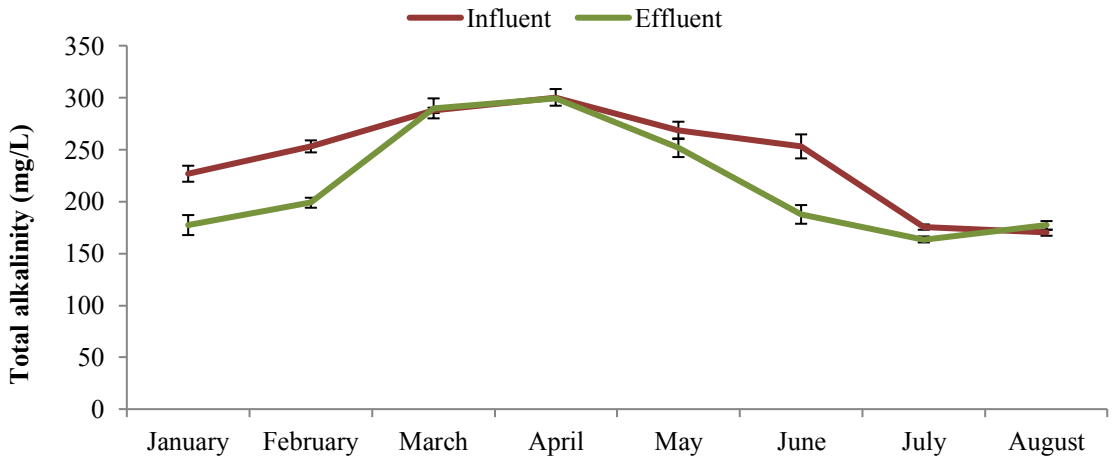

Figure 3.37 The time averaged Total alkalinity for Influent and Effluent at the GWWTP for 2013. The error bars represent the standard error, where, n = 6 for January, February, March, May &June; n =3 for April; n = 7 for July & August

[57]Morris, a small town in Minnesota, USA, discharges its water from the sewage treatment ponds to the Pomme de Terre River. http://environment.umn.edu/wp-content/uploads/2014/02/MS-0008-12-Final-Addendum.pdf
[58]http://water.epa.gov/type/rsl/monitoring/vms510.cfm
[59]Total alkalinity is expressed as $CaCO_3$ equivalents and ions responsible for total alkalinity are given in the link. http://www.usbr.gov/pmts/water/publications/reportpdfs/Primer%20Files/08%20-%20Alkalinity.pdf

The IN TA ranged from 170 - 300 mg/L whereas it ranged from 163 - 299 mg/L in the EF. The highest reduction of TA achieved was 26% in June, the average reduction was found to be 10%. However, on a few sampling occasions, it increased slightly in the EF. However, the value recorded is in the range of that observed for natural water, though it is significantly higher than the value (200 mg/L) observed in typical medium strength wastewaters in the United States (Tchobanoglous et al. 2003).

To summarize the performance efficiency of the GWWTP, the % change in the observed parameters (from IN to EF), is presented in **Table 3.11.** The system was found to reduce COD, turbidity, TSS, TDS, chloride and total alkalinity by 78 %, 75 %, 72 %, 35 %, 17 % and 10 %, respectively. Moreover, it was found to reduce conductivity, NH_3-N, NO_2-N, NO_3-N, Cu, Fe and Zn by 15 %, 18 %, 28 %, 34 %, 64 %, 42 % and 56 %, respectively. The DO was found to increase up to 4.2 mg/L. The treatment system is quite effective in removing organic matter from the wastewater; evident by a significant removal of COD, turbidity and TSS from the IN. However, it cannot be considered as effective in reducing other parameters such as chloride and total alkalinity.

Table 3.11 The % change in the Effluent = (Influent – Effluent)/Influent*100. The October data is from this study and the rest are from the HPCIDBC.

	Temp. (°C)	pH	TSS (mg/L)	Turbidity (NTU)	TDS (mg/L)	COD (mg/L)	Chloride (mg/L)	Total alkalinity (mg/L)
January	9	-7	69	-	-	77	29	22
February	-1	-9	64	74	45	76	30	21
March	0	-11	67	73	29	76	10	-1
April	-5	-11	74	74	33	77	3	0
May	-5	-11	72	71	39	74	15	6
June	-5	-9	72	69	35	79	19	26
July	-5	-9	76	-	32	82	19	7
August	-5	-10	74	83	32	84	11	-4
October	3	-12	83	82	-	-	-	-

3.4 Conclusions and suggested further research

3.4.1 Conclusions and comments

- The Bagmati River, fed by a number of tributaries and spring / monsoon rainfall, is a holy river that originates in the Shivapuri hill and flows through the Kathamdnu valley.

- Many cultural and traditional rituals are performed along the bank of the river, thus the Bagmati River is of great cultural and religious importance to the Nepalese people.

- With the intervention of human activities, due to urbanization and industrialization, the river basin is facing various environmental and ecological challenges.

- This study has identified two major issues which contribute most in the deterioration of the river environment - the discharge of untreated sewage wastewater into the river environment and the solid waste dumping along the river bank.

- The Bagmati River water monitoring in the upper part of the river, from Sundarijal (upstream) to Chovar (downstream), found that the river water quality diminishes as it flows downstream to more populated area.

- Upstream, in rural areas, human sewage from open defecation and fertilizer from the agricultural land are found as major contaminants, whereas downstream, in urban areas, municipal sewage is the major contaminant.

- The direct discharge of sewage wastewater through the pipeline starts at the Tilganga and Pashupati dham areas. The sewage wastewater discharge piping continues throughout the municipality as well as through individual residential buildings along the river segment up to Sundarighat.

- When the river flows up to Thapathali and Sundarighat, it becomes biologically dead. The DO level was found to be too low at these two sampling stations to support the survival of any aquatic.

- The encroachment of the river bank for squatter settlement, road construction, and commercial buildings has reduced the aesthetic value of the river.

- In Sundarijal, the DO was fond to be > 8 mg/L and the river water was clear (transparent).

- In Gorkarna, the temperature, pH, conductivity, DO, turbidity and TSS was found to be 23.1°C, 6.2, 53.5 µS/cm, 7.3 mg/L, 20 NTU and 19 mg/L, respectively.

- In Pashupati dham, the river water quality was investigated upstream as was the pipeline discharge from the GWWTP downstream. The river quality was also investigated upstream at Aaryeght and downstream at Tilganga.

- In Aaryeghat, the temperature, pH, conductivity, DO, turbidity and TSS were found to be 21.1 °C, 6.5, 87.7 µS/cm, 7.4 mg/L, 28.5 NTU and 26.0 mg/L, respectively. The concentrations of NH_3-N, NO_2-N, NO_3-N, Cu, Fe and Zn were found to be 10.2, 1.4, 1.1, 0.01, 0.33 and 0.57 mg/L, respectively.

- In Tilganga, the temperature, pH, conductivity, DO, turbidity and TSS were found to be 23.1 °C, 6.8, 431.6 µS/cm, 3.1 mg/L, 241 NTU and 225 mg/L, respectively. The concentrations of NH_3-N, NO_2-N, NO_3-N, Cu, Fe and Zn were found to be 21.2, 2.3, 3.0, 0.05, 0.95 and 0.95 mg/L, respectively.

- A comparison between the values for the observed parameters at Aaryeghat and Tilganga shows that the river water quality diminishes significantly at Tilganga.

- In Sankhamul, the temperature, pH, conductivity, DO, turbidity and TSS were found to 19.9 °C, 7.4, 249.3 µS/cm, 6.6 mg/L, 59 NTU and 51 mg/L, respectively.

- In Thapathali, the temperature, pH, conductivity, DO, turbidity and TSS were found to 22.8 °C, 7.0, 316.0 µS/cm, 1.4 mg/L, 188 NTU and 186 mg/L, respectively.

- In Sundarighat, the temperature, pH, conductivity, DO, turbidity and TSS were found to 23.8 °C, 7.1, 418.3 µS/cm, 10.8 mg/L, 35 NTU and 27 mg/L, respectively.

- In Chovar, the temperature, pH, conductivity, DO, turbidity and TSS were found to 22.3°C, 7.2, 388.7 µS/cm, 6.4 mg/L, 70 NTU and 46 mg/L, respectively.

- The Guheshwori Wastewater treatment Plant (GWWTP) has been constructed with a view to treat municipal wastewater before discharging it into the river. This centralized treatment system utilizes a large quantity of electrical energy (2.3 unit/kg BOD_5) and has high operational and maintenance costs.

- Due to the shortage of electricity supply and proper maintenance, the treatment plant is functional only up to part of its efficiency potential.

- The treatment plant was found to remove 78 % COD, 75 % turbidity, 72 % TSS, 35 % TDS, 17 % chloride and 10 % total alkalinity, from the Influent. Moreover, it reduced 15 % conductivity, 18 % NH_3-N, 28 % NO_2-N, 34 % NO_3-N, 64 % Cu, 42 % Fe and 56 % Zn.

- Though the treatment efficiency of the GWWTP seems to be generally satisfactory, the discharge from this plant to Tilganga has highly deteriorated the river water quality. The temperature, pH, conductivity, turbidity, TSS, NH_3-N, NO_2-N, NO_3-N measurements and the Cu, Fe and Zn increased, whereas DO decreased. This might be due to the mixing of bypassed Influent, diverted from the GWWTP, with the treated Effluent. Thus, it can be concluded that the river water quality worsened as the wastewater was discharged via a large pipeline directly into the river at Tilganga.

- Therefore, the construction of simple, low cost decentralized wastewater treatment systems at the community level is indicated.

- The sewage treatment at the local level reduces the need of the construction of highly technical and expensive centralized systems. Moreover, the treated water might have the potential for reuse such as in irrigation, which might also reduce the diversion of river water.

3.4.2 Suggested further research

- Data on the quantity of the sewage water and catchment area covered by the GWWTP is not available. Thus, a study could be conducted to obtain this information, so that a plan could be developed to upgrade the treatment plant.

- An investigation should be carried out on potential management systems to stop the bypassing of received Influent in order to minimize the impact on the Bagmati River water quality. Either the treatment of sewage at the local level or the upgrading of the treatment plant, or both, could be the viable options.

- An alternative solution(s) for the solid waste dumping and land encroachment for other purposes should be investigated in order to retain and restore the river environment.

- The possibility of running the GWWTP with an alternative power supply such as renewable energy (solar power, wind turbine) should be piloted, in order to increase its reliability and efficiency.

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CHAPTER 4: The design and operation of a pilot scale Vermifiltration unit for domestic wastewater management

4.1 Introduction

4.1.1 Vermifiltration for domestic wastewater (sewage) management[60]

In developing countries, increasing development and population growth in urban areas is presenting a major challenge with respect to the introduction of appropriately viable and affordable technologies for the management of sewage. In developing countries, approximately 90 per cent of the wastewater generated is discharged directly into waterways, without any kind of treatment - and almost half of the population of the developing world does not have access to sufficient sanitation (Corcoran 2010; Rammont and Amin 2010). This leads to contaminated freshwater and reduced access to safe drinking water, posing a threat to both environmental and human health. Considering the threat to freshwater availability, and the fact that globally more than 1.8 million children under 5 years of age die every year due to waterborne diseases, it is essential to develop and implement appropriate technologies to treat wastewater before discharging it to waterways. Many developing countries are not able to build or maintain wastewater treatment plants based on advanced technologies and therefore need to consider economical, small-scale, and less technically sophisticated, treatment systems. One such technology is Vermifiltration (VF) that uses earthworms and microorganisms to treat wastewater, preferably at a community level. This kind of treatment system also provides opportunities to recognize wastewater as a resource and encourages its reuse for different applications as well as for return to waterways. This will ultimately reduce the stress on freshwater availability and improve environmental and public health (Kivaisi 2001; Massoud et al. 2009).

Municipal wastewater, that is sewage from communities as in this study, contains organic contaminants in the form of dissolved/suspended solids (TDS/TSS) and presents both COD and BOD_5. Similarly, it contains nutrients, pathogens and toxic compounds in moderate to

[60] The author is aware that some part of this section (review on vermifiltration technology) is the same as **Section 1.2.6.3**. However, it is repeated for continuity and for the convenience of the reader.

trace levels. Generally, such sewage is 99.9 % water and only 0.1 % solids. Of the solids, 30 % is inorganic such as grit, salts and metals, and 70 % is organic such as protein, carbohydrates and fats (Mara 2013). High loads of organics as well as other contaminants should be removed from wastewater prior to being discharged into surface water, otherwise it could be fatal to aquatic fauna and flora due to the depletion of DO as a result of being consumed by aerobic bacteria (Sinha et al. 2008b). Moreover, wastewater should also be treated before its discharge to the environment to reduce the transmission of excreta-related diseases (Mara 2013).

Conventional centralized wastewater treatment plants are highly technical and expensive to build and maintain, making such plants problematic for developing countries. In contrast, decentralized biological treatment systems such as vermifiltration tend to have low construction and maintenance costs and such technology presents alternative options to conventional centralized systems. Many researchers have presented vermifiltration as a novel technology which uses earthworms as a biofilter to remove organic contaminants from influent water from different sources. Thus this treatment system has been effectively applied on a pilot scale for the treatment of municipal, domestic and rural sewage and sludge (Xing et al. 2010a; Wang et al. 2011b; Xing et al. 2011) effluents from gelatine industry (Ghatnekar et al. 2010) and the swine industry (Li et al. 2008b).

VF technology, which is also called lumbrifiltration, was first introduced in 1992 by Prof. José Toha at the University of Chile. A full scale VF sewage treatment plant, known as the TOHA vermifiltration system, has been constructed with a treatment capacity of 1000 persons per day (Sinha and Valani 2011). Since then, many studies have been conducted on the optimization and performance of VF technology.

VF is defined as ‘a process that separates wastewater solids by allowing wastewater to be gravity-fed over the filtration material (Wang et al. 2011a). *Eisenia fetida* (the Indian tiger worm) is a common species of earthworm chosen for wastewater treatment due to the ability of its body performing as a ‘biofilter’. Other worm species used in VF are versatile ‘waste-eating’ earthworm species such as the Red Tiger Worm (*Eisenia andrei*) and the Indian Blue Worm (*Perionyx excavates*) (Sinha et al. 2008b; Li et al. 2012; Wang et al. 2013). These worms act as ‘an aerator, grinder, crusher, chemical degrader and a biological stimulator’ (Sinha et al. 2010a). They are also capable of bio-accumulating metals, including heavy

metals such as cadmium, mercury, lead, copper, manganese, calcium, iron and zinc, in high concentration. Sinha et al. (2010b) defines vermiculture technology as 'economically viable, environmentally sustainable and socially acceptable' technology. Furthermore he adds that technology based on earthworms is 'self-promoted, self-regulated, self-improved and self-enhanced, with low or no-energy requirements, with zero-waste, easy to construct, operate and maintain'. Various studies have been conducted using earthworms in a filter bed as a 'biofilter'. A VF system set up by (Wang et al. 2011b) in China used cubic stages and a tank, each stage comprised of four layers of filter bed; soil, silver sand, fine detritus and cobblestones. They argue that the system efficiency might be influenced by parameters such as the running time (residence time), the increasing nitrification ability between the "stages" and the chemistry of the metal (Al, Fe, Ca) oxides. Taylor et al. (2003), in his study on a commercial on-site domestic wastewater treatment system 'Biolytix', observed that earthworms are capable of colonizing the filter bed and that this defines the efficiency of the filtration process. The comparative study conducted by Sinha et al. (2008b) found that filtration with earthworms was more effective in removing contaminants than filtration without worms. The Vermifiltration technology is like 'killing two birds with a single stone' - on one hand, it is a safe wastewater management technology and on the other, it helps sustainable agriculture by producing compost (Sinha et al. 2008a). Whilst the use of earthworms for the management of organic solid waste by means of 'vermicomposting' has been in practice for many years, its use in wastewater management is a relatively new approach.

Some researchers have applied vermicomposting for sludge stabilization. For instance, research conducted at Murdoch University (Bajsa et al. 2004) utilized vermicomposting technology to destabilize sludge from wastewater treatment plants. The vermicomposting reduces the quantity of sludge delivered to landfills by using the compost produced as a fertilizer. This study has listed the advantages of large-scale vermicomposting for sewage sludge stabilization that could make it a viable option for developing countries.

Previous studies showed that the VF has many applications for the treatment of a wide range of wastewaters, such as swine wastewater (Li et al. 2008a), rural sewage (Xing et al. 2010b), and household wastewater (Xing et al. 2010a). Only a few known scientific studies have been conducted on vermifiltration technology in countries that include Chile, India, China,

Zimbabwe and Australia. **Tables 4.1** and **4.2** provide an overview on some of these VF technology.

Table 4.1 Characteristic of various vermifiltration units studied previously, using different filter media, worm species and operating conditions - for different types of wastewater.

Reference	Type of wastewater	Worm species	No. of worms introduced	Filter material
Kumar et al. (2015)	Synthetic domestic wastewater	*Eisenia fetida*	150 ind, Stocking density of 1000/m^3	River bed material Wood coal Glass balls Mud balls
Li et al. (2012)	Raw sewage	*Eisenia andrei*		Quartz sand Turf Wood chips Fibre
Tomar & Suthar (2011)		*P. sansibaricus*	22-24.5 g/L	Large & small stones Gravel Pebble Plastic net Saw dust Dry leaves Sand
Yang et al. (2011)	Municipal	*Eisenia fetida*	11440 ind/m^2	Quartz sand Zeolite
Fang et al. (2010)	Domestic	*Eisenia fetida*	1.69 kg	Artificial soil Sand Gravel Cobble
Wang et al. (2010)	Rural domestic	*Eisenia fetida*		Converter slag Coal cinder
Xing et al. (2010a)	Domestic	*Eisenia foetida*	21000 ind/m^2	Quartz sands Ceramsite
Lu et al. (2009)	Municipal sewage-sludge	*Eisenia fetida*		Quartz sand
Li et al. (2008b)	Swine wastewater	*Eisenia andrei*		Wood chip Bark Peat Straw Vermicompost
Sinha et al. (2008b)	Sewage	*Eisenia fetida* *Eisenia andrei* *Perionyx excavaus* *Eudrilus euginae* *Lumbricus rubellus*	20000 ind/m^2	Gravel Garden Soil

In China, Wang et al. (2011b) investigated a VF system with four filter media; namely, soil mixed with saw dust in a 3:1 ratio by volume, earthworms, sand, detritus and cobblestone. These workers found that the VF layer (in which the soil was mixed with the saw dust) was

more effective than the other layers due to higher porosity and larger surface area[61]. This finding is supported by Kumar et al. (2015), who argued that the filter media with larger surface area) help to accumulate biomass and perform to higher treatment efficiency. Many filter media beds have been used in VF by various researchers, including the use of ceramsite (Liu et al. 2009), soil mixed with saw dust (Wang et al. 2011b) and quartz sand (Xing et al. 2010a). **Table 4.1** lists a wide variety of materials used as a packing material and/or filter media in VF such as ceramsite, gravels, stones, cobblestones, pebbles, saw dust, quartz soil, zeolite, sand, silver sand, soil, detritus, wood coal, mud balls etc. Based on these studies of different filter media, it may be concluded that the type of media used can affect the treatment efficiency. It is noteworthy to mention that particle size ratio of the filter media can also make a difference as it influences on microbial activity and flow rates of the filter.

Here, a comparative study conducted by Xing et al. (2011) on filter media suitability suggests ceramsite to be a more suitable filter media because of its low sludge yield and good vermicast sludge stabilization. Moreover, cuticle injury of worms in a ceramsite bed was found to be less than that in a quartz sand bed. Similarly, a laboratory-scale study by Wang et al. (2011b) provides evidence for the effective removal of COD by the soil sawdust-earthworm layer, and the authors argue that their four-layer VF could be effective for domestic wastewater treatment.

In Australia, Sinha et al. (2008b) conducted a comparative study of a vermifiltration system with and without earthworms in the top layer of the filter media. The study found that the VF with earthworms was more effective in removing contaminants than the one without earthworms. Taylor et al. (2003) observed that the removal rate of COD and BOD_5 from the influent was more efficient as it passed through the filter bed, showing that the filter-depth plays significant role in reducing the oxygen demand.

[61] More information on the effect of porosity and surface area is provided in a practice guide by Klobes, P., Meyer, K. & Munro, R. G. 2006. *Porosity and specific surface area measurements for solid materials,* US Department of Commerce, Technology Administration, National Institute of Standards and Technology. http://www.glb.nist.gov/customcf/get_pdf.cfm?pub_id=854263 22/03/2014

Table 4.2 A comparative study on the performance efficiency of some vermifiltration units. Parameters are reported in % removal.

Filter system	HLR, $m^3/m^2/d$	BOD_5	COD	TDS	TSS	NH_4-N	TP	TN	Reference
Lab-scale vermifilter, VFR	1.5	81	72	56	73	76	-248	-	Kumar et al. (2015)
Lab-scale vermifilter, VFC		75	65	54	61	74	-219	-	
Lab-scale vermifilter, VFG		73	62	50	38	58	-156	-	
Lab-scale vermifilter, VFM		71	60	49	36	54	-165	-	
Four-layered vermifilter	0.93	98	70	95	95	-	-	-	Manyuchi et al. (2013)
Three-stage tower earthworm ecofilter	0.25	-	88	-	-	99	99	90	Fang et al. (2010)
	0.5	-	84	-	-	99	99	84	
Three-stage tower vermifiltration	-	-	81	-	-	98	98	60	Wang et al. (2011c)
	-	> 90	80 - 90	90 - 92	90 - 95	-	-	-	Sinha et al. (2010c)
Pilot-scale vermifilter	-	55 - 66	47 - 65	-	57 - 78	21 - 62	-	8 - 15	Xing et al. (2010b)
	-	-	-	-	-	60	30	50	Li et al. 2008b
	-	90 - 98	80 - 86	-	95 - 98	30 - 60	-	-	Xing et al. (2005)

With respect to the effect of HRT and HLR on the treatment efficiency of the VF, previous studies illustrated that high HRT and low HLR are favourable for better treatment efficiency. For instance, a study conducted by Fang et al. (2010) on the effect of HLR on the removal of contaminants from synthetic domestic water, showed a variance in nutrient removal efficiency; better removal efficiency being observed with low HLR, **Table 4.2**. Similarly, a study on the variability with HRT also resulted in a variation in removal efficiency. Xing et al. (2010a) observed that the BOD_5, COD, SS, TN and NH_4-N removal rates decreased with an increase in HLR and a decrease in HRT.

In terms of the performance of VF, worm density and their health is another factor which will affect the treatment efficiency. Xing et al. (2010b) observed a reduction in adult and clitellated earthworms and found that the density of hatchling and cocoon was relatively

higher (which is evident that worms are capable of breeding and incubating in the filter). This study also reported that adult worms play a more significant role than younger ones in removing contaminants; hence a decline in adult worms in the filter decreased the efficiency of the system. Thus it is imperative to maintain optimum conditions in the environment where these worms reside. Hughes et al. (2007) reported that the optimum pH for earthworm survival ranges from 6.2 to 9.7. A detail discussion on the basic environmental requirements/factors affecting the worms, is provided in **Section 4.1.2.2**.

Studies have revealed that this simple and low-tech biological process is actually capable of handling a large variation in wastewater characteristics. A series of studies conducted by Wang et al. (2010; 2011a; 2011b; 2013) investigated the various physical, chemical and biological processes in VF and how these efficiently remove organic matter from the influent. In this regard, in various VF processes the majority of N removal has been shown to be due to nitrification followed by denitrification (Sinha et al. 2008b; Wang et al. 2010). Thus, Li et al. (2012) has dubbed the vermifiltration process as a 'sponge' and Sinha et al. (2008b) described the earthworm as a 'biofilter'. The biological wastewater treatment process involves the removal of organic pollutants by ingestion, absorption through body walls and through a biodegradation process carried out together with other living organisms – i.e. microbes (Sinha et al. 2008b; Tomar and Suthar 2011).

Various designs and combinations have been used to enhance the treatment efficiency of VF. For instance, Xing et al. (2005) studied VF combined with an up-flow anaerobic sludge blanket (UASB) via a pilot plant based at the Shanghai Quyang Wastewater Plant operating over a period of around one year. The combination of UASB with VF produces fertilizer (soil conditioner) as a sludge which only requires to be removed from the system every six months. This system is suitable for developing countries because it fulfils sustainable wastewater management criteria. Tomar and Suthar (2011) successfully investigated the combined VF and constructed wetland system, at a pilot scale.

Considering the simplicity and potential efficiency of the technology, many researchers argue that this decentralized biological wastewater treatment technology is economical and suitable for developing countries. Xing et al. (2010a) suggested that VF is suitable for the rural community of China to treat wastewater on-site. They claimed that the earthworm bio-filter saves almost 48.7 % in costs compared to the conventional activated sludge method. Another

investigation carried out by a group of researchers in China (Wang et al. 2011a) claimed that VF is the most economical technology for treating domestic wastewater - among other proposed solutions such as constructed wetland, soil infiltration and vegetation-based wastewater treatment. Bajsa et al. (2004) has listed the benefits of such innovative technology as being pollution free, odourless, low cost, and with no requirement for the transportation of raw sludge. It also complements other waste generated in the region and produces a valuable end product instead of sludge.

The potential for the reuse of the treated wastewater for various applications is another issue that was investigated. Liu et al. (2009) claimed that the effluent from ceramsite vermifilter is suitable for reuse in toilet flushing, floor washing and garden/crop irrigation. This being said, a major concern for the reuse of such wastewater relates to the presence of nutrients and pathogens in the system. This is discussed further in **Section 4.3.8**.

4.1.2 Earthworms as a filter media

An earthworm is a bilaterally symmetrical oligochaeta with an elongated and segmented body and is commonly found in soil and feeds within living or dead organic matter. Such an invertebrate has very simple body metabolism. Its digestive system runs throughout the length of its body and it respires through the skin. It has a central and a peripheral nervous system with a closed blood circulatory system. Earthworms are hermaphrodite (also referred to as a bisexual organism) i.e., each individual carries both female and male sex organs.. It can multiply very rapidly and each worm can replicate 256 worms every 6 months under favourable conditions of moisture, temperature and food. During the reproduction system, each worm produces up to 3 cocoons which contain approximately 10 - 12 tiny worms. Each adult worm is capable of producing 300 - 400 young worms in its life span (Hand et al. 1988). 2000 adult worms weigh approximately 1 kg and usually an individual can live 3 to 7 years, depending upon the species.

An earthworm is highly sensitive to light, touch and temperature. Its activities slow down significantly in cold temperature but high temperatures and dryness kills it almost instantly. Thus, a dark and moist habitat is ideal for this burrowing animal. Similarly, soil with low pH (acidic, < 4) and coarse textures are not suitable for worms. However, it can live in soil with

high salinity and can tolerate high concentrations of toxins in the environment such as heavy metals and endocrine disrupting chemicals (Sinha et al. 2008b). Vermifiltration uses versatile 'waste-eating' earthworm species such as the Indian Tiger Worm (*Eisenia fetida*), the Red Tiger Worm (*Eisenia andrei*) and the Indian Blue Worm (*Perionyx excavates*), as the major filter media (Sinha et al. 2008b; Li et al. 2012; Wang et al. 2013). Such species have the capability to survive in harsh environments (Hughes et al. 2007) and to multiply rapidly. Moreover, these worms have a characteristic organic waste eating and biodegrading capacity.

4.1.2.1 The Nature of Earthworms

Earthworms are one of the most ancient of the terrestrial animal groups and fossils of polychaete worms, believed to be ancestral to the oligochaete species, have been found in South Australia in pre-Cambrian sediments that are 650 - 570 million years old (Glaessner et al. 1969).

4.1.2.2 Basic environmental requirements of earthworms

a) *An adequate and suitable food supply*: Generally, worms feed on organic matters including dead organic tissues, microorganisms, fungi, micro/meso fauna. Worms are capable of ingesting plant litter only when this is partly decomposed. Microorganisms and fungi ingested with such decomposing organic matter plays a vital role in digestion. The *detritivores* species feeds at or near the soil surface (plant litter, dead roots, plant debris, and mammalian dung) while the *geophages* species feeds deeper beneath the surface (Lee 1985, p. 17). Richards and Arme (1982) reported that *Eisenia fetida* is capable of absorbing nutrients such as amino acids, monosaccharides and fatty acids from the solution via the integument.
b) *Adequate moisture*: Lee (1985) mentioned that earthworms can live in aerated fresh water continuously for a long time. The optimum soil moisture content for earthworm activity varies with worm species. However, excess soil water content causes respiratory stress.
c) *A suitable temperature*: Lee (1985) has listed lethal temperature limits and optimum temperatures for earthworms under varied conditions of exposure (p. 41- 43). Different factors affect the body temperature of worms For instance, high temperature causes dehydration resulting in moisture stress or extraction of water from the soil. An

earthworm's metabolic rate changes with a change in body temperature. The temperature range within which most earthworms can be "active" is 25 - 35 °C. However, the actual optimum temperature is species dependent. For example, the optimum temperature for tropical and sub-tropical species is 20 - 30 °C, whereas for cool temperature species, it is 10 - 20 °C (Curry 1994 in Edwards 1998). In terms of temperate species such as *Eisenia fetida and Eisenia andrei*, although they are able to tolerate a temperature range of 0 - 35 °C, the optimum temperature for their survival and growth is 25 °C (Edwards 1998).

d) *Respiratory exchange*: Earthworms have very a simple respiratory system involving inspiration of oxygen and expiration of carbon dioxide through cuticles, this is generally known as the cutaneous respiratory system. Despite such a simple respiratory system, they are able to respire oxygen obtained from air or oxygenated water, and possess a survival capability for anaerobic metabolism in an antagonistic environment. They are tolerant to a short period of anaerobiosis (Lee 1985, p. 52) i.e, they can survive for a while in anaerobic condition. Mucus secreted by gland cells acts as a lubricant and helps to keep the body surface moist for respiration to occur effectively.
e) *Protection from light*: prolonged exposure to light may injure or kill the worms.
f) *Suitable soil texture*: Earthworms are rarely found in materials with a coarse texture as this injures their body surface.

4.1.2.3 Worm mechanism of action in Vermifiltration

- In a vermifiltration system, the first soil layer usually traps suspended solids by an adsorption process, which is synchronously fed by worms and the microorganisms present in the soil. Organic and inorganic suspended solids are then stabilized through a complex biodegradation process involving worm and microbe activity, which also results in soil aeration, enhancing the effectiveness of the filtration (Tomar and Suthar 2011).
- Symbiotic and synchronous activity of earthworms and microorganisms for the enzymatic degradation of solid organic matters present in the wastewater. The earthworms secrete enzymes such as proteases, lipases, amylases, cellulases and chitinases in the gizzard and intestine. These enzymes are biological catalysts that convert the cellulosic and the proteinaceous materials in the organic matter rapidly by enhancing biological reactions.

- A Vermifilter provides a large specific area – up to 800 m^2/g and a voidage of up to 60 %. Earthworms process the suspended particles trapped at the top of the vermifilter and soil microorganisms feed on the processed particles.
- Earthworms granulate the clay particles, which increases the hydraulic conductivity of the system. They grind the silt and sand particles, which provide a large total specific surface area for the adsorption of the organic and inorganic particles in the wastewater.
- Earthworms feed on solid particles in the wastewater and excrete vermicast, which is also called vermicompost and contains 'hydrophilic' groups in the 'lignin content' and humus. Vermicompost adsorbs the heavy metals and pollutants present in the wastewater. Vermicompost shows similar properties to both sand and clay; having hydraulic conductivity like that of sand and the high adsorptive power of clay.
- Earthworms feed on inactive and harmful microorganism in the wastewater, which prevents the system from clogging and provides a better working environment for the biodegrading microorganisms.

4.1.2.4 Factors affecting Vermifiltration

4.1.2.4.1 Worm density (population)

The number and population density of earthworms in the soil layer is a vital factor in the removal of pollutants in a wastewater treatment process. About 8,000 - 10,000 worms per square meter of worm bed or 10 kg per cubic meter is considered the optimal worm population or density for effective worm activities (Komarowski 2001). In addition, the maturity and health of the earthworms affect the worm action.

4.1.2.4.2 Hydraulic Retention Time

Hydraulic retention time is defined as 'the time taken by the wastewater to flow through the soil profile (vermifilter bed) which earthworms inhabit (Sinha and Valani 2011, p.185). The flow rate of wastewater to the vermifiltration unit, volume of the soil profile and quality of the soil used, determine the HRT. It is a prerequisite for wastewater to remain in contact with the earthworms for a certain period so that they get enough time to extract the organic matter

from the wastewater. This retention time provides an opportunity for earthworms to remove nutrients from the wastewater by physical and biological actions, which eventually decreases the BOD_5, COD and TSS levels. Thus, the efficiency of removal of pollutants increases with a longer HRT. HRT can be calculated according to equation (4.1).

$$HRT = (\rho \times V_s) / Q_{wastewater} \quad (4.1)$$

Where, HRT = theoretical hydraulic retention time (hr)

V_s = volume of the soil profile (vermifilter bed), through which the wastewater flows and which contains live earthworms (m^3)

ρ = porosity of the entire medium (soil, sand and gravel) through which the wastewater flows

$Q_{wastewater}$ = flow rate of wastewater through the vermifilter bed (m^3/hr)

4.1.2.4.3 Hydraulic Loading Rate

Hydraulic loading rate is defined as 'the volume of wastewater applied per unit area of the soil profile (vermifilter bed) per unit time' (Sinha and Valani 2011, p.186). The efficiency of pollutant removal from the wastewater decreases with an increase in HLR, due to a corresponding decrease in HRT. Xing et al. (2010b) found that an increase in HLR reduced the abundance of adult worms in the VF. HLR can be calculated according to equation (4.2).

$$HLR = V_{wastewater} / (A \times t) \quad (4.2)$$

Where, HLR = hydraulic loading rate (m/hr)

$V_{wastewater}$ = volumetric flow rate of wastewater (m^3)

A = area of soil profile exposed (m^2)

t = time taken by the wastewater to flow through soil profile (hr)

The HRT and the HLR are inversely interrelated; i.e. when one increases the other decreases.

4.1.3 Parameters monitored for wastewater quality assessment

The following parameters, relevant to the wastewater quality, were investigated.

4.1.3.1 Temperature

Temperature is a critical factor for the survival of earthworms and their activity. They are able to perform better in a cold and moist environment than in hot and dry conditions. They usually become inactive above 29 °C. As discussed previously in **Section 4.1.2.2**, temperature is a major factor that determines the metabolic rate of an earthworm. For different species, the temperature tolerance range and the optimum temperature at which the worms can survive and grow varies.

4.1.3.2 pH

pH is a measurement of the hydronium ion concentration in the wastewater, mathematically expressed as

$$pH = - \log_{10} [H_3O^+]$$

Usually, neutral pH is desired for the treated wastewater. Earthworms are sensitive towards pH. Though they are able to survive in a pH range of 4.5 to 9, they perform better at a neutral pH of 7.

4.1.3.3 Conductivity

Conductivity is a measurement of the ability of wastewater to conduct an electric current. The concentration and type of soluble salts in the wastewater (as well as the temperature) determines the conductivity of the wastewater. Generally it is used as a substitute value for the measurement of TDS, although these two do not have any linear relationship.

4.1.3.4 Turbidity

Turbidity is a measurement of the cloudiness of the wastewater caused by suspended and colloidal particles, such as fine organic matter, clay and slit. The measurement of turbidity may be affected by colouration of the wastewater caused by dissolved material[62]. It is expressed in NTU (Number of Transfer Units) and defined as 'expression of optical properties of a liquid that causes light rays to be scattered and absorbed rather than transmitted in straight lines through a sample' (Sinha and Valani 2011, p. 188).

4.1.3.5 Total Suspended Solids

In wastewater, solids exist in the form of dissolved or suspended particles, which consist of organic and inorganic compounds. Chemical and biological pollutants in the wastewater are adsorbed onto such suspended solid particles. A high concentration of TSS in wastewater increases turbidity.

4.1.3.6 Biochemical Oxygen Demand

The BOD_5 is defined as the amount of oxygen required to decompose organic matter by aerobic microorganisms in a specified volume of wastewater. Thus, BOD_5 represents the amount of oxygen consumed by bacteria and other microorganisms under aerobic conditions at a specified temperature during the decomposition of organic matter. Generally, "five days BOD_5" is measured at 20 °C, by determining the DO of the sample before and after the five-day incubation period. The value of BOD_5 depends on the amount of organic matter present in the wastewater; i.e. the higher the amount of organic matter, the higher is the BOD_5 (Sinha and Valani 2011). The method for the measurement is discussed further in **Section 4.2.4.2**.

4.1.3.7 Chemical Oxygen Demand

The COD is the amount of oxygen required to chemically oxidize the organic matter present in wastewater to carbon dioxide, ammonia and water, in the presence of strong oxidizing

[62] http://water.epa.gov/type/rsl/monitoring/vms55.cfm

agents such as dichromate (Cr_2O_7) in acidic media. This may be represented by the following equation.

$$C_nH_aO_bN_c + (n + a/4 – b/2 – 3c/4)\ O_2 \rightarrow nCO2 + (a/2 – 3c/2)\ H_2O + cNH_3$$

The presence of inorganic substances in wastewater might result in a high COD value, due to the reaction between such compounds and dichromate. In vermifiltration, the organic substances that microorganisms cannot oxidize are ingested by the earthworms. This reduces the organic matter in the wastewater and results in a lowering of the COD.

4.1.3.8 Total Nitrogen

Nitrogen and phosphorus are nutrients which, in excessive amounts, may cause nutrient enrichment in water bodies. Thus, the nutrient level in wastewater should be taken care of before discharging it into any water bodies. The biological processes that remove nitrogen in wastewater involve ammonification, nitrification and denitrification[63]. Ammonification is a process in which microbes convert organic nitrogen into the ammonium cation. Then, ammonium is converted to nitrite and nitrate by the nitrification process. Ammonium nitrogen converts into nitrite with via *Nitrosomonas spp.* and nitrite coverts into nitrate via *Nitrobacter spp.*. Finally, the denitrification process reduces nitrate back into nitrogen gas via denitrifying bacteria.

The nitrification reaction is given as follows:

$$NH_4^+ + 3/2\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O + Energy$$

$$NO_2^- + 1/2\ O_2 \rightarrow NO_3^- + Energy$$

The complete reaction is:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O + Energy$$

The denitrification reaction is given as follows:

Oxidation reaction:

$$C_xH_yO_z + (2x-z)\ H_2O \rightarrow x\ CO_2 + (4x+y-2z)\ H^+ + (4x+y-2z)\ e \text{ or}$$

[63] http://www.wastewaterhandbook.com/documents/nitrogen_removal/411_NR_forms_and_reactions.pdf

Reduction reaction:

$e + 6/5\ H^+ + 1/5\ NO_3^- \rightarrow 1/10\ N_2 + 3/5\ H_2O$

Overall redox reaction:

$C_xH_yO_z + (4x+y-2z)/5\ H^+ + (4x+y-2z)/5\ NO_3^- \rightarrow x\ CO_2 + (2x+3y-z)/5\ H_2O + (4x+y-2z)/10\ N_2$

Where, $C_xH_yO_z$ is a general structural formula for organic matter.

In terms of nitrogen removal in VF, nitrogen transformation caused specifically by the earthworms could be responsible for changing the nitrogen content in the effluent. Lee (1985) has discussed the contribution of earthworm metabolism on the nitrogenous products in soil. Four pathways have been discussed; namely, through cast, urine, mucoproteins and dead earthworm tissue. A relatively higher concentration of exchangeable and soluble nitrogen was found in earthworm casts than what it had ingested before (Barley and Jennings 1959). Urine contains nitrogen in the form of ammonia, urea and uric acid. The body of an earthworm also secretes mucoproteins. Most of the nitrogen in earthworm tissue is incorporated into proteins and the protein content of earthworm is of the order of 60 – 80 % (60 – 61 % for *E. fetida*).

4.1.3.9 Total Phosphorus

As stated in **Section 4.1.3.8**, total phosphorus is one of the nutrients that causes eutrophication of water bodies. This lowers the DO level in water and could be detrimental to aquatic flora and fauna. Thus, it is essential to reduce the TP level in wastewater before discharging it into a water body, certainly up to the standard limit as proposed by reputable environmental organizations/or authorities. Though many physical and chemical processes have been proposed for TP removal from wastewater, biological processes are at the forefront, due to their low cost (with no chemical use) and the potential of phosphorus recovery (Nimali Gunasekara 2011). In biological phosphorus removal processes, specific microorganisms have an enhanced capacity for cellular phosphorus uptake[64] (Sathasivan 2009). However, phosphorus removal has not been found to be satisfactory in vermifiltration,

[64] http://www.desware.net/sample-chapters/d13/e6-144-10.pdf 27/03/2015

as the phosphorus retained in particulate form in the filter media biotransforms to soluble phosphorus (Wang et al. 2010).

4.1.3.10 Heavy metals

Heavy metals in municipal wastewater originate mainly from industrial discharge, including mining and smelting, energy and fuel production, the fertilizer and pesticide industries, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, leatherworking, photography, electric appliance manufacturing, metal surface treating, etc (Wang and Chen 2009). However, in the absence of discharges from such industries, heavy metal concentration in municipal influent is generally not considered significant. Though these elements do not have harmful effects below certain tolerance limits, if present in excess amounts they are considered as toxic contaminants in wastewater, posing risks to aquatic life, (Demirbas 2008). The heavy metals of most concern are cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) (Wang and Chen 2006). Though many physical, chemical and biological technologies are in use to remove heavy metals from wastewater, the application of biotechnology such as biosorption is attracting greater attention (Ahluwalia and Goyal 2007; Demirbas 2008; Ngah and Hanafiah 2008). Microorganisms are capable of actively binding heavy metals by intracellular accumulation, extracellular precipitation and chemical transformation via processes such as oxidation, reduction, methylation or demethylation (Kulbat et al. 2003). Previous studies (Hartenstein et al. 1980; Azizi et al. 2013) have also shown that earthworms are capable of bio-accumulate high concentrations of heavy metals in their tissues. Contreras-Ramos et al. (2005) reported that the concentration of heavy metals such as Cr, Cu, Zn and Pb, in sewage sludge after processing by earthworms, was found to be in line with USEPA limits.

4.1.3.11 Microorganisms in wastewater

In wastewater, the microorganisms of main concern are the pathogens originating from human faeces, which pose a threat to the public. Generally, wastewater is discharged into water ways, so that pathogens contaminate water that is used for recreation, fishing, drinking etc. Moreover, reuse of wastewater, e.g. in the garden, for toilet flushing or for irrigation, is

common in many countries due to water scarcity. Thus, the removal of pathogens is essential prior to the discharge of wastewater into the environment.

Previous studies reported that bacteria such as *Salmonella spp*. and *Campylobacter spp*., enteric viruses such as *Enteroviruses*, *Rotavirus*, *Norovirus*, *Adenovirus*, and protozoa such as *Giardia cysts*, *Crystosporidium oocysts* are the pathogens commonly found in wastewater (Ottoson 2005). However, it is members of the coliform group, such as *E. Coli*, i.e. *faecal coliforms* are used as an indicator of faecal contamination in wastewater. In a biological wastewater treatment process, the microbes feed on organic matter, and in due course pathogens are also removed as a result of competition (for space and nutrients, with indigenous soil microflora (Kadam et al. 2008), digestion and sedimentation. The commonly used method for pathogen removal in wastewater is disinfection with chlorine or ultraviolet radiation (Ottoson 2005).

Previous studies have reported an enhanced efficiency of pathogen removal from sewage and sludge when earthworms are used in the treatment system (Sinha et al. 2008c; Arora et al. 2014a). These findings support the research of Bajsa et al. (2004), where a significant reduction in *E. coli* and *Salmonella* are observed when *E. fetida* earthworms are present in the VF. In this regard, earthworms are considered to feed upon all types of pathogens and are also capable of nurturing some bacteria and fungi to produce antibiotic effects. Moreover, a celeomic fluid secreted by worms has been confirmed to possess anti-bacterial properties.

4.1.4 Research objectives in relation to Vermifiltration

This research aims to further develop and explore vermifiltration as an alternative approach to wastewater management involving localized community-based systems that are low-cost, technologically simple and which can be easily operated and maintained. Another important objective is to extend the scientific knowledge of such systems by investigating the relative pollutant removal efficiencies of the individual layers of the multilayer vermifiltration system under different conditions, **Figure 4.1**. This is with a view to optimizing the performance. Thus some broad aims are as follows:

- To design and construct a vermifiltration unit based on previous studies carried out by various researchers. Here, filter media to be used in the vermifiltration unit has been chosen so as to be easily available locally and cost effective. City West Water (CWW), a water retailer company based in Melbourne, provided in-kind support through access to infrastructure and expertise for accessing sewage influent from the influent tank of a sewer mining facility.

- To design an experimental plan, including a sample collection and comprehensive statistical strategy, from measuring the physical, chemical and biological properties of the unit - and for analysing the resultant data. This has been conducted within the scope and limitations of the study. The scope of the study was to explore viable alternatives for overall waste (solid and liquid) management in representative Nepalese communities (**Section 1.6**). Therefore, that part of this PhD project devoted to vermifiltration was necessarily subject to time constraints and had to be designed accordingly. In addition, the time provided by the industry partner CWW in order to access their influent and facilities also had to be taken in account. Thus the pilot-scale unit (sampling site) was set-up remote from the University campus and laboratories and sampling was limited to once a week; although the frequency was increased in the later phase in order to ameliorate such constraints.

- To compare the treatment efficiency of the vermifiltration unit over time, with and without earthworms in the system, with two different soil types and at different hydraulic retention times and loading rates. Sinha and Valani (2011) have defined a vermifiltration unit as 'a logical extension of soil filtration'. Therefore, it is important to also assess the vermifiltration unit *without* earthworms, which is considered to be the 'control' for this study. Due to resource and space constrictions only one, rather than two parallel vermifiltration units, was constructed - which was tested as a control initially and, after a certain time, earthworms were introduced to the system as discussed in **Section 4.2.2**.

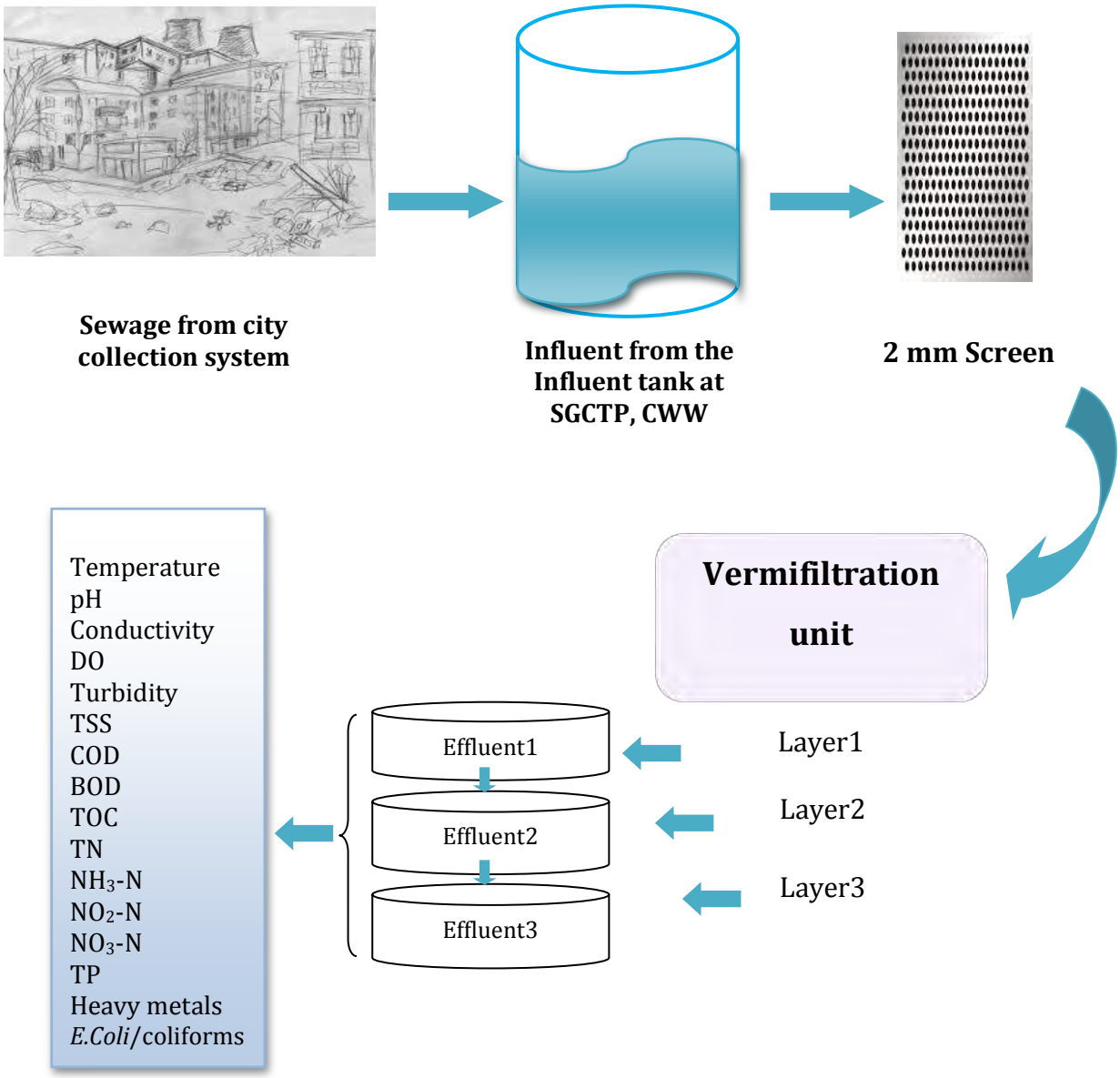


Figure 4.1 Wastewater treatment strategy for domestic sewage from communities in Melbourne.

4.2 Materials and Methods

4.2.1 The Vermifiltration unit set-up

A pilot-scale vermifiltration unit was designed and constructed on-site at City West Water's Sunshine Golf Course Treatment Plant (SGCTP), **Figure 4.2**. A schematic diagram and a photograph of the completed unit are shown in **Figure 4.3**. The main body of the unit was a commercially available Intermediate Bulk Container (IBC) consisting of a high density polyethylene blow moulded tank of dimensions 1200 mm x 1000 mm x 1100 mm, giving a 1000 L capacity. This was surrounded by a steel cage mounted on a wooden pallet, **Figure 4.3**. This tank was filled with distinct layers of soil, sand and gravel as described below. Sample collection pipes were fitted at the bottom of each layer as shown in order to allow systematic sampling and analysis of the effluent as it passes through each layer.

A submersible pump was used to supply previously screened and homogenised raw sewage from the influent tank of the SGCTP sewer mine to the unit. For uniform distribution of influent into the system, a 90 cm x 76 cm rectangular frame with six vertical 15 mm diameter PVC pipes with 8 mm diameter perforated holes was used, **Figure 4.2 (f)**. The tank was filled with the different filter materials as follows: bottom layer - 280 mm of a mixture of 20 mm and 14 mm gravel; middle layer - 280 mm cm of sand (mixture of premium washed sand and coarse sand), a 1 cm layer of cardboard and sawdust; top layer - 280 mm of garden soil. Each layer was separated by a thin perforated plastic net. The sample collection pipes were positioned at the bottom of the gravel, sand and soil layers. An overflow outlet and retention-level pipe were also included.

Figure 4.2 Vermifiltration unit set-up process on the premises of the SGCTP.

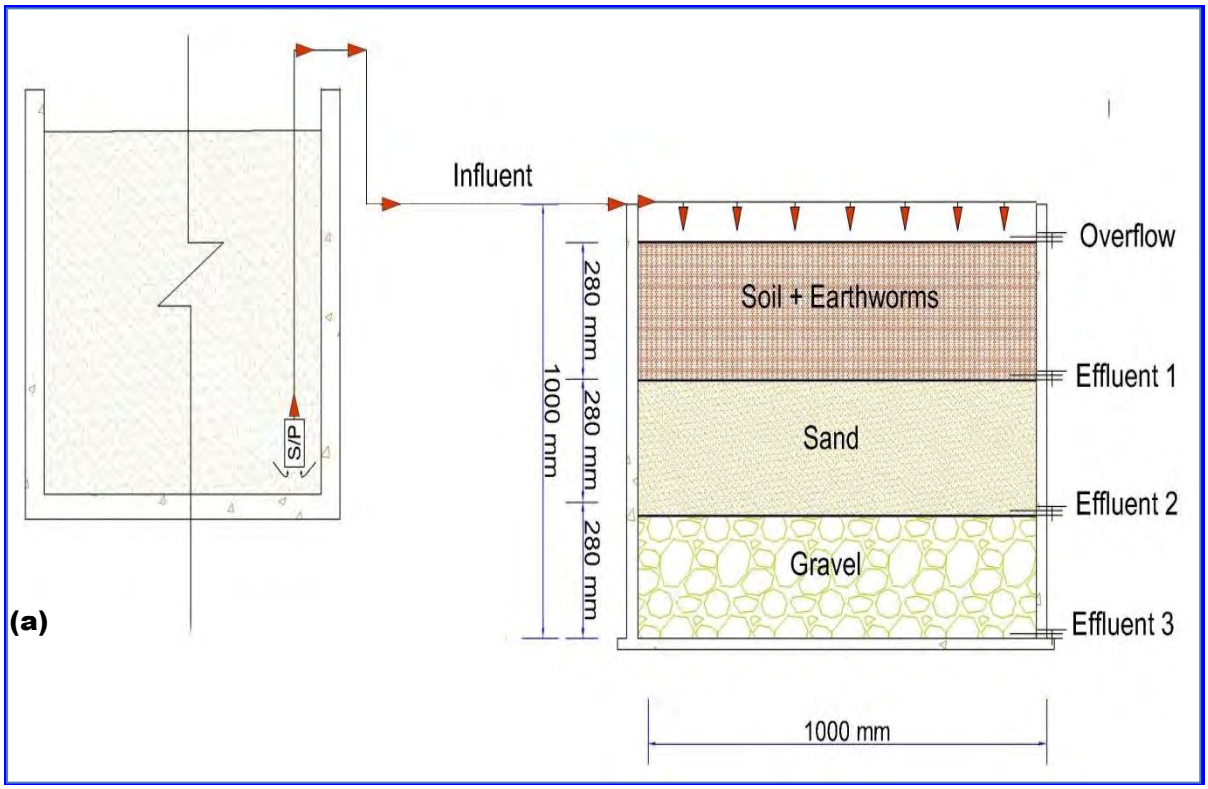


Figure 4.3 (a) Schematic diagram for the Vermifiltration unit (b) the Vermifiltration unit set-up at the SGCTP; a, b & c represents the outlets from Layer 1, Layer 2 & Layer 3 respectively. (S/P submersible pump)

4.2.2 Unit operation

At first, raw sewage from the influent tank of the SGCTP was fed into the treatment unit and left for 50 days for biofilm culturing. The unit was then operated in a steady state for two months with three different operating conditions: influent feeding every 1 week, every 12 hours and every 8 hours[65]. The influent feeding rate was controlled by using a submersible pump (TLE-370WS) and a digital mains timer (Powertech MS-61100). The system before introducing worms (without worms) was taken as the control. Thus the unit was operated for a period of two months prior to the introduction of worms. Subsequently, approximately 5.5 kg (> 10,000) of earthworms (*Eisenia andrei*)[66] were introduced into the first layer of the unit. After ten days of an acclimatization period, the influent was then fed into the unit. The system was then operated in steady state with the three different operating conditions (OCs) for Phase I and one operational condition for Phase II, **Table 4.3**.

Table 4.3 Operating conditions (OCs) for the Vermifiltration unit for Phase I and Phase II

	Phase I (Jan-Dec, 2013)	**Phase II (Jan-May, 2014)**
Filter medium		
Layer 1	Garden soil and worms	Garden soil + Pine bark saw dust + straw + sand + worms
Layer 2	Sand	Sand
Layer 3	Gravel	Gravel
Hydraulic retention time (HRT)		
OC1	12.96 hours	
OC2	19.52 hours	19.52 hours
OC3	182 hours	
Hydraulic loading rate (HLR)		
OC1	1.43 m/hour	
OC2	0.91 m/hour	0.91 m/hour
OC3	0.6 m/hour	
Earthworm population		
	>10,000	>10,000

The OCs was derived using the equations (4.1) and (4.2) as follows:

For OC1,

$$HRT = (\rho \times V_s) / Q_{wastewater} = (0.54 \times 0.3)/ 0.0125 = 12.96 \text{ hrs}$$

[65]Three different operational conditions were chosen as Influent was fed on 1week for 15 mins, 12 hours and 8 hours for 10 mins, so that comparison for the wastewater treatment efficiency could be done between wide variation and short variation of retention time difference. For all operational conditions, flow rate was 10L/min.

[66] The species of the earthworms was identified based on the supplier and verified based on the phenotype as described in Baker and Barrett 1995, and Lee 1985.

$$HLR = V_{wastewater} / (A \times t) = 0.600\ m^3 / (1\ m^2 \times 0.42\ hrs) = 1.43\ m/hrs$$

For OC2,

$$HRT = (\rho \times V_s) / Q_{wastewater} = (0.54 \times 0.3) / 0.0083 = 19.52\ hrs$$

$$HLR = V_{wastewater} / (A \times t) = 0.300\ m^3 / (1\ m^2 \times 0.33\ hrs) = 0.91\ m/hrs$$

For OC3,

$$HRT = (\rho \times V_s) / Q_{wastewater} = (0.54 \times 0.3) / 0.00089 = 182\ hrs$$

$$HLR = V_{wastewater} / (A \times t) = 0.200\ m^3 / (1\ m^2 \times 0.33\ hrs) = 0.6\ m/hrs$$

The porosity of soil (ρ) was assumed to be 0.54 (Salama et al. 2000; Shukla 2013) for HRT calculation and the flow rate ($Q_{wastewater}$) was calculated using the relation volume/time (10 L/min).

Phase I and Phase II differ only in terms of the combination of filter material used in the first layer of the vermifiltration unit, **Table 4.3**. In Phase I, only garden soil was used as a filter media to introduce earthworms, which encountered clogging problems more frequently. Therefore, a mixture of garden soil, pine bark saw dust, straw and sand (4:1:1:1 v/v) was used in Phase II. Otherwise, all other specifications were kept the same.

4.2.3 Sampling techniques and the preservation and handling of samples

The main objective of the water quality monitoring is to collect samples for analysis to provide data on the treatment process efficiency of the VF unit at the Sunshine Golf Course Treatment Plant (SGCTP).

After the retention of influent from the influent tank in the VF unit for 50 days, the Influent (IN) and Effluents (EFs) from three layers; outlets a, b & c in **Figure 4.3 (a)** & **(b)**, was sampled out once in January 2013. Then, the sampling program was carried out from February 2013 to December 2013 (Phase I) and from January 2014 to May 2014 (Phase II), **Figure 4.4**.

a) *Sampling points*: The influent sample was collected just before it enters the vermifiltration system, **Figure 4.3 (b)**. Effluent samples were collected from three different filter layers in the system i.e., soil, sand and gravel. In the vermifiltration unit, three different outlets have been installed at the bottom of each layer for the purpose of sampling as discussed in **Section 4.2.1**.

b) *Frequency and patterns of sampling*: From February 2013, wastewater samples from the influent point and the three effluent points were collected once every week from week 1 to week 6 before the introduction of worms into the system. This is considered as the 'control' and reported data as 'without worms'. After the introduction of worms into the system on week 6, the samples from the influent and the three effluent points were collected once every week from week 7 to week 34. Data collected after the introduction of worms in the system was reported as being 'with worms'. It was planned to carry out the sampling program every Tuesday of the week to eliminate diurnal and daily variations (AS/NZS 5667.10: 1998, Clause 5.2.3).

c) *Field measurement*: DO, temperature, pH and conductivity were measured on-site as soon as the sample was collected, as these analytes quickly degrade and the concentrations can significantly change during transportation and storage (EPA South Australia 2007, Clause 5.2).

d) *Sampling procedure*: The grab sample was taken to provide a 'snapshot' of the sample collected at a specific point and time. Samples were collected directly into the sampling container as far as possible. Whenever it was not possible to do so, an intermediate container was used. Moreover, sampling for different analytes, such as dissolved oxygen, nutrients, metals, organic and microbes, followed the protocol as mentioned in EPA South Australia, 2007, Clause 5.4.

In the vermifiltration unit, IN and EF sampling points have been fitted with taps. Hence, a protocol for microbiological sampling from taps was followed (AS 2031- 2012, Clause 4.4). Thus all the sampling outlets were disinfected with sodium hypochlorite solution before taking samples. Specifically, the outside and inside (as much possible) of the outlet was swabbed and sprayed with disinfectant and left for 2 to 3 minutes. Then water

(influent and effluent) from the outlet was run for 3 minutes - providing sufficient time to remove all the traces of disinfectant before taking a further sample.

e) *Subsequent treatment and preservation of samples*: The capped sample containers (bottles) were placed in a snap-lock bag and transported in an "esky" packed with ice. Sample containers suitable for the collection and storage of the different analytes, as indicated in standard methods (APHA 1998), were used. Different preservation techniques – refrigeration, freezing and chemical addition were adopted (AS/NZS 5667.1:1998; APHA 1998).

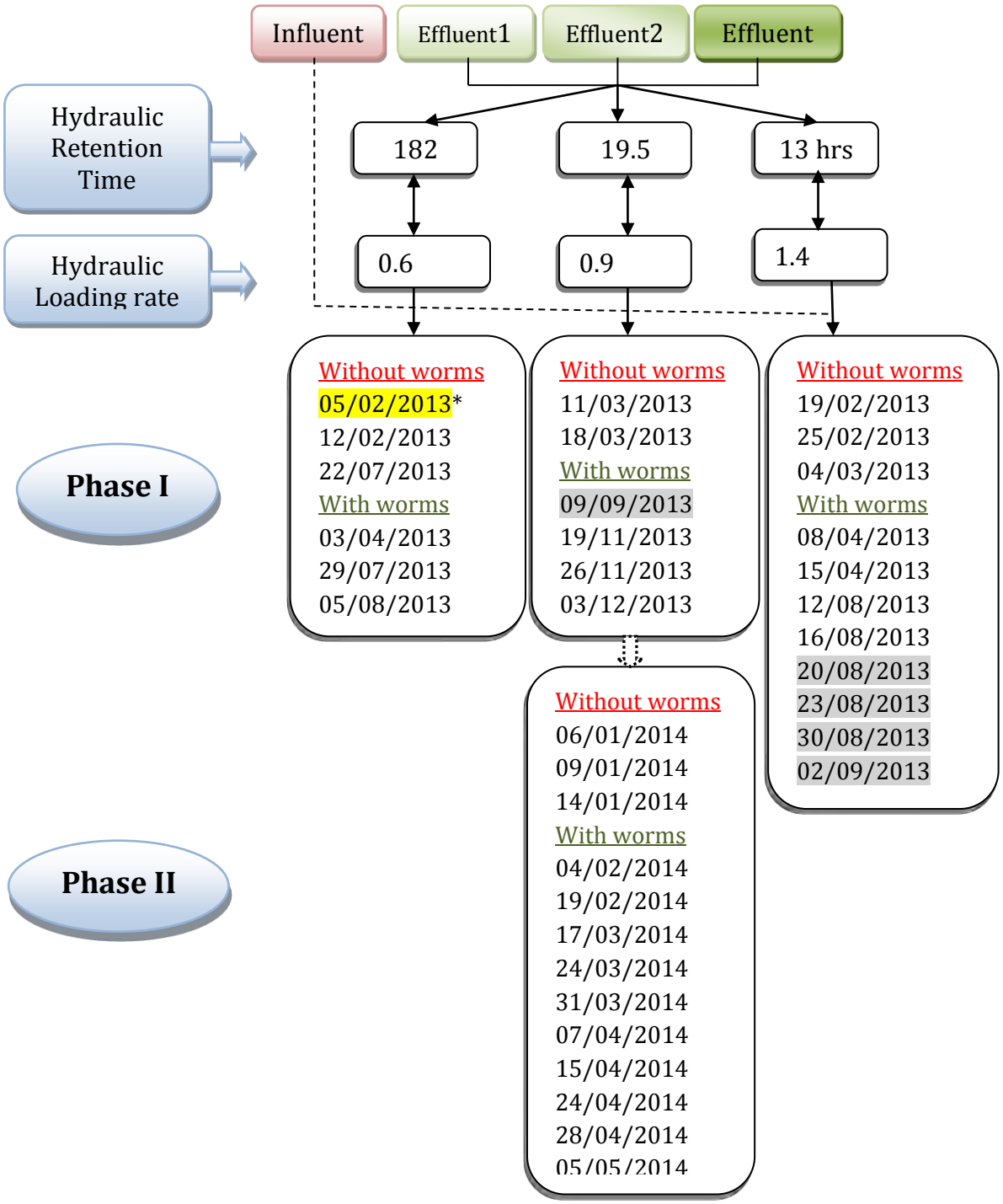


Figure 4.4 Influent and effluent sampling protocol over the experimental period for Phases I & II. Data sampled on 05/02/2013* is the first sampling set, which is analysed after retaining the Influent in the VF for 50 days. Data from 20/08/2013 to 09/09/2013, highlighted in grey, are data sampled at the time when the CWW mining project was under maintenance. Thus, the VF did not receive fresh influent sample at this time period. This dataset is included in the overall dataset with a view to investigate the treatment efficiency of the VF for influent with variation in pollution loading.

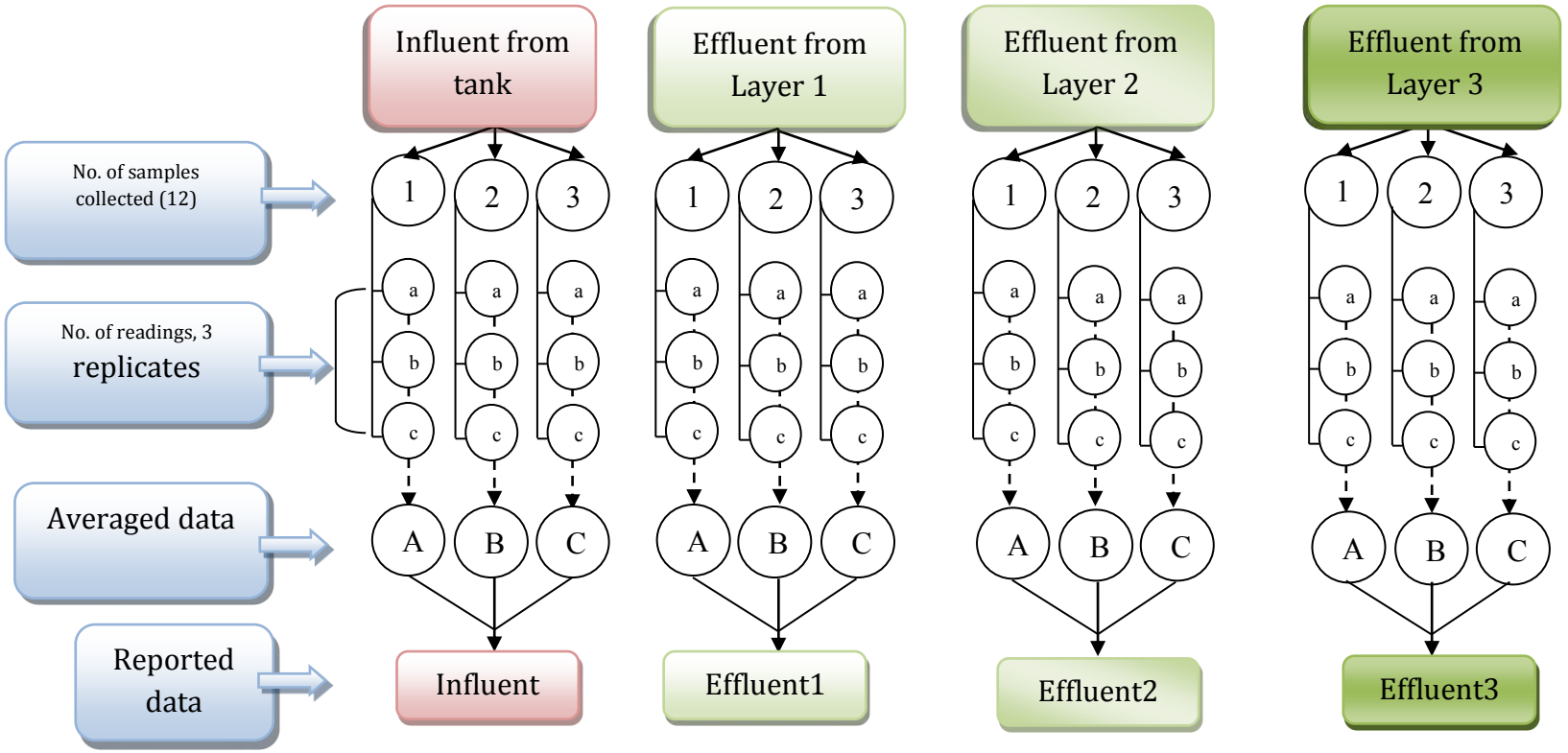


Figure 4.5 Sampling frequency and data presentation. Where, 1, 2 and 3 represents three samples (triplicate) collected from each sampling point, they are influent from the tank and effluent from first, second and third layers of Vermifiltration unit. a, b and c refers to three readings for each sample. A, B and C are averaged data for three readings of three samples for each sampling point, presented now onwards as Influent (IN), Effluent 1 (EF1), Effluent 2 (EF2) and Effluent 3 (EF3).

Case Study: Sample collection and analysis

Figure 1 (a) The SGCTP sewer mining site (b) the vermifiltration unit beside the sand filtration tank and (c) on-site measurement of some parameters. Photos taken by Anu Joshi.

To provide an insight on the sample collection and analysis procedure, a representative case study is presented. On a given sample collection day, e.g. April 8, 2013, the field trip started from the Victoria University, Werribee Campus, 240 Hoppers Lane, Werribee to the the Sunshine Golf Club, 475 Mt. Derrimut Rd, Derrimut (the site of the CWW SGCTP) - a drive of approximately 19 km. Since the site of the pilot unit is in a remote location and is under high security, the researcher (AJ) was allowed to go there only accompained by the supervisor/associate supervisor or one of the University's technical staff. At the SGCTP, a timer was programmed in such a way to supply influent to the vermifiltration unit at 11 am. Therefore, it was required to reach there by this time, so that the effluent samples could be collected before the influent supply commenced into the unit. The supplies taken included 500 mL brown plastic bottles, sterile falcon tubes, beakers, a milliQ water bottle along with an "esky" with ice and a multipurpose meter. As shown in **Figure 4.5**, three replicate samples from each effluent point (Effluents 1, 2 & 3) and from the influent point were collected, **Figure 4.3**, to give a total 12 individual samples. On alternate occasions, an identical sampling regime was also carried out for microbial analysis, where the samples were collected in sterile falcon tubes. Some of the collected samples were monitored at the on-site SCGTP facility for temperature, pH, conductivity and DO. The samples were then stored in an esky with ice and transported to the University for further investigation/analysis. At the University laboratory, approximately 50 mL of each sample was acidified with conc. H_2SO_4 to below pH 2 and stored in a freezer (< -20 °C) for the long term. Microbial analysis was carried out on the same day as sampling and a complete analysis was done within 24 hours.

4.2.4 Analytical methods

In the pilot-scale unit, IN and EF samples were collected regularly once a week. The IN sample was collected from a point just before the influent inlet. The EF samples were collected from three different sample points located at the bottom of three different layers of the treatment unit. Thus the collected water samples were analyzed for the parameters listed in **Table 4.4**, based in standard methods (APHA 1998) and/or Hach methods. From each sampling point, three different samples were collected and analyzed in triplicate, **Figure 4.3**. Finally, the average for each sampling point was used for data analysis. Moreover, a microbial examination was carried out for *E.Coli*/coliform count using a 3M petrifilm.

Table 4.4 Test parameters and methods used for analysis.

Parameters	Method (APHA 1998)	Equipment/Method
Temperature (°C)	2550	Hach HQ40d
pH	2310/2320	Hach HQ40d
Conductivity (mS/cm)	2510	Hach HQ40d
Turbidity (NTU)	2130	Hach Turbidimeter
TSS (mg/L)	2540 D	Hach DR890 / 8006
DO (mg/L)	4500-O	Hach LDO Probe/ 10360
COD (mg/L)	5220D	Hach DR5000 / 8000
NH_3-N (mg/L)	4500-NH_3	Hach DR5000/ 10031
NO_2-N (mg/L)	4500-NO_2	Hach DR890 / 8153
NO_3-N (mg/L)	4500-NO_3	Hach DR890 / 8039
TN (mg/L)	4500-N	Shimazdu TOC/TN Analyser
TOC (mg/L)	5310	Shimazdu TOC/TN Analyser
TP (mg/L)	4500 -P	Hach DR5000 / 8190
Heavy metals (mg/L)	3125	Shimazdu ICP OES
Microbial examination	9000	Biolog/ 3M petriplate

The % change in the IN and the EFs, for these parameters, was used for the assessment of the treatment efficiency, and calculated using equation (4.3):

$$\% \text{ Change} = ((IN - EF)/IN) \times 100 \quad (4.3)$$

4.2.4.1 Physicochemical measurements

The pH, conductivity and Dissolved Oxygen (DO) of the IN and the EFs were determined using a multipurpose Hach HQ40d portable meter with a gel-filled pH electrode, conductivity probe and a DO probe, respectively. The temperature and pH of the sample was recorded with the same equipment. The equipment was calibrated prior to measurement using the method outlined in the manufacturer's manual. These parameters were measured directly by immersing the electrode into the respective samples.

The turbidity was measured using a Hach Turbidimeter. The instrument was first calibrated with standard turbidity solutions of a range 0 – 1000 NTU based on the manufacturer's manual. Then, the vial was filled with a 10 mL sample, placed on vortex and measured by placing the vial in the turbidimeter.

The total Suspended Solids (TSS) was measured with a DR 890 spectrophotometer according to the procedure described in Hach method 8006. The vial was filled with a 25 mL sample, placed on a vortex and measured spectrophotometrically.

4.2.4.2 Organic matter

4.2.4.2.1 Biochemical Oxygen Demand

BOD_5 was determined by a standard method (APHA 1998) or the HACH method 8043 (dilution method) using a Hach HQ40d multipurpose meter connected to a DO probe. A 300 mL BOD_5 bottle with a rubber stopper was used for BOD_5 determination. For IN, 2, 3, 4 and 5 mL, and for EF, 2, 3, 6 and 9 mL, sample volumes were chosen based on the estimated BOD_5 (mg/L), **Tables 4.5** & **4.6**.

As shown in **Table 4.6**, IN, EF1, EF2 and EF3 samples were added to the designated BOD bottles. Each bottle was filled with dilution water[67] just below the lip. The bottles were fitted tightly with stoppers and inverted many times to mix the solutions. The initial DO concentration was determined from each bottle. Then, the bottles were fitted with rubber stoppers, doused with dilution water and covered with plastic caps. At the same time, two BOD bottles were filled with dilution water as a blank. All these bottles were incubated at 20 ± 1°C for five days. The final DO was measured in each bottle after five days.

Table 4.5 Minimum sample volume required for BOD_5 determination.

Sample type	Estimated BOD_5 (mg/L)	Minimum sample volume (mL)
Raw & settled sewage	300	2
	200	3
	150	4
	120	5
	100	6
	75	8
	60	10

(Source: Hach Method 8043)

Table 4.6 Sample volume added in each BOD bottle for BOD_5 determination.

Sample type	Influent				Effluent1				Effluent2				Effluent3			
Bottle No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Volume of sample (mL)	2	3	4	5	2	3	6	9	2	3	6	9	2	3	6	9

BOD_5 was calculated using the equation,

$$BOD_5 = ((B_2 - D_2) - (B_1 - D_1) / P) \quad - (B_1 - B_2) \qquad (4.4)$$

[67] Preparation of dilution water: 3 L of the MilliQ water was filled in 5 L capacity plastic jug, then shaken vigorously so that the water became saturated with air (oxygen) and this was stored at 20 ± 1°C for two days. The contents of a Hach BOD_5 nutrient buffer pillow (Catalogue No.1486166) were then added to the stored MilliQ water and shaken vigorously for at least one minute to saturate the water with air, before use.

Where, D_1 = initial dissolved oxygen of sample (mg/L)

D_2 = final dissolved oxygen of sample (mg/L)

B_1 = initial dissolved oxygen of blank (mg/L)

B_2 = final dissolved oxygen of blank (mg/L)

P = decimal volumetric fraction of sample

Accuracy check for BOD_5

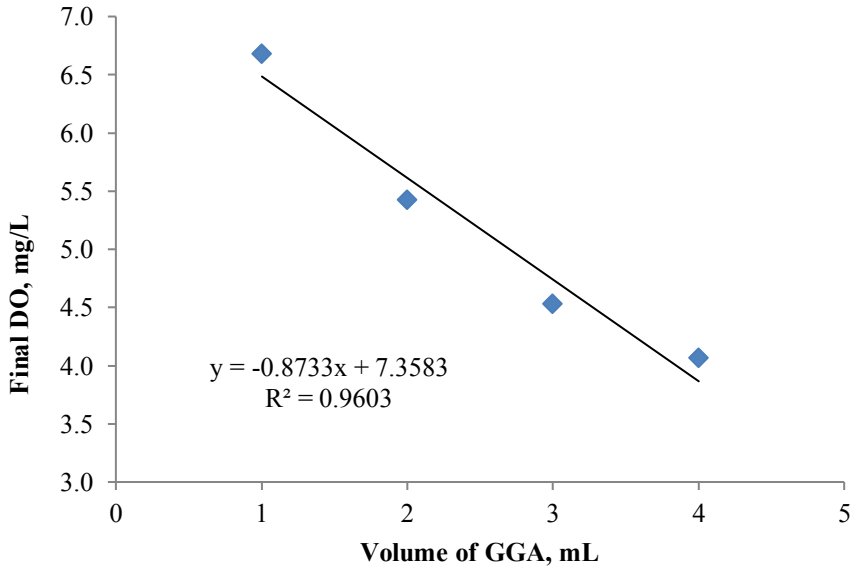


Figure 4.6 Accuracy check for BOD_5.

4.2.4.2.2 Chemical Oxygen Demand

COD was determined via a standard method (APHA, 1998) or the HACH method 8000 (reactor digestion method) using a Hach DR5000 colorimeter. 2 mL of ten-fold diluted wastewater sample was added to a COD digestion reagent vial (Catalog No. 21258-25). The vial was heated with a strong oxidizing agent, potassium dichromate $K_2Cr_2O_7$, for two hours at 150 °C in a DRB200 reactor. The COD reagent also contains silver and mercury ions. Silver is a catalyst and mercury is used to complex chloride interferences. Oxidizable organic compounds react, reducing the dichromate ion ($Cr_2O_7^{2-}$) to the green chromic ion (Cr^{3+}). The low range COD digestion reagent vial i.e. the 3–150 mg/L colorimetric method, was used, so the amount of Cr^{6+} remaining was determined. The test results for the 3 to 150 mg/L range were measured at 420 nm. Here, the mg/L COD results are defined as mg of O_2 consumed per litre of sample, see **Appendix 4.1**.

4.2.4.2.3 Total Organic Carbon

TOC was analyzed using a Shimazdu TOC Analyzer, **Figure 1** in **Appendix 4.1**, by the method described in standard methods (APHA, 1998). The sample was diluted ten-fold prior to measurement.

Preparation of the standard solution for TOC analysis: Potassium hydrogen phthalate was dried by heating in an incubator at 105 - 110 ℃ for 1 hr and allowed to cool in a desiccator. 2.125 g of this was dissolved in 1 L of milliQ water to prepare a 1000 ppm solution. 100 mL of a 100 ppm solution was prepared from this by a tenfold dilution. The 100 ppm standard solution was used to measure the accuracy of the equipment.

4.2.4.3 Nutrients

4.2.4.3.1 Total Nitrogen

TN was analyzed using a Shimazdu TOC Analyser coupled with nitrogen analyser, **Figure 1** in **Appendix 4.1**, by the method described in standard methods (APHA 1998). The sample was diluted ten-fold prior to measurement.

Preparation of the standard solution for TN analysis: Potassium nitrate (KNO_3) was dried by heating in an incubator at 110 °C for 1 hr and allowed to cool in a desiccator. 7.219 gm of this was dissolved in 1 L of milliQ water to prepare a 1000 ppm solution. 100 mL of a 50 ppm solution was prepared from this by a twenty-fold dilution. The 50 ppm standard solution was used to measure the accuracy of the equipment.

4.2.4.3.2 Ammonia nitrogen

NH_3-N was determined via standard method 4500 - NH_3 (APHA, 1998) or the HACH method 10031 (salicylate method), using a Hach DR5000 colorimeter. 0.1 mL of a ten-fold diluted sample was added to one AmVer™ diluent reagent testNtube for higher range ammonia nitrogen (Catalog No. 26069-45). Then, the content of one ammonia salicylate and

one ammonia cyanurate reagent powder pillow was added to the tube, mixed, and left to stand for 20 minutes of reaction period before measurement. A blank reference was prepared by replacing the sample with ammonia-free deionised water. Here, ammonia compounds combine with chlorine to form monochloramine, which reacts with salicylate to form 5-aminosalicylate. In the presence of a sodium nitroprusside catalyst, the 5-aminosalicylate oxidizes to form a blue colored compound. The blue color is hindered by the yellow color from the excess reagent present to give a green-colored solution. The solutions were measured at 655 nm.

Accuracy check for NH_3-N:

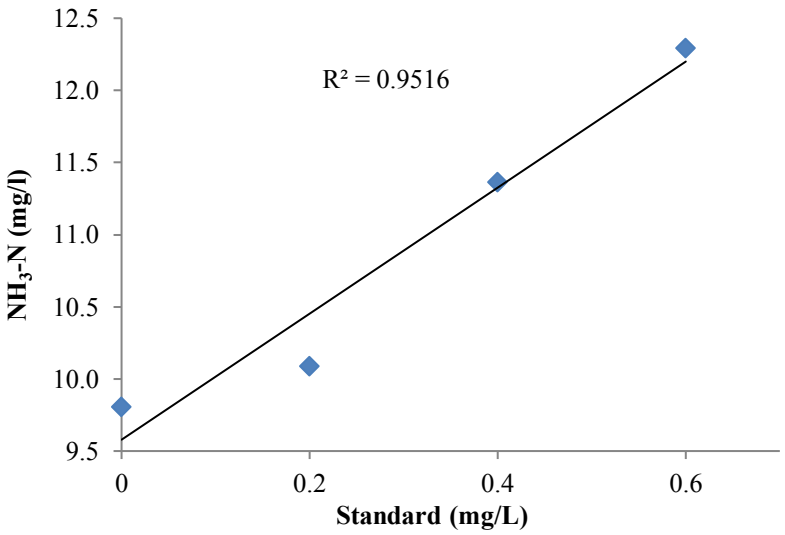


Figure 4.7 Accuracy check for NH_3-N.

Three sample spikes were prepared by adding 0.2, 0.4 and 0.6 mL of ammonia standard solution to the 25 mL of three individual samples, which had already been tested for NH_3-N concentration. Then, the spiked samples were measured as mentioned above and a graph was drawn to view the best fit line through the standard additions data points, **Figure 4.7**.

4.2.4.3.3 Nitrite Nitrogen

NO_2-N was determined via a standard method 4500 - NO_2 (APHA, 1998) or the HACH method 8153 (ferrous sulfate method), using a Hach DR890 colorimeter. A sample cell was filled with 10 mL of the pre-filtered sample and the content of one NitriVer 2 Nitrite reagent

powder pillow (Catalog No. 21075-69) was added to it. The content of the sample cell was well mixed and allowed for 10 minutes reaction time before measurement. A blank reference was also prepared by filling a 10 mL sample by itself. Here, acidified ferrous sulphate reduces nitrite to nitrous oxide and the ferrous ions react with the nitrous oxide to give a greenish-brown complex.

Accuracy check for NO_2-N:

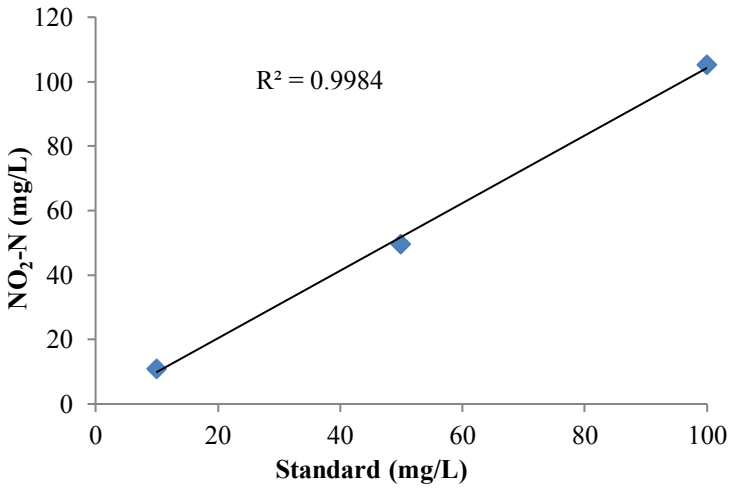


Figure 4.8 Accuracy check for NO_2-N.

A standard solution of 100 mg/L was prepared by dissolving 0.150 gm of fresh sodium nitrite ($NaNO_2$) in 1 L of milliQ water. The 100 mg/L standard solution was diluted to prepare 50 mg/L and 10 mg/L standard solutions. These three standards were measured as mentioned above and a graph was drawn to view the accuracy, **Figure 4.8**.

4.2.4.3.4 Nitrate Nitrogen

The Nitrate nitrogen (NO_3-N) was determined via a standard method (APHA, 1998) or a HACH method 8039 (cadmium reduction method) using a Hach DR890 colorimeter. One NitraVer 5 Nitrate reagent powder pillow (Catalog No. 21061-69) was added to the sample cell filled with 10 mL of the filtered sample, shaken vigorously and left to stand for 5 minutes, to complete reaction time. A blank reference was prepared by filling the sample cell with 10 mL of the sample by itself. Here, cadmium metal reduces nitrates present in the

sample to nitrite. Then, the nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt, which couples to gentisic acid to form an amber-coloured product.

Accuracy check for NO_3-N:

Three sample spikes were prepared by adding 0.1, 0.2 and 0.3 mL of nitrate nitrogen standard solution to the 25 mL of three individual samples. Then, sample cells were filled with 10 mL of each spiked samples and were measured as mentioned above. A graph was drawn to view the best fit line through the standard additions data points, **Figure 4.9**.

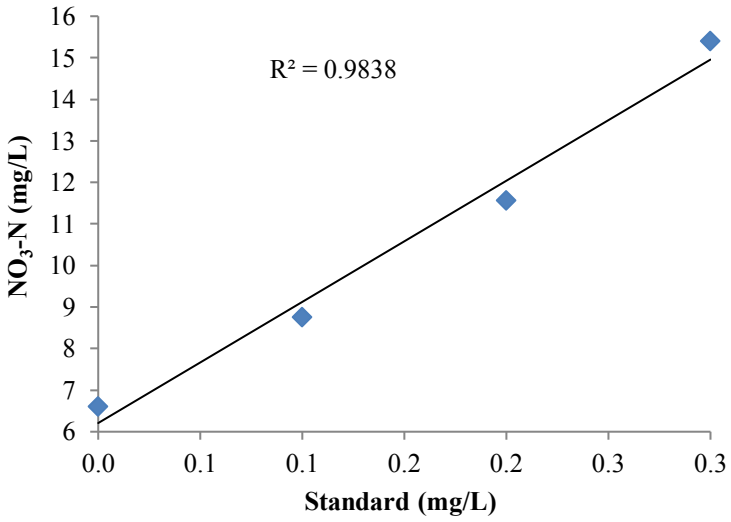


Figure 4.9 Accuracy check for NO_3-N.

