The Creation and Interpretation of an Elemental Database for Chinese Traditional Herbal Remedies

A thesis submitted for the degree of Master of Science

by

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January 2013

Declaration

I, Hongli Wu, declare that the Master by Research thesis entitled “The Creation and Interpretation of an Elemental Database for Chinese Traditional Herbal Remedies” is no more that 60,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, 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.

Signature:..Date:...............................

Publications relevant to the scope of this thesis

Conference proceedings

Wu, H, Ngeh, LN, Xu, H, Orbell, JD, Adorno, P, Tzardis, S, Buddhadasa, S, 2009, 'Heavy metals in Chinese herbs'. In: Progress in Environmental Science and Technology, 2, (Pt. A), 203-209. Language: English, Database: CAPLUS, Publisher: Science Press, CODEN: PESTC4.

Conference presentations

Wu, H, Kodituwakka, D, Xu, H, Ngeh, LN, Orbell, JD, 'The Concentration and Bioavailability of Transition Metals in Blood Tonifying Herbs'. Poster presentaion, 11th Government Food Analysts Conference, 22nd – 24th February 2009, Melbourne, Australia.

ABSTRACT

Scientific advances based on the study of natural products and materials are an active and fruitful area of research. For example, the discovery of drug leads from Traditional Chinese Medicine (TCM), in particular, has resulted in a number of successful therapeutic agents that are in use worldwide. However, most of the active molecules that have been identified and further developed have been *organic*. Given the emerging area of bioinorganic chemistry/medicinal inorganic chemistry, it is now recognized that small molecule *inorganic* species play an equally important role in biology. In this regard, there has been very little research, if any, conducted into the search for bioactive, small molecule, metallo-species in natural products or traditional medicines, that might contribute to their reputed therapeutic value and that might have potential for drug development. This project represents a first step towards correcting this imbalance. Therefore, a strategy has been devised and is presented whereby existing bioinorganic/medical inorganic chemistry knowledge may be reconciled with Traditional Chinese Medical information that might facilitate the identification of such bioactive, small molecule, metallo-species in such natural products. Central to this strategy is the creation of an appropriate elemental database across a carefully selected library of well-characterized Chinese medicinal herbal substances.

From more than 300 Chinese herbal medicines reported in the authoritative literature, 191 commonly used medicinal herbs were obtained from local pharmaceutical groceries and University-based sources. All of this material originated from different regions of mainland China. The 191 samples were each ground to a fine powder, oven dried, catalogued and stored - in order to establish a collection for further study. A subset of 103 representative samples were chosen for replicate quantitative analysis with respect to twenty-four chemical elements, including metallic elements which are considered essential elements to humans and also some elements that are not considered to be essential. These elements include those that are of relevance to medical inorganic chemistry.

Thus a global database was generated by using inductively coupled plasma spectrometry (ICP) to determine (in triplicate) the total and bioavailable levels of the elements, V, Cr, Mn, Fe, Co. Ni, Cu, Zn, Li, Bi, As, Se, Mo, Cd, Al, Sn, Sb, Pb. Hg, K, Na, Ca, Mg. and Ba, in *each* of these 103 samples. Of these, the macrominerals (K, Na, Ca, and Mg), microminerals (Fe, Cu, Zn, Mn, Mo, Se, Cr, and Co) and trace elements (Sn, Ni, V, Li and Bi) are

represented. A toxicity investigation was undertaken by specifically examining the data for the elements: Pb, As, Cd, Hg, Al, Sb and Ba, across the 103 herbs. Enrichment factors were also calculated for all twenty-four elements (Al was taken as the reference element) across the database and consideration was also given to whether the herbal material was derived from the leaf, stem, root, seed, fruit or flower of the plant.

This investigation has generated a large amount of data for analysis. Crucial to demonstrating the utility of this approach is to devise a strategy to systematically interrogate this resource. There is obviously more than one conceivable way of doing this. The approach that has been adopted here is to consider the relative total and bioavailable elemental levels for the 103 herbal substances - on a metal by metal basis. Thus the data is represented in this thesis as a series of twenty-four comparative histograms. Each histogram has been critically analysed in terms of the relevance of the metal to medical inorganic chemistry, in the context of the Traditional Chinese Medical applications for each herb. This allows associations and possible drug leads to be proposed - with a view to subjecting those herbal substances of interest to metal speciation analysis – although the latter work is beyond the scope of this thesis. During the analyses it has also been found that clues for the discovery or development of interesting metal ligand systems, as well as information relating to nutritional and toxicological factors, may also be gleaned.

The establishment of this resource and its subsequent interrogation according to the strategy described above has resulted in a set of recommendations whereby candidate herbal materials are suggested for further metal speciation/chemical analysis, and possible biochemical/biological investigations, with respect to individual metals. These outcomes are presented in matrix form in the conclusions.

Acknowledgments

I would like to thank my supervisors, Prof. John Orbell, Dr. Lawrence Ngeh and Dr. Hong Xu for all their assistance and advice. In particular I would like to sincerely thank my principal supervisor Prof. John Orbell - his expert advice and guidance, strong encouragement and great support guided me through this research.

The majority of the testing for this project was undertaken at the National Measurement Institute (NMI), Australia. I would like to thank all the staff at NMI, particularly Mr Paul Adorno, Mr Stavros Tzardis, Dr. Saman Buddhadasa, Ms Devika Kodituwakka, Dr. Nunzio Llimongiello, Mr Trong Le for their ongoing support and willingness to discuss project related issues.

Thanks are due to Victoria University for supplying the postgraduate scholarship. Also thanks to the staff of the School of Engineering and Science, Victoria University for their assistance.

Finally I would like to thank my family and friends for their encouragement and unwavering support throughout my research.

List of abbreviations

AAS	Atomic absorption spectrometry
ATP	Adenosine triphosphate
BBB	Brain blood barrier
BF	Biconcentration factor
D-AT-FAAS	Derivative atom trapping flame atomic absorption spectrometry
DNA	Deoxyribo nucleic acid
DRC	Dynamic reaction cell
EF	Enrichment factor
FAAS	Flame-atomic absorption spectrometry
FAO	Food and Agricultural Organisation
GC-MS	Gas chromatography-mass spectrometry
GF-AAS	Graphite furnace-atomic absorption spectrometry
ICP	Inductively coupled plasma spectrometry
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ICP-MS	Inductively coupled plasma - mass spectrometry
ICP-OES	Inductively coupled plasma - optical emission spectrometry
INAA	Instrumental neutron activation analysis
LC-MS	Liquid chromatography-mass spectrometry
MF	Mobility factor
NMI	National Measurement Institute
OEC	Oxygen-evolving complex
PIXE	Particle induced X-ray emission
PTWI	Provisional Tolerable Weekly Intake
QA	Quality assurance
RNA	Ribo nucleic acid
RPD	Relative percentage difference
SD	Standard deviation
SE	Standard errors
SOD	Superoxide dismutase
SRM	Standard Reference Materials
TCM	Traditional Chinese Medicine

TOF	Time-of-flight
WHO	World Health Organisation

Note: Throughout this thesis the chemical symbol for the relevant metal is used to represent its common cation unless a particular oxidation state needs to be specified.

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Chapter 1: Preamble

In the “western world” and in countries other than those that already have a long history of using traditional herbal medicines, there has been an increasing use of botanicals, botanical extracts, and purified natural compounds for the treatment and prevention of disease, especially chronic disease [1-5]. In China, the use of such herbal remedies in Traditional Chinese Medicine (TCM) has always been highly valued and TCM enjoys equal status