4.2.4.3.5 Total Phosphorous

The Total phosphorous (TP) was determined via a standard method 4500 - P (APHA, 1998) or the HACH method 8190 (acid persulfate digestion method) using a Hach DR5000 colorimeter. 5 mL of the ten-fold diluted sample was added to one Total and Acid Hydrolyzable test vial. Then, the content of one potassium persulfate powder pillow (Catalog No. 20847-66) was added to the vial, mixed well and heated for 30 minutes in DRB 200 at 150 °C. When the vials cooled down, 2 mL of 1.54 N sodium hydroxide standard solution was added to each vial, mixed and measured in the colorimeter as a blank reference. Then, the content of one PhosVer 3 powder pillow (Catalog No. 21060-46) was added to the vial

and measured for the TP after 2 minutes reaction time. Here, the pre-treatment of the sample with acid and heat provides the favourable conditions for hydrolysis of the condensed inorganic forms of phosphates (meta-, pyro-, or other polyphosphates). Before the analysis of the samples, the phosphates present in the sample, in the form of organic and condensed inorganic forms, should be converted to reactive orthophosphate. Organic phosphates are converted to orthophosphates by heating with acid and persulfate. Then, orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex, which is reduced by ascorbic acid to give an intense molybdenum blue colour, see **Figure 4** in **Appendix 4.1**. The test results were measured at 880 nm.

Accuracy check for TP

Three spiked samples were prepared by adding 0.1, 0.2 and 0.3 mL of phosphate standard solution to 25 mL of three individual samples, respectively. The, sample cells were filled with 5 mL of each spiked sample and were measured colorimetrically as mentioned above. A standard addition graph was prepared, **Figure 4.10**.

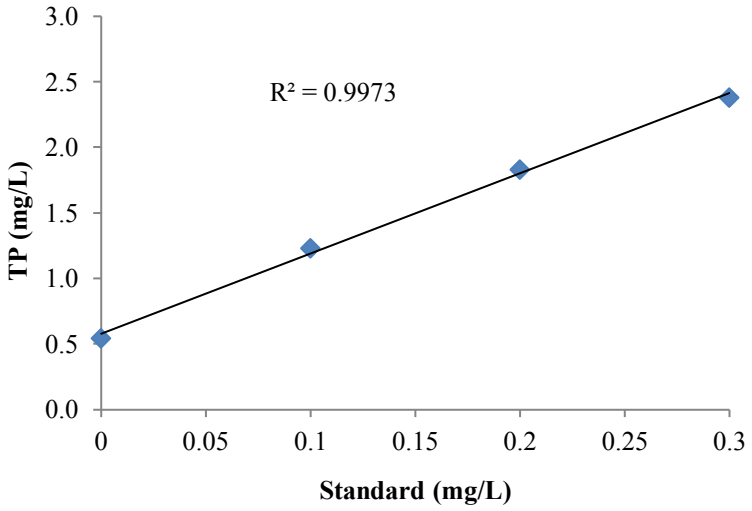


Figure 4.10 Accuracy check for TP.

4.2.4.4 Heavy metal analysis

The concentration of the heavy metals in IN and EFs samples were determined using APHA standard method 3125 and Inductively Coupled Plasma Spectrophotometer (Shimazdu ICP OES), (see **Appendix 4.1**). 10 mL of each sample was digested with 1 mL 1:1 HNO_3 in a 15 mL falcon tube for 2 hours by placing it in water bath at 99 °C. The digested samples were

centrifuged at 4000 rpm for 30 minutes at 4 °C and the supernatant was filtered through a 0.45 µm filter disc. For calibration, six different standards of each heavy metal to be investigated were prepared and calibration curves obtained by running standards using ICP OES. Thus obtained calibration curves are presented in **Appendix 4.2**. Then, the samples were run to obtain the concentration of each element.

4.2.4.5 Microbial analysis

Isolation of bacteria: Microbes isolated from the IN and EFs were collected in sterile vials, following the protocol described in **Section 4.2.3**. The IN and EFs samples were diluted 10 times using 0.1 % (w/v) sterile peptone water (Sigma-Aldrich, Australia) and cultured (0.1 mL aliquot) on nutrient agar, using the spread plate technique (APHA 1998). The plates were incubated at 35 ± 2 °C for 24 hours. Bacteria with distinct colony morphology were identified, isolated and sub-cultured in the nutrient agar until a pure culture was obtained.

Identification of bacteria: Identification of isolated bacteria was done using a Biolog GEN III microplate™ system, which analyzes microorganisms in 96 phenotypic tests[68]. The system utilizes redox tetrazolium dyes to detect the respiration of carbon sources (Garland and Mills 1991). Biolog GENIII 96 well plates were inoculated with 150.0 µL bacterial suspensions, which were prepared using a fraction of a colony in a special 'gelling' inoculating fluid, IFA or IFB, and adjusted to the appropriate cell density, according to the manufacturer's protocol. The inoculated plates were incubated at 30 ± 2 °C or 25 ± 2 °C for 18 - 24 hours (for slow growing microorganisms). Development of colour reactions was observed at every 18 hours, 22 hours and 24 hours intervals, using an automated microplate reader at 590 nm until a similarity index (SIM) of ≥ 0.500 was obtained. The Biolog's Microbial Identification System's software (OmniLog® Data collection) was used for species identification, which uses the reference metabolic profiles available in the MicroLog GEN III database (release 3.01A) (Holmes et al. 1994; Hashimoto et al. 2013; Vithanage et al. 2014).

Identification of E.Coli/ coliforms: The 3M™ petrifilm was inoculated with 1 mL of the IN and EFs samples that were diluted 10 times using 0.1 % (w/v) sterile peptone water (Sigma-Aldrich, Australia). It was then incubated at 25 ± 2 °C for 24 hours. The red and blue

[68] http://biolog.com/pdf/milit/00P%20185rA%20GEN%20III%20MicroPlate%20IFU%20Mar2008.pdf 19/06/2014

colonies associated with gas bubbles are counted and recorded as a coliform count, blue colonies are *E.Coli*[69].

Here, the pathogen removal efficiency (K) is reported as the log removal value (LRV) and is calculated using the equation (5)[70] (Arora et al. 2014a) as follows:

$$\text{Log removal value (K)} = \log10(C_{in}/C_{out}) \quad (4.5)$$

Where, C_{in} = the pathogen concentration in Influent and Effluent

C_{out} = the pathogen concentration in Effluent

4.2.4.6 Biofilm extraction and Scanning Electron Microscopy (SEM) analysis

The filter media from two different layers were collected from two different sampling points for each layer at different heights. The sand samples (sand 1 & sand 2) were collected from the front left corner and sand samples (3 & 4) from the back right corner at 50 cm and 44 cm from the bottom, respectively. Similarly, soil samples (1 & 2) were collected from the front left corner and samples (soil 3 & soil 4) from the back right corner at 75 cm and 70 cm from the bottom, respectively. Collected samples were stored below 4°C till further analysis.

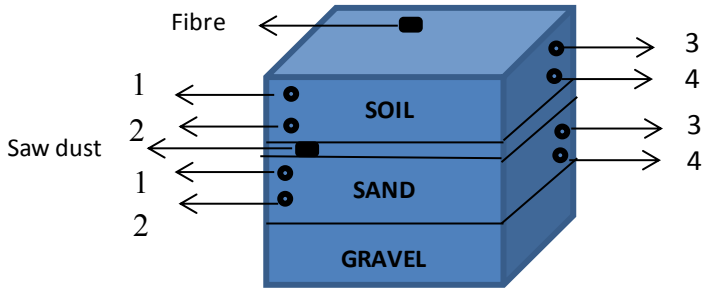


Figure 4.11 Sampling points for biofilm analysis.

All the sand samples were rinsed with sterile water several times and the biofilm in the water was collected by centrifuging for 30 minutes at 8000 rpm and 4 °C. Settled biofilm samples were freeze-dried and filtered through 0.15 mm sieves, and were used for further analysis. Moreover, all the collected samples were freeze-dried and used for further analysis.

[69] http://solutions.3m.com/wps/portal/3M/en_US/Microbiology/FoodSafety/product-information/product-catalog/?PC_Z7_RJH9U523003DC023S7P92O3O87000000_nid=C0WJ62882Vbe29BDXSBJ7Fgl 03/05/2013

[70] http://www.filtsep.com/view/829/pathogen-removal-from-water-technologies-and-techniques/ 30/03/2015

The humic acid-like (HAL) fractions were extracted from the biofilms collected from the sand and gravel. Samples for fluorescence analysis were prepared as follows: 1 gm of sample was 0.5-mm sieved and air dried before being extracted with 10 mL 0.1 M sodium diphosphate ($Na_4P_2O_7$) and 10 mL 0.5 mM sodium hydroxide (NaOH). The mixture was agitated on a rotary shaking platform in a capped plastic bottle for 24 hours at room temperature (25 ºC). The supernatant solution was separated from the residue by centrifugation at 9600 ×g for 30 minutes. The combined alkaline supernatant was acidified to pH 1 with 6 M HCl and allowed to stand for 24 hours at 4 ºC and centrifuged at 10,000 × g for 30 minutes. The extracted HAL fraction was purified in a 5 mL volume of 0.3 M KCl and 5 mL of 0.2 M KOH. Finally, it was washed several times with water until the last rinse yielded a negative chloride test with silver nitrate. The residues were freeze-dried and stored in plastic vials that were placed in a desiccators containing phosphorus pentoxide (P_2O_5) (Li et al. 2011). For the Scanning Electron Microscope (SEM) micrographs, the freeze-dried samples were coated with 10 nm gold and the SEM image was taken using a Scanning Electron Microscope.

4.2.4.7 The organization and management of the data

Figure 4.12 depicts the overall organization and management of the data, as sampled according to **Figure 4.4**. Thus, the data for Phase I consists of 22 sample sets consisting of mean values from triplicate samples for all relevant parameters and the data for Phase II consists of 13 sample sets consisting of mean values from triplicate samples for all relevant parameters. These datasets have been subdivided into 6 subsets from Phase I (shaded pink) and 2 subsets from Phase II (shaded green).

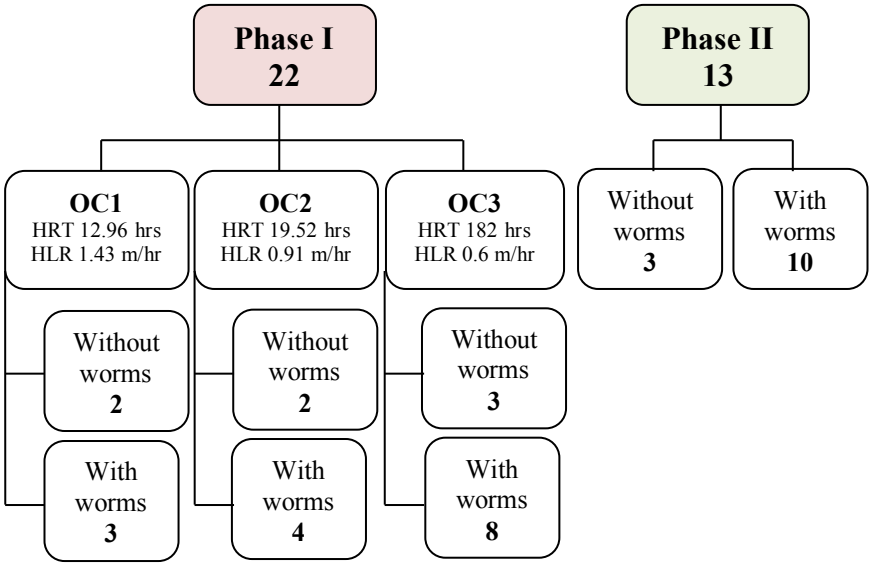


Figure 4.12 The organization and management of the data. This schematic depicts the datasets and subsets that have been selected for statistical analyses and subsequently employed for the testing of the hypotheses. Note: the number of sample sets in each subset is indicated by the bolded number.

4.2.4.8 The statistical strategy

In relation to the datasets depicted in **Figure 4.12**, a statistical strategy has been designed and implemented and is outlined in **Figure 4.13**, below.

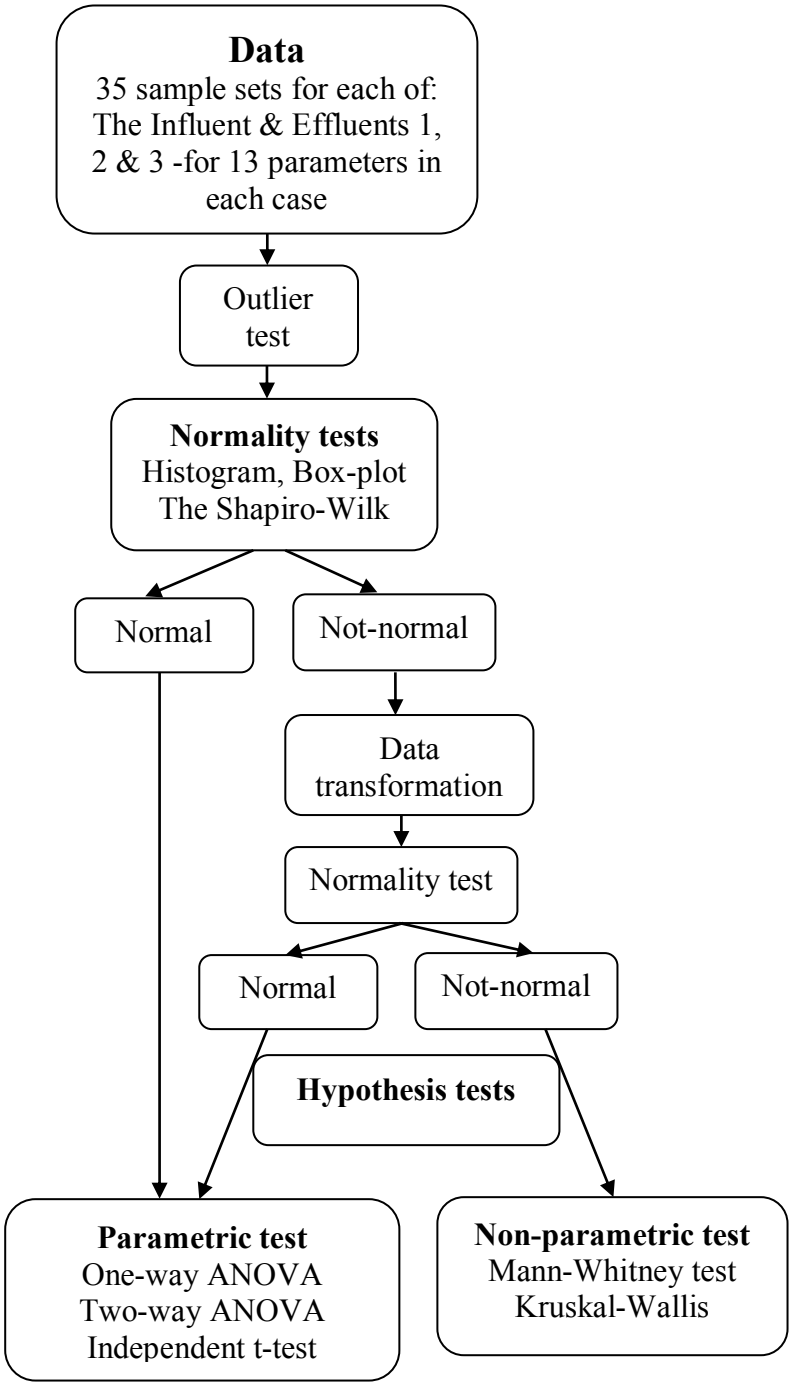


Figure 4.13 The statistical strategy adopted for the analysis of the data. This schematic relates to the 2 datasets (includes 8 subsets), Figure 4.12, that have been selected for statistical analyses and subsequently employed for the hypothesis testing.

4.2.4.8.1 Testing for outliers

The datasets, both normally and non-normally distributed, were tested for outliers. Outliers were determined using the box-plot and the Outlier Labelling Technique (Hoaglin et al. 1986; Hoaglin and Iglewicz 1987). A specific representative example of the application of this test is shown in **Figure 4.14**. Other representative examples of this test are shown in **Figure 4.15**.

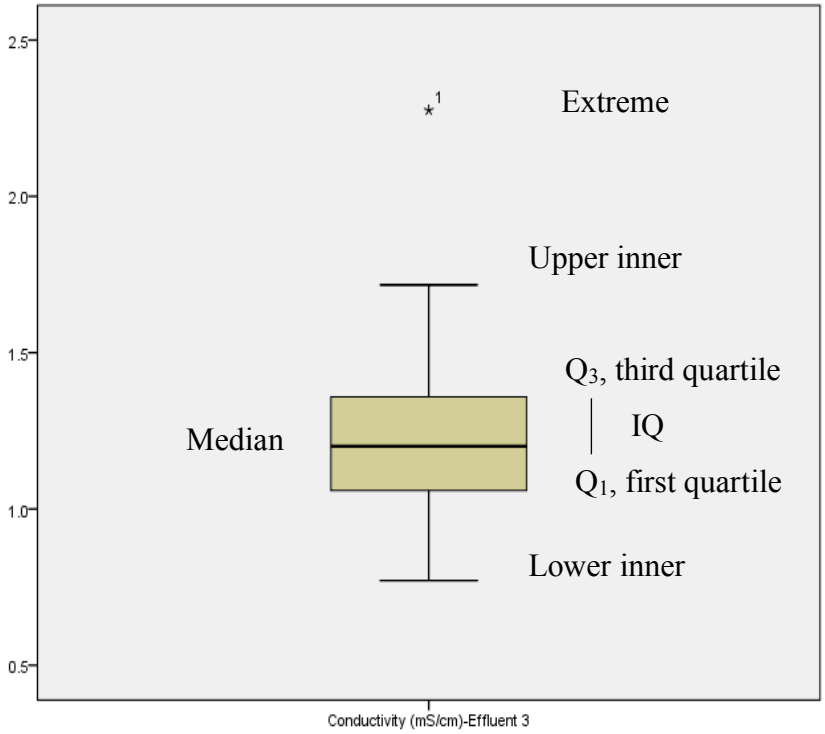


Figure 4.14 The Outlier Labelling Technique box plot showing the distribution of data for the conductivity of Effluent 3 – as a representative example. The central rectangle box is a span from the first quartile, Q_1, to the third quartile, Q_3, which is the Inter Quartile Range (IQR), (Q_3 - Q_1). A line in the rectangle box is median and the whiskers above and below the box represents the upper inner fence and the lower inner fence respectively. An outlier, which is assumed not to be a part of normal observation, is indicated by asterisk sign.

The first quartile (Q_1) and the third quartile (Q_3) values were used to calculate the inner fence and the probable outliers were obtained from SPSS version 20. The relation, Q3 + 2.2* (Q_3 - Q_1) was used to get the upper inner fence and Q_1 - 2.2* (Q_3 - Q_1) was used to get the lower inner fence. The values beyond the inner fences were considered as extreme outliers, which is usually 3 times the IQR (Carver and Nash 2011). The box-plots obtained for outlier tests are presented in **Appendix 4.3** & **4.4 (CD)**.

4.2.4.8.2 Testing for normality

As depicted in **Figure 4.12** and **4.13**, the sample sets of the datasets and subsets were tested for normality using the box-plot test and Shapiro-Wilk (S-W) test, within SPSS, version 20 (Greasley 2008). The p-value < 0.05 was considered to determine the normality. The probability Q-Q plots and histogram distribution curves obtained from the S-W tests for all datasets are presented in **Appendix 4.4 (CD)**. The datasets satisfy the normality test or not, is provided in a Table, **Appendix 4.5**. Four such representative Q-Q plots and the histograms are presented in **Figure 4.15**. These represent datasets that are normally distributed, with and without outliers, and datasets that are non-normally distributed, with and without outliers.

4.2.4.8.3 Transformation of non-normal data

In this study, some extreme outliers were retained in the dataset when this did not affect the normality, since it was decided that in these cases the outliers might provide important information. However, there is also the possibility that such outliers might be the result of either sampling error, experimental error, instrumental error or changing conditions associated with the supply of the sewage. For example, as referred to in **Figure 4.4**, the SGCTP sewer mining plant was undergoing maintenance during the sample collection period 20/08/2013 to 09/09/2013. The data for this period was found to be affected only with respect to the DO parameter. The datasets that were not normally distributed, were tested for lognormal distribution, and where these were used in statistical comparisons (hypothesis testing), all other datasets were also transformed (Fay and Gerow 2012).

4.2.4.8.4 Parametric versus non-parametric hypothesis testing

The normally distributed datasets were compared using the following parametric tests. A Two-way ANOVA was employed to assess the interaction between worms and the HRT. The independent t-test was employed to assess the effect of the 3 different layers (EFs 1, 2 & 3) in the treatment of the IN, to compare the effect of worms in the VF and the effect of the different first soil layers in the Phase I (soil type 1) and Phase II (soil type 2). A One-way ANOVA was used to assess the effect of the HRT in the treatment efficiency, which was then further analysed with the Tukey's test. For those datasets which were not normally

distributed, nonparametric tests were run separately for independent samples (Mann-Whitney test and Kruskal-Wallis test).

4.2.4.9 Influent characteristics

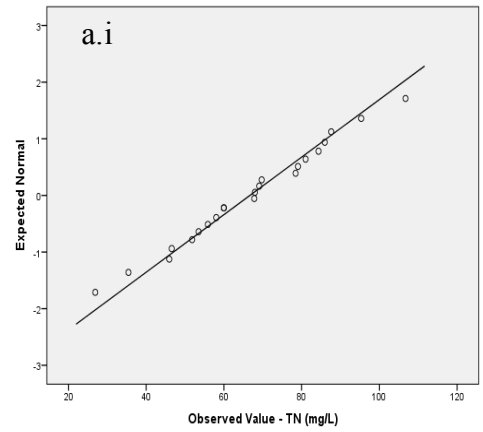
The sewage wastewater for feeding into the vermifiltration unit (i.e. the IN) was accessed from the influent tank at the City West Water's Sunshine Golf Course Treatment Plant (SGCTP), "The Sewer Mine", **Figure 4.1**[71]. The IN samples were collected on 35 sampling occasions, of which 22 during Phase I and 13 during Phase II, **Figure 4.4** over the period 05/02/2013 to 05/05/2014. For comparison purposes or testing the hypotheses, data from specific run period were judiciously combined with the IN in order to provide the best possible parametric information. This was considered to be justified since there was found to be no apparent seasonal variations in the IN parameters, during a specific run period. This Influent dataset was tested for normality and outliers, with respect to each of the 13 parameters. As an extreme variation could occur in the IN characteristic based on the end use or any other factor, the outliers were not excluded from the dataset. For instance, **Figure 4.19** displays an extreme pH value of an IN (5.6) on September 9, 2013, which was due to a lack of fresh sewage supply in the influent tank since this coincided with the sewer mine maintenance period, from 10/08/2013 to 09/09/2013. The IN values for the parameters investigated for the specific run period is provided in the Tables under the respective parameters discussed below.

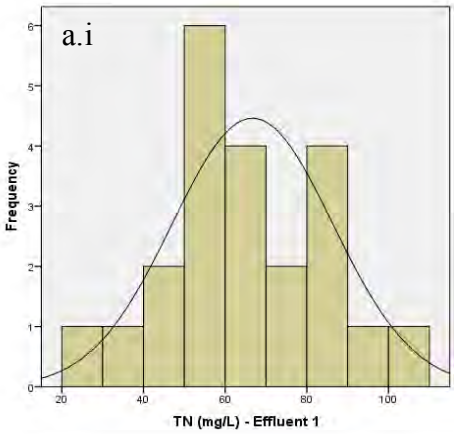
To compare the characteristics of the IN in Melbourne, Australia and in Kathmandu, Nepal, the IN collected throughout the experimental period is considered, in both cases, **Table 4.7**. This data shows that the strength of IN in Kathmandu, Nepal is higher than the one in Melbourne, which might be due to less water use in Kathmandu than in Melbourne, as well as the variation in the end use of available water by the customers.

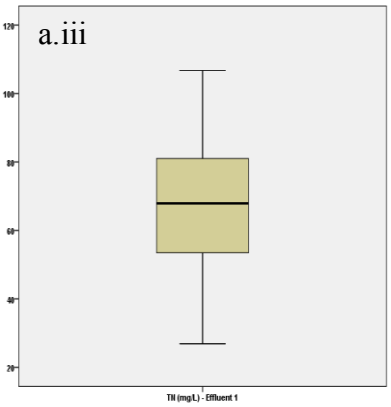
[71]Information on the Sunshine Golf Club Sewer Mining Project
http://www.citywestwater.com.au/our_assets/sunshine_golf_club_sewer_mining_project.aspx

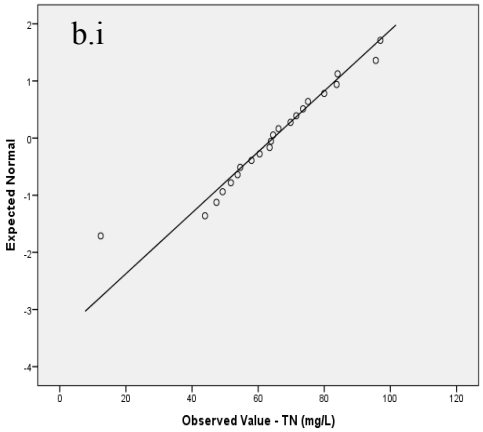
Table 4.7 A comparison between the characteristics of Influent in Melbourne and Kathmandu.

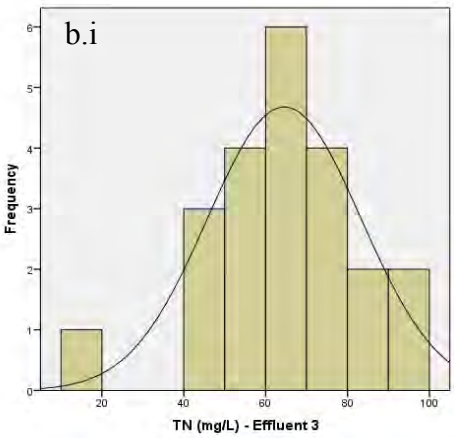
Observed parameters	Mean		Range	
	Melbourne	**Kathmandu**	**Melbourne**	**Kathmandu**
Temperature (°C)	19.1 ± 0.5	21.7 ± 0.3	12.4	13.3
pH	7.7 ± 0.1	6.7 ± 0.01	2.9	1.0
Conductivity (mS/cm)	1.02 ± 0.03	0.74 ± 0.01	0.89	0.44
Turbidity (NTU)	336 ± 30	441 ± 9	651	499
TSS (mg/L)	288 ± 22	338 ± 8	508	411
DO (mg/L)	1.69 ± 0.43	0.01 ± 0.01	8.16	0.65
COD (mg/L)	347 ± 23	1031 ± 42	560	854
NH_3-N (mg/L)	80 ± 5	78 ± 12	131	48
NO_2-N (mg/L)	6.6 ± 0.7	1.7 ± 0.6	20.0	2.6
NO_3-N (mg/L)	10.5 ± 1.3	0.9 ± 0.4	26.2	2.0

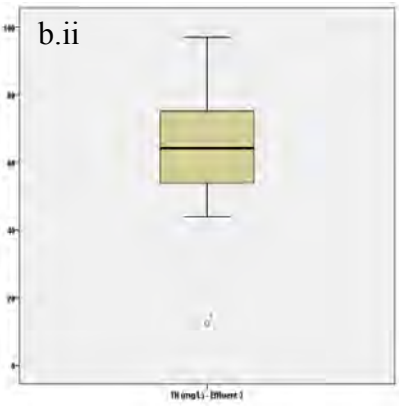

a.i
Expected Normal
Observed Value - TN (mg/L)

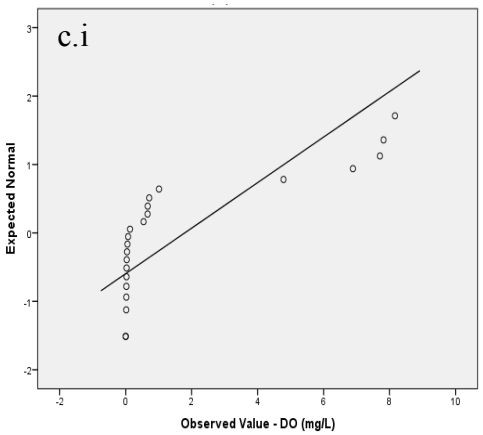

a.i
Frequency
TN (mg/L) - Effluent 1

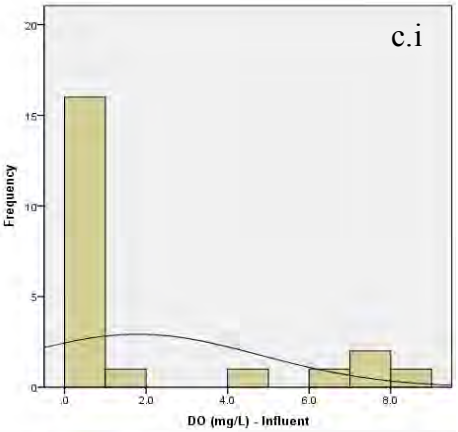

a.iii

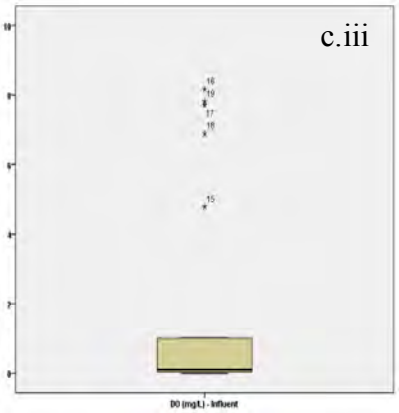

b.i
Expected Normal
Observed Value - TN (mg/L)

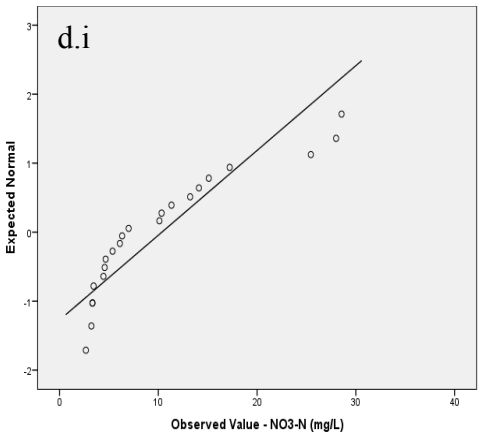

b.i
Frequency
TN (mg/L) - Effluent 3

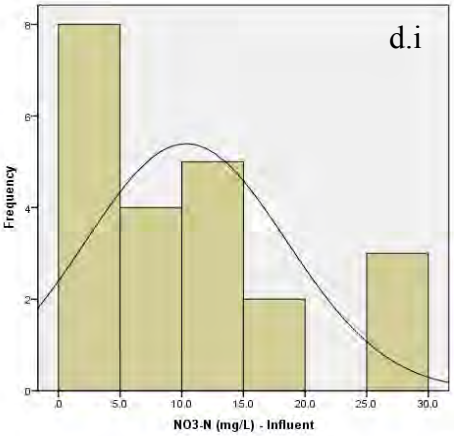

b.ii


c.i
Expected Normal
Observed Value - DO (mg/L)


c.i
Frequency
DO (mg/L) - Influent


c.iii


d.i
Expected Normal
Observed Value - NO3-N (mg/L)


d.i
Frequency
NO3-N (mg/L) - Influent

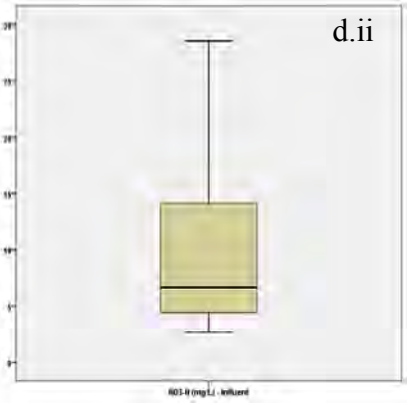

d.ii

Figure 4.15 (a.i) Representative normally distributed Q-Q plot for the Effluent 1 of TN (p-value = 0.999) (a.ii) Histogram for normally distributed Effluent 1 of TN (a.iii)The respective box-plot for a (without any outlier). (b.i) Representative normally distributed Q-Q plot for the Effluent 3 of TN (p-value=0.410) (b.ii) Histogram for normally distributed Effluent 3 TN (b.iii) The respective box-plot for (a) (with an outlier). (c.i) Representative not-normally distributed Q-Q plot of the Influent DO (p-value=0.000) (c.ii) Histogram for not-normally distributed Influent DO (c.iii) The respective box-plot for (a) (with outliers).(d.i) Representative normally distributed Q-Q plot of the Influent NO_3-N (p-value = 0.001) (d.ii) Histogram for not-normally distributed Influent NO_3-N (d.iii) The respective box-plot for a (without any outlier).

4.2.4.10 Formulation of null and experimental hypotheses

Null hypotheses and experimental hypotheses were derived for appropriate testing to assess the effect of VF on the IN pollution factors[72] and the relevant physico-chemical parameters[73].

4.2.4.10.1 Null hypotheses

A. The effect of filter layers

The hypothesis was tested separately for the VF 'without worms' and 'with worms' in the Phase I and Phase II, **Appendix 4.6**, to examine whether each layer of soil, sand and gravel in the VF contribute in the reduction of the IN pollution factors and to observe whether these layers has any effect on the relevant physico-chemical parameters (12 hypotheses for each parameter).

1. The soil, sand and gravel layers (EFs 1, 2 & 3) do not have significantly different temperatures compared to the IN. (12 hypotheses)
2. The soil, sand and gravel layers (EFs 1, 2 & 3) do not have significantly different pHs compared to the IN pH.
3. The soil, sand and gravel layers (EFs 1, 2 & 3) do not have significantly different conductivities compared to the IN.

[72]The "pollution factors" are those parameters that are considered desirable to reduce in sewage. Namely, turbidity, TSS, COD, NH_3-N, NO_2-N, NO_3-N, TN, TOC and TP.
[73]The relevant "physico-chemical parameters" are the temperature, the pH, the conductivity and the DO.

4. The soil, sand and gravel layers do not have significantly reduced turbidities compared to the IN.
5. The soil, sand and gravel layers do not have significantly reduced TSS compared to the IN.
6. The soil, sand and gravel layers do not have significantly increased DO levels compared to the IN.
7. The soil, sand and gravel layers do not significantly reduce COD levels compared to the IN COD level.
8. The soil, sand and gravel layers do not have reduced NH_3-N levels compared to the IN.
9. The soil, sand and gravel layers do not have reduced NO_2-N levels compared to the IN.
10. The soil, sand and gravel layers do not have reduced NO_3-N levels compared to the IN.
11. The soil, sand and gravel layers do not have reduced TN levels compared to the IN.
12. The soil, sand and gravel layers do not have reduced TOC levels compared to the Influent.
13. The soil, sand and gravel layers do not have reduced TP levels compared to the IN.

B. The effect of worms in the VF

The hypothesis was tested separately for the VF in the Phase I and Phase II, **Appendix 4.7**, to examine whether worms play any significant role in the reduction of the IN pollution factors and to observe whether worms has any effect on the relevant physico-chemical parameters.

1. The worms do not have any effect on the temperature of the EFs compared to the temperature of the IN.
2. The worms do not have any effect on the pH of the EFs compared to the pH of the IN.
3. The worms do not have any effect on the conductivity of the EFs compared to the IN.
4. The worms do not significantly reduce the turbidity of the EFs compared to the IN.
5. The worms do not significantly reduce the TSS of the EFs compared to the IN.
6. The worms do not contribute to increasing the DO of the EFs compared to the IN.
7. The worms do not contribute to reducing the COD of the EFs compared to the IN.
8. The worms do not contribute to reducing the NH_3-N of the EFs compared to the IN.
9. The worms do not contribute to reducing the NO_2-N levels of the EFs compared to the IN.

10. The worms do not contribute to reducing the NO_3-N levels of the EFs compared to the IN.
11. The worms do not contribute to reducing the TN of the EFs compared to the IN.
12. The worms do not contribute to reducing the TOC of the EFs compared to the IN.
13. The worms do not contribute to reducing the TP of the EFs compared to the IN.

C. The effect of soil type in the VF

The hypothesis was tested separately for the VF 'without worms' and 'with worms', **Appendix 4.8**, to examine whether the soil type ('soil type 1' in Phase I and 'soil type 2' in Phase II) has any effect in the reduction of the IN pollution factors and on the relevant physico-chemical parameters.

1. The soil type does not have any effect on the temperature of the EFs compared to the temperature of the IN.
2. The soil type does not have any effect on the pH of the EFs compared to the pH of the IN.
3. The soil type does not have any effect on the conductivity of the EFs compared to the IN.
4. The soil type does not significantly reduce the turbidity of the EFs compared to the IN.
5. The soil type does not significantly reduce the TSS of the EFs compared to the IN.
6. The soil type does not contribute to increasing the DO of the EFs compared to the IN.
7. The soil type does not contribute to reducing the COD of the EFs compared to the IN.
8. The soil type does not contribute to reducing the NH_3-N of the EFs compared to the IN.
9. The soil type does not contribute to reducing the NO_2-N levels of the EFs compared to the IN.
10. The soil type does not contribute to reducing the NO_3-N levels of the EFs compared to the IN.
11. The soil type does not contribute to reducing the TN of the EFs compared to the IN.
12. The soil type does not contribute to reducing the TOC of the EFs compared to the IN.
13. The soil type does not contribute to reducing the TP of the EFs compared to the IN.

D. The effect of Hydraulic Retention Time (HRT) and the Hydraulic Loading Rate (HLR)

The hypothesis was tested separately for the VF 'without worms' and 'with worms' in Phase I, **Appendix 4.9**, to examine whether the variation in Hydraulic Retention Time (HRT) and Hydraulic Loading Rate (HLR) has any effect in the reduction of the IN pollution factors and the change on the relevant physico-chemical parameters.

1. The temperature of the EFs do not change significantly when the HRT increases and the HLR decreases.
2. The pH of the EFs do not change significantly when the HRT increases and the HLR decreases.
3. The conductivity of the EFs do not change significantly when the HRT increases and the HLR decreases.
4. The turbidity of the EFs do not reduce significantly when the HRT increases and the HLR decreases.
5. The TSS of the EFs do not reduce significantly when the HRT increases and the HLR decreases.
6. The DO of the EFs do not increase significantly when the HRT increases and the HLR decreases.
7. The COD of the EFs do not reduce significantly when the HRT increases and the HLR decreases.
8. The NH_3-N of the EFs do not reduce significantly when the HRT increases and the HLR decreases.
9. The NO_2-N of the EFs do not reduce significantly when the HRT increases and the HLR decreases.
10. The NO_3-N of the EFs do not reduce significantly when the HRT increases and the HLR decreases.
11. The TN of the EFs do not reduce significantly when the HRT increases and the HLR decreases.
12. The TOC of the EFs do not reduce significantly when the HRT increases and the HLR decreases.
13. The TP of the EFs do not reduce significantly when the HRT increases and the HLR decreases.

4.2.4.10.2 Experimental (Alternative) hypotheses

A. The effect of filter layers

The experimental hypothesis, an alternative hypothesis to the null hypothesis, was formulated separately for the VF 'without worms' and 'with worms' in the Phase I and Phase II, **Appendix 4.6**, to examine whether each layer of soil, sand and gravel in the VF contribute in the reduction of the IN pollution factors and to observe whether these layers have any effect on the relevant physico-chemical parameters.

1. The soil, sand and gravel layers (EFs 1, 2 & 3) have significantly different temperatures compared to the IN. (12 hypotheses)
2. The soil, sand and gravel layers (EFs 1, 2 & 3) have significantly different pHs compared to the IN pH.
3. The soil, sand and gravel layers (EFs 1, 2 & 3) have significantly different conductivities compared to the IN.
4. The soil, sand and gravel layers have significantly reduced turbidities compared to the IN.
5. The soil, sand and gravel layers have significantly reduced TSS compared to the IN.
6. The soil, sand and gravel layers have significantly increased DO levels compared to the IN.
7. The soil, sand and gravel layers significantly reduce COD levels compared to the IN COD level.
8. The soil, sand and gravel layers have reduced NH_3-N levels compared to the IN.
9. The soil, sand and gravel layers have reduced NO_2-N levels compared to the IN.
10. The soil, sand and gravel layers have reduced NO_3-N levels compared to the IN.
11. The soil, sand and gravel layers have reduced TN levels compared to the IN.
12. The soil, sand and gravel layers have reduced TOC levels compared to the Influent.
13. The soil, sand and gravel layer have reduced TP levels compared to the IN.

B. The effect of worms in the VF

The experimental hypothesis, was formulated separately for the VF in the Phase I and Phase II, **Appendix 4.7**, to examine whether worms play any significant role in the reduction of the

IN pollution factors and to observe whether worms has any effect on the relevant physico-chemical parameters.

1. The worms effect on the temperature of the EFs compared to the temperature of the IN.
2. The worms effect on the pH of the EFs compared to the pH of the IN.
3. The worms effect on the conductivity of the EFs compared to the IN.
4. The worms significantly reduce the turbidity of the EFs compared to the IN.
5. The worms significantly reduce the TSS of the EFs compared to the IN.
6. The worms contribute to increasing the DO of the EFs compared to the IN.
7. The worms contribute to reducing the COD of the EFs compared to the IN.
8. The worms contribute to reducing the NH_3-N of the EFs compared to the IN.
9. The worms contribute to reducing the NO_2-N levels of the EFs compared to the IN.
10. The worms contribute to reducing the NO_3-N levels of the EFs compared to the IN.
11. The worms contribute to reducing the TN of the EFs compared to the IN.
12. The worms contribute to reducing the TOC of the EFs compared to the IN.
13. The worms contribute to reducing the TP of the EFs compared to the IN.

C. The effect of soil type in the VF

The experimental hypothesis was formulated separately for the VF 'without worms' and 'with worms', **Appendix 4.8**, to examine whether the soil type ('soil type 1' in Phase I and 'soil type 2' in Phase II) has any effect in the reduction of the IN pollution factors and on the relevant physico-chemical parameters.

1. The soil type effects on the temperature of the EFs compared to the temperature of the IN.
2. The soil type effects on the pH of the EFs compared to the pH of the IN.
3. The soil type effects on the conductivity of the EFs compared to the IN.
4. The soil type contributes to significantly reduce the turbidity of the EFs compared to the IN.
5. The soil type contributes to significantly reduce the TSS of the EFs compared to the IN.
6. The soil type contributes to increasing the DO of the EFs compared to the IN.
7. The soil type contributes to reducing the COD of the EFs compared to the IN.
8. The soil type contributes to reducing the NH_3-N of the EFs compared to the IN.
9. The soil type contributes to reducing the NO_2-N levels of the EFs compared to the IN.

10. The soil type contributes to reducing the NO_3-N levels of the EFs compared to the IN.
11. The soil type contributes to reducing the TN of the EFs compared to the IN.
12. The soil type contributes to reducing the TOC of the EFs compared to the IN.
13. The soil type contributes to reducing the TP of the EFs compared to the IN.

D. The effect of Hydraulic Retention Time (HRT) and the Hydraulic Loading Rate (HLR)

The experimental hypothesis was formulated separately for the VF 'without worms' and 'with worms' in Phase I, **Appendix 4.9**, to examine whether the variation in Hydraulic Retention Time (HRT) and Hydraulic Loading Rate (HLR) has any effect in the reduction of the IN pollution factors and the change on the relevant physico-chemical parameters.

1. The temperature of the EFs change significantly when the HRT increases and the HLR decreases.
2. The pH of the EFs change significantly when the HRT increases and the HLR decreases.
3. The conductivity of the EFs change significantly when the HRT increases and the HLR decreases.
4. The turbidity of the EFs reduce significantly when the HRT increases and the HLR decreases.
5. The TSS of the EFs reduce significantly when the HRT increases and the HLR decreases.
6. The DO of the EFs increase significantly when the HRT increases and the HLR decreases.
7. The COD of the EFs reduce significantly when the HRT increases and the HLR decreases.
8. The NH_3-N of the EFs reduce significantly when the HRT increases and the HLR decreases.
9. The NO_2-N of the EFs reduce significantly when the HRT increases and the HLR decreases.
10. The NO_3-N of the EFs reduce significantly when the HRT increases and the HLR decreases.
11. The TN of the EFs reduce significantly when the HRT increases and the HLR decreases.
12. The TOC of the EFs reduce significantly when the HRT increases and the HLR decreases.
13. The TP of the EFs reduce significantly when the HRT increases and the HLR decreases.

4.3 Results and Discussions

4.3.1 Physicochemical measurements

4.3.1.1 Data presentation

Influent from the supply tank is referred to as the 'Influent' (IN), effluent from the soil layer (Layer 1), the sand layer (Layer 2) and the gravel layer (Layer 3) are referred to as 'Effluent 1' (EF1), 'Effluent 2' (EF2) and 'Effluent 3' (EF3), respectively, throughout, see **Figures 4.3** & **4.4**. For Phase I, the experimental period was from January 2013 to December 2013. For Phase II, the experimental period was from January 2014 to May 2014, **Figure 4.4**. The difference between the Phase I and Phase II experiments is described in **Table 4.3**, *vide supra*. For the parameters of interest with respect to the IN and EFs 1-3, temporal data are shown by line graphs and time-averaged data are presented as column graphs (histograms) as follows. For the column graphs, for each parameter of interest, the blue bar represents the time-averaged data recorded for the IN before introducing earthworms into the system and the pink bar represents the time-averaged data for the IN after introducing worms into the system. While red bars represent EFs without worms and green bars represents EFs with worms. In Phase I for, 'without worms', the bars present the average of 7 sets of data which is taken as a 'control' and for, 'with worms', the bars present the average of 15 sets of data. In Phase II, for, 'without worms', the bars present the average of 3 sets of data and for, 'with worms', the bars present the average of 10 sets of data, **Figure 4.4**. Note: In the line graphs for Phase I, data on the NH_3-N is missing for 25 February and data on the TP is missing for 12 February, 11 & 18 March and 22 July, due to errors in the analyses.

Full comparative time-averaged data, giving the mean and SE values for all relevant parameters over the specific run periods, is given in the respective Tables under the respective parameters, as discussed below. To facilitate discussion, these Tables include the range and the percentage differences between the time-averaged parameters of the different EFs and the IN.

4.3.1.2 The temperature profile

An overview of the temperature profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.8**. Temporal graphical representations of this data for IN and EFs 1-3, throughout the Phase I and Phase II experimental periods, are presented in **Figures 4.16** & **4.17**, respectively. The figure shows that there was a variation in temperature throughout the experimental period. The temperature of the IN was generally slightly higher than the EF temperatures but follows the same general pattern. This suggests that the ambient temperature[74] affects both the IN and the EFs in a similar way. There does not appear to be a heating process occurring within the unit.

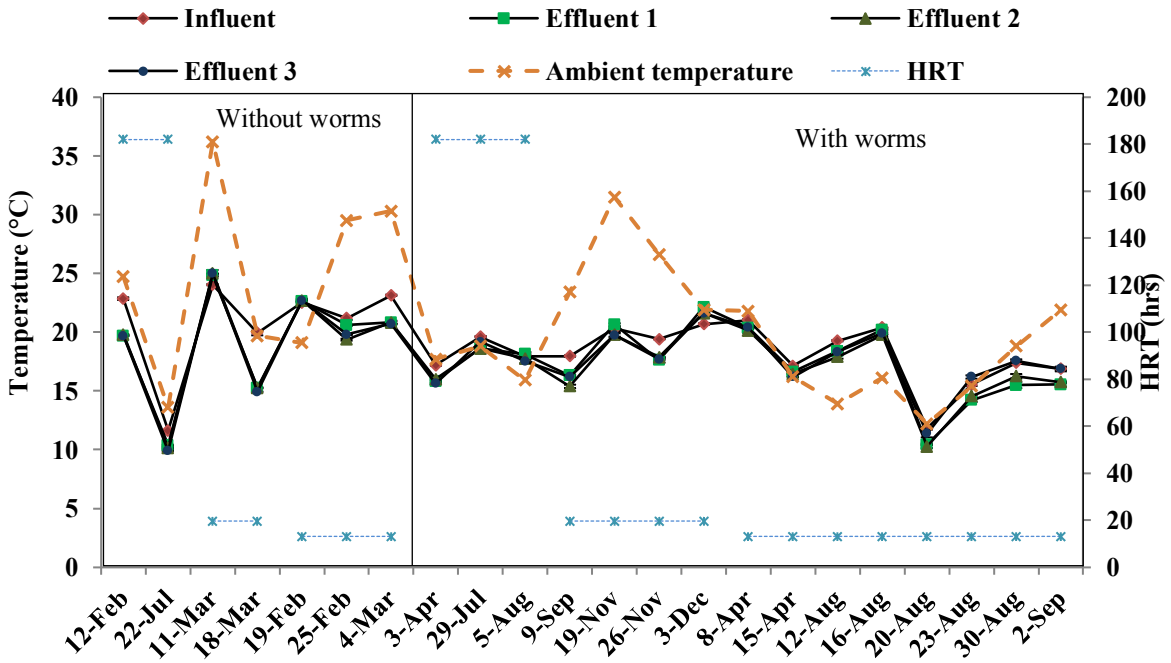


Figure 4.16 Temperature profile throughout the experimental period during Phase I. Error bars are standard errors, n = 3. The dotted amber line represents the maximum ambient temperature recorded on the sampling day.

[74] The maximum temperature recorded at Laverton weather station (the closest to the SGCTP), by the Bureau of Meteorology, on the sampling day of observation, in 2013, is provided in this link: http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_nccObsCode=122&p_display_type=dailyDataFile&p_startYear=2013&p_c=-1514882515&p_stn_num=087031 or, http://www.bom.gov.au/tmp/cdio/IDCJAC0010_087031_2013.pdf 16/03/2015

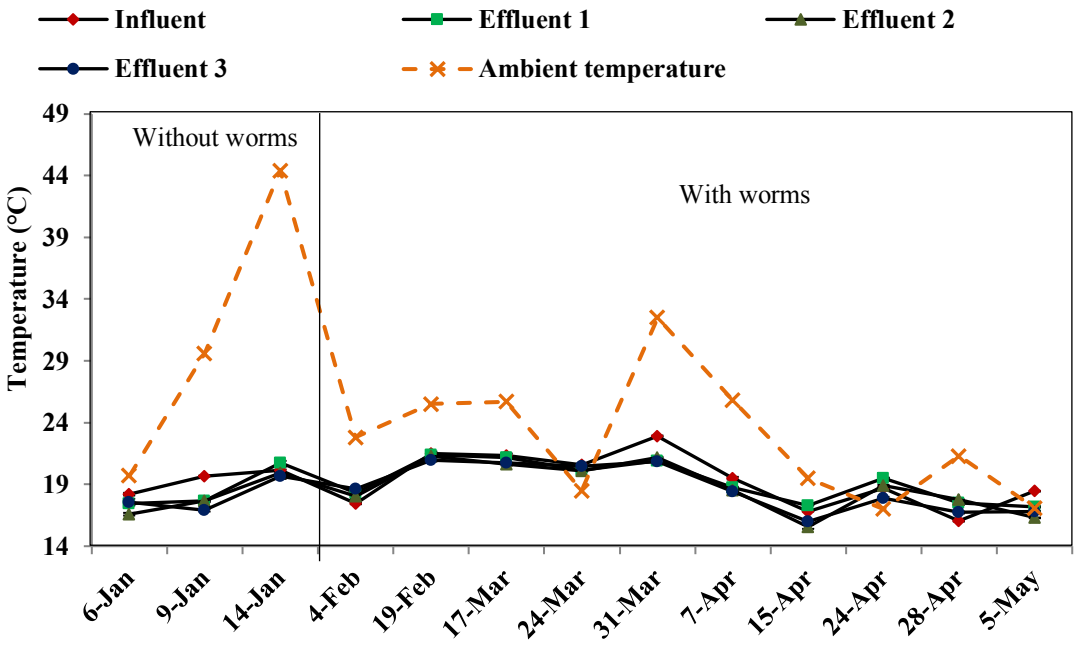


Figure 4.17 Temperature profile throughout the experimental period during Phase II. Error bars are the standard errors, n = 3.

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.18 (a)** & **(b)** depicting the time-averaged temperature for the VF with and without worms, over Phases I and II, respectively.

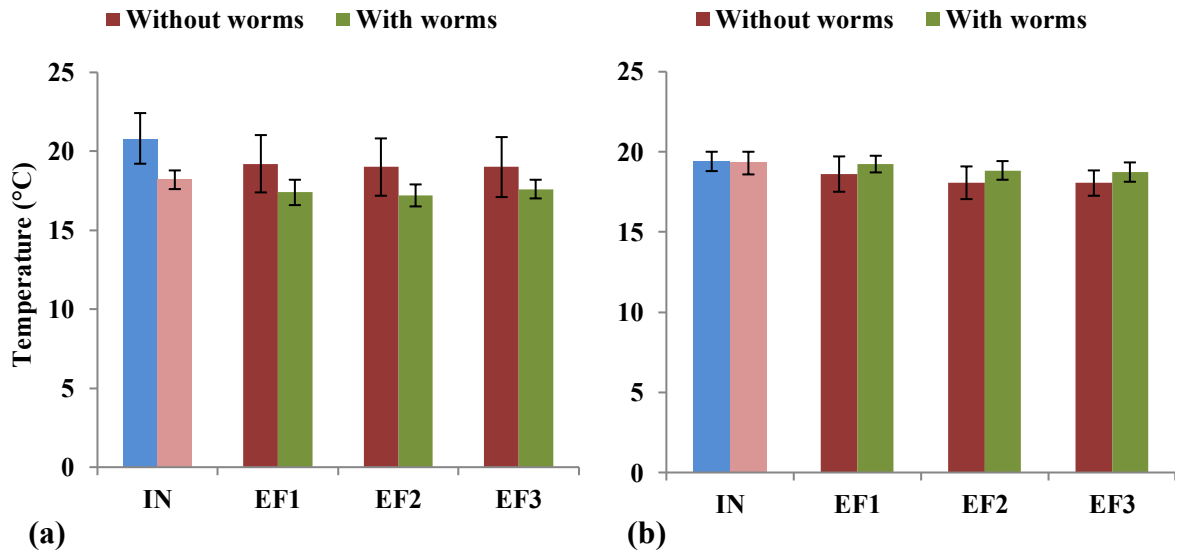


Figure 4.18 Time-averaged temperature comparisons for (a) Phase I and (b) Phase II. Error bars represent standard errors. In Phase I, n = 7, for 'without worms'& n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms'& n = 10, for 'with worms'. Note: For the IN, pink bar represents the periods of time when the system was run with worms and blue bar for without worms), and the % change in this parameter was related to the average IN temperature for the specific run period.

Table 4.8 An overview on the temperature profile during the experimental period, Phase I and Phase II. The % change reported for the EFs is calculated with respect to the average IN pH for the specific run period.

		Without worms				With worms			
Temperature	**OCs**	IN	EF1	EF2	EF3	IN	EF1	EF2	EF3
Phase I									
Mean ± SE	1	22.3 ± 0.6	21.4 ± 0.6	21.0 ± 1.0	21.1 ± 0.9	17.4 ± 1.0	16.4 ± 1.2	16.4 ± 1.1	17.1 ± 1.0
	2	22.0 ± 2.1	20.1 ± 4.8	20.1 ± 4.9	20.0 ± 5.0	19.6 ± 0.6	19.2 ± 1.3	18.7 ± 1.3	18.8 ± 1.2
	3	17.2 ± 5.6	15 ± 4.7	15.0 ± 4.8	14.8 ± 4.9	18.2 ± 0.7	17.5 ± 0.9	17.5 ± 0.8	17.5 ± 1.0
Range	1	21.2 - 23.1	20.6 - 22.6	19.4 - 22.7	19.8 - 22.7	11.8 - 21.0	10.4 - 20.4	10.3 - 20.1	11.4 -20.4
	2	19.9 - 24.0	15.3 - 24.8	15.2 - 25.0	14.9 - 25.0	18.0 - 20.7	16.3 - 22.1	15.4 - 21.6	16.2 - 21.6
	3	11.6 - 22.8	10.4 - 19.7	10.1 - 19.8	9.9 - 19.7	17.2 - 19.6	15.8 - 18.6	16.0 - 18.6	15.6 - 19.2
% Change	1	-	4	6	5	-	6	6	2
	2	-	9	9	9	-	2	5	4
	3	-	13	13	14	-	4	4	4
Phase II									
Mean SE	2	19.4 ± 0.6	18.6 ± 1.1	18.1 ± 1	18.1 ± 0.8	19.3 ± 0.7	19.2 ± 0.5	18.8 ± 0.6	18.8 ± 0.6
Range	2	18.2 - 20.2	17.4 - 20.8	16.6 - 19.9	16.9 - 19.7	16.0 - 22.9	17.2 - 21.4	15.6 - 21.3	16.0 - 21.0
% Change	2	-	4	7	7	-	0.4	2	3

Here, though the graphs illustrate the two different phases showing two different patterns of temperature change in the system, it is noteworthy to mention here that the IN and the EFs temperature seems to be dependent on the ambient temperature. Hence, our concern with respect to temperature is only with the IN temperature that directly affects the earthworms, that reside on the top layer of the vermifiltration unit. The Influent temperature ranged from 11.6 - 24.0 °C during Phase I and 16.0 - 23.0 °C during Phase II, which is within the temperature range of 0 - 35 °C that the earthworms are able to tolerate (Edwards 1998), further discussed in **Section 4.1.2.2**., and the higher limit being the approximate optimum temperature for survival and growth of *E. Andrei* (25 °C).

4.3.1.3 The pH profile

An overview of the pH profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.9**. Temporal graphical representations of this data for IN and EFs 1-3, throughout the Phase I and Phase II experimental periods, are presented in **Figures 4.19** & **4.20**, respectively. It is interesting to note that the pH of the EFs tend to stabilize and approach neutrality both with and without worms, regardless of the degree of acidity or alkalinity in the IN. This effect appears to be quite robust as is evidenced by the neutralization of the highly acidic IN observed on 9 September, 2013. The low pH value on this occasion is difficult to explain but such extreme variations in IN parameters are sometimes observed and could be due to maintenance activities or irregular deposition into the sewage system. High acidity might arise from putrescible wastes, while high alkalinity might be from products containing caustic soda and alkaline salts. A study carried out by Hughes et al. (2007) showed worms are pH sensitive, e.g. in an environment with a pH < 5.5 and > 10.5, worms are not able to survive. However, here, the pH of the IN ranged from 5.6 – 8.8, which is within the favourable range during the entire experimental period, i.e. over Phases I & II. The pH was observed to stabilize and approach neutrality after passage through the first filter media (soil) and remained stable when passed through the second and the third layers of the VF, for both Phases.

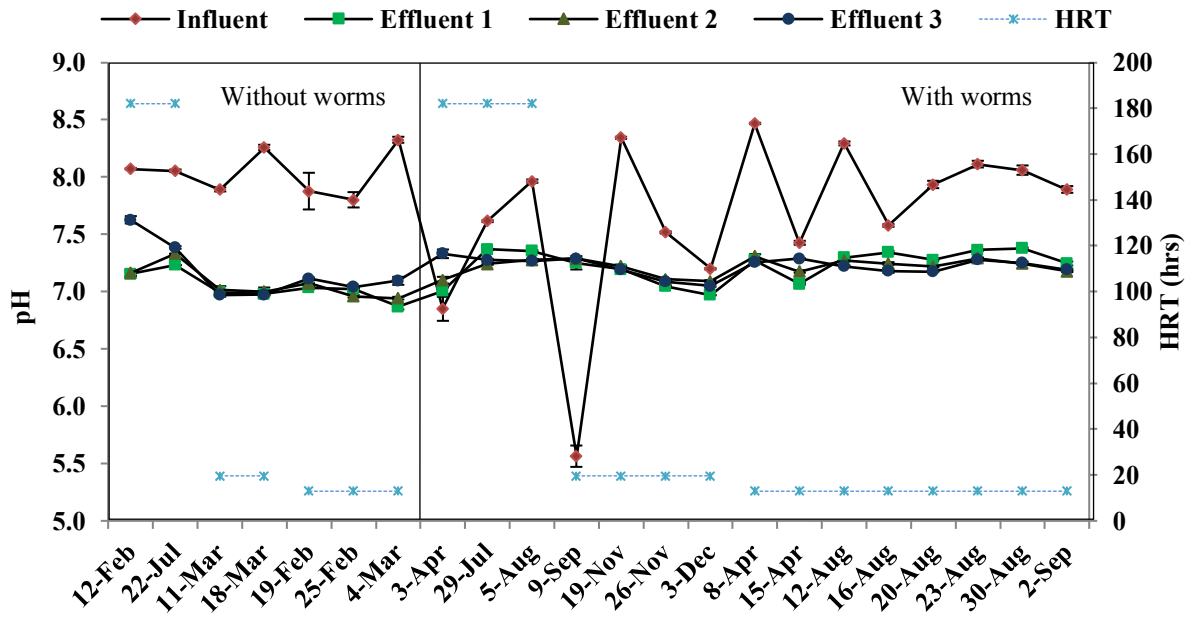


Figure 4.19 pH profile throughout the experimental period in Phase I. Error bars are standard errors, n =3.

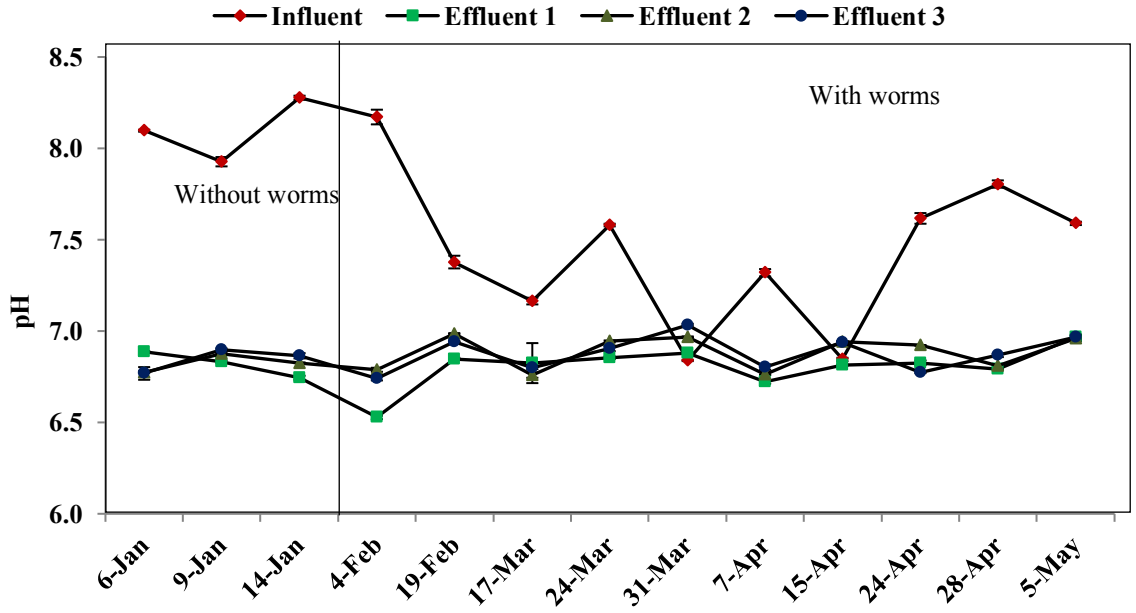


Figure 4.20 pH profile throughout the experimental period in Phase II. Error bars are standard errors, n =3.

Table 4.9 An overview of the pH profile for Phase I and Phase II. The reported % change is calculated with respect to the average IN pH for each specific run period.

		Without worms				With worms			
pH	**OC**	**IN**	**EF1**	**EF2**	**EF3**	**IN**	**EF1**	**EF2**	**EF3**
Phase I									
Mean ± SE	1	8.00 ± 0.16	6.98 ± 0.05	6.99 ± 0.04	7.08 ± 0.02	7.97 ± 0.12	7.28 ± 0.03	7.24 ± 0.02	7.23 ± 0.02
	2	8.07 ± 0.18	6.99 ± 0.01	7.01 ± 0.01	6.97 ± 0.00	7.16 ± 0.58	7.12 ± 0.07	7.18 ± 0.05	7.15 ± 0.05
	3	8.06 ± 0.01	7.19 ± 0.04	7.25 ± 0.08	7.51 ± 0.12	7.48 ± 0.32	7.24 ± 0.12	7.21 ± 0.05	7.29 ± 0.02
Range	1	7.80 - 8.32	6.87 - 7.04	6.94 - 7.07	7.04 - 7.11	7.43 - 8.47	7.07 - 7.38	7.17 - 7.31	7.18 - 7.29
	2	7.89 - 8.26	6.98 - 6 99	7.00 - 7.01	6.97 - 6.97	5.56 - 8.34	6.97 - 7.25	7.09 - 7.29	7.05 - 7.29
	3	8.05 - 8.07	7.15 - 7.23	7.16 - 7.33	7.38 - 7.63	6.85 - 7.96	7.00 - 7.37	7.10 - 7.28	7.27 - 7.33
% Change	1	-	13	13	12	-	9	9	9
	2	-	13	13	14	-	1	0	0
	3	-	11	10	7	-	3	4	2
Phase II									
Mean SE	2	8.10 ± 0.10	6.82 ± 0.04	6.83 ± 0.03	6.84 ± 0.04	7.43 ± 0.13	6.81 ± 0.04	6.88 ± 0.03	6.88 ± 0.03
Range	2	7.93 - 8.28	6.74 - 6.89	6.78 - 6.88	6.77 - 6.90	6.84 - 8.17	6.53 - 6.97	6.76 - 6.99	6.74 - 7.03
% Change	2	-	16	16	16	-	8	7	7

With respect to the effect of Operating Conditions (OCs) on the treatment efficiency of the VF; in Phase I, **Table 4.9** shows that OC2 is more favourable in moving the pH value towards neutrality, than OC1 and OC3. However, a comparatively higher pH of the EFs for the VF without worms for OC3 might be attributed to the filter materials, as was observed during the initial phase of the experiment. Thus, a low HRT seems to be better for achieving neutral pH than a high HRT.

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.21 (a)** & **(b)** depicting the time-averaged pH for the VF with and without worms, over Phases I and II, respectively. These plots highlight the significant pH stabilization for both types of first layer (soil types 1 and 2), irrespective of whether worms are present or not. Although it would appear that the presence of worms in **Figure 4.18 (a)** has a significant effect on the pH stabilization, this is more likely to be due to the variation in OCs (HRT and HLR) during Phase 1, see **Table 4.9**. In this regard, the worms do not significantly affect the pH stabilization for Phase 2.

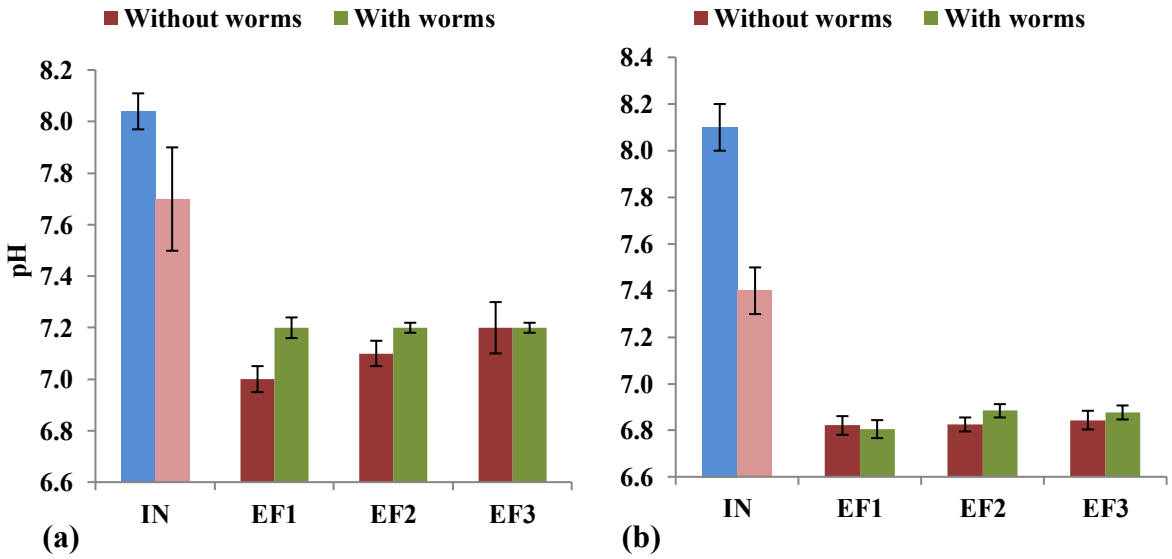


Figure 4.21 Time averaged pH comparisons for (a) Phase I and (b) Phase II. Error bars represent standard errors. In Phase I, n = 7, for 'without worms' & n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms' & n = 10, for 'with worms'. Note: For the IN, it was observed that for the different experimental runs (i.e. for the periods of time when the system was run with worms (pink bar) and without worms (blue bar), the average pH was found to be significantly different as is indicated in the above graphs. Therefore, the % change in this parameter was related to the average IN pH for the specific run period.

4.3.1.4 Conductivity

An overview of the conductivity profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.10**. Temporal graphical representations of this data for IN and EFs 1-3, throughout the Phase I and phase II experimental periods, are presented in **Figures 4.22** & **4.23**, respectively. Here, a significantly high value of the conductivity in the EFs than in the IN was observed on 12 February, which suggests that the washing of the filter materials may contain higher ionic concentration. However, it could also be due to the longer retention period. In Phase I, a trend of conductivity was not clear without worms, but it increased dramatically as worms were introduced in the VF and had a tendency to stabilize in the later stage. In Phase II, the conductivity decreased slightly without worms, but increased significantly while worms were introduced and tended to stabilize after a certain time period, similar as in Phase I. This conductivity trend with worms, to increase during the initial stage and to stabilize over time (later stage) might be attributed to worm activity. Here, interestingly, soil type 2 seems to adsorb ions rather than releasing it like soil type 1. Thus, soil type 1 seems to be more favourable in reducing conductivity.

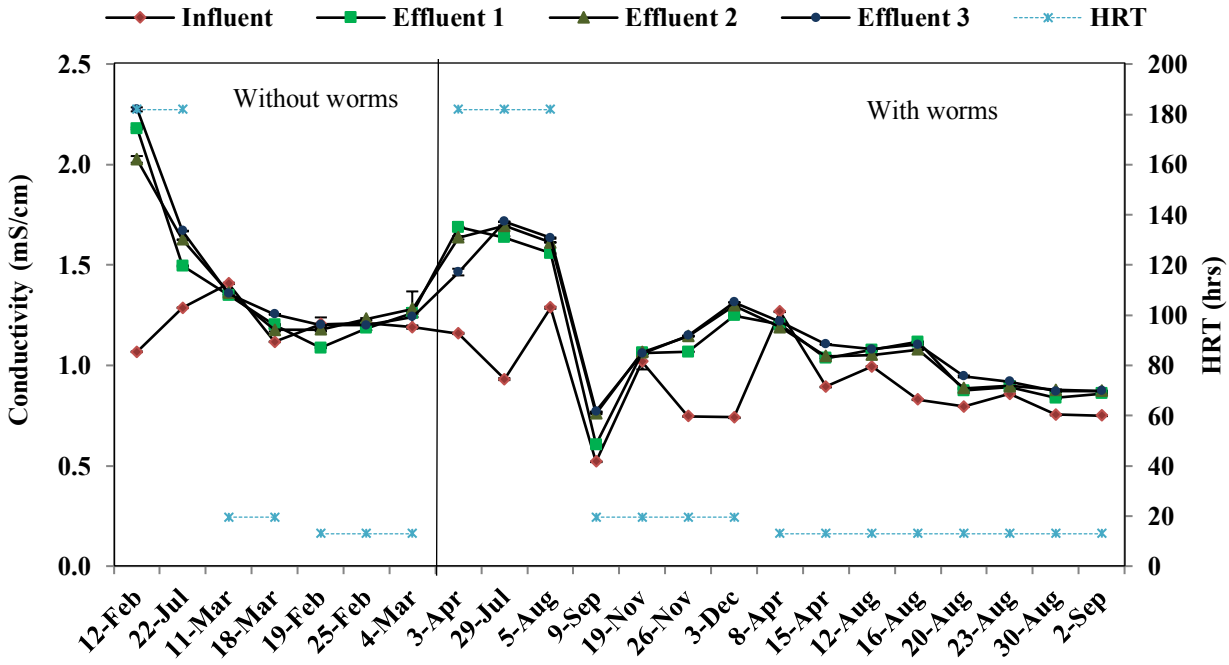


Figure 4.22 Conductivity profile, throughout the experimental period in Phase I. Error bars are standard errors, n =3.

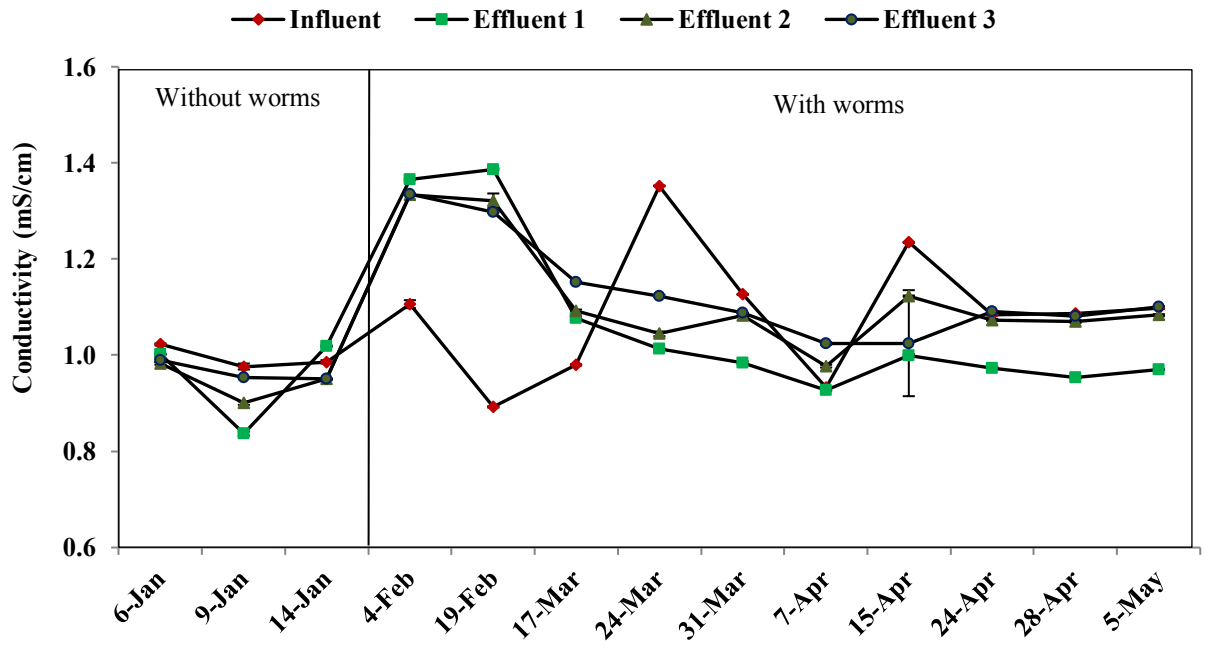


Figure 4.23 Conductivity profile, throughout the experimental period in Phase II. Error bars are standard errors, n =3.

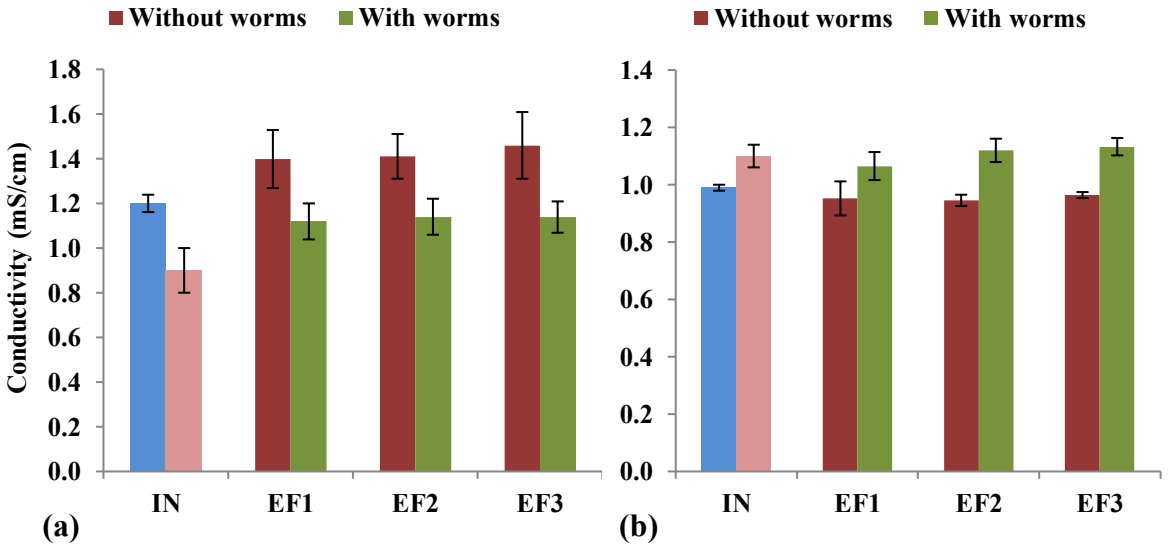


Figure 4.24 Time-averaged conductivities for (a) Phase I and (b) Phase II. Error bars represent the standard errors. In Phase I, n = 7, for 'without worms' & n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms' & n = 10, for 'with worms'. Note: For the IN, it was observed that for the different experimental runs (i.e. for the periods of time when the system was run with worms (pink bar) and without worms (blue bar), the average conductivity was found to be significantly different as is indicated in the above graphs. Therefore, the % change in this parameter was related to the average IN conductivity for the specific run period.

Table 4.10 An overview of the conductivity profile for Phase I and Phase II. The reported % change is calculated with respect to the average IN conductivity for each specific run period.

		Without worms				With worms			
Conductivity	**OC**	IN	EF1	EF2	EF3	IN	EF1	EF2	EF3
Phase I									
Mean ± SE	1	1.20 ± 0.10	1.18 ± 0.05	1.23 ± 0.03	1.21 ± 0.10	0.89 ± 0.06	0.99 ± 0.05	0.99 ± 0.04	1.02 ± 0.05
	2	1.26 ± 0.15	1.28 ± 0.07	1.27 ± 0.09	1.31 ± 0.05	0.76 ± 0.10	1.00 ± 0.14	1.07 ± 0.11	1.07 ± 0.11
	3	1.18 ± 0.11	1.84 ± 0.34	1.83 ± 0.20	1.97 ± 0.30	1.13 ± 0.10	1.63 ± 0.04	1.65 ± 0.02	1.61 ± 0.07
Range	1	1.19 - 1.21	1.09 - 1.26	1.18 - 1.28	1.20 - 1.24	0.75 - 1.27	0.84 - 1.20	0.87 - 1.19	0.87 - 1.22
	2	1.12 - 1.41	1.20 - 1.35	1.18 - 1.36	1.25 - 1.36	0.52 - 1.02	0.61 - 1.25	0.76 - 1.30	0.77 - 1.31
	3	1.07 - 1.29	1.50 - 2.18	1.63 - 2.03	1.67 - 2.28	0.93 - 1.29	1.56 - 1.69	1.61 - 1.70	1.46 - 1.72
% Change	1	-	2	-3	-1	-	-11	-11	-14
	2	-	-2	-1	-4	-	-32	-41	-42
	3	-	-56	-55	-67	-	-45	-46	-43
Phase II									
Mean SE	2	0.99 ± 0.01	0.95 ± 0.06	0.94 ± 0.02	0.96 ± 0.01	1.09 ± 0.04	1.07 ± 0.05	1.12 ± 0.04	1.13 ± 0.03
Range	2	0.98 - 1.02	0.84 - 1.02	0.90 - 0.98	0.95 - 0.99	0.89 - 1.35	0.93 - 1.39	0.98 - 1.33	1.02 - 1.34
% Change	2	-	4	5	3	-	2	-3	-4

With respect to the effect of OCs on the conductivity, it seems that OC1 is more suitable in achieving low conductivity in the EFs. The observation in Phase I suggests that higher HRT and low HLR contributes high ionic concentration in the EFs, irrespective to the presence or absence of worms.

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.24 (a)** & **(b)** depicting the time-averaged conductivity for the VF with and without worms, over Phases I and II, respectively.

These plots highlight the significance of the first layer soil type in changing the conductivity in EFs. With soil type 1, regardless of the presence or the absence of the worms, the average conductivity increased. However, not much difference was observed with soil type 2.

4.3.1.5 Dissolved Oxygen

An overview of the DO profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.11**. Temporal graphical representations of this data for IN and EFs 1-3, throughout the Phase I and phase II experimental periods, are presented in **Figures 4.25** & **4.26**, respectively. The DO in the IN was found to be too low, except during the maintenance period – August 20 to September 9, 2013. Usually IN DO is considered to have zero value, which is also evidenced by this observation. The DO was found to be comparatively higher in Phase II, irrespective to the presence or absence of worms, which is attributed to the soil type rather than worm activity. However, in Phase I, though the increase in DO after the introduction of worms for OC1 and OC2 was not high, it is significantly higher for OC3. Thus, the OC3 was more suitable in increasing DO significantly. This observation suggests that the high HRT and less HLR contributed dissolved oxygen in the treatment process. Here, this increasing effect may be due to a synchronous effect of worms and microbes.

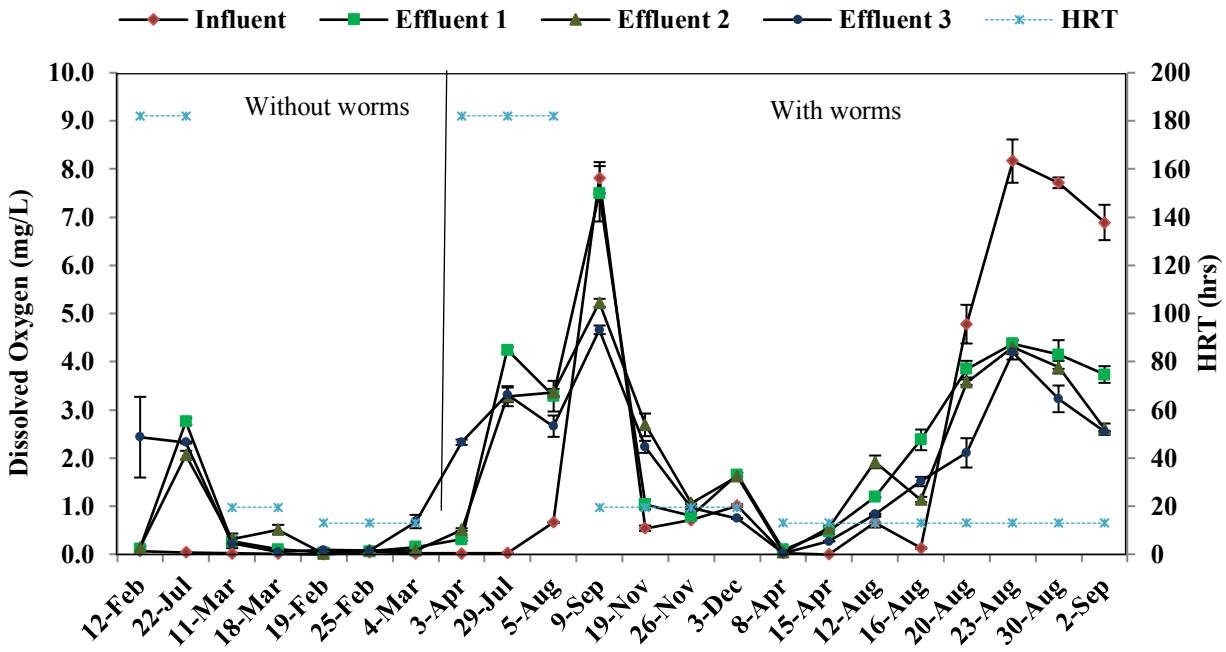


Figure 4.25 Dissolved oxygen profile throughout the experimental period in Phase I. Error bars are standard errors, n =3.

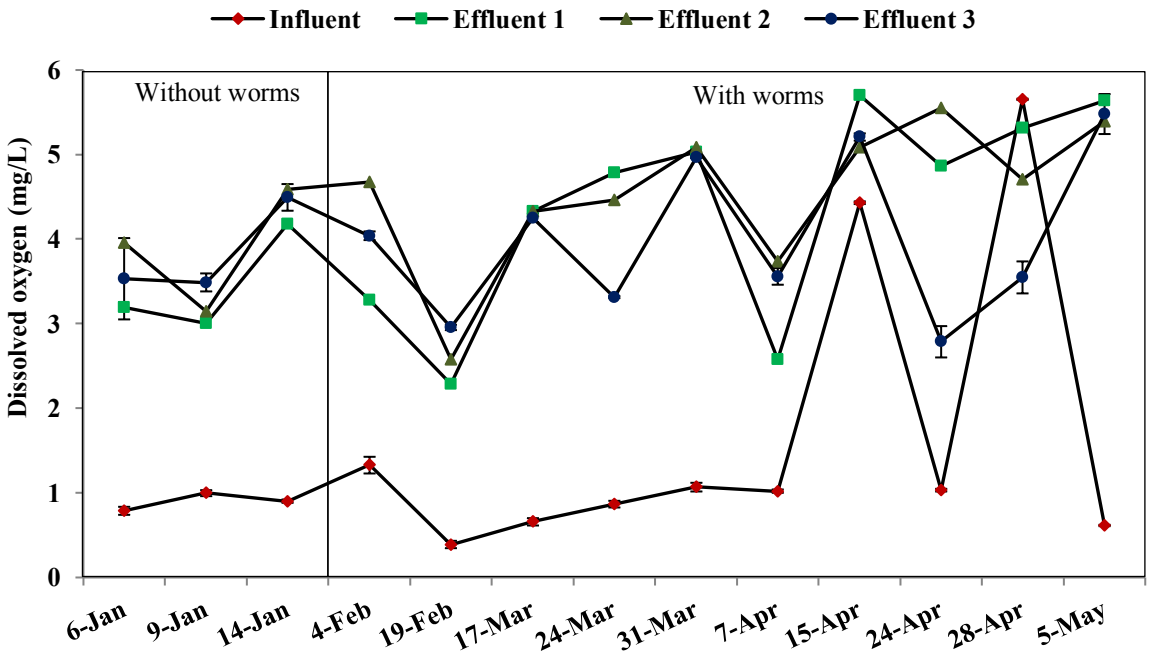


Figure 4.26 Dissolved oxygen profile throughout the experimental period in Phase II. Error bars are standard errors, n =3.

Table 4.11 An overview of the DO profile for Phase I and Phase II. The reported % change is calculated with respect to the average IN DO for each specific run period.

		Without worms				With worms			
DO	**OCs**	IN	EF1	EF2	EF3	IN	EF1	EF2	EF3
Phase I									
Mean ± SE	1	0.02 ± 0.02	0.08 ± 0.03	0.06 ± 0.02	0.28 ± 0.20	3.55 ± 1.31	2.53 ± 0.61	2.25 ± 0.56	1.80 ± 0.52
	2	0.02 ± 0.00	0.19 ± 0.08	0.42 ± 0.10	0.14 ± 0.10	2.52 ± 1.77	2.74 ± 1.59	2.65 ± 0.92	2.15 ± 0.90
	3	0.06 ± 0.02	1.4 ± 1.32	1.1 ± 0.96	2.38 ± 0.06	0.24 ± 0.21	2.61 ± 1.20	2.39 ± 0.94	2.77 ± 0.29
Range	1	0.00 - 0.05	0.04 - 0.15	0.02 - 0.08	0.08 - 0.68	0.00 - 8.16	0.10 - 4.37	0.04 - 4.30	0.02 - 4.19
	2	0.02 - 0.02	0.11 - 0.27	0.32 - 0.51	0.05 - 0.23	0.54 - 7.82	0.79 - 7.49	1.07 - 5.22	0.75 - 4.66
	3	0.04 - 0.07	0.12 - 2.76	0.14 - 2.06	2.32 - 2.44	0.02 - 0.66	0.31 - 4.24	0.51 - 3.36	2.33 - 3.32
% Change	1	-	-243	-146	-1066	-	29	37	48
	2	-	-819	-1923	-583	-	-9	-5	15
	3	-	-2461	-1859	-4142	-	-996	-902	-1061
Phase II									
Mean SE	2	0.89 ± 0.06	3.46 ± 0.36	3.90 ± 0.42	3.84 ± 0.33	1.70 ± 0.57	4.38 ± 0.39	4.56 ± 0.28	4.01 ± 0.30
Range	2	0.79 - 1.00	3.00 - 4.17	3.14 - 4.59	3.49 - 4.49	0.39 - 5.65	2.29 - 5.70	2.58 - 5.55	2.79 - 5.47
% Change	2	-	-288	-338	-331	-	-158	-168	-136

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.24 (a)** & **(b)** depicting the time-averaged DO for the VF with and without worms, over Phases I and II, respectively.

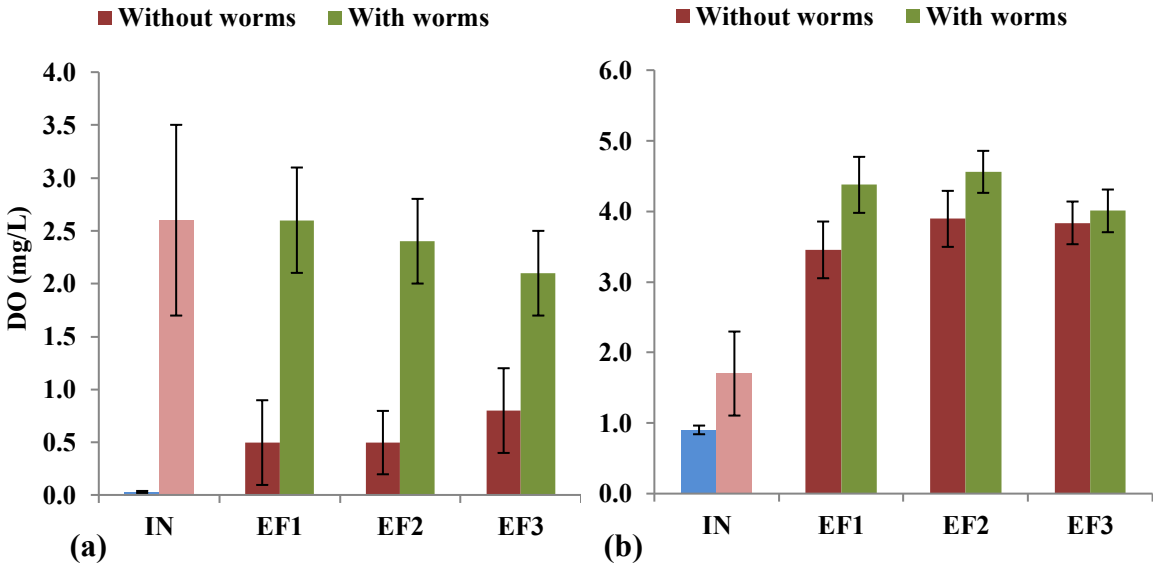


Figure 4.27 Time-averaged DO levels for (a) Phase I and (b) Phase II. Error bars represent standard errors. In Phase I, n = 7, for 'without worms' & n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms' & n = 10, for 'with worms'. Note: For the IN, it was observed that for the different experimental runs (i.e. for the periods of time when the system was run with worms (pink bar) and without worms (blue bar), the average DO was found to be significantly different as is indicated in the above graphs. Therefore, the % change in this parameter was related to the average IN DO for the specific run period.

These plots highlight the significance of the soil layers and worms in increasing DO in the EFs. The soil type 2 was found to be more effective in increasing DO in the EFs, which might be attributed to the nature of the soil type used, where the worms' activity could be enhanced hence creating an aerobic environment in the VF.

4.3.1.6 Turbidity

An overview of the turbidity profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.12**. Temporal graphical representations of this data for IN and EFs 1-3, throughout the Phase I and phase II experimental periods, are presented in **Figures 4.28** & **4.29**, respectively. It is interesting to observe that though a high variation in IN turbidity was observed, there was a significant removal of turbidity in the EFs. More

interestingly, the turbidity increased just after the introduction of worms on February 4, 2014, which stabilized on further experimental runs. This observation suggests that the worms are effective in reducing turbidity and this may be due to the removal of suspended organic materials (as worms feed on them). The system appears to be quite robust as is evidenced by the removal efficiency performed, during Phase I, on highly turbid IN on September 2 & 9, 2013. In terms of turbidity removal, the second (sand) and third (gravel) layers are also seen to be effective, though the first (soil) layer contributes most. For instance, the average IN turbidity in OC1 was reduced by 73% in EF1, by 10% further in EF2 and by 5% further in EF3.

In terms of the effect of OCs on the reduction of IN turbidity, in Phase I, **Table 4.12** and **Figure 4.28** suggests that the OC3 is more suitable than OC1 and OC2. Though the % removal is higher for OC1 and OC2 in the final effluent (EF3) with worms, the removal efficiency is higher for OC3 in EF1; 73% in OC1, 71% in OC2 and 77% in OC3 for EF1. Thus, higher HRT and low HLR remove turbidity better than low HRT and high HLR.

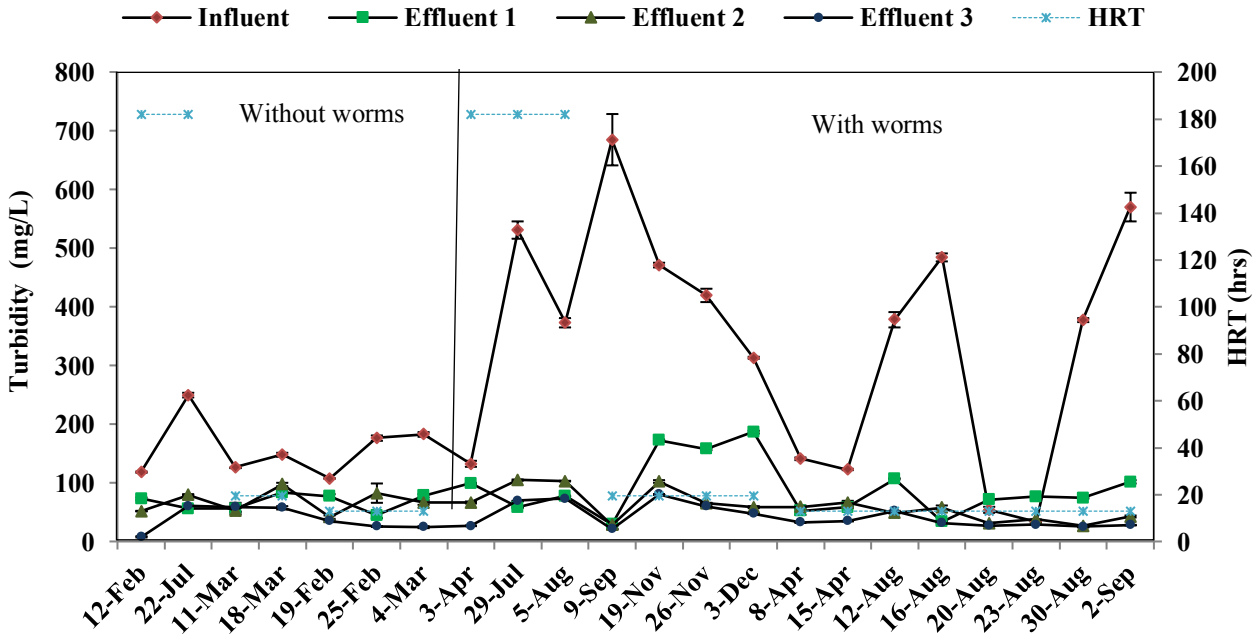


Figure 4.28 Turbidity profile throughout the experimental period in Phase I. Error bars are standard error, n =3.

Table 4.12 An overview of the turbidity profile for Phase I and Phase II. The reported % change is calculated with respect to the average IN turbidity for each specific run period.

		Without worms				With worms			
Turbidity	**OCs**	IN	EF1	EF2	EF3	IN	EF1	EF2	EF3
Phase I									
Mean ± SE	1	156 ± 24	67 ± 11	64 ± 12	29 ± 3	271 ± 73	72 ± 9	47 ± 5	33 ± 3
	2	138 ± 11	70 ± 14	76 ± 22	59 ± 10	472 ± 78	137 ± 36	64 ± 15	53 ± 12
	3	184 ± 66	65 ± 9	66 ± 14	35 ± 26	346 ± 116	79 ± 12	92 ± 13	57 ± 15
Range	1	108 - 183	45 - 79	42 - 83	25 - 35	34 - 570	35 - 107	27 - 67	26 - 53
	2	127 - 148	57 - 84	53 - 98	58 - 60	313 - 685	30 - 187	28 - 103	22 - 81
	3	119 - 250	57 - 74	52 - 80	8 - 61	132 - 531	59 - 99	67 - 105	27 - 74
% Change	1	-	57	59	81	-	73	83	88
	2	-	49	45	57	-	71	86	89
	3	-	65	64	81	-	77	73	84
Phase II									
Mean ± SE	2	504 ± 82	153 ± 33	122 ± 28	109 ± 20	406 ± 37	141 ± 54	70 ± 7	68 ± 8
Range	2	388 - 662	106 - 217	86 - 176	79 - 146	138 - 563	62 - 623	45 - 113	47 - 124
% Change	2	-	70	76	78	-	65	83	83

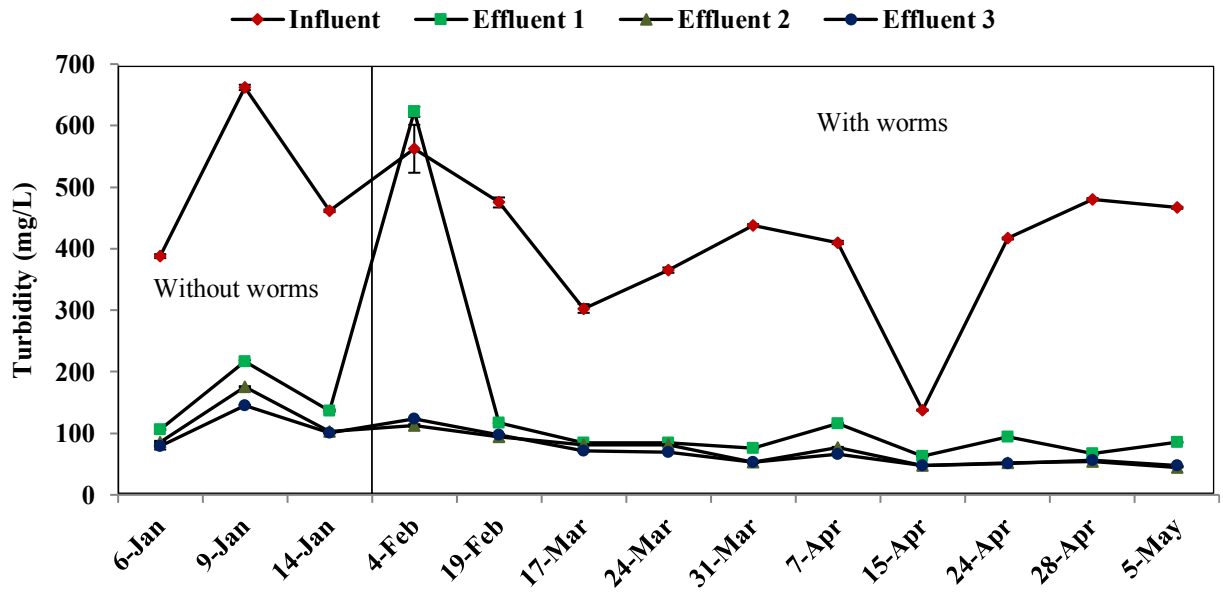


Figure 4.29 Turbidity profile throughout the experimental period in Phase II. Error bars are standard error, n =3.

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.30 (a)** & **(b)** depicting the time-averaged turbidity for the VF with and without worms, over Phases I and II, respectively.

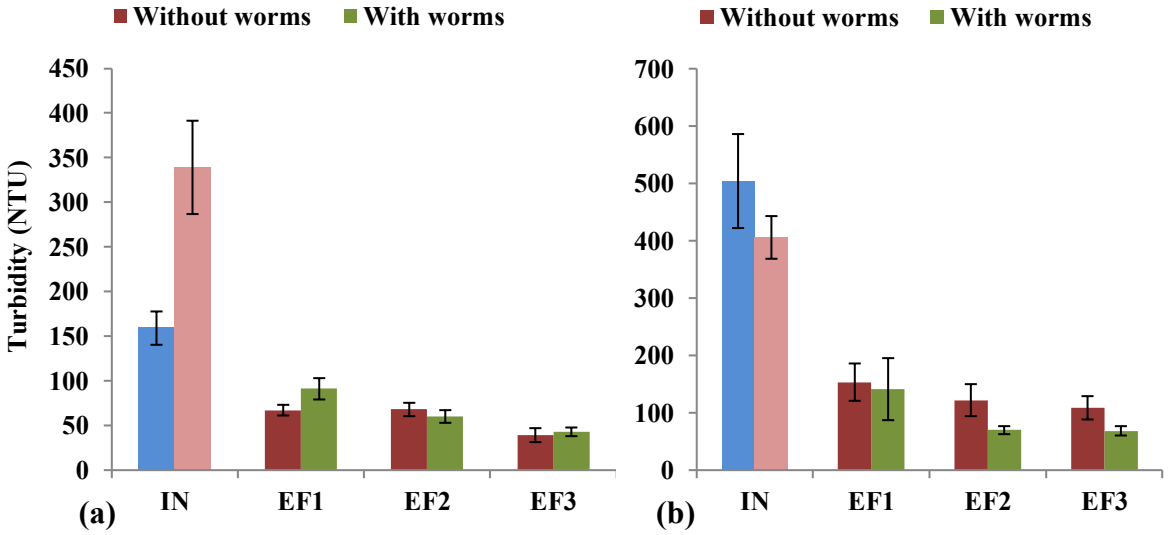


Figure 4.30 Time-averaged turbidity levels for (a) Phase I and (b) Phase II. Error bars represent standard errors. In Phase I, n = 7, for 'without worms' & n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms' & n = 10, for 'with worms'. Note: For the IN, it was observed that for the different experimental runs (i.e. for the periods of time when the system was run with worms (pink bar) and without worms (blue bar), the average turbidity was found to be significantly different as is indicated in the above graphs. Therefore, the % change in this parameter was related to the average IN turbidity for the specific run period.

These plots highlight the significance of worms in stabilizing the turbidity in the EFs, as is evidenced in **Figure 4.30 (b)**. Though this effect is not clear in **Figure 4.30 (a)**, it may be hindered by different OCs. Moreover, these plots depict the significance of soil type in the VF and other geo-layers (sand and gravel) in reducing turbidity from the IN. The finding in this study reflects the observation by Chaudhari (2006), which showed that the vermifiltration system achieved more than 98 % removal of turbidity in both systems, with and without earthworms. Here, the geological system in the unit could have played a vital role in the reduction of turbidity by adsorption of suspended particles on the surface of soil, sand and gravel.

4.3.1.7 Total Suspended Solids

An overview of the TSS profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.13**. Temporal graphical representations of this data for IN and EFs 1-3, throughout the Phase I and phase II experimental periods, are presented in **Figures 4.31** & **4.32**, respectively.

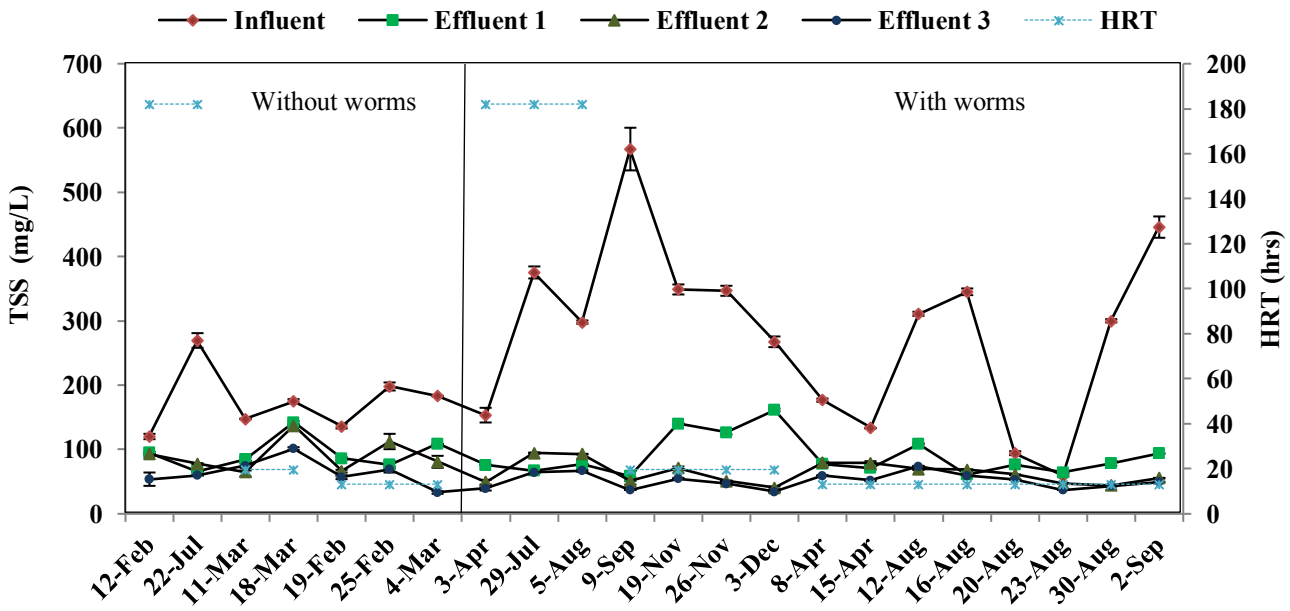


Figure 4.31 Total suspended solids profile throughout the experimental period in Phase I. Error bars are standard errors, n =3.

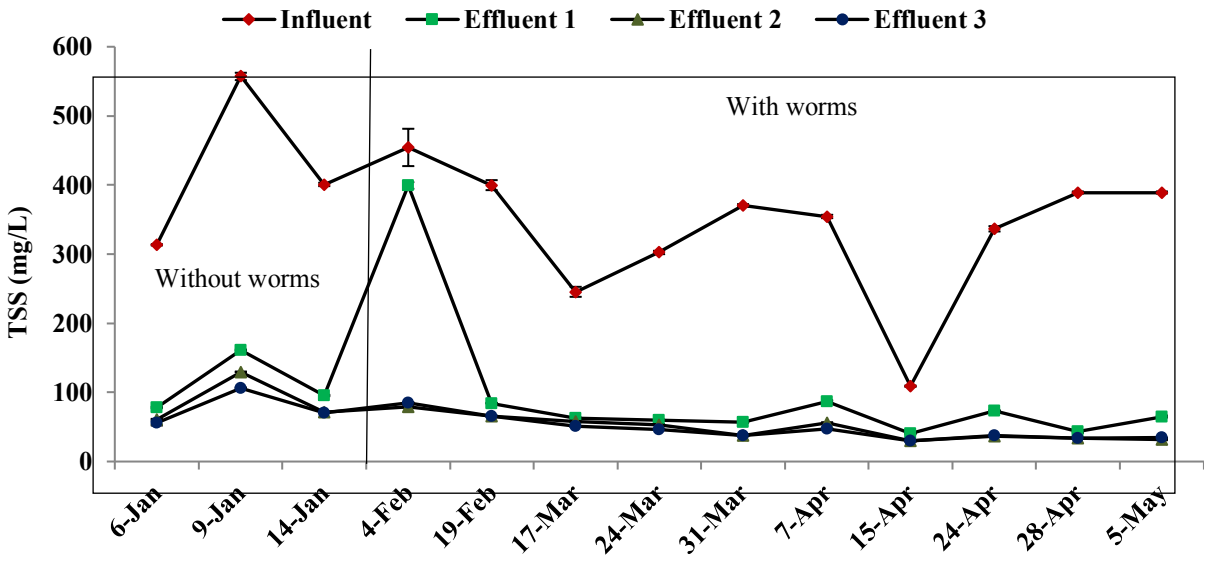


Figure 4.32 Total suspended solids profile throughout the experimental period in Phase II. Error bars are standard errors, n =3.

Similar to the turbidity removal, the VF was found to reduce TSS significantly in EFs, irrespective of the high variation of TSS in the IN. Again, interestingly, the TSS was found to be high in EF1 on February 4, 2014 - just after the introduction of worms. However, it stabilized on further experimental runs. In terms of the effect of OCs, the OC3 is more favourable in reducing TSS in EFs. This suggests that a high HRT and low HLR is more effective for increased removal efficiency, for instance, the TSS was reduced by 66 % for OC1 but reduced by 73 % for OC3 in EF1 with worms.

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.33 (a)** & **(b)** depicting the time-averaged TSS for the VF with and without worms, over Phases I and II, respectively.

These plots highlight a significant reduction of TSS in the EFs and shows that the TSS tends to stabilize with worms in soil type 2. Moreover, these plots reflect that soil type 2 seems to reduce TSS better than soil type 1. For instance, **Table 4.13**, shows that soil type 1 reduced TSS in EF1 by only 30 % whereas soil type 2 reduced it by 74 %. This observation supports the notion that the worms feed on the solid and improve the adsorption performance of the soil profile.

Table 4.13 An overview of the TSS profile for Phase I and Phase II. The reported % change is calculated with respect to the average IN TSS for each specific run period.

		Without worms				With worms			
TSS	**OCs**	IN	EF1	EF2	EF3	IN	EF1	EF2	EF3
Phase I									
Mean ± SE	1	172 ± 19	90 ± 10	86 ± 14	53 ± 10	233 ± 48	79 ± 6	62 ± 5	53 ± 4
	2	161 ± 14	113 ± 29	101 ± 36	88 ± 13	383 ± 64	122 ± 22	53 ± 6	43 ± 5
	3	195 ± 75	80 ± 15	85 ± 8	57 ± 3	275 ± 65	73 ± 3	79 ± 15	57 ± 9
Range	1	136 - 198	76 - 109	66 - 112	33 - 69	59 - 446	61 - 108	44 - 80	37 - 74
	2	147 - 175	85 - 142	65 - 137	75 - 101	267 - 567	59 - 161	40 - 71	34 - 54
	3	120 - 269	66 - 95	78 - 93	54 - 60	153 - 375	67 - 77	49 - 94	39 - 67
% Change	1	-	48	50	69	-	66	73	77
	2	-	30	37	45	-	68	86	89
	3	-	59	56	71	-	73	71	79
Phase II									
Mean SE	2	424 ± 71	112 ± 25	87 ± 21	77 ± 15	335 ± 31	97 ± 34	48 ± 5	47 ± 5
Range	2	313 - 557	78 - 161	61 - 129	55 - 107	109 - 455	41 - 399	30 - 79	30 - 85
% Change	2	-	74	79	82	-	71	86	86

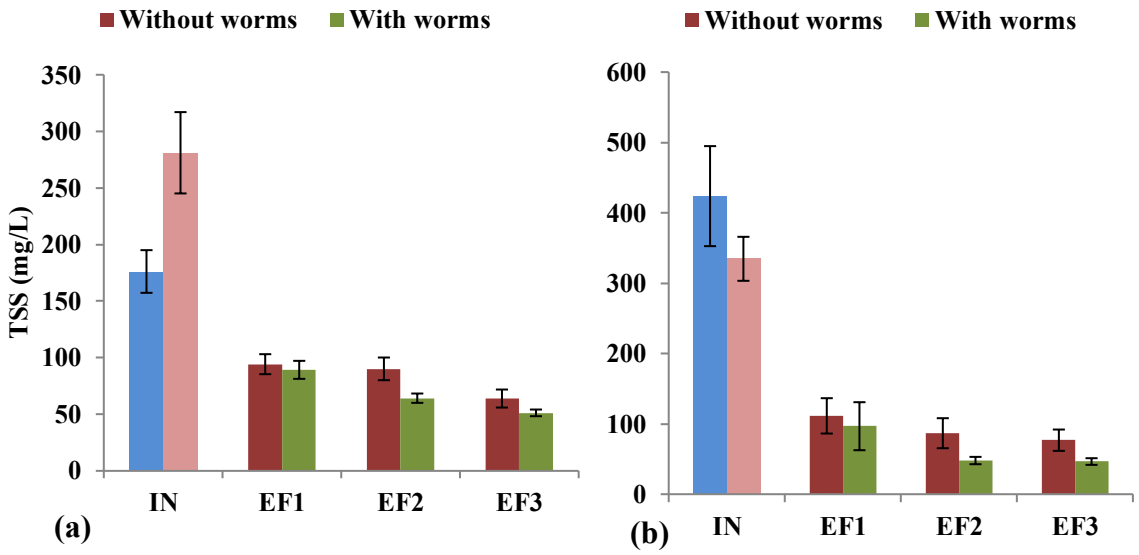


Figure 4.33 Time-averaged TSS levels for (a) Phase I and (b) Phase II. Error bars represent the standard errors. Phase I, n = 7, for 'without worms' & n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms' & n = 10, for 'with worms'. Note: For the IN, it was observed that for the different experimental runs (i.e. for the periods of time when the system was run with worms (pink bar) and without worms (blue bar), the average TSS was found to be significantly different as is indicated in the above graphs. Therefore, the % change in this parameter was related to the average IN TSS for the specific run period.

4.3.2 Organic matter removal

4.3.2.1 Chemical Oxygen Demand

An overview of the COD profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.14**. Temporal graphical representations of this data for IN and EFs 1-3, throughout the Phase I and phase II experimental periods, are presented in **Figures 4.34** & **4.35**, respectively. There was a high variation in IN COD, similar to turbidity and TSS, for both Phases. However, it was reduced significantly in EFs, especially in the presence of worms. It is interesting to observe that though the COD increased dramatically in EF1 on February 4, 2014, just after introducing worms in the VF, it was well taken care of by the second and third geo-layers. Thus, the VF appears to be a quite robust technology. With respect to the effect of worms in reducing COD, the observations suggest that a higher removal was achieved in presence of worms, as shown in **Table 4.14**, e.g., in Phase I, COD reduction was found to be only by 5% without worms and by 27% with worms, for EF1.

In terms of the effect of the OCs on the removal of COD, the OC3 was more favourable in reducing COD in EFs. Thus, a high HRT and Low HLR were more effective in obtaining EFs with less COD. This finding reflects the observation of Malek et al. (2013), who reported that less HLR and high worm density is the most suitable condition for the removal of COD for palm oil mill effluent where 82 - 96 % COD removal was achieved, using *Eudrilus eugeniae*.

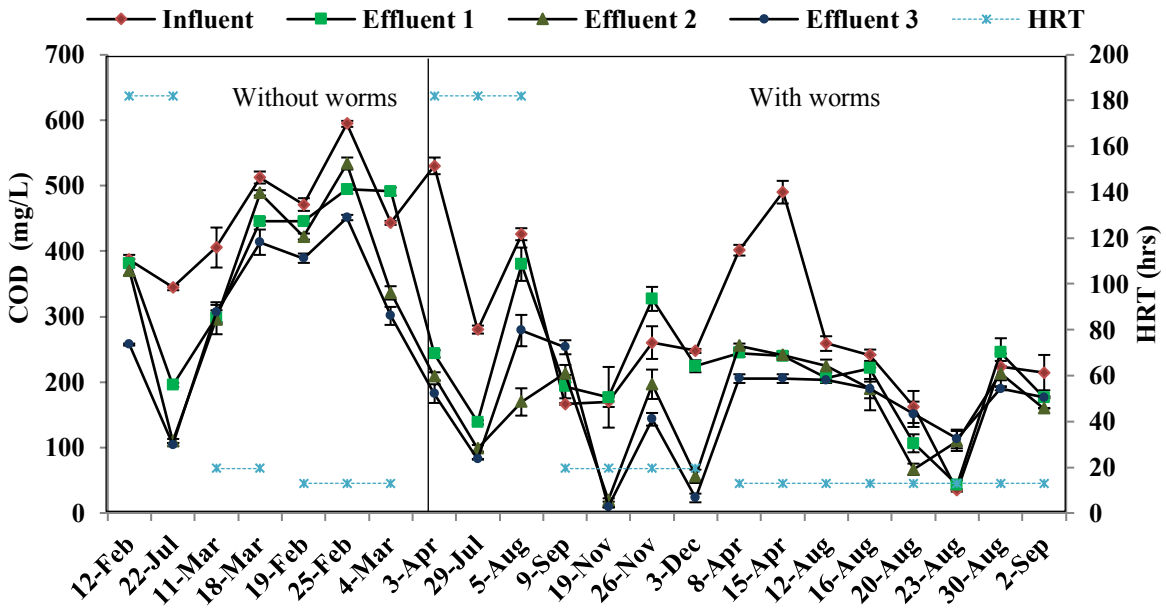


Figure 4.34 Chemical oxygen demand profile throughout the experimental period in Phase I. Error bars are standard errors, n =3.

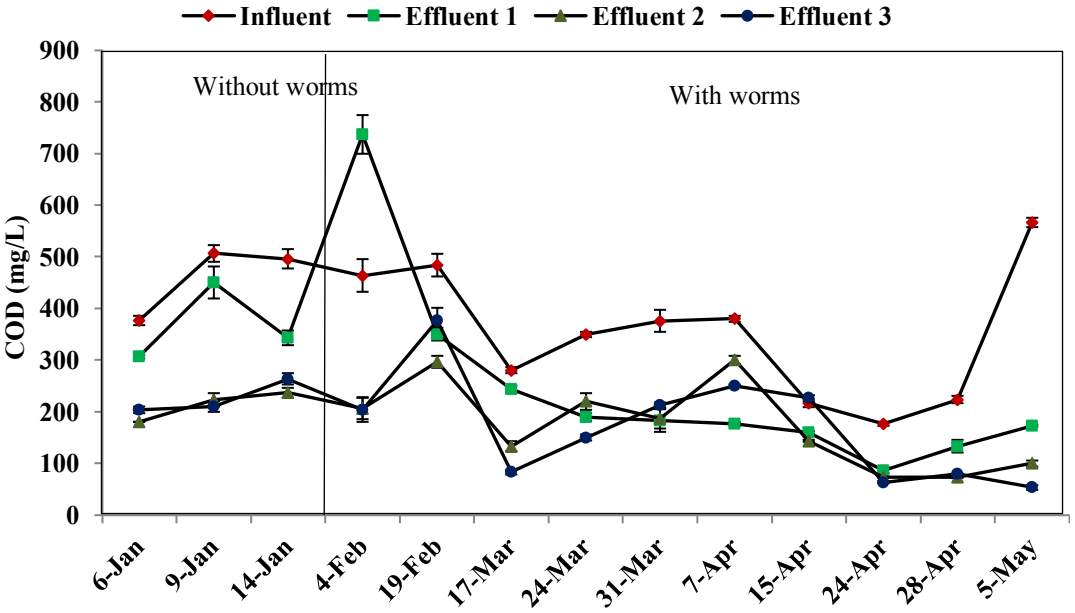


Figure 4.35 Chemical oxygen demand profiles throughout the experimental period in Phase II. Error bars are standard errors, n =3.

Table 4.14 An overview of the COD profile for Phase I and Phase II. The reported % change is calculated with respect to the average IN COD for each specific run period.

		Without worms				With worms			
COD	**OCs**	IN	EF1	EF2	EF3	IN	EF1	EF2	EF3
Phase I									
Mean ± SE	1	503 ± 46	477 ± 16	431 ± 57	380 ± 44	253 ± 49	186 ± 26	183 ± 23	179 ± 11
	2	459 ± 53	372 ± 73	392 ± 97	360 ± 53	211 ± 25	230 ± 34	122 ± 49	107 ± 57
	3	366 ± 21	288 ± 93	240 ± 130	181 ± 77	412 ± 72	254 ± 70	159 ± 32	181 ± 57
Range	1	443 - 594	446 - 494	337 - 533	301 - 451	34 - 490	43 - 246	67 - 256	113 - 206
	2	406 - 512	299 - 446	296 - 489	307 - 413	167 - 260	177 - 327	20 - 213	10 - 253
	3	344 - 387	196 - 381	110 - 370	104 - 258	280 - 530	139 - 380	99 - 209	82 - 279
% Change	1	-	5	14	24	-	27	28	29
	2	-	19	15	22	-	-9	42	49
	3	-	21	34	50	-	38	61	56
Phase II									
Mean SE	2	460 ± 42	367 ± 43	213 ± 17	226 ± 19	352 ± 41	197 ± 23	173 ± 26	170 ± 33
Range	2	377 - 507	307 - 450	180 - 237	203 - 263	177 - 567	87 - 350	73 - 300	53 - 377
% Change	2	-	20	54	51	-	44	51	52

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.36 (a)** & **(b)** depicting the time-averaged COD for the VF, with and without worms, over Phases I and II, respectively.

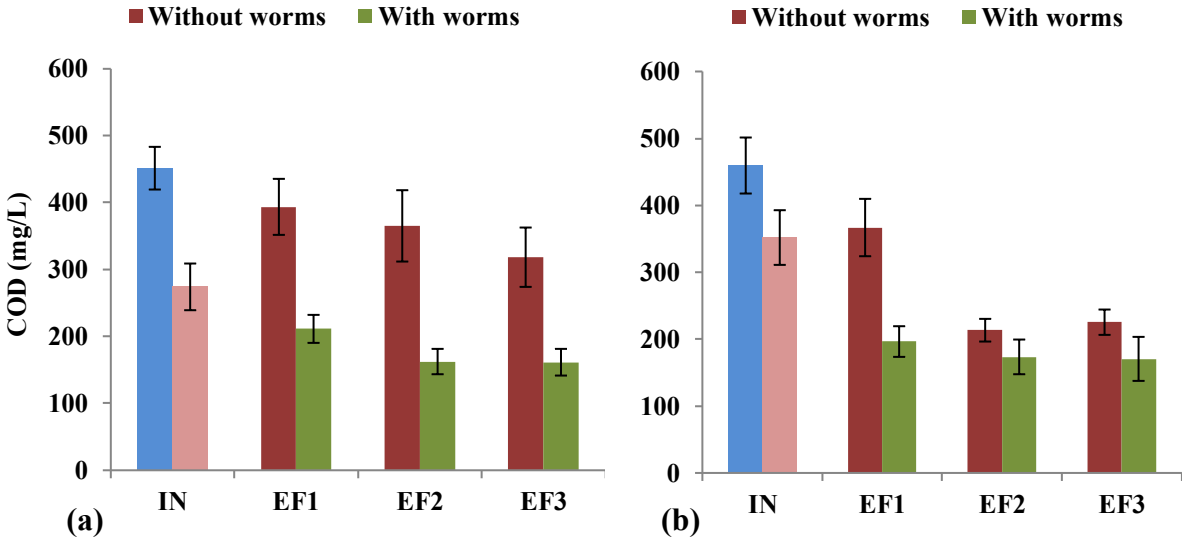


Figure 4.36 Time-averaged COD levels for (a) Phase I and (b) Phase II. Error bars represent standard errors. In Phase I, n = 7, for 'without worms' & n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms' & n = 10, for 'with worms'. Note: For the IN, it was observed that for the different experimental runs (i.e. for the periods of time when the system was run with worms (pink bar) and without worms (blue bar), the average COD was found to be significantly different as is indicated in the above graphs. Therefore, the % change in this parameter was related to the average IN COD for the specific run period.

These plots highlight the effect of worms in the removal and stabilization of COD in EFs, irrespective to the soil type. The results indicate that the worms played a significant role in decomposing the organic matter in the sewage via enzymatic action - biological catalysis. This finding reflects the findings of Chaudhari 2006, which report that the average COD removal from sewage (municipal wastewater) in their vermifiltration system was more than 45 % with worms, while without earthworms it was only 18 %, at a HRT of 1-2 hours. It was argued that COD removal in the system with earthworms was much higher than the 'geo-microbial system' i.e. without worms. Here, the enzymatic activity in the gut of earthworms was implicated in the degradation of the chemicals which cannot be achieved by microbial activity alone.

4.3.2.2 Biological Oxygen Demand

BOD_5 measurement in wastewater samples were performed only on a few sampling occasions. Here, only the measurements carried out, for the IN and EFs, collected on April 3, 2013 are presented. This is during the Phase I experimental run, with soil type 1, with worms. **Figure 3.37** suggests that there was a significant removal of BOD_5 and all three geo-layers had a significant effect on the overall removal. The BOD was observed to be reduced by 63 % after passage through the first filter media (soil), which was subsequently reduced by 17 % and 14 % when passed through the second (sand) and third (gravel) layers, respectively. Therefore, 274 mg/L of BOD_5 in IN reduced to 15 mg/L in EF3, which is a reduction of 94 %. On this sampling occasion, the COD was reduced by 66 % and the TOC was reduced by 64 %. Thus, the BOD_5 removal was significantly higher than COD and TOC removal.

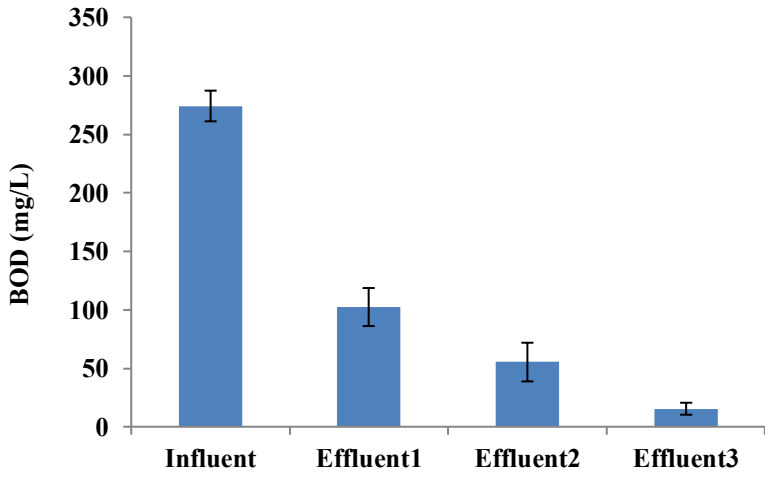


Figure 4.37 The BOD_5 level observed in the sample collected on April 3, 2013. Error bars represent standard errors, where, n = 4.

A significant decrease of BOD_5 in EFs may be attributed to the removal of organic compounds present in the IN during the treatment process. The high removal of BOD_5 in EFs may be attributed to the enzymatic action of the worms in decomposing the organic matter present in IN, as described by Sinha et al. (2008b), highlighting the difference between microbial degradation and vermin-degradation. This indicates the efficacy of a synchronous action of worms and microbes over the action of a microbial system only in the geological system (Arroyo et al. 2010; Rajpal et al. 2012; Kumar et al. 2014).

4.3.2.3 Total Organic Carbon

An overview of the TOC profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.15**. Temporal graphical representations of this data for IN and EFs 1-3, throughout the Phase I and phase II experimental periods, are presented in **Figures 4.38** & **4.39**, respectively. Similar to other parameters observed, there was also a high variation in IN TOC. Though a clear trend was not observed without worms, it was reduced significantly in EFs with worms and has a tendency to stabilize. Interestingly, the TOC increased dramatically on February 4, 2014, like turbidity, TSS and COD, after the introduction of worms. However, it was taken care of by the other two geo-layers – sand and gravel. This observation suggests that the soil type 1 with worms seems to perform better in reducing TOC.

With respect to the effect of OCs in reducing TOC in EFs, OC3 was found to be more suitable with worms. Thus, a high HRT and low HLR is effective in achieving a higher reduction of TOC in EFs.

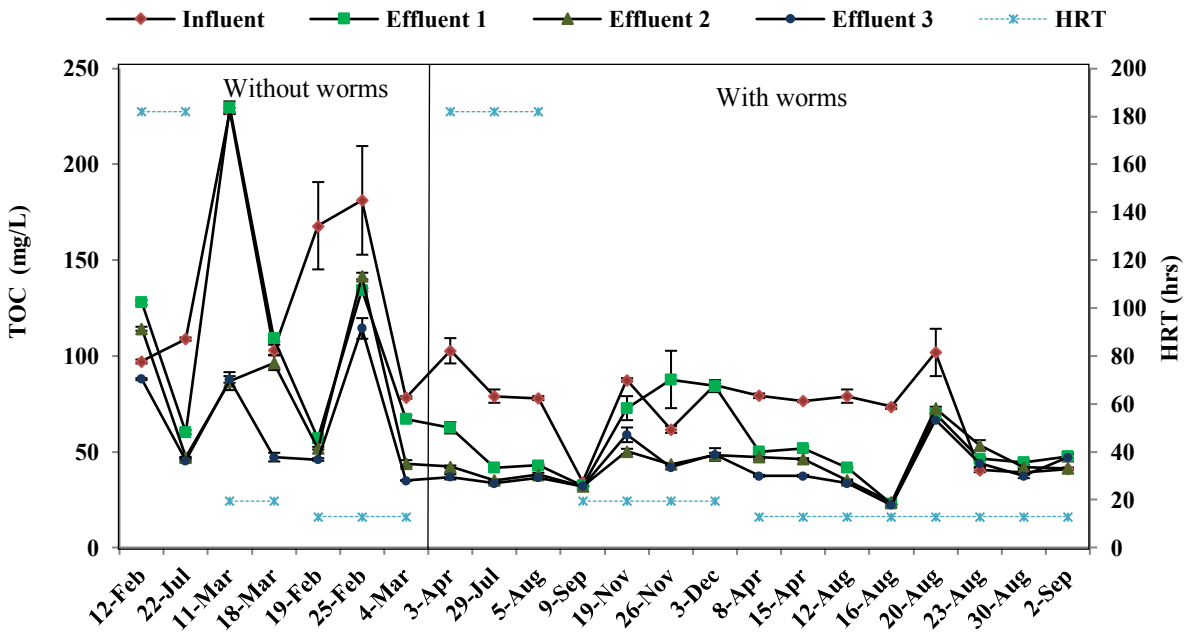


Figure 4.38 TOC profile throughout the experimental period in Phase I. Error bars are standard errors, n = 3.

Table 4.15 An overview of the TOC profile for Phase I and Phase II. The reported % change is calculated with respect to the average IN TOC for each specific run period.

		Without worms				With worms			
TOC	**OCs**	IN	EF1	EF2	EF3	IN	EF1	EF2	EF3
Phase I									
Mean ± SE	1	143 ± 32	86 ± 24	79 ± 31	65 ± 25	66 ± 8	47 ± 5	45 ± 5	41 ± 5
	2	165 ± 62	169 ± 60	92 ± 5	67 ± 20	67 ± 12	69 ± 13	44 ± 4	45 ± 6
	3	103 ± 6	94 ± 34	81 ± 34	67 ± 21	87 ± 8	49 ± 7	39 ± 2	36 ± 1
Range	1	78 - 181	57 - 134	44 - 142	35 - 114	39 - 102	23 - 70	24 - 73	22 - 67
	2	103 - 228	109 - 230	87 - 96	47 - 88	34 - 88	32 - 88	32 - 50	32 - 59
	3	97 - 109	60 - 128	47 - 114	45 - 88	78 - 103	42 - 63	35 - 42	34 - 37
% Change	1	-	40	44	54	-	29	32	38
	2	-	-2	45	60	-	-3	34	33
	3	-	9	22	35	-	43	55	59
Phase II									
Mean SE	2	104 ± 30	96 ± 31	76 ± 22	64 ± 24	71 ± 5	54 ± 11	41 ± 4	41 ± 4
Range	2	46 - 150	58 - 157	44 - 118	34 - 111	28 - 92	20 - 133	24 - 63	24 - 60
% Change	2	-	8	27	39	-	24	42	43

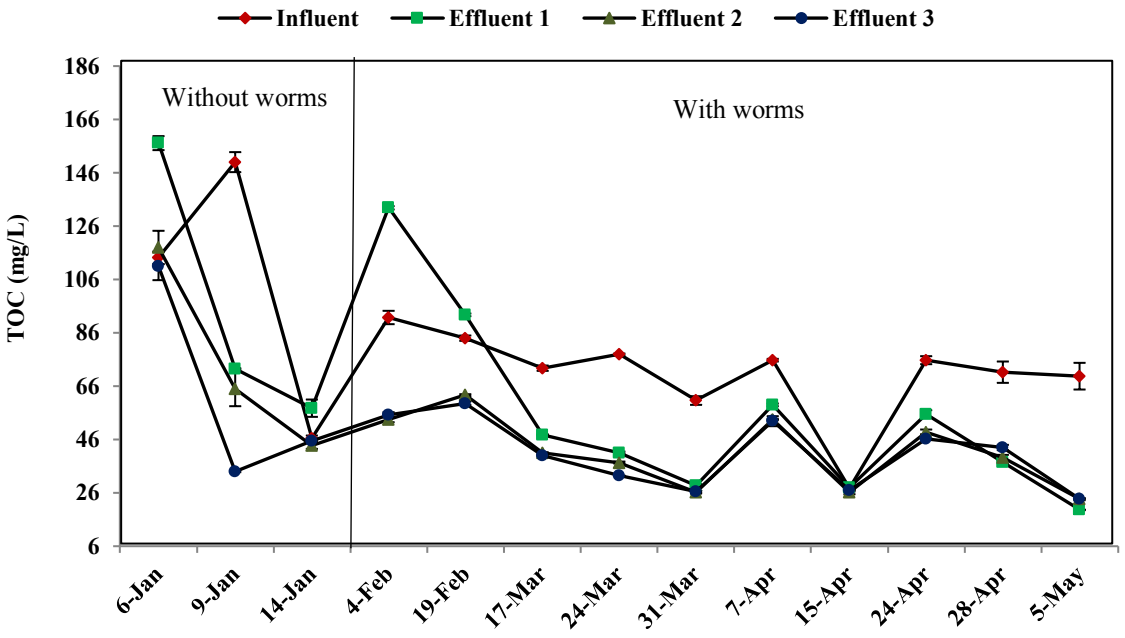


Figure 4.39 TOC profile throughout the experimental period in Phase II. Error bars are standard errors, n = 3.

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.40 (a)** & **(b)** depicting the time-averaged TOC for the VF with and without worms, over Phases I and II, respectively.

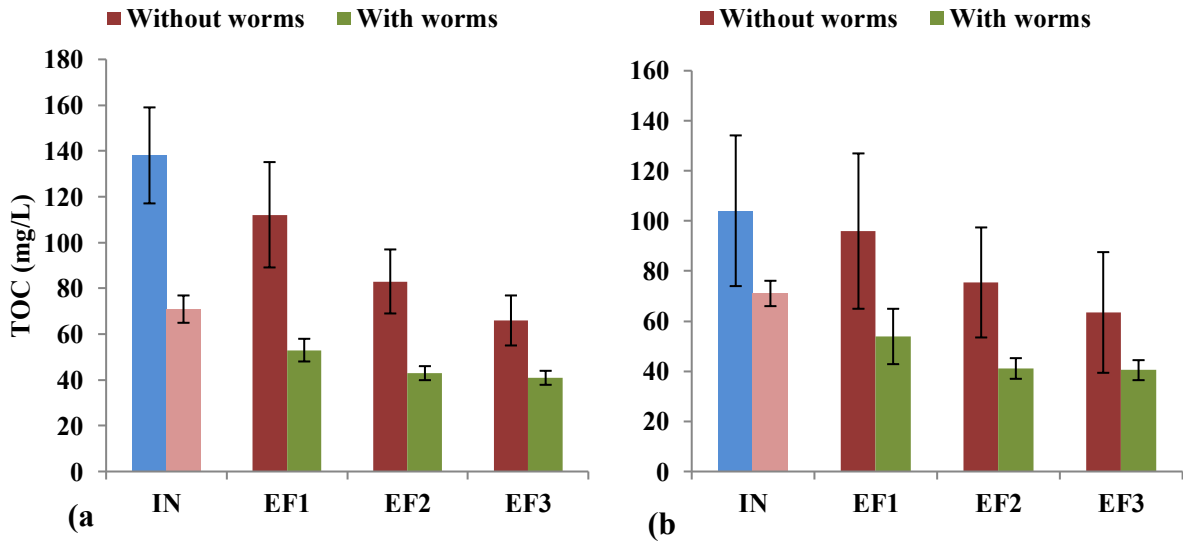


Figure 4.40 Time-averaged TOC levels for (a) Phase I and (b) Phase II. Error bars represent standard errors. In Phase I, n = 7, for 'without worms' & n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms' & n = 10, for 'with worms'. Note: For the IN, it was observed that for the different experimental runs (i.e. for the periods of time when the system was run with worms (pink bar) and without worms (blue bar), the average TOC was found to be significantly different as is indicated in the above graphs. Therefore, the % change in this parameter was related to the average IN TOC for the specific run period.

These plots highlight the significant reduction of TOC in the EFs. Without worms, both Phases follow a similar trend of a gradual decrease of TOC, as IN is passed through three consecutive filter layers. It is clear from the plots that the worms play a significant role in stabilization, which does not appear in the VF without worms.

4.3.3 Nutrients

During the overall test period, values of two different nutrients - TN and TP decreased steadily throughout the three different layers. Similarly, the significant removal of three different forms of nitrogen - NH_3-N, NO_2-N and NO_3-N has been achieved in the final effluent.

4.3.3.1 Total Nitrogen

An overview of the TN profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.16**. Temporal graphical representations of this data for IN and EFs 1-3, throughout the Phase I and phase II experimental periods, are presented in **Figures 4.41** & **4.42**, respectively. These figures show that though there was a high variation in IN TN, a significant reduction was observed in EF1, which was reduced further by second (sand) and third (gravel) layers. Here, the reduction in the efficiency of TN removal after the introduction of worms in the VF might be attributed to the nutrient contributed by worms. This is more distinct in Phase II on February 4 and 19, 2014, where TN increased in EF1, whereas reduced by sand and gravel layers. Moreover, the contribution of TN in EF1 during the later stage, in Phase I, November 26 and December 3, 2013 is evidence of the contribution to the TN by worms. This might be due to the accumulation of vermicast in the soil layer as the VF becomes mature. Furthermore, the soil type 2 seems to be more favourable in stabilizing TN in the system, and this characteristic was not well defined in soil type 1.

With respect to the effect of OCs on the removal efficiency of TN, the OC3 was found to be more favourable. Thus, a high HRT and low HLR has better TN removal efficiency, e.g., for

OC1 the TN removal efficiency was only 28 % whereas it was 51 % for OC3, without worms.

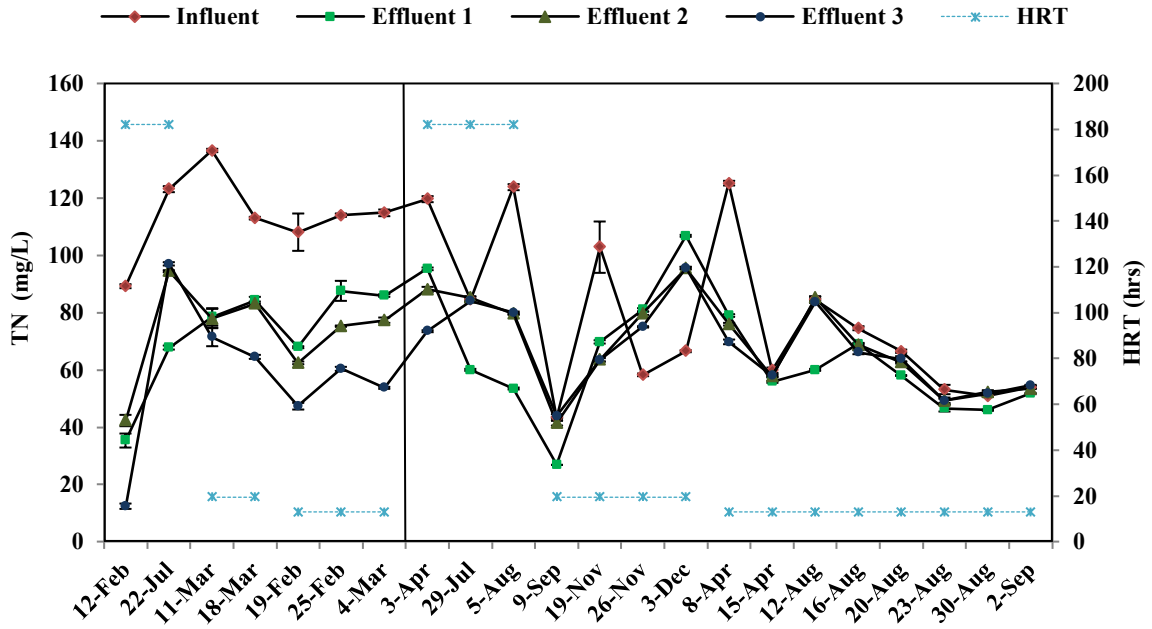


Figure 4.41 Total nitrogen profile throughout the experimental period in Phase I. Error bars are standard errors, n = 3.

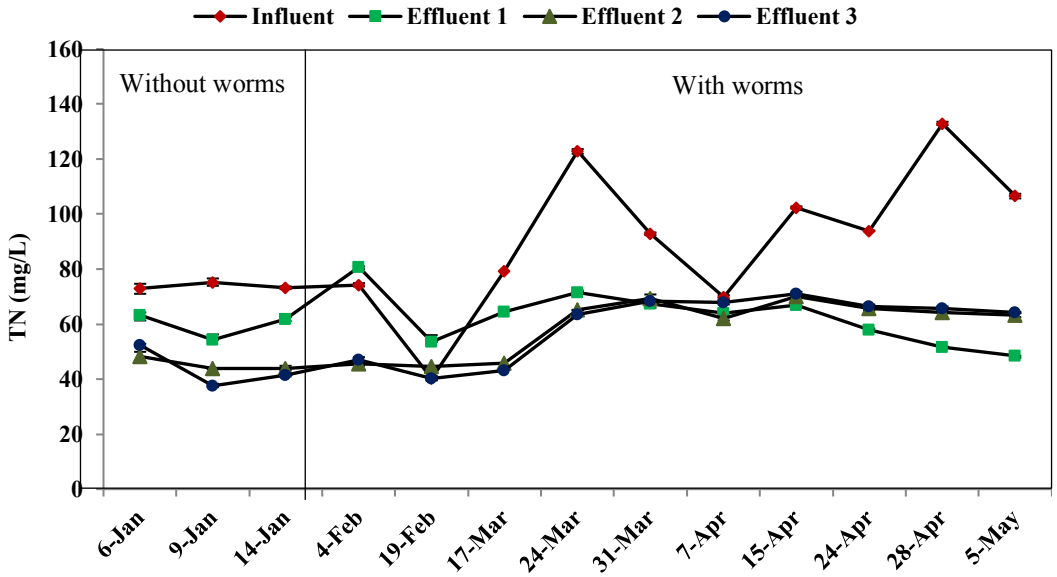


Figure 4.42 Total nitrogen profile throughout the experimental period in Phase II. Error bars are standard errors, n = 3.

Table 4.16 An overview of the TN profile for Phase I and Phase II. The reported % change is calculated with respect to the average IN TN for each specific run period.

		Without worms				With worms			
TN	**OCs**	IN	EF1	EF2	EF3	IN	EF1	EF2	EF3
Phase I									
Mean ± SE	1	112 ± 2	81 ± 5	72 ± 5	54 ± 4	71 ± 9	59 ± 4	63 ± 5	62 ± 4
	2	125 ± 12	82 ± 3	81 ± 3	68 ± 4	68 ± 13	71 ± 17	70 ± 12	70 ± 11
	3	106 ± 17	52 ± 16	68 ± 26	55 ± 42	109 ± 12	70 ± 13	84 ± 2	79 ± 3
Range	1	108 - 115	68 - 88	62 - 77	47 - 60	51 - 125	46 - 79	49 - 85	49 - 84
	2	113 - 137	78 - 84	78 - 83	65 - 72	43 - 103	27 - 107	41 - 96	44 - 96
	3	89 - 123	35 - 68	42 - 95	12 - 97	85 - 124	52 - 95	80 - 88	74 - 84
% Change	1	-	28	36	52	-	17	11	13
	2	-	34	35	46	-	-5	-3	-3
	3	-	51	36	48	-	36	23	28
Phase II									
Mean SE	2	74 ± 1	60 ± 3	45 ± 1	44 ± 4	92 ± 9	63 ± 3	60 ± 3	60 ± 4
Range	2	73 - 75	54 - 63	44 - 48	38 - 52	40 - 133	49 - 81	45 - 70	40 - 71
% Change	2	-	19	39	41	-	32	35	35

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.43 (a)** & **(b)** depicting the time-averaged TN for the VF with and without worms, over Phases I and II, respectively.

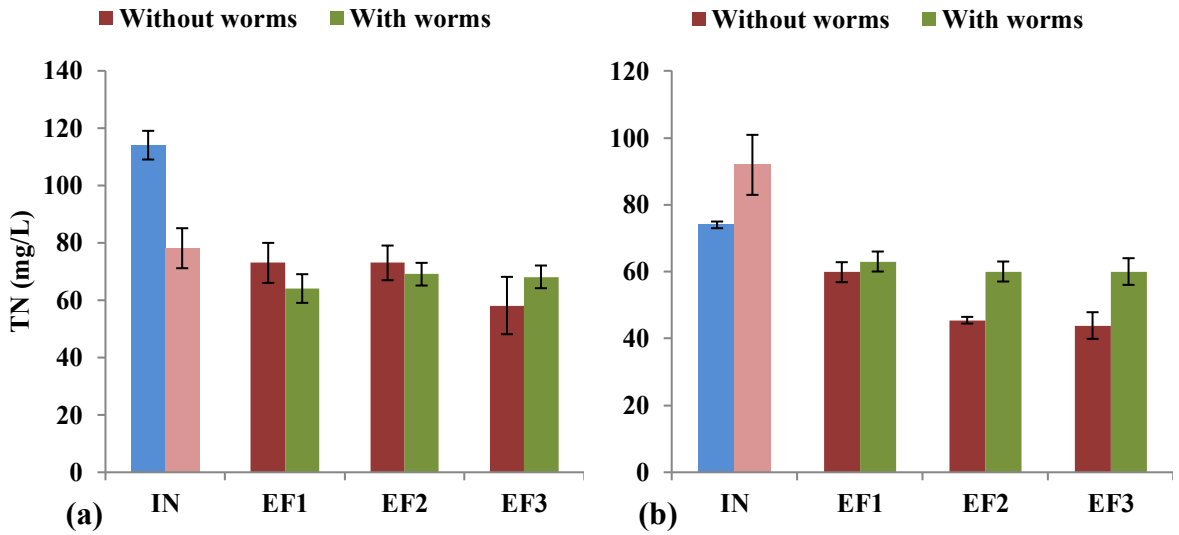


Figure 4.43 Time-averaged TN levels for (a) Phase I and (b) Phase II. Error bars represent standard errors. In Phase I, n = 7, for 'without worms' & n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms' & n = 10, for 'with worms'. Note: For the IN, it was observed that for the different experimental runs (i.e. for the periods of time when the system was run with worms (pink bar) and without worms (blue bar), the average TN was found to be significantly different as is indicated in the above graphs. Therefore, the % change in this parameter was related to the average IN TN for the specific run period.

These plots highlight the significance of geo-layers in reducing TN in EFs, irrespective of presence or absence of worms. Here, worms seem to stabilize the TN, with soil type 2. However, it is difficult to say the same for soil type 1.

4.3.3.2 Ammonium Nitrogen

An overview of the NH_3-N profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.17**. Temporal graphical representations of this data for IN and EFs 1-3, throughout the Phase I and phase II experimental periods, are presented in **Figures 4.44** & **4.45**, respectively. A much higher variation in IN NH_3-N was observed during Phase I than in Phase II. The observation shows that the VF without worms was more favourable in removing NH_3-N than with worms. It is interesting to observe that worms contribute NH_3-N

in EFs as the unit gets mature (see experimental run on November 26 and December 3, 2013), which may be due to a release of NH_3-N from vermicasts accumulated on the top.

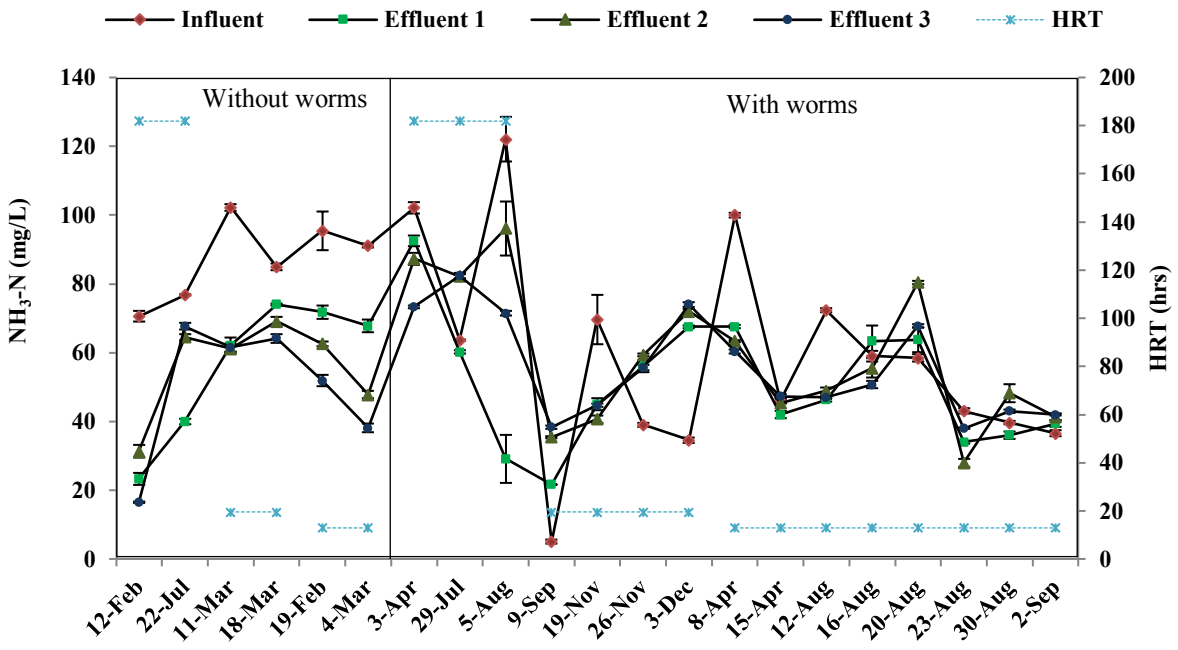


Figure 4.44 NH_3-N profile throughout the experimental period in Phase I. Error bars are standard errors, n = 3.

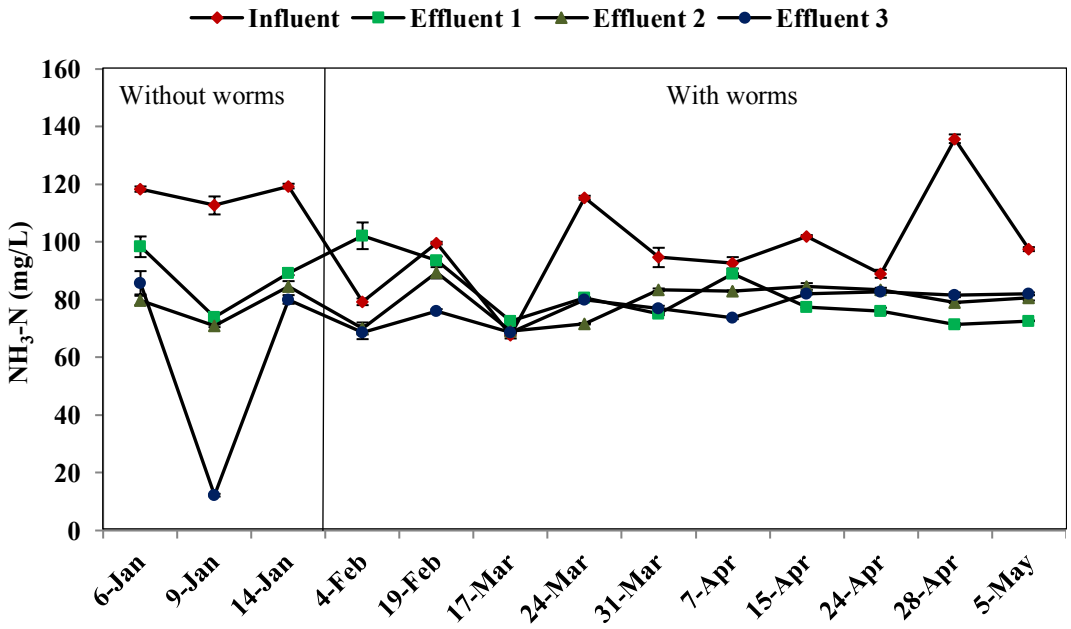


Figure 4.45 NH_3-N profile throughout the experimental period in Phase II. Error bars are standard errors, n = 3.

Table 4.17 An overview of the NH_3-N profile for Phase I and Phase II. The reported % change is calculated with respect to the average IN NH_3-N for each specific run period.

		Without worms				With worms			
NH_3-N	**OCs**	IN	EF1	EF2	EF3	IN	EF1	EF2	EF3
Phase I									
Mean ± SE	1	93 ± 2	70 ± 2	55 ± 7	45 ± 7	57 ± 7	49 ± 5	52 ± 6	50 ± 4
	2	94 ± 9	68 ± 6	65 ± 4	63 ±1	37 ± 13	48 ± 10	52 ± 8	53 ± 8
	3	74 ± 3	32 ± 8	48 ± 17	42 ± 26	96 ± 17	61 ± 18	89 ± 4	76 ± 3
Range	1	91 - 95	68 - 72	48 - 63	38 - 52	37 - 100	34 - 68	28 - 80	38 - 70
	2	85 - 102	62 - 74	61 - 69	62 - 64	5 - 70	22 - 68	35 - 72	38 - 74
	3	71 - 77	23 - 40	31 - 65	16 - 68	64 - 122	29 - 93	82 - 96	71 - 82
% Change	1	-	25	41	52	-	14	9	13
	2	-	27	30	33	-	-30	-40	-43
	3	-	57	35	43	-	37	8	21
Phase II									
Mean SE	2	117 ± 2	87 ± 7	78 ± 4	59 ± 24	97 ± 6	81 ± 3	79 ± 2	77 ± 2
Range	2	113 - 119	74 - 98	71 - 85	12 - 86	68 - 136	71 - 102	69 - 83	69 - 83
% Change	2	-	26	33	49	-	16	19	20

Moreover, in Phase II, though NH_3-N was found to increase in EF1 just after the introduction of worms, it was subsequently reduced by the second and third layers and the NH_3-N was stabilized on further runs. In terms of the effect of OCs of the reduction of NH_3-N in EFs, OC3 was found to be more favourable; though the sand layer contributed more NH_3-N to the EFs that was removed by the soil layer.

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.46 (a)** & **(b)** depicting the time-averaged NH_3-N for the VF with and without worms, over Phases I and II, respectively.

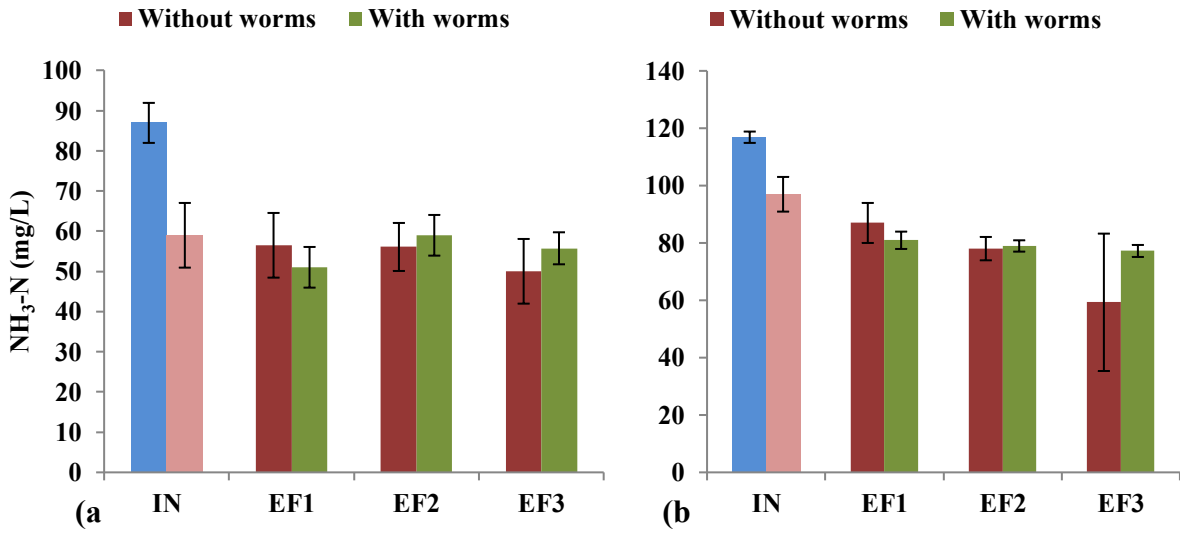


Figure 4.46 Time-averaged NH_3-N levels for (a) Phase I and (b) Phase II. Error bars represent standard errors. In Phase I, n = 7, for 'without worms' & n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms' & n = 10, for 'with worms'. Note: For the IN, it was observed that for the different experimental runs (i.e. for the periods of time when the system was run with worms (pink bar) and without worms (blue bar), the average NH_3-N was found to be significantly different as is indicated in the above graphs. Therefore, the % change in this parameter was related to the average IN NH_3-N for the specific run period.

These plots highlight the significant reduction of NH_3-N in EFs, for both types of soil layer, especially without worms. The effect of sand and gravel layers to reduce NH_3-N, are clearly visible in Phase II, which is not the case for Phase I.

4.3.3.3 Nitrite Nitrogen

An overview of the NO_2-N profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.18**. Temporal graphical representations of this data for IN and

EFs 1-3, throughout the Phase I and phase II experimental periods, are presented in **Figures 4.47** & **4.48**, respectively. There was a high variation in the IN NO_2-N during both Phases I and II. This observation suggests a significant reduction of NO_2-N in the EFs, especially in the VF with worms. In terms of the removal of NO_2-N, this VF system seems to be quite robust as is evidenced by the observation on 29 July, 2013, see **Figure 4.47**, where a very high NO_2-N was significantly reduced.

With respect to the soil layers, soil layer 2 was found to be more suitable in reducing NO_2-N from IN than soil type 1, e.g soil type 1 was found to reduce it by 63 % whereas the soil type 2 reduced it by 91 %, without worms. For the effect of OCs on the treatment efficiency with regard to the removal of NO_2-N, OC3 was found to be more favourable than other OCs. Both with and without worms, a high HRT and low HLR reduced NO_2-N better than a low HRT and high HLR.

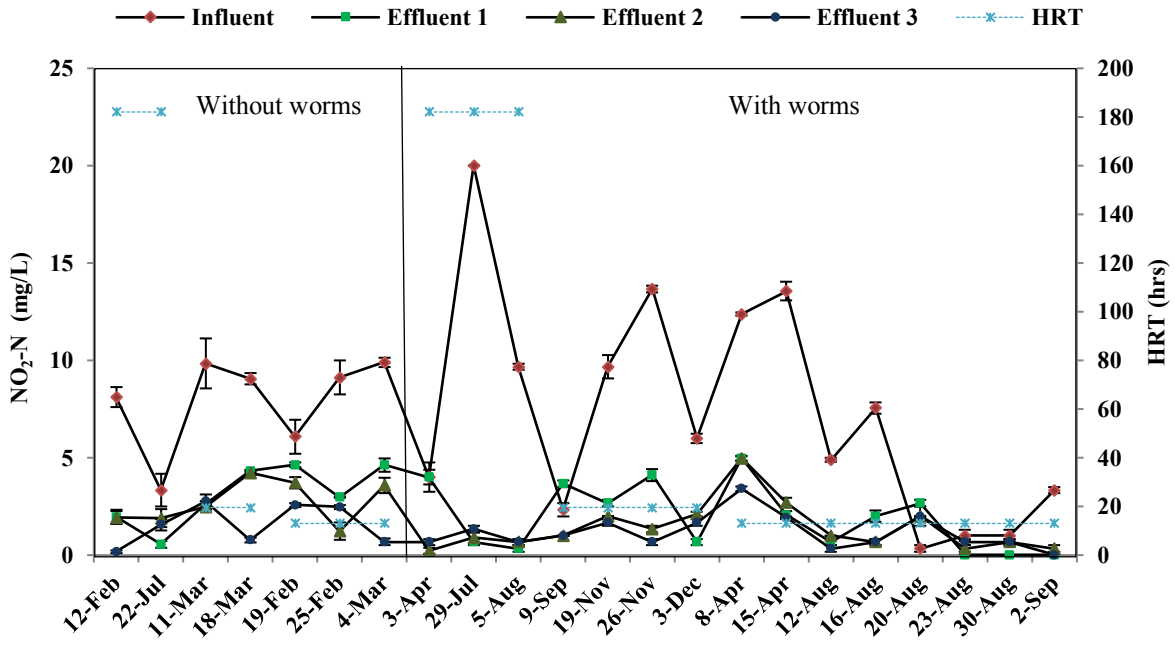


Figure 4.47 NO_2-N profile throughout the experimental period in Phase I. Error bars are standard errors, n = 3.

Table 4.18 An overview of the NO_2-N profile for Phase I and Phase II. The reported % change is calculated with respect to the average IN NO_2-N for each specific run period.

		Without worms				With worms			
NO_2-N	**OCs**	IN	EF1	EF2	EF3	IN	EF1	EF2	EF3
Phase I									
Mean ± SE	1	8.4 ± 1.2	4.1 ± 0.6	2.8 ± 0.8	1.9 ± 0.6	5.5 ± 1.8	1.5 ± 0.6	1.6 ± 0.6	1.2 ± 0.4
	2	9.4 ± 0.4	3.5 ± 0.9	3.3 ± 0.9	1.8 ± 1.0	7.9 ± 2.4	2.8 ± 0.8	1.6 ± 0.3	1.3 ± 0.3
	3	5.7 ± 2.4	1.3 ± 0.7	1.9 ± 0.0	0.9 ± 0.7	11.2 ± 4.7	1.7 ± 1.2	0.6 ± 0.2	0.9 ± 0.2
Range	1	6.1 - 9.9	3.0 - 4.6	1.2 - 3.7	0.7 - 2.6	0.3 - 13.5	0.0 - 5.0	0.3 - 5.0	0.0 - 3.1
	2	9.1 - 9.8	2.6 - 4.3	2.5 - 4.2	0.8 - 2.8	2.3 - 13.7	0.7 - 4.1	1.0 - 2.1	0.7 - 1.7
	3	3.3 - 8.1	0.6 - 2.0	1.9 - 1.9	0.2 - 1.7	4.0 - 20.0	0.3 - 4.0	0.2 - 0.9	0.7 - 1.3
% Change	1	-	51	66	77	-	72	71	78
	2	-	63	65	81	-	65	80	84
	3	-	77	67	85	-	85	95	92
Phase II									
Mean SE	2	2.4 ± 1.4	0.2 ± 0.2	0.6 ± 0.6	0.1 ± 0.1	5.9 ± 0.6	1.0 ± 0.2	0.03 ± 0.03	0.1 ± 0.1
Range	2	0.0 - 4.7	0.0 - 0.7	0.0 - 1.7	0.0 - 0.3	3.0 - 10.0	0.0 - 2.3	0.0 - 0.3	0.0 - 0.7
% Change	2	-	91	77	95	-	83	99	98

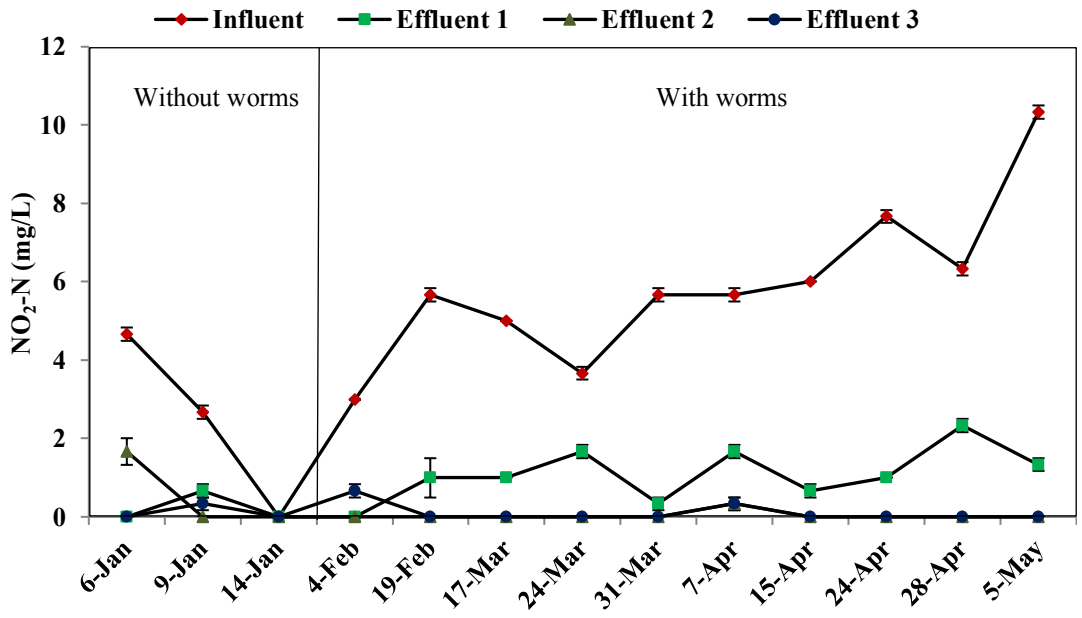


Figure 4.48 NO_2-N profile throughout the experimental period in Phase II. Error bars are standard errors, n = 3.

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.49 (a)** & **(b)** depicting the time-averaged NO_2-N for the VF with and without worms, over Phases I and II, respectively.

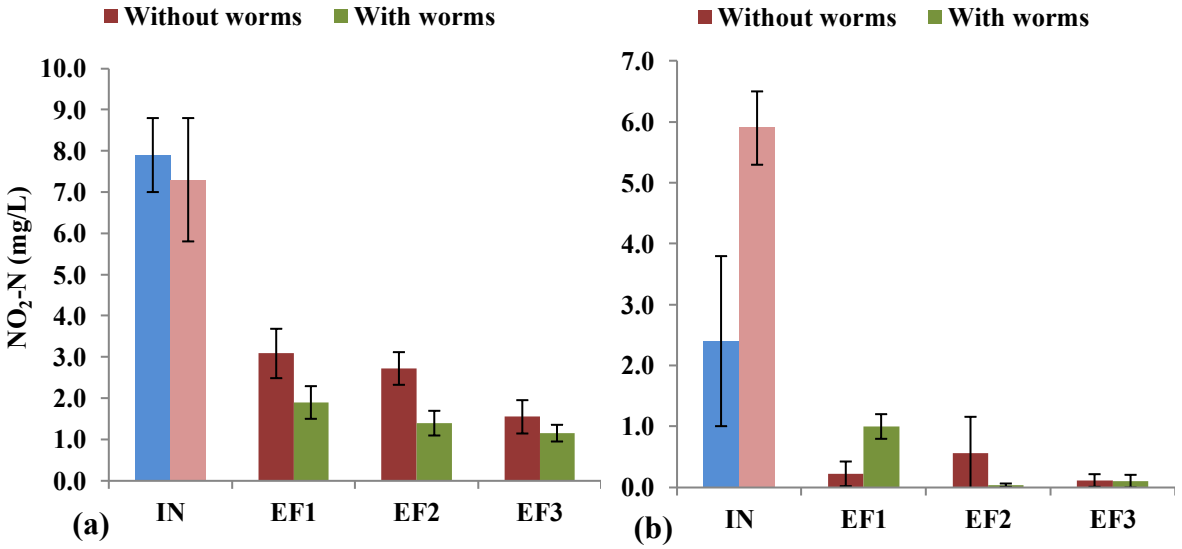


Figure 4.49 Time-averaged NO_2-N levels for (a) Phase I and (b) Phase II. Error bars represent standard errors. In Phase I, n = 7, for 'without worms' & n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms' & n = 10, for 'with worms'. Note: For the IN, it was observed that for the different experimental runs (i.e. for the periods of time when the system was run with worms (pink bar) and without worms (blue bar), the average NO_2-N was found to be significantly different as is indicated in the above graphs. Therefore, the % change in this parameter was related to the average IN NO_2-N for the specific run period.

These plots highlight the significance of geo-layers and worms in the reduction of NO_2-N, for both Phases I and II. A significant reduction in NO_2-N may be a synchronous effect of worms and microorganism (*Nitrobacter* and *Nitrospira*)[75] in oxidising NO_2-N to NO_3-N, in the nitrification process.

4.3.3.4 Nitrate Nitrogen

An overview of the NO_3-N profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.19**. Temporal graphical representations of this data for IN and EFs 1-3, throughout the Phase I and phase II experimental periods, are presented in **Figures 4.50** & **4.51**, respectively.

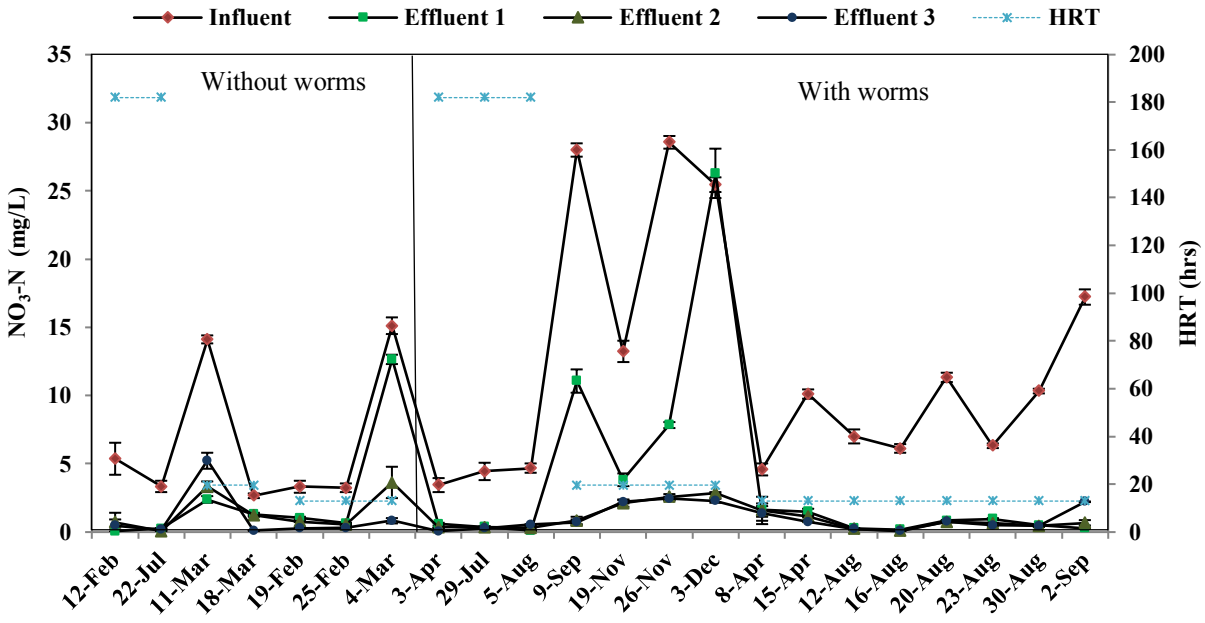


Figure 4.50 NO_3-N profile throughout the experimental period in Phase I. Error bars are standard errors, n =3.

[75] http://en.wikipedia.org/wiki/Nitrification 22/03/2015

Table 4.19 An overview of the NO_3-N profile for Phase I and Phase II. The reported % change is calculated with respect to the average IN NO_3-N for each specific run period.

		Without worms				With worms			
NO_3-N	**OCs**	IN	EF1	EF2	EF3	IN	EF1	EF2	EF3
Phase I									
Mean ± SE	1	7.2 ± 3.9	4.8 ± 3.9	1.6 ± 1.0	0.5 ± 0.2	9.1 ± 1.4	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.2
	2	8.4 ± 5.7	1.8 ± 0.5	2.3 ± 1.1	2.7 ± 2.6	23.8 ± 3.6	12.2 ± 4.9	2.1 ± 0.4	1.9 ± 0.4
	3	4.4 ± 1.0	0.2 ± 0.1	0.4 ± 0.3	0.3 ± 0.1	4.2 ± 0.4	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1
Range	1	3.2 - 15.1	0.6 - 12.7	0.5 - 3.6	0.3 - 0.8	4.6 - 17.2	0.2 - 1.6	0.1 - 1.6	0.1 - 2.2
	2	2.7 - 14.1	1.3 - 2.4	1.2 - 3.3	0.1 - 5.2	13.2 - 28.6	3.8 - 26.3	0.8 - 2.9	0.7 - 2.5
	3	3.3 - 5.4	0.1 - 0.3	0.1 - 0.7	0.2 - 0.5	3.4 - 4.7	0.1 - 0.6	0.3 - 0.3	0.0 - 0.5
% Change	1	-	34	77	94	-	92	92	91
	2	-	78	73	68	-	49	91	92
	3	-	96	91	93	-	92	92	93
Phase II									
Mean SE	2	17.0 ± 2.9	1.6 ± 0.9	0.1 ± 0.1	0.5 ± 0.3	8.7 ± 2.1	1.4 ± 0.3	0.7 ± 0.1	0.6 ± 0.3
Range	2	13 - 22.6	0.1 - 3.3	0.0 - 0.4	0.0 - 1.0	2.3 - 18.7	0.0 - 2.3	0.0 - 1.7	0.0 - 2.7
% Change	2	-	91	99	97	-	84	92	93

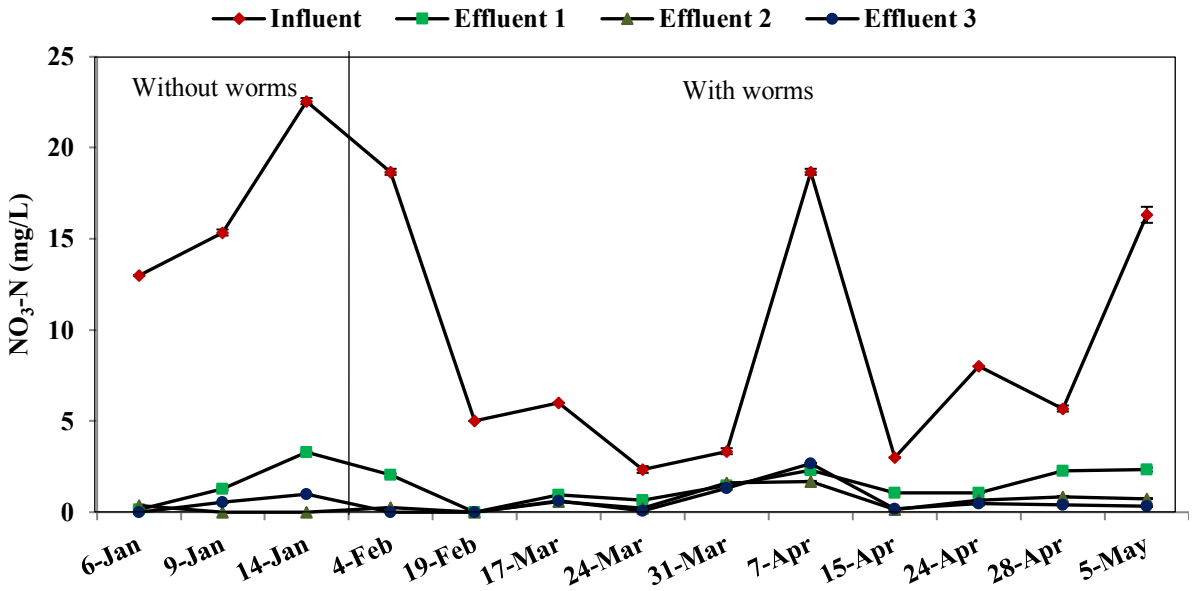


Figure 4.51 NO_3-N profile throughout the experimental period in Phase II. Error bars are standard errors, n =3.

Though a high variation was observed in the IN NO_3-N, for both Phases I and II, there was a significant reduction of NO_3-N in the EFs. Interestingly, a tendency for stabilization was observed, irrespective of soil type and whether worms are present or not. However, soil type 2 seems to be more favourable in reducing NO_3-N in EFs. In terms of the effect of OCs in the treatment efficiency, OC3 was found to be more favourable than OC1 and OC2. Thus, a high HRT and low HLR significantly reduce NO_3-N in the VF.

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.52 (a)** & **(b)** depicting the time-averaged NO_3-N for the VF, with and without worms, over Phases I and II, respectively.

These plots highlight the significance of the geo-layers in reducing NO_3-N in effluents. It is clear from the plots that worms do not significantly reduce the NO_3-N, as the reduction was higher in the VF without worms. The presence of denitrifying microorganisms in the geo-layers may be performing a denitrification process[76]. Denitrifying microorganisms are a large group of heterotrophic facultative anaerobic bacteria, which are responsible for the reduction of NO_3-N to N_2.

[76] http://en.wikipedia.org/wiki/Denitrification 23/03/2015

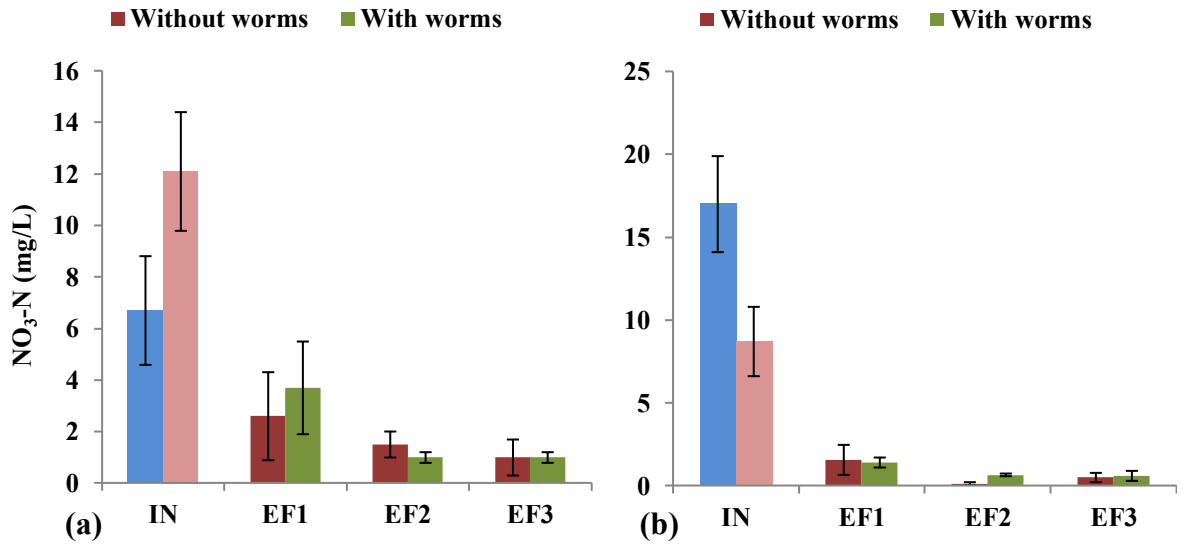


Figure 4.52 Time-averaged NO_3-N levels for (a) Phase I and (b) Phase II. Error bars represent standard errors. In Phase I, n = 7, for 'without worms' & n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms' & n = 10, for 'with worms'. Note: For the IN, it was observed that for the different experimental runs (i.e. for the periods of time when the system was run with worms (pink bar) and without worms (blue bar), the average NO_3-N was found to be significantly different as is indicated in the above graphs. Therefore, the % change in this parameter was related to the average IN NO_3-N for the specific run period.

4.3.3.5 Total Phosphorus

An overview of the TP profiles during the overall experimental period, for both Phases I and II, is presented in **Table 4.20**. Temporal graphical representations of this data for IN and EFs 1-3, throughout the Phase I and Phase II experimental periods, are presented in **Figures 4.53** & **4.54**, respectively. Surprisingly, the variation in IN TP was not as high as for other parameters. However, the effect of worms and soil type in the EFs is quite distinctive. These observations suggest that soil type 1 reduces the TP from the IN whereas soil type 2 contributes TP in the EFs. The increase of TP in the EFs for soil type 2 is much higher with worms than without worms. Even for soil type 1, the TP removal efficiency appears to be affected by the worms, reducing the % removal.

In terms of the effect of OCs in the treatment efficiency in relation to the TP removal, OC3 was found to be more favourable than OC1 and OC2. Thus, a high HRT and low HLR better remove the TP in the EFs.

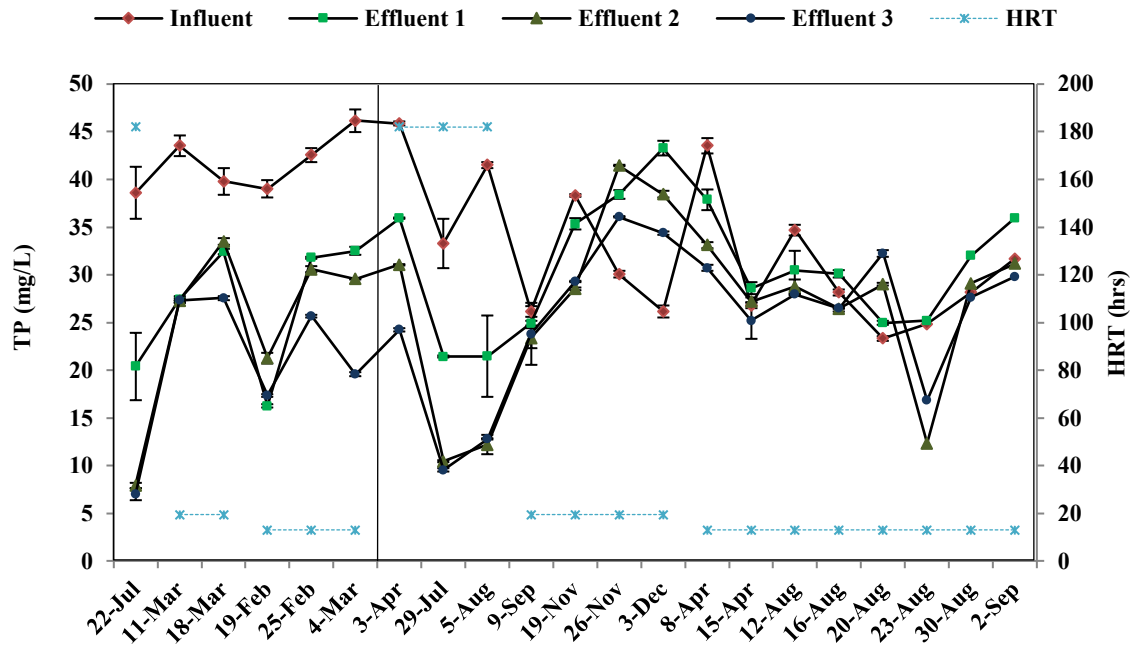


Figure 4.53 Total phosphorus profile throughout the experimental period in Phase I. Error bars represent standard errors, n =3.

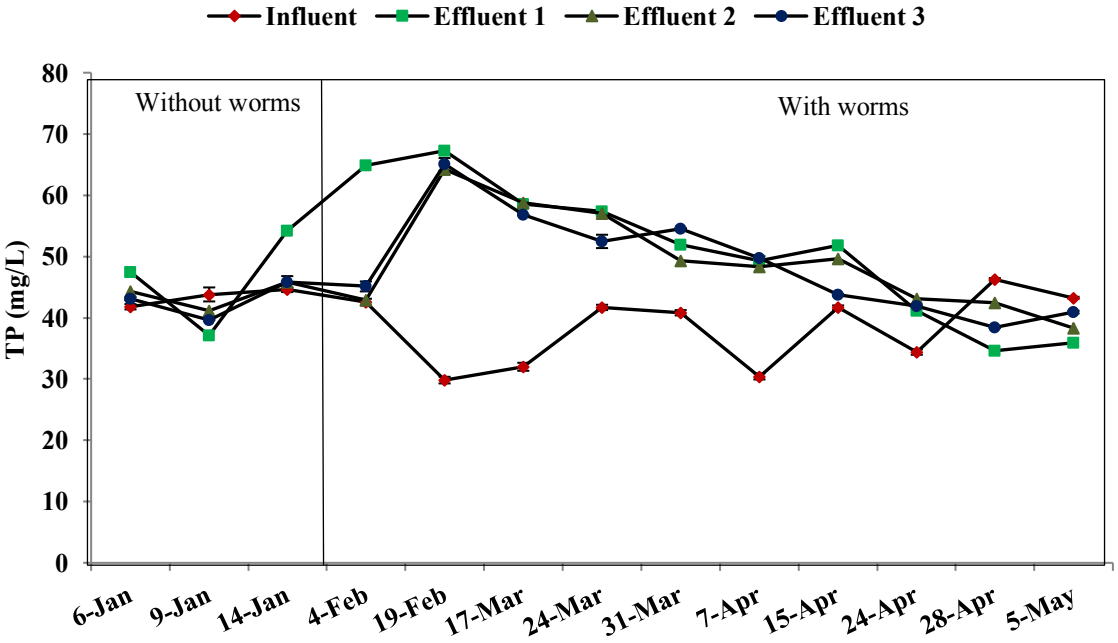


Figure 4.54 Total phosphorus profile throughout the experimental period in Phase II. Error bars are standard errors, n = 3.

Table 4.20 An overview of the TP profile for Phase I and Phase II. The reported % change is calculated with respect to the average IN TP for each specific run period.

		Without worms				With worms			
TP	**OCs**	IN	EF1	EF2	EF3	IN	EF1	EF2	EF3
Phase I									
Mean ± SE	1	43 ± 2	27 ± 5	27 ± 3	21 ± 3	30 ± 2	31 ± 2	27 ± 2	27 ± 2
	2	42 ± 2	30 ± 3	30 ± 3	27 ± 0	30 ± 3	35 ± 4	33 ± 4	31 ± 3
	3	39 ± 0	20 ± 0	8 ± 0	7 ± 0	40 ± 4	26 ± 5	18 ± 7	16 ± 5
Range	1	39 - 46	16 - 33	21 - 31	17 - 26	23 - 44	25 - 38	12 - 33	17 - 32
	2	40 - 44	27 - 32	27 - 34	27 - 28	26 - 38	25 - 43	23 - 41	24 - 36
	3	39 - 39	20 - 20	8 - 8	7 - 7	33 - 46	21 - 36	10 - 31	10 - 24
% Change	1	-	37	36	51	-	-2	10	10
	2	-	28	27	34	-	-18	-9	-2
	3	-	47	79	82	-	35	55	61
Phase II									
Mean SE	2	43 ±1	46 ± 5	44 ± 1	43 ± 2	38 ± 2	51 ± 4	49 ± 3	49 ± 3
Range	2	42 - 45	37 - 54	41 - 46	40 - 46	30 - 46	35 - 67	38 - 64	38 - 65
% Change	2	-	-8	-2	0	-	-35	-30	-29

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.55 (a)** & **(b)** depicting the time-averaged TP for the VF with and without worms, over Phases I and II, respectively.

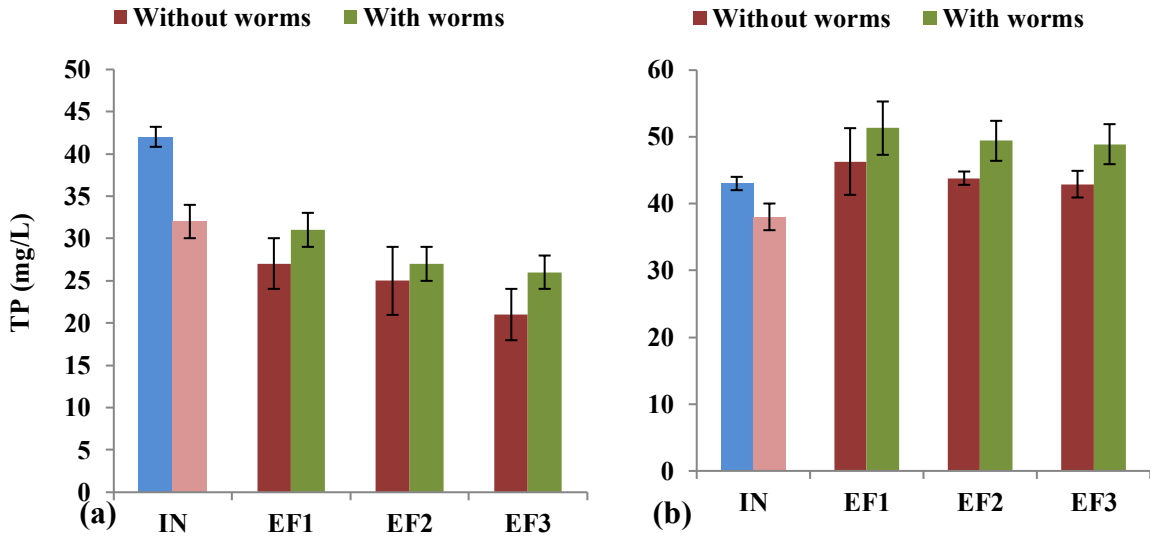


Figure 4.55 Time-averaged Total phosphorus levels for (a) Phase I and (b) Phase II. Error bars represent standard errors. In Phase I, n = 6, for 'without worms' & n = 15, for 'with worms'. In Phase II, n = 3, for 'without worms' & n = 10, for 'with worms'. Note: For the IN, it was observed that for the different experimental runs (i.e. for the periods of time when the system was run with worms (pink bar) and without worms (blue bar), the average TP was found to be significantly different as is indicated in the above graphs. Therefore, the % change in this parameter was related to the average IN TP for the specific run period.

These plots highlight the significance of worms and soil types in the VF to reduce TP in the EFs. The observation clearly suggests that soil types 1 and 2 have contradictory effects on TP; the soil type 1 reduced TP whereas the soil type 2 increased TP in the EFs. The increase in TP reflects the finding of Kumar et al. (2015), in **Table 4.2**, however the decrease in TP reflect the rest. This observation shows that the filter media affects the removal efficiency (Wang et al. 2010). Kim et al. (2006) and Yim and Kim (2004) reported that the converter slag-coal cinder filters removed phosphorus efficiently. In this study, phosphorus removal was not as effective as other nutrients because when worms feed on organic matter, worm gut enzymes convert phosphorus to more soluble forms acid phosphatases and alkaline phosphatases (Tomar and Suthar 2011). Earthworm feaces (vermicast) are rich in nutrients (Xing et al. 2005) and contribute nutrients in the effluent. Moreover, phosphorus retained in particulate forms may have leached from the filter media, as discussed in **Section 4.1.3.9**.

4.3.3.6 Laboratory-based nutrient removal experiment

A laboratory based nutrient removal experiment was designed and implemented accordingly to validate the finding on the efficiency of the various layers used in the VF system in nutrients removal in terms of total nitrogen (TN) and total phosphorus (TP).

The experiment was set up as shown in **Figure 4.56**. Six glass columns were filled with (A) soil (B) soil & worms (C) sand (D) gravel (E) mixture of soil, sand, straw, pine bark saw dust (4:1;1:1 v/v) and (F) mixture of soil, sand, straw, pine bark saw dust (4:1;1:1 v/v) & worms, to resemble the filter layers used in the pilot scale VF. Synthetic water (SW) was prepared, by dissolving 680 mg KNO_3 and 219 mg KH_2PO_4 in 1 L deionised water (DIW), to obtain artificial IN with 100 mg/L TN and 50 mg/L TP standard. At first, 100 mL DIW was filled in each column and retained for 24 hours, then voided to get EF. This is to obtain the data for blank, as DIW do not contain any TN and TP. In the next step, each column was filled with 100 mL SW, retained for 24 hours and voided to get EF. Both experiments were done in triplicate and the IN and EF data was analyzed in triplicate as well. The data from this experiment is summarized in **Table 4.21**.

Figure 4.56 Laboratory-based nutrient removal experiments. Glass column containing (A) Soil (B) Soil & earthworms (C) Sand (D) Gravel (E) Mixture of soil, sand, straw, pine bark saw dust (4:1;1:1 v/v) and (F) Mixture of soil, sand, straw, pine bark saw dust (4:1;1:1 v/v) & earthworms.

Table 4.21 An overview on the laboratory based nutrient-removal experiment that resembles the pilot scale VF. * Initial value of the distilled water (DIW) and synthetic water (SW) for the measured parameters before feeding in the glass column. Values reported for A, B, C, D, E and F are those after feeding DIW and SW in the respective glass columns.

Sample ID	Phase	Layer corresponding to the VF unit	Temperature (°C)		pH		Conductivity (mS/cm)		TN (mg/L)		TP (mg/L)	
			DIW	SW	DIW	SW	DIW	SW	DIW	SW	DIW	SW
Initial *			21.5	22.0	8.1	6.3	0.1	1.2	0.0	100.0	0.0	50.0
A	I	1 - Soil[a]	20.0	20.8	3.6	5.2	2.4	1.4	26.4	95.9	62.2	45.6
B	I	1 - Soil + worms[a]	19.5	20.4	4.1	4.9	1.9	0.4	23.3	16.1	55.6	56.6
C	I, II	2 - Sand	19.5	20.4	6.2	6.0	0.1	1.0	0.1	81.2	0.4	59.8
D	I, II	3 - Gravel	20.3	20.4	9.8	7.4	1.0	1.0	0.1	86.9	0.8	65.6
E	II	1 - Soil + Sand + Straw + Pine bark saw dust[b]	20.1	20.6	4.6	4.2	2.2	3.4	53.7	91.5	67.1	76.0
F	II	2 - Soil + Sand + Straw + Pine bark saw dust+ worms[b]	21.4	21.2	4.5	4.8	2.5	1.3	47.5	73.0	62.8	69.6

[a] soil type 1
[b] soil type 2

It is interesting to observe that soil type 2 contributed a more significant amount of TN and TP than soil type 1, though both leached TN and TP, while DIW was fed into the glass columns. However, sand and gravel did not contribute any TN and TP. However, when SW was fed into the column, soil type 1 with worms (B) reduced TN significantly, which was not a case for the soil type 2 (F). Moreover, the reduction of TN was found to be significant with worms (B & F) than without worms (A & E). TN reduced in sand (C) and (D) as well, which suggests that TN was absorbed or transformed to other forms, in these two filter materials. Conversely, TP was found to be increased in EF from all filter layers except a slight decline in (A). For soil type 1, worms seem to contribute TP in EF whereas for soil type 2 it was not the case. Here, C and D seem to leach TP in EF, which may be due to the transformation of particulate phosphorus into water soluble phosphorus. The trend of the change in TN and TP is difficult to explain, as Wang et al. (2014) argue that out of all the contaminants in wastewater, nutrients are of much concern due to their capacity for complex transformations and interactions.

4.3.4 Toxic contaminant removal

4.3.4.1 Heavy Metals

Some heavy metals such as zinc, magnesium, and manganese are useful if present in trace amounts. However, in excess, they are toxic to aquatic animals/plants and pose a hazard to the environment. **Table 4.22** provides an overview of the heavy metals profiles investigated during the overall experimental period, for both Phases I and II. The heavy metals investigated in this study are cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn). It may be seen from **Table 4.22** that Mg is the predominant metal in the IN. The concentration of heavy metals in the IN was found to be present in the following order: Mg > Fe > Zn > Cu > Mn > Pb > Ni > Cd > Cr > Co.

The concentrations of all these heavy metals in the IN were found to be within the range for the NWQMS 2000 standard, see **Table 4.22**. However, Fe was found to be significantly higher in the EFs, with soil type 2 whereas it was within range for soil type 1. Apart from Fe,

all other elements in the EFs were found in line with the NWQMS 2000 standard. Moreover, this study could detect only trace amount of heavy metals such as Cd, Co, Cr and Pb in the IN as well as in the EFs. Interestingly, the study also revealed that the concentration of elements such as Mg, Mn, Ni and Fe increased in the EFs compared to the IN. This observation suggests that the filter layers leach these elements in the EFs.

Earthworms are capable of the bioaccumulation of heavy metals in the chloragogen cell[77] without affecting their physiology. Sinha et al. (2008c) has reported that worms can accumulate Cd, Hg, Pb, Cu, Mn, Ca, Fe and Zn that are readily absorbed by their gut, and this is supported by Hartenstein et al. (1980). Bajsa et al. (2004) also argue that, if worms are exposed to high concentrations of heavy metals, it will reduce their weight and result in a decline in the reproduction rate. Hartenstein et al. (1980, p.24) claimed that - "Accumulation of a heavy metal in animal tissue may be said to occur when animal tissues contain increasingly higher levels of the heavy metal in the environment over a period of time, or at one given time the tissues show increasing levels in proportion to the concentration in the environment."

Another perspective on the data may be obtained by the comparative histograms shown in **Figure 4.57** & **4.58,** depicting the time-averaged heavy metals (individual histograms for each element investigated) for the VF with and without worms, over Phases I and II, respectively. These plots highlight the significance of soil type and worms in VF in changing heavy metal concentration in the EFs when the IN is passed through the different layers (i.e. the effect of worms and soil type on heavy metals in the IN.) Here, the histogram for Co is not provided in **Figure 4.57,** as it was not detected in either the IN or the EFs, for Phase II. Here, the treatment efficiency has been discussed by comparing the concentration of elements in the IN and in the final effluent, EF3. Though Cd was detected in trace amounts, the observation showed that worms were effective in removing Cd. With worms, it reduced by 37 % and 78 % in Phases I and II, respectively. However, without worms, soil type 1 seems to contribute Cd in the EFs. In terms of Co, again, worms significantly removed it by 41 %. A different trend was observed for Cr in Phases I & II, soil type 1 reducing it better than soil type 2, in the presence of worms.

[77]Chloragogen cells are cells in annelids that function similarly to the liver in vertebrates. https://en.wikipedia.org/wiki/Chloragogen_cell

Table 4.22 Heavy metals observed in IN and EFs, with and without worms, in Phases I and II. The unit is mg/L for all the observed heavy metals.

	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Zn
Phase I										
Without worms										
Influent	0.0011	0.0006	0.0008	0.018	0.041	4.20	0.011	0.005	0.001	0.045
Effluent 1	0.0013	0.0006	0.0002	0.008	0.566	9.62	0.120	0.003	0.001	0.018
Effluent 2	0.0008	0.0006	0.0005	0.004	2.135	13.80	0.535	0.008	0.001	0.014
Effluent 3	0.0015	0.0006	0.0006	0.004	1.779	15.22	0.642	0.006	0.001	0.012
% Change	-38	1	29	76	-4208	-262	-5789	-36	-11	74
With worms										
Influent	0.0004	0.0069	0.0009	0.028	0.140	3.26	0.035	0.026	0.007	0.277
Effluent 1	0.0001	0.0000	0.0000	0.006	0.635	12.02	0.492	0.022	0.004	0.057
Effluent 2	0.0001	0.0043	0.0002	0.001	3.605	17.17	2.069	0.032	0.004	0.044
Effluent 3	0.0003	0.0041	0.0002	0.001	3.467	17.86	2.953	0.029	0.003	0.037
% Change	37	41	79	95	-2384	-448	-8254	-9	53	87
Phase II										
Without worms										
Influent	0.0061	0.0000	0.0029	0.030	2.806	4.28	0.001	0.014	0.013	0.079
Effluent 1	0.0047	0.0000	0.0021	0.005	21.411	3.98	0.084	0.014	0.007	0.000
Effluent 2	0.0052	0.0000	0.0084	0.000	88.000	9.22	0.771	0.019	0.000	0.000
Effluent 3	0.0058	0.0000	0.0017	0.000	102.533	10.49	0.941	0.016	0.000	0.000
% Change	4	0	43	100	-3555	-145	-141050	-13	100	100
With worms										
Influent	0.0104	0.0000	0.0014	0.030	1.898	4.23	0.014	0.010	0.051	0.082
Effluent 1	0.0022	0.0000	0.0017	0.005	15.307	3.81	0.071	0.012	0.052	0.006
Effluent 2	0.0027	0.0000	0.0012	0.000	40.431	8.07	0.623	0.013	0.058	0.001
Effluent 3	0.0022	0.0000	0.0012	0.000	42.486	8.89	0.746	0.011	0.066	0.001
% Change	78	0	15	100	-2138	-110	-5364	-12	-29	99
NWQMS 2000 Standard	*0.01 – 0.05*	*0.05 – 0.10*	*0.1 – 1.0*	*0.2 – 5.0*	*0.2 – 10.0*	-	*0.2 – 10.0*	*0.2 - 2.0*	*2 - 5*	*2 - 5*

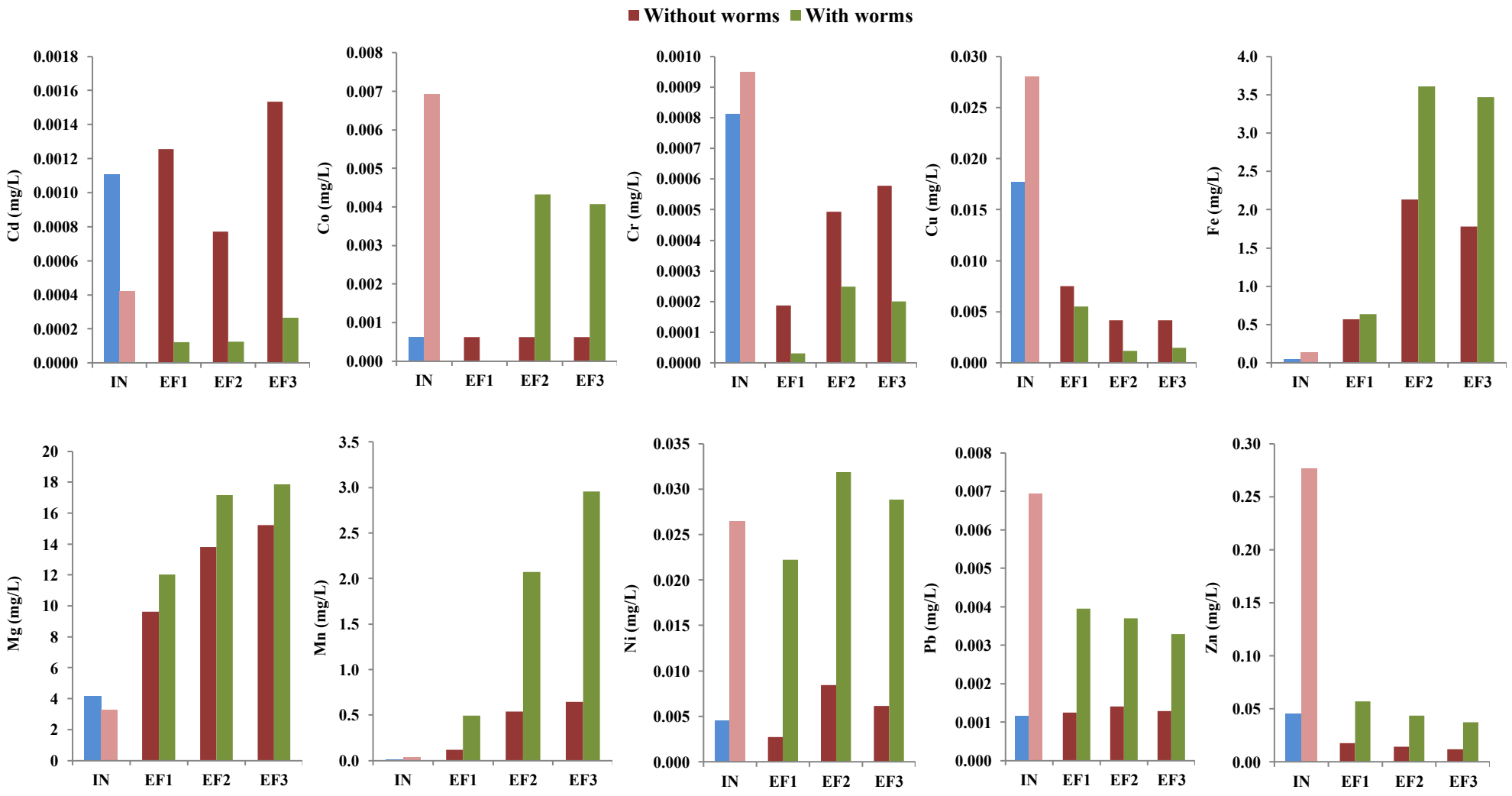


Figure 4.57 The heavy metals – Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn, recorded in IN, EF1, EF2 and EF3, in Phase I. The data is an average of 7 experimental runs for without worms and 13 experimental runs for with worms.

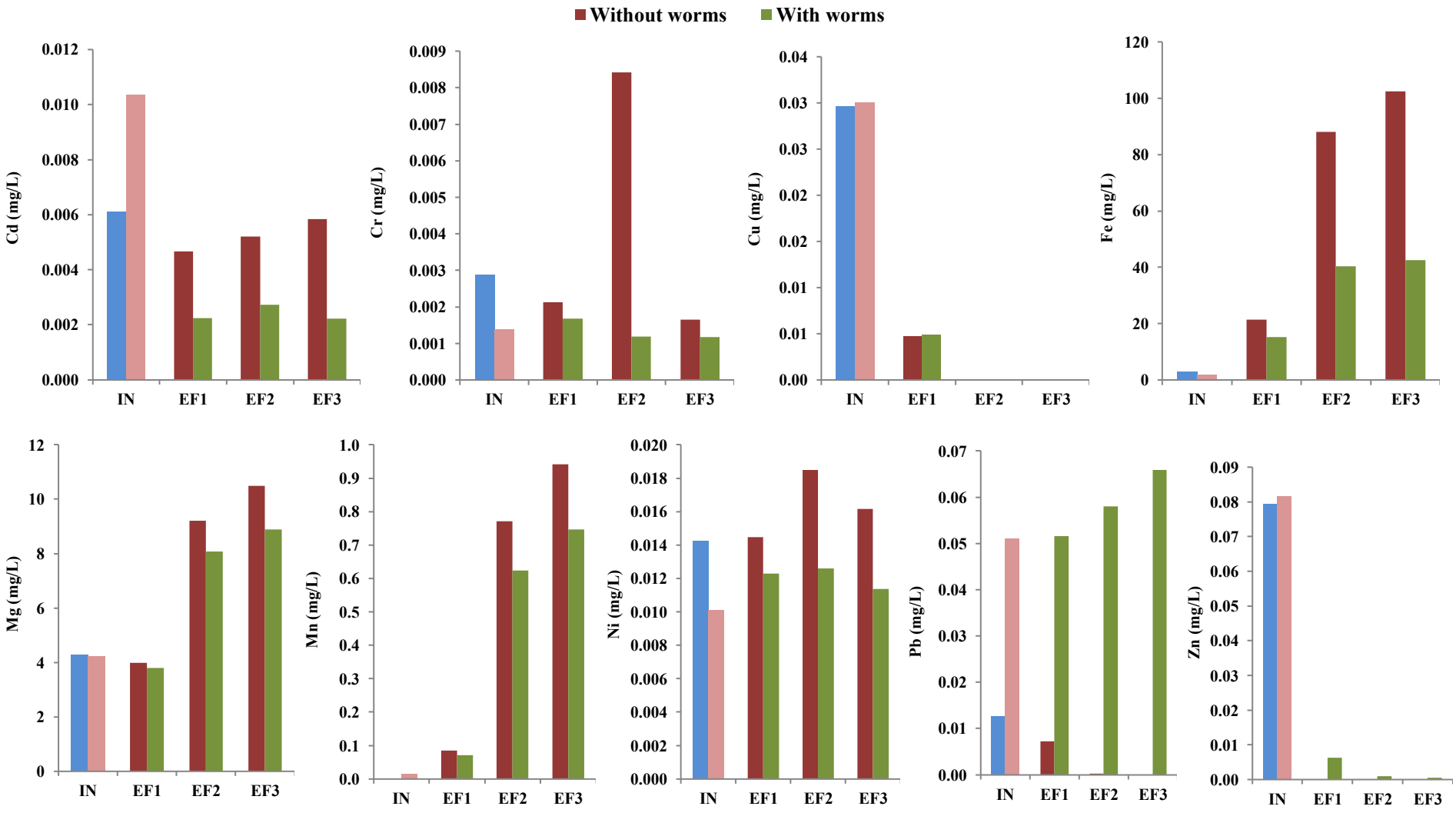


Figure 4.58 The heavy metals – Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn, recorded in IN, EF1, EF2 and EF3, in Phase II. The data is an average of 3 experimental runs for without worms and 7 experimental runs for with worms.

It is interesting to observe that Cu was removed significantly, irrespective of whether worms were present or absent. However, Fe, Mg, Mn and Ni was found to be accumulated significantly in the EFs, irrespective to the soil type and worms. A similar observation was made for Mg and Mn, for Phase II. Here, the increase in Fe is comparatively very high with soil type 2. In terms of Pb, it was found to decrease with soil type 1 and increase with soil type 2. However, Zn decreased significantly, irrespective of the soil type and presence of worms.

4.3.5 Biological observations

4.3.5.1 Isolation and identification of microorganisms (microbial diversity within the VF)

The microorganisms isolated from the influent (IN) and the effluents from three different layers (EF1, EF2 and EF3) are presented in **Table 4.23**. This includes only those microorganisms that could be isolated and identified within the researchers capability and does not represents an exhaustive determination of the population of microorganisms in the system. A range of representative microorganisms were isolated from the IN (raw sewage), namely *Acinetobacter*[78] *junii, Acinetobacter genomospecies 6, Aeromonas*[79] *encheleia, Citrobacter*[80] *koseri/ youngie, Citrobacter braakii, Comamonas denitrificans, Escherichia coli*[81]*, Enterobacter*[82] *aerogenes, Enterobacter asburiae, Klebsiella oxytoca*[83]*, Raoultella planticola/ ornithinolytica*[84]. The microbes isolated from the EFs are *Aeromonas caviae DNA Group 4, Aeromonas hydrophila DNA Group 1*[85]*, Aeromonas salmonicida ss salmonicida, Acinetobacter guillouiae, Bacillus ginsegi, Brachymonas denitrificans, Pseudomonas*

[78] More information on *Acinetobacter* spp. http://medind.nic.in/iau/t01/i1/iaut01i1p30g.pdf
[79] More information on *Aeromonas* spp. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC229582/pdf/350369.pdf
[80] More information on *Citrobacter* spp. http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/citrobacter-eng.php 30/03/2015
[81] More information on *Escherichia coli* http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/escherichia-coli-pa-eng.php
[82]More information on *Enterobacter* spp. http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/enterobacter-eng.php
[83] More information on *Klebsiella* spp. http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/klebsiella-eng.php
[84] More information on *Raoultella* spp. http://www.uobabylon.edu.iq/uobcoleges/fileshare/articles/R.%20ornithinolytica-Univ%20website.pdf
[85] More information on *Aeromonas hydrophila* http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/aeromonas-hydrophila-eng.php

alcaligenes, Pseudomonas citronellolis, Pseudomonas pseudoalcaligenes, Pseudomonas stutzeri. Most of these are gram negative aerobic/facultative microorganisms[86] and are found to be either pathogenic in nature or support the wastewater treatment process by forming a biofilm.

Table 4.23 An overview on the microorganisms (bacteria) isolated from IN and EFs, over Phase I (soil type 1). These are reported as identified with Biolog system.

Microbial diversity isolated and identified in Influent (Raw Sewage)	
Acinetobacter junii *Aeromonas encheleia* *Citrobacter koseri/ youngae* *Acinetobacter genospecies 6* *Comamonas denitrificans*	*Enterobacter aerogenes* *Enterobacter asburiae* *Klebsiella oxytoca* *Citrobacter braakii* *Raoultella planticola/ ornithinolytica* *Escherichia coli*
Microbial diversity isolated and identified before introducing worms into the system	**Microbial diversity isolated and identified after introducing worms into the system**
Effluent from layer 1 (soil) *Aeromonas salmonicida ss salmonicida* *Bacillus ginsegi* *Pseudomonas stutzeri*	Effluent from layer 1 (soil) *Aeromonas hydrophila DNA Group 1* *Pseudomonas alcaligenes*
Effluent from layer 2 (sand) *Acinetobacter guillouiae* *Klebsiella oxytoca*	Effluent from layer 2 (sand) *Klebsiella oxytoca* *Aeromonas caviae DNA Group 4*
Effluent from layer 3 (Gravel) *Pseudomonas citronellolis*	Effluent from layer 3 (Gravel) *Brachymonas denitrificans* *Pseudomonas stutzeri* *Pseudomonas pseudoalcaligenes*

According to Andersson (2009), some of the microorganisms identified here such as *Comamonas denitrificans, Brachymonas denitrificans and Aeromonas hydrophila* have efficient nutrient removal capability. The initial two are denitrifying organisms as their names` indicate, while the latter one is capable of storing polyphosphates in its cell under

[86] Microbial identification databases for Biolog systems. http://biolog.com/pdf/milit/00A%20005rC%20Biolog%20Database%20Book.pdf 28/03/2015

aerobic condition. Moreover, some of the members of genera *Acinetobacter, Bacillus* and *Pseudomonas* are denitrifying bacteria. Cakmakci et al. (1981) found that *Klebsiella oxytoca* is capable of fixing atmospheric nitrogen. Igbinosa et al. (2012) investigated *Pseudomonas* spp., isolated from wastewater, and found that they are opportunistic pathogens and pose a threat to public health due to their antibacterial resistance.

4.3.5.2 *E.Coli* / Coliforms

As discussed earlier in **Section 4.1.3.11**, the faecal contamination in wastewater is usually indicated by the presence of pathogens such as *E.Coli*/coliforms in the sample. This study was thus focused on the coliforms and *E.Coli* count in the IN and EFs, which is discussed below.

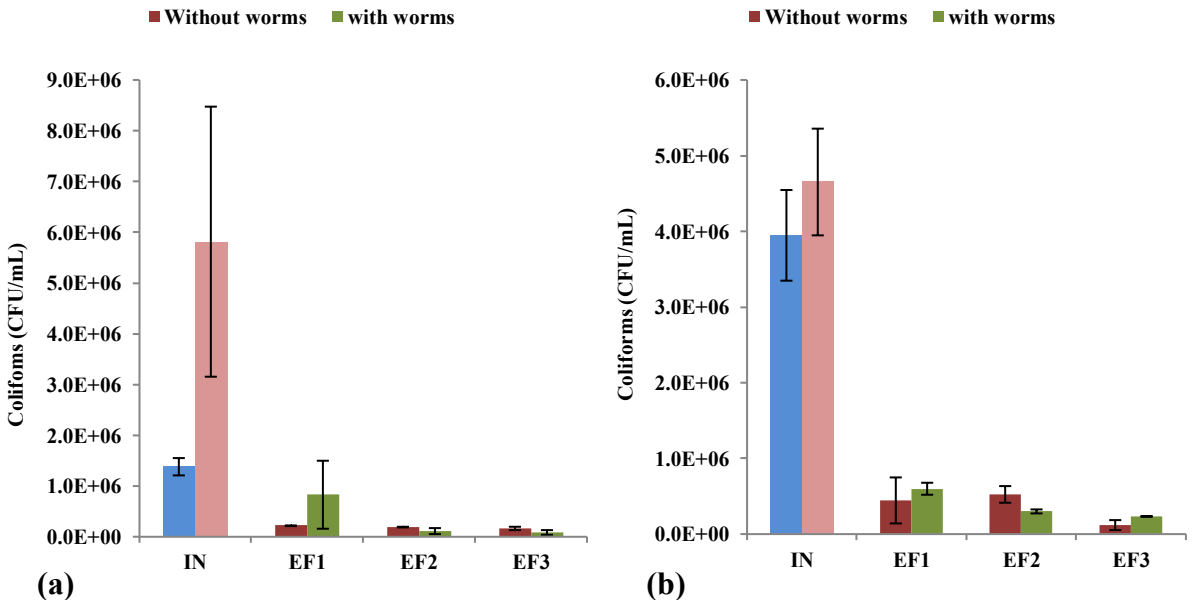


Figure 4.59 Time averaged coliforms for (a) Phase I and (b) Phase II, both at HRT 19.5 hours. Error bars represent standard errors. In Phase I, for without worms n = 2 and for with worms n = 3. In Phase II, for both with and without worms n = 3.

Figure 4.59 (a) & **(b)** depicts the coliforms profile throughout the filter layers, with and without worms, in Phase I and II, respectively. These plots highlight the significance of the geo-layers and worms in the removal of coliforms in the VF. A significant log removal of coliforms was observed in the EFs for both Phases, irrespective to the soil type, and whether worms are present or absent. However, the log removal efficiency, reported as a log removal value (LRV), K, was found to be higher in the VF with worms than without worms. In Phase

I, the coliforms in EF3 reduced by 0.9 log units (88 %) without worms whereas it reduced by 1.8 log units (98 %) with worms. But, in Phase II, a significant difference was not observed as in Phase I, as coliforms reduced by 1.8 log units (98 %) without worms and it reduced by 1.9 log units (99 %) with worms. This observation clearly shows that soil type 2 is more efficient for the removal coliforms than soil type 1.

Figure 4.60 (a) & **(b)** depicts the *E.Coli* profile throughout the filter layers with and without worms, in Phase I and II, respectively.

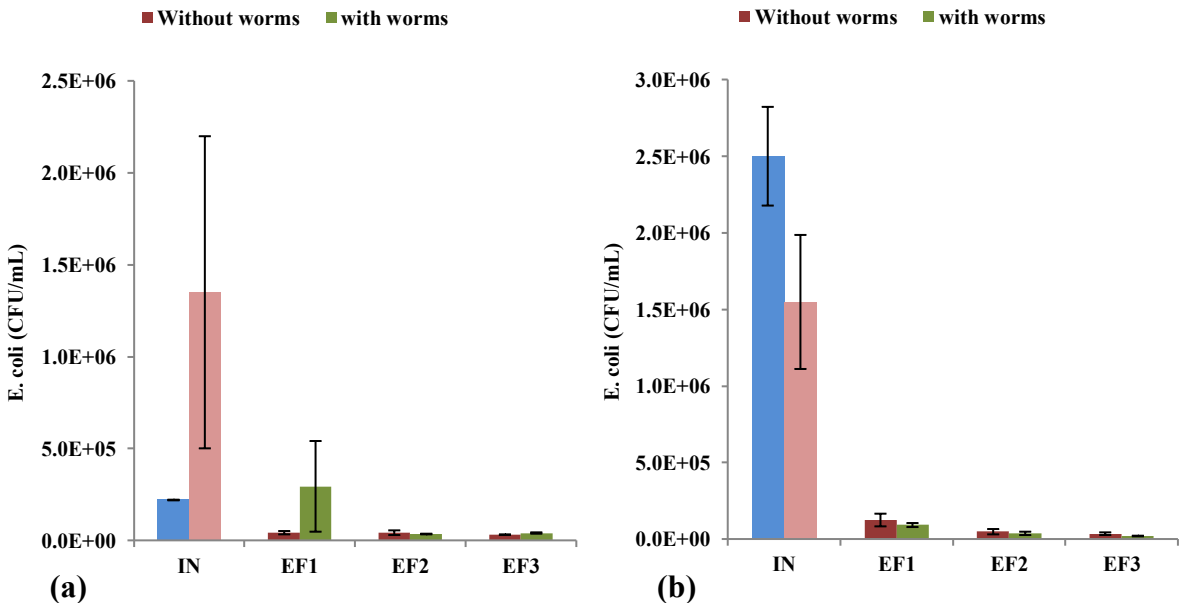


Figure 4.60 Time averaged *E.Coli* for (a) Phase I and (b) Phase II, both at HRT 19.5 hours. Error bars represent standard errors. In Phase I, for both with and without worms n = 2. In Phase II, for both with and without worms n = 3.

These plots highlight the significance of the geo-layers and worms in the removal of E. coli in the VF. Similar to coliforms, a significant log removal of *E.Coli* was observed in the EFs for both Phases, irrespective to the soil type and worms. However, the log removal efficiency was found to be higher in the VF with worms than without worms. In Phase I, the *E.Coli* in EF3 reduced by 0.8 log units (85 %) without worms whereas it reduced by 1.5 log units (97 %) with worms. But, in Phase II, there was not a significant difference, as *E.Coli* reduced by 1.9 log units (99 %) both with and without worms. Thus, this observation shows a similar trend of *E.Coli* removal as for coiforms removal, i.e. soil type 2 is more efficient for the removal coliforms than soil type 1.

In terms of the effect of HRT on pathogen removal efficiency, **Table 4.24** clearly illustrates that the VF with higher HRT performs comparatively better. For instance, the performance

efficiency of the VF with worms increased with the increase in the LRV as, 2.4 (182 hrs) > 1.8 (19.5 hrs) > 0.9 (13 hrs). This observation also indicates that the worms have a significant impact in reducing pathogens in VF.

The observation of this study is consistent with those of Arora et al. (2014a; 2014b), that reported that the log removal of coliforms and *E.Coli* was higher in VF (with worms) than in GF (without worms). The K value (see **Table 4.24**) for coliforms was found to be 3.15 log units in VF and 2.24 in GF and for *E.Coli* it was 2.03 log units for VF and 1.0 log units for GF.

Table 4.24 The performance of the VF with respect to coliforms removal efficiency, depending upon the variation in the HRT.*LRV, represents the Log removal value (K) = $\log_{10}$ (IN/EF3), and % removal = ((IN – EF3)/IN)*100.

HRT (hours)	**182**		**19.5**		**13**	
Sample ID	**Without worms**	**with worms**	**Without worms**	**with worms**	**Without worms**	**with worms**
IN	1.7E+06	5.3E+06	1.4E+06	5.8E+06	1.1E+07	4.4E+06
EF1	2.0E+05	4.3E+04	2.3E+05	8.3E+05	1.1E+06	6.9E+05
EF2	4.4E+05	2.4E+04	2.0E+05	1.1E+05	1.3E+06	6.3E+05
EF3	4.4E+04	2.1E+04	1.6E+05	8.7E+04	1.3E+06	5.0E+05
% Removal	97	100	88	98	89	89
LRV (K)*	1.6	2.4	0.9	1.8	1.0	0.9

Therefore the observation in this study are consistent and complementary to previous studies (Bajsa et al. 2004; Rajpal et al. 2012), which observed a significant removal of pathogens such as *E. Coli* and faecal coliforms in VF. These studies found that worms are capable of consuming all types of pathogens such as bacteria, fungus, protozoa and nematodes. Thus, the reduction of pathogens in the EFs might be attributed to a synchronous effect of the action of enzymes and antibacterial microbes, which is prevalent in the intestines of worms. These enzymes could have toxic effects towards pathogens and antibacterial microbes prevent their growth in the system, thus significantly reducing pathogens (Khwairakpam and Bhargava 2009). Bilej et al. (2000) argues that the coelomic fluid excreted by worms has a variety of biological effects including an effective defensive mechanisms against invaders. In addition to the worm effect, a significant removal of these microbes by the filter layers themselves is attributed to the retention capacity of the filter media via the adsorption of pathogens.

Moreover, these filter media create unfavourable conditions for pathogens to survive (Kadam et al. 2008).

4.3.5.3 Biofilm Growth

A collection of microorganisms attached to a solid surface and enclosed by a matrix of extracellular polymeric substances (EPS) is termed a biofilm. Biofilms contain water, EPS, cells, entrapped particles and precipitates, sorbed ions and polar/apolar organic molecules (Hans-Curt Flemming 1999). The microorganisms could be bacteria, diatoms, fungi, algae and/or protozoa (Sebastian Cohn et al. 2010). In biological wastewater treatment systems, biofilms are exploited to remove organic and inorganic pollutants from the water.

4.3.6 Hypotheses Testing Outcomes

The statistics associated with all the individual hypothesis tests as shown in **Appendices 4.6** to **4.9**, are given in Tables in **Appendix 4.10**. Whether these hypotheses have been accepted or rejected is indicated in the matrices of Tables in **Appendices 4.6** to **4.9 (colour coded – red for rejected and green for accepted).** These outcomes allow the following deductions to be made.

4.3.6.1 The effect of filter layers

1. *The soil, sand and gravel layers (EFs 1, 2 & 3) do not have significantly different temperatures compared to the IN.* This hypothesis was **accepted**. In Phase I, both with and without worms, the Influent (IN) temperature was not significantly different to the temperatures in Effluents 1, 2 and 3 (EF1, EF2 & EF3). Thus, without worms, the temperature in EF1 was found to decrease by 8 %, whereas it decreased by 9 % for both EF2 and EF3, compared to the IN temperature. However, with worms, the temperature decreased by 4 %, 5 % and 3 % for EF1, EF2 & EF3 respectively, compared to the IN temperature. Although this might seem to demonstrate that the worms, rather than the layers, are affecting the EF temperatures, it is more likely that these slight variations in temperature are the result of variations in the ambient temperature at the time of

sampling. The maximum ambient temperature recorded during the experimental period ranged from 12.2 °C to 36.2 °C, **Figure 4.16**. Again, in Phase II, with and without worms, the IN temperature was not significantly different to the temperatures in EF1, EF2 & EF3. Thus, without worms, the temperature in EF1 was found to decrease by 4 % and it decreased by 7 % for EF2 and EF3, compared to the IN temperature. With worms, the temperature in EF1 was found to decrease by 0.4 % whereas it decreased by 2 % and 3 % for EF2 and EF3, compared to the IN temperature. Again, these changes were likely to be due to the effect of the ambient temperature at the time of sampling.

2. *The soil, sand and gravel layers (EFs 1, 2 & 3) do not have significantly different pHs compared to the IN pH.* This hypothesis was **rejected**. In Phase I, both with and without worms, there was a significant difference between the IN pH and the pHs of EF1, EF2 and EF3, at the 99 % probability level. Without worms, the pHs of EF1, EF2 and EF3 decreased by 13 %, 12 % and 10 % respectively, compared to the IN. With worms, it decreased by 6 % for EF1, EF2 and EF3. Here, the soil layer without worms seemed to perform better than the other layers, attaining a neutral pH of 7. The soil layer with worms attained a slightly basic pH of 7.2, which remained constant and was not affected by the sand and gravel layers. In Phase II, both with and without worms, there was a significant difference between the IN pH and the pHs of EF1, EF2 and EF3, at the 99 % probability level. Without worms, the pHs of EF1, EF2 and EF3 decreased by 16 % compared to the IN. With worms, the pH decreased by 8 % for EF1 and by 7 % for both EF2 and EF3. The resulting pHs of the EFs showed that the soil layer without worms attained a slightly acidic pH of 6.8.

3. *The soil, sand and gravel layers (EFs 1, 2 & 3) do not have significantly different conductivities compared to the IN.* This hypothesis was **rejected** for Phase I with worms but **accepted** otherwise. In Phase I, without worms, the IN conductivity did not change significantly. Thus, the IN conductivity was found to be increased by 17 %, 18 % and 22 % for EF1, EF2 and EF3, respectively. However, with worms, a significant difference was observed between the conductivity of the IN and EF1 and EF2, at the 95 % probability level - the significance difference was found to be at the 99 % level for EF3. Thus, EF1 conductivity increased by 24 % and by 27 % for both EF2 and EF3. This observation demonstrated that the soil layer with worms does have a significant effect on increasing the conductivity - from 1.2 mS/cm for the IN to 1.4 mS/cm for EF1. In Phase

II, both with and without worms, the conductivity changes were not significant. The conductivity decreased by 4 %, 5 % and 3 % for EF1, EF2 and EF3, respectively, compared to the IN. With worms, it decreased by 3 % for EF1 whereas it increased by 2 % and 3 % for EF2 and EF3, respectively. The soil layer in Phase I 'without worms' contributed the most to increasing the conductivity.

4. *The soil, sand and gravel layers do not have significantly reduced turbidity compared to the IN.* This hypothesis was **rejected**. In Phase I, both with and without worms, the turbidity was significantly decreased, at the 99 % probability level, for EF1, EF2 and EF3, compared to the IN. Thus, without worms, the turbidity of EF1 and EF2 were both reduced by 58 % and by 75 % for EF3. With worms, the turbidity of EF1, EF2 and EF3 were reduced by 73 %, 82 % and 87 %, respectively. This outcome showed that although the sand layer contributes to removing turbidity from the IN, the soil layer with worms performs better than any other layer (i.e. a reduction from 339 mg/L to 91 mg/L). Again in Phase II, without worms, the turbidity was significantly decreased, at the 95 % probability level for EF1 and at the 99 % probability level for EF2 and EF3. Thus, without worms, the turbidity in EF1, EF2 and EF3 was reduced by 73 %, 76 % and 78 %, respectively. With worms, it was reduced by 65 % for EF1 and by 83 % for both EF2 and EF3. Again, although the sand layer contributed to removing turbidity from the IN, the soil layer without worms removed turbidity more effectively than all the other layers.

5. *The soil, sand and gravel layers do not have significantly reduced TSS compared to the IN*. This hypothesis was **rejected**. In Phase I, both with and without worms, the TSS was significantly lower, at the 99 % probability level, for EF1, EF2 and EF3, compared to the IN. Thus, without worms, the TSS of EF1, EF2 and EF3 was reduced by 47 %, 49 % and 64 %, respectively. With worms, the TSS of EF1, EF2 and EF3 was reduced by 68 %, 77 % and 82 %, respectively. This outcome demonstrated that although the sand and gravel layers contributed in removing TSS from the IN, the soil layer with worms performed better than any other layer (i.e. a reduction from 281 mg/L to 94 mg/L). Again in Phase II, without worms, the TSS was significantly lower, at the 95 % probability level, for EF1, whereas it was significantly lower, at the 95 % level, for EF2 and EF3. Thus, without worms, the TSS in EF1, EF2 and EF3 was reduced by 74 %, 79 % and 82 %, respectively. With worms, the TSS was significantly decreased, at the 99 % probability level, for EF1, EF2 and EF3, compared to the IN. Thus; it was reduced by 71 % for EF1

and by 86 % for both EF2 and EF3. Again, although the sand layer contributed to the removal of TSS from the IN, the soil layer without worms removed TSS more effectively than the other layers (i.e. 424 mg/L to 112 mg/L).

6. *The soil, sand and gravel layers do not have significantly increased DO levels compared to the IN*. This hypothesis was **accepted** for Phase I, except EF3 (without worms), but **rejected** otherwise. In Phase I, without worms, the DO in EF1 and EF2 did not change significantly, whereas it increased significantly, at the 90 % probability level, in EF3. Thus, the DO in EF1 and EF2 increased in up to 0.50 mg/L (1567 %) and in EF3 up to 0.80 mg/L (2567 %), compared to the IN (0.03 mg/L) However, with worms, the DO did not change significantly in the EF1, EF2 and EF3. Interestingly, the IN DO decreased as it passed through the sand and gravel layers, from 2.6 mg/L for EF2 to 2.1 mg/L for EF3. In Phase II, both with and without worms, the DO in the EFs increased significantly, at the 99 % probability level. Thus, without worms, the DO in EF1, EF2 and EF3 increased by 284 %, 333 % and 326 %, respectively. With worms, the DO in the EF1, EF2 and EF3 increased by 158 %, 168 % and 136 %, respectively. These observations suggested that the soil was more effective in increasing DO than the worms.

7. *The soil, sand and gravel layers do not significantly reduce COD levels compared to the IN COD level.* This hypothesis was **accepted** for Phase I EF1 and EF2 (without worms), EF1 (with worms) and for Phase II EF1 (without worms) and **rejected** otherwise. In Phase I, without worms, the COD did not decrease significantly for EF1 and EF2 but it did decrease significantly for EF3, at the 95 % probability level. Thus, without worms, the COD of EF1, EF2 and EF3 were decreased by 13 %, 19 % and 29 %, respectively. With worms, the COD did not decrease significantly for EF1 whereas it decreased significantly for EF2 and EF3, at the 99 % probability level. Thus, the COD of EF1 was found to decrease by 23 %, whereas it was found to be decrease by 41 % for EF2 and EF3. This observation suggests that the soil layer with worms contributed most to the decrease of COD. Microbes in the geo-layers and the worms could have a synchronous effect in reducing COD. Again, in Phase II, without worms, the COD did not change significantly for EF1, whereas it decreased significantly for EF2 and EF3, at the 99 % probability level, compared to the IN COD. Thus, without worms, the COD of EF1, EF2 and EF3 was decreased by 20 %, 54 % and 51 %, respectively. With worms, the COD was decreased significantly, by 44 %, 51 % and 52 % for EF1, EF2 and EF3,

respectively, at the 99 % probability level. Again, this significantly higher reduction of COD by the soil layer with worms might be due to a synchronous effect of microbes in the geo-layers and worms.

8. *The soil, sand and gravel layers do not have reduced NH_3-N levels compared to the IN*. This hypothesis was **accepted** for Phase I with worms but **rejected** otherwise. In Phase I, without worms, the ammonia nitrogen (NH_3-N) decreased significantly at the 99 % level in all the EFs. Thus, the NH_3-N decreased by 35 %, 36 % and 43 % in EF1, EF2 and EF3, respectively. However, with worms, the NH_3-N did not decrease significantly in the EFs. Thus, it decreased only by 14 %, 0.3 % and 6 % in EF1, EF2 and EF3, respectively. This observation shows that NH_3-N removal from the IN was more effective with a soil layer without worms. In Phase II, both with and without worms, the NH_3-N was significantly decreased, at the 95 % probability level for EF1, whereas it decreased, at the 99 % level for EF2 and EF3. Thus, without worms, the NH_3-N for EF1, EF2 and EF3 was found to decrease by 26 %, 33 % and 49 %, respectively. With worms, it was found to decrease by 16 %, 19 % and 20 % for EF1, EF2 and EF3, respectively. In terms of NH_3-N removal, the geo-layers themselves seemed to be the effective factor rather than the worms.

9. *The soil, sand and gravel layers do not have reduced NO_2-N levels compared to the IN*. This hypothesis was **accepted** for Phase II without worms but **rejected** otherwise. In Phase I, both with and without worms, the nitrite nitrogen (NO_2-N) was significantly decreased for EF1, EF2 and EF3, at the 99 % probability level. Thus, without worms, the NO_2-N of EF1, EF2 and EF3 was found to decrease by 61 %, 66 % and 80 %, respectively. With worms, it was found to decrease by 74 %, 81 % and 84 % for EF1, EF2 and EF3, respectively. Although the layers are likely to remove the NO_2-N, the soil layer with worms seems to reduce the NO_2-N more effectively than the other layers. In Phase II, without worms, although the NO_2-N was found to decrease by 91 %, 77 % and 95 % for EF1, EF2 and EF3, respectively, it was not statistically significant. However, with worms, the NO_2-N was significantly decreased by 83 %, 99 % and 98 % for EF1, EF2 and EF 3, respectively, at the 99 % probability level. Here, the changes were more likely to be due to geo-layers themselves rather than the worms.

10. *The soil, sand and gravel layers do not have reduced NO_3-N levels compared to the IN*. This hypothesis was **accepted** for the soil layer, EF1, in Phase I without worms, but

rejected otherwise. In Phase I, without worms, the nitrate nitrogen (NO_3-N) did not decrease significantly for EF1, whereas it decreased significantly for EF2 and EF3, at the 95 % probability level. Thus, the NO_3-N was found to decrease by 61 %, 78 % and 85 % EF1, EF2 and EF3, respectively. With worms, there was a significant decrease in the NO_3-N for all Effluents, at 99 % probability level. Thus, it was found to decrease by 69 % for EF1 and by 92 % for EF2 and EF3. The observation demonstrates that although the layers themselves are effective in removing NO_3-N, the worms are also likely to make a contribution to this. Again, in Phase II, without worms, the NO_3-N was significantly decreased for EF1, at the 99 % probability level, and for EF2 and EF3 at the at 95 % level. Thus, the NO_3-N for EF1, EF2 and EF3 was found to decrease by 91 %, 99 % and 97 %, respectively. With worms, the NO_3-N was significantly decreased by 84%, 92 % and 93 % for Effluents 1, 2 and 3, respectively, at the 99 % probability level.

11. *The soil, sand and gravel layers do not have reduced TN levels compared to the IN*. This hypothesis was **accepted** for Phase I with worms but **rejected** otherwise. In Phase I, without worms, the total nitrogen (TN) was significantly decreased for all Effluents at the 99 % probability level. Thus, the TN for EF1 and EF2 was found to decrease by 36 % whereas it decreased by 49 % for EF3. With worms, the TN did not significantly decrease in the Effluents. It was found to decrease by 18 %, 12 % and 13 % for EF1, EF2 and EF3, respectively. This observation suggests that the layers themselves are effective in removing TN and that the worms are likely to contribute to the TN. In Phase II, both with and without worms, the TN was significantly decreased for EF1, EF2 and EF3 at 99 % probability level. Thus, without worms, the TN of EF1, EF2 and EF3 was found to decrease by 19 %, 39 % and 41 % respectively. With worms, it was found to decrease by 32 % for EF1 and EF2 whereas it decreased by 35 % for EF3. This showed that whereas the layers themselves were likely to decrease the TN, the worms were likely to stabilise it.

12. *The soil, sand and gravel layers do not have reduced TOC levels compared to the IN*. This hypothesis was **accepted** for EF1 (without worms) in Phase I, all layers (without worms) and EF1 (with worms) in Phase II but **rejected** otherwise. In Phase I, without worms, the total organic carbon (TOC) did not decrease significantly for EF1 whereas it was significantly decreased for EF2, at the 95 % probability level, and for EF3 at the 99 % level, Thus, the TOC for EF1, EF2 and EF3 was found to decrease by 19 %, 40 % and 52 %, respectively. With worms, the TOC decreased significantly for EF1 at the 95 %

probability level, and for EF2 and EF3. at the 99 % level. It was found to decrease by 25 %, 39 % and 42 % for EF1, EF2 and EF3, respectively. This observation suggests that a synchronous effect of microbes in the soil layer and worms results in better TOC removal efficiency. However in Phase II, without worms, there was no significant decrease of TOC for all the EFs. Thus, the TOC decreased by 8 %, 27 % and 39 % for EF1, EF2 and EF3, respectively. With worms, the TOC did not decrease significantly for EF1 whereas it did decrease significantly for EF2 and EF3, at the 99 % level of probability. Thus, the TOC decreased by 24 %, 42 % and 43 % for EF1, EF2 and EF3, respectively. Again, these changes were likely due to the synchronous effect of microbes and worms in the soil layer.

13. *The soil, sand and gravel layers do not have reduced TP levels compared to the IN*. This hypothesis was **accepted** for the EF1 in Phase I with worms and all layers in Phase II without worms but **rejected** otherwise. In Phase I, without worms, there was a significant decrease in the total phosphorus (TP) for EF1, EF2 and EF3, at the 99 % probability level. Thus, the TP was found to decrease by 36 %, 40 % and 50 % for EF1, EF2 and EF3, respectively. However, with worms, the TP did not significantly decrease for EF1, whereas it decreased significantly for EF2 at the 90 % probability level and at the 95 % level for EF3. Thus, it was found to decrease by 3 %, 16 % and 19 % for EF1, EF2 and EF3, respectively. In Phase II, without worms, the TP did not change significantly in EFs. Thus, the TP increased by 8 % and 2 % for EF1 and EF2 whereas it decreased by 0.3 % for EF3. With worms, the TP increased significantly at the 99 % level for all EFs. Thus, it increased by 35 %, 30 % and 29 % for EF1, EF2 and EF3, respectively. Here, the increase in TP is more likely to be due to the effect of worms. The soil layer without worms in Phase I was the most effective layer in removing TP from the IN. However, the sand and gravel layers also contributed to removing TP.

4.3.6.2 The effect of worms in the VF

The comparison was made between the values of (a) EF1 with worms and EF1 without worms, (b) EF2 with worms and EF2 without worms, and (c) EF3 with worms and EF3 without worms, for both Phases (Phase I and Phase II), to see if worms have any significant effect in the system.

1. *The worms do not have any effect on the temperature of the EFs compared to the temperature of the IN*. This hypothesis was **accepted**. In both phases, the worms did not have any significant effect in changing the EF temperatures compared to the IN. In Phase I, while comparing the difference between the EF temperatures and the IN temperature, without worms and with worms, it was found to be reduced by 9 % for EF1 and EF2 and by 7 % for the EF3. However, in Phase II, it was found to be higher by 3 % for EF1 and by 4 % for EF2 and EF3. These results demonstrated that although the temperature could be affected by the presence of worms in the soil layer, it was insignificant compared to the effects of the ambient temperature.

2. *The worms do not have any effect on the pH of the EFs compared to the pH of the IN.* This hypothesis was **rejected** for EF1 and EF2 in Phase I, but **accepted** otherwise. In Phase I, the worms have significantly increased the pH for EF1 and EF2, at the 99 % level of probability, but there was no significant difference in pH for EF3. The pH was higher by 3 % and 1 % for EF1 and EF2, respectively, for the VF with worms. In Phase II, no significant difference was observed for the respective EFs. The pH was found to be less by 0.2 % for EF1 and it was higher by 1 % and 0.5 % for EF2 and EF3, respectively. Although the difference in pH could be due to the effect of worms, it might also be due to the effect of layers themselves.

3. *The worms do not have any effect on the conductivity of the EFs compared to the IN*. This hypothesis was **accepted** for EF1 in Phase II but **rejected** otherwise. In Phase I, the worms have significantly lowered the conductivity for the respective EFs, at the 95 % level of probability for EF1 and at the 99 % level for EF2 and EF3. The difference was found to be by 20 %, 19 % and 22 % for EF1, EF2 and EF3, respectively. In Phase II, the worms did not significantly increase the conductivity for EF1, whereas the worms significantly increased the conductivity for EF2 and EF3, at the 95 % level of probability. Thus, it was raised by 12 %, 19 % and 17 % for EF1, EF2 and EF3, respectively. Although the change in the conductivity was probably due to the worms' action, the alternative patterns of the worm action in Phase I and Phase II was probably due to the different soil types.

4. *The worms do not significantly reduce the turbidity of the EFs compared to the IN*. This hypothesis was **rejected** for EF2 and EF3 in Phase II but **accepted** otherwise. In Phase I, there was no significant difference between the turbidity for the respective EFs, with or without worms. The turbidity was found to be higher by 36 % and 10 % for EF1 and EF3, respectively, for the VF with worms and, conversely, it was found to be lower by 12 % for EF2. In Phase II, no significant difference was observed for EF1, but there was a significant difference for EF2 and EF3, at the 95 % probability level. Thus, the turbidity was lower by 8 %, 42 % and 37 % for EF1, EF2 and EF3, respectively, in the VF with worms.

5. *The worms do not significantly reduce the TSS of the EFs compared to the IN*. This hypothesis was **accepted** for EF1 in both Phase I and Phase II but **rejected** otherwise. In both Phases I and II, there was no significant difference in the TSS of EF1, whereas there was a significant difference for the TSS of EF2 and EF3, at the 95 % probability level. In Phase I, the TSS was found to be less by 5 %, 29 % and 20 % for EF1, EF2 and EF3, respectively, in the VF with worms. In Phase II, it was found to be less by 13 % 45 % and 39 %, respectively. Thus, worms appeared to contribute in reducing the TSS of the IN.

6. *The worms do not contribute to increasing the DO of the EFs compared to the IN*. This hypothesis was **accepted** for Phase II but **rejected** for Phase I. In Phase I, there was a significant difference between the DO levels for the respective EFs, at the 99 % probability level for EF1 and EF2 and at the 95 % level for EF3. The DO was found to be higher by 420 %, 380 % and 163 % for EF1, EF2 and EF3, respectively, in the VF with worms. In Phase II, there was no significant increase in DO for the VF with worms compared to without worms. The DO was found to be higher by only 27 %, 17 % and 4 % for EF1, EF2 and EF3, respectively. Here, although the value of DO is higher in Phase II (2.1 ± 0.4 mg/L for Phase I and 4.0 ± 0.3 mg/L for Phase II, in the VF with worms), it appeared that the soil type in the first layer was responsible for the higher DO value in Phase II (0.8 ± 0.4 mg/L for Phase I and 3.8 ± 0.3 mg/L for Phase II, in the VF without worms).

7. *The worms do not contribute to reducing the COD of the EFs compared to the IN*. This hypothesis was **accepted** for EF2 and EF3 in Phase II but **rejected** otherwise. In Phase I, significant differences were found for the COD in the respective EFs, at the 99 %

probability level. The COD was found to be less by 46 %, 56 % and 49 % for EF1, EF2 and EF3, respectively, in the VF with worms. In Phase II, a significant difference was found for EF1, at the 99 % probability level. Conversely, there was no statistically significant difference for EF2 and EF3. Thus, the COD was less, in the VF with worms, by 46 %, 19 % and 25 % EF1, EF2 and EF3, respectively.

8. *The worms do not contribute to reducing the NH_3-N of the EFs compared to the IN*. This hypothesis was **accepted**. In both Phase I and Phase II, there was no significant difference between the NH_3-N levels for the respective EFs. In Phase I, the NH_3-N was lower by 10 % for EF1, but it was higher by 5 % and 11 % for EF2 and EF3, respectively. In Phase II, the NH_3-N was less by 7 % for EF1, but it was higher by 1 % and 30 % for EF2 and EF3, respectively.

9. *The worms do not contribute to reducing the NO_2-N levels of the EFs compared to the IN*. This hypothesis was **accepted** for EF1 and EF3 in Phase I and EF3 in Phase II and **rejected** otherwise. In Phase I, there was no significant difference between the NO_2-N levels for EF1 and EF3 but there was a significant difference for EF2, at the 95 % probability level. Thus, the NO_2-N, in the VF with worms, was found to be lower by 39 %, 49 % and 26 % for EF1, EF2 and EF3, respectively. In Phase II, there was a significant difference between the NO_2-N in EF1, at the 95 % probability level and EF2, at the 90 % level, but no significant difference was observed for EF3. The NO_2-N, in the VF with worms, was found to be higher by 350 % for EF1; conversely, it was found to be lower by 94 % and 10 % for EF2 and EF3, respectively.

10. *The worms do not contribute to reducing the NO_3-N levels of the EFs compared to the IN.* This hypothesis was **accepted**. In both Phase I and Phase II, there was no statistically significant difference in the NO_3-N levels for the respective EFs. In Phase I, the NO_3-N, in the VF with worms, was found to be higher by 42 % for EF1 but it was found to be lower by 33 % for EF2 and EF3. In Phase II, the NO_3-N was found to be lower by 10 % for EF1 but higher by 440 % and 19 % for EF2 and EF3 respectively.

11. *The worms do not contribute to reducing the TN of the EFs compared to the IN*. This hypothesis was **rejected** for EF2 and EF3 in Phase II but **accepted** otherwise. In Phase I, there was no significant difference between the TN values for the respective EFs. Thus,

the TN was found to be less by 12 % and 5 % for EF1 and EF2, respectively but it was found to be higher by 17 % for EF3. In Phase II, there was no significant difference between the TN value for EF1 but there was a significant difference for EF2 and EF3, at the 95 % probability level. The TN was found to be higher by 5 %, 32 % and 37 % for EF1, EF2 and EF3, respectively.

12. *The worms do not contribute to reducing the TN of the EFs compared to the IN*. This hypothesis was **accepted** for EF1 and EF3 in Phase II but **rejected** otherwise. In Phase I, a significant difference was found between the TOC values for EF1 and EF2, at the 99 % probability level and for EF3, at the 95 % level. The TOC, in the VF with worms, was found to be less by 53 %, 48 % and 38 % for EF1, EF2 and EF3 respectively. However, in Phase II, there was no significant difference for EF1 and EF3. Interestingly, a significant difference was observed for EF2. The TOC was found to be less by 44 %, 46 % and 36 % for EF1, EF2 and EF3 respectively.

13. *The worms do not contribute to reducing the TP of the EFs compared to the IN*. This hypothesis was **rejected** for EF3 in Phase I but **accepted** otherwise. In Phase I, there was no significant difference between the TP values for EF1 and EF2. Conversely, a significant difference was observed for EF3, at the 95 % probability level. The TP, in the VF with worms, was found to be 15 %, 8 % and 24 % for EF1, EF2 and EF3 respectively. In Phase II, there was no significant difference between the TP values for the respective EFs. The TP was found to be higher by 11 %, 13 % and 14 % for EF1, EF2 and EF3 respectively.

4.3.6.3 The effect of soil type in the VF

1. *The soil type does not have any effect on the temperature of the EFs compared to the temperature of the IN*. This hypothesis was **accepted** except for EF1 with worms. In the VF without worms, there was no significant difference in the temperature between the respective EFs. Thus, in Phase II, the temperature was found to be less by 3 % for EF1 and 5 % for EF1 and EF2, compared to Phase I. With worms, there was a significant difference in temperature for EF1, at the 90 % probability level, but no significant difference was observed for EF2 and EF3. In Phase II, the temperature was found to be

higher by 11 %, 10 % and 7 % for EF1, EF2 and EF3, respectively. Thus, the opposite trend was observed for the temperature in the VF with and without worms, and for Phase I and Phase II. Though the effect seemed to be only low, it may be due to a synchronous effect of worms and soil type.

2. *The soil type does not have any effect on the pH of the EFs compared to the pH of the IN*. This hypothesis was **rejected**. In the VF without worms, a significant difference in pH between 'soil type 1' and 'soil type 2' was observed for EF1, EF2 and EF3, at the 99%, 95 % and 90 % probability level, respectively. Thus, the pH was found to be less in Phase II for EF1, EF2 and EF3 by 3 %, 4 % and 5 %, respectively. In the VF with worms, there was a significant difference in pH between soil type 1 and soil type 2 for EF1, EF2 and EF3, at the 99 % level. Thus, the pH was found to be higher in Phase II by 5 % for EF1 and 4 % for EF2 and EF3. The pH was found to be slightly alkaline in soil type 1 (7.2 ± 0.02 for EF3 with worms) but slightly acidic in soil type 2 (6.9 ± 0.03 for EF3 with worms).

3. *The soil type does not have any effect on the conductivity of the EFs compared to the IN*. This hypothesis was **accepted** for the VF with worms but **rejected** for the VF without worms. Without worms, a significant difference in the conductivity between soil type 1 and soil type 2 was observed at the 90 % probability level for EF1 and EF3 and at the 95 % level for EF2. Thus, the conductivity was found to be less in soil type 2 by 32 %, 33 % and 34 % for EF1, EF2 and EF3, respectively. On the contrary, with worms, no significant difference was observed. Thus, the conductivity in soil type 2 was found to be lower by only 5 %, 25 and 1 % for EF1, EF2 and EF3, respectively. Therefore, it seemed that soil type 2 was more effective in reducing the conductivity than soil type 1. However, worms were responsible for raising the conductivity in the VF.

4. *The soil type does not significantly reduce the turbidity of the EFs compared to the IN.* This hypothesis was **accepted** for EF1 and EF2 in the VF with worms but **rejected** otherwise. Without worms, a significant difference in the turbidity was observed for EF1 and EF3 at the 99 % probability level and at the 95 % level for EF2. Thus, the turbidity was found to be higher in soil type 2 by 129 %, 79 % and 179 % for EF1, EF2 and EF3, respectively. With worms, there was no significant difference for EF1 and EF2, but a significant difference at the 99 % probability level was observed for EF3. The turbidity

was found to be higher for EF1, EF2 and EF3 by 55 %, 17 % and 59 %, respectively. This observation showed that soil type 1 removed turbidity from the IN better than soil type 2.

5. *The soil type does not significantly reduce the TSS of the EFs compared to the IN*. This hypothesis was **rejected** for EF2 with worms but **accepted** otherwise. Without worms, there was no significant difference observed for the respective EFs. Thus, the TSS was found to be higher by 19 % and 21 % for EF1 and EF3 but less by 3 % for EF2. With worms, there was no significant difference observed for EF1 and EF3 but a significant difference at the 95 % probability level was observed for EF2. Here, the TSS was found to be higher in 'soil type 2' by 9 % only, but less by 25 % and 8 % for EF2 and EF3 respectively. Therefore, this observation showed that the soil type 2 was more efficient in reducing TSS from the IN.

6. *The soil type does not contribute to increasing the DO of the EFs compared to the IN*. This hypothesis was **rejected**. Without worms, there was a significant increase in DO in soil type 2 for the relevant EFs, at the 99 % probability level. The DO was found to be higher in soil type 2 by 591 %, 679 % and 380 % for EF1, EF2 and EF3, respectively. With worms, a significant increase in DO was observed for EF1 at the 95 % probability level and at the 99 % level for EF2 and EF3. The DO was found to be higher in soil type 2 by 68 %, 90 % and 91 % for EF1, EF2 and EF3, respectively. This observation showed that the soil type 2 was preferred in the VF in order to achieve a higher DO. The DO in the VF with worms for EF1 increased up to 4.0 ± 0.3 mg/L with soil type 2, however it was only 2.1 ± 0.4 mg/L with soil type 1.

7. *The soil type does not contribute to reducing the COD of the EFs compared to the IN.* This hypothesis was **rejected** for EF3 without worms but **accepted** otherwise. Without worms, there was a significant difference in COD for EF3 at the 99 % probability level but no significant difference was observed for EF1 and EF2. The COD was found to be less in soil type 2 by 7 %, 42 % and 29 % for EF1, EF2 and EF3, respectively. With worms, no significant difference was observed in COD for the relevant EFs. The COD was found to be less in soil type 2 by 7 % for EF1 whereas it was higher in EF2 and EF3 by 7 % and 6 % respectively. Thus, the soil type 2 seemed to be more efficient in reducing COD from the IN.

8. *The soil type does not contribute to reducing the NH_3-N of the EFs compared to the IN*. This hypothesis was **accepted** for EF3 without worms but **rejected** otherwise. Without worms, the NH_3-N was significantly higher in soil type 2 for EF1 and EF2 at the 95 % probability level but it was not significant for EF3. The NH_3-N was higher by 54 %, 39 % and 19 % for EF1, EF2 and EF3, respectively. With worms, the NH_3-N was significantly higher in soil type 2 for the respective EFs, at the 99 % probability level. The NH_3-N was higher 59 %, 34 % and 39 % for EF1, EF2 and EF3, respectively. Thus, this observation showed that soil type 1 was efficient in reducing NH_3-N from the IN.

9. *The soil type does not contribute to reducing the NO_2-N levels of the EFs compared to the IN.* This hypothesis was **accepted** for EF1 with worms but **rejected** otherwise. Without worms, NO_2-N was significantly less in soil type 2 at the 99 % probability level for EF1 and at the 95 % for EF2 and EF3. The NO_2-N was found to be less by 93 % for EF1 and EF3 and by 80 % for EF2. With worms, there was no significant difference for EF1 but a significantly lower NO_2-N was observed for EF2 and EF2, both at the 99 % level. The NO_2-N was found to be less in soil type 2 by 47 %, 98 % and 91 % for EF1, EF2 and EF3, respectively. Thus, this observation showed that soil type 2 was efficient in reducing the NO_2-N from IN.

10. *The soil type does not contribute to reducing the NO_3-N levels of the EFs compared to the IN*. This hypothesis was **rejected** for EF1 with worm and **accepted** otherwise. Without worms, there was no significant difference in NO_3-N between soil type 1 and 2. Though NO_3-N was found to be less in soil type 2 by 40 %, 92 % and 50 % for EF1, EF2 and EF3 respectively, it was not statistically significant. With worms, a significant difference was observed for EF1, at the 99 % probability level, but for EF2 and EF3, the difference was not significant. The NO_3-N was less in soil type 2 by 62 %, 34 % and 30 % for EF1, EF2 and EF3, respectively. Again, this observation showed that soil type 2 was efficient in reducing NO_3-N from the IN.

11. *The soil type does not contribute to reducing the TN of the EFs compared to the IN*. This hypothesis was **rejected** for EF2 without worms and **accepted** otherwise. Without worms, there was a significant difference in TN between soil type 1 and 2 for EF2 but no significant difference was observed for EF1 and EF3. The TN was found to be less by 18%, 38 % and 24 % for EF1, EF2 and EF3, respectively. With worms, there was no

significant difference in TN between soil type 1 and 2. It was found to be less by 2 %, 13 % and 12 % for EF1, EF2 and EF3, respectively. Similar to NO_2-N and NO_3-N, the soil type 2 seemed to be better in reducing TN from the IN.

12. *The soil type does not contribute to reducing the TOC of the EFs compared to the IN*. This hypothesis was **accepted**. There was no significant difference in TOC between soil type 1 and 2 for the respective EFs, with and without worms. Without worms, the TOC was found to be less in soil type 2 by 14 %, 9 % and 4 % for EF1, EF2 and EF3, respectively. With worms, the TOC was higher in soil type 2 for EF1 by 2 % and less by 4 % and 1 % for EF2 and EF3, respectively. Thus, in terms of TOC removal, soil type did not seem to have any significant effect.

13. *The soil type does not contribute to reducing the TP of the EFs compared to the IN*. This hypothesis was **rejected**. The TP was significantly higher in soil type 2 compared to soil type 1, at the 99 % probability level for the respective EFs, with and without worms. The TP was higher in soil type 2 by 71 %, 75 % and 104 % for EF1, EF2 and EF3, respectively in the VF without worms. With worms, it was higher by 65 %, 83 % and 88 % for EF1, EF2 and EF3, respectively. Thus, soil type 1 may be better in reducing TP. Conversely, if we are looking for higher TP (as a nutrient supply) in EFs, soil type 2 may be better.

4.3.6.4 The effect of Hydraulic Retention Time and the Hydraulic Loading Rate

1. *The temperatures of the EFs do not change significantly when the HRT increases and the HLR decreases*. This hypothesis was **accepted**. There was no significant difference in temperature among OC1, OC2 and OC3. The temperature in OC2 and OC3 was higher by 10 % and 2% respectively compared to OC1. The temperature in OC3 was lower by 7.3 % than in OC2. Although the temperature seems to be increase by higher HRT and less HLR, it was not statistically significant.

2. *The pH of the EFs do not change significantly when the HRT increases and the HLR decreases*. This hypothesis was **rejected**. There was a significant difference in pH between OC1 & OC2, at the 90 % probability level whereas at the 99 % level, between

OC1 & OC3 and OC2 & OC3. The pH in OC2 was lower by 1.1 % but higher by 0.9 % in OC3 compared to OC1. With respect to OC2 and OC3, it was found to be higher by 1.9% in the latter. This showed the pH became more alkaline with higher HRT and lower HLR, as pH increased from 7.2 in OC1 to 7.3 in OC3.

3. *The conductivity of the EFs do not change significantly when the HRT increases and the HLR decreases*. This hypothesis was **rejected**. Though there was no significant difference in conductivity between OC1 & OC2, a significant difference at the 99 % probability level was observed for OC1 & OC3 and OC2 & OC3. The conductivity in OC2 and OC3 was found to be higher by 5.2 % and 57.4 %, respectively compared to OC1. Similarly, it was higher in OC3 by 49.6 % compared to OC2. Thus, the conductivity increased with increasing HRT and decreasing HLR, it increased from 1.02 in OC1 to 1.61 mS/cm in OC3.

4. *The turbidity of the EFs do not reduce significantly when the HRT increases and the HLR decreases*. This hypothesis was **accepted**. There was a significant increase in turbidity between OC1 & OC2 and OC1 & OC3, at the 99 % probability level. The turbidity was higher in OC2 and OC3 by 59.3 % and 71.5 % respectively compared to OC1. The increasing trend of turbidity with higher HRT and lower HLR showed that the efficiency of turbidity removal decreased in OC3.

5. *The TSS of the EFs do not reduce significantly when the HRT increases and the HLR decreases*. This hypothesis was **accepted**. There was a significant increase in TSS between OC1 & OC3, at the 99 % probability level. Although the TSS was lower in OC2 by 6.7 % compared to OC1, it was higher by 32 % in OC3 compared to OC2. Similar to turbidity, the increasing trend of TSS with higher HRT and lower HLR reduced the TSS removal efficiency.

6. *The DO of the EFs do not increase significantly when the HRT increases and the HLR decreases.* This hypothesis was **rejected**. There was as significant increase in DO, at the 90 % probability level, in OC3 compared to OC1. Although the DO was higher in OC2 by 16.9 % compared to OC1 and higher in OC3 by 28.8 % compared to OC2, it was not statistically significant. It seemed that OC3 was better in achieving higher DO.

7. *The COD of the EFs do not reduce significantly when the HRT increases and the HLR decreases*. This hypothesis was **accepted**. There was no significant difference in COD with variation in the OCs. The COD in OC2 and OC3 was lowered by 40.1 % and increased by 0.9 %, respectively compared to OC1. With respect to OC2 & OC3, it was increased by 68.6 %. Thus, this increasing trend of COD with higher HRT and lower HLR (OC3) suggested that OC2 reduced COD better than the other OCs.

8. *The NH_3-N of the EFs do not reduce significantly when the HRT increases and the HLR decreases*. This hypothesis was **accepted**. There was no significant difference in NH_3-N with variation in the OCs. The NH_3-N in OC2 and OC3 was higher by 7.4 % and 52.9 %, respectively, compared to OC1. With respect to OC2 & OC3, it was higher in the latter by only 42.4 %. Thus, the tendency of NH_3-N to increase with higher HRT and lower HLR, i.e. from 50 mg/L in OC1 to 76 mg/L in OC3, though not statistically significant, might suggest that OC1 lowers the NH_3-N better than under the other OCs.

9. *The NO_2-N of the EFs do not reduce significantly when the HRT increases and the HLR decreases.* This hypothesis was **accepted**. There was no significant difference in NO_2-N with variation in the OC. The NO_2-N in OC2 and OC3 was found to be higher by 3.4 % and lower by 26.5 %, respectively compared to OC1. With respect to OC2 & OC3, it was lower in the latter by 28.9 %. Thus, a clear decreasing trend of NO_2-N with higher HRT and lesser HLR, 1.2 mg/L in OC1 to 0.9 mg/L in OC3, though not statistically significant, might suggest that OC3 reduces NO_2-N better than other OCs.

10. *The NO_3-N of the EFs do not reduce significantly when the HRT increases and the HLR decreases*. This hypothesis was **rejected**. There was a significant difference in NO_3-N between OC2 & OC3, at the 90 % probability level. The NO_3-N was higher by 141.5 % in OC2 and lower by 64.4 % in OC3 compared to OC1. In terms of OC2 & OC3, it was lower in OC3 by 85.3 %. Here, the trend of NO_3-N seemed to be ambiguous but it decreased with increasing HRT and less HLR - 0.8 mg/L in OC1 to 0.3 mg/L in OC3, although this was not statistically significant.

11. *The TN of the EFs do not reduce significantly when the HRT increases and the HLR decreases.* This hypothesis was **accepted**. There was no significant difference in TN with variation in the OCs. The TN in OC2 and OC3 was higher by 12.9 % and 27.4 %,

respectively, compared to OC1. With respect to OC2 & OC3, it was higher in the latter by 12.9 %. Thus, TN increased with increasing HRT and less HLR - 62 mg/L in OC1 to 79 mg/L in OC3, although this was not statistically significant.

12. *The TOC of the EFs do not reduce significantly when the HRT increases and the HLR decreases*. This hypothesis was **accepted**. There was no significant difference in TOC with variation in the OCs. The TOC in OC2 was found to be higher by 9.8 % and lower by 13 % in OC3 compared to OC1. With respect to OC2 & OC3, it was lower in the latter by 20.7 %. Similar to TN, the trend of TOC seems to be ambiguous but it decreased with increasing HRT and less HLR - 41 mg/L in OC1 to 36 mg/L in OC3, though this was not statistically significant.

13. *The TP of the EFs do not reduce significantly when the HRT increases and the HLR decreases*. This hypothesis was **rejected**. A significant decrease in TP was observed with higher HRT and lower HLR, at the 99 % probability level, between OC1 & OC3 and OC2 & OC3. The TP in OC2 was found to be higher by 13.9 % and lower by 42.7 % in OC3 compared to OC1. With respect to OC2 & OC3, it was lower in the latter by 49.7 %. Again the trend of TP seems to be ambiguous but it did decrease with increasing HRT and lower HLR - 27 mg/L in OC1 to 16 mg/L in OC3. This finding suggested that OC3 was better for lower nutrients in the EFs.

4.3.7 Challenges in the design of a community-based VF system

The above vermifiltration pilot plant for the treatment of municipal wastewater was operated for almost a year and half. During this period, different challenges emerged that provided significant insight into problems that might be expected to impact on the effective operation of such technology. Detailed scientific testing was conducted that provided new information of the effect of different soil types, the influence of each individual layer and the role of the worms themselves - and some of the factors that are required to ensure a healthy worm population. Environmental conditions such as temperature, pH, moisture, aerobic environment, hydraulic retention time and hydraulic loading rate were identified as major parameters to be examined in order to optimize the system. Moreover, the nature of the

influent was also found to be of importance and, in this regard, for the influent used here, system clogging was observed to be a particular problem - due to fibre in the influent resulting in a decreased water percolation rate through the top soil layer.

Notably, during the entire testing period, the vermifiltration system itself was found not to create any foul odour. Worms create aerobic conditions in the system due to their burrowing action; hence the action of anaerobic microorganisms that are responsible for releasing foul odours (caused by hydrogen sulphide and mercaptans) is suppressed. Odour problems were only experienced during the feeding process from the influent tank to the vermifiltration unit. As alluded to previously, problems encountered with the feeding system were mainly due to the presence of highly fibrous material in the influent even though it was passed through a 2 mm screen and the accumulation of fibrous material on the surface of the top layer of the vermifiltration unit created a problem for the percolation of the wastewater through the soil bed.

4.3.7.1 Environmental conditions

In a vermifiltration unit, earthworms are used as a major filter media. They usually live on/in the soil (top) layer and it has been observed that the influent water quality (IN) has a direct impact on them. Therefore, it is important to maintain optimum environmental conditions to keep the worms healthy and active in the system (Baumgartner 2013). In this study, a particular focus was made on the influent water quality and the effluent water quality from the soil layer (EF1) as they are determinative/indicative of the condition of the soil layer.

Temperature*:* The optimal temperature range for worms to remain active in the system is considered to be 15 °C to 25 °C (Klein 2008). During our study period, the IN and EF1 temperatures ranged 11.6 - 24.0 °C and 10.4 - 22.6 °C respectively.

pH: The best pH to keep worms active is neutral (7). However, they can survive in the pH range of 4.5 to 9. During the study period, the IN and EF1 pH ranged 5.6 - 8.3 and 6.5 - 7.4 respectively. Thus, there was not a requirement to adjust pH during this study.

Aerobic environment: Keeping the system aerobic is a major challenge. Dissolved oxygen in the influent was negligible, and the DO increase in EF1 is likely to be due to the synchronous activity of microbes in the soil and the worms. The death of worms when the VF became flooded is probably due to the lack of DO in the water. Otherwise, worms are capable of surviving in fresh water (DO > 8 mg/L) for more than 3 days, which was confirmed by a lab-based experiment.

Population and density of worms: The unit started with a large population and density of worms (~12,000 /m^2) since this provides a better removal rate for organic contaminants (Klein 2008).

Hydraulic Retention Time and Hydraulic Loading Rate: Higher HRT and lower HLR is found to be more favourable for the removal of the pollutants by the worms. **Figure 4.61** depicts what can happen when these parameters are not regulated properly.

Figure 4.61 Dead worms on the soil layer due to pooling of influent. Worms were observed to come to the surface and tried to escape on the side of the unit. These pictures are from the few occasions when the timer failed and the VF unit received the IN in an uncontrolled way. Photos taken by Anusuya Joshi.

4.3.7.2 System design and operation

During the set up of the filter unit and operation period, we experienced the following problems in terms of system design:

Wastewater application method: To minimize the impact of the water pressure on the worms and to evenly distribute the IN in the system, a frame with 2 mm diameter holes was employed, which was found to become blocked due to the presence of fibre in the influent. Hence, the diameter of holes was increased up to 8 mm to promote a better flow. However, regular cleaning of the holes in the frame was found to be required. This aspect could benefit from further development.

***Clogging*:** This study detected fibre in the influent (even though the influent was passed through a 2 mm screen). This, and soil compaction are the two major reasons for the clogging of the system. The fibre created a thin firm layer on the top of soil, as shown in **Figure 4.61**, blocking the passage of water through the soil. Scanning electron microscopy **(**SEM) micrographs of the vermicast, soil, HAL fraction isolated from sand, and the fibre deposited on the surface of soil (which impeded flow through the top layer) are presented in **Figure 4.62**. According to Li et al. (2011), a compacted lumpy structure provides better permeability to water, hence the vermicast seems to be perform better with respect to percolation of water than soil.

The large amount of fibrous material deposited on the surface of the VF appeared to be coming from toilet paper, one of the flushable consumer products (FCPs), that is used frequently and in a large amount. Eren and Karadagli (Eren and Karadagli 2012) reported that toilet paper discharge rates per person per day can be as low as 12 sheets/30 L = 0.4 sheets/L or as high as 30 sheets/6 L = 5 sheets/L. In sewer systems, their movement and breakup can be complex as the number of sheets per flush increases. This might not be a case in developing countries due to the tradition of using water after defecation rather than toilet paper - except for a few places with foreign influence (Giri et al. 2006). These FCPs are susceptible to absorbing fat, oil and grease (FOG) from the wastewater and form a FOG deposit, which may cause blockage (He et al. 2011). Nimali Gunakesara (2011) has investigated the potential for the utilization of toilet paper present in wastewater as a carbon source in a post-anoxic denitrification process. This might help to reduce the fibrous material in the influent and minimize the clogging problem.

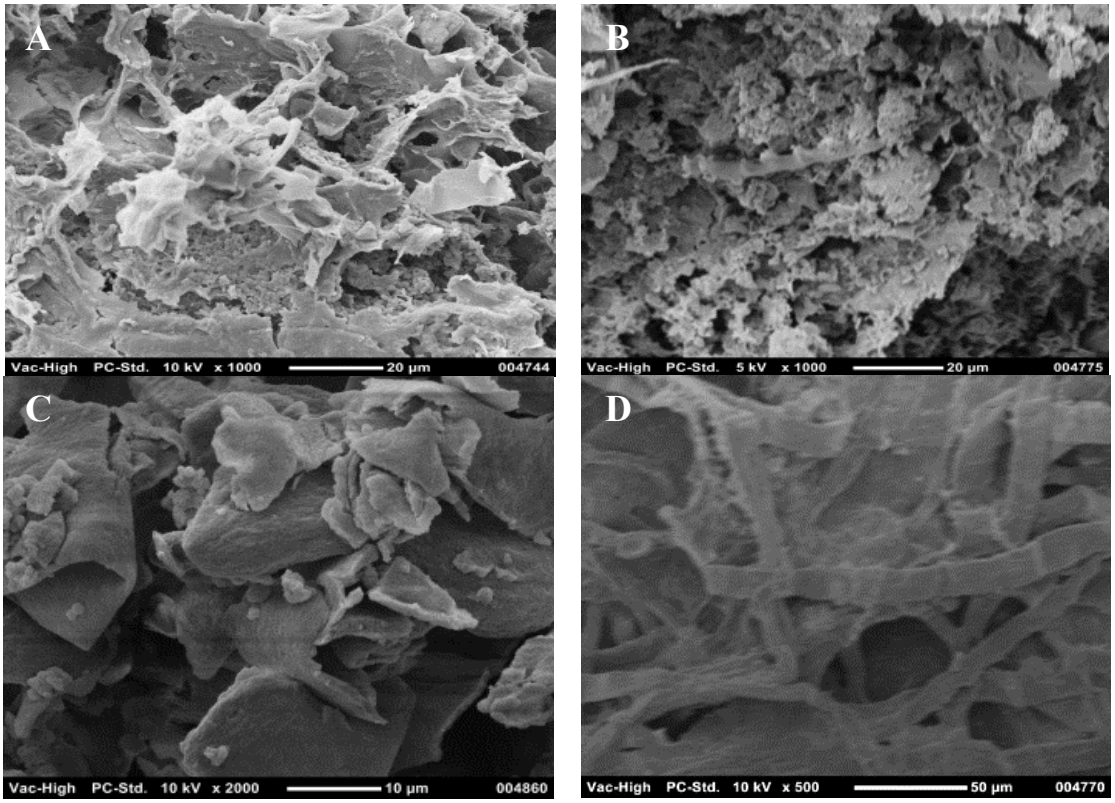


Figure 4.62 Scanning electron microscopy of A. Vermicast; B. Soil; C. HAL fraction isolated from sand; D. Fibre on the soil layer.

4.3.8 Potential reuse of the effluent

With current global concern over fresh water scarcity, the potential reuse of treated wastewater has been emerged as an alternative source. Wu et al. (2013) argues that reused water should be considered a new water resource. However, the risk associated with the application of the treated wastewater for non-potable use on the public health should not be underestimated. Previous studies (Liu et al. 2009; Manyuchi et al. 2013) have shown that vermifiltered wastewater meet the standard for irrigation water quality, and may be suitable for other uses such as toilet flushing, floor washing, or watering garden/parks .

Water quality, based on the parameters investigated in this study, has been assessed in terms of the standards for irrigation, as presented in **Table 4.25**. The final effluent (EF3) obtained from the VF, over Phases I and II seems to be in-line with the threshold standards, except for TN, TOC and TP. The major concern is pathogen removal, which can be addressed by applying disinfection methods such as chlorination or ultraviolet (UV) irradiation. For

example, the Peschiera del Garda municipal WWTP, Verona, Italy applies UV technology for the disinfection of effluent wastewater, **Figure 4.63**. It is arguable that, in developing countries, where a direct application of wastewater, without any kind of treatment, for irrigation (Qadir et al. 2010; Scheierling et al. 2010) is in practice, a slightly higher value of these parameters than standard may be tolerable. The application of wastewater in agriculture is a traditional practice in Nepal, like in other developing countries (Rutkowski et al. 2006). Thus, vermifiltered wastewater could be a better alternative to untreated wastewater.

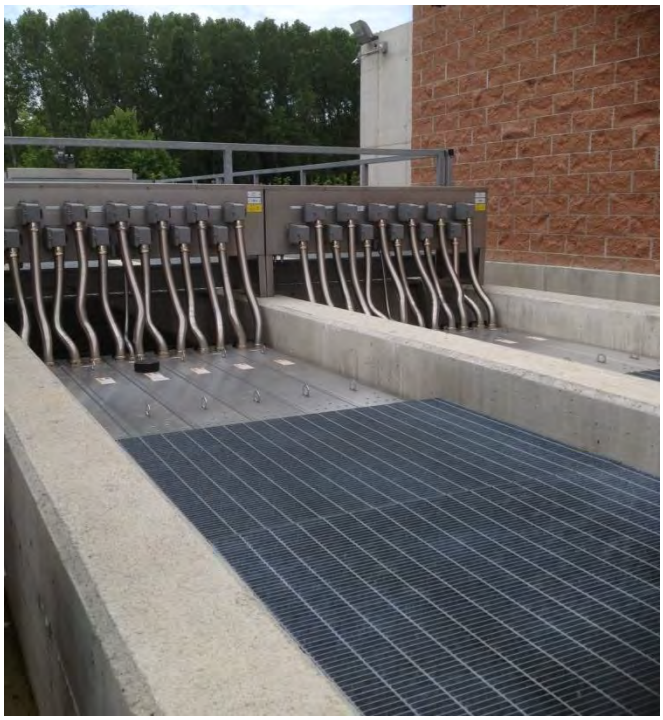


Figure 4.63 An application of UV technology for the disinfection of effluent wastewater at the Peschiera del Garda municipal WWTP, Verona, Italy (Photo taken by Prof. John Orbell).

Table 4.25 A potential reuse of vermifiltered wastewater for irrigation. The final effluent (EF3) obtained from Phases I and II were compared with the standards for irrigation, from various organizations.

Observed parameters (unit)	Observed values for EF3		NWQS (2008)	NWQMS (ANZECC/ARMCANZ 2000)	USEPA (2004)	WHO (2006)[a]	GB5084-2005 China[b]
	Phase I	*Phase II*					
pH	*7.20 ± 0.02*	*6.90 ± 0.03*	6 - 8.5	6 - 8.5	-	6.5 - 8	5.5 - 8.5
Conductivity (mS/cm)	*1.14 ± 0.07*	*1.10 ± 0.03*	< 0.40	0.65 - 1.30*	-	0.70 - 3.00	-
Turbidity (NTU)	*43 ± 5*	*68 ± 8*	-	-	< 0.1 - 30	-	-
TSS (mg/L)	*51 ± 3*	*47 ± 5*	< 50	-	< 5 - 30	50 - 100	15 - 80
COD (mg/L)	*161 ± 20*	*170 ± 33*	-	-	< 20 -90	-	60 - 150
BOD_5 (mg/L)	*15.5 ± 5*	-	-	-	< 10 - 45	-	15 - 60
TN (mg/L)	*68 ± 4*	*60 ± 4*	-	25 – 125	< 1 - 30	5 - 30	-
TOC (mg/L)	*41 ± 3*	*41 ± 4*	-	-	< 1 - 10	-	-
TP (mg/L)	*26 ± 2*	*49 ± 3*	-	0.8 – 12	< 1 - 20	-	-
Coliforms (CFU/mL)	*8.7E+04*	*9.5E+04*	-	<1000 **	< 1 - 1000	-	1000 - 4000
***E.Coli* (CFU/mL)**	*4.0E+04*	*2.1E+04*	-	-	-	-	-
Heavy Metals (mg/L)[#]							
Cadmium	*0.0003*	*0.0022*	< 0.01	0.01 - 0.05[#]	< 0.01	0.01	0.01
Chromium	*0.004*	*0.000*	< 0.1	0.1 – 1	-	0.1	0.1
Cobalt	*0.0002*	*0.0012*	< 0.05	0.05 - 0.1	-	0.05	-
Copper	*0.001*	*0.000*	< 0.2	0.2 – 5	-	0.2	-
Iron	*3.5*	*42.5*	< 5.0	0.2 – 10	-	5	-
Manganese	*3*	*1*	< 0.02	0.2 – 10	-	0.2	-
Lead	*0.03*	*0.01*	< 0.2	2 – 5	-	5	0.2
Nickel	*0.003*	*0.066*	< 0.2	0.2 – 2	< 0.1 - 0.02	0.2	-
Zinc	*0.0373*	*0.0005*	< 1.0	2 – 5	-	2	-

***Moderately sensitive crop**
****Raw human food crops not in direct contact with irrigation water.**
[#]Long term trigger value - Short term trigger value
[a]Slight to moderate degree of restriction on use
[b]Lu et al. (2009)

4.4 Concluding remarks and suggested further research

4.4.1 Concluding remarks

Detailed conclusions are provided in the body of the text and within the hypothesis outcomes, *vide supra*. However, some overall concluding remarks are warranted - as follows.

The overall performance of the vermifiltation (VF) system was evaluated based on the quality of the final effluent (EF3), with worms.

- The VF investigated in this study was found to be effective in reducing the "pollution factors", namely - turbidity, TSS, COD, BOD_5, NH_3-N, NO_2-N, NO_3-N, TN, TOC and TP, from the Influent (sewage wastewater). In addition, the VF was found to alter relevant "physico-chemical parameters" such as the temperature, pH, conductivity and DO, in the resulting Effluents.

- The VF performance was found to be significantly effective in removing turbidity, TSS, COD, NO_2-N and NO_3-N with removal efficiencies of 87 %, 82 %, 45 %, 85 % and 92 %, respectively, for Phase I and 83 %, 86 %, 52 %, 98 % and 93 %, respectively, for Phase II.

- The TOC removal efficiency was found to be satisfactory with 43% reduction in both Phases I and II.

- The VF was not considered to perform satisfactorily for the reduction of TN and TP, in Phase I, which reduced by 13 % and 23 %, respectively. However, the NH_3-N increased by 3 %.

- In Phase II, the VF was found to be moderately effective in reducing NH_3-N and TN by 20 % and 35 %, respectively. However, the TP actually increased by 29 %.

- In the VF, soil type 1 increased the conductivity whereas soil type 2 did not have much effect on the conductivity.

- The pH of the effluent was found to approach neutrality, in both Phases I and II, and this may be attributed to a buffering capacity of the worms.

- The DO change was considered satisfactory for Phase I, whereas it increased significantly in Phase II, suggesting that soil type 2 enhances the microbial and worm activity so as to increase the DO.

- In terms of the concentration of heavy metals in EF3, this was found to be in line with the NWQMS 2000.

- Coliforms were reduced by 1.8 log units and 1.9 log units in Phases I and II, respectively. *E.Coli* was reduced by 1.5 log units and 1.9 log units, respectively.

Generally, the performance of the VF was found to be affected by various factors such as the geo-layers used, the presence or absence of worms in the system, the characteristics of the first layer (i.e. either soil types 1 or 2) and the HRT/HLR.

The effect of the different layers in the VF, without worms: In Phase I, significant changes were observed for all the monitored parameters, except for the DO, which shows that the layers themselves were effective in removing contaminants from the IN. The effect of the first layer (soil type 1) was more significant than the second (sand) layer and the third (gravel) layer. In Phase II, again, there was a significant change in all the parameters, except the conductivity and the TP. Like Phase I, the first layer (soil type 2) was found to be more effective than other two layers. The pathogen removal by layers was also significant in both Phases.

The effect of worms in the VF: In Phase I, the presence of worms in the VF was found to be significant in reducing conductivity, TSS, COD, NO_2-N and TOC. The worms were also effective in changing the pH towards neutrality. Worm action was found not to be significant in reducing turbidity, NH_3-N, NO_3-N, TN and TP. In Phase II, worms were found to be

effective in reducing turbidity, TSS, COD, NO_2-N and TOC. The presence of worms significantly increased the conductivity and TN. Conversely, there was no significant change in the pH, DO, NH_3-N, NO_3-N and TP. The worm effect was significant in pathogen removal in Phase I but did not play a pivotal role in Phase II.

The effect of the first soil layer in the VF: Without worms, the effect of the first layer (soil type 1 or soil type 2), (**Section 4.2.2, Table 4.3**), was found to be significant for the pH, conductivity, turbidity, DO, COD, NH_3-N, NO_2-N, NO_3-N, TN and TP. These observations showed that the reduction in conductivity, COD, NO_2-N and TN was better for soil type 2. But, the reduction in turbidity, NH_3-N and TP was better for soil type 1. However, soil type 2 increased DO significantly. There was not any significant effect of soil type on TSS, and TOC. With worms, the effect of soil type was significant for pH, turbidity, TSS, DO, NH_3-N, NO_2-N, NO_3-N and TP. Soil type 2 was more efficient in reducing pH, TSS, NO_2-N and NO_3-N whereas soil type 1 was effective in reducing turbidity, NH_3-N and TP. The soil type did not have any effect on conductivity COD, TN or TOC. In terms of pathogen removal, soil type 2 performed better than soil type 1.

***The effect of the HRT and HLR in the VF*:** The HRT and HLR are interrelated, when HRT increases, the HLR decreases. Here, the variation in HRT/HLR was found to affect pH, conductivity, turbidity, TSS, DO, NO_3-N and TP. The pH, conductivity, DO, turbidity and TSS was found to increase with increasing HRT, whereas, NO_3-N and TP decreased with increasing HRT. Variation in HRT/HLR did not have any effect on COD, NH_3-N, NO_2-N, TN and TOC. In terms of pathogen removal, a higher HRT performed better in reducing coliforms and *E.Coli*.

VF appeared to produce an effluent rich in nutrients – and more so as the system matured. Over time, the worms deposit vermicast, which is rich in nitrogen and phosphorus, on the top of the first layer. This observation suggests that the worms are more effective in the removal of organic matter rather than other contaminants.

Most of the parameters, of the final effluent produced from the treatment system, are in line with the irrigation water quality standards of the Government of Nepal and other organizations. This low cost and environmentally friendly technology, which is also less technical and requires energy only for pumping (when gravity feeding is not possible) can be

a satisfactory alternative to centralised systems in developing countries like Nepal. Moreover, effluent that is rich in nutrients can be used for irrigation purposes which will help to address the water scarcity problem of the city and will result in less diversion of drinking water from the water cycle. In addition, transformation of waste sludge (sewage) into useful resources (e.g. vermicast on the top layer) has potential social, economic and ecological benefits.

Thus, the accolade provided by Sir Charles Darwin, to these earthworms, as 'unheralded soldiers of mankind' and 'friends of farmers', seems to be appropriate. According to Darwin, 'there may not be any other creature in world that has played so important a role in the history of life on earth'. Dr. Anatoly Igonin also admired these little creatures as, 'Nobody and nothing can be compared with earthworms and their positive influence on the whole living nature. They create soil and everything that lives in it. They are the main creatures converting all organic matter into soil humus providing soil's fertility and biosphere's functions: disinfecting, neutralizing, protective and productive' (Sinha and Valani 2011).

4.4.2 Suggested further research

Further research on VF is warranted to further optimize the performance of the system and the reuse potential of the vermifiltered wastewater.

- Different filter media, which are locally available and easy to obtain, could also be trialled. This will minimize the cost of importing the filter media from other places. For example, Kumar et al. (2015) used ranges of filter media that are easily available in India.

- The design of the VF could be modified to make it more sophisticated. For instance, it could be modularized so that the filter media could be replaced as it gets exhausted (although this was not observed in this study). Moreover, some voidage could be provided, by placing gaps in between layers, to provide a more aerobic environment.

- To meet the stringent water quality guidelines, the VF could be combined with some other novel technologies for further treatment. For instance, the vermifiltered water

could be further treated with ultraviolet rays (UV) or ozone for pathogen removal. However, the cost associated with such technologies should also be considered.

- With respect to the reuse potential of vermifiltered wastewater and vermicast as bio-fertilizer, of most concern is its adverse effect on human health and the environment due to pathogens and heavy metals. Therefore, the bioaccumulation of heavy metals and the fate of pathogens should be investigated further.

- This study, as well as previous studies, showed that the worms have anti-bacterial properties. Thus, further investigations should be carried out to explore the potential enhancement of this capability.

- Finally, VF could be a practical solution in developing countries, especially in communities without toilets and where open defecation is prevalent. It could be piloted in such areas and a health assessment could be carried out to evaluate the effects of improved sanitation.

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CHAPTER 5: The Takakura Composting Method - towards optimization for household and community implementation

5.1 Introduction

5.1.1 Composting for organic waste management

Many alternatives have been investigated to find a viable technique to reduce the waste generated in and around cities. Composting is a simple and traditionally used technology that is popular worldwide for the management of the organic waste. Composting is environmentally sound, does not need complex technical knowledge and can be practiced at the household or community level. Various kinds of composting technologies have been developed and are in practice such as bin compost (Akinbile and Yusoff 2012), Rotary drum and Windrow pile compost (Bhatia et al. 2012) and vermicomposting (Sinha 2009, 2010; Sinha et al. 2012). Composting recycles the organic portion of solid waste and produces a valuable product for gardeners and farmers alike in the form of mature compost. Until the twentieth century, the use of compost was an integral part of traditional farming which was gradually superseded by the use of synthetic fertilizers (Bernai et al. 1998). More specifically, composting is a process of biologically decomposing and stabilising organic matter under favourable conditions to produce a stable product which is beneficial for land application (Kianirad et al. 2010). The USEPA (1994, p.2) defines Composting as "... ***controlled*** decomposition of organic (or carbon-containing) matter by micro-organisms (mainly bacteria and fungi) into a stable humus material that is dark brown or black and has an earthy smell".

5.1.1.1 The composting process

Generally two major steps are involved in the composting process. In the first step, the composting feedstock breaks down into simple compounds via microbial activities and this metabolism release heat making the compost pile characteristically hot. This phase is called '**active period**'. A wide range of microorganisms are responsible for this process and play a vital role in enhancing the mechanisms of biodegradation during the different stages of the process (Ishii and Takii 2003; Rebollido et al. 2008; Partanen et al. 2010). In the active period or first step, bacteria are responsible for breaking down readily decomposable nutrients such as proteins, carbohydrates and sugar. In the second step, curing or finishing of the compost product occurs, in which the microbial activities slow down due to lack of readily available nutrients. This phase is called '**curing period**'. In this stage, fungi take over since, unlike bacteria, they can decompose cellular components and are able to survive in a low-moisture and less nutrient-rich environment (USEPA 1994).

The structure and diversity of the microbial community, their activity and the physico-chemical features of the compost are highly affected by the initial aerobic exothermic microbial decomposition of the organic matter, which causes a change in temperature, moisture content, nutrient availability and oxygen concentration during the overall composting process (Bhatia et al. 2012). In the initial stage of the composting process, mesophilic microorganisms, which can survive at temperatures between 25 - 45 °C, are active. These mesophiles produce carbon dioxide, water and energy in the form of heat, which gets trapped in the compost pile. Eventually, with the rise in temperature, thermophilic microorganisms, which can survive at temperatures between 45 - 70 °C, become dominant.

5.1.1.2 Factors affecting the composting process

The extent of microbial activity depends on the environmental conditions, which also determines the rate of composting. Conditions such as temperature, pH, particle size of feedstock material, oxygen content, moisture level and carbon to nitrogen ratio are interconnected (Goyal et al. 2005). The rate of composting increases with a smaller "particle" size of feedstock material - as

smaller particles provide a larger surface area for microbial action (Richard 1992). At the same time, a smaller particle size provides favourable conditions for maintaining a stable temperature since the feedstock material may be mixed more homogenously. However, the size should not be so small so that it becomes compact and creates anaerobic conditions in the pile. An optimum moisture content, usually 40 - 60 % by weight, provides most favourable environment for oxygen supply and microbial activity. Thus, moisture content in a compost pile is a vital factor for microbes to decompose the organic matter, as it occurs in thin liquid films on the surface of the particulate material. However, excess moisture can create anaerobic condition in the system, by inhibiting oxygen supply to the system by filling the pores between particles and low moisture will also inhibit microbial activity (Cornwell Waste Management Institute 1996).

Temperature is one of the most significant parameters in the composting process for tracking microbial activity (Sundberg 2005). The optimum temperature for efficient composting is reported to be between 45 and 59 °C. Microorganisms cannot grow in temperatures less than 20 °C and this inhibits the decomposition of organic matter. Similarly, microorganisms die off in temperatures above 59 °C also inhibiting the decomposition. In terms of pH, the optimum pH for composting is between 6 and 7.5. If the pH goes below 6 or above 9, bacteria die off and decomposition declines. In contrast, fungi can survive over a wider range of pH (Megan 2007). Sundberg (2005) has discussed three different acid-base systems that influence pH in the composting process. The first is the carbonic system; carbon dioxide (CO_2) is produced during the decomposition of organic matter (OM), which is either volatilized as a gas or forms carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) in aqueous solution. This system tends to neutralize the pH of the compost, with two relevant acid dissociation constants (pK_a = 6.35 and 10.33 at 25 °C). The second system is the ammonium (NH_4^+) and ammonia (NH_3) system, attained by the decomposition of proteins and amines, which increases the pH, with a relevant pK_a value of 9.24 at 25 °C. The final system is the carboxylic organic acid system, especially acetic acid and lactic acid, which will decrease pH, with a relevant pK_a value of 4.14 at 25 °C.

Generally, composting can be carried out in the presence (aerobic) or absence (anaerobic) of oxygen. The application of anaerobic composting is diminishing since it is too slow and creates an unpleasant odour by the release of nuisance gases including methane, hydrogen sulphide and

amines. Aerobic conditions can be maintained by supplying oxygen via turning or mixing of the compost pile. The aeration rate is one of the main factors that affect the nitrogen dynamics of a composting process (de Guardia et al. 2008). In this regard, nitrogen availability in the compost is one of the most significant factors that determine its final quality. Thus, nitrogen transformation provides information on the availability of nitrogen during the different stages of the process. Many researchers (Fricke and Vogtmann 1994; Sánchez-Monedero et al. 2001;Said-Pullicino et al. 2007) have reported that most of the nitrogen found in composting is organic nitrogen - from proteins, amino acids and peptides. When the composting process starts, the microbial activities mineralize organic nitrogen to produce ammonia by the "ammonification" process. Depending on the properties of the compost matrix, the ammonia undergoes different reactions. It either dissolves to form ammonium, which can be utilized further by microorganisms as a source of nitrogen, which transform it to organic nitrogen, or the ammonia could be volatilized when the temperature of the system becomes thermophilic (above 40° C) with the system attaining a pH greater than 7.5. Ammonium can undergo a nitrification process, carried out by nitrifying bacteria, when the temperature of the system changes from thermophilic to mesophilic (below 40° C) and this results in a lower pH due to hydrogen ion release (Sánchez-Monedero et al. 2001; De Guardia et al. 2010). The nitrification process is summarized as follows:

Nitrosomonas bacteria: $2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$

Nitrobacter bacteria: $2NO_2^- + O_2 \rightarrow 2NO_3^-$

Carbon and nitrogen are the two most significant elements in the composting process and the carbon to nitrogen ratio (C:N ratio) is a key environmental parameter along with temperature, moisture and oxygen. Carbon supplies energy to the microbes whereas nitrogen is vital for microbial growth. Limited nitrogen availability will inhibit microbial growth resulting in a slow decomposition of the available carbon. The initial C:N ratio of the material to be used to start the composting affects the progress of the overall process (this is further discussed in **Section 5.3.3.7**). By applying the following relationship to the individual materials to be composted and by solving/rearranging the equation, the appropriate masses of materials can be calculated so that an optimum C:N ratio can be maintained (Richard and Trautmann 2014).

$$R = \sum Q_n(C_n \times (100 - M_n) / Q_n(C_n \times (100 - M_n) \quad (5.1)$$

$$Q_2 = [Q_1 \times N_1 \times (R - C_1/N_1) \times (100 - M_1)] / [N_2 \times (C_2/N_2 - R) \times (100 - M_2)] \quad (5.2)$$

Where, R = C:N ratio of the compost mixture

Q_n = mass of material n

C_n = Carbon (%) of material n

N_n = Nitrogen(%) of material n

M_n = Moisture (%) of material n

This project aims to scientifically investigate a more recent composting method called the Takakura Composting Method (TCM) for which there is a paucity of scientific information. This method has been practiced in the target community of this study for a number of years for the management of organic solid waste, and it will be to the advantage of these communities to optimize this method on the basis of scientific enquiry.

5.1.2 Takakura composting

The Takakura Composting Method (TCM) was invented in Japan by Mr. Koji Takakura of J-POWER group/JPec and is one of the fastest and most efficient methods of composting domestic and municipal organic waste (JICA). According to Takakura and Yaoya (2011), the organic waste degrades in 7 days and compost is stabilized by 3 weeks - as described in the original method. As such, it represents a significant advance on existing composting techniques. TCM, being relatively simple, can (and has) be adopted with limited investment and has a low operational/maintenance cost (Premakumara et al. 2011). This composting method can be applied either at the household or community level. TCM was first piloted by the KitaQ Composting System in one of Surabaya's[87] urban communities; namely, Kampeng Rungkot Lor (UNEP 2011). This technology is different to other composting technologies in the sense that it is based on exploiting locally available fermentation microorganisms, known as native microorganisms (NM), rather than so-called 'effective' microorganisms (EM) (Maeda 2009). EM is "a combination of useful regenerated micro-organisms that exist freely in nature and are

[87]Indonesia's second largest city after Jakarta.

not manipulated in any way" (http://www.effectivemicro-organisms.co.uk/). Thus EM is a mixture of five different kinds of microorganisms – lactic acid bacteria, yeast, actinomycetes, photosynthetic bacteria and fungi. EM is available commercially, but NM are produced in same locality where the TCM composting is carried out. TCM utilizes fermentation microorganisms isolated from locally available materials such as fruit skins, fermented foods, rice husks, rice bran, leaf mould etc. For example, Ying and Ibrahim (2013) used local fermented food such as *tempeh*, *tempoyak* and *tapai* to isolate such microorganisms. These isolated microorganisms intensify the decomposition of organic matters via natural fermentation (Ying and Ibrahim 2013). As the composting process progresses, the dominant microbial community changes with each stage. Generally, TCM has three different transitional stages (Takakura and Yaoya 2011). In the first stage, useful microorganisms decompose the most easily degradable organic matter. To proliferate such microorganisms in the system, the large quantity of harmless or beneficial moulds and bacilli present in the compost are further increased by adding a fermented food such as *Aspergillus oryazae* fungus and lactic acid bacteria (*Lactobacillus* spp.). In the second stage, the majority of cellulose and hemi-cellulose (plant material such as vegetables) are decomposed by actinomycetes bacteria. In the third stage, basidiomycetes fungi slowly decompose the decomposable lignin that is present in the plant material. The microbial activities in the different stages of TCM have been characterized by Chi and Ibrahim (2012), which complements the research of Partanen et al. (2010) on the bacterial diversity at different stages of the combined drum and tunnel composting process. The latter study estimated more than 2000 bacterial species of different phylotypes. Such fermentation microorganisms are very effective in the decomposition of the different varieties of materials present in the organic matter. In the feed material from household waste, Partanen et al. (2010) found mostly mesophilic bacteria such as different species of the *Lactobacillus*, *Leuconostoc* and *Pseudomonas* genera. As the composting process progresses, the abundance of *Bacillus* spp. shows a transition from the mesophilic to the thermophilic stage. The presence of bacteria such as *Actinobacter* and *Thermoactinomyces spp.* reveal the aerobic condition in the process. The enzymatic activity also increases during this process with concomitant biodegradation of organic matter - indicating the microbes' ability to synthesize enzymes that hydrolyse complex organic matter (Ying and Ibrahim 2013).

Composting is a process in which complex organic matters break down into simple compounds under different interconnected environmental conditions such as temperature, pH, moisture content, microbial diversity, oxygen level and the consistency (e.g. particle size) of the substrate used (USEPA 1994). Chi and Ibrahim (2012) have reported that the temperature of the TCM composting pile increases from 28 to 30 °C in day 1 and reaches a maximum of 50 °C on day 4, followed by a subsequent decline. Goyal et al. (2005) observed a similar trend for conventional composting; however, the highest temperature of 46 °C was attained after 14 days, which demonstrates TCM to be relatively faster than the conventional method. Takakura and Yaoya (2011) suggested that a high moisture level in the TCM composting pile will create anaerobic conditions leading to rotting and that too low moisture level will slow down microbial activities. Thus the *optimum* moisture level is recommended (Takakura and Yaoya 2011) to be between 40 to 60 % (v/v) - which can be simply tested by taking the compost into the palm of the hand and squeezing it; the moisture level is considered just right if a lump is formed and no water drips through it (JICA). Admittedly, this remains to be quantified scientifically.

The Japan International Cooperation Agency (JICA, p.3) has described TCM as "a composting technology in the KitaQ Composting System, that is simple, easy-to-follow, locally-relevant and has great potential to be transferred, adopted and replicated without too many outside sources." In addition, this method has other benefits such as the process not emitting foul odours and leachate, and having readily available waste materials that are required as raw material. Indeed, JICA reported a 30 % waste reduction in Surabaya city after adopting the KitaQ Composting System over a 6 year time period.

The Sibu (Indonesia) Municipal Council has outlined the benefits of the Takakura Home Method (THM) over other composting methods that have been practice in Sibu such as pot composting, plastic bag composting, tower tyre composting, compost pits, windrow (batas) composting, wire hoop composting, heap composting and bottomless bin composting (SMC 2010). Considering the success of the Surabaya model, it has been replicated in seven other Indonesian cities; namely, Semarang, Medan, Makassar, Palembang, Central Jakarta, Balikpapan and Tarakan. From Indonesia, it was extended to six Philippino cities; namely, Bago, Cebu, Cavite, Talisay, Puerto and Princesa; and two Thai cities – Bangkok and Sankamphaeng. Following the

successful Takakura composting practice in Bangkok, it was subsequently replicated in Nepal (Lalitpur) and Malaysia (Sibu, Kuala Lumpur) (IGES 2010b; Maeda 2013). Chi and Ibrahim (2012) attribute the popularity of TCM in South East Asia to the simplicity of the technology which may be practically implemented using readily and locally available materials. An added advantage is the promotion of on-site waste segregation which, in itself, will reduce waste generation and improve household sanitation leading to social, economic and environmental benefits (Kurniawan et al. 2013; Ying and Ibrahim 2013).

Generally, the five steps are involved in TCM are as follows (JICA; IGES 2010a;Ying and Ibrahim 2013):

a) *Preparation of the fermentation solution:* By using locally available vegetables, fruits, soil, leaves and other materials, an effective native microorganism (NM) solution is prepared to generate the fermentative microbes to be used.
b) *Preparation of seed compost:* Native microorganisms isolated in the fermenting solution are used along with rice husk and rice/wheat bran for the preparation of the seed compost, which takes 4 - 5 days for good fermentation. Here, rice husk is used to provide a habitat for microorganisms and rice bran provides nutrients for the microbes to grow (Takakura - personnel communication)[88].
c) *Selection of compost bin:* A vessel in which aerobic conditions can easily be maintained is selected for composting. The inner part of the bin is lined with cardboard or carpet, so that the compost will be free from harmful insects and the seed compost will not spill out of the bin.
d) *The Compost Making Process*: Biodegradable organic wastes are finely chopped and mixed with the seed compost. The moisture level, temperature, pH and air circulation level are maintained for best results. Note that with respect to: moisture – if dry, sprinkle more water with mixing, if wet, add some dry materials such as paper pieces, dry fruit skins or rice/wheat bran; temperature – if too high a temperature, mix/agitate to release heat and if temperature is too low, place some hot waste bottles in the pile and cover; pH – if acidic, add ash or limestone to correct. The whole bin is covered with a cloth to

[88]The method by Takakura, and other reports, do not refer to any scientific research that has been done, although it does mention the names of the microbes.

protect it from insects. The waste is added daily and mixed well until the bin becomes full. Note that smaller pieces of waste provide greater surface area for microbes to act on them, resulting in faster biodegradation.

e) *Use of compost*: When the bin is full, 2/3 of the compost is left in the bin as return compost and 1/3 is taken out and stored in another vessel (cardboard box or sack). The stored compost can be used after 2 weeks, being the time required for it to become stable and mature.

As discussed, minimal scientific research has been reported to characterise and optimise TCM. The research that has been reported on some biotechnical and microbial aspects by Ying and Ibrahim, 2013 and Chi and Ibrahim 2013, respectively, are the only known published scientific investigations to date. The present study effectively initiates such detailed scientific enquiry into the step-by-step composting process. Thus the objective of this research is to carry out physico-chemical and microbial analysis of a) fermenting solutions trialled with three different proportions of substrate, b) seed compost trialled with three different compositions of media and c) compost mixed with three different proportions of seed compost. This is described in more detail as follows:

5.1.3 Research objectives in relation to Takakura composting

In 2009, the Takakura Home Method[89] (THM) (Maeda 2013; Ying and Ibrahim 2013) was introduced into Nepalese target communities (including the target community that is the subject of this thesis) by a local non-government organization called "LUZZA Nepal". This was carried out in co-operation with the local government's Lalitpur Sub-metropolitan City Office and resourced by the Japan Overseas Cooperation Volunteers (JOCV). Officials of LUZZA and JOCV were educated in this method via attendance at a three day conference in Bangkok on "*Workshop-Training on Organic Waste Composting: Waste Recovery for Sustainable Solid Waste Management*", that was also attended by the inventor himself, namely, Kouji Takakura.

[89]Maeda 2013 has mentioned three different types of Takakura composting - the Takakura Home Method (THM) for household waste, Takakura Susun Method (TSM) for Community waste and New Windrow method for market waste. This study mainly deals with the Takakura Home Method.

With this short training experience, many trials were subsequently conducted in Nepal by Sayaka Yaoya (JOCV) and the author of this current thesis using local domestic organic waste to produce compost. Thus the process was qualitatively controlled by JOCV via liaison between Sayaka Yaoya and Kouji Takakura to ensure that it was correct prior to it being introduced into the community. Subsequently, members of several Women's groups[90] were trained and encouraged to carry out composting at home. The training on composting was embedded with a waste management workshop. Thus, they were first educated on types of waste, waste hierarchy, waste segregation at the source and the consequences of improper waste management. Then they were provided with compost bins and waste bins (to segregate waste) at nominal cost. A regular monitoring in the community (by LUZZA) and the field observation under this study demonstrated the effective implementation of the Takakura composting at the household level in the target community.

- Though the Takakura composting method is being implemented in the target community and many other places around the world, only a few researchers have investigated this method scientifically. Thus some limited research on the biotechnical and microbial aspects of TCM has been carried out by Ying and Ibrahim, 2013 and Chi and Ibrahim 2013, respectively. To the best of our knowledge these are the only published scientific investigations to date. Therefore, there is an obvious need to scientifically characterize the various factors involved in this composting method. Thus this study aims to carry out an extensive scientific investigation on the different stages involved during the Takakura composting process – namely, the preparation of the fermentation solution, the seeding compost (inoculate) and the composting process itself. Therefore the more specific objectives are:

- To construct a composting set up based on existing community-based Takakura Home Method (THM) composting systems, for which key parameters (e.g. amount of substrate and retention time - in terms of the fermentation solution) are varied and measurable (quantifiable) subsequent outcomes are scientifically evaluated. The original method itself

[90] Woman's group refers to a group with women members, formed within certain communities with a view to empower women by participating in social and community activities. Anyone who is interested in contributing to the community can be a member and so a range of women in the community is involved including - senior citizens, housewives, working women, young women without any limitations on age and occupation.

does not quantify the amount of substrate to be used in preparing the fermentation solution. It just mentions that any food and vegetable waste can be used in a salt-based solution, and that fermented food can be used in a sugar-based solution. In fact, in the case of the target community, any amount is actually used (not any defined amount). This study aims to assess the effect of substrate variation on the resulting fermentation solution. Similarly, the method as it stands, only advises that the solution should be incubated from three to five days prior to preparing seed compost. This study aims to investigate the effect(s) of longer periods of incubation.

- To devise and carry out an experimental plan to measure key parameters of the fermentation solution and to relate these to retention time. Thus an experimental plan was developed to test various physical, chemical and biological parameters of the fermentation solution prepared with three different amounts of substrate (100 g, 150 g and 200 g – salt based solution, 10 g, 25 g and 50 g – sugar based solution), with the volume of water and amount of salt and sugar kept constant. At the same time, the fruit to vegetable ratio was maintained to be the same in each of the three sets, with only the weight being increased. The amount of substrate was chosen randomly with a view to provide some baseline information for further investigation.

- To select an "ideal" fermentation solution from which to prepare inoculate variants. Note that for small-scale production of fermentation solutions, factors such as substrate and retention time might not be so important. However, for large-scale production of fermentation solutions these factors might contribute to reducing costs and time. By quantifying the substrate for an 'ideal' fermentation solution, there could be the possibility for its commercialization – in commercial effective microorganism solutions, the amount of the substrate is well quantified!

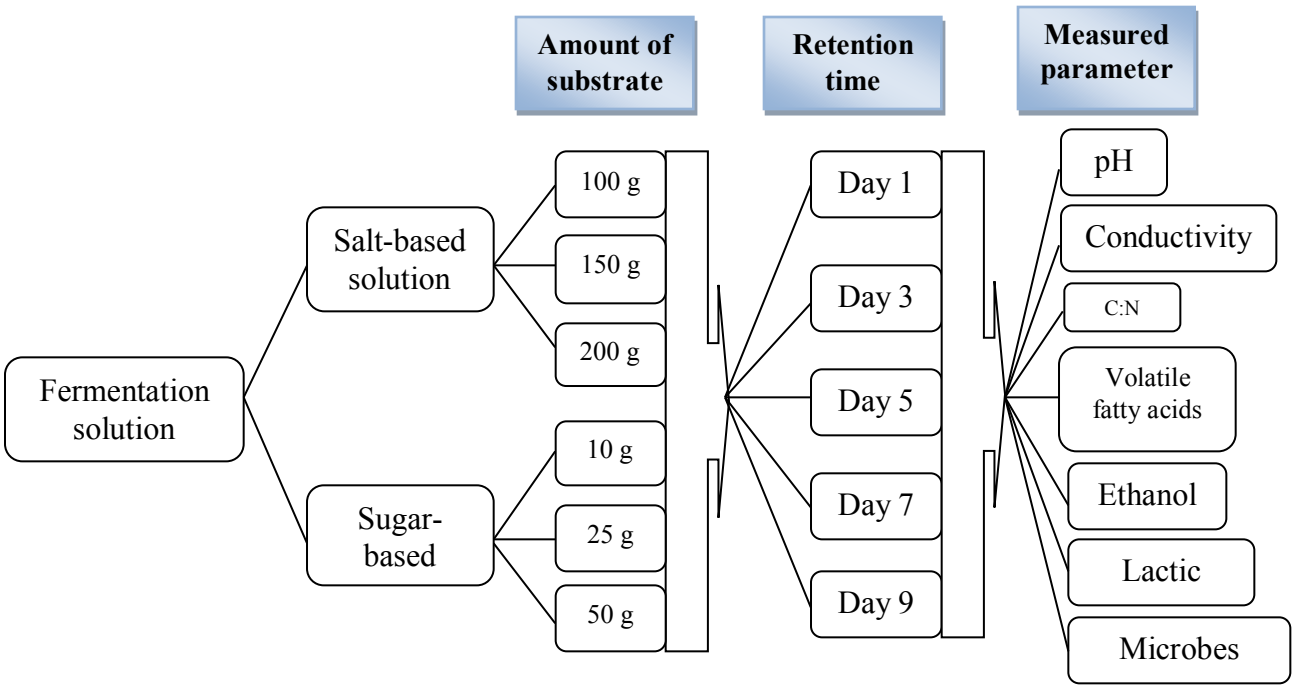


Figure 5.1 Schematic diagram of the experimental plan for fermentation solution optimization.

- To prepare seeding inoculates with different composition of materials for microbial growth and to compare the suitability of their use. The solid materials used for the preparation of seeding inoculate for the further growth of microorganisms are usually rice bran and rice husk in equal ratio (1:1). This study aims to investigate the effect of variation in the proportion of these materials and the source of nutrient on the compost quality. (Here only ideal fermentation solution was chosen for the preparation of seeding inoculates). To investigate the effect of all the prepared fermentation solutions on the seeding inoculates is outside the scope of this study.

- To prepare compost using three variants and compare the quality of the three different composts in terms of nutrients and maturity.

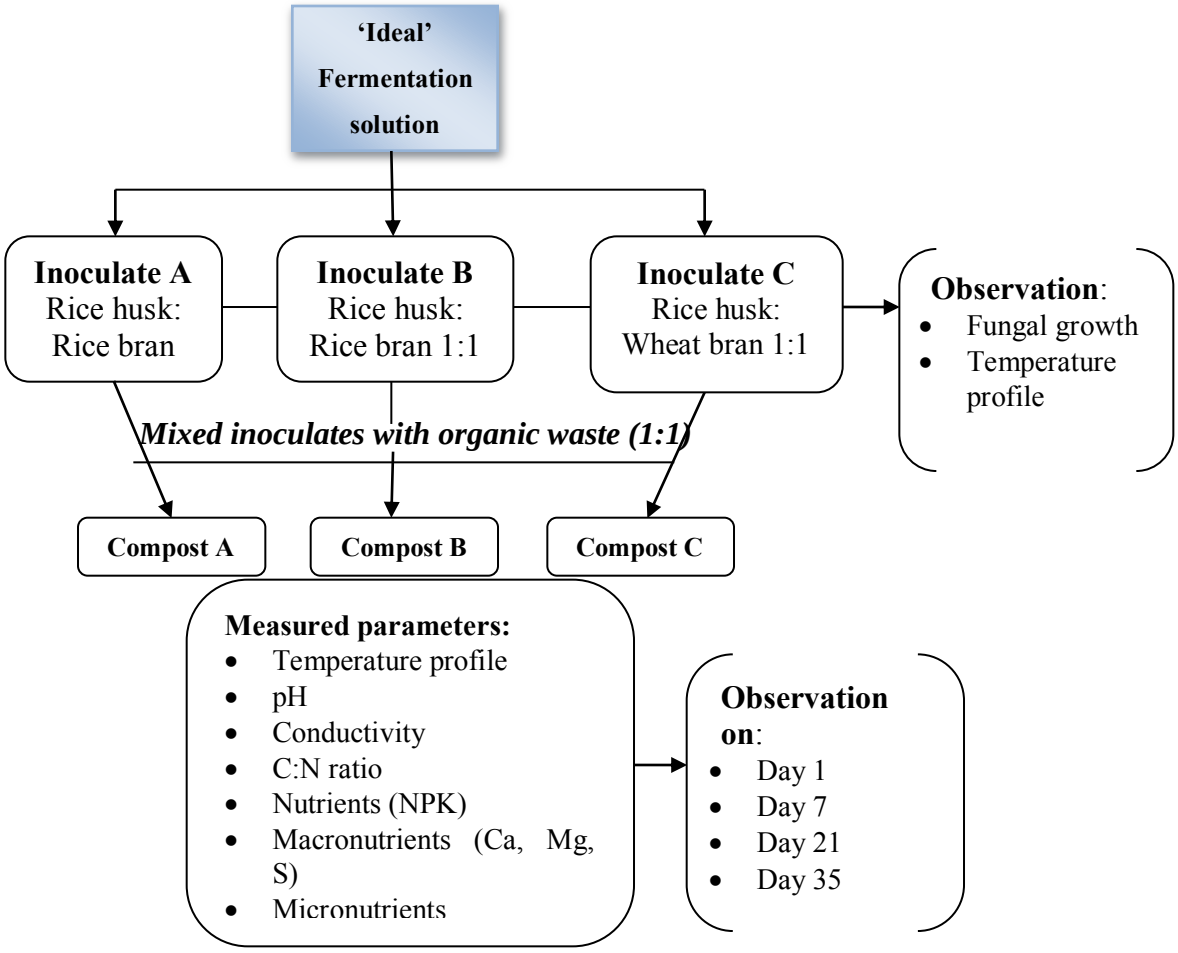


Figure 5.2 Schematic diagram of the experimental plan for seeding inoculate optimization.

5.2 Materials and Methods

5.2.1 The preparation and characterization of the fermentation solution(s)

Salt-based and sugar-based fermentation solutions were prepared as stated in the original TCM, in order to isolate appropriate fermentative microorganisms, which are subsequently utilized for the composting process. A salt-based solution promotes the growth of those microbes which can even survive in high salt concentration, by providing an ionic environment to the system (Brown 1964) and prohibits the growth of unwanted microbes. Similarly, sugar based solution promotes microbial fermentation.

5.2.1.1 Preparation of salt (NaCl)-based fermentation solution(s)

Three 2 L capacity glass bottles were taken for preparing the salt-based fermentation solutions, as shown in **Figure 5.4**. Each bottle was filled with 1 L of tap water (tap water was used with a view to exploit the native microbes for NM/natural process) and approximately 10 g of NaCl was added to each bottle. The pH and conductivity of the tap water and salt solution were measured and noted, so that the change in the pH and conductivity could be monitored after the addition of substrate to it.100 g of substrate (i.e. a mixture of chopped fruit and vegetables, listed in **Table 5.1** and depicted in **Figure 5.3**) was added in Bottle A, 150 g was added in Bottle B and 200 g was added in Bottle C. Fruits and vegetables are used since the surface of these represent a good habitat for the microorganisms that are beneficial for the composting process such as lactic acid Bacillus and yeast fungus (Takakura and Yaoya 2011). The variation and weight of each substrate used in the three different solutions is presented in **Table 5.1**. Any combination of locally available fruits and vegetables can be selected to make the substrate – in order to isolate the fermentation bacteria (IGES).

Figure 5.3 Chopped fruit and vegetables used in the preparation of the salt-based fermentation solutions.

The substrate is then mixed in the salt solution using a magnetic stirrer for 10 minutes. The bottles can also be shaken manually to affect this mixing. After mixing, a liquid suspension sample was taken from each bottle and stored at 4 ºC for analysis as controls. The three bottles were each covered with a lid (not screwed tightly as gas generates during the fermentation process) and stored in a water bath set at 35 ± 2 ºC in a fume hood.

Table 5.1 Substrate compositions in the salt-based solutions. Note that each bottle has the same relative proportions of different substrate materials but different relative total substrate amounts.

	Substrate Materials	Bottle A (g)	Bottle B (g)	Bottle C(g)
1	Carrot	25	37.5	50
2	Cauliflower	15	22.5	30
3	Banana peel	15	22.5	30
4	Celery	5	7.5	10
5	Cabbage	5	7.5	10
6	Lettuce	5	7.5	10
7	Orange peel	10	15	20
8	Apple peel	5	7.5	10
9	Pears	10	15	20
10	Kiwi fruit	5	7.5	10
Relative Total amounts of Substrate		***100***	***150***	***200***

5.2.1.2 Preparation of sugar-based fermentation solution(s)

Three 2 L capacity glass bottles were taken for preparing the sugar-based fermentation solution, as shown in **Figure 5.5**. Each bottle was filled with 1 L of tap water and approximately 10 g sugar was added into each bottle. The pH and conductivity of the tap water and sugar solution were measured and noted.10 g of substrate was added in Bottle 1, 25 g was added in Bottle 2 and 50 g of substrate was added in Bottle 3. Yogurt, yeast, and mushroom were used as a substrate to

be added into the solution. The variation and weight of each substrate used in the three different solutions is presented in **Table 5.2**.

Here, yogurt, yeast, and mushroom were chosen to isolate fermentation microbes to be utilized in preparing seeding inoculate. In fact, any combination of locally available fermented food can be selected to be used as substrate to isolate fermentation bacteria from it (IGES).

Table 5.2 Substrate compositions in the sugar-based solutions. Note that each bottle has the same relative proportions of different substrate materials but different relative total substrate amounts.

	Substrate materials	Bottle 1(g)	Bottle 2 (g)	Bottle 3 (g)
1	Yoghurt	5	12.5	25
2	Yeast	0.5	1.25	2.5
3	Mushroom	4.5	11.25	22.5
Relative Total amounts of Substrate		***10***	***25***	***50***

The substrate was then mixed into the sugar solution using a magnetic stirrer for 10 minutes. It can also be shaken manually to affect this mixing. After mixing, a liquid suspension was taken from each bottle and preserved at 4 ºC for analysis as control. All three bottles were covered with lid (not screwed tightly as gas generates during the fermentation process) and stored in water bath set at 35 ± 2 ºC in a fume hood.

Liquid suspension from each bottle was then taken on Day 3, Day 5, Day 7 and Day 9, and were stored at 4 ºC for subsequent analysis. The pH and conductivity of all the samples were measured and recorded on the same day of sampling. However, the carbon, nitrogen and ethanol, low-chain volatile fatty acids (acetic acid, propanoic acid, iso-butyric acid, n-butyric acid and valeric acid) and lactic acid were analyzed after the nine day period.

Figure 5.5 Salt-based fermentation solutions.

Figure 5.4 Sugar-based fermentation solutions.

5.2.2 Preparation and characterization of seeding inoculates

The suggested method for the preparation of the seeding inoculate for the TCM (IGES 2010a) involves the mixing of rice husk (RH) and rice bran (RB), as materials for the 'fermenting bed', in a 1:1 proportion by weight. It has also been suggested (personal communication with Kouji Takakura) that wheat bran (WB) could be substituted for RB. For these investigations, other proportions by weight of these three materials have been trialled and WB has also been introduced. Thus, three variations of seeding inoculate for the compost were prepared using different combinations of RH, RB and WB, as shown in **Table 5.3**.

Table 5.3 Relative proportions of fermenting bed materials for the seeding inoculate variants. RH – Rice husk; RB – Rice bran; WB – Wheat bran; FS – Optimal fermentation solution (i.e. 150 g of substrate in salt sol + 25 g of substrate in sugar solution).

Seeding inoculate	Fermenting bed combination	Proportion (by weight)	Mixed fermentation solution	Compost
Basket A (SI-A)	RH:RB	1:2	2 L optimal FS + 2 L water	**Compost A (CA)**
Basket B (SI-B)	RH:RB	1:1	2 L optimal FS + 2 L water	**Compost B (CB)**
Basket C (SI-C)	RH:WB	1:1	2 L optimal FS + 2 L water	**Compost C (CC)**

For each variant the relative times required to achieve a good growth of fungus (i.e. the qualitative observation of a white cotton-like growth, as shown in **Figure 5.21**, accompanied by a pleasant wine-like smell), were measured. The temperature profiles have also been measured over these time periods.

5.2.3 Preparation and characterization of Takakura compost

The three seeding inoculate matrices prepared in **Section 5.2.2** were each mixed with the same volume (1:1 v/v) of organic waste, as shown in **Figure 5.6 (a)** & **(b)**. The organic waste that was collected from a household kitchen was cut into small pieces before mixing so that it could provide larger surface area for the microbes to react. (The practice of reducing the size of the waste pieces is prevalent in the Nepalese community - as mentioned in the original TCM.)

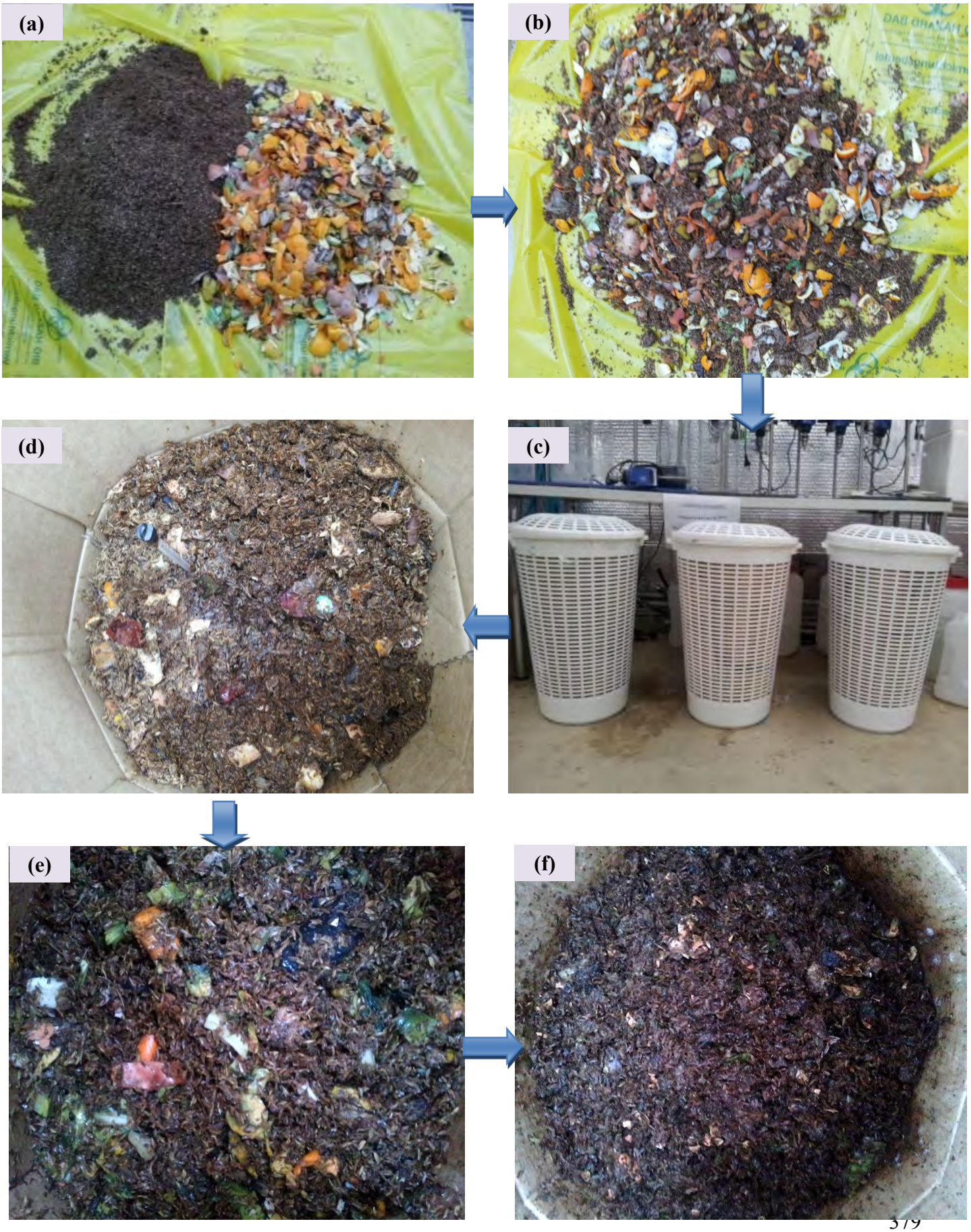


Figure 5.6 Compost making process (a) 1:1 v/v seeding inoculate and organic waste (b) after mixing (c) 3 compost variants in compost bin (d) compost on Day 2 (e) compost on Day 4 (f) compost on Day 7.

This waste consisted of orange peel, apple peel/core, banana peel, broccoli, cauliflower, kiwi fruit, carrot, cucumber, tea bags, potato peel, egg shells etc. The three mixed compost matrices, namely CA, CB and CC, **Table 5.3**, were incubated in three different baskets – Basket A, Basket B and Basket C, **Figure 5.6 (c)**. The composting baskets were covered with lid and placed in 'shed' where the temperature was maintained at 25 ± 2 °C.

The temperatures of CA, CB and CC were measured, in the centre of the compost pile, followed by mixing, every day for a period of seven days. During the seven days, the moisture level was monitored using squeezing technique - i.e. taking the compost into the palm of the hand and squeezing it. The moisture level is considered just right if a lump is formed and no water drips through it. After seven days of incubation, the baskets were stored without any further mixing. Samples from the 3 baskets were taken on Day 7, Day 21, and Day 35 for analysis. The analytical parameters and results are discussed in **Section 5.3.2**.

5.2.4 Preparation and characterization of vermicompost

A worm farm called "TUMBLEWEED Worm Farm", otherwise referred to as Can-O-Worms®, was purchased from Bunnings Pty Ltd. This product is a 51 x 51 x 65 cm^3 sized container made from black (100 per cent recycled) plastic and consists of a round fly-proof ventilated lid, two large capacity working trays, a ventilated collector tray, a tap with mound/sump and legs. The set-up and assembly of this equipment is shown in **Appendix 5.1**. To prepare the working tray, a worm farm bedding block (provided as 'coir brick') was soaked in 6-7 L of water, along with its packaging. As the block soaks with water it expands and mixes evenly, a process which takes ~15 minutes. The cardboard packaging provided was then folded in a circle and placed onto the base of the working tray. The remaining cardboard packaging was torn into small pieces and placed on top of the round cardboard. The expanded worm bedding block was spread over the top of the cardboard pieces so as to cover the tray evenly. Then, ~1000 worms were introduced onto the top of the bedding, along with some vermicompost, which was also purchased from Bunnings. The set-up was kept in a natural environment away from direct sunlight.

At first the worms were fed only once a week, assuming that they need some time to acclimatize to new environment and are able to consume only the same amount of waste of their body weight (Sinha and Valani 2011). Approximately 100 g of kitchen waste, of similar composition as referred in **Section 5.2.3** (excluding citrus[91]), was fed to the worms. After a two month period, the feeding rate was increased to twice a week, assuming the worms might now be well adapted to the new environment and that there might be an increase in the worm population. Unused kitchen waste in the working tray was separated and set aside. The vermicompost was collected after six months according to a literature method. Thus the vermicompost, along with worms, was taken out of the tray, placed in a plastic sheet and arranged into small portions of trapezoid piles. Due to light stimulation the worms hide under the middle of a pile. Therefore, the upper part of a pile can be easily separated and stored in a box. The worms are then returned to the working tray to continue the composting process. The vermicompost was stored in a box for two weeks and was sampled for analysis as matured compost.

5.2.5 Analytical methods

5.2.5.1 Physicochemical measurements

The pH, conductivity and temperature of the fermentation solutions, seeding inoculate and compost were determined using a multipurpose HACH HQd Portable Meter with a gel-filled pH electrode and a conductivity probe. The pH, conductivity and temperature of the fermentation solutions were measured directly by immersing the electrode into the respective solutions. For the measurement of the pH and conductivity of the seeding inoculate and compost samples, 10 g of an air dried and 2 mm - sieved sample was suspended in 50 mL of deionised water in a 250 mL Erlenmeyer flask. The 1:5 solid: liquid slurry was then shaken on rotary shaker at 180 rpm for 20 mins. Subsequently, the pH and conductivity of the slurry was measured by direct immersion of the electrode until the reading becomes stable (Thompson et al. 2001).

[91]Citrus components could affect the health of worms and it could have detrimental effect on them Sinha, R. K. & Valani, D. 2011. *Vermiculture Revolution: The Technological Revival of Charles Darwin's Unheralded Soldiers of Mankind,* Nova Science Publishers..

TOC and TN were analyzed using a Shimazdu TOC Analyzer by the methods described in Standard methods (APHA 1998). The fermentation solution sample was filtered through a 0.45 μm membrane filter and diluted 10 fold prior to measurement. TOC and TN analysis of the seeding inoculate and compost was carried out on slurry prepared by suspending 5 g of an air dried and 2 mm - sieved sample. The 1:10 solid:liquid slurry was shaken on rotary shaker for 2 hours at 125 rpm at room temperature. Then the slurry was centrifuged (centrifuge used is SORVELL®RT7) at 4000 rpm for 30 minutes and the supernatant was filtered through a 0.45 μm membrane filter and diluted 10 fold prior to measurement.

Preparation of the standard solution for TOC analysis: Potassium hydrogen phthalate was dried by heating in an incubator at 105 - 110 °C for 1 hr and allowed to cool in a dessicator. 2.125 g of this was dissolved in 1 L of milliQ water to prepare a 1000 ppm solution. 100 mL of a 100 ppm solution was prepared from this by a tenfold dilution.

Preparation of the standard solution for TN analysis: Potassium nitrate was dried by heating in an incubator at 110 °C for 1 hr and allowed to cool in a dessicator. 7.219 gm of this was dissolved in 1 L of milliQ water to prepare a 1000 ppm solution. 100 mL of a 50 ppm solution was prepared from this by a twentyfold dilution.

5.2.5.2 Determination of ethanol, volatile fatty acids and lactic acid in the fermentation solution(s)

5.2.5.2.1 Analytical methods

A Shimazdu GC – 2010 Gas Chromatograph, equipped with a flame ionisation detector (FID), a SGE BP20 column (12 m x 0.22 mm internal diameter x 0.25 μm film thickness) and an auto sampler, was used to determine ethanol and various volatile fatty acid concentrations (**Table 5.4**). Nitrogen was used as the carrier gas. The analysis was performed at 54.7 kPa pressure, 56.7 mL/min total flow, 1.05 mL/min column flow, 137.2 cm/sec linear velocity and a 1:50 split ratio.

The injector and detector temperatures were 200 ºC and 250 ºC respectively. The temperature was programmed as follows: 1 min at 35 ºC, raised to 200 ºC at 10 ºC/min and retained for 6 mins (SGE Manual). The total analysis time for each sample was 22.50 minutes.

A Shimadzu LCMS-2010 EV High Performance Liquid Chromatography - Mass Spectrophotometer was used to determine lactic acid concentration in the fermentation solution(s). The chromatographic system was consisted of: 0.6 mL/min, 5mM H_2SO_4 mobile phase, UV = 220 nm detector. The analytical column used was Aminex HPX-87H (300 mm x 7.8 mm and 9 m particle size) supplied by BIO-RAD, California, USA (de Sá et al. 2011).

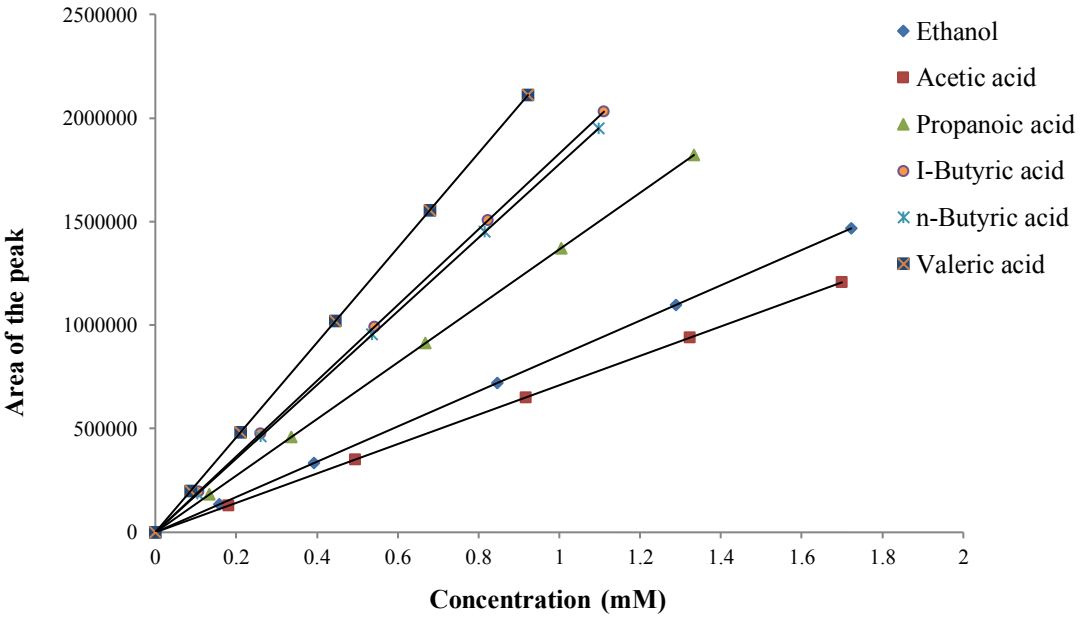
5.2.5.2.2 Preparation of standard solutions

All chemicals used were of analytical grade and were sourced as shown in **Table 5.4**.

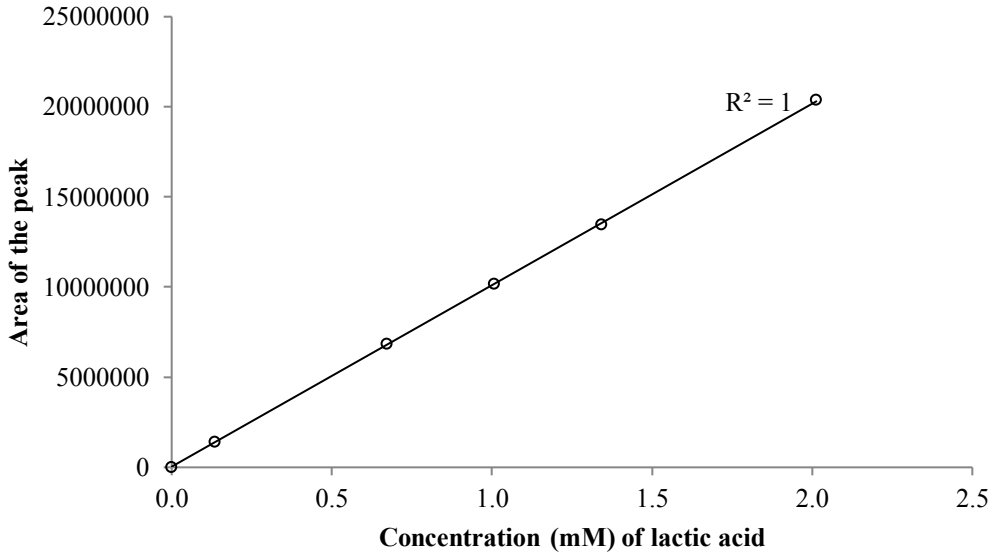
Table 5.4 VFAs employed in this study, together with their source.

Chemical	Abbreviation	Source
Ethanol	E	Chem-Supply
Acetic acid	AA	Merck
Propanoic acid	PA	Sigma-Aldrich
iso-Butyric acid	iBA	Sigma-Aldrich
n-Butyric acid	nBA	Sigma-Aldrich
Iso-Valeric acid	VA	Aldrich
Lactic acid	LA	BDH AnalaR

1% v/v stock solutions of the individual compounds were prepared. Standard solutions (0.1, 0.25, 0.5, 0.75 and 1% v/v) of the compounds listed in **Table 5.4** were prepared in order to obtain the GC calibration curves as shown in **Figure 5.7(a)** and standard solutions (0.1, 0.5, 0.75, 1.0 and 1.5 % v/v) of lactic acid were prepared in order to obtain the HPLC calibration curve (for LA) as shown in **Figure 5.7(b)**.



(a)



(b)

Figure 5.7 Calibration curve of **(a)** Ethanol, acetic acid, propanoic acid, i-butyric acid, n-butyric acid and valeric acid[92] and **(b)** Lactic acid, obtained for GC and HPLC analyse respectively. For the calibration curves the % v/v units were converted to mM.

[92]Note that the relative sensitivities of GC detection are: VA > iBA > nBA > PA > E > AA, as indicated by the relative slopes of the lines.

5.2.5.2.3 Sample preparation

For GC and HPLC analysis of the fermentation solution(s), a 50 mL sample was taken from each bottle of the salt-based and sugar-based solution(s) corresponding to Day 1, Day 3, Day 5, Day 7 and Day 9, see **Tables 5.1** & **5.2**. Before drawing the samples, the bottles were shaken and the suspension was pipetted out and stored in 50 mL falcon tubes. The collected samples were centrifuged at 4000 rpm for 30 minutes and the supernatant was filtered through a 0.45 µm membrane filter.

5.2.5.3 Nutrients, macronutrient, micronutrients and trace metals analysis in the compost(s)

The presence of nutrients, macronutrients, micronutrients and trace metals in the compost(s) determines the compost quality. The major nutrients in the compost known as the nitrogen, phosphorus, potassium (NPK) value and other macronutrients such as calcium (Ca), magnesium (Mg), sodium (Na), sulphur (S), barium (Ba) and boron (B) were measured using a Shimazdu Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP) instrument as mentioned in the Test Methods for the Examination of Compost and Composting, TMECC (Thompson et al. 2001) and standard methods (APHA 1998). In addition, some of the micronutrients such as copper (Cu), manganese (Mn), iron (Fe), zinc (Zn) and trace metals such as cadmium (Cd), chromium (Cr), cobalt (Co), molybdenum (Mo), nickel (Ni) were also analyzed. All the compost and seeding inoculate were digested by an aqua regia method as described in TMECC (Thompson et al. 2001). Such water-soluble mineral content was reported as mg/L material.

5.2.5.3.1 Aqua regia procedure

10 g of an air-dried and 2 mm sieved sample was wetted with deionised water (DIW). 10 mL of concentrated nitric acid (HNO_3) was then added and heated slowly to evaporate the HNO_3. The slow heating was continued for approximately 2 hours (the set up was covered with a watch glass to condense the vapour). 2 hours of slow heating brought the sample to dryness. Once the set up was cooled, 20 mL of 3 N hydrochloric acid (HCl) was added, covered with a watch glass and

gently refluxed for 2 hours. Finally, the digested sample was filtered through a 0.45 µm Whatman filter paper together with washing the remaining material with 0.1 N HCl. The volume was made up to 50 mL in a volumetric flask.

5.2.5.4 Compost maturity test

An assessment of the maturity of the compost was carried out using four different methods: (a) germination percentage (b) plant bioassay (c) C:N ratio and (d) Fourier Transform Infrared (FTIR) spectroscopy (Ouatmane 2000; Goyal et al. 2005; Said-Pullicino et al. 2007; Khan and Fouzia 2011; Fourti 2013).

Measurement of the germination process can be complex as explained in a review by (Ranal and Santana 2006). However, for this study, the simplest method was selected.

Figure 5.8 Controlled environment provided for the determination of germination percentage and for the plant bioassay test. Photo taken by Anu Joshi.

For the germination percentage and plant bioassay tests (Khan et al. 2011), 10 pots were filled with CA, CB, CC, VC and garden soil (GS) in duplicate (2 pots for each type of compost matrix). Here, GS is considered as a control. 5 radish (*Raphanus sativs*) seeds (Ko et al. 2008)

were planted at the depth of 5 cm in each pot and placed at 25 ± 2 °C in a controlled environment, **Figure 5.8**. The pots were observed for plant germination at defined time intervals. The germination percentage (GP) was calculated using the following equation (Ahmadloo et al. 2011):

$$GP = (\text{number of seeds germinated/number of seeds planted}) \times 100\% \quad (5.3)$$

The pots were then observed on Week 1, Week 2, Week 3 and Week 4 to record the height of the plant and the number of leaves. The carbon to nitrogen ratio was calculated using the equation (Fourti 2013):

$$C{:}N \text{ ratio} = \text{Total organic carbon} / \text{Total nitrogen} \quad (5.4)$$

Two different spectroscopic methods - Fourier transform infrared (FTIR) and Diffuse Reflectance Infrared Fourier transform (DRIFT) spectroscopy were trialled to study the transformation of organic matter during the composting process and to assess the maturity of the composts (Provenzano et al. 2014). These two methods have different techniques of sample preparation. The FTIR spectra of CA, CB, CC and VA were recorded on KBr pellets in the 4000 to 400 cm^{-1} wavelength range using IRAffinity-1 Shimadzu spectrophotometer. The KBr pellet was prepared by pressing a mixture of 2 mg samples and 200 mg KBr (both dried at 105°C) and compressed under vacuum at 10 tonne for 10 minutes (Chefetz et al. 1998). At the same time, DRIFT spectroscopy has been used directly on compost samples prepared by mixing/grinding the same proportion of the sample and KBr as mentioned for FTIR. Due to time constraints only one representative TCM (CA) and VC was subjected to FTIR analysis.

5.2.5.5 Biological observation

Microbes were isolated from the salt and sugar-based solutions after the solution was incubated at 35 ± 2 °C for five days by the method described in the Standard Methods (APHA, 1998). Bacteria were identified using Biolog equipment (GEN III MicroPlate™) as reported by Bochner (Bochner 2009). Fungus was identified under a light microscope.

5.3 Results and Discussions

5.3.1 Optimizing the fermentation solution(s)

5.3.1.1 pH

Figure 5.9 (a) & **(b)** presents the pH profiles of the fermentation solutions from Day 1 to Day 9 on every alternative day, in salt-based and sugar-based solutions, respectively. The pH profile presented on Day 0 depicts the pH of the salt and sugar solutions before the addition of substrate. The average pH of 7.87, decreased as the substrate was added to the salt solution. The pH of the fermentation solution tended to decrease with increasing substrate and retention time as shown in **Figure 5.9 (a)**, although a levelling out was observed after Day 1.

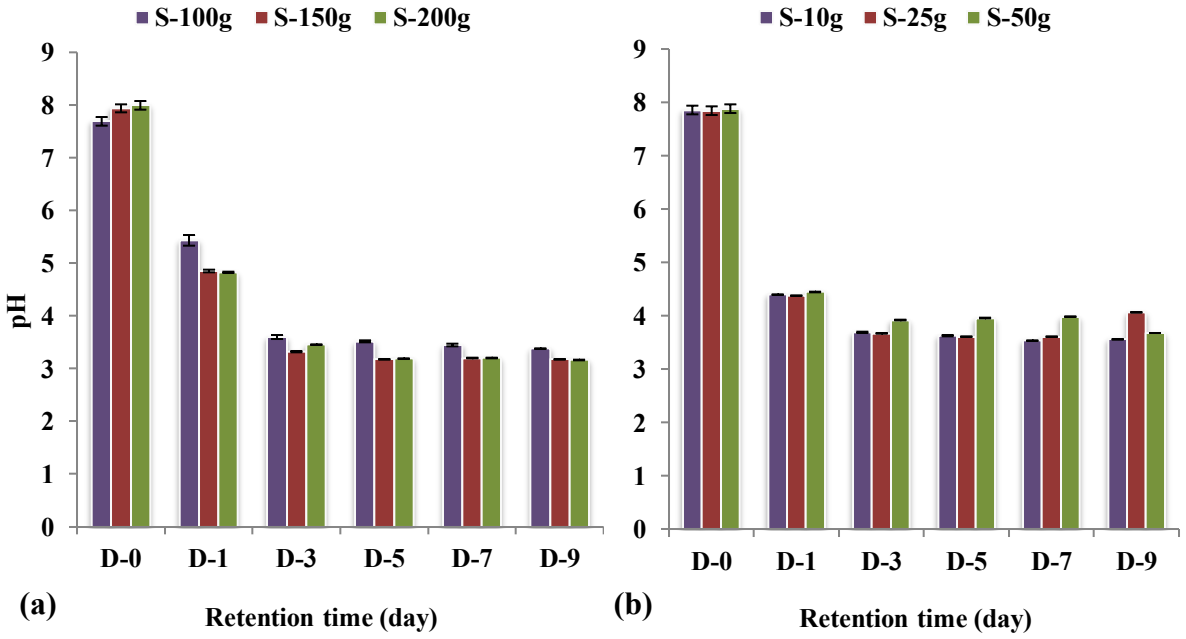


Figure 5.9 pH profile over time in (a) Salt-based solution and (b) Sugar-based solution. S-100g: salt-based solution with 100 g substrate; S-150 g: salt-based solution with 150 g substrate; S-200 g: salt-based solution with 200 g substrate; S-10 g: sugar-based solution with 10 g substrate; S-25 g: sugar-based solution with 25 g substrate; and S-50 g: sugar-based solution with 50 g substrate.

In the solution with 100 g substrate, the pH decreased from 5.43 on Day 1 to 3.38 on Day 9. Similarly, the pH dropped from 4.86 on Day 1 to 3.18 on Day 9 in the solution with 150 g substrate. A similar trend was observed with the solution with 200 g substrate. The initial pH of

4.82 on Day 1 dropped to 3.17 on Day 9. The low pH is likely to be due to the formation of VFAs and lactic acid during the fermentation process (Bolzonella et al. 2005; Bolzonella et al. 2007). Organic acid formation is due to the degradation of soluble and easily degradable materials in substrate such as monosaccharide, starch and lipids (Nair and Okamitsu 2010). Thus, it can be concluded that the pH decreased with the lactic acid and VFAs produced with longer retention time, as shown in **Figure 5.10**.

A similar observation was obtained for the sugar solution. The average pH of 7.86 decreased with increasing substrate loading and retention time. In the solution with 10 g substrate loading, the pH decreased from 4.40 on Day 1 to 3.56 on Day 9. Similarly, the pH dropped from 4.37 on Day 1 to 4.07 on Day 9 in the solution with 25 g substrate loading. A similar trend was observed with the sugar solution with 50 g substrate loading. The Initial pH of 4.45 on Day 1 dropped to 3.68 on Day 9, as in **Figure 5. 9 (b)**.

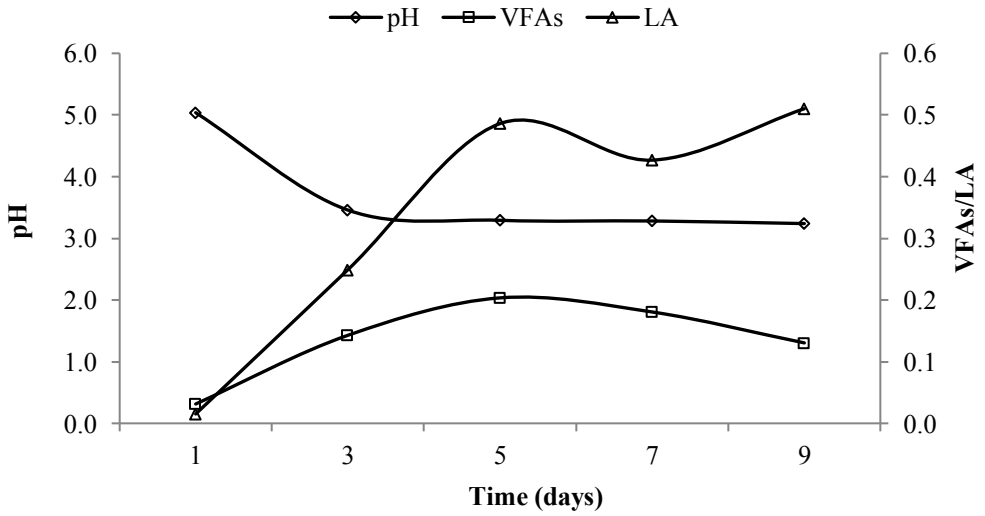


Figure 5.10 Relation between pH and VFAs/LA for salt-solution. The values presented here is an average of the pH and VFAs/LA value observed for the salt solution with 100 g, 150 g and 200 g substrates.

The average pH of the salt and sugar solution was 3.3 and 3.7 respectively on Day 5, which is just in the range at which acidogens can survive (Lee et al. 2014). However, the observation shows that the pH of sugar solution is higher than that of salt solution. As discussed by Sundberg (2005), the acid-base system called the organic acid system, could be responsible for the drop in pH, especially due to acetic acid and lactic acid production, **Figure 5.10**.

5.3.1.2 Conductivity

Figure 5.11 (a) & **(b)** presents the conductivity profiles of the fermentation solutions from Day 1 to Day 9 on every alternative day, in salt and sugar-based solutions, respectively. The conductivity of the salt solution with 100 g substrate decreased notably from 14.31 mS/cm on Day 1 to 11.11 mS/cm on Day 3, and only slightly increased on Day 5 and Day 7 with conductivity 11.50 mS/cm and 11.00 mS/cm respectively. However, for the salt solution with 150 g and 200 g substrate loadings, the conductivity remained moderately constant. Moreover, it may be observed that the conductivity of the salt solution increased with an increase in substrate loadings. A similar observation was noted for the salt solution with 150 g and 200 g substrate loadings, **Figure 5.11 (a)**.

The conductivity of the sugar solution with 10 g substrate increased from 0.11 mS/cm on Day 1 to 0.46 mS/cm on Day 7, and then it increased slightly on Day 9 to 0.47 mS/cm. In contrast, the conductivity of the solution with 25 g substrate increased gradually from 0.34 mS/cm on Day 1 to 0.68 mS/cm on Day 7, and then increased to 1.67 mS/cm on Day 9. However, a different trend was observed with the solution containing 50 g substrate.

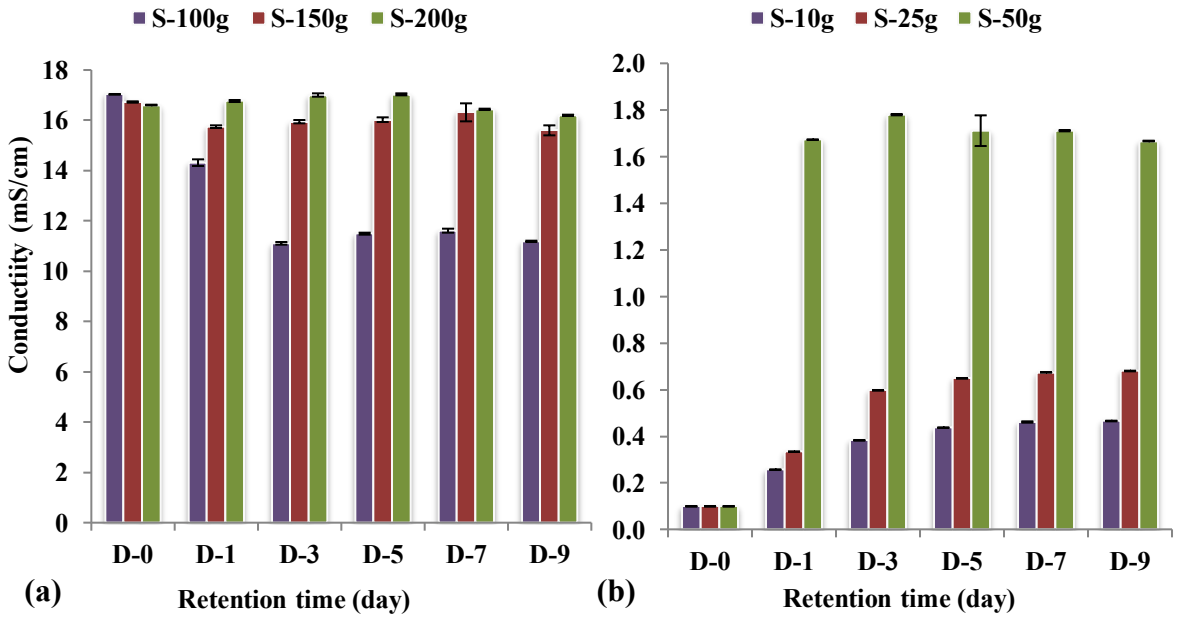


Figure 5.11 Conductivity profile over time in (a) Salt-based solution and (b) Sugar-based solution.

The conductivity changed from 1.67 mS/cm on Day 1 to 1.71 mS/cm on Day 7, and then it dropped to 1.69 mS/cm on Day 9 as in **Figure 5.11 (b)**. The increase in conductivity could be due to the release of mineral salts and ammonium ions in the solution due to the decomposition of organic matter from the substrate and the decrease could be due to the precipitation of such salts and ions (Nair and Okumitsu 2010).

Higher conductivity implies a high concentration of soluble salts in solution, which is due to the ionisation of soluble nutrients in the substrate. Possible salts are chlorides, nitrates, sulphates and carbonates of sodium, calcium, potassium and magnesium. The nature of the substrate used in the solution defines the predominance of the type of salt ions in the solution (Watson 2013).

5.3.1.3 Dissolved oxygen

Figure 5.12 (a) & **(b)** present the dissolved oxygen profiles of the fermentation solutions from Day 1 to Day 9 on every alternative day, in salt-based and sugar-based solutions, respectively. The fermentation process is anaerobic, and the DO in the salt based solution decreased significantly with the addition of substrate and further incubation. **Figure 5.12 (a)** & **(b)** shows that the fermentation process effectively maintained anaerobic conditions in the fermentation solutions(s) during the incubation period. There is no significant correlation observed between DO and substrate as well as retention time. The low DO values are evident of an anaerobic process. However, the increase in DO on Day 9 might be due to aerobic microbes starting to become active in the system.

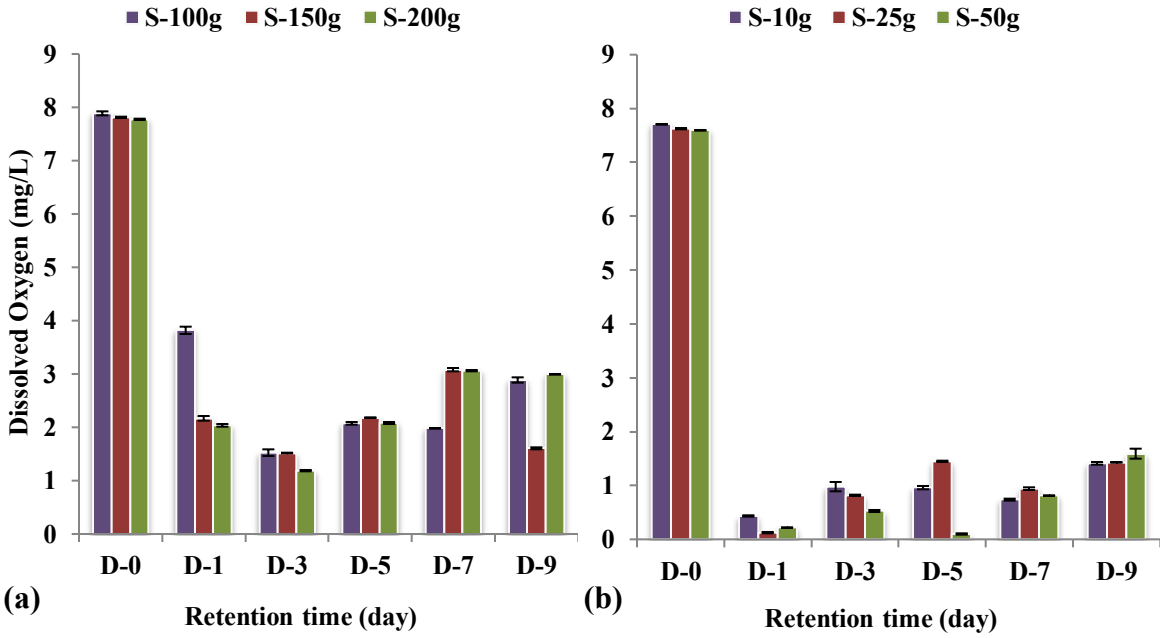


Figure 5.12 Dissolved oxygen profile over time in (a) Salt-based solution and (b) Sugar-based solution.

5.3.1.4 Total Organic Carbon

Figure 5.13 (a) & **(b)** presents the TOC profiles of the fermentation solutions from Day 1 to Day 9 in every alternative day, in salt-based and sugar-based solutions, respectively. The TOC increased from 4769 mg/L on Day 1 to 5497 mg/L on Day 3 in the salt solution with 100 g substrate, which increased to 5571 mg/L on Day 5, then decreased to 5353 and 4685 mg/L on Day 7 and 9, respectively. A similar trend was observed for the solution with 150 g substrate. However, the TOC increased gradually until Day 7, and then decreased slightly on Day 9 for 200 g substrate solution, **Figure 5.13 (a)**. In the sugar-based solution with 10 g substrate loading, the TOC decreased from 3216 mg/L on Day 1 to 2913 mg/L on Day 3 which started to increase gradually up to 3438 mg/L on Day 7, and then decreased again to 3136 mg/L on Day 9. A similar observation was obtained for the solution with 25 g substrate. In contrast, TOC increased with longer retention time with 50 g substrate as shown in **Figure 5.13 (b)**.

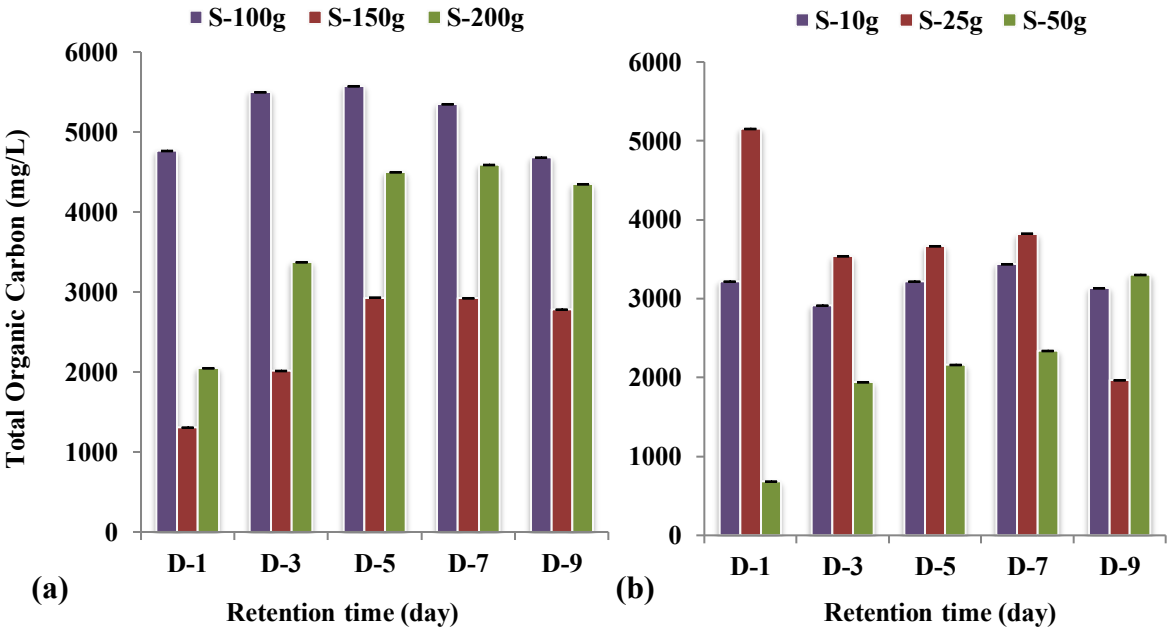


Figure 5.13 TOC profile over time in (a) Salt-based solution and (b) Sugar-based solution.

5.3.1.5 Total Nitrogen

Figure 5.14 (a) & (b) presents the TN profiles of the fermentation solutions from Day 1 to Day 9 in every alternative day, in salt-based and sugar-based solutions, respectively. The TN increased notably from 22.13 mg/L on Day 1 to 39.89 mg/L on Day 3 in salt solution with 100 g substrate, which decreased slightly to 39.70 mg/L on Day 5 and further decreased to 31.81 mg/L on Day 9, **Figure 5.14 (a)**. Similar observation of sharp increase at first and gradual decrease was obtained for substrate 150 g and 200 g.

In the sugar-based solution, the TN increased substantially from 18.73 g/L on Day 1 to 46.58 mg/L in salt solution with 10 g, which increased to 69.60 and 84.75 mg/L on Day 5 and Day 7 respectively. Then, it increased further to 88.45 mg/L on Day 9. A similar observation was obtained for 25 g, however there was decrease on Day 9 for 50 g substrate as, **Figure 5.14 (b)**.

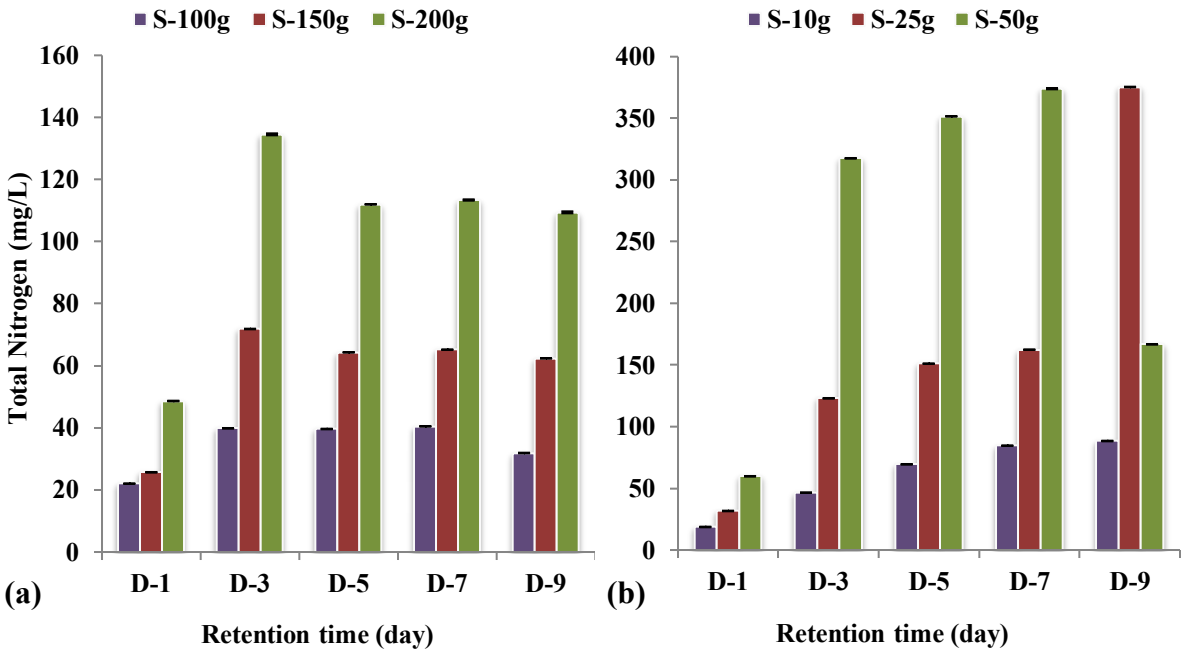


Figure 5.14 TN profile over time in (a) Salt-based solution and (b) Sugar-based solution.

The salt-based and sugar-based solutions were incubated at a mesophilic temperature (35 ± 2 °C), thus nitrogen transformations within the closed system might have varied the nitrogen concentration in the solutions.

5.3.1.6 The fermentation products (ethanol, volatile fatty acids and lactic acid)

Ethanol, volatile fatty acids (VFAs) and lactic acids are the major fermentation products obtained in a fermentation process (Playne 1985). The initial fermentation step of acidogenesis involves the hydrolysis of proteins, lipids and carbohydrates and provides metabolic energy for the growth of microbes that decompose organic substrates. The oxidation of these compounds produces VFAs, including lactic acid and ethanol, as fermentation products (Scoma et al. 2013).

Volatile fatty acids (VFAs) are short chain fatty acids containing C_2-C_6 carbon atoms which are also known as low-molecular mass carboxylic acids. In biological processes, these VFAs are significant intermediates produced by acidogenesis or acidogenic fermentation or dark

fermentation (Lee et al. 2014). The existence of VFAs in a sample usually indicates biological activity due to the presence of a range of microorganisms (Siedlecka et al. 2008) such *as Bacteriocides*, *Clostridia*, *Bifidobacteria*, *Streptococci* and *Enterobacteriaceae* (Weiland 2010).

Generally, sugar is used as the main source of carbon for VFA production. However, it can also be produced using waste as raw material - such as food waste, organic municipal solid waste, landfill leachates, sewage sludge and industrial wastewater (Ruan et al. 2003). Different wastes used for VFA production are discussed extensively by Lee et al. (2014). Using waste as feedstock for VFA production reduces the waste quantity as well as minimizes the requirement of fresh food. In the present context, there is a wide use of VFAs in different sectors such as nutrient removal in wastewater (Obaja et al. 2005), bioplastics (Pijuan et al. 2009) and bioenergy (Uyar et al. 2009). For the commercial production of VFAs, biological routes are gaining interest over chemical routes (Huang et al. 2002). This study adopts a prudent waste management approach of resource recovery and utilizes fruit and vegetable waste from the kitchen to produce the fermentation solution and to isolate native microorganisms for composting.

Previous studies (Kathirvale et al. 2004; Elbeshbishy et al. 2011) claim that food or kitchen waste is appropriate for VFA production as it is a major component in municipal solid waste and contains a high COD value. Nevertheless, different concentrations of VFAs are found in various environmental substances (Siedlecka et al. 2008). VFA production using food waste is influenced by certain operational parameters such as pH, temperature, retention time, substrate concentration, organic load and additives (Hong and Haiyun 2010; Lee et al. 2014). Most acidogens are capable of living only in the pH range of 3 to 12; the optimum pH value being 5.25 - 11 for the production of VFAs. However, the pH range depends on the waste type used as well. For example, for the hydrolysis of kitchen waste, acidogenesis occurs well at pH 7 due to the high solubility of carbohydrate, protein and lipid, with the production of high VFA concentrations. In addition, the pH determines the type of VFA produced by acidogenesis, especially acetic acid, propanoic acid or butyric acid.

Previous studies carried out on VFA production revealed that production increased with increasing temperature. For example, Zhang et al. (2009) showed that VFA concentration was

significantly higher under mesophilic (10 °C) conditions rather than psychrophilic conditions (35 °C). Mengmeng et al. (2009) reported that at thermophilic temperatures (60 °C), the rate of acidogenesis was higher than under mesophilic conditions (35 °C), with higher VFA production. Similarly, Lu and Ahring (2005) found that the VFA production was notably higher at hyper-thermophilic temperatures (70 °C) than at thermophilic temperatures (55 °C).

Besides pH and temperature, retention time is also a vital parameter for the production of VFAs during the acidogenesis of organic waste. Previous studies found that higher retention time increased VFAs production, whereas extended retention time did not change the production significantly (Lim et al. 2008). A higher retention time provides a longer time for microbial activities. However, the extended period might also promote methanogens to dominate the process. In this study the term retention time (RT) has been used for the time given for the waste in the solution to ferment.

Different analytical methods have been used by different researchers to determine VFA production in a sample. Siedlecka et al. (2008) has recommended gas chromatography (GC) as a reliable method based on its precision and accuracy. In his comparative study, gas chromatography was found to be the only method which could measure individual VFAs even in trace amounts compared to other methods such as distillation and spectrophotometric methods.

During the initial stages in the composing process, mesophilic organic acid produces bacteria such as *Lactobacillus* spp. and *Acetobacter* spp. which are found to be dominant amongst identified bacterial communities (Partanen et al. 2010). Considering their dominance, it can be concluded these are the major microbial communities responsible for stimulating the composting process. *Lactobacillus* spp. and *Acetobacter* spp. are responsible for producing lactic acid and acetic acid respectively. Thus, the fermentation solution prepared in the Takakura composting method was assessed in terms of ethanol, volatile fatty acids and lactic acid to characterize the fermentation solutions, considering an incubating time period of 3 to 5 days - as suggested by Takakura (IGES Home Composting Method).

In the salt-based fermentation processes studied here, lactic acid and acetic acid are the major products, whereas in the sugar based process lactic acid and ethanol are the major products. For the fermentation solutions of this study, a GC method (**Section 5.2.5.2**) has been applied to analyse for ethanol, VFAs (**Table 5.4**) and the lactic acid formed. Although, as revealed by the calibration plots of **Figure 5.7 (a)**, the sensitivities of detection vary in the order VA > iBA > nBA > PA > E > AA, this GC method as applied to a mixture of all of the compounds listed in **Table 5.4** and the fermentation solution was found to detect and produce well-resolved peaks - except for LA. Indeed, it was concluded that this method was of insufficient sensitivity to detect this particular analyte (LA) in the sample. To challenge this method with respect to LA, a high concentration of LA, i.e. of 10 % v/v, was analysed. This gave a sharp peak with a relatively long tail as shown in **Appendix 5.2**. For this reason another method was developed for the *specific* detection of LA, namely HPLC, **Figure 5.7 (b)**, as described in **Section 5.2.5.2**. This method was found to be able to satisfactorily detect and quantify the LA present in the samples (**Figure 5.16**).

During these experiments, the HPLC method was used selectively for the detection of LA and the GC method was used for ethanol and the remaining fatty acids. This approach was adopted for a number of reasons, including the fact that when GC was applied to all the samples, including LA, according to the original experimental plan, it was found that the relative sensitivity of the method for LA was inadequate. This was in spite of a number of different columns being trialled. Thus, HPLC was adopted specifically for LA detection. It was not considered practical to use HPLC for ethanol and the remaining VFAs since alternative columns would have to be employed. An additional factor with respect to the preferential use of GC is that only one column was required and the instrument was more freely available in our laboratory - and is an easier method to operate for routine analysis. It is anticipated that this might be generally the case.

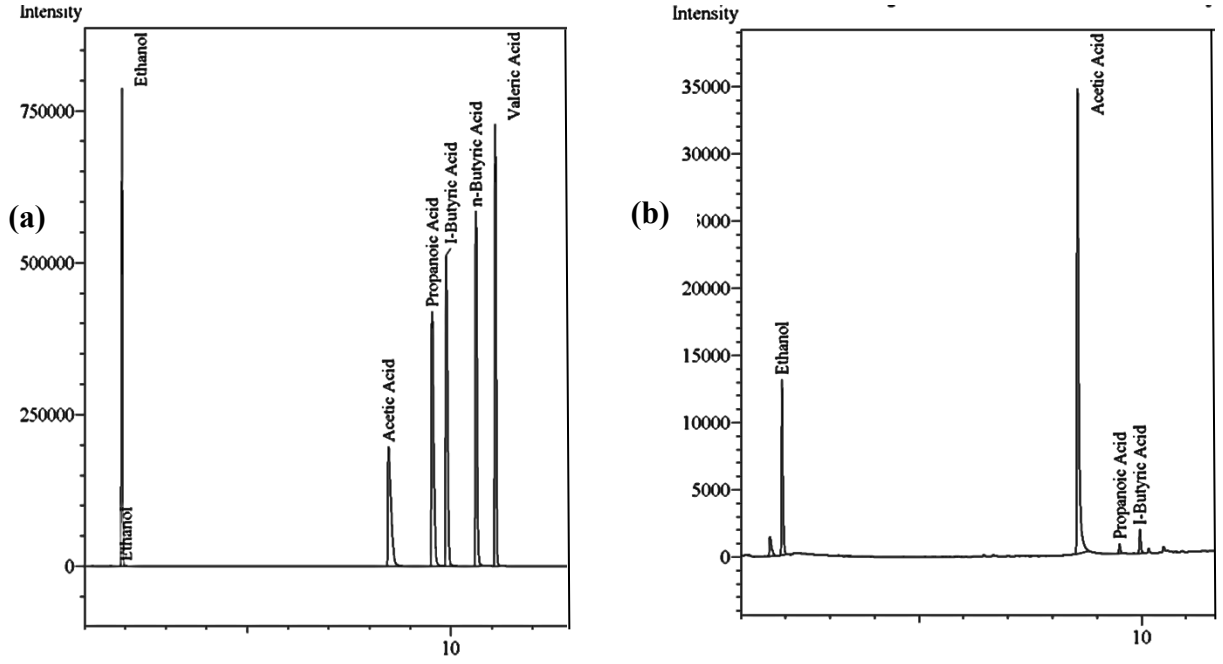


Figure 5.15 GC peak obtained for (a) 1 % mix standard and (b) Salt-based solution with 150 g substrate on Day 5.

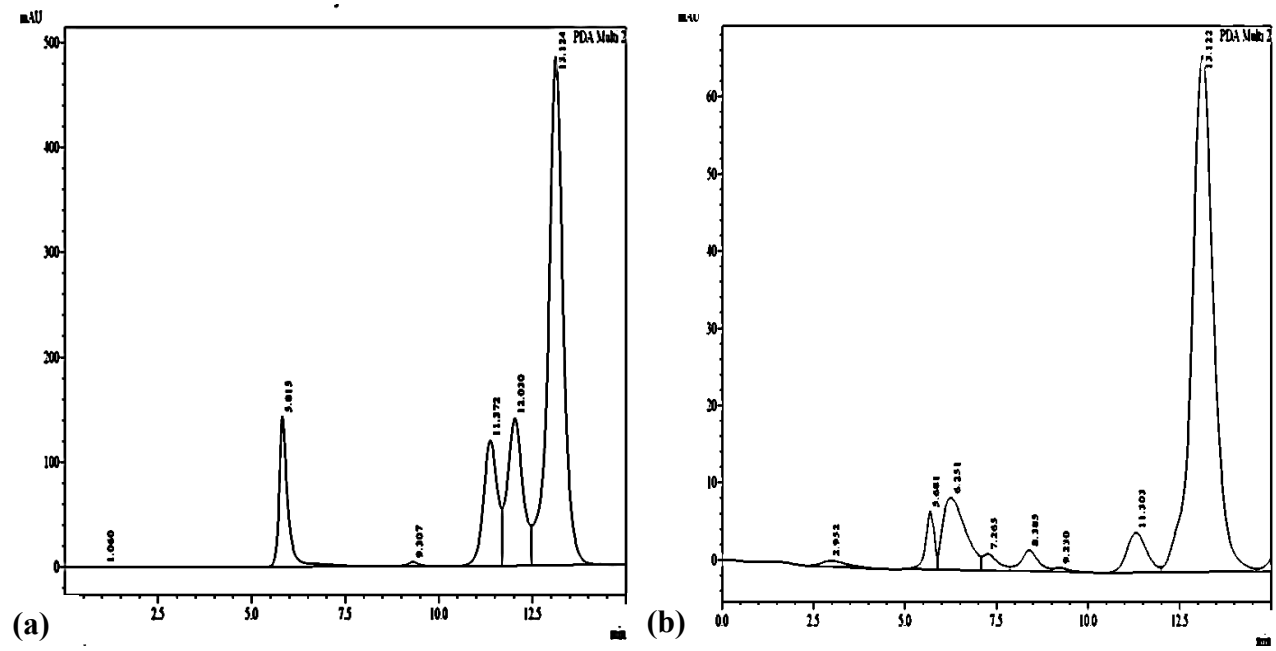


Figure 5.16 HPLC peak obtained for (a) 1% standard LA and (b) Sugar-based solution with 25 g substrate on Day 5.

Table 5.5 The amount of fermentation products in the salt-based solution over different retention time reported in mM unit. Total VFAs is the sum of AA, PA, I-BA, n-BA and VA.

Time	Substrate loading (g)	Ethanol	AA	PA	I-BA	n-BA	VA	LA	Total VFAs
Day-1	**100**	0.005	0.024	0.000	0.000	0.000	0.000	0.012	0.024
Day-1	**150**	0.007	0.019	0.000	0.000	0.000	0.000	0.009	0.019
Day-1	**200**	0.012	0.049	0.000	0.000	0.000	0.003	0.024	0.052
Day-3	**100**	0.377	0.238	0.001	0.004	0.000	0.008	0.114	0.252
Day-3	**150**	0.034	0.052	0.000	0.004	0.000	0.001	0.256	0.058
Day-3	**200**	0.051	0.112	0.000	0.003	0.001	0.003	0.378	0.119
Day-5	**100**	0.043	0.391	0.003	0.006	0.002	0.009	0.180	0.411
Day-5	**150**	0.055	0.080	0.000	0.008	0.000	0.000	0.430	0.088
Day-5	**200**	0.069	0.103	0.000	0.007	0.001	0.000	0.849	0.111
Day-7	**100**	0.000	0.320	0.001	0.004	0.000	0.009	0.140	0.335
Day-7	**150**	0.046	0.079	0.000	0.011	0.000	0.000	0.560	0.090
Day-7	**200**	0.072	0.108	0.000	0.008	0.001	0.000	0.580	0.117
Day-9	**100**	0.221	0.187	0.000	0.004	0.000	0.002	0.181	0.193
Day-9	**150**	0.015	0.075	0.000	0.007	0.000	0.000	0.582	0.082
Day-9	**200**	0.039	0.110	0.000	0.006	0.001	0.000	0.769	0.117

Table 5.6 The amount of fermentation products in the sugar-based solution over different retention times reported in mM unit. Total VFAs is the sum of AA, PA, I-BA, n-BA and VA.

Time	Substrate loading (g)	Ethanol	AA	PA	I-BA	n-BA	VA	LA	Total VFAs
Day-1	**10**	0.009	0.100	0.000	0.000	0.000	0.006	0.008	0.106
Day-1	**25**	0.024	0.098	0.000	0.000	0.000	0.005	0.016	0.103
Day-1	**50**	0.010	0.030	0.000	0.000	0.000	0.002	0.118	0.032
Day-3	**10**	1.024	0.014	0.000	0.000	0.000	0.001	0.052	0.015
Day-3	**25**	1.045	0.017	0.001	0.001	0.000	0.000	0.260	0.020
Day-3	**50**	0.067	0.031	0.004	0.006	0.000	0.000	0.087	0.041
Day-5	**10**	1.070	0.021	0.001	0.001	0.000	0.000	0.082	0.023
Day-5	**25**	1.022	0.019	0.001	0.001	0.000	0.000	0.276	0.022
Day-5	**50**	0.091	0.012	0.001	0.001	0.000	0.000	0.157	0.015
Day-7	**10**	1.084	0.026	0.001	0.001	0.000	0.000	0.137	0.029
Day-7	**25**	1.086	0.024	0.001	0.001	0.000	0.000	0.117	0.027
Day-7	**50**	0.055	0.014	0.001	0.001	0.000	0.000	0.209	0.016
Day-9	**10**	0.421	0.026	0.001	0.001	0.000	0.000	0.120	0.029
Day-9	**25**	0.026	0.021	0.001	0.001	0.000	0.000	0.220	0.023
Day-9	**50**	0.589	0.031	0.001	0.001	0.000	0.000	0.147	0.034

The individual fermentation products and their relative levels during the fermentation process are discussed as follows.

According to the experimental plan depicted in **Figure 5.1**, and utilizing the techniques of GC and HPLC described herein, the levels of the seven components listed in **Table 5.4** have each been tracked over a nine day period for the three different amounts of substrate in both the salt-based and sugar-based solutions as shown in **Tables 5.5** and **5.6**. The sum of acetic acid (AA), propanoic acid (PA), iso-butyhric acid (i-BA) and n-butyric acid (n-BA) is reported as total volatile fatty acids (TVFA). Lactic acid was assessed separately as the study was motivated to investigate on the isolation of *Lactobacillus spp*. associated with LA.

5.3.1.6.1 Change in volatile fatty acids produced

Figure 17 (a) & **(b)** shows the TVFA level during the fermentation period for the salt-based and sugar-based solutions, respectively, with respect to different substrate loadings. The highest concentration of total VFA (0.411 mM) in the salt solution was achieved on Day 5 with the lowest 100 g substrate loading. A maximum TVFA level was observed on Day 5, which gradually decreases until Day 9. Notably, during the acidogenic digestion of dephenolized olive mill wastewaters (Scoma et al. 2013) the highest yield of VFA was also reported to be on Day 5 of retention time.

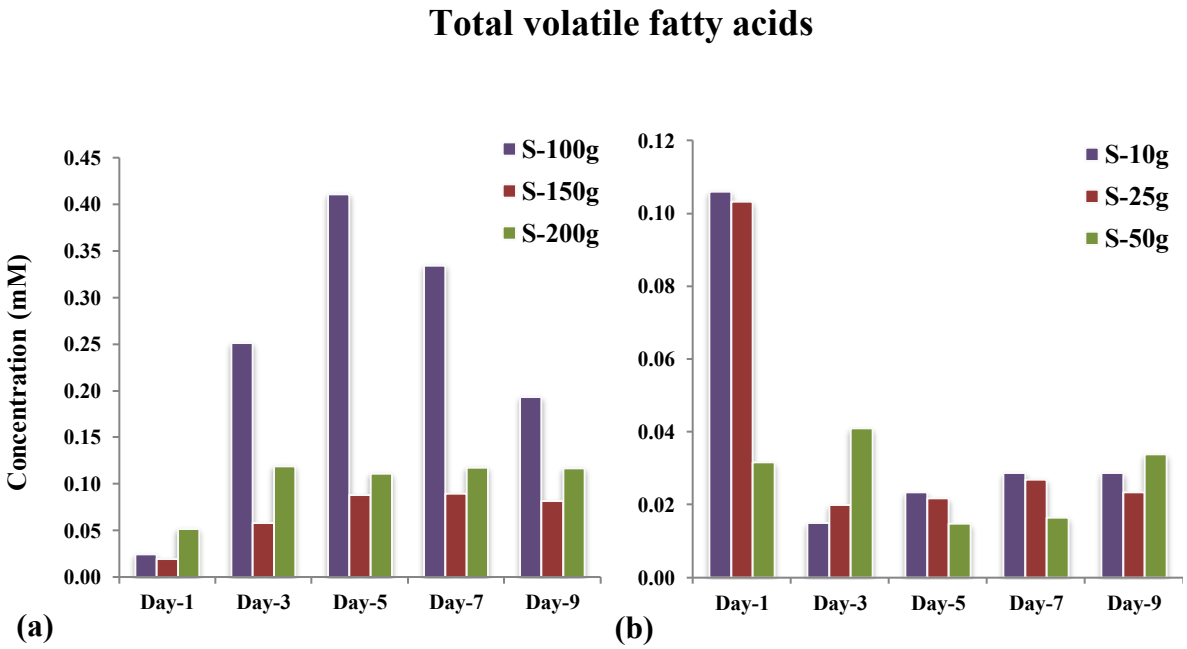


Figure 5.17 The amount of total VFAs produced over time in (a) Salt-based solution and (b) Sugar-based solution. S-100 g: salt-based solution with 100 g substrate; S-150 g: salt-based solution with 150 g substrate; S-200 g: salt-based solution with 200 g substrate; S-10 g: sugar-based solution with 10 g substrate; S-25 g: sugar-based solution with 25 g substrate; and S-50 g: sugar-based solution with 50 g substrate.

These findings show that the better proliferation of bacteria occurs on Day 5 with low substrate. In the solutions with 150 g and 200 g substrate loadings, the total VFAs increased after Day 1 to a relatively lower level compared to the 100 g loading and stabilized at that level. This suggests a threshold substrate loading in the TCM for the production of TVFAs during the fermentation process. This is consistent with other non-TCM fermentation studies (Xiong et al. 2012) which reveal that a lower substrate loading produces higher concentrations of VFAs. In anaerobic fermentation, higher substrate loadings can promote methanogen dominance (Ferrer et al. 2010) leading to a suppression of VFA production. With reference to **Table 5.5**, it may be seen that the individual component AA dominates the TVFA surge with respect to the lower substrate loading of 100 g. This is consistent with the finding of Feng et al. (2009) and Scoma et al. (2013); 100 % on Day 1 with 100 and 150 g substrate and 81.82 % on Day 7 with 150 g substrate. The quantity of other VFAs produced namely propanoic acid, iso-butyric acid, n-butyric acid and valeric acid ranged from 0.51 % to 18.18 %.

On the other hand, the TVFA produced in the sugar based solution were less than that observed in the salt based solution. The highest concentration of 0.041 mM was observed on Day 3 with 50 g substrate loading, **Figure 5.17 (b)**. With further retention of the solution, it decreased to 0.016 mM by Day 7 but increased again on Day 9. In terms of 10 g and 25 g substrate loadings, TVFA decreased on Day 3 and stabilized at that level. On the other hand, there was a fluctuation in the VFA production with a longer retention time. Similar to the salt solution, AA was the major VFA produced during the fermentation process followed by iso-butyric acid, propanoic acid and valeric acid. In the total VFAs produced, the acetic acid concentration ranged from 90.32 % on Day 1 with 25 g substrate loading to 69.23 % on Day 3 with 50 g substrate loading whereas i-BA ranged from 19.23 % on Day 3 with 50 g substrate loading to 5% on Day 9 with 50 g substrate loading. The findings obtained in this study are supported by the previous study of Bolzonella et al. (2005). The comparatively higher concentration of VFAs production on Day 1 could be due to the acidogenic microorganisms reacting with substrate with rapid acidification of the fruit and vegetable decreasing the pH (Bouallagui et al. 2009), followed by methoanogens dominating the process and with further incubation resulting a decrease in VFA concentration .

5.3.1.6.2 Change in lactic acid produced

Figure 5.18 (a) & **(b)** presents the LA level during the fermentation period for the salt-based and sugar-based solutions, respectively, with respect to different substrate loadings. As shown in **Figure 5.18 (a)** LA was the major product during the salt solution fermentation. The highest quantity of LA (0.849 mM) was produced on Day 5 with 200 g substrate loading. This shows the LA produced increased with an increase in substrate quantity and longer retention time. The highest concentration of lactic acid production on Day 5 during the fermentation process shows the growth of lactic acid bacteria (LAB) was high on Day 5 (Tang et al. 2008). Notably, LABs are capable of preventing the growth of putrefactive bacteria and fungi, which helps to preserve the fermentation solution.

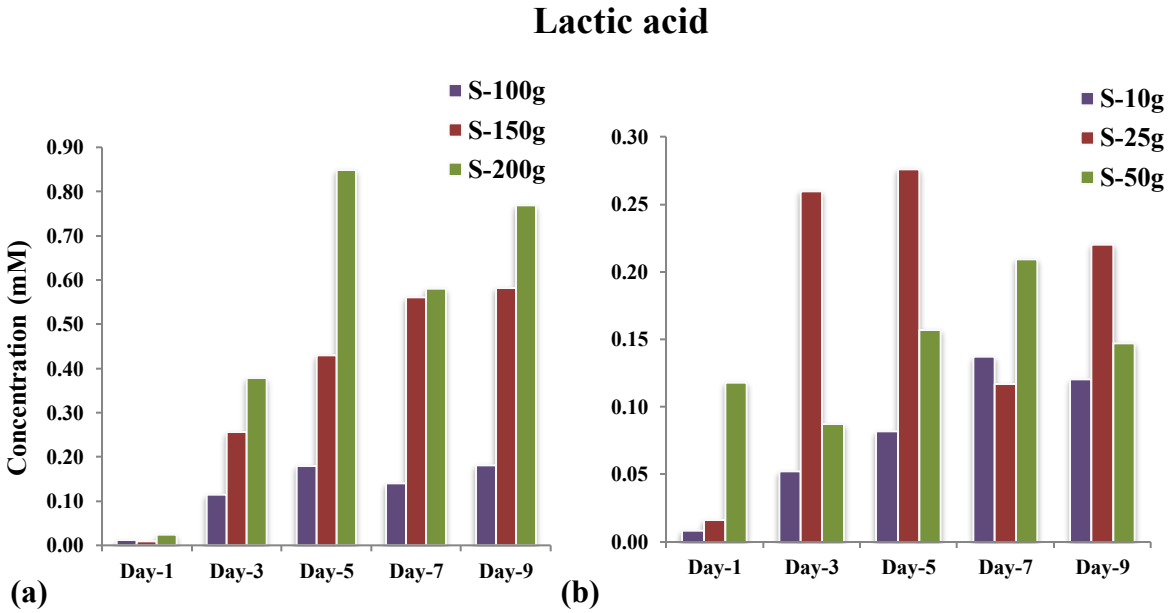


Figure 5.18 The amount of total LA produced over time in (a) Salt-based solution and (b) Sugar-based solution.

Similar to total VFAs, lactic acid produced was less in the sugar solution shown in **Figure 5.18 (b)**. The highest amount of LA (0.276mM) was produced on Day 5 with 25 g substrate loading. The low concentration of LA indicates that there was less growth of acid-producing bacteria in this process.

5.3.1.6.3 Change in ethanol produced

Figure 5.19 (a) & **(b)** presents the ethanol produced during the fermentation period for the salt-based and sugar-based solutions, respectively, with respect to different substrate loadings. During the fermentation process, for the salt-based solution, the ethanol produced in the solution with 100 g substrate loading increased from 0.005 mM on Day 1 up to 0.377 mM on Day 3, and then it started to decrease with longer retention time. In contrast to the higher level of TVFA and LA in the salt-based solution than in the sugar-based solution, the ethanol level was less in the salt-based solution and higher in sugar-based solution. For the sugar-based solution, the ethanol production increased from 0.009 mM on Day 1 with 10 g substrate loading up to 1.024 mM on Day 3, which increased further to 1.084 mM on Day 7 and then started to decrease. The

comparatively higher ethanol concentration in sugar-based solution is due to the ethanol fermentation of sugar and yeast (Tang et al. 2008).

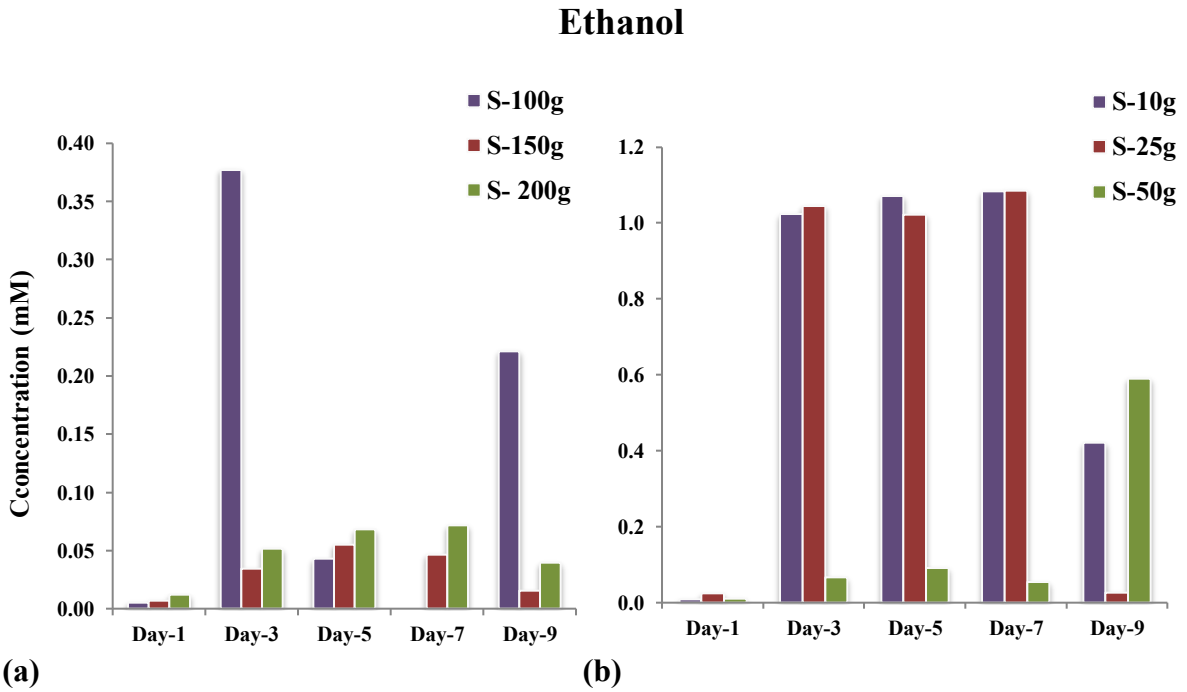


Figure 5.19 The amount of total ethanol produced over time in (a) Salt-based solution and (b) Sugar- based solution.

5.3.1.7 Microorganisms observed in the fermentation solutions

A diverse range of microorganisms have been isolated and identified by previous (general composting) studies at different stages of the fermentation process. In this study, *Lactobacillus plantarum* was isolated from the salt based solution and identified with a Biolog identification system. *Lactobacillus plantarum* is a rod shaped gram-positive bacteria, which is a member of the lactic acid bacteria (LAB) and commonly used in food fermentation. It is a facultative bacteria; capable to grow in both aerobic and anaerobic conditions. Similarly, yeast was isolated from the sugar-based solution and identified with light microscopy.

5.3.2 Optimizing the seeding inoculate(s)

5.3.2.1 The temperature profile

As discussed previously in **Section 5.2.2**, the temperature profiles of three different seeding inoculate matrices (SI-A, SI-B and SI-C) as listed in **Table 5.3** were investigated and are presented in **Figure 5.20**.

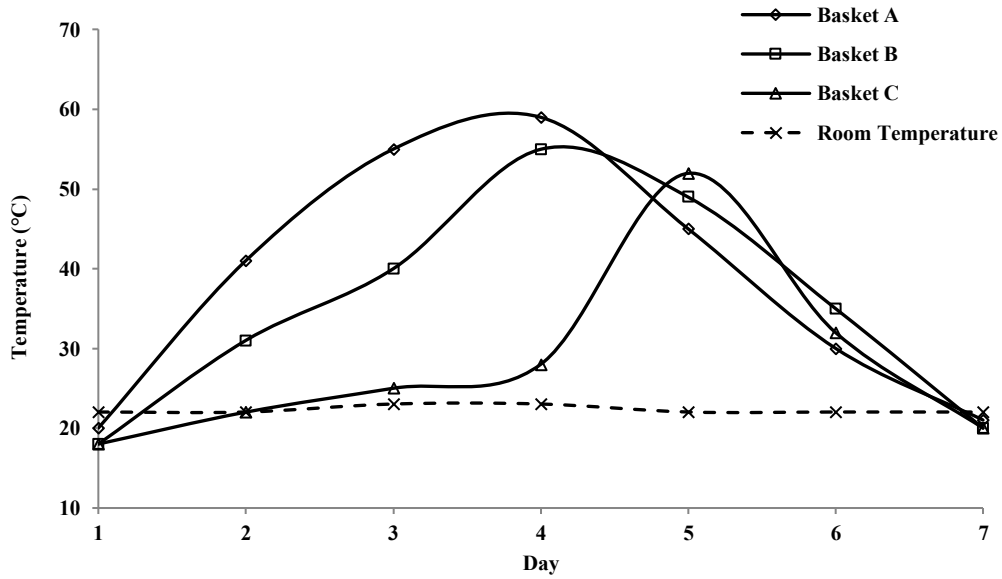


Figure 5.20 Temperature profiles of SI-A (Basket A), SI-B (Basket B) and SI-C (Basket C). The dotted line represents the measured ambient temperature on a given day. The mean ambient temperature in the 'shed' over the course of the experiments was 22.3 ± 0.2 °C.

The temperature of the SI-A matrix increased gradually from 20 °C on Day 1 and achieved the peak temperature of 60.5 °C after 3.5 days, which was ≥ 45 °C for almost 2.5 days. A temperature higher than 45 °C is considered to be the best temperature for the elimination of pathogens (Fornes et al. 2012). Then there was a gradual decline in temperature and thermophilic conditions were maintained until Day 5.8. After Day 6, the temperature stabilized at around ambient temperature. In terms of SI-B, the temperature increased gradually from 18 °C on Day 1 and achieved the peak temperature of 55 °C after 4.2 days. The SI-B matrix maintained a thermophilic temperature until Day 6, which was ≥ 45 °C for almost 2.1 days, and then stabilized at around ambient temperature on Day 7. For SI-C, the temperature increased from 18 °C on Day

1 and maintained thermophilic conditions almost until 6 days. All relevant parameters are summarized in **Table 5.7**. All 3 seeding inoculate matrices achieved a temperature higher than 45 °C and maintained thermophilic conditions for a time, with relative maximum temperatures of SI-A > SI-B > SI-C.

Table 5.7 Characteristic of temperature profile in seeding inoculate matrices.

	Basket A	**Basket B**	**Basket C**
Peak temperature achieved, °C	60.5	55	52
Time taken to achieve peak temperature, days	3.5	4.2	5
Time the matrix remained at ≥ 45 °C, days	2.5	2.1	1
Active period, days	6	6	6
Lag period, days	< 1	< 1	< 1

5.3.2.2 Fungal growth and odour analysis

The fungal growth in all three seeding inoculate matrices, namely SI-A, SI-B and SI-C on Day 5 of incubation, is presented qualitatively in **Figure 5.21 (a)**, **(b)** and **(c)**, respectively. The incubation of the seeding inoculate should be terminated once the white fluffy cotton like fungus grows on the surface of the pile, as depicted in **Figure 5.21**. In this study, it was observed on Day 5. Notably, the healthy looking fungal growth was visibly seen on SI-C, whereas in rest of the seeding inoculates the fungal growth was dense inside the pile though it was not visible as in SI-C. There was no grey/black fungal growth in the piles, which is an indication of no unwanted fungal growth. These seeding inoculate matrices produced a “sweet wine-like” odour during the incubation, which suggested a successful fermentation process. A foul rotten egg-like odour would suggest the failure of the process.

Figure 5.21 Fungus growth on seeding inoculates on day 5 (a) RH:RB 1:2 (SI-A) (b) RH:RB 1:1 (SI-B) (c) RH:WB 1:1 (SI-C). Photos taken by Anu Joshi.

5.3.3 Compost analysis

In general, the composting process is an intense biological activity facilitated by individual microorganisms at different stages (e.g. thermophilic microbes in the active phase and mesophilic microbes in the curing phase) and it is vital to provide an optimum environment for those microbes such as temperature, moisture, pH, oxygen and nutrients, as discussed previously in **Section 5.1.1**. The effect of these parameters on the composting process and the final compost product is discussed as follows.

5.3.3.1 The temperature profile

As discussed previously (**Section 5.1.1**), the temperature profile of a compost matrix is a major composting indicator, which provides information on the microbial degradation of the organic matter.

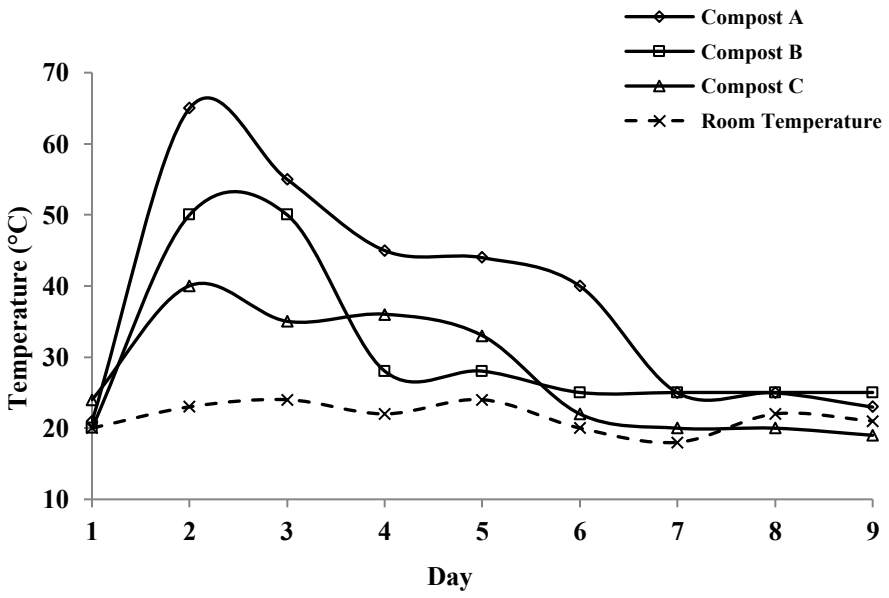


Figure 5.22 Temperature profile of Compost A, Compost B and Compost C. The dotted line represents the measured ambient temperature on a given day. The mean ambient temperature in the 'shed' over the course of the experiments was 21.6 ± 0.7 °C.

Figure 5.22 presents the change in temperature at different stages of the composting process for the three variants: Compost A (designated CA), Compost B (CB) and Compost C (CC). The

temperature of the CA matrix increased rapidly from 21 °C on Day 1 to 65 °C on Day 2 and maintained thermophilic conditions (≥ 45 °C) until Day 6, thus providing ideal conditions for thermophilic microbial growth. After Day 6, there was a gradual decline and after Day 7 the temperature stabilized at around ambient temperature. The peak temperature of 67 °C was achieved after 2.2 days and was ≥ 45 °C for almost 2.5 days, which is considered to be the best temperature for the elimination of pathogens from waste (Fornes et al. 2012). In terms of CB, the temperature increased from 20 °C to 50 °C on Day 2 and maintained thermophilic conditions until Day 4, and then stabilized at around ambient temperature. For CC, the temperature increased from 24 °C on Day 1 to 40 °C on Day 2 and maintained thermophilic condition until Day 4, and then the temperature decreased slowly. All relevant parameters are given in **Table 5.8**. Thus all three TCM compost matrices achieved peak temperatures within 2.5 days, with the maximum temperature of CA > CB > CC. Said-Pullicino et al. (2007) have suggested that the thermophilic condition in compost matrices is due to the heat released by the decomposition of organic matter by aerobic microorganisms. In terms of the three variants, CA achieved both the highest peak temperature (66.2 °C) and maintained a thermophilic temperature for a relatively longer period. This could be due to the comparatively high amount of nutrients provided by the relatively higher proportion of rice bran (as discussed above in **Section 5.3.2**).

Table 5.8 Characteristic of temperature profile in compost matrices.

	Compost A	**Compost B**	**Compost C**
Peak temperature achieved, °C	66.2	53	40.4
Time taken to achieve peak temperature, days	1.1	1.5	1.1
Time the compost matrix remained at ≥ 45 °C, days	2.5	1.5	0
Active period, days	6	3	5
Lag period, days	< 1	< 1	< 1

The temperature profiles observed in this study generally resemble those in other, non-TCM, composting studies and reflect standard bacterial growth curves (Goyal et al. 2005); in terms of

an increase in temperature towards a maximum followed by a decline towards ambient temperature. However, there are two important differences that are characteristic of TCM that are demonstrated in these experiments. Thus the lag time here is less than 1 day as compared to a minimum of 4 days for conventional composting. Also, in this method, the completion of biodegradation (composting) was relatively short, being from four to seven days, whereas for conventional composting it usually takes months (Said-Pullicino et al. 2007).

5.3.3.2 pH

In compost, both nutrient availability and microbial activity are influenced by pH (**Section 5.1**). **Figure 5.23** shows the pH profiles of the individual seeding inoculates, SI-A, SI-B & SI-C (**Section 5.2.2**) used to prepare the three different TCMs CA, CB& CC. The pH profiles of the common organic matter (OM) that is mixed with the seeding inoculates (SI) in order to prepare CA, CB& CC are also shown as are the time dependent pH profiles of the resulting composts CA, CB& CC. These data are benchmarked to the pH of Vermicompost (VC), as shown. **Figure 5.23 (a)** & **(b)** presents the same data from different perspectives with (a) comparing the data within an individual compost matrix over time and (b) with emphasizing the differences between the individual compost matrices.

The approximately neutral pH of the SIs and the acidic pH of the OM were recorded on Day 1, before the mixing of the two to form the composts. After mixing the OM with the SIs, all three compost variants attained an alkaline pH over the course of organic matter decomposition ranging from 7.43 - 8.76. On Day 7, the pHs of the three variants were almost equivalent (8.25 to 8.34). However, by Day 21, whereas CA and CC had become even more alkaline, the pH of CB decreased. On Day 35, when the composting process is considered to be complete, a clear pattern emerged whereby the pH of CA (8.5) > VC (8.2) > CB (7.7) > C.C (7.4). The overall increasing pH during the composting process is as expected and is due to the decomposition of organic matter in the compost matrix – organic-N-mineralization. Thus microorganisms decompose proteins, amino acids and peptides resulting in basic amine compounds such as ammonia being formed. The increased pH value in CA and CC until Day 21 suggests that the nitrification process was inhibited by then and nitrifying microbes started to act only afterwards, which was

much faster in case of CC than in CA and CB. The process being aerobic, it is assumed that the frequent aeration supports volatilization of pH raising ammonia, as found by Sundberg and Jönsson (2008) - up until Day 21. In terms of VC, the alkaline pH value could be due to a limited ammonia volatilization as aeration occurs in the system only via the burrowing activity of the earthworms.

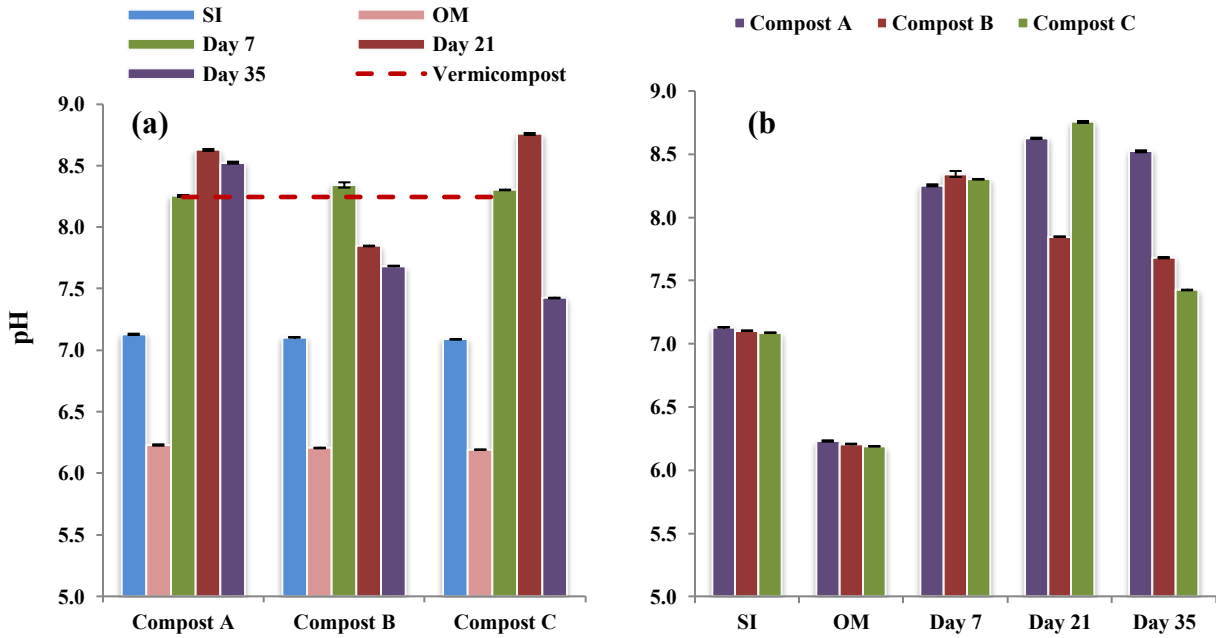


Figure 5.23 (a) pH in the different seeding innoculates (SI) and for the common organic matter (OM) for Composts A, B and C. The green histograms track the pH over the course of the composting period for Composts A, B and C. The reference pH for the vermicompost is represented by the dashed red line (b) Thus the pH values for the individual SIs and for the common OM that make up the three composts are compared in the first two sets of histograms. The remaining three sets of histograms compare the pH levels for composts A, B and C at different stages of the process. Error bars represent standard error, n = 3.

Different plant species require different levels of acidity/alkalinity though most plants prefer around neutral pH (Hoffmann 2010). In addition, pH affects nutrient availability. For example, in alkaline conditions, Ca and Mg react with phosphates and forms less soluble compounds. Similarly, in acidic condition, phosphates reacts with Al and Fe to form less soluble compound, which impedes the availability of nutrients (Jenson 2014). Thus, the various levels of pH attained by CA, CB and CC at maturity provides a basis for "tuning" the compost according to a particular plant's requirements - by selecting the desired seeding inoculate.

5.3.3.3 Conductivity

Figure 5.24 shows the conductivity profile of the individual seeding inoculates, SI-A, SI-B & SI-C (**Section 5.2.2**) used to prepare the three different TCMs CA, CB & CC. The conductivity profiles of the common organic matter (OM) that is mixed with the seeding inoculates (SI) in order to prepare CA, CB & CC are also shown as are the time dependent conductivity profiles of the resulting composts CA, CB & CC. These data are benchmarked to the conductivity of Vermicompost (VC), as shown. **Figure 5.24 (a) & (b)** presents the same data from different perspectives with (a) comparing the data within an individual compost matrix over time and (b) with emphasizing the differences between the individual compost matrices.

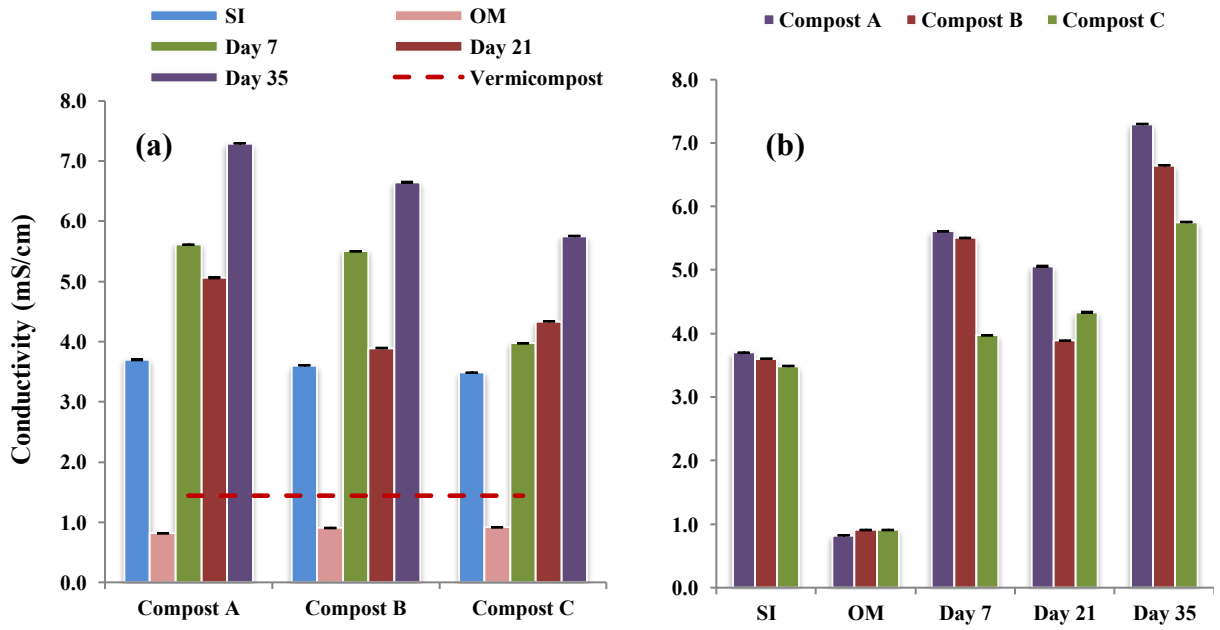


Figure 5.24 (a) Conductivity in the different seeding innoculates (SI) and for the common organic matter (OM) for Composts A, B and C. The green histograms track the conductivity over the course of the composting period for Composts A, B and C. The reference conductivity for the vermicompost is represented by the dashed red line (b) Thus the conductivity values for the individual SIs and for the common OM that make up the three composts are compared in the first two sets of histograms. The remaining three sets of histograms compare the conductivity levels for composts A, B and C at different stages of the process. Error bars represent standard errors, n = 3.

It is clear from this data that the conductivity of all compost matrices increased significantly on Day 7, then fluctuated somewhat up to Day 21 and appears to establish a pattern by Day 35

whereby CA > CB > CC > VC. Interestingly, for the TCM variants this order reflects the relative pH values at 35 days. This could suggest that the increase in conductivity is more related to those ions that have acid/base characteristics (e.g. carboxylates, NH_4^+ etc.). The higher value of nutrients and macronutrients present in CA (**Section 5.3.3.6**) also attribute to relatively higher value of conductivity. Also, nitrification of composts after Day 21 promotes the higher conductivity (Sánchez-Monedero et al. 2001). This data also suggests that a mineralization process was in progress until Day 35. The fluctuations in conductivity after the active phase suggest that mineralization processes are continuing into the curing process. The extent of this activity appears to be matrix composition-dependent. Vermicompost has a relatively low conductivity (1.4 mS/cm) compared to the other three composts (7.3, 6.7 and 5.8 mS/cm for CA, CB and CC respectively). The comparatively higher conductivity in CA might be due to more release of soluble salts by a higher microbial action. Nutrients provided by a seeding inoculate prepared with double the amount of rice bran than in CB might well lead to higher microbial activities. A similar explanation could be given for the relatively higher conductivity in CB than in CC. Different plants have different salt tolerances and a very high salt level in compost may be toxic to some plants (Thompson et al. 2001). Thus, again, the seeding inoculate can be selected accordingly to 'tune' the compost based upon its proposed use in a specific plant growth.

Figure 5.25 shows the TOC profiles of the individual seeding inoculates, SI-A, SI-B & SI-C (**Section 5.2.2**) used to prepare the three different TCMs CA, CB & CC. The TOC profiles of the common organic matter (OM) that is mixed with the seeding inoculates (SI) in order to prepare CA, CB & CC are also shown as are the time dependent TOC profiles of the resulting composts CA, CB & CC. These data are benchmarked to the TOC of Vermicompost (VC), as shown. **Figure 5.25 (a) & (b)** presents the same data from different perspectives with (a) comparing the data within an individual compost matrix over time and (b) with emphasizing the differences between the individual compost matrices.

5.3.3.4 Total organic carbon

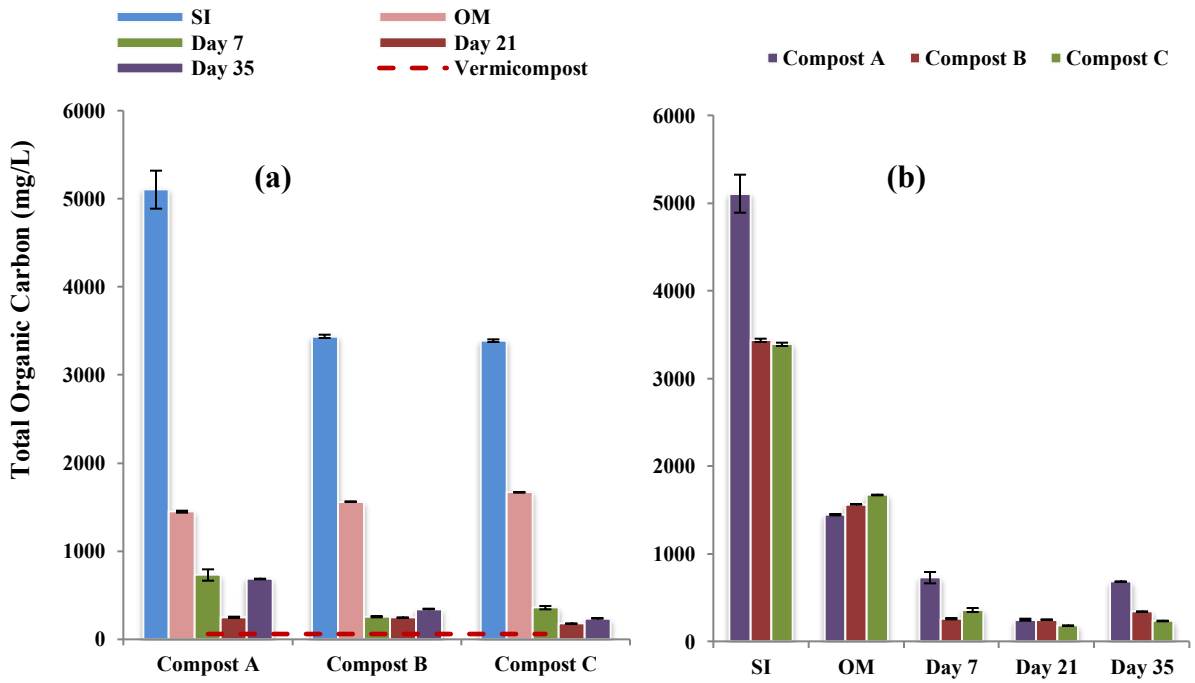


Figure 5.25 (a) Total organic carbon (TOC) in the different seeding innoculates (SI) and for the common organic matter (OM) for Composts A, B and C. The green histograms track the TOC over the course of the composting period for Composts A, B and C. The reference TOC for the vermicompost is represented by the dashed red line (b) Thus the TOC values for the individual SIs and for the common OM that make up the three composts are compared in the first two sets of histograms. The remaining three sets of histograms compare the TOC levels for composts A, B and C at different stages of the process. Error bars represent standard errors, n = 3.

A remarkable decrease in TOC was observed by Day 7 that decreased further until Day 21 and increased slightly up to Day 35. The large decrease up to Day 7 is due to the accelerated decomposition of organic matter over this period and is consistent with the temperature profile over the active period presented in **Figure 5.22**. The slight fluctuation in TOC later in the composting process (Day 21 to Day 35) may be attributed equilibrium between various reactions which increase or decrease the amount of dissolved organic matter. Notably, for matured vermicompost, the final TOC is comparatively less than that for all three TCM composts. The decrease in TOC throughout the composting process could be due to the organic carbon lost in the form of carbon dioxide, as the organic matter is decomposed and heat is generated.

5.3.3.5 Total Nitrogen

Nitrogen is one of the most important elements for plant growth. **Figure 5.26** shows the TN profile of the individual seeding inoculates, SI-A, SI-B & SI-C (**Section 5.2.2**) used to prepare the three different TCMs CA, CB & CC. The TN profiles of the common organic matter (OM) that is mixed with the seeding inoculates (SI) in order to prepare CA, CB & CC are also shown as are the time dependent TN profiles of the resulting composts CA, CB & CC. These data are benchmarked to the TN of Vermicompost (VC), as shown. **Figure 5.26 (a)** & **(b)** presents the same data from different perspectives with (a) comparing the data within an individual compost matrix over time and (b) with emphasizing the differences between the individual compost matrices.

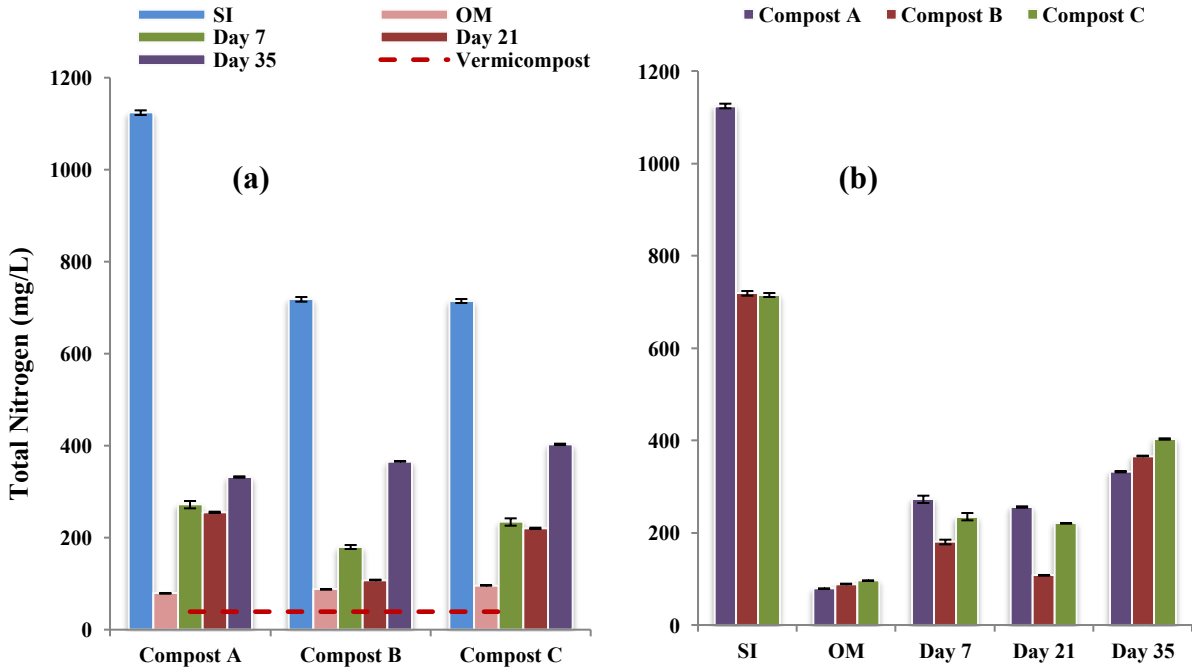


Figure 5.26 (a) Total nitrogen (TN) in the different seeding innoculates (SI) and for the common organic matter (OM) for Composts A, B and C. The green histograms track the TN over the course of the composting period for Composts A, B and C. The reference TN for the vermicompost is represented by the dashed red line (b) Thus the TN values for the individual SIs and for the common OM that make up the three composts are compared in the first two sets of histograms. The remaining three sets of histograms compare the TN levels for composts A, B and C at different stages of the process. Error bars represent standard errors, n = 3.

It is clear from the figure that the TN in seeding inoculate A is significantly higher than in seeding inoculate B and C. Regardless of the type of seeding inoculate, the total nitrogen decreased significatly in all three variants of compost on Day 7, once organic waste was mixed with these seeding inoculates. Interestingly, TN loss in CA was higher than in CB and CC, though TN in SI-A was comparatively very high on Day 35. Moreover, there was an increase in TN after Day 21.

Nitrogen undergoes various transformation during composting. As discussed earlier in **Section 5.1.1.2**, less value of TN on Day 7 and further decrease upto Day 21 could be due to the production of complex compounds by the microbial action on amino acids and proteins. As a result of microbial activity during the thermophilic phase, which is until Day 7, nitrogen in the form of (NH_3-N, NH_4^+-N) gets progressively included into humic substances with aromatic structures. At the later mesophilic phase, the NH_4-N content, after an initial increase, starts to decline as a result of its eventual volatilization and oxidation into NO_3-N, NO_2-N. Similarly, nitrogen loss might be due to the volatilization of ammonia (NH_3) in the atmosphere with mixing (Fourti 2013). The comparatively low value of TN in VC can be explained as TN loss through lechate, which is restricted in case of TCM as its caried out in closed system.

5.3.3.6 Nutrients, macronutrients, micronutrients and trace metals in the compost(s)

N, P and K, usually referred to as the major nutrients or the NPK value in composting, are essential nutrients which are utilized by plants for growth. Macronutrients, micronutrients and trace metals (usually reffered as heavy metals), listed in **Table 5.9**, are required for plant growth but could have phytotoxic effect if present in too higher quantities. The availability of these elements varies in finished compost depending upon the variation in the nature of the compost feedstock (U S Composting Council 2012; Washington State University 2014).

Of the three innoculates employed in these studies, Innoculate A was selected as a representative example for nutrient anlaysis, including NPK. Thus the NPK analysis along with macronutrients, micronutrients and trace metals for this innoculate is given in **Table 5.9**.

Table 5.9 Nutrients, macronutrients, micronutrients and trace metals concentration in Seeding Inoculate A (SI-A).

Nutrients		Macronutrients		Micronutrients		Trace metals	
Element	Quantity (mg/L)	Element	Quantity (mg/L)	Element	Quantity (mg/L)	Element	Quantity (mg/L)
N	1124.5	Ca	2700.0	Al	15.0	Cd	< 0.1
P	650.0	Mg	340.0	Cu	1.5	Cr	< 0.1
K	1600.0	Na	295.0	Fe	21.5	Co	< 1.0
		Ba	2.5	Mn	30.0	Mo	< 1.0
		S	265.0	Zn	5.0	Ni	< 0.1

Table 5.9 is consistent with the expectation that nutrients > macronutrients > micronutrient > trace metals (with the exception of calcium which has the highest absolute level). The abundance of NPK suggests that SI-A is rich in nutrients and the low value of trace metals in the SI-A suggests that SI-A do not contribute any trace metal in the composting process. Here, SI-A was investigated for the elements in **Table 5.9** with a view to comparing these data with those in CA. It was assumed that the comparison helps to understand the transformation of these elements in the composting process. The comparison was limited to SI-A and CA only. The comparison between SI-B & CB and SI-C & CC was beyond the scope of this study.

Figure 5.27 (a) presents the NPK nutrients available in all three TCM variants on Day 35 and for VC, considered as matured compost i.e, finished product in this study. Nitrogen content was found to be too low in VC, only 40 mg/L, compared to CC (403 mg/L), CB (366 mg/L) and CA (333 mg/L). Similarly, phosphorous content was comparatively higher in all three TCM variants than in VC; CA > CB > CC > VC. The lower values of N and P in VC could be due to the loss of a significant volume of lechate through vermiwash[93], which is restricted in TCM. In regard to potassium, except in CC, other two TCM variants and VC contained relatively higher concentration of K. The NPK ratio derived for CA, CB, CC and VC are 1.0:4.35:9.91, 1.0:3.28:9.15, 1.0:2.48:3.23 and 1.0:4.13:50.0 respectively. It is interesting to observe that CC

[93]Vermibed is regularly flushed with water to keep it moist for providing a cool and moist environment for the worms.

has exceptional nurient content among TCM variants (slightly higher N and significantly low P & K).

Figure 5.27 (b) presents the concentration of macronutrients, such as calcium (Ca), magnesium (Mg), sodium (Na), barium (Ba), sulphur (S), content in all three TCM variants on Day 35 and for VC. Ca was the most abundant macronutrient found amongst compost variants, except in CC. However, B was not detected on any of the compost variants. Interestingly, the concentration of Ca was relatively low in CC and the concentration of Na was relatively high in CB.

Figure 5.27 (c) presents the concentration of micronutrients, such as copper (Cu), manganese (Mn), iron (Fe), zinc (Zn), content in all three TCM variants on Day 35 and for VC. The significantly high concentration of Fe in VC than in TCM, is an interesting observation. Similar to nutrients and macronutrients, the concentration of micronutrients was found to be relatively less in CC among TCM. In terms of trace metals such as cadmium (Cd), chromium (Cr), cobalt (Co), molybdenum (Mo), nickel (Ni), these elements could not be detected for all compost variants using ICP-OES.

The findings of the study on nutrients, macronutrients, micronutrients and trace metals in all three TCM variants on Day 35 and for VC, show that the nutrient content in VC, with few exceptions, is less than in TCM. ForTCM itself, the nutrient content is higher in CA > CB > CC.

While comparing NPK in SI-A (seeding inoculate used to prepare CA, **Section 5.2.2**) and CA, the concentration of N decreased and the concentration of P and K increased significantly in CA. In terms of Ca, Mg, Na, Ba and S, there was a significant increase in concentration except for a slight increase in Ba. Only a minor increase was observed for Mn, Cu, Fe and Zn. These quantitative observations suggest that the mixed organic matter has contributed a significant quantity of nutrients in CA, whereas it did not significantly contribute any trace metals.

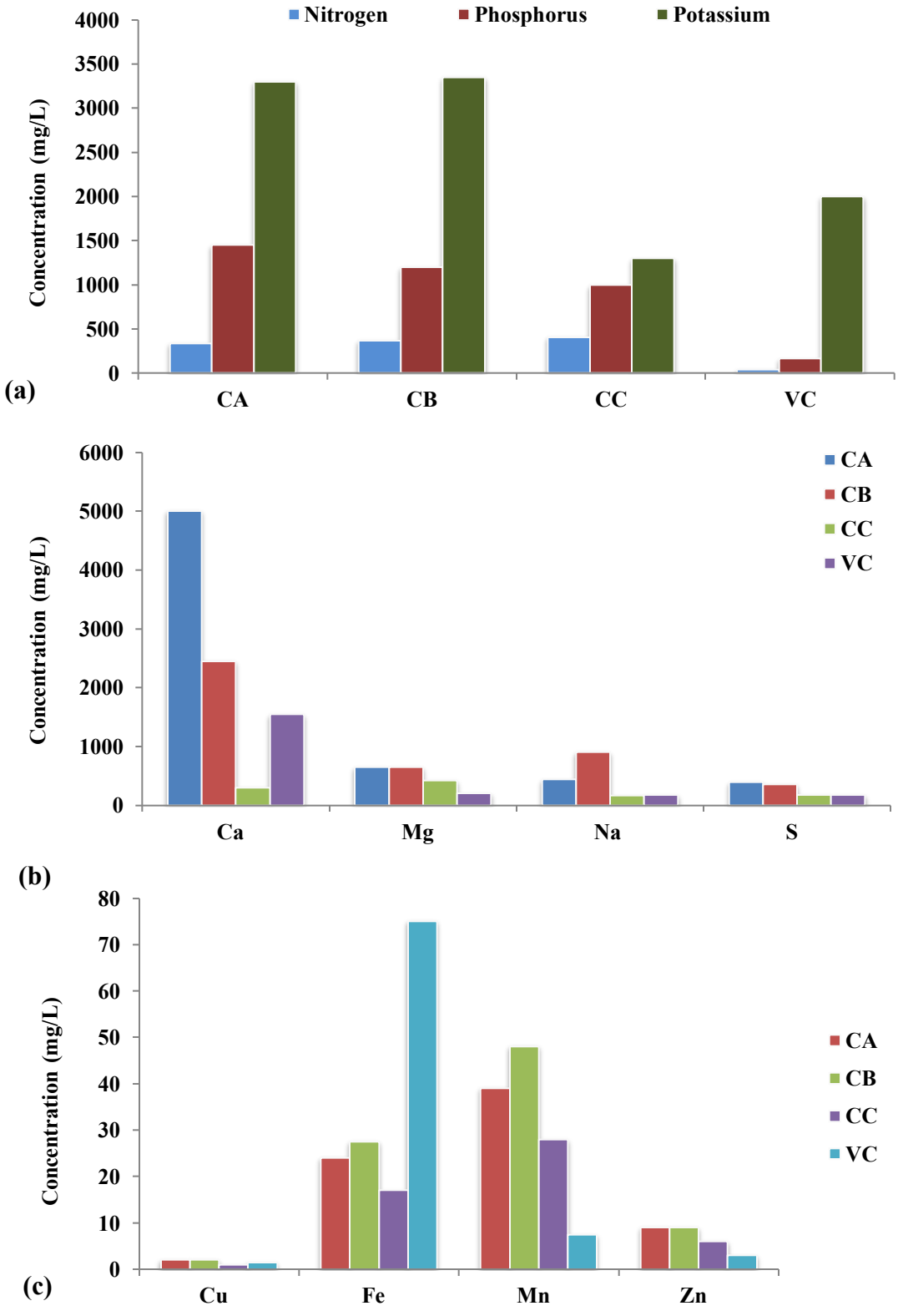


Figure 5.27 (a) Nutrient (NPK) (b) Macronutrient and (c) Micronutrient concentration in matured (Day 35) CA, CB, CC and VC.

5.3.3.7 Compost maturity tests

Compost maturity tests are generally based on the assessment of changes in organic matter compostition during the course of the composting process up until its stabilization (Ouatmane et al. 2000). In this regard, various methods have been proposed for compost maturity testing (Goyal et al. 2005; Jouraiphy et al. 2005; Said-Pullicino et al. 2007;Provenzano et al. 2014). As suggested by Kuo et al.(2004), C:N ratio should be used along with at least one other maturity test. Here, all four methods (**Section 5.2.5.4**); namely: the Germination Percentage (GP), the plant bioassay, the C:N ratio and FTIR spectroscopy, have been adopted.

5.3.3.7.1 Carbon to nitrogen ratio

The C:N ratio of the materials to be composted and of the final compost is a major parameter that characterizes the composting process as well as the final quality of the compost (Handreck 1986; Fricke and Vogtmann 1994). During the composting process, carbon is converted into carbon dioxide and humic substances, whereas nitrogen is converted into ammonia, nitrite or nitrate. In these transformation processes, when the carbon loss is greater than the nitrogen loss, it results in reduction in the C:N ratio. Thus the change in C:N ratio is used as a significant indicator of compost maturity and stability (Fourti 2013) and, as such, it has also been used in this study. Generally in a compost pile, a C:N ratio in the range of 25 - 30 is considered ideal for microbial activity. Higher ratios diminish the microbial activity while a low ratio is associated with nitrogen loss as ammonia. The C:N ratio of the final compost is dependent on the initial raw material used and the composting technique itself. Out of the many optimum values suggested in the literature, we have adopted the theoretical value of ~ 10 that is reflective of humic substances (Cheng et al. 2013).

Figure 5.28 shows the C:N ratios of the individual seeding inoculates, SI-A, SI-B & SI-C (**Section 5.2.2**) used to prepare the three different TCMs CA, CB & CC. The C:N ratios of the common organic matter (OM) that is mixed with the seeding inoculates (SI) in order to prepare CA, CB & CC are also shown as the time dependent C:N profiles of the resulting composts CA, CB & CC. These data are benchmarked to the C:N ratio of Vermicompost (VC), as shown.

Figure 5.28 (a) & **(b)** presents the same data from different perspectives with (a) comparing the data within an individual compost matrix over time and (b) with emphasizing the differences between the individual compost matrices.

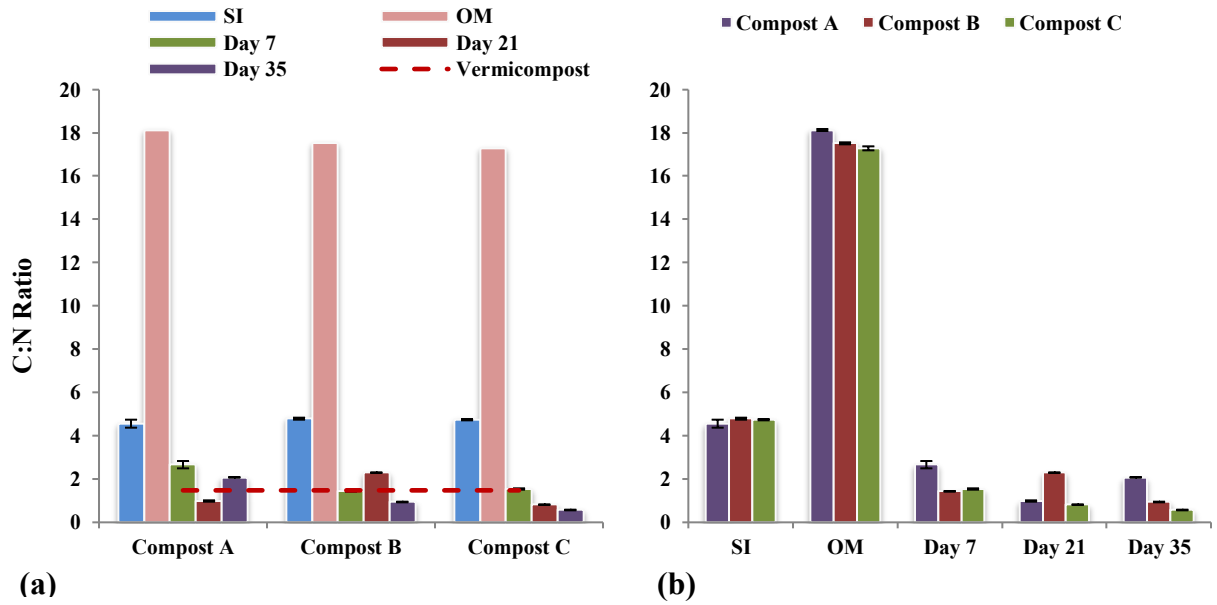


Figure 5.28 (a) Carbon to nitrogen (C:N) ratio in the different seeding innoculates (SI) and for the common organic matter (OM) for Composts A, B and C. The green histograms track the C:N ratio over the course of the composting period for Composts A, B and C. The reference C:N ratio for the vermicompost is represented by the dashed red line (b) Thus the C:N ratio values for the individual SIs and for the common OM that make up the three composts are compared in the first two sets of histograms. The remaining three sets of histograms compare the C:N ratio levels for composts A, B and C at different stages of the process. Error bars represent standard errors, n = 3.

The initial C:N ratio for SIs ranged from 4.5 - 4.7 and from 17.3 - 18.1 for OM, which are less than the expected range of 25 - 30 for a start-up compost (Handreck 1986; Rynk 1992). Of the four different compost matrices discussed here, the C:N ratio is CA > CB > CC > VC at Day 35 for TCM (well-matured) and for matured VC. The final C:N ratios can be seen to differ based on the different SIs used. As the composting process progresses in the TCM composts, the final C:N ratio is significanly reduced after 35 days; however, there is some fluctuation for CA and CB along the way. On Day 35, the the trend of the C:N ratio for all three compost matrices (that differ from each other based on the different SIs used) follow a similar trend to the TOC but

follow an opposite trend to TN, which reflects the fact a higher value of nitrogen and a lower value of TOC gives a lower C:N ratio.

Chanyasak and Kubota (1981) proposed the C:N ratio of 5 - 6 as the desired maturity index for compost prepared with different materials. However, different values are suggested elsewhere (Ouatmane 2000; Cabañas-Vargas et al. 2005). The values achieved here for the TCM are considerably less than the expected value of ~ 10 and are less than for vermicompost with respect to CB and CC. Therefore, other tests to assess the quality of the TCM have been conducted as follows.

5.3.3.7.2 The Germination Percentage

Figure 5.29 depicts the GPs determined for radish seeds planted in GS (garden soil control), CA, CB, CC and VC, as described in **Section 5.2.5.4**, and the time taken to germinate the seeds.

It was observed that seeds planted in VC germinated faster than in the TCM composts and the garden soil (control). Thus, the relative times taken for the germination of the seeds was recorded as VC (< 72 hours), control (< 96 hours), CC and CB (< 120 hours). The germination percentage calculated using equation (5.1), **Section 5.2.5.4**, showed that control (GS), VC and CC has similar GP of 80 % however CB has only 60 % GP. In contrast, the seeds did not germinate at all in CA, which was verified with three replicates.

For a further investigation of the germination issue in CA, two approaches were taken. In the first approach, radish seeds were geminated in the garden soil (GS) and then transfered/planted in CA. In the second approach, CA was mixed with GS in 1:1 v/v and radish seeds were sown to observe the germination process. In the former case, plant growth was not satisfactory, though it didn't completely die off. In the later case, the seeds germminated in < 96 hours with 80 % GP, similar to the use of GS itself.

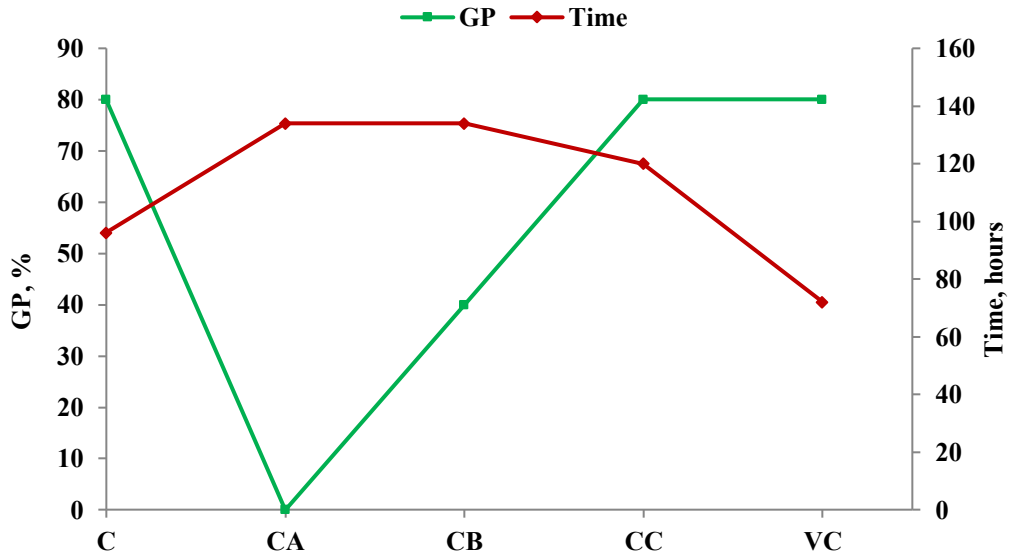


Figure 5.29 Seed Germination Percentage Vs. time taken to geminate seed for garden soil as Control (C), CA, CB, CC & VC.

5.3.3.7.3 The Plant Bioassay

To investigate the effect of nutrient availability on plant growth for different composts, observations were made of the relative plant growth (using radish seed, **Section 5.2.5.4**) for six different types of matrices, namely GS, CA, CB, CC, VC & CA:GS, over a four week period, as expressed in terms of plant height and leaf number (Khan and Fouzia 2011). The results are presented in **Table 5.10**.

Figure 5.30.a (i) & **(ii)** illustrates the average height of the plants grown in GS, CA, CB, CC, VC and CA:GS over a time period of four weeks, from two different perspectives - in order to track and compare the plant height growth rate over time. It is clear from the presented figures that the average plant height was higher in GS, CA:GS and VC rather than in TCM by Week 4. However, the height was relatively higher in CA:GS until Week 2. In terms of TCM, the relative heights achieved were CC > CA > CB. **Figure 5.30.b (i)** & **(ii)** presents the differential and double differential plot for the data presented in **Figure 5.30 (a)**, respectively. It clearly shows two distinct profiles of plant growth rate in terms of average plant height. Those compost matrices in the first category, namely CA, CC and CA:GS, have earlier maximum growth and level off at a

later stage. Those in the second category, namely VC, GS and CB give maximum growth only at a later stage.

Table 5.10 The average height and the average number of leaves of radish plant in different composts and control (GS). Data reported for CA:GS is the mixtuer of 1:1 v/v GS & CA, as discussed in GP section.

Time period	Measured	Control (Garden soil)	Compost A*	Compost A: Garden soil 1:1 v/v	Compost B	Compost C	Vermicompost
Week 1	**Height (cm)**	2.0	2.3	2.8	1.8	1.9	2.7
	No. of leaves	2.0	2.0	2.0	2.0	2.0	2.0
Week 2	**Height (cm)**	3.4	4.9	5.5	2.9	4.0	3.6
	No. of leaves	2.0	2.3	4.0	4.0	3.3	3.7
Week 3	**Height (cm)**	6.5	5.3	6.4	4.9	4.6	6.1
	No. of leaves	4.0	3.3	5.3	4.7	5.3	5.0
Week 4	**Height (cm)**	6.8	5.5	6.8	5.3	5.7	6.6
	No. of leaves	4.0	4.0	6.0	5.3	6.3	6.0

* Data reported for CA is for radish seeds germinated in garden soil and planted in Compost A.

Figure 5.31.a (i) & **(ii)** illustrates the average number of leaves of the plants in GS, CA, CB, CC,VC and CA:GS over a time period of four weeks, from two different perspectives - in order to track and compare the plant leaf growth rate over time. In Week 1, plants in all six matrices had 2 leaves each, which grew faster in CA:GS and CB on Week 2 - and at the end of the Week 4, CC achieved the highest number of leaves. **Figure 5.31 (b)** presents the differential and double differential plots for the data presented in **Figure 5.31 (a)**, respectively. It clearly shows two distinct profiles of the plant growth rate in terms of average number of leaves. Those compost matrices in the first category, namely VC, CB and CA:GS start earlier maximum leaf production and level off at a later stage. Those in the second category, namely GS, CA and CC, start leaf production initially, drop down and give maximum leaf production at a later stage.

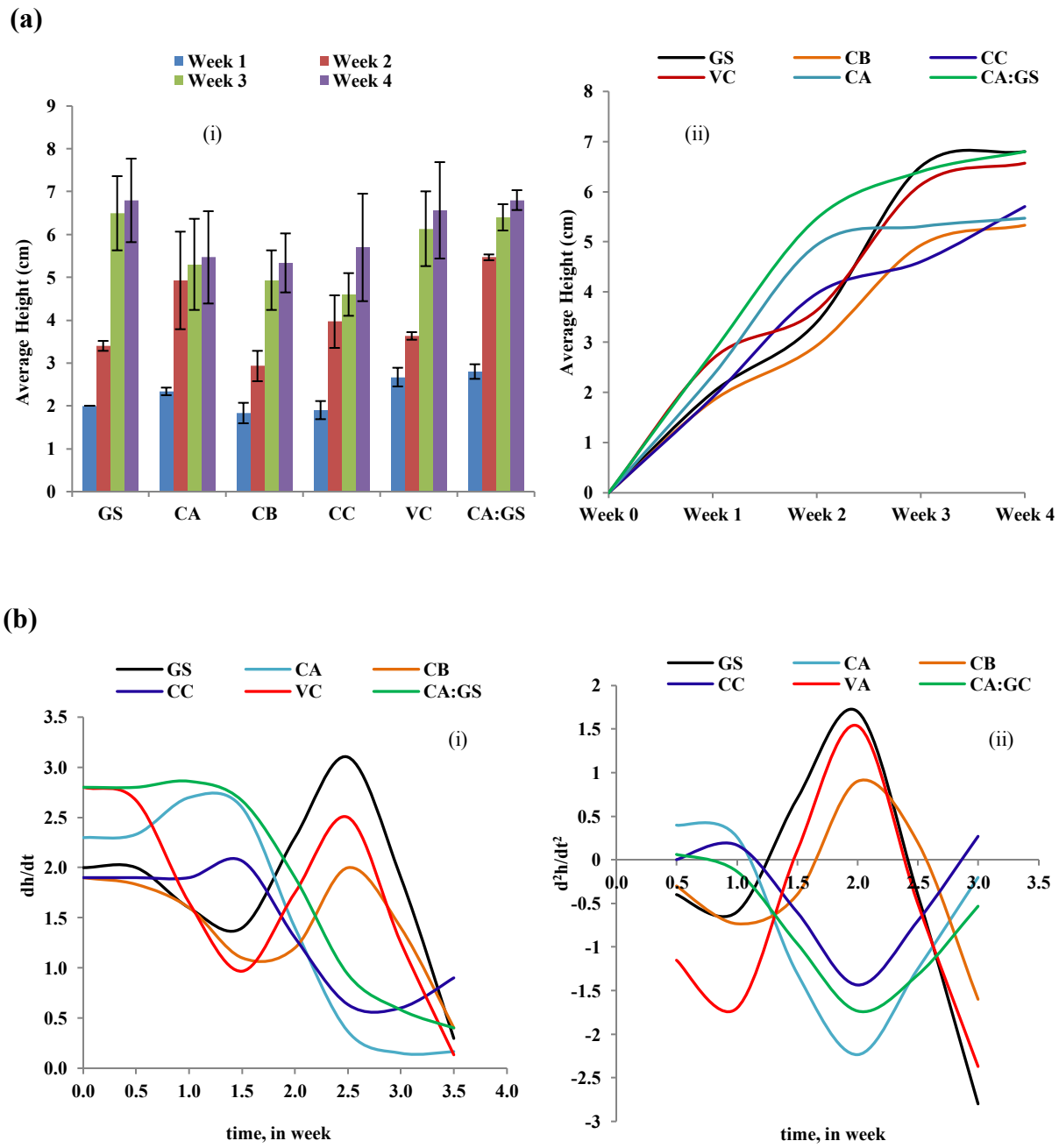


Figure 5.30 a.(i) The average height of the radish plants over time in GS, CA, CB, CC, VC & CA:GS. The error bars represents standard error with n = 3. (ii) The same data presented with different perspective to compare the plant average height growth rate **b**.(i) Differential plot and (ii) Double differential plot, for data presented in (a).

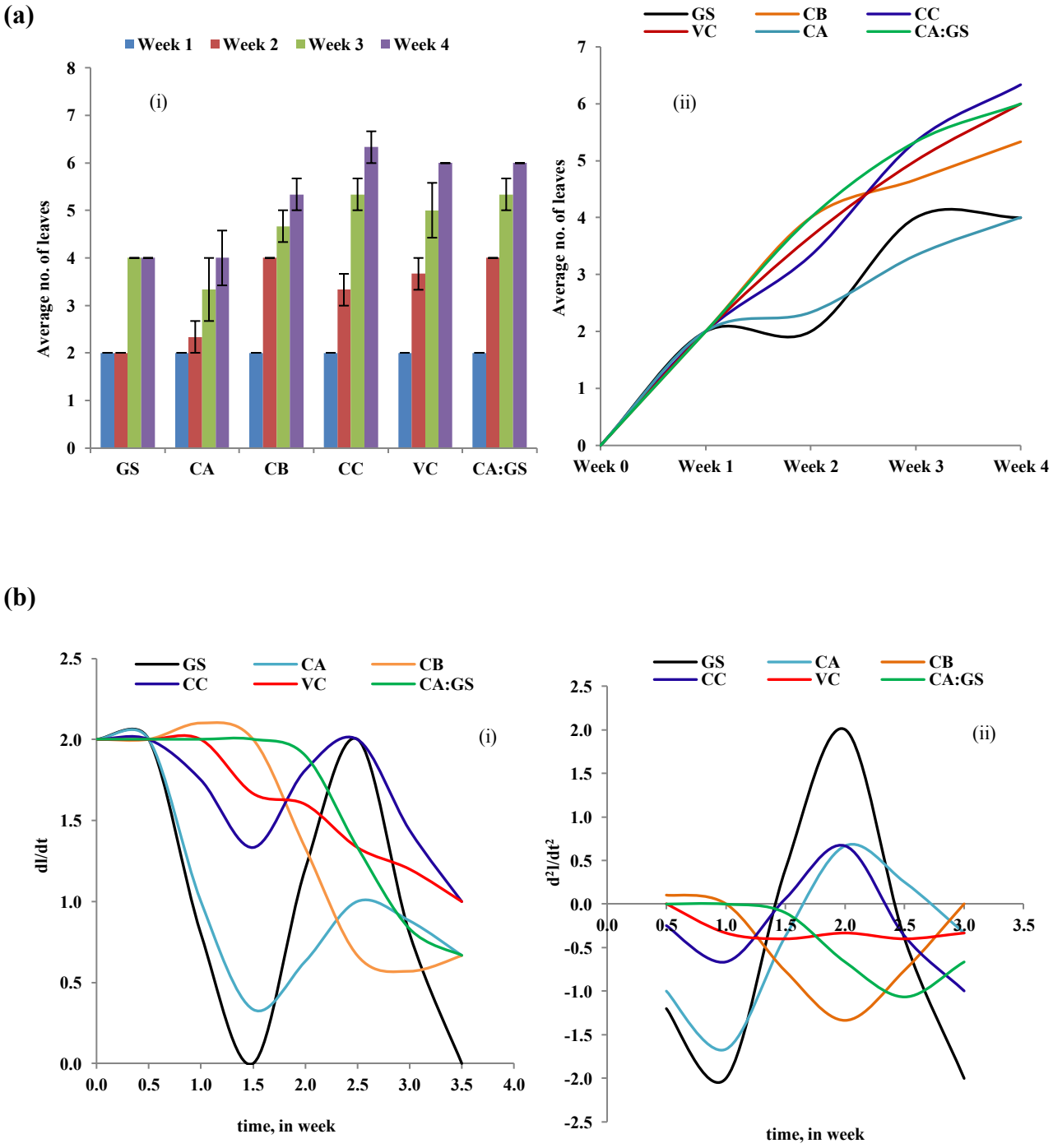


Figure 5.31 a.(i) The average number of leaves of the radish plants over time GS, CA, CB, CC, VC & CA:GS. The error bars represent standard errors with n = 3. (ii) The same data presented with different perspective to compare the plant average leaves growth rate **b**.(i) Differential plot and (ii) Double differential plot, for data presented on (a).

Thus, the above data give two distinct categories for the plant growth rate for the both average height and the average number of leaves. The variation in the plant growth rate with respect to different TCM matrices are due to the variation in the seeding inoculate used to prepare TCM. At the meantime, both VC and GS shows growth profiles different to the TCM variants. This shows that for TCM, the compost can be tuned, based on the specific plant requirements, by selecting the preferred SIs. The desirable plant growth rate for this study is considered as the most number of leaves produced in the least time and with the smallest height. While we consider Week 4 as the minimum time required to assess the plant growth, it seems that the plant grown in CC best fits the criteria to get the desired plant growth. **Figures 5.32.c (iii)** and **Figure 5.33** reflects the qualitative observations made during this study. The observation shows a better plant growth in CC with a preferred average height of the plant and average number of leaves..

To define a "healthy plant growth", the concept of a "bushiness index" has been introduced by incorporating both the average height and the average leaf production.

In terms of CA, the above observation suggests that plants did not germinate and plant seedlings could not grow well in CA by itself, however the plant growth in CA:GS was as prominent as in VC itself. The relatively high nutrient content in CA (**Section 5.3.3.6**) might have a phytotoxic effect on seed germination and seedling growth. In addition, the abundance of volatile organic acids and soluble salts in the compost matrix due to immaturity, could also have a phytotoxic effect (Kuo et al. 2004; Ko et al. 2008). However, an enhanced plant growth in CA:GS, as dipicted in **Figure 5.34**, suggests that the dilution of the high nutrient content CA with soil, impart nutrients to the soil and provides just the right nutrient content for plant growth. The realtively better plant growth in CC, than in other TCM variants, supports the argument that the nutrient available in CC is more favourable for plant growth than in other compsot matrices.

Figure 5.32 (a) Radish seed sown in pots with VC, CA, CB and CC (b) Germination in VC and CC (c) Plant growth in (i) VC, (ii) CB and (iii) CC. Photos by Anu Joshi.

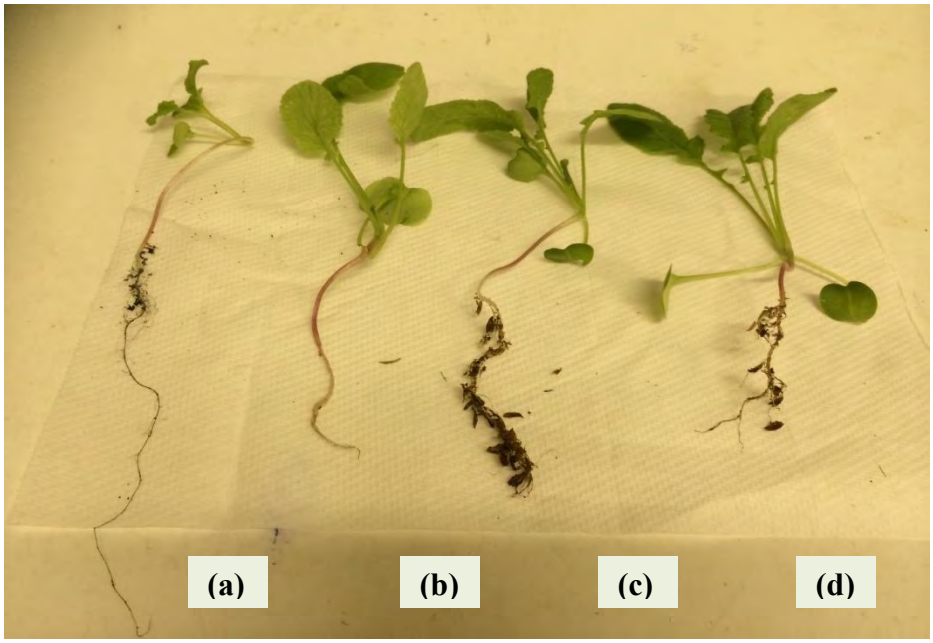


Figure 5.33 Plants with root grown in (a) GS (control), (b) VC, (c) CB and (d) CC. Photo by Anu Joshi.

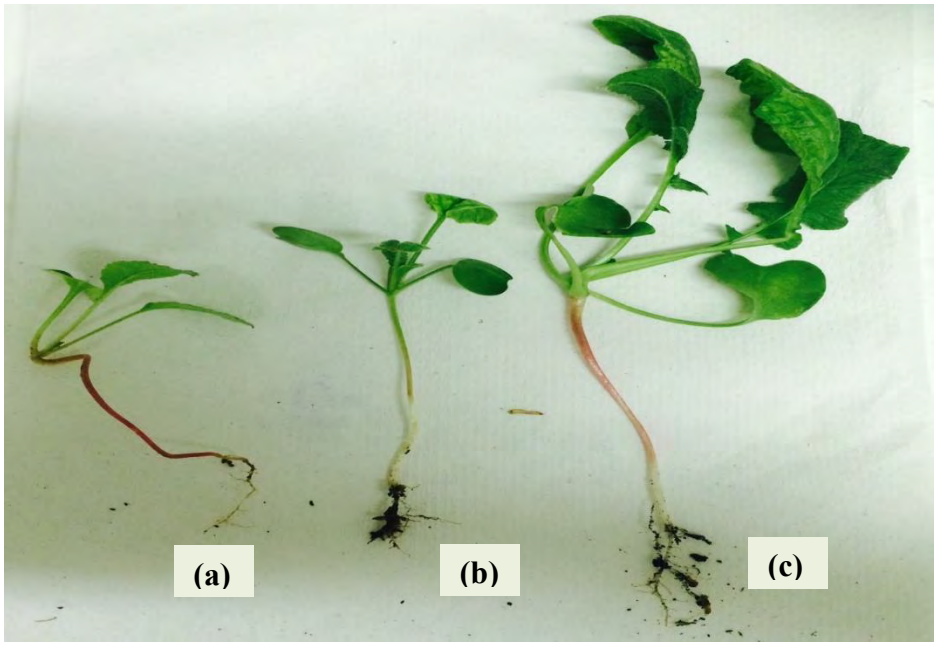


Figure 5.34 Plants with root grown in (a) CA (b) GS (control) and (c) CA:GS. Photo by Anu Joshi.

5.3.3.7.4 The Bushiness Index (BI)

The "Bushiness Index", BI, is defined as the ratio of the average number of leaves to the average plant height. The higher the value of BI, the bushier the plant. A bushier plant has the appearance of being healthier and more substantial.

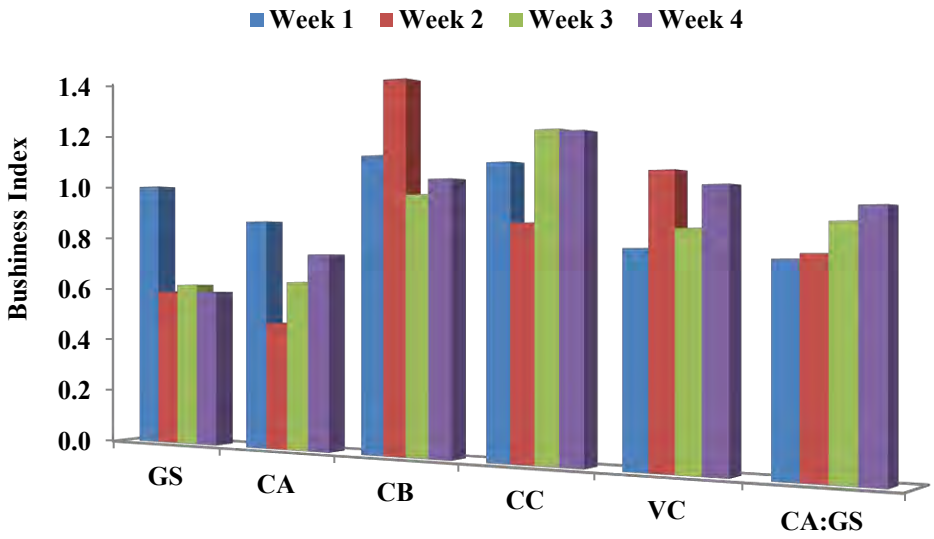


Figure 5.35 Relative Bushiness Indices as a function of time for GS, CA, CB, CC, VC and CA: GS.

Figure 5.35 presents the 'Bushiness Index' (BI) for GS, GS,CA, CB, CC, VC and CA: GS, for the respective compost matrices. It is clear from the figure that the plant grown on CB was the bushiest on Week 2. However, at the end of the observation i.e. on Week 4, plant grown on CC was the most bushiest. A qualitative observation on the plant growth for the various compost matrices suggests that the TCM, particularly CB and CC promotes a bushier plant.

5.3.3.7.5 Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analysis of compost over time provides maturity information (Ouatmane 2000). **Figure 5.35 (a)** presents the FTIR spectra of TCM Compost A (CA) over time; i.e. on Day 7 (CA D-7),

Day 21 (CA D-21) and Day 35 (CA D-35). **Figure 5.35 (b)** presents a comparative FTIR spectra for CA on Day 35 and vermicompost (VC) - assuming both to be matured. For both **Figure 5.35 (a)** & **(b)** most of the major peaks were around the same wave number - suggesting there was no significant qualitative change in these compost matrices. However, there was a variation in the relative intensity of the peaks, as summarized in **Figure 5.37**, which reflects the expected degradation of certain compounds during the composting process (Carballo et al. 2008).

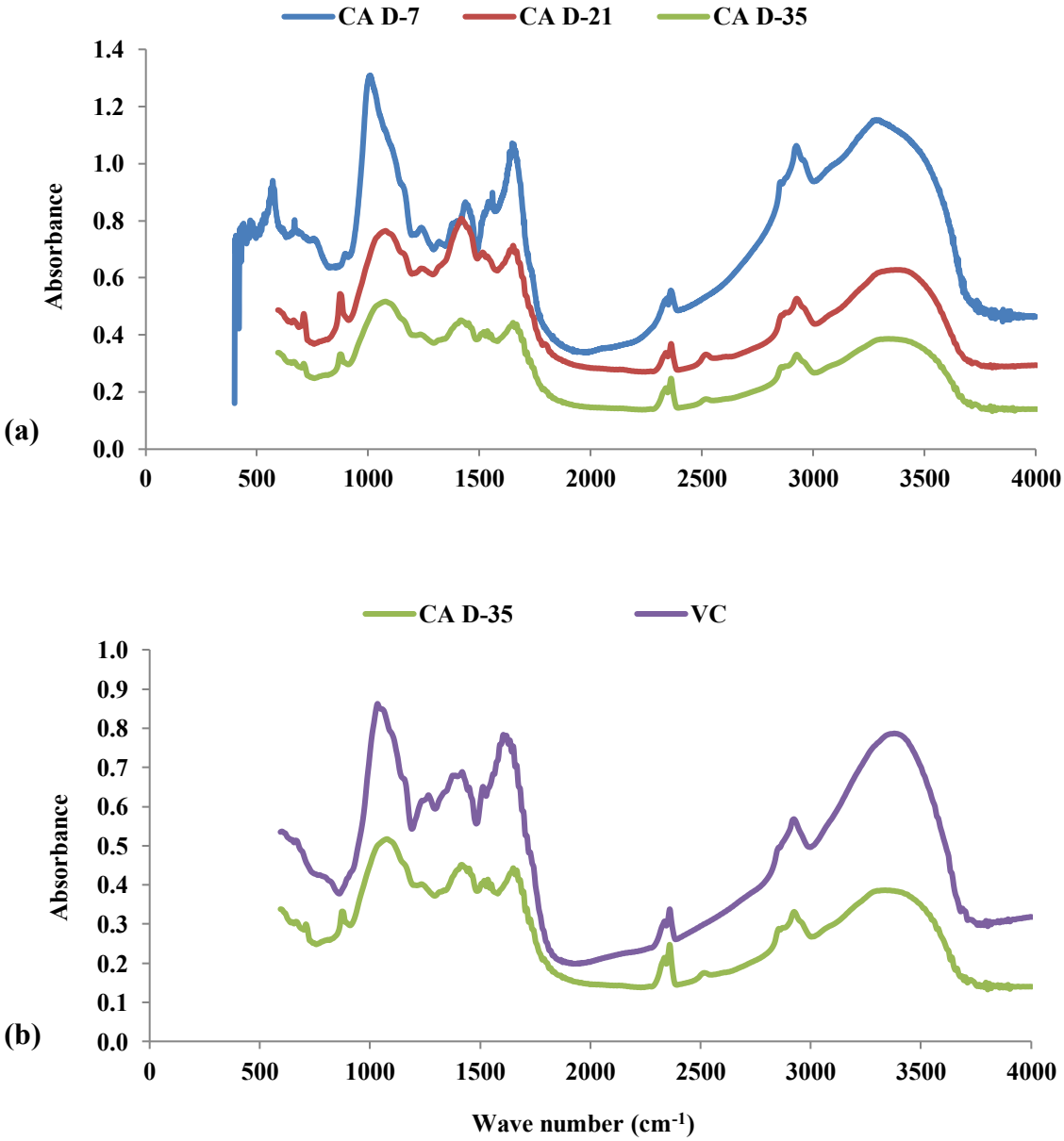


Figure 5.36 FTIR spectra observed for (a) CA on Day 7, Day 21 and Day 35 (b) Comparative spectra for matured CA and VC. The original spectra are provided in **Appendix 5.3.**

Figure 5.36 (a) shows that as the compost approached towards maturity, more uniform and smooth spectra were recorded than those on premature compost, and the recorded spectra become more similar over time. The infrared spectra reflect the biodegradation of dissolved organic matter during the composting process, characteristic of a homogeneous mixture of minerals and humic matter (Ouatmane 2000; Sanmanee et al. 2010).

The original FTIR spectra obtained by using DRIFT FTIR method as described in **Section 5.2.5.4** are provided in **Appendix 5.4**.

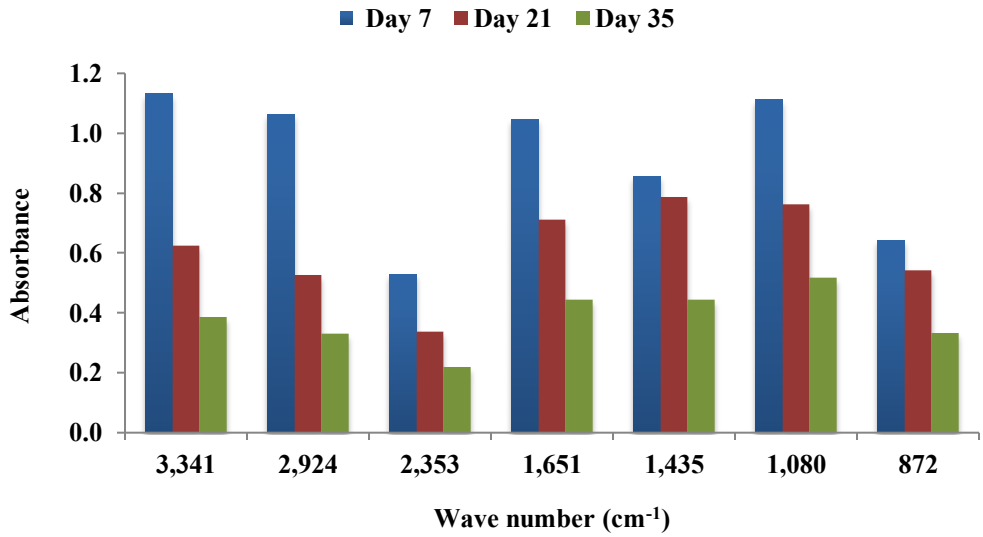


Figure 5.37 FTIR relative intensities obtained for CA on Day 7, Day 21 and Day 35 in relation to compost maturity assessment. The assignments of the relevant absorbance are as follows (in cm^{-1}): 3341 - hydroxyl group, alcohols and carboxylic functions, amides and amines; 2924 - aliphatic methylene group; 2353 - alkynes; 1651 - amide I, carboxylates, aromatic ring modes, bonded conjugated ketones, quinones, carboxylic acid, esters; 1435 - bending frequencies of carboxylic acids, carboxylates and the aliphatic CH_2 group of alkanes, carbonates; 1080 - polysaccharides or polysaccharide-like substances, aromatic ether and carbohydrates; 872 - primary amine group.

It may be observed from **Figure 5.37** that all of the significant intensities decrease over the maturation time for the TCM CA system. This is a characteristic found in all composting systems, including VC. Thus it may be concluded that in terms of the compounds present and their behaviour during a composting process, the TCM behaves in a similar manner to other methods including VC. However, the advantage of TCM is that the point of maturity is reached

relatively sooner. Thus these experiments demonstrate that, even though the TCM accelerates the relative maturation time, the chemical processes necessary for this to occur are not compromised.

The main absorbance bands of relevance for compost matrices are presented in **Table 5.11**. The interpretation of the compost spectra was based on Chefetz et al. (1998), Coates (2000), Ouatmane et al. (2000), Jouraiphy et al. (2005), Said-Pullicino et al. (2007), Carballo et al. (2008), Pavia (2009) and Li et al. (2011).

Table 5.11 FTIR absorbance bands of relevance for the compounds present in compost, e.g. polysaccharides, humid matter, protein material etc.

Compost A-Day 7		Compost A – Day 21		Compost A – Day 35		Vermicompost		Vibration	Functional group
Wave number (cm^{-1})	Intensity	Wave number (cm^{-1})	Intensity	Wave number (cm^{-1})	Intensity	Wave number (cm^{-1})	Intensity		
3298.42	1.15	3371.57	0.63	3340.71	0.39	3379.29	0.79	O-H stretching N-H stretching	Hydroxyl group, alcohols and carboxylic functions Amides and amines
2924.21 2854.77	1.06 0.94	2924.09 2854.77	0.53 0.47	2924.09 2854.77	0.33 0.29	2924.09 2854.77	0.57 0.50	C-H stretching	Aliphatic methylene group
2359.04	0.55	2360.85	0.37	2353.16	0.22	2360.87	0.34	C≡C stretching C≡N stretching	Alkyl group
1647.28	1.07	1635.64	0.70	1651.07	0.44	1604.77	0.78	C=O stretching C=C stretching	Amide I, carboxylates Aromatic ring modes, alkenes, bonded conjugated ketones, quinine, carboxylic acid & esters
1570.00	0.85	-	-			-	-	N-H in plane	Amides II
1512.00	0.80	1512.19	0.69	1535.34	0.41	1512.19	0.65	Aromatic skeletal	Lignin
1433.17	0.86	1419.61	0.81	1427.32	0.45	1419.61	0.69	O-H in plane CO_2 stretching C-O stretching	(bend of) Carboxylic acids Carboxylates and the aliphatic CH_2 group of alkanes carbonates
1396.52	0.80	-	-	-	-	1388.75	0.68	Anti-symmetric COO^- stretching	Aliphatic C-H deformation
1319.37	0.73							C-N stretching	Aromatic primary and secondary amines
1238.35	0.78	1234.44	0.63	1234.44	0.40	1265.30	0.63	C-O stretching O-H deformation	Carboxylic acids Aryl ethers and phenols
1080.14	1.11	1080.14	0.76	1080.14	0.52	1033.85	0.86	C-O stretching Si-O	Polysaccharides or polysaccharide-like substances, aromatic ether & carbohydrate Silica impurities
871.82	0.64	871.82	0.5416	871.82	0.331	-	-	NH_2 out of plane C-O out of plane	Primary amine group Bend of carbonates

5.4 Conclusions and suggested further research

The Takakura Composting Method (TCM), invented in Japan by Mr. Kouji Takakura is a technology that has been adopted in a number of developing countries for the management of solid organic waste at the domestic and community levels. The target community, in this study, has been successfully practicing this technology to manage household solid organic waste since 2009. However, in spite of favourable anecdotal evidence to support this method, to date, there has been very little scientific investigation into this composting method. Therefore this study has attempted to redress this by characterizing different varieties of Takakura compost in comparison to vermicompost and garden soil. The findings of this study and suggestions for further work on the TCM may be summarized as follows:

- These laboratory based pilot-scale experiments suggest that TCM could be a preferred option for managing solid organic waste, via conversion into valuable compost by a controlled biodegradation process. Currently, solid waste management is a global challenge, due to its increasing volume with rising population and changes in living standards. In developing countries, high costs are involved in its segregation, transport, storage and final disposal - and in constructing and maintaining engineered landfill sites. This points to the alternative of managing solid waste at the local level. TCM provides such an alternative for the management of a significant portion of such organic waste, at the household level, which reduces the cost associated with its disposal and which also provides valuable high quality compost.

- The initial step in the TCM is the preparation of the salt-based and the sugar-based solution to isolate microbes for utilization in the composting process. The study shows that the desired quality of fermentation solution referred as 'ideal fermentation solution', in terms of physico-chemical properties and the amount of fermentation products such as ethanol, VFAs and lactic acid, can be obtained by varying the relative amount of substrates used and the retention/incubation time. Thus a degree of "tuning" is inherent in this method.

- The ideal fermentation solution is defined in terms of the production of the highest amount of fermentation products such as ethanol, VFAs and lactic acid, with a view to

provide a high population of the microbes associated with the acidogenesis process. The study shows that the salt-based solution containing less substrate (100g) produced a higher amount of total VFAs on Day 5; acetic acid (AA) being a major product among the VFAs (acetic acid, propanoic acid, butyric acid and valeric acid). However, in terms of lactic acid (LA), the salt-based solution containing high substrate (200 g) produced a higher amount of LA on Day 5. While considering both AA and LA, the salt-based solution with higher substrate on Day 5 produced a higher concentration. This finding provides a basis for the selection of an ideal fermentation solution depending upon whether microbes associated with acetic acid or lactic acid (or both) is desired.

- The second step in the TCM is the preparation of seeding inoculates (SIs) utilizing the "ideal" fermentation solutions. In terms of seeding inoculate, a qualitative observation shows that the SI-C, prepared with 1:1 v/v rice husk and wheat bran, achieved a white fluffy healthy looking fungal growth that was superior to that of SI-A and SI-B, prepared with 1:2 and 1:1 v/v rice husk and rice bran respectively. The TCM assumes that a SI, which produces better white fluffy healthy looking fungal growth, without an objectionable odour, is more desirable for the production of quality compost. "Quality compost" is that which produces healthy plants in the matured compost (TCM on Day 35). In this study, the health of plants has been defined in terms of the "bushiness index" (BI); i.e. the highest number of leaves produced for a given height.

- The final step of the composting process is the preparation of compost utilizing three different SIs. The study shows that among three TCM matrices, CA contained a higher concentration of nutrients (NPK) than CB and CC, and that CA was not favourable in plant growth. Interestingly, CC contained the least nutrient and was the most favourable in terms of healthy plant growth. While comparing TCM with vermicompost, the overall nutrient content was relatively less in VC than in TCM.

- The germination percentage and plant bioassay test for plant maturity test revealed that CA was not favourable for seed germination and seedling growth. However, when CA was mixed with garden soil (GS), a synergy was observed whereby relatively healthier plants were produced in CA:GS than in CA or GS individually. This finding reflects the

fact that high nutrient levels in compost can exhibit phytotoxic effects and that the level and balance of nutrients is critical.

- The plant bioassay study revealed an interesting observation that plant growth rates varied in the three TCM variants. This variation is due to the difference in the characteristics of the composts produced with SI-A, SI-B and SI-C. Thus, this study demonstrates that the compost is "tunable" with respect to plant growth - based on the selection of the seeding inoculate.

- Fourier transform infrared spectroscopy (FTIR) analysis to study the change in organic matter composition for compost maturity assessment in CA gave seven well-defined peaks. The FTIR intensities obtained for CA on Day 7, Day 21 and Day 35 are as follows (in cm-1): 3341 - hydroxyl group, alcohols and carboxylic functions, amides and amines; 2924 - aliphatic methylene group; 2353 - alkynes; 1651 - amide I, carboxylates, aromatic ring modes, bonded conjugated ketones, quinones, carboxylic acid, esters; 1435 –bend of carboxylic acids, carboxylates and the aliphatic CH_2 group of alkanes, carbonates; 1080 – polysaccharides or polysaccharides like substances, aromatic ether and carbohydrates; 872 – primary amine group. The intensity of these peaks decreased with time, which reflects the degradation of organic matter over time.

Therefore, the study supports the claim of the inventor that TCM is an innovative technology, which is simple, fast and easy to adopt in the communities, especially at the household level. The fermentation solution was confirmed to be appropriate to use on Days 3 to 5 for the preparation of seeding inoculate. Similarly, the seeding inoculate was ready to use in the composting process by Day 5. This scientific investigation of the Takakura composting method, revealed the possibility of for "tuning" the fermentation solution, seeding inoculate and the compost itself for desired outcomes. Furthermore, TCM provides a favourable alternative for solid waste management in the communities of developing countries, as illustrated by its adoption by the target community of this study.

Initially, in agriculture, the application of chemical fertilizer was the preferred method for enhancing crop production. Recent work on micro flora and its application to crop cultivation (Reisch 2014) is more consistent with this study on the TCM, which prefers to utilize

microbes for enhanced plant growth. The bushiness index, which has been used as an indicator of plant health, shows encouraging outcomes for TCM as compared to, VC and GS, which is likely to be associated with the specific microbes isolated in the fermentation solution preparation stage and the presence of the diversity of microbes in the seeding inoculates. Due to the limitation of the scope of this study, detailed microbial analysis in the fermentation solutions, seeding inoculates and compost, could not be conducted – this is an obvious direction for future research. Future research can also be directed at further controlled variation substrate materials, the relative volume of these materials and incubation times. This project has set the stage for such research to continue.

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CHAPTER 6: Conclusions and Recommendations[94]

6.1 Overview

- Appropriate technologies for the sustainable management of the Nepalese community's domestic waste and sewerage were identified and assessed for their efficacy.

- An eco-audit conducted in a representative Nepalese community, i.e. Ward Number 20 of Lalitpur Sub-metropolitan City, Lalitpur, Nepal, provided an update on the current status of their waste management practices, in terms of solid waste and wastewater, and its impact on the surrounding environment, as discussed in Chapter 2.

- Monitoring of the Bagmati River characterized the extent of the pollution along the stretch within the Kathmandu valley. Moreover, an assessment of the Guheshwori Wastewater treatment Plant (GWWTP), the centralized wastewater treatment plant situated in the valley, revealed its current efficiency and potential improvements, and pointed to the importance of small decentralized wastewater treatment plants in developing countries like Nepal, as delineated in Chapter 3.

- 'Vermifiltration' was chosen as the most appropriate technology for wastewater treatment in Nepalese communities with a particular emphasis being placed on simple, less technical, cost effective and innovative technologies that also have the potential to contribute to the local economy and which engage and involve the community. The vermifiltration technology was investigated scientifically by assessing the influent and effluent characteristics, as outlined in Chapter 4.

- 'Takakura composting', that has already been introduced in the representative community, was chosen as the most appropriate technology for organic solid waste management in Nepalese communities. This has been scientifically investigated and its

[94] Some conclusions and recommendations for further research have also been presented in the individual chapters (Chapter 2, 3, 4 and 5). Here, a brief synopsis of these has been presented.

potential for optimization demonstrated - with a view to characterizing the compost and its efficiency for waste management, as outlined in Chapter 5.

6.2 Conclusions

The main objective of this research was to identify and assess appropriate technologies for solid waste management and sewage treatment, with the potential to be adopted in Nepalese communities. Thus, the following conclusions were drawn based on the findings of the project.

- The "Towards ZERO Waste" program, which was introduced in the representative target community in 2008, is an innovative waste management system that addressed the problem and the challenges associated with the sustainable management of municipal waste. This program can be considered a viable approach based on the data collected from the community survey conducted in this study.

- The average solid waste generation was found to be 499.6 gm per HH per day and the average wastewater generation was found to be 200.6 L per HH per day, in the representative community.

- More than 65 % of solid waste generated in the community can be diverted from landfill sites and has the potential to be recovered as a resource.

- The discharge of untreated sewage wastewater into the river environment and the solid waste dumping along the river bank are the two major issues which contribute the most to the deterioration of the Bagmati River environment.

- Therefore, the river water quality diminishes as it flows downstream to more populated areas, as observed by the Bagmati River water monitoring in the upper part of the river, from Sundarijal (upstream) to Chovar (downstream).

- Upstream, in rural areas, human sewage from open defecation and fertilizer from agricultural land are found as major contaminants; whereas downstream, in urban areas, municipal sewage is the major contaminant.

- $_5$ Thus, the treatment plant is functional only up to its partial efficiency due to electricity outages and lack of proper maintenance.

- Based on the assessment of the centralized GWWTP, and the performance review of existing decentralized systems (Ellingsen 2012; Regmi 2013; Jha and Bajracharya 2014), it is appropriate to suggest that small biological systems are viable alternatives for wastewater treatment in a country like Nepal.

- The Vermifiltration system (VF), investigated as an alternative to the conventional wastewater treatment in this study, was found to be effective in reducing "pollution factors" namely, turbidity, TSS, COD, BOD_5, NH_3-N, NO_2-N, NO_3-N, TN, TOC and TP, from the Influent (sewage wastewater). In addition, VF was found to alter the relevant "physico-chemical parameters" such as the temperature, pH, conductivity and DO, in the resulting Effluents.

- Earthworms were found to be effective in the removal of turbidity, TSS, organic matter and *E.Coli*/Coliforms from the influent and to increase the DO. However, a variation in treatment efficiency was observed between two different soil types that were trialled in the VF. Moreover, the performance of the VF was influenced by the operating conditions. The higher the hydraulic retention time and the lower the hydraulic loading rate, the better the performance, see Table 4.8 to 4.20, and 4.24.

- The resulting VF treated effluent quality was found to satisfy the irrigation water quality standards of the Government of Nepal and other organizations (except for a few parameters), as discussed in Chapter 4. Thus, the effluent rich in nutrients can be used for irrigation purposes that would minimise the fresh water demand from the water cycle. This less technical, low cost and environmentally friendly technology is, therefore, a viable alternative to centralised systems in developing countries.

- With respect to solid waste management, laboratory based pilot-scale experiments showed that the Takakura Composting Method (TCM) could be one of the best options to manage the organic portion of municipal solid waste (MSW). More than 65 % of municipal waste is comprised of organic waste, which can be converted into valuable compost by a biodegradation process. Thus, a major volume of MSW can be managed at the household or community level, reducing the cost associated with its collection, transportation and disposal - and reducing the land required for landfill sites.

- The compost produced with TCM was found to be rich in nutrients, macronutrients, micronutrients and trace metals, which promotes plant health and growth. The process itself was simple, easy and fast, thus suitable to be adopted in the community.

6.3 Recommendations and further suggestions for research

Based on this research, the following recommendations can be made and issues can be identified which could be further investigated to further the findings of this study.

- The zero waste approach taken by the representative target community, by introducing the 'Towards ZERO waste' program, could be transferred to other communities. The outcome of this program could be promoted as a model to inspire policy makers and local authorities.
- With respect to sustainable solid waste management, similar surveys to explore current waste management practices, and the willingness to participate in new approach to waste management, could be conducted in other Nepalese communities.

- The study has shown that most of the community people are segregating organic and inorganic waste at source, and those who are not currently separating waste based on type, are willing to do so. However, there does seem to be dissatisfaction due to a lack of a separate collection system. Thus, the local authority should develop such system which could collect organic and inorganic waste separately. Alternatively, a community-based collection centre, where people can drop off separated waste, could be a preferred option.

This practice reduces the cost and effort associated with waste disposal. On the other hand, community people will be motivated to manage their waste at source.

- With respect to wastewater management, existing sophisticated data on wastewater generation, collection and treatment is not available, and this is the key in the decision making process for wastewater management. Moreover, data on the quantity of sewage water and the catchment area covered by the GWWTP is not available. Thus, a further study could be conducted to obtain this information, so that planning can be done in an efficient way.

- The GWWTP could be run to its full efficacy by utilizing renewable sources of power such as solar power so that power outages would not affect its performance. Again, community-based decentralized wastewater systems in the catchment area may address the issue of by-passing influent at the GWWTP. This will reduce the impact of by-passed influent on the Bagmati River at Pashupati dham.

- To meet stringent water quality guidelines, the VF could be combined with other novel technologies for further treatment. For instance, the vermifiltered water could be further treated with ultraviolet rays (UV) or ozone for pathogen removal. However, the cost associated with such technologies would need to be considered.

- With respect to the reuse potential of vermifiltered wastewater and vermicast as bio-fertilizer, of most concern is its adverse effect on human health and the environment due to pathogens and heavy metals. Therefore, the bioaccumulation of heavy metals by plants and the fate of pathogens should be investigated further.

- VF could be a practical solution in developing countries, in communities without toilets and where sanitation is poor due to open defecation, as discussed by some researchers (Spears 2013; Greenslade 2014). It could be piloted in such areas and health assessments could be done to establish the effect of better sanitation.
- Finally, the VF studied in this research could be modified, in terms of design and operating conditions. This system for wastewater treatment could be integrated with solid waste management and investigated for its efficacy.

6.4 References

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Greenslade, L. 2014. The Nutritional Values of Toilets. Available: http://www.everywomaneverychild.org/news-events/news/968-the-nutritional-value-of-toilets [Accessed 06/11/2014].

Jha, A. K. & Bajracharya, T. r. 2014. Wastewater Treatment Technologies in Nepal. *Institute of Engineering Graduate Conference.* Kathmandu, Nepal: Institute of Engineering.

Regmi, S. 2013. *Wastewater Treatment in Kathmandu: Management, Treatment and Alternative.* Bachelor's Thesis, Mikkeli University of Applied Science.

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Appendix 2.1

Eco-Audit in Ward No. 20, Lalitpur Summetropolitan City

House No: _____ Date: _____

Adult	Child	Female	Male	Total

1) Waste segregation:

Yes ☐ No ☐

2) Waste volume (gm):

Organic wastes		Rubber and leather	
Plastics		Textiles	
Paper and paper products		Dirt and construction debris	
Metals		Hazardous wastes	
Glass		Others: _____	

3) Composting of organic waste: Yes ☐ No ☐

If yes, the method used _____

4) Management of waste generated:

Collection by Municipality	Collection by Private Sector	Dump in public place	Other

5) Power usage:

Meter reading for a month (Kwh) - ☐

6) Water usage:

Water use (L/D)	Source of water supply	Capacity of Water tank (underground)	Capacity of Water tank (above ground)

7) Property connected to sewer: Yes ☐ No ☐

8) On-site Wastewater Treatment System: Yes ☐ No ☐

If yes, the method used _____

9) Solar power:

Yes	No	Quantity	Application

10) Rainwater/Stormwater Harvesting facility:

Yes	No	Quantity	Application

11) Other Comments:

Appendix 3.1

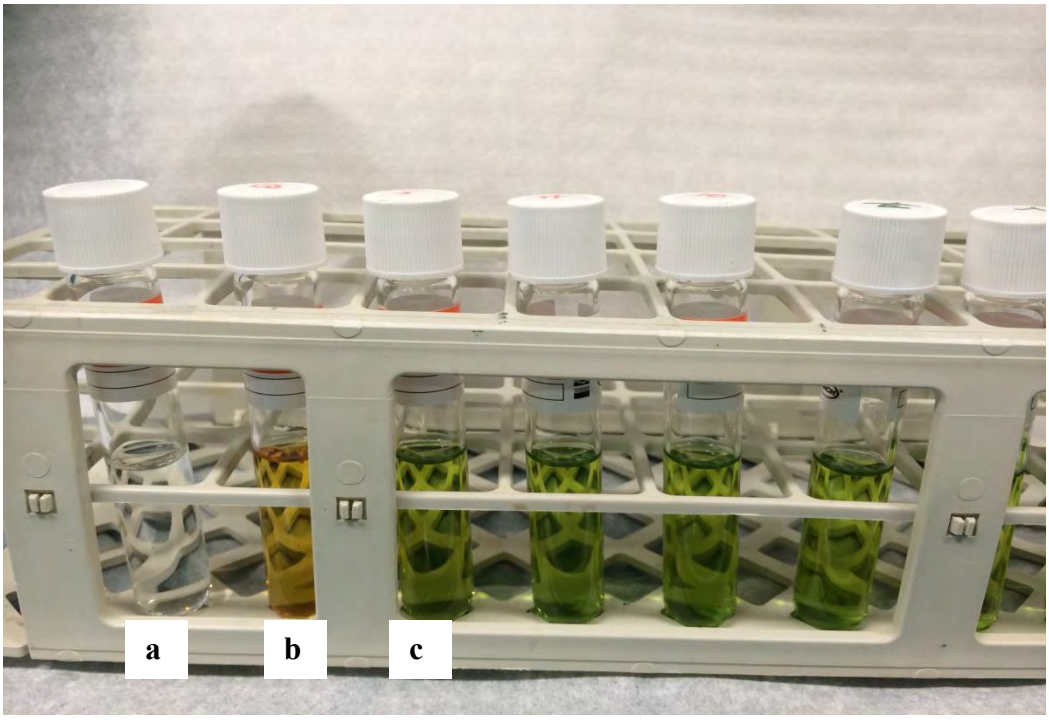


Figure 1 The ammonium nitrogen (NH_3-N) test in wastewater (Influent and Effluents). The vial (a) is transparent before the test, the vial (b) is for blank test and the vial (c) with green colour indicates the presence of NH_3-N in samples. Photo by Anusuya Joshi.

Figure 2 The nitrite nitrogen (NO_2-N) and the nitrate nitrogen (NO_3-N) test in wastewater (Influent and Effluents). The equipment used is DR890 colorimeter. Photo by Anusuya Joshi.

The performance of Guheshwori Wastewater Treatment Plant (GWWTP)

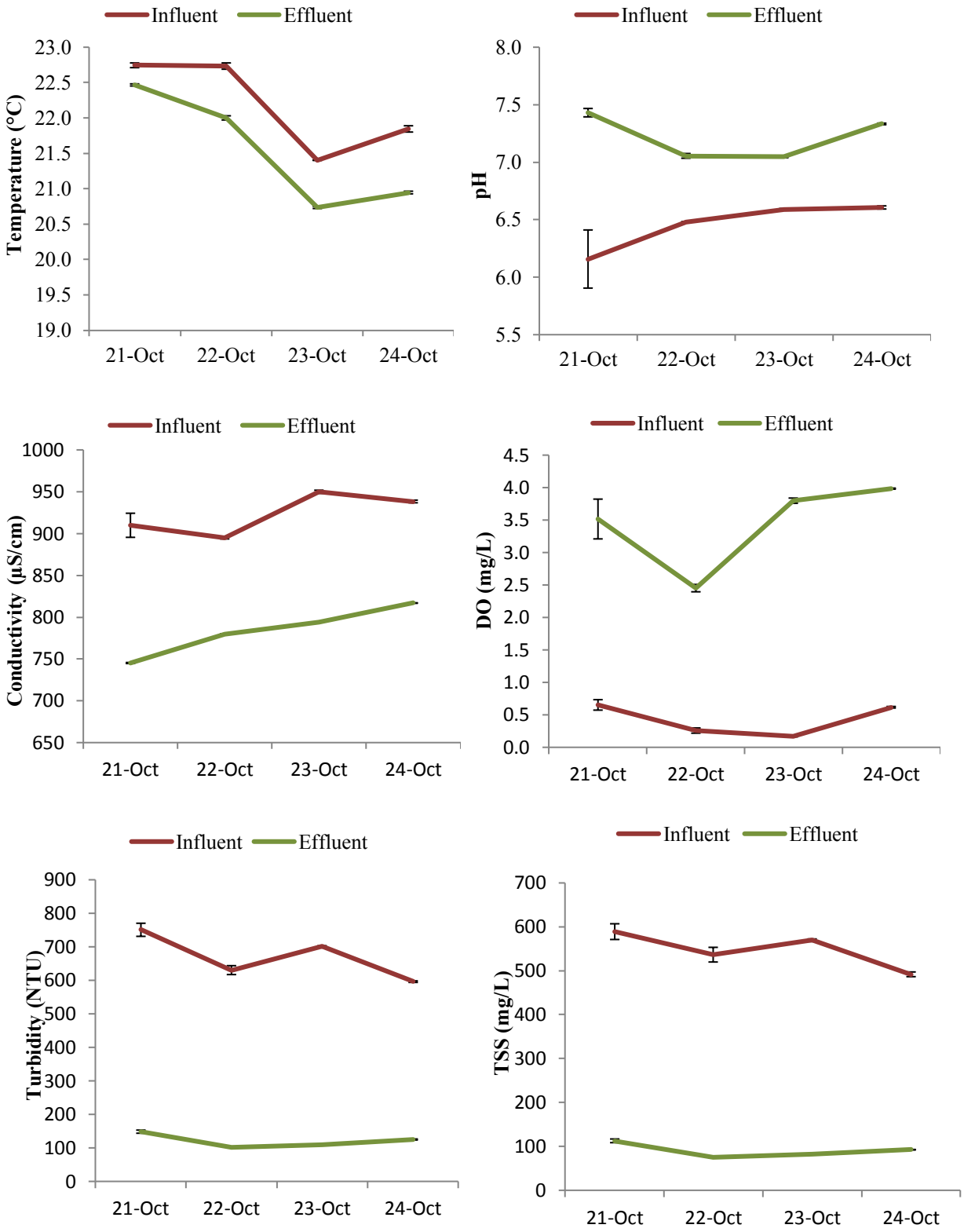


Figure 1 The temperature, pH, conductivity, DO, turbidity and TSS profile for Influent and Effluent at the GWWTP for October 2013 (this study). The error bars represent the standard error, where n=3.

Figure 1 The total organic carbon (TOC) on analyser, used for the determination of the TOC and total nitrogen (TN). Photo by Anusuya Joshi.

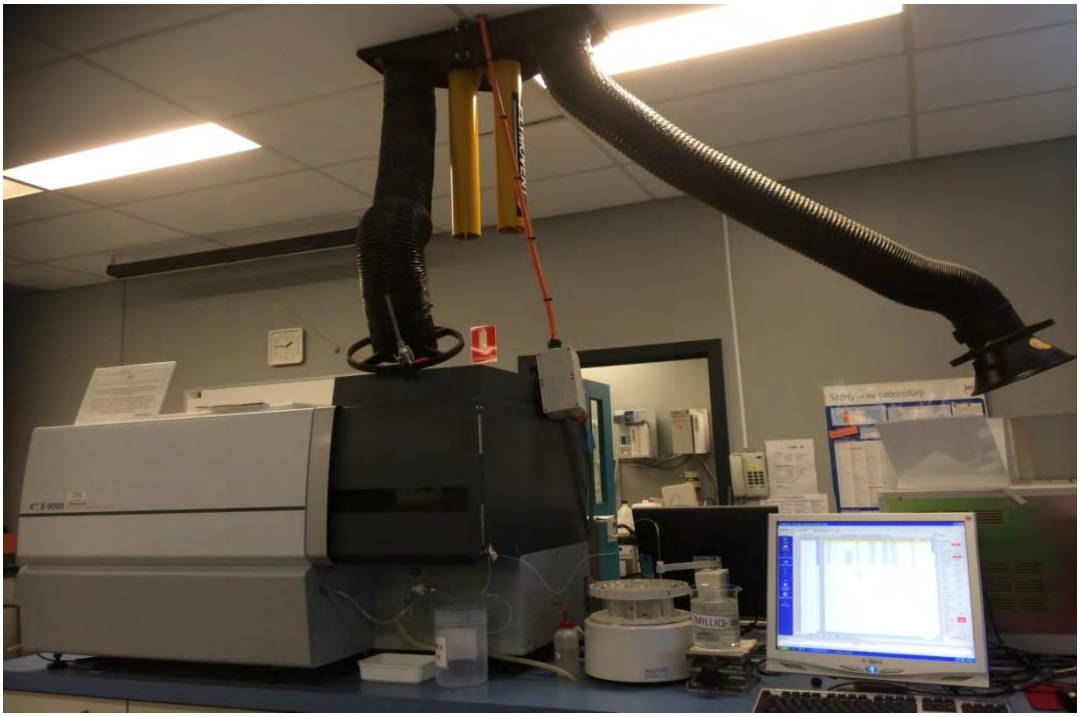


Figure 2 The inductively coupled plasma – optical emission spectrophotometer used for the determination of heavy metals, nutrients and macronutrients. Photo by Anusuya Joshi.

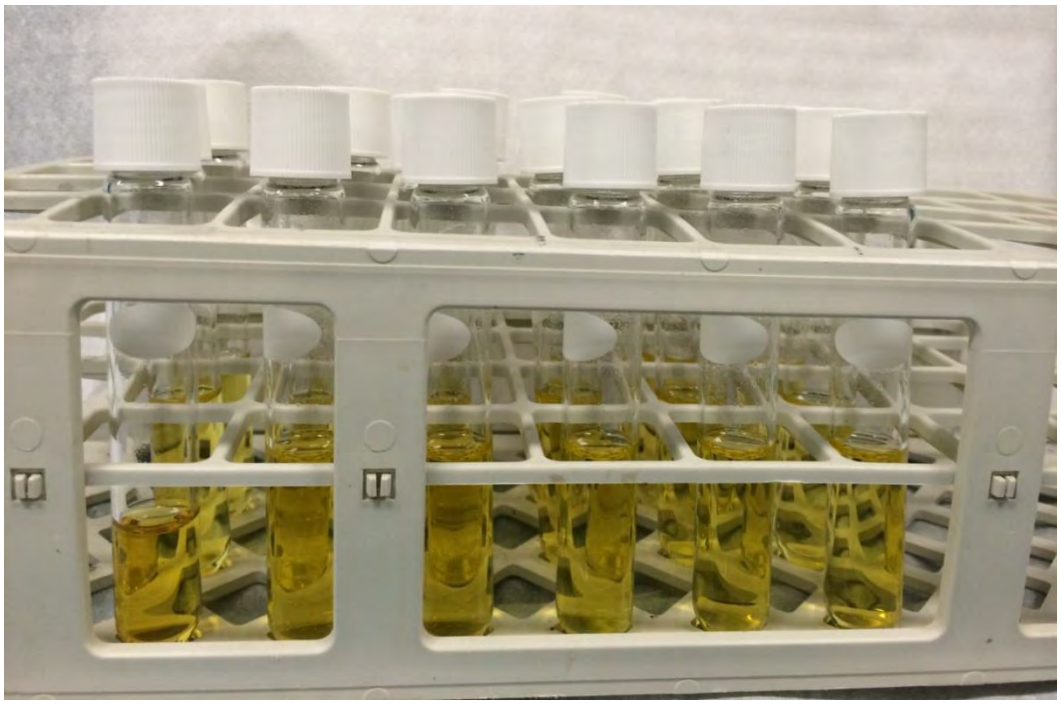
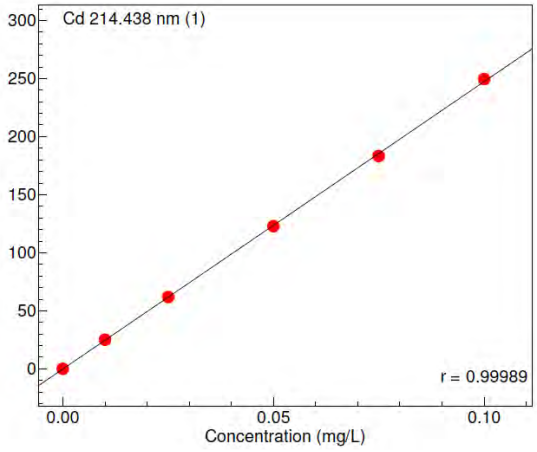
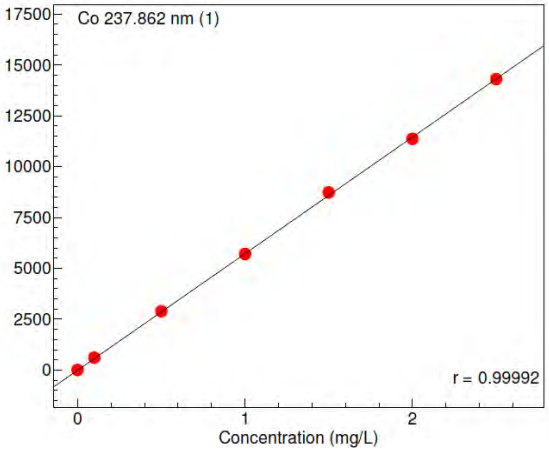


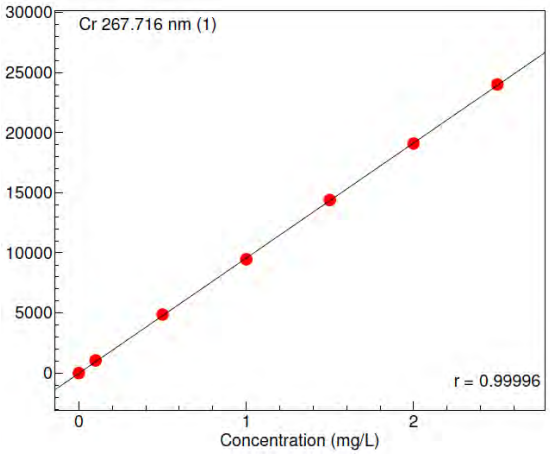
Figure 3 The chemical oxygen demand (COD) test in wastewater (Influent and Effluents). Photo by Anusuya Joshi.

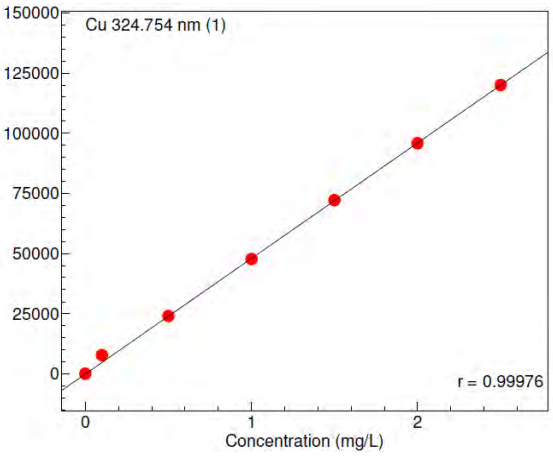
Figure 4 The total phosphorus (TP) test in wastewater (Influent and Effluents). The equipment used is DR5000 spectrophotometer. Photo by Anusuya Joshi.

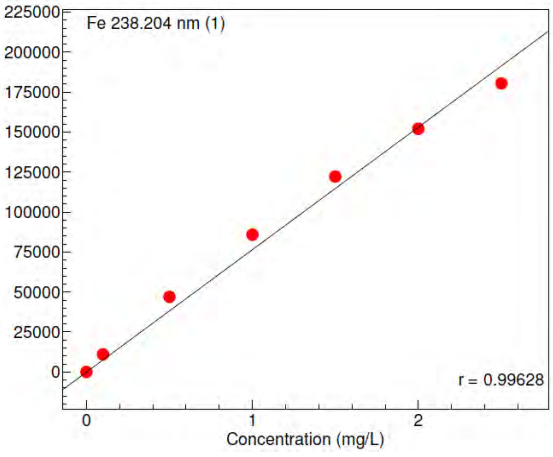
Appendix 4.2

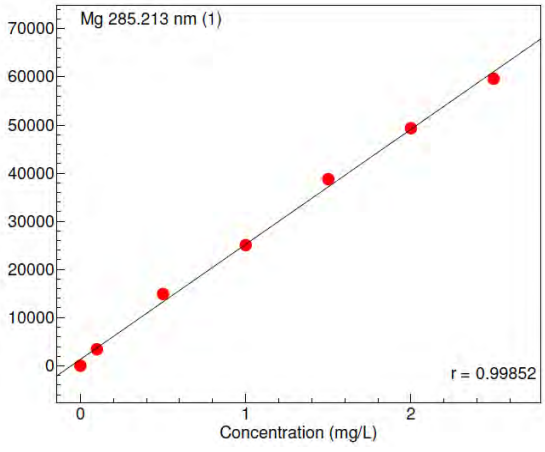

Cd 214.438 nm (1)
r = 0.99989
Concentration (mg/L)


Co 237.862 nm (1)
r = 0.99992
Concentration (mg/L)


Cr 267.716 nm (1)
r = 0.99996
Concentration (mg/L)


Cu 324.754 nm (1)
r = 0.99976
Concentration (mg/L)


Fe 238.204 nm (1)
r = 0.99628
Concentration (mg/L)


Mg 285.213 nm (1)
r = 0.99852
Concentration (mg/L)

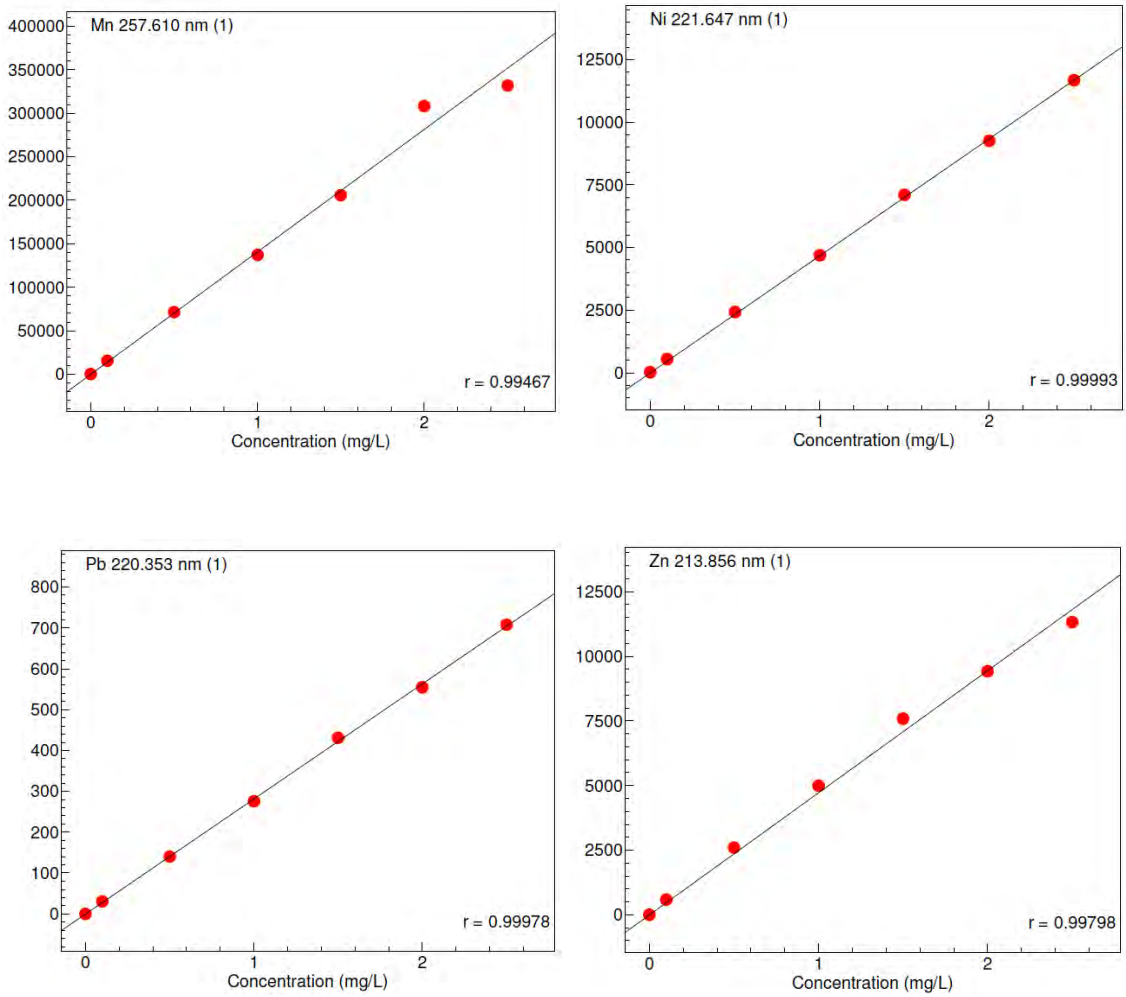


Figure 1 Calibration curves for Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn. Altogether six standards were prepared (0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L). The wave length at which the measurement was taken is indicated in the respected calibration curves.

Appendix 4.5

Table 1 Normality test for Phase I datasets, which contains 22 sampling sets including 7 sets "without worms" and 15 sets "with worms". Here, green colour represents the normal dataset and red colour represents the non-normal dataset. Asterisk (*) represents the datasets with extreme outlier/s.

Phase I	Overall datasets - 22 subsets				"Without worms" datasets - 7 subsets				"With worms" datasets - 15 subsets			
	Influent	Effluent 1	Effluent 2	Effluent 3	Influent	Effluent 1	Effluent 2	Effluent 3	Influent	Effluent 1	Effluent 2	Effluent 3
Temperature (°C)												
pH	*								*			
Conductivity (mS/cm)				*								
Turbidity (NTU)												
TSS (mg/L)												
DO (mg/L)	*					*	*					
COD (mg/L)												
NH_3-N (mg/L)												
NO_2-N (mg/L)											*	
NO_3-N (mg/L)		*		*		*		*		*		
TN (mg/L)												
TOC (mg/L)	*	*	*	*								
TP (mg/L)												

Table 2 Normality test for Phase I datasets, which contains 22 sampling sets including 11 sets with 13 hours HRT, 6 sets with 19.5 hours HRT and 5 sets with 182 hours HRT. These sets do not consider "without worms" and "with worms". Here, green colour represents the normal dataset and red colour represents the non-normal dataset. Asterisk (*) represents the datasets with extreme outlier/s.

Phase I	HRT 13 hrs				HRT 19.5 hrs				HRT 182 hrs			
	Influent	Effluent 1	Effluent 2	Effluent 3	Influent	Effluent 1	Effluent 2	Effluent 3	Influent	Effluent 1	Effluent 2	Effluent 3
Temperature (°C)												
pH												
Conductivity (mS/cm)										*	*	*
Turbidity (NTU)				*								
TSS (mg/L)							*					
DO (mg/L)					*	*			*			
COD (mg/L)												
NH_3-N (mg/L)												*
NO_2-N (mg/L)												
NO_3-N (mg/L)		*	*								*	
TN (mg/L)											*	*
TOC (mg/L)		*	*	*		*				*	*	*
TP (mg/L)			*									

Table 3 Normality test for Phase II datasets, which contains 13 sampling sets including 3 sets "without worms" and 10 sets "with worms". Here, green colour represents the normal dataset and red colour represents the non-normal dataset. Asterisk (*) represents the datasets with extreme outlier/s.

Phase II	Overall datasets - 13 subsets				"Without worms" datasets - 3 subset				"With worms" datasets - 10 subsets			
	Influent	Effluent 1	Effluent 2	Effluent 3	Influent	Effluent 1	Effluent 2	Effluent 3	Influent	Effluent 1	Effluent 2	Effluent 3
Temperature (°C)												
pH		*										
Conductivity (mS/cm)		*										
Turbidity (NTU)		*								*		
TSS (mg/L)		*								*		
DO (mg/L)	*								*			
COD (mg/L)												
NH_3-N (mg/L)				*								
NO_2-N (mg/L)			*	*							*	*
NO_3-N (mg/L)				*								*
TN (mg/L)												
TOC (mg/L)			*	*					*			
TP (mg/L)												

Hypothesis outcomes **Appendix 4.6**

1. The effect of filter layers

A. Phase I, Without worms

Observed Parameters	Layer 1	Layer 2	Layer 3
1. Temperature	H_0: $\mu_{In.T} - \mu_{A\text{-}Eff1.T} = 0$ H_1: $\mu_{In.T} - \mu_{A\text{-}Eff1.T} \neq 0$	H_0: $\mu_{In.T} - \mu_{A\text{-}Eff2.T} = 0$ H_1: $\mu_{In.T} - \mu_{A\text{-}Eff2.T} \neq 0$	H_0: $\mu_{In.T} - \mu_{A\text{-}Eff3.T} = 0$ H_1: $\mu_{In.T} - \mu_{A\text{-}Eff3.T} \neq 0$
2. pH	H_0: $\mu_{In.pH} - \mu_{A\text{-}Eff1.pH} = 0$ H_1: $\mu_{In.pH} - \mu_{A\text{-}Eff1.pH} \neq 0$	H_0: $\mu_{In.pH} - \mu_{A\text{-}Eff2.TpH} = 0$ H_1: $\mu_{In.pH} - \mu_{A\text{-}Eff2.TpH} \neq 0$	H_0: $\mu_{In.pH} - \mu_{A\text{-}Eff3.TpH} = 0$ H_1: $\mu_{In.pH} - \mu_{A\text{-}Eff3.TpH} \neq 0$
3. Conductivity	H_0: $\mu_{In.C} - \mu_{A\text{-}Eff1.C} = 0$ H_1: $\mu_{In.C} - \mu_{A\text{-}Eff1.C} \neq 0$	H_0: $\mu_{In.C} - \mu_{A\text{-}Eff2.C} = 0$ H_1: $\mu_{In.C} - \mu_{A\text{-}Eff2.C} \neq 0$	H_0: $\mu_{In.C} - \mu_{A\text{-}Eff3.C} = 0$ H_1: $\mu_{In.C} - \mu_{A\text{-}Eff3.C} \neq 0$
4. Turbidity	H_0: $\mu_{In.Tur} - \mu_{A\text{-}Eff1.Tur} = 0$ H_1: $\mu_{In.Tur} - \mu_{A\text{-}Eff1.Tur} \neq 0$	H_0: $\mu_{In.Tur} - \mu_{A\text{-}Eff2.Tur} = 0$ H_1: $\mu_{In.Tur} - \mu_{A\text{-}Eff2.Tur} \neq 0$	H_0: $\mu_{In.Tur} - \mu_{A\text{-}Eff3.Tur} = 0$ H_1: $\mu_{In.Tur} - \mu_{A\text{-}Eff3.Tur} \neq 0$
5. Total Suspended Solids	H_0: $\mu_{In.TSS} - \mu_{A\text{-}Eff1.TSS} = 0$ H_1: $\mu_{In.TSS} - \mu_{A\text{-}Eff1.TSS} \neq 0$	H_0: $\mu_{In.TSS} - \mu_{A\text{-}Eff2.TSS} = 0$ H_1: $\mu_{In.TSS} - \mu_{A\text{-}Eff2.TSS} \neq 0$	H_0: $\mu_{In.TSS} - \mu_{A\text{-}Eff3.TSS} = 0$ H_1: $\mu_{In.TSS} - \mu_{A\text{-}Eff3.TSS} \neq 0$
6. Dissolved Oxygen	H_0: $\mu_{In.DO} - \mu_{A\text{-}Eff1.DO} = 0$ H_1: $\mu_{In.DO} - \mu_{A\text{-}Eff1.DO} \neq 0$	H_0: $\mu_{In.DO} - \mu_{A\text{-}Eff2.DO} = 0$ H_1: $\mu_{In.DO} - \mu_{A\text{-}Eff2.DO} \neq 0$	H_0: $\mu_{In.DO} - \mu_{A\text{-}Eff3.DO} = 0$ H_1: $\mu_{In.DO} - \mu_{A\text{-}Eff3.DO} \neq 0$
7. Chemical Oxygen Demand	H_0: $\mu_{In.COD} - \mu_{A\text{-}Eff1.COD} = 0$ H_1: $\mu_{In.COD} - \mu_{A\text{-}Eff1.COD} \neq 0$	H_0: $\mu_{In.COD} - \mu_{A\text{-}Eff2.COD} = 0$ H_1: $\mu_{In.COD} - \mu_{A\text{-}Eff2.COD} \neq 0$	H_0: $\mu_{In.COD} - \mu_{A\text{-}Eff3.COD} = 0$ H_1: $\mu_{In.COD} - \mu_{A\text{-}Eff3.COD} \neq 0$
8. Ammonium Nitrogen	H_0: $\mu_{In.NH3\text{-}N} - \mu_{A\text{-}Eff1.NH3\text{-}N} = 0$ H_1: $\mu_{In.NH3\text{-}N} - \mu_{A\text{-}Eff1.NH3\text{-}N} \neq 0$	H_0: $\mu_{In.NH3\text{-}N} - \mu_{A\text{-}Eff2.NH3\text{-}N} = 0$ H_1: $\mu_{In.NH3\text{-}N} - \mu_{A\text{-}Eff2.NH3\text{-}N} \neq 0$	H_0: $\mu_{In.NH3\text{-}N} - \mu_{A\text{-}Eff3.NH3\text{-}N} = 0$ H_1: $\mu_{In.NH3\text{-}N} - \mu_{A\text{-}Eff3.NH3\text{-}N} \neq 0$
9. Nitrite Nitrogen	H_0: $\mu_{In.NO2\text{-}N} - \mu_{A\text{-}Eff1.NO2\text{-}N} = 0$ H_1: $\mu_{In.NO2\text{-}N} - \mu_{A\text{-}Eff1.NO2\text{-}N} \neq 0$	H_0: $\mu_{In.NO2\text{-}N} - \mu_{A\text{-}Eff2.NO2\text{-}N} = 0$ H_1: $\mu_{In.NO2\text{-}N} - \mu_{A\text{-}Eff2.NO2\text{-}N} \neq 0$	H_0: $\mu_{In.NO2\text{-}N} - \mu_{A\text{-}Eff3.NO2\text{-}N} = 0$ H_1: $\mu_{In.NO2\text{-}N} - \mu_{A\text{-}Eff3.NO2\text{-}N} \neq 0$
10. Nitrate Nitrogen	H_0: $\mu_{In.NO3\text{-}N} - \mu_{A\text{-}Eff1.NO3\text{-}N} = 0$ H_1: $\mu_{In.NO3\text{-}N} - \mu_{A\text{-}Eff1.NO3\text{-}N} \neq 0$	H_0: $\mu_{In.NO3\text{-}N} - \mu_{A\text{-}Eff2.NO3\text{-}N} = 0$ H_1: $\mu_{In.NO3\text{-}N} - \mu_{A\text{-}Eff2.NO3\text{-}N} \neq 0$	H_0: $\mu_{In.NO3\text{-}N} - \mu_{A\text{-}Eff3.NO3\text{-}N} = 0$ H_1: $\mu_{In.NO3\text{-}N} - \mu_{A\text{-}Eff3.NO3\text{-}N} \neq 0$
11. Total Nitrogen	H_0: $\mu_{In.TN} - \mu_{A\text{-}Eff1.TN} = 0$ H_1: $\mu_{In.TN} - \mu_{A\text{-}Eff1.TN} \neq 0$	H_0: $\mu_{In.TN} - \mu_{A\text{-}Eff2.TN} = 0$ H_1: $\mu_{In.TN} - \mu_{A\text{-}Eff2.TN} \neq 0$	H_0: $\mu_{In.TN} - \mu_{A\text{-}Eff3.TN} = 0$ H_1: $\mu_{In.TN} - \mu_{A\text{-}Eff3.TN} \neq 0$
12. Total Organic Carbon	H_0: $\mu_{In.TOC} - \mu_{A\text{-}Eff1.TOC} = 0$ H_1: $\mu_{In.TOC} - \mu_{A\text{-}Eff1.TOC} \neq 0$	H_0: $\mu_{In.TOC} - \mu_{A\text{-}Eff2.TOC} = 0$ H_1: $\mu_{In.TOC} - \mu_{A\text{-}Eff2.TOC} \neq 0$	H_0: $\mu_{In.TOC} - \mu_{A\text{-}Eff3.TOC} = 0$ H_1: $\mu_{In.TOC} - \mu_{A\text{-}Eff3.TOC} \neq 0$
13. Total Phosphorus	H_0: $\mu_{In.TP} - \mu_{A\text{-}Eff1.TP} = 0$ H_1: $\mu_{In.TP} - \mu_{A\text{-}Eff1.TP} \neq 0$	H_0: $\mu_{In.TP} - \mu_{A\text{-}Eff2.TP} = 0$ H_1: $\mu_{In.TP} - \mu_{A\text{-}Eff2.TP} \neq 0$	H_0: $\mu_{In.TP} - \mu_{A\text{-}Eff3.TP} = 0$ H_1: $\mu_{In.TP} - \mu_{A\text{-}Eff3.TP} \neq 0$

B. Phase I, With worms

Observed Parameters	Layer 1	Layer 2	Layer 3
1. Temperature	H_0: $\mu_{In.T} - \mu_{B\text{-}Eff1.T} = 0$ H_1: $\mu_{In.T} - \mu_{B\text{-}Eff1.T} \neq 0$	H_0: $\mu_{In.T} - \mu_{B\text{-}Eff2.T} = 0$ H_1: $\mu_{In.T} - \mu_{B\text{-}Eff2.T} \neq 0$	H_0: $\mu_{In.T} - \mu_{B\text{-}Eff3.T} = 0$ H_1: $\mu_{In.T} - \mu_{B\text{-}Eff3.T} \neq 0$
2. pH	H_0: $\mu_{In.pH} - \mu_{B\text{-}Eff1.pH} = 0$ H_1: $\mu_{In.pH} - \mu_{B\text{-}Eff1.pH} \neq 0$	H_0: $\mu_{In.pH} - \mu_{B\text{-}Eff2.TpH} = 0$ H_1: $\mu_{In.pH} - \mu_{B\text{-}Eff2.TpH} \neq 0$	H_0: $\mu_{In.pH} - \mu_{B\text{-}Eff3.TpH} = 0$ H_1: $\mu_{In.pH} - \mu_{B\text{-}Eff3.TpH} \neq 0$
3. Conductivity	H_0: $\mu_{In.C} - \mu_{B\text{-}Eff1.C} = 0$ H_1: $\mu_{In.C} - \mu_{B\text{-}Eff1.C} \neq 0$	H_0: $\mu_{In.C} - \mu_{B\text{-}Eff2.C} = 0$ H_1: $\mu_{In.C} - \mu_{B\text{-}Eff2.C} \neq 0$	H_0: $\mu_{In.C} - \mu_{B\text{-}Eff3.C} = 0$ H_1: $\mu_{In.C} - \mu_{B\text{-}Eff3.C} \neq 0$
4. Turbidity	H_0: $\mu_{In.Tur} - \mu_{B\text{-}Eff1.Tur} = 0$ H_1: $\mu_{In.Tur} - \mu_{B\text{-}Eff1.Tur} \neq 0$	H_0: $\mu_{In.Tur} - \mu_{B\text{-}Eff2.Tur} = 0$ H_1: $\mu_{In.Tur} - \mu_{B\text{-}Eff2.Tur} \neq 0$	H_0: $\mu_{In.Tur} - \mu_{B\text{-}Eff3.Tur} = 0$ H_1: $\mu_{In.Tur} - \mu_{B\text{-}Eff3.Tur} \neq 0$
5. Total Suspended Solids	H_0: $\mu_{In.TSS} - \mu_{B\text{-}Eff1.TSS} = 0$ H_1: $\mu_{In.TSS} - \mu_{B\text{-}Eff1.TSS} \neq 0$	H_0: $\mu_{In.TSS} - \mu_{B\text{-}Eff2.TSS} = 0$ H_1: $\mu_{In.TSS} - \mu_{B\text{-}Eff2.TSS} \neq 0$	H_0: $\mu_{In.TSS} - \mu_{B\text{-}Eff3.TSS} = 0$ H_1: $\mu_{In.TSS} - \mu_{B\text{-}Eff3.TSS} \neq 0$
6. Dissolved Oxygen	H_0: $\mu_{In.DO} - \mu_{B\text{-}Eff1.DO} = 0$ H_1: $\mu_{In.DO} - \mu_{B\text{-}Eff1.DO} \neq 0$	H_0: $\mu_{In.DO} - \mu_{B\text{-}Eff2.DO} = 0$ H_1: $\mu_{In.DO} - \mu_{B\text{-}Eff2.DO} \neq 0$	H_0: $\mu_{In.DO} - \mu_{B\text{-}Eff3.DO} = 0$ H_1: $\mu_{In.DO} - \mu_{B\text{-}Eff3.DO} \neq 0$
7. Chemical Oxygen Demand	H_0: $\mu_{In.COD} - \mu_{B\text{-}Eff1.COD} = 0$ H_1: $\mu_{In.COD} - \mu_{B\text{-}Eff1.COD} \neq 0$	H_0: $\mu_{In.COD} - \mu_{B\text{-}Eff2.COD} = 0$ H_1: $\mu_{In.COD} - \mu_{B\text{-}Eff2.COD} \neq 0$	H_0: $\mu_{In.COD} - \mu_{B\text{-}Eff3.COD} = 0$ H_1: $\mu_{In.COD} - \mu_{B\text{-}Eff3.COD} \neq 0$
8. Ammonium Nitrogen	H_0: $\mu_{In.NH3\text{-}N} - \mu_{B\text{-}Eff1.NH3\text{-}N} = 0$ H_1: $\mu_{In.NH3\text{-}N} - \mu_{B\text{-}Eff1.NH3\text{-}N} \neq 0$	H_0: $\mu_{In.NH3\text{-}N} - \mu_{B\text{-}Eff2.NH3\text{-}N} = 0$ H_1: $\mu_{In.NH3\text{-}N} - \mu_{B\text{-}Eff2.NH3\text{-}N} \neq 0$	H_0: $\mu_{In.NH3\text{-}N} - \mu_{B\text{-}Eff3.NH3\text{-}N} = 0$ H_1: $\mu_{In.NH3\text{-}N} - \mu_{B\text{-}Eff3.NH3\text{-}N} \neq 0$
9. Nitrite Nitrogen	H_0: $\mu_{In.NO2\text{-}N} - \mu_{B\text{-}Eff1.NO2\text{-}N} = 0$ H_1: $\mu_{In.NO2\text{-}N} - \mu_{B\text{-}Eff1.NO2\text{-}N} \neq 0$	H_0: $\mu_{In.NO2\text{-}N} - \mu_{B\text{-}Eff2.NO2\text{-}N} = 0$ H_1: $\mu_{In.NO2\text{-}N} - \mu_{B\text{-}Eff2.NO2\text{-}N} \neq 0$	H_0: $\mu_{In.NO2\text{-}N} - \mu_{B\text{-}Eff3.NO2\text{-}N} = 0$ H_1: $\mu_{In.NO2\text{-}N} - \mu_{B\text{-}Eff3.NO2\text{-}N} \neq 0$
10. Nitrate Nitrogen	H_0: $\mu_{In.NO3\text{-}N} - \mu_{B\text{-}Eff1.NO3\text{-}N} = 0$ H_1: $\mu_{In.NO3\text{-}N} - \mu_{B\text{-}Eff1.NO3\text{-}N} \neq 0$	H_0: $\mu_{In.NO3\text{-}N} - \mu_{B\text{-}Eff2.NO3\text{-}N} = 0$ H_1: $\mu_{In.NO3\text{-}N} - \mu_{B\text{-}Eff2.NO3\text{-}N} \neq 0$	H_0: $\mu_{In.NO3\text{-}N} - \mu_{B\text{-}Eff3.NO3\text{-}N} = 0$ H_1: $\mu_{In.NO3\text{-}N} - \mu_{B\text{-}Eff3.NO3\text{-}N} \neq 0$
11. Total Nitrogen	H_0: $\mu_{In.TN} - \mu_{B\text{-}Eff1.TN} = 0$ H_1: $\mu_{In.TN} - \mu_{B\text{-}Eff1.TN} \neq 0$	H_0: $\mu_{In.TN} - \mu_{B\text{-}Eff2.TN} = 0$ H_1: $\mu_{In.TN} - \mu_{B\text{-}Eff2.TN} \neq 0$	H_0: $\mu_{In.TN} - \mu_{B\text{-}Eff3.TN} = 0$ H_1: $\mu_{In.TN} - \mu_{B\text{-}Eff3.TN} \neq 0$
12. Total Organic Carbon	H_0: $\mu_{In.TOC} - \mu_{B\text{-}Eff1.TOC} = 0$ H_1: $\mu_{In.TOC} - \mu_{B\text{-}Eff1.TOC} \neq 0$	H_0: $\mu_{In.TOC} - \mu_{B\text{-}Eff2.TOC} = 0$ H_1: $\mu_{In.TOC} - \mu_{B\text{-}Eff2.TOC} \neq 0$	H_0: $\mu_{In.TOC} - \mu_{B\text{-}Eff3.TOC} = 0$ H_1: $\mu_{In.TOC} - \mu_{B\text{-}Eff3.TOC} \neq 0$
13. Total Phosphorus	H_0: $\mu_{In.TP} - \mu_{B\text{-}Eff1.TP} = 0$ H_1: $\mu_{In.TP} - \mu_{B\text{-}Eff1.TP} \neq 0$	H_0: $\mu_{In.TP} - \mu_{B\text{-}Eff2.TP} = 0$ H_1: $\mu_{In.TP} - \mu_{B\text{-}Eff2.TP} \neq 0$	H_0: $\mu_{In.TP} - \mu_{B\text{-}Eff3.TP} = 0$ H_1: $\mu_{In.TP} - \mu_{B\text{-}Eff3.TP} \neq 0$

C. Phase II, Without worms

Observed Parameters	Layer 1	Layer 2	Layer 3
1. Temperature	H_0: $\mu_{In.T} - \mu_{C\text{-}Eff1.T} = 0$ H_1: $\mu_{In.T} - \mu_{C\text{-}Eff1.T} \neq 0$	H_0: $\mu_{In.T} - \mu_{C\text{-}Eff2.T} = 0$ H_1: $\mu_{In.T} - \mu_{C\text{-}Eff2.T} \neq 0$	H_0: $\mu_{In.T} - \mu_{C\text{-}Eff3.T} = 0$ H_1: $\mu_{In.T} - \mu_{C\text{-}Eff3.T} \neq 0$
2. pH	H_0: $\mu_{In.pH} - \mu_{C\text{-}Eff1.pH} = 0$ H_1: $\mu_{In.pH} - \mu_{C\text{-}Eff1.pH} \neq 0$	H_0: $\mu_{In.pH} - \mu_{C\text{-}Eff2.TpH} = 0$ H_1: $\mu_{In.pH} - \mu_{C\text{-}Eff2.TpH} \neq 0$	H_0: $\mu_{In.pH} - \mu_{C\text{-}Eff3.TpH} = 0$ H_1: $\mu_{In.pH} - \mu_{C\text{-}Eff3.TpH} \neq 0$
3. Conductivity	H_0: $\mu_{In.C} - \mu_{C\text{-}Eff1.C} = 0$ H_1: $\mu_{In.C} - \mu_{C\text{-}Eff1.C} \neq 0$	H_0: $\mu_{In.C} - \mu_{C\text{-}Eff2.C} = 0$ H_1: $\mu_{In.C} - \mu_{B\text{-}Eff2.C} \neq 0$	H_0: $\mu_{In.C} - \mu_{C\text{-}Eff3.C} = 0$ H_1: $\mu_{In.C} - \mu_{C\text{-}Eff3.C} \neq 0$
4. Turbidity	H_0: $\mu_{In.Tur} - \mu_{C\text{-}Eff1.Tur} = 0$ H_1: $\mu_{In.Tur} - \mu_{C\text{-}Eff1.Tur} \neq 0$	H_0: $\mu_{In.Tur} - \mu_{B\text{-}Eff2.Tur} = 0$ H_1: $\mu_{In.Tur} - \mu_{B\text{-}Eff2.Tur} \neq 0$	H_0: $\mu_{In.Tur} - \mu_{C\text{-}Eff3.Tur} = 0$ H_1: $\mu_{In.Tur} - \mu_{C\text{-}Eff3.Tur} \neq 0$
5. Total Suspended Solids	H_0: $\mu_{In.TSS} - \mu_{C\text{-}Eff1.TSS} = 0$ H_1: $\mu_{In.TSS} - \mu_{C\text{-}Eff1.TSS} \neq 0$	H_0: $\mu_{In.TSS} - \mu_{B\text{-}Eff2.TSS} = 0$ H_1: $\mu_{In.TSS} - \mu_{B\text{-}Eff2.TSS} \neq 0$	H_0: $\mu_{In.TSS} - \mu_{C\text{-}Eff3.TSS} = 0$ H_1: $\mu_{In.TSS} - \mu_{C\text{-}Eff3.TSS} \neq 0$
6. Dissolved Oxygen	H_0: $\mu_{In.DO} - \mu_{C\text{-}Eff1.DO} = 0$ H_1: $\mu_{In.DO} - \mu_{C\text{-}Eff1.DO} \neq 0$	H_0: $\mu_{In.DO} - \mu_{B\text{-}Eff2.DO} = 0$ H_1: $\mu_{In.DO} - \mu_{B\text{-}Eff2.DO} \neq 0$	H_0: $\mu_{In.DO} - \mu_{C\text{-}Eff3.DO} = 0$ H_1: $\mu_{In.DO} - \mu_{C\text{-}Eff3.DO} \neq 0$
7. Chemical Oxygen Demand	H_0: $\mu_{In.COD} - \mu_{C\text{-}Eff1.COD} = 0$ H_1: $\mu_{In.COD} - \mu_{C\text{-}Eff1.COD} \neq 0$	H_0: $\mu_{In.COD} - \mu_{B\text{-}Eff2.COD} = 0$ H_1: $\mu_{In.COD} - \mu_{B\text{-}Eff2.COD} \neq 0$	H_0: $\mu_{In.COD} - \mu_{C\text{-}Eff3.COD} = 0$ H_1: $\mu_{In.COD} - \mu_{C\text{-}Eff3.COD} \neq 0$
8. Ammonium Nitrogen	H_0: $\mu_{In.NH3\text{-}N} - \mu_{C\text{-}Eff1.NH3\text{-}N} = 0$ H_1: $\mu_{In.NH3\text{-}N} - \mu_{C\text{-}Eff1.NH3\text{-}N} \neq 0$	H_0: $\mu_{In.NH3\text{-}N} - \mu_{B\text{-}Eff2.NH3\text{-}N} = 0$ H_1: $\mu_{In.NH3\text{-}N} - \mu_{B\text{-}Eff2.NH3\text{-}N} \neq 0$	H_0: $\mu_{In.NH3\text{-}N} - \mu_{C\text{-}Eff3.NH3\text{-}N} = 0$ H_1: $\mu_{In.NH3\text{-}N} - \mu_{C\text{-}Eff3.NH3\text{-}N} \neq 0$
9. Nitrite Nitrogen	H_0: $\mu_{In.NO2\text{-}N} - \mu_{C\text{-}Eff1.NO2\text{-}N} = 0$ H_1: $\mu_{In.NO2\text{-}N} - \mu_{C\text{-}Eff1.NO2\text{-}N} \neq 0$	H_0: $\mu_{In.NO2\text{-}N} - \mu_{B\text{-}Eff2.NO2\text{-}N} = 0$ H_1: $\mu_{In.NO2\text{-}N} - \mu_{B\text{-}Eff2.NO2\text{-}N} \neq 0$	H_0: $\mu_{In.NO2\text{-}N} - \mu_{C\text{-}Eff3.NO2\text{-}N} = 0$ H_1: $\mu_{In.NO2\text{-}N} - \mu_{C\text{-}Eff3.NO2\text{-}N} \neq 0$
10. Nitrate Nitrogen	H_0: $\mu_{In.NO3\text{-}N} - \mu_{C\text{-}Eff1.NO3\text{-}N} = 0$ H_1: $\mu_{In.NO3\text{-}N} - \mu_{C\text{-}Eff1.NO3\text{-}N} \neq 0$	H_0: $\mu_{In.NO3\text{-}N} - \mu_{B\text{-}Eff2.NO3\text{-}N} = 0$ H_1: $\mu_{In.NO3\text{-}N} - \mu_{B\text{-}Eff2.NO3\text{-}N} \neq 0$	H_0: $\mu_{In.NO3\text{-}N} - \mu_{C\text{-}Eff3.NO3\text{-}N} = 0$ H_1: $\mu_{In.NO3\text{-}N} - \mu_{C\text{-}Eff3.NO3\text{-}N} \neq 0$
11. Total Nitrogen	H_0: $\mu_{In.TN} - \mu_{C\text{-}Eff1.TN} = 0$ H_1: $\mu_{In.TN} - \mu_{C\text{-}Eff1.TN} \neq 0$	H_0: $\mu_{In.TN} - \mu_{B\text{-}Eff2.TN} = 0$ H_1: $\mu_{In.TN} - \mu_{B\text{-}Eff2.TN} \neq 0$	H_0: $\mu_{In.TN} - \mu_{C\text{-}Eff3.TN} = 0$ H_1: $\mu_{In.TN} - \mu_{C\text{-}Eff3.TN} \neq 0$
12. Total Organic Carbon	H_0: $\mu_{In.TOC} - \mu_{C\text{-}Eff1.TOC} = 0$ H_1: $\mu_{In.TOC} - \mu_{C\text{-}Eff1.TOC} \neq 0$	H_0: $\mu_{In.TOC} - \mu_{B\text{-}Eff2.TOC} = 0$ H_1: $\mu_{In.TOC} - \mu_{B\text{-}Eff2.TOC} \neq 0$	H_0: $\mu_{In.TOC} - \mu_{C\text{-}Eff3.TOC} = 0$ H_1: $\mu_{In.TOC} - \mu_{C\text{-}Eff3.TOC} \neq 0$
13. Total Phosphorus	H_0: $\mu_{In.TP} - \mu_{C\text{-}Eff1.TP} = 0$ H_1: $\mu_{In.TP} - \mu_{C\text{-}Eff1.TP} \neq 0$	H_0: $\mu_{In.TP} - \mu_{B\text{-}Eff2.TP} = 0$ H_1: $\mu_{In.TP} - \mu_{B\text{-}Eff2.TP} \neq 0$	H_0: $\mu_{In.TP} - \mu_{C\text{-}Eff3.TP} = 0$ H_1: $\mu_{In.TP} - \mu_{C\text{-}Eff3.TP} \neq 0$

D. Phase II, With worms

Observed Parameters	Layer 1	Layer 2	Layer 3
1. Temperature	H_0: $\mu_{In.T} - \mu_{D\text{-}Eff1.T} = 0$ H_1: $\mu_{In.T} - \mu_{D\text{-}Eff1.T} \neq 0$	H_0: $\mu_{In.T} - \mu_{D\text{-}Eff2.T} = 0$ H_1: $\mu_{In.T} - \mu_{D\text{-}Eff2.T} \neq 0$	H_0: $\mu_{In.T} - \mu_{D\text{-}Eff3.T} = 0$ H_1: $\mu_{In.T} - \mu_{D\text{-}Eff3.T} \neq 0$
2. pH	H_0: $\mu_{In.pH} - \mu_{D\text{-}Eff1.pH} = 0$ H_1: $\mu_{In.pH} - \mu_{D\text{-}Eff1.pH} \neq 0$	H_0: $\mu_{In.pH} - \mu_{D\text{-}Eff2.TpH} = 0$ H_1: $\mu_{In.pH} - \mu_{D\text{-}Eff2.TpH} \neq 0$	H_0: $\mu_{In.pH} - \mu_{D\text{-}Eff3.TpH} = 0$ H_1: $\mu_{In.pH} - \mu_{D\text{-}Eff3.TpH} \neq 0$
3. Conductivity	H_0: $\mu_{In.C} - \mu_{D\text{-}Eff1.C} = 0$ H_1: $\mu_{In.C} - \mu_{D\text{-}Eff1.C} \neq 0$	H_0: $\mu_{In.C} - \mu_{D\text{-}Eff2.C} = 0$ H_1: $\mu_{In.C} - \mu_{D\text{-}Eff2.C} \neq 0$	H_0: $\mu_{In.C} - \mu_{D\text{-}Eff3.C} = 0$ H_1: $\mu_{In.C} - \mu_{D\text{-}Eff3.C} \neq 0$
4. Turbidity	H_0: $\mu_{In.Tur} - \mu_{D\text{-}Eff1.Tur} = 0$ H_1: $\mu_{In.Tur} - \mu_{D\text{-}Eff1.Tur} \neq 0$	H_0: $\mu_{In.Tur} - \mu_{D\text{-}Eff2.Tur} = 0$ H_1: $\mu_{In.Tur} - \mu_{D\text{-}Eff2.Tur} \neq 0$	H_0: $\mu_{In.Tur} - \mu_{D\text{-}Eff3.Tur} = 0$ H_1: $\mu_{In.Tur} - \mu_{D\text{-}Eff3.Tur} \neq 0$
5. Total Suspended Solids	H_0: $\mu_{In.TSS} - \mu_{D\text{-}Eff1.TSS} = 0$ H_1: $\mu_{In.TSS} - \mu_{D\text{-}Eff1.TSS} \neq 0$	H_0: $\mu_{In.TSS} - \mu_{D\text{-}Eff2.TSS} = 0$ H_1: $\mu_{In.TSS} - \mu_{D\text{-}Eff2.TSS} \neq 0$	H_0: $\mu_{In.TSS} - \mu_{D\text{-}Eff3.TSS} = 0$ H_1: $\mu_{In.TSS} - \mu_{D\text{-}Eff3.TSS} \neq 0$
6. Dissolved Oxygen	H_0: $\mu_{In.DO} - \mu_{D\text{-}Eff1.DO} = 0$ H_1: $\mu_{In.DO} - \mu_{D\text{-}Eff1.DO} \neq 0$	H_0: $\mu_{In.DO} - \mu_{D\text{-}Eff2.DO} = 0$ H_1: $\mu_{In.DO} - \mu_{D\text{-}Eff2.DO} \neq 0$	H_0: $\mu_{In.DO} - \mu_{D\text{-}Eff3.DO} = 0$ H_1: $\mu_{In.DO} - \mu_{D\text{-}Eff3.DO} \neq 0$
7. Chemical Oxygen Demand	H_0: $\mu_{In.COD} - \mu_{D\text{-}Eff1.COD} = 0$ H_1: $\mu_{In.COD} - \mu_{D\text{-}Eff1.COD} \neq 0$	H_0: $\mu_{In.COD} - \mu_{D\text{-}Eff2.COD} = 0$ H_1: $\mu_{In.COD} - \mu_{D\text{-}Eff2.COD} \neq 0$	H_0: $\mu_{In.COD} - \mu_{D\text{-}Eff3.COD} = 0$ H_1: $\mu_{In.COD} - \mu_{D\text{-}Eff3.COD} \neq 0$
8. Ammonium Nitrogen	H_0: $\mu_{In.NH3\text{-}N} - \mu_{D\text{-}Eff1.NH3\text{-}N} = 0$ H_1: $\mu_{In.NH3\text{-}N} - \mu_{C\text{-}Eff1.NH3\text{-}N} \neq 0$	H_0: $\mu_{In.NH3\text{-}N} - \mu_{D\text{-}Eff2.NH3\text{-}N} = 0$ H_1: $\mu_{In.NH3\text{-}N} - \mu_{D\text{-}Eff2.NH3\text{-}N} \neq 0$	H_0: $\mu_{In.NH3\text{-}N} - \mu_{D\text{-}Eff3.NH3\text{-}N} = 0$ H_1: $\mu_{In.NH3\text{-}N} - \mu_{D\text{-}Eff3.NH3\text{-}N} \neq 0$
9. Nitrite Nitrogen	H_0: $\mu_{In.NO2\text{-}N} - \mu_{C\text{-}Eff1.NO2\text{-}N} = 0$ H_1: $\mu_{In.NO2\text{-}N} - \mu_{D\text{-}Eff1.NO2\text{-}N} \neq 0$	H_0: $\mu_{In.NO2\text{-}N} - \mu_{D\text{-}Eff2.NO2\text{-}N} = 0$ H_1: $\mu_{In.NO2\text{-}N} - \mu_{D\text{-}Eff2.NO2\text{-}N} \neq 0$	H_0: $\mu_{In.NO2\text{-}N} - \mu_{D\text{-}Eff3.NO2\text{-}N} = 0$ H_1: $\mu_{In.NO2\text{-}N} - \mu_{D\text{-}Eff3.NO2\text{-}N} \neq 0$
10. Nitrate Nitrogen	H_0: $\mu_{In.NO3\text{-}N} - \mu_{D\text{-}Eff1.NO3\text{-}N} = 0$ H_1: $\mu_{In.NO3\text{-}N} - \mu_{D\text{-}Eff1.NO3\text{-}N} \neq 0$	H_0: $\mu_{In.NO3\text{-}N} - \mu_{D\text{-}Eff2.NO3\text{-}N} = 0$ H_1: $\mu_{In.NO3\text{-}N} - \mu_{D\text{-}Eff2.NO3\text{-}N} \neq 0$	H_0: $\mu_{In.NO3\text{-}N} - \mu_{D\text{-}Eff3.NO3\text{-}N} = 0$ H_1: $\mu_{In.NO3\text{-}N} - \mu_{D\text{-}Eff3.NO3\text{-}N} \neq 0$
11. Total Nitrogen	H_0: $\mu_{In.TN} - \mu_{D\text{-}Eff1.TN} = 0$ H_1: $\mu_{In.TN} - \mu_{D\text{-}Eff1.TN} \neq 0$	H_0: $\mu_{In.TN} - \mu_{D\text{-}Eff2.TN} = 0$ H_1: $\mu_{In.TN} - \mu_{D\text{-}Eff2.TN} \neq 0$	H_0: $\mu_{In.TN} - \mu_{D\text{-}Eff3.TN} = 0$ H_1: $\mu_{In.TN} - \mu_{D\text{-}Eff3.TN} \neq 0$
12. Total Organic Carbon	H_0: $\mu_{In.TOC} - \mu_{D\text{-}Eff1.TOC} = 0$ H_1: $\mu_{In.TOC} - \mu_{D\text{-}Eff1.TOC} \neq 0$	H_0: $\mu_{In.TOC} - \mu_{D\text{-}Eff2.TOC} = 0$ H_1: $\mu_{In.TOC} - \mu_{D\text{-}Eff2.TOC} \neq 0$	H_0: $\mu_{In.TOC} - \mu_{D\text{-}Eff3.TOC} = 0$ H_1: $\mu_{In.TOC} - \mu_{D\text{-}Eff3.TOC} \neq 0$
13. Total Phosphorus	H_0: $\mu_{In.TP} - \mu_{D\text{-}Eff1.TP} = 0$ H_1: $\mu_{In.TP} - \mu_{D\text{-}Eff1.TP} \neq 0$	H_0: $\mu_{In.TP} - \mu_{D\text{-}Eff2.TP} = 0$ H_1: $\mu_{In.TP} - \mu_{D\text{-}Eff2.TP} \neq 0$	H_0: $\mu_{In.TP} - \mu_{D\text{-}Eff3.TP} = 0$ H_1: $\mu_{In.TP} - \mu_{D\text{-}Eff3.TP} \neq 0$

Note: μ_A – Mean (Phase I, without worms), μ_B – Mean (Phase I, with worms), μ_C – Mean (Phase II, without worms), μ_D – Mean (Phase II, with worms), H_0 – Null hypothesis and H_1 – Alternative hypothesis

Appendix 4.7

2. The effect of worms (comparison between 'without worms' and 'with worms')

Phase I

Observed Parameters	Layer 1	Layer 2	Layer 3
1. Temperature	H_0: $\mu_{A\text{-}Eff1.T} = \mu_{B\text{-}Eff1.T}$ H_1: $\mu_{A\text{-}Eff1.T} \neq \mu_{B\text{-}Eff1.T}$	H_0: $\mu_{A\text{-}Eff2.T} = \mu_{B\text{-}Eff2.T}$ H_1: $\mu_{A\text{-}Eff2.T} \neq \mu_{B\text{-}Eff2.T}$	H_0: $\mu_{A\text{-}Eff3.T} = \mu_{B\text{-}Eff3.T}$ H_1: $\mu_{A\text{-}Eff3.T} \neq \mu_{B\text{-}Eff3.T}$
2. pH	H_0: $\mu_{A\text{-}Eff1.pH} = \mu_{B\text{-}Eff1.pH}$ H_1: $\mu_{A\text{-}Eff1.pH} \neq \mu_{B\text{-}Eff1.pH}$	H_0: $\mu_{A\text{-}Eff2.pH} = \mu_{B\text{-}Eff2.pH}$ H_1: $\mu_{A\text{-}Eff2.pH} \neq \mu_{B\text{-}Eff2.pH}$	H_0: $\mu_{A\text{-}Eff3.pH} = \mu_{B\text{-}Eff3.pH}$ H_1: $\mu_{A\text{-}Eff3.pH} \neq \mu_{B\text{-}Eff3.pH}$
3. Conductivity	H_0: $\mu_{A\text{-}Eff1.C} = \mu_{B\text{-}Eff1.C}$ H_1: $\mu_{A\text{-}Eff1.C} \neq \mu_{B\text{-}Eff1.C}$	H_0: $\mu_{A\text{-}Eff2.C} = \mu_{B\text{-}Eff2.C}$ H_1: $\mu_{A\text{-}Eff2.C} \neq \mu_{B\text{-}Eff2.C}$	H_0: $\mu_{A\text{-}Eff3.C} = \mu_{B\text{-}Eff3.C}$ H_1: $\mu_{A\text{-}Eff3.C} \neq \mu_{B\text{-}Eff3.C}$
4. Turbidity	H_0: $\mu_{A\text{-}Eff1.Tur} = \mu_{B\text{-}Eff1.Tur}$ H_1: $\mu_{A\text{-}Eff1.Tur} \neq \mu_{B\text{-}Eff1.Tur}$	H_0: $\mu_{A\text{-}Eff2.Tur} = \mu_{B\text{-}Eff2.Tur}$ H_1: $\mu_{A\text{-}Eff2.Tur} \neq \mu_{B\text{-}Eff2.Tur}$	H_0: $\mu_{A\text{-}Eff3.Tur} = \mu_{B\text{-}Eff3.Tur}$ H_1: $\mu_{A\text{-}Eff3.Tur} \neq \mu_{B\text{-}Eff3.Tur}$
5. Total Suspended Solids	H_0: $\mu_{A\text{-}Eff1.TSS} = \mu_{B\text{-}Eff1.TSS}$ H_1: $\mu_{A\text{-}Eff1.TSS} \neq \mu_{B\text{-}Eff1.TSS}$	H_0: $\mu_{A\text{-}Eff2.TSS} = \mu_{B\text{-}Eff2.TSS}$ H_1: $\mu_{A\text{-}Eff2.TSS} \neq \mu_{B\text{-}Eff2.TSS}$	H_0: $\mu_{A\text{-}Eff3.TSS} = \mu_{B\text{-}Eff3.TSS}$ H_1: $\mu_{A\text{-}Eff3.TSS} \neq \mu_{B\text{-}Eff3.TSS}$
6. Dissolved Oxygen	H_0: $\mu_{A\text{-}Eff1.DO} = \mu_{B\text{-}Eff1.DO}$ H_1: $\mu_{A\text{-}Eff1.DO} \neq \mu_{B\text{-}Eff1.DO}$	H_0: $\mu_{A\text{-}Eff2.DO} = \mu_{B\text{-}Eff2.DO}$ H_1: $\mu_{A\text{-}Eff2.DO} \neq \mu_{B\text{-}Eff2.DO}$	H_0: $\mu_{A\text{-}Eff3.DO} = \mu_{B\text{-}Eff3.DO}$ H_1: $\mu_{A\text{-}Eff3.DO} \neq \mu_{B\text{-}Eff3.DO}$
7. Chemical Oxygen Demand	H_0: $\mu_{A\text{-}Eff1.COD} = \mu_{B\text{-}Eff1.COD}$ H_1: $\mu_{A\text{-}Eff1.COD} \neq \mu_{B\text{-}Eff1.COD}$	H_0: $\mu_{A\text{-}Eff2.COD} = \mu_{B\text{-}Eff2.COD}$ H_1: $\mu_{A\text{-}Eff2.COD} \neq \mu_{B\text{-}Eff2.COD}$	H_0: $\mu_{A\text{-}Eff3.COD} = \mu_{B\text{-}Eff3.COD}$ H_1: $\mu_{A\text{-}Eff3.COD} \neq \mu_{B\text{-}Eff3.COD}$
8. Ammonium Nitrogen	H_0: $\mu_{A\text{-}Eff1.NH3\text{-}N} = \mu_{B\text{-}Eff1.NH3\text{-}N}$ H_1: $\mu_{A\text{-}Eff1.NH3\text{-}N} \neq \mu_{B\text{-}Eff1.NH3\text{-}N}$	H_0: $\mu_{A\text{-}Eff2.NH3\text{-}N} = \mu_{B\text{-}Eff2.NH3\text{-}N}$ H_1: $\mu_{A\text{-}Eff2.NH3\text{-}N} \neq \mu_{B\text{-}Eff2.NH3\text{-}N}$	H_0: $\mu_{A\text{-}Eff3.NH3\text{-}N} = \mu_{B\text{-}Eff3.NH3\text{-}N}$ H_1: $\mu_{A\text{-}Eff3.NH3\text{-}N} \neq \mu_{B\text{-}Eff3.NH3\text{-}N}$
9. Nitrite Nitrogen	H_0: $\mu_{A\text{-}Eff1.NO2\text{-}N} = \mu_{B\text{-}Eff1.NO2\text{-}N}$ H_1: $\mu_{A\text{-}Eff1.NO2\text{-}N} \neq \mu_{B\text{-}Eff1.NO2\text{-}N}$	H_0: $\mu_{A\text{-}Eff2.NO2\text{-}N} = \mu_{B\text{-}Eff2.NO2\text{-}N}$ H_1: $\mu_{A\text{-}Eff2.NO2\text{-}N} \neq \mu_{B\text{-}Eff2.NO2\text{-}N}$	H_0: $\mu_{A\text{-}Eff3.NO2\text{-}N} = \mu_{B\text{-}Eff3.NO2\text{-}N}$ H_1: $\mu_{A\text{-}Eff3.NO2\text{-}N} \neq \mu_{B\text{-}Eff3.NO2\text{-}N}$
10. Nitrate Nitrogen	H_0: $\mu_{A\text{-}Eff1.NO3\text{-}N} = \mu_{B\text{-}Eff1.NO3\text{-}N}$ H_1: $\mu_{A\text{-}Eff1.NO3\text{-}N} \neq \mu_{B\text{-}Eff1.NO3\text{-}N}$	H_0: $\mu_{A\text{-}Eff2.NO3\text{-}N} = \mu_{B\text{-}Eff2.NO3\text{-}N}$ H_1: $\mu_{A\text{-}Eff2.NO3\text{-}N} \neq \mu_{B\text{-}Eff2.NO3\text{-}N}$	H_0: $\mu_{A\text{-}Eff3.NO3\text{-}N} = \mu_{B\text{-}Eff3.NO3\text{-}N}$ H_1: $\mu_{A\text{-}Eff3.NO3\text{-}N} \neq \mu_{B\text{-}Eff3.NO3\text{-}N}$
11. Total Nitrogen	H_0: $\mu_{A\text{-}Eff1.TN} = \mu_{B\text{-}Eff1.TN}$ H_1: $\mu_{A\text{-}Eff1.TN} \neq \mu_{B\text{-}Eff1.TN}$	H_0: $\mu_{A\text{-}Eff2.TN} = \mu_{B\text{-}Eff2.TN}$ H_1: $\mu_{A\text{-}Eff2.TN} \neq \mu_{B\text{-}Eff2.TN}$	H_0: $\mu_{A\text{-}Eff3.TN} = \mu_{B\text{-}Eff3.TN}$ H_1: $\mu_{A\text{-}Eff3.TN} \neq \mu_{B\text{-}Eff3.TN}$
12. Total Organic Carbon	H_0: $\mu_{A\text{-}Eff1.TOC} = \mu_{B\text{-}Eff1.TOC}$ H_1: $\mu_{A\text{-}Eff1.TOC} \neq \mu_{B\text{-}Eff1.TOC}$	H_0: $\mu_{A\text{-}Eff2.TOC} = \mu_{B\text{-}Eff2.TOC}$ H_1: $\mu_{A\text{-}Eff2.TOC} \neq \mu_{B\text{-}Eff2.TOC}$	H_0: $\mu_{A\text{-}Eff3.TOC} = \mu_{B\text{-}Eff3.TOC}$ H_1: $\mu_{A\text{-}Eff3.TOC} \neq \mu_{B\text{-}Eff3.TOC}$
13. Total Phosphorus	H_0: $\mu_{A\text{-}Eff1.TP} = \mu_{B\text{-}Eff1.TP}$ H_1: $\mu_{A\text{-}Eff1.TP} \neq \mu_{B\text{-}Eff1.TP}$	H_0: $\mu_{A\text{-}Eff2.TP} = \mu_{B\text{-}Eff2.TP}$ H_1: $\mu_{A\text{-}Eff2.TP} \neq \mu_{B\text{-}Eff2.TP}$	H_0: $\mu_{A\text{-}Eff3.TP} = \mu_{B\text{-}Eff3.TP}$ H_1: $\mu_{A\text{-}Eff3.TP} \neq \mu_{B\text{-}Eff3.TP}$

Phase II

Observed Parameters	Layer 1	Layer 2	Layer 3
1. Temperature	H_0: $\mu_{C\text{-}Eff1.T} = \mu_{D\text{-}Eff1.T}$ H_1: $\mu_{C\text{-}Eff1.T} \neq \mu_{D\text{-}Eff1.T}$	H_0: $\mu_{C\text{-}Eff2.T} = \mu_{D\text{-}Eff2.T}$ H_1: $\mu_{C\text{-}Eff2.T} \neq \mu_{D\text{-}Eff2.T}$	H_0: $\mu_{C\text{-}Eff3.T} = \mu_{D\text{-}Eff3.T}$ H_1: $\mu_{C\text{-}Eff3.T} \neq \mu_{D\text{-}Eff3.T}$
2. pH	H_0: $\mu_{C\text{-}Eff1.pH} = \mu_{D\text{-}Eff1.pH}$ H_1: $\mu_{C\text{-}Eff1.pH} \neq \mu_{D\text{-}Eff1.pH}$	H_0: $\mu_{C\text{-}Eff2.pH} = \mu_{D\text{-}Eff2.pH}$ H_1: $\mu_{C\text{-}Eff2.pH} \neq \mu_{D\text{-}Eff2.pH}$	H_0: $\mu_{C\text{-}Eff3.pH} = \mu_{D\text{-}Eff3.pH}$ H_1: $\mu_{C\text{-}Eff3.pH} \neq \mu_{D\text{-}Eff3.pH}$
3. Conductivity	H_0: $\mu_{C\text{-}Eff1.C} = \mu_{D\text{-}Eff1.C}$ H_1: $\mu_{C\text{-}Eff1.C} \neq \mu_{D\text{-}Eff1.C}$	H_0: $\mu_{C\text{-}Eff2.C} = \mu_{D\text{-}Eff2.C}$ H_1: $\mu_{C\text{-}Eff2.C} \neq \mu_{D\text{-}Eff2.C}$	H_0: $\mu_{C\text{-}Eff3.C} = \mu_{D\text{-}Eff3.C}$ H_1: $\mu_{C\text{-}Eff3.C} \neq \mu_{D\text{-}Eff3.C}$
4. Turbidity	H_0: $\mu_{C\text{-}Eff1.Tur} = \mu_{D\text{-}Eff1.Tur}$ H_1: $\mu_{C\text{-}Eff1.Tur} \neq \mu_{D\text{-}Eff1.Tur}$	H_0: $\mu_{C\text{-}Eff2.Tur} = \mu_{D\text{-}Eff2.Tur}$ H_1: $\mu_{C\text{-}Eff2.Tur} \neq \mu_{D\text{-}Eff2.Tur}$	H_0: $\mu_{C\text{-}Eff3.Tur} = \mu_{D\text{-}Eff3.Tur}$ H_1: $\mu_{C\text{-}Eff3.Tur} \neq \mu_{D\text{-}Eff3.Tur}$
5. Total Suspended Solids	H_0: $\mu_{C\text{-}Eff1.TSS} = \mu_{D\text{-}Eff1.TSS}$ H_1: $\mu_{C\text{-}Eff1.TSS} \neq \mu_{D\text{-}Eff1.TSS}$	H_0: $\mu_{C\text{-}Eff2.TSS} = \mu_{D\text{-}Eff2.TSS}$ H_1: $\mu_{C\text{-}Eff2.TSS} \neq \mu_{D\text{-}Eff2.TSS}$	H_0: $\mu_{C\text{-}Eff3.TSS} = \mu_{D\text{-}Eff3.TSS}$ H_1: $\mu_{C\text{-}Eff3.TSS} \neq \mu_{D\text{-}Eff3.TSS}$
6. Dissolved Oxygen	H_0: $\mu_{C\text{-}Eff1.DO} = \mu_{D\text{-}Eff1.DO}$ H_1: $\mu_{C\text{-}Eff1.DO} \neq \mu_{D\text{-}Eff1.DO}$	H_0: $\mu_{C\text{-}Eff2.DO} = \mu_{D\text{-}Eff2.DO}$ H_1: $\mu_{C\text{-}Eff2.DO} \neq \mu_{D\text{-}Eff2.DO}$	H_0: $\mu_{C\text{-}Eff3.DO} = \mu_{D\text{-}Eff3.DO}$ H_1: $\mu_{C\text{-}Eff3.DO} \neq \mu_{D\text{-}Eff3.DO}$
7. Chemical Oxygen Demand	H_0: $\mu_{C\text{-}Eff1.COD} = \mu_{D\text{-}Eff1.COD}$ H_1: $\mu_{C\text{-}Eff1.COD} \neq \mu_{D\text{-}Eff1.COD}$	H_0: $\mu_{C\text{-}Eff2.COD} = \mu_{D\text{-}Eff2.COD}$ H_1: $\mu_{C\text{-}Eff2.COD} \neq \mu_{D\text{-}Eff2.COD}$	H_0: $\mu_{C\text{-}Eff3.COD} = \mu_{D\text{-}Eff3.COD}$ H_1: $\mu_{C\text{-}Eff3.COD} \neq \mu_{D\text{-}Eff3.COD}$
8. Ammonium Nitrogen	H_0: $\mu_{C\text{-}Eff1.NH3\text{-}N} = \mu_{D\text{-}Eff1.NH3\text{-}N}$ H_1: $\mu_{C\text{-}Eff1.NH3\text{-}N} \neq \mu_{D\text{-}Eff1.NH3\text{-}N}$	H_0: $\mu_{C\text{-}Eff2.NH3\text{-}N} = \mu_{D\text{-}Eff2.NH3\text{-}N}$ H_1: $\mu_{C\text{-}Eff2.NH3\text{-}N} \neq \mu_{D\text{-}Eff2.NH3\text{-}N}$	H_0: $\mu_{C\text{-}Eff3.NH3\text{-}N} = \mu_{D\text{-}Eff3.NH3\text{-}N}$ H_1: $\mu_{C\text{-}Eff3.NH3\text{-}N} \neq \mu_{D\text{-}Eff3.NH3\text{-}N}$
9. Nitrite Nitrogen	H_0: $\mu_{C\text{-}Eff1.NO2\text{-}N} = \mu_{D\text{-}Eff1.NO2\text{-}N}$ H_1: $\mu_{C\text{-}Eff1.NO2\text{-}N} \neq \mu_{D\text{-}Eff1.NO2\text{-}N}$	H_0: $\mu_{C\text{-}Eff2.NO2\text{-}N} = \mu_{D\text{-}Eff2.NO2\text{-}N}$ H_1: $\mu_{C\text{-}Eff2.NO2\text{-}N} \neq \mu_{D\text{-}Eff2.NO2\text{-}N}$	H_0: $\mu_{C\text{-}Eff3.NO2\text{-}N} = \mu_{D\text{-}Eff3.NO2\text{-}N}$ H_1: $\mu_{C\text{-}Eff3.NO2\text{-}N} \neq \mu_{D\text{-}Eff3.NO2\text{-}N}$
10. Nitrate Nitrogen	H_0: $\mu_{C\text{-}Eff1.NO3\text{-}N} = \mu_{D\text{-}Eff1.NO3\text{-}N}$ H_1: $\mu_{C\text{-}Eff1.NO3\text{-}N} \neq \mu_{D\text{-}Eff1.NO3\text{-}N}$	H_0: $\mu_{C\text{-}Eff2.NO3\text{-}N} = \mu_{D\text{-}Eff2.NO3\text{-}N}$ H_1: $\mu_{C\text{-}Eff2.NO3\text{-}N} \neq \mu_{D\text{-}Eff2.NO3\text{-}N}$	H_0: $\mu_{C\text{-}Eff3.NO3\text{-}N} = \mu_{D\text{-}Eff3.NO3\text{-}N}$ H_1: $\mu_{C\text{-}Eff3.NO3\text{-}N} \neq \mu_{D\text{-}Eff3.NO3\text{-}N}$
11. Total Nitrogen	H_0: $\mu_{C\text{-}Eff1.TN} = \mu_{D\text{-}Eff1.TN}$ H_1: $\mu_{C\text{-}Eff1.TN} \neq \mu_{D\text{-}Eff1.TN}$	H_0: $\mu_{C\text{-}Eff2.TN} = \mu_{D\text{-}Eff2.TN}$ H_1: $\mu_{C\text{-}Eff2.TN} \neq \mu_{D\text{-}Eff2.TN}$	H_0: $\mu_{C\text{-}Eff3.TN} = \mu_{D\text{-}Eff3.TN}$ H_1: $\mu_{C\text{-}Eff3.TN} \neq \mu_{D\text{-}Eff3.TN}$
12. Total Organic Carbon	H_0: $\mu_{C\text{-}Eff1.TOC} = \mu_{D\text{-}Eff1.TOC}$ H_1: $\mu_{C\text{-}Eff1.TOC} \neq \mu_{D\text{-}Eff1.TOC}$	H_0: $\mu_{C\text{-}Eff2.TOC} = \mu_{D\text{-}Eff2.TOC}$ H_1: $\mu_{C\text{-}Eff2.TOC} \neq \mu_{D\text{-}Eff2.TOC}$	H_0: $\mu_{C\text{-}Eff3.TOC} = \mu_{D\text{-}Eff3.TOC}$ H_1: $\mu_{C\text{-}Eff3.TOC} \neq \mu_{D\text{-}Eff3.TOC}$
13. Total Phosphorus	H_0: $\mu_{C\text{-}Eff1.TP} = \mu_{D\text{-}Eff1.TP}$ H_1: $\mu_{C\text{-}Eff1.TP} \neq \mu_{D\text{-}Eff1.TP}$	H_0: $\mu_{C\text{-}Eff2.TP} = \mu_{D\text{-}Eff2.TP}$ H_1: $\mu_{C\text{-}Eff2.TP} \neq \mu_{D\text{-}Eff2.TP}$	H_0: $\mu_{C\text{-}Eff3.TP} = \mu_{D\text{-}Eff3.TP}$ H_1: $\mu_{C\text{-}Eff3.TP} \neq \mu_{D\text{-}Eff3.TP}$

3. The effect of soil type (comparison between 'soil type 1' in Phase I and 'soil type 2' in Phase II)

'Without worms'

Observed Parameters	Layer 1	Layer 2	Layer 3
1. Temperature	H_0: $\mu_{A\text{-}Eff1.T} = \mu_{C\text{-}Eff1.T}$ H_1: $\mu_{A\text{-}Eff1.T} \neq \mu_{C\text{-}Eff1.T}$	H_0: $\mu_{A\text{-}Eff2.T} = \mu_{C\text{-}Eff2.T}$ H_1: $\mu_{A\text{-}Eff2.T} \neq \mu_{C\text{-}Eff2.T}$	H_0: $\mu_{A\text{-}Eff3.T} = \mu_{C\text{-}Eff3.T}$ H_1: $\mu_{A\text{-}Eff3.T} \neq \mu_{C\text{-}Eff3.T}$
2. pH	H_0: $\mu_{A\text{-}Eff1.pH} = \mu_{C\text{-}Eff1.pH}$ H_1: $\mu_{A\text{-}Eff1.pH} \neq \mu_{C\text{-}Eff1.pH}$	H_0: $\mu_{A\text{-}Eff2.pH} = \mu_{C\text{-}Eff2.pH}$ H_1: $\mu_{A\text{-}Eff2.pH} \neq \mu_{C\text{-}Eff2.pH}$	H_0: $\mu_{A\text{-}Eff3.pH} = \mu_{C\text{-}Eff3.pH}$ H_1: $\mu_{A\text{-}Eff3.pH} \neq \mu_{C\text{-}Eff3.pH}$
3. Conductivity	H_0: $\mu_{A\text{-}Eff1.C} = \mu_{C\text{-}Eff1.C}$ H_1: $\mu_{A\text{-}Eff1.C} \neq \mu_{C\text{-}Eff1.C}$	H_0: $\mu_{A\text{-}Eff2.C} = \mu_{C\text{-}Eff2.C}$ H_1: $\mu_{A\text{-}Eff2.C} \neq \mu_{C\text{-}Eff2.C}$	H_0: $\mu_{A\text{-}Eff3.C} = \mu_{C\text{-}Eff3.C}$ H_1: $\mu_{A\text{-}Eff3.C} \neq \mu_{C\text{-}Eff3.C}$
4. Turbidity	H_0: $\mu_{A\text{-}Eff1.Tur} = \mu_{C\text{-}Eff1.Tur}$ H_1: $\mu_{A\text{-}Eff1.Tur} \neq \mu_{C\text{-}Eff1.Tur}$	H_0: $\mu_{A\text{-}Eff2.Tur} = \mu_{C\text{-}Eff2.Tur}$ H_1: $\mu_{A\text{-}Eff2.Tur} \neq \mu_{C\text{-}Eff2.Tur}$	H_0: $\mu_{A\text{-}Eff3.Tur} = \mu_{C\text{-}Eff3.Tur}$ H_1: $\mu_{A\text{-}Eff3.Tur} \neq \mu_{C\text{-}Eff3.Tur}$
5. Total Suspended Solids	H_0: $\mu_{A\text{-}Eff1.TSS} = \mu_{C\text{-}Eff1.TSS}$ H_1: $\mu_{A\text{-}Eff1.TSS} \neq \mu_{C\text{-}Eff1.TSS}$	H_0: $\mu_{A\text{-}Eff2.TSS} = \mu_{C\text{-}Eff2.TSS}$ H_1: $\mu_{A\text{-}Eff2.TSS} \neq \mu_{C\text{-}Eff2.TSS}$	H_0: $\mu_{A\text{-}Eff3.TSS} = \mu_{C\text{-}Eff3.TSS}$ H_1: $\mu_{A\text{-}Eff3.TSS} \neq \mu_{C\text{-}Eff3.TSS}$
6. Dissolved Oxygen	H_0: $\mu_{A\text{-}Eff1.DO} = \mu_{C\text{-}Eff1.DO}$ H_1: $\mu_{A\text{-}Eff1.DO} \neq \mu_{C\text{-}Eff1.DO}$	H_0: $\mu_{A\text{-}Eff2.DO} = \mu_{C\text{-}Eff2.DO}$ H_1: $\mu_{A\text{-}Eff2.DO} \neq \mu_{C\text{-}Eff2.DO}$	H_0: $\mu_{A\text{-}Eff3.DO} = \mu_{C\text{-}Eff3.DO}$ H_1: $\mu_{A\text{-}Eff3.DO} \neq \mu_{C\text{-}Eff3.DO}$
7. Chemical Oxygen Demand	H_0: $\mu_{A\text{-}Eff1.COD} = \mu_{C\text{-}Eff1.COD}$ H_1: $\mu_{A\text{-}Eff1.COD} \neq \mu_{C\text{-}Eff1.COD}$	H_0: $\mu_{A\text{-}Eff2.COD} = \mu_{C\text{-}Eff2.COD}$ H_1: $\mu_{A\text{-}Eff2.COD} \neq \mu_{C\text{-}Eff2.COD}$	H_0: $\mu_{A\text{-}Eff3.COD} = \mu_{C\text{-}Eff3.COD}$ H_1: $\mu_{A\text{-}Eff3.COD} \neq \mu_{C\text{-}Eff3.COD}$
8. Ammonium Nitrogen	H_0: $\mu_{A\text{-}Eff1.NH3\text{-}N} = \mu_{C\text{-}Eff1.NH3\text{-}N}$ H_1: $\mu_{A\text{-}Eff1.NH3\text{-}N} \neq \mu_{C\text{-}Eff1.NH3\text{-}N}$	H_0: $\mu_{A\text{-}Eff2.NH3\text{-}N} = \mu_{C\text{-}Eff2.NH3\text{-}N}$ H_1: $\mu_{A\text{-}Eff2.NH3\text{-}N} \neq \mu_{C\text{-}Eff2.NH3\text{-}N}$	H_0: $\mu_{A\text{-}Eff3.NH3\text{-}N} = \mu_{C\text{-}Eff3.NH3\text{-}N}$ H_1: $\mu_{A\text{-}Eff3.NH3\text{-}N} \neq \mu_{C\text{-}Eff3.NH3\text{-}N}$
9. Nitrite Nitrogen	H_0: $\mu_{A\text{-}Eff1.NO2\text{-}N} = \mu_{C\text{-}Eff1.NO2\text{-}N}$ H_1: $\mu_{A\text{-}Eff1.NO2\text{-}N} \neq \mu_{C\text{-}Eff1.NO2\text{-}N}$	H_0: $\mu_{A\text{-}Eff2.NO2\text{-}N} = \mu_{C\text{-}Eff2.NO2\text{-}N}$ H_1: $\mu_{A\text{-}Eff2.NO2\text{-}N} \neq \mu_{C\text{-}Eff2.NO2\text{-}N}$	H_0: $\mu_{A\text{-}Eff3.NO2\text{-}N} = \mu_{C\text{-}Eff3.NO2\text{-}N}$ H_1: $\mu_{A\text{-}Eff3.NO2\text{-}N} \neq \mu_{C\text{-}Eff3.NO2\text{-}N}$
10. Nitrate Nitrogen	H_0: $\mu_{A\text{-}Eff1.NO3\text{-}N} = \mu_{C\text{-}Eff1.NO3\text{-}N}$ H_1: $\mu_{A\text{-}Eff1.NO3\text{-}N} \neq \mu_{C\text{-}Eff1.NO3\text{-}N}$	H_0: $\mu_{A\text{-}Eff2.NO3\text{-}N} = \mu_{C\text{-}Eff2.NO3\text{-}N}$ H_1: $\mu_{A\text{-}Eff2.NO3\text{-}N} \neq \mu_{C\text{-}Eff2.NO3\text{-}N}$	H_0: $\mu_{A\text{-}Eff3.NO3\text{-}N} = \mu_{C\text{-}Eff3.NO3\text{-}N}$ H_1: $\mu_{A\text{-}Eff3.NO3\text{-}N} \neq \mu_{C\text{-}Eff3.NO3\text{-}N}$
11. Total Nitrogen	H_0: $\mu_{A\text{-}Eff1.TN} = \mu_{C\text{-}Eff1.TN}$ H_1: $\mu_{A\text{-}Eff1.TN} \neq \mu_{C\text{-}Eff1.TN}$	H_0: $\mu_{A\text{-}Eff2.TN} = \mu_{C\text{-}Eff2.TN}$ H_1: $\mu_{A\text{-}Eff2.TN} \neq \mu_{C\text{-}Eff2.TN}$	H_0: $\mu_{A\text{-}Eff3.TN} = \mu_{C\text{-}Eff3.TN}$ H_1: $\mu_{A\text{-}Eff3.TN} \neq \mu_{C\text{-}Eff3.TN}$
12. Total Organic Carbon	H_0: $\mu_{A\text{-}Eff1.TOC} = \mu_{C\text{-}Eff1.TOC}$ H_1: $\mu_{A\text{-}Eff1.TOC} \neq \mu_{C\text{-}Eff1.TOC}$	H_0: $\mu_{A\text{-}Eff2.TOC} = \mu_{C\text{-}Eff2.TOC}$ H_1: $\mu_{A\text{-}Eff2.TOC} \neq \mu_{C\text{-}Eff2.TOC}$	H_0: $\mu_{A\text{-}Eff3.TOC} = \mu_{C\text{-}Eff3.TOC}$ H_1: $\mu_{A\text{-}Eff3.TOC} \neq \mu_{C\text{-}Eff3.TOC}$
13. Total Phosphorus	H_0: $\mu_{A\text{-}Eff1.TP} = \mu_{C\text{-}Eff1.TP}$ H_1: $\mu_{A\text{-}Eff1.TP} \neq \mu_{C\text{-}Eff1.TP}$	H_0: $\mu_{A\text{-}Eff2.TP} = \mu_{C\text{-}Eff2.TP}$ H_1: $\mu_{A\text{-}Eff2.TP} \neq \mu_{C\text{-}Eff2.TP}$	H_0: $\mu_{A\text{-}Eff3.TP} = \mu_{C\text{-}Eff3.TP}$ H_1: $\mu_{A\text{-}Eff3.TP} \neq \mu_{C\text{-}Eff3.TP}$

'With worms'

Observed Parameters	Layer 1	Layer 2	Layer 3
1. Temperature	H_0: $\mu_{B\text{-}Eff1.T} = \mu_{D\text{-}Eff1.T}$ H_1: $\mu_{B\text{-}Eff1.T} \neq \mu_{D\text{-}Eff1.T}$	H_0: $\mu_{B\text{-}Eff2.T} = \mu_{D\text{-}Eff2.T}$ H_1: $\mu_{B\text{-}Eff2.T} \neq \mu_{D\text{-}Eff2.T}$	H_0: $\mu_{B\text{-}Eff3.T} = \mu_{D\text{-}Eff3.T}$ H_1: $\mu_{B\text{-}Eff3.T} \neq \mu_{D\text{-}Eff3.T}$
2. pH	H_0: $\mu_{B\text{-}Eff1.pH} = \mu_{D\text{-}Eff1.pH}$ H_1: $\mu_{B\text{-}Eff1.pH} \neq \mu_{D\text{-}Eff1.pH}$	H_0: $\mu_{B\text{-}Eff2.pH} = \mu_{D\text{-}Eff2.pH}$ H_1: $\mu_{B\text{-}Eff2.pH} \neq \mu_{D\text{-}Eff2.pH}$	H_0: $\mu_{B\text{-}Eff3.pH} = \mu_{D\text{-}Eff3.pH}$ H_1: $\mu_{B\text{-}Eff3.pH} \neq \mu_{D\text{-}Eff3.pH}$
3. Conductivity	H_0: $\mu_{B\text{-}Eff1.C} = \mu_{D\text{-}Eff1.C}$ H_1: $\mu_{B\text{-}Eff1.C} \neq \mu_{D\text{-}Eff1.C}$	H_0: $\mu_{B\text{-}Eff2.C} = \mu_{D\text{-}Eff2.C}$ H_1: $\mu_{B\text{-}Eff2.C} \neq \mu_{D\text{-}Eff2.C}$	H_0: $\mu_{B\text{-}Eff3.C} = \mu_{D\text{-}Eff3.C}$ H_1: $\mu_{B\text{-}Eff3.C} \neq \mu_{D\text{-}Eff3.C}$
4. Turbidity	H_0: $\mu_{B\text{-}Eff1.Tur} = \mu_{D\text{-}Eff1.Tur}$ H_1: $\mu_{B\text{-}Eff1.Tur} \neq \mu_{D\text{-}Eff1.Tur}$	H_0: $\mu_{B\text{-}Eff2.Tur} = \mu_{D\text{-}Eff2.Tur}$ H_1: $\mu_{B\text{-}Eff2.Tur} \neq \mu_{D\text{-}Eff2.Tur}$	H_0: $\mu_{B\text{-}Eff3.Tur} = \mu_{D\text{-}Eff3.Tur}$ H_1: $\mu_{B\text{-}Eff3.Tur} \neq \mu_{D\text{-}Eff3.Tur}$
5. Total Suspended Solids	H_0: $\mu_{B\text{-}Eff1.TSS} = \mu_{D\text{-}Eff1.TSS}$ H_1: $\mu_{B\text{-}Eff1.TSS} \neq \mu_{D\text{-}Eff1.TSS}$	H_0: $\mu_{B\text{-}Eff2.TSS} = \mu_{D\text{-}Eff2.TSS}$ H_1: $\mu_{B\text{-}Eff2.TSS} \neq \mu_{D\text{-}Eff2.TSS}$	H_0: $\mu_{B\text{-}Eff3.TSS} = \mu_{D\text{-}Eff3.TSS}$ H_1: $\mu_{B\text{-}Eff3.TSS} \neq \mu_{D\text{-}Eff3.TSS}$
6. Dissolved Oxygen	H_0: $\mu_{B\text{-}Eff1.DO} = \mu_{D\text{-}Eff1.DO}$ H_1: $\mu_{B\text{-}Eff1.DO} \neq \mu_{D\text{-}Eff1.DO}$	H_0: $\mu_{B\text{-}Eff2.DO} = \mu_{D\text{-}Eff2.DO}$ H_1: $\mu_{B\text{-}Eff2.DO} \neq \mu_{D\text{-}Eff2.DO}$	H_0: $\mu_{B\text{-}Eff3.DO} = \mu_{D\text{-}Eff3.DO}$ H_1: $\mu_{B\text{-}Eff3.DO} \neq \mu_{D\text{-}Eff3.DO}$
7. Chemical Oxygen Demand	H_0: $\mu_{B\text{-}Eff1.COD} = \mu_{D\text{-}Eff1.COD}$ H_1: $\mu_{B\text{-}Eff1.COD} \neq \mu_{D\text{-}Eff1.COD}$	H_0: $\mu_{B\text{-}Eff2.COD} = \mu_{D\text{-}Eff2.COD}$ H_1: $\mu_{B\text{-}Eff2.COD} \neq \mu_{D\text{-}Eff2.COD}$	H_0: $\mu_{B\text{-}Eff3.COD} = \mu_{D\text{-}Eff3.COD}$ H_1: $\mu_{B\text{-}Eff3.COD} \neq \mu_{D\text{-}Eff3.COD}$
8. Ammonium Nitrogen	H_0: $\mu_{B\text{-}Eff1.NH3\text{-}N} = \mu_{D\text{-}Eff1.NH3\text{-}N}$ H_1: $\mu_{B\text{-}Eff1.NH3\text{-}N} \neq \mu_{D\text{-}Eff1.NH3\text{-}N}$	H_0: $\mu_{B\text{-}Eff2.NH3\text{-}N} = \mu_{D\text{-}Eff2.NH3\text{-}N}$ H_1: $\mu_{B\text{-}Eff2.NH3\text{-}N} \neq \mu_{D\text{-}Eff2.NH3\text{-}N}$	H_0: $\mu_{B\text{-}Eff3.NH3\text{-}N} = \mu_{D\text{-}Eff3.NH3\text{-}N}$ H_1: $\mu_{B\text{-}Eff3.NH3\text{-}N} \neq \mu_{D\text{-}Eff3.NH3\text{-}N}$
9. Nitrite Nitrogen	H_0: $\mu_{B\text{-}Eff1.NO2\text{-}N} = \mu_{D\text{-}Eff1.NO2\text{-}N}$ H_1: $\mu_{B\text{-}Eff1.NO2\text{-}N} \neq \mu_{D\text{-}Eff1.NO2\text{-}N}$	H_0: $\mu_{B\text{-}Eff2.NO2\text{-}N} = \mu_{D\text{-}Eff2.NO2\text{-}N}$ H_1: $\mu_{B\text{-}Eff2.NO2\text{-}N} \neq \mu_{D\text{-}Eff2.NO2\text{-}N}$	H_0: $\mu_{B\text{-}Eff3.NO2\text{-}N} = \mu_{D\text{-}Eff3.NO2\text{-}N}$ H_1: $\mu_{B\text{-}Eff3.NO2\text{-}N} \neq \mu_{D\text{-}Eff3.NO2\text{-}N}$
10. Nitrate Nitrogen	H_0: $\mu_{B\text{-}Eff1.NO3\text{-}N} = \mu_{D\text{-}Eff1.NO3\text{-}N}$ H_1: $\mu_{B\text{-}Eff1.NO3\text{-}N} \neq \mu_{D\text{-}Eff1.NO3\text{-}N}$	H_0: $\mu_{B\text{-}Eff2.NO3\text{-}N} = \mu_{D\text{-}Eff2.NO3\text{-}N}$ H_1: $\mu_{B\text{-}Eff2.NO3\text{-}N} \neq \mu_{D\text{-}Eff2.NO3\text{-}N}$	H_0: $\mu_{B\text{-}Eff3.NO3\text{-}N} = \mu_{D\text{-}Eff3.NO3\text{-}N}$ H_1: $\mu_{B\text{-}Eff3.NO3\text{-}N} \neq \mu_{D\text{-}Eff3.NO3\text{-}N}$
11. Total Nitrogen	H_0: $\mu_{B\text{-}Eff1.TN} = \mu_{D\text{-}Eff1.TN}$ H_1: $\mu_{B\text{-}Eff1.TN} \neq \mu_{D\text{-}Eff1.TN}$	H_0: $\mu_{B\text{-}Eff2.TN} = \mu_{D\text{-}Eff2.TN}$ H_1: $\mu_{B\text{-}Eff2.TN} \neq \mu_{D\text{-}Eff2.TN}$	H_0: $\mu_{B\text{-}Eff3.TN} = \mu_{D\text{-}Eff3.TN}$ H_1: $\mu_{B\text{-}Eff3.TN} \neq \mu_{D\text{-}Eff3.TN}$
12. Total Organic Carbon	H_0: $\mu_{B\text{-}Eff1.TOC} = \mu_{D\text{-}Eff1.TOC}$ H_1: $\mu_{B\text{-}Eff1.TOC} \neq \mu_{D\text{-}Eff1.TOC}$	H_0: $\mu_{B\text{-}Eff2.TOC} = \mu_{D\text{-}Eff2.TOC}$ H_1: $\mu_{B\text{-}Eff2.TOC} \neq \mu_{D\text{-}Eff2.TOC}$	H_0: $\mu_{B\text{-}Eff3.TOC} = \mu_{D\text{-}Eff3.TOC}$ H_1: $\mu_{B\text{-}Eff3.TOC} \neq \mu_{D\text{-}Eff3.TOC}$
13. Total Phosphorus	H_0: $\mu_{B\text{-}Eff1.TP} = \mu_{D\text{-}Eff1.TP}$ H_1: $\mu_{B\text{-}Eff1.TP} \neq \mu_{D\text{-}Eff1.TP}$	H_0: $\mu_{B\text{-}Eff2.TP} = \mu_{D\text{-}Eff2.TP}$ H_1: $\mu_{B\text{-}Eff2.TP} \neq \mu_{D\text{-}Eff2.TP}$	H_0: $\mu_{B\text{-}Eff3.TP} = \mu_{D\text{-}Eff3.TP}$ H_1: $\mu_{B\text{-}Eff3.TP} \neq \mu_{D\text{-}Eff3.TP}$

4. The effect of HRT(comparison Effluent 3 among retention time of 13 hours, 19.5 hours and 182 hours)

Observed Parameters	Phase I, Without worms	Phase I, With worms
1. Temperature	H_0: $\mu_{A\text{-}Eff3.T.13} = \mu_{A\text{-}Eff3.T.19.5} = \mu_{A\text{-}Eff3.T.182}$ H_1: $\mu_{A\text{-}Eff3.T.13} \neq \mu_{A\text{-}Eff3.T.19.5} \neq \mu_{A\text{-}Eff3.T.182}$	H_0: $\mu_{B\text{-}Eff3.T.13} = \mu_{B\text{-}Eff3.T.19.5} = \mu_{B\text{-}Eff3.T.182}$ H_1: $\mu_{B\text{-}Eff3.T.13} \neq \mu_{B\text{-}Eff3.T.19.5} \neq \mu_{B\text{-}Eff3.T.182}$
2. pH	H_0: $\mu_{A\text{-}Eff3.pH.13} = \mu_{A\text{-}Eff3.pH.19.5} = \mu_{A\text{-}Eff3.pH.182}$ H_1: $\mu_{A\text{-}Eff3.pH.13} \neq \mu_{A\text{-}Eff3.pH.19.5} \neq \mu_{A\text{-}Eff3.pH.182}$	H_0: $\mu_{B\text{-}Eff3.pH.13} = \mu_{B\text{-}Eff3.pH.19.5} = \mu_{B\text{-}Eff3.pH.182}$ H_1: $\mu_{B\text{-}Eff3.pH.13} \neq \mu_{B\text{-}Eff3.pH.19.5} \neq \mu_{B\text{-}Eff3.pH.182}$
3. Conductivity	H_0: $\mu_{A\text{-}Eff3.C.13} = \mu_{A\text{-}Eff3.C.19.5} = \mu_{A\text{-}Eff3.C.182}$ H_1: $\mu_{A\text{-}Eff3.C.13} \neq \mu_{A\text{-}Eff3.C.19.5} \neq \mu_{A\text{-}Eff3.C.182}$	H_0: $\mu_{B\text{-}Eff3.C.13} = \mu_{B\text{-}Eff3.C.19.5} = \mu_{B\text{-}Eff3.C.182}$ H_1: $\mu_{B\text{-}Eff3.C.13} \neq \mu_{B\text{-}Eff3.C.19.5} \neq \mu_{B\text{-}Eff3.C.182}$
4. Turbidity	H_0: $\mu_{A\text{-}Eff3.Tur.13} = \mu_{A\text{-}Eff3.Tur.19.5} = \mu_{A\text{-}Eff3.Tur.182}$ H_1: $\mu_{A\text{-}Eff3.Tur.13} \neq \mu_{A\text{-}Eff3.Tur.19.5} \neq \mu_{A\text{-}Eff3.Tur.182}$	H_0: $\mu_{B\text{-}Eff3.Tur.13} = \mu_{B\text{-}Eff3.Tur.19.5} = \mu_{B\text{-}Eff3.Tur.182}$ H_1: $\mu_{B\text{-}Eff3.Tur.13} \neq \mu_{B\text{-}Eff3.Tur.19.5} \neq \mu_{B\text{-}Eff3.Tur.182}$
5. Total Suspended Solids	H_0: $\mu_{A\text{-}Eff3.TSS.13} = \mu_{A\text{-}Eff3.TSS.19.5} = \mu_{A\text{-}Eff3.TSS.182}$ H_1: $\mu_{A\text{-}Eff3.TSS.13} \neq \mu_{A\text{-}Eff3.TSS.19.5} \neq \mu_{A\text{-}Eff3.TSS.182}$	H_0: $\mu_{B\text{-}Eff3.TSS.13} = \mu_{B\text{-}Eff3.TSS.19.5} = \mu_{B\text{-}Eff3.TSS.182}$ H_1: $\mu_{B\text{-}Eff3.TSS.13} \neq \mu_{B\text{-}Eff3.TSS.19.5} \neq \mu_{B\text{-}Eff3.TSS.182}$
6. Dissolved Oxygen	H_0: $\mu_{A\text{-}Eff3.DO.13} = \mu_{A\text{-}Eff3.DO.19.5} = \mu_{A\text{-}Eff3.DO.182}$ H_1: $\mu_{A\text{-}Eff3.DO.13} \neq \mu_{A\text{-}Eff3.DO.19.5} \neq \mu_{A\text{-}Eff3.DO.182}$	H_0: $\mu_{B\text{-}Eff3.DO.13} = \mu_{B\text{-}Eff3.DO.19.5} = \mu_{B\text{-}Eff3.DO.182}$ H_1: $\mu_{B\text{-}Eff3.DO.13} \neq \mu_{B\text{-}Eff3.DO.19.5} \neq \mu_{B\text{-}Eff3.DO.182}$
7. Chemical Oxygen Demand	H_0: $\mu_{A\text{-}Eff3.COD.13} = \mu_{A\text{-}Eff3.COD.19.5} = \mu_{A\text{-}Eff3.COD.182}$ H_1: $\mu_{A\text{-}Eff3.COD.13} \neq \mu_{A\text{-}Eff3.COD.19.5} \neq \mu_{A\text{-}Eff3.COD.182}$	H_0: $\mu_{B\text{-}Eff3.COD.13} = \mu_{B\text{-}Eff3.COD.19.5} = \mu_{B\text{-}Eff3.COD.182}$ H_1: $\mu_{B\text{-}Eff3.COD.13} \neq \mu_{B\text{-}Eff3.COD.19.5} \neq \mu_{B\text{-}Eff3.COD.182}$
8. Ammonium Nitrogen	H_0: $\mu_{A\text{-}Eff3.NH3\text{-}N.13} = \mu_{A\text{-}Eff3.NH3\text{-}N.19.5} = \mu_{A\text{-}Eff3.NH3\text{-}N.182}$ H_1: $\mu_{A\text{-}Eff3.NH3\text{-}N.13} \neq \mu_{A\text{-}Eff3.NH3\text{-}N.19.5} \neq \mu_{A\text{-}Eff3.NH3\text{-}N.182}$	H_0: $\mu_{B\text{-}Eff3.NH3\text{-}N.13} = \mu_{B\text{-}Eff3.NH3\text{-}N.19.5} = \mu_{B\text{-}Eff3.NH3\text{-}N.182}$ H_1: $\mu_{B\text{-}Eff3.NH3\text{-}N.13} \neq \mu_{B\text{-}Eff3.NH3\text{-}N.19.5} \neq \mu_{B\text{-}Eff3.NH3\text{-}N.182}$
9. Nitrite Nitrogen	H_0: $\mu_{A\text{-}Eff3.NO2\text{-}N.13} = \mu_{A\text{-}Eff3.NO2\text{-}N.19.5} = \mu_{A\text{-}Eff3.NO2\text{-}N.182}$ H_1: $\mu_{A\text{-}Eff3.NO2\text{-}N.13} \neq \mu_{A\text{-}Eff3.NO2\text{-}N.19.5} \neq \mu_{A\text{-}Eff3.NO2\text{-}N.182}$	H_0: $\mu_{B\text{-}Eff3.NO2\text{-}N.13} = \mu_{B\text{-}Eff3.NO2\text{-}N.19.5} = \mu_{B\text{-}Eff3.NO2\text{-}N.182}$ H_1: $\mu_{B\text{-}Eff3.NO2\text{-}N.13} \neq \mu_{B\text{-}Eff3.NO2\text{-}N.19.5} \neq \mu_{B\text{-}Eff3.NO2\text{-}N.182}$
10. Nitrate Nitrogen	H_0: $\mu_{A\text{-}Eff3.NO3\text{-}N.13} = \mu_{A\text{-}Eff3.NO3\text{-}N.19.5} = \mu_{A\text{-}Eff3.NO3\text{-}N.182}$ H_1: $\mu_{A\text{-}Eff3.NO3\text{-}N.13} \neq \mu_{A\text{-}Eff3.NO3\text{-}N.19.5} \neq \mu_{A\text{-}Eff3.NO2\text{-}N.182}$	H_0: $\mu_{B\text{-}Eff3.NO3\text{-}N.13} = \mu_{B\text{-}Eff3.NO3\text{-}N.19.5} = \mu_{B\text{-}Eff3.NO3\text{-}N.182}$ H_1: $\mu_{B\text{-}Eff3.NO3\text{-}N.13} \neq \mu_{B\text{-}Eff3.NO3\text{-}N.19.5} \neq \mu_{B\text{-}Eff3.NO2\text{-}N.182}$
11. Total Nitrogen	H_0: $\mu_{A\text{-}Eff3.TN.13} = \mu_{A\text{-}Eff3.TN.19.5} = \mu_{A\text{-}Eff3.TN.182}$ H_1: $\mu_{A\text{-}Eff3.TN.13} \neq \mu_{A\text{-}Eff3.TN.19.5} \neq \mu_{A\text{-}Eff3.TN.182}$	H_0: $\mu_{B\text{-}Eff3.TN.13} = \mu_{B\text{-}Eff3.TN.19.5} = \mu_{B\text{-}Eff3.TN.182}$ H_1: $\mu_{B\text{-}Eff3.TN.13} \neq \mu_{B\text{-}Eff3.TN.19.5} \neq \mu_{B\text{-}Eff3.TN.182}$
12. Total Organic Carbon	H_0: $\mu_{A\text{-}Eff3.TOC.13} = \mu_{A\text{-}Eff3.TOC.19.5} = \mu_{A\text{-}Eff3.TOC.182}$ H_1: $\mu_{A\text{-}Eff3.TOC.13} \neq \mu_{A\text{-}Eff3.TOC.19.5} \neq \mu_{A\text{-}Eff3.TOC.182}$	H_0: $\mu_{B\text{-}Eff3.TOC.13} = \mu_{B\text{-}Eff3.TOC.19.5} = \mu_{B\text{-}Eff3.TOC.182}$ H_1: $\mu_{B\text{-}Eff3.TOC.13} \neq \mu_{B\text{-}Eff3.TOC.19.5} \neq \mu_{B\text{-}Eff3.TOC.182}$
13. Total Phosphorus	H_0: $\mu_{A\text{-}Eff3.TP.13} = \mu_{A\text{-}Eff3.TP.19.5} = \mu_{A\text{-}Eff3.TP.182}$ H_1: $\mu_{A\text{-}Eff3.TP.13} \neq \mu_{A\text{-}Eff3.TP.19.5} \neq \mu_{A\text{-}Eff3.TP.182}$	H_0: $\mu_{B\text{-}Eff3.TP.13} = \mu_{B\text{-}Eff3.TP.19.5} = \mu_{B\text{-}Eff3.TP.182}$ H_1: $\mu_{B\text{-}Eff3.TP.13} \neq \mu_{B\text{-}Eff3.TP.19.5} \neq \mu_{B\text{-}Eff3.TP.182}$

The significance test outcomes

1. The effect of filter layers - Mean difference

Phase I	Without worms			With worms		
	ΔIn - Eff1	ΔIn - Eff2	ΔIn - Eff3	ΔIn - Eff1	ΔIn - Eff2	ΔIn - Eff3
Temperature (°C)	1.6	1.7	1.8	0.8	1.0	0.5
pH	1***	1***	0.9***	0.4**	0.4**	0.4**
Conductivity (mS/cm)	-1.8	-0.2	-0.2	-0.2**	-0.2**	-0.3***
Turbidity (NTU)	91***	91***	120***	249***	279***	296***
TSS (mg/L)	82***	85***	111***	192***	218***	230***
DO (mg/L)	-0.5	-0.4	-0.8*	0.01	0.2	0.5
COD (mg/L)	58	86	134**	63	112***	113***
NH_3-N (mg/L)	30***	31***	37***	9	0.5	4
NO_2-N (mg/L)	4.8***	5.2***	6.4***	5.4***	5.9***	6.1***
NO_3-N (mg/L)	4.1	5.3**	5.7**	8.3***	11.1***	11.1***
TN (mg/L)	42***	41***	56***	14	9	10
TOC (mg/L)	26	55**	72***	17**	27***	30***
TP (mg/L)	15***	17***	21***	1	5*	6**

Phase II	Without worms			With worms		
	ΔIn - Eff1	ΔIn - Eff2	ΔIn - Eff3	ΔIn - Eff1	ΔIn - Eff2	ΔIn - Eff3
Temperature (°C)	0.7	1.3	1.3	0.1	0.5	0.6
pH	1.3***	1.3***	1.3***	0.6***	0.5***	0.6***
Conductivity (mS/cm)	0.04	0.05	0.03	0.02	-0.03	-0.04
Turbidity (NTU)	351**	382***	395***	265***	336***	337***
TSS (mg/L)	312**	337***	346***	238***	287***	288***
DO (mg/L)	-2.6***	-3***	-2.9***	-2.7***	-2.9***	-2.3***
COD (mg/L)	93	246***	234***	155***	178***	182***
NH_3-N (mg/L)	30**	38***	57***	16**	18***	20***
NO_2-N (mg/L)	2.2	1.9	2.3	4.9***	5.9***	5.8***
NO_3-N (mg/L)	15.4***	16.8**	16.5**	7.3***	8.0***	8.1***
TN (mg/L)	14***	28***	30***	29***	32***	32***
TOC (mg/L)	8	28	40	17	30***	30***
TP (mg/L)	-3	-0.4	0.5	-13***	-11***	-11***

2. The effect of worms

Observed parameters	Phase I			Phase II		
	ΔEff1	ΔEff2	ΔEff3	ΔEff1	ΔEff2	ΔEff3
Temperature (°C)	1.1	1.2	0.8	-0.6	-0.8	-0.7
pH	-0.2***	-0.1***	-0.04	0.02	-0.06	-0.03
Conductivity (mS/cm)	0.2**	0.2***	0.3***	-0.1	-0.2**	-0.2**
Turbidity (NTU)	-28	1	-7	12	52**	40**
TSS (mg/L)	3	26**	15**	15	39**	30**
DO (mg/L)	-2.1***	-2***	-1.3**	-0.9	-0.7	-0.2
COD (mg/L)	156***	200***	151***	170***	40	56
NH_3-N (mg/L)	4	-8	-9	6	-0.9	-18
NO_2-N (mg/L)	0.9	1.4**	0.4	-0.7**	0.5*	0.01
NO_3-N (mg/L)	-2.2	0.4	0.2	0.1	-0.5	-0.1
TN (mg/L)	5	1	-11	-2	-14**	-16**
TOC (mg/L)	61***	42***	26**	42	34**	23
TP (mg/L)	-5	-4	-6**	-6	-6	-6

3. The effect of soil type

Observed parameters	Without worms			With worms		
	ΔEff1	ΔEff2	ΔEff3	ΔEff1	ΔEff2	ΔEff3
Temperature (°C)	0.5	0.9	0.9	-1.9*	-1.6	-1
pH	0.2***	0.2**	0.3*	0.4***	0.3***	0.4***
Conductivity (mS/cm)	0.4*	0.5**	0.5*	0.05	0.02	0.02
Turbidity (NTU)	-86***	-54**	-70***	-50	-10	-25***
TSS (mg/L)	-18	3	-13	-8	15**	4
DO (mg/L)	-3***	-3.4***	-3***	-1.8**	-2.2***	-2***
COD (mg/L)	27	152	92***	15	-12	-9
NH_3-N (mg/L)	-31**	-22**	-9	-30***	-20***	-22***
NO_2-N (mg/L)	2.8***	2.1**	1.5**	0.9	1.4***	1***
NO_3-N (mg/L)	1.1	1.3	0.5	2.3***	0.3	0.4
TN (mg/L)	13	28**	14	1	10	8
TOC (mg/L)	16	8	2.7	-1	2.3	0.4
TP (mg/L)	-19***	-19***	-22***	-20***	-23***	-23***

4. The effect of HRT/HLR

Observed parameters	ΔEff3		
	13 -- 19.5	13 -- 182	19.5 -- 1182
Temperature (°C)	-0.3	2.9	3.3
pH	0.1*	-0.2***	-0.3***
Conductivity (mS/cm)	-0.1	-0.7***	-0.6***
Turbidity (NTU)	-31***	-7	24***
TSS (mg/L)	-35***	-4	32***
DO (mg/L)	-0.06	-1.9*	-1.8
COD (mg/L)	46	99	53
NH_3-N (mg/L)	-11	-12	-1
NO_2-N (mg/L)	-0.03	0.52	0.55
NO_3-N (mg/L)	-0.9	0.8	1.8*
TN (mg/L)	-11	-9	2
TOC (mg/L)	-4	2	5
TP (mg/L)	-5	13***	18***

Appendix 5.1

Setting up of worm farm

Figure 1 Can-O-Worms® TUMBLEWEED Worm Farm set up at Werribee campus. Photos taken by Anusuya Joshi

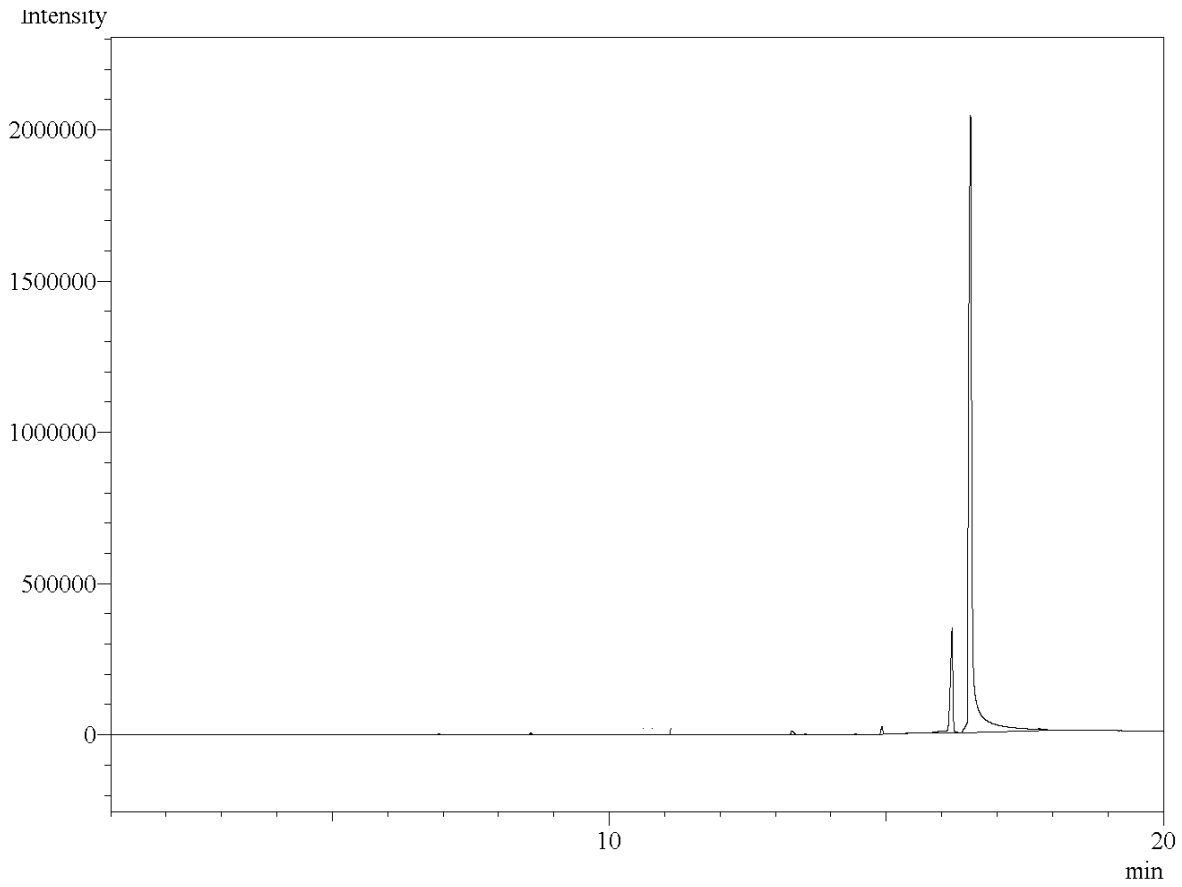
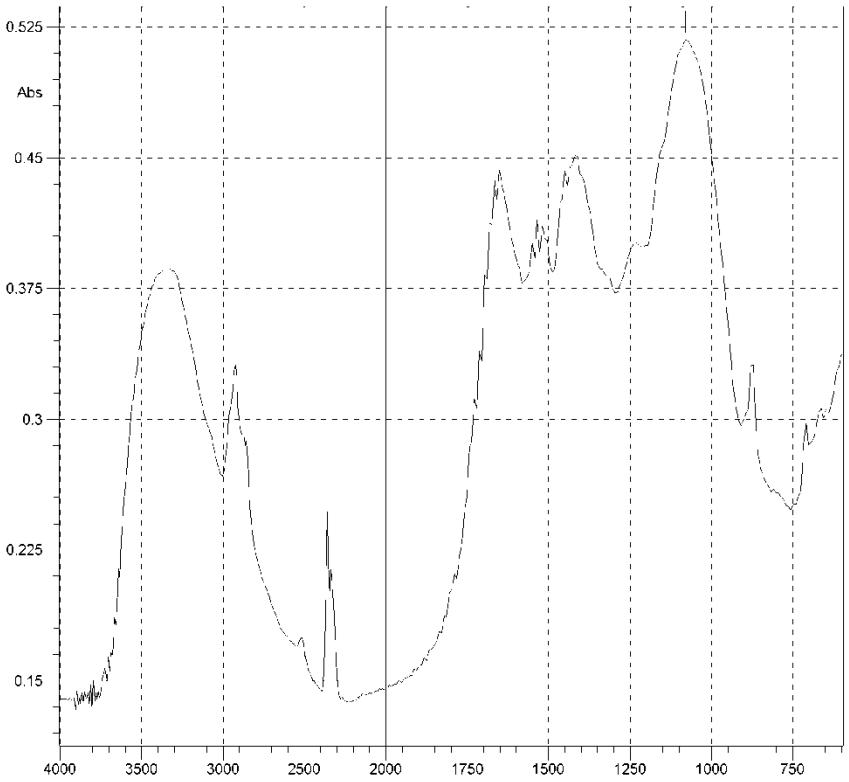


Figure 1 GC peak for 10% DL Lactic acid.

(a)



(b)

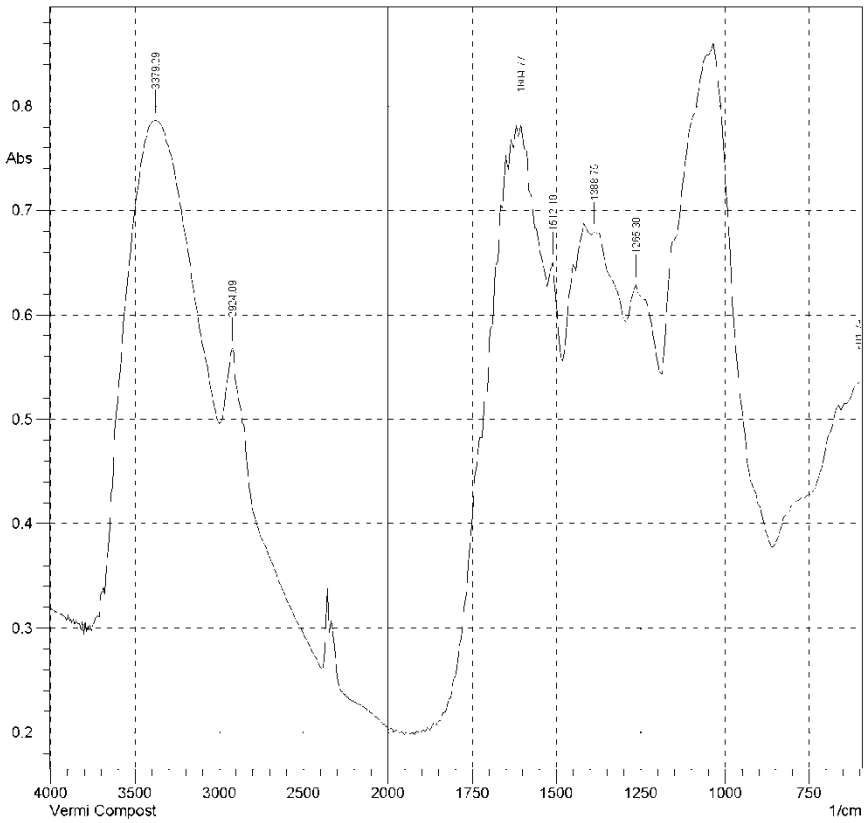
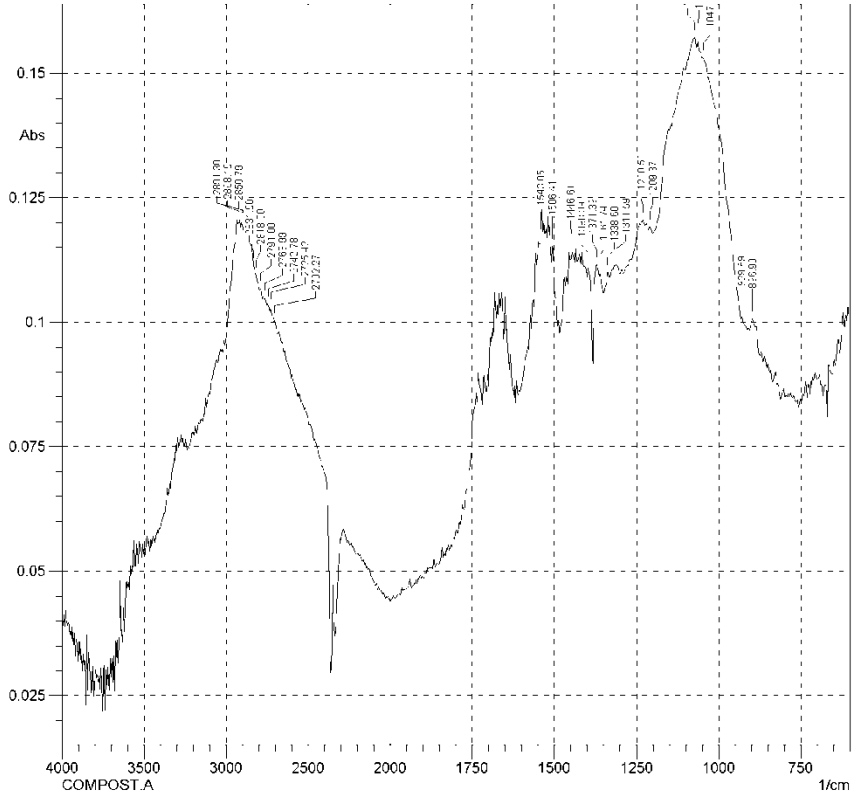


Figure 1 FTIR spectra for (a) Compost A – day 35 and (b) vermicompost (see Appendix 5.3 in CD for details).

(a)



(b)

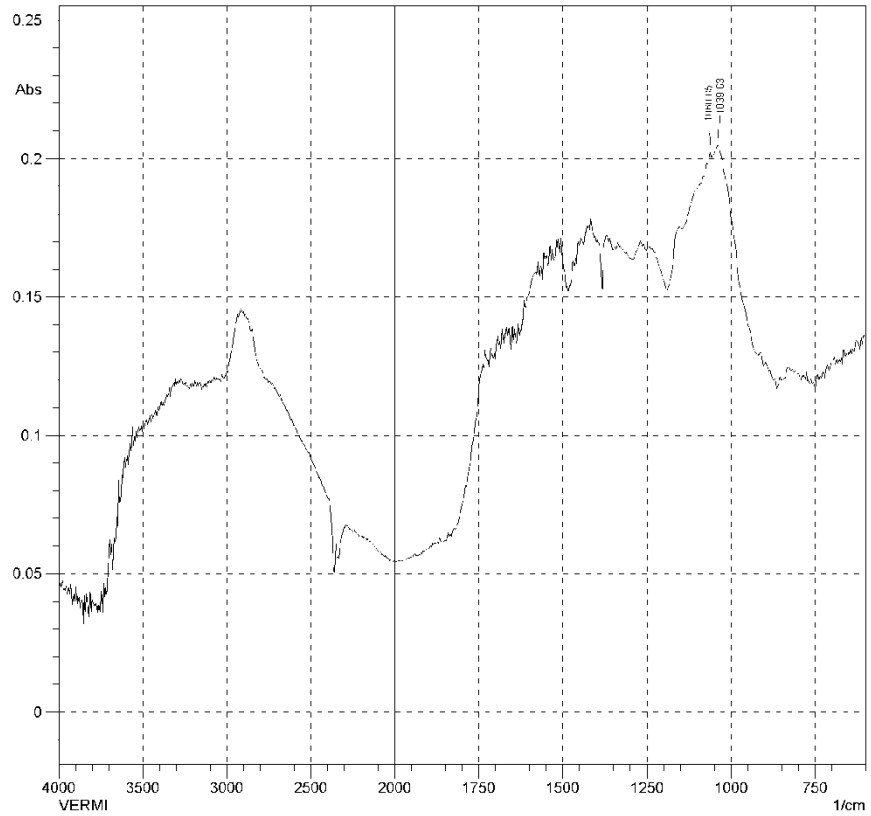


Figure 1 DRIFT FTIR spectra for (a) Compost A – day 35 and (b) vermicompost (see Appendix 5.4 in CD for details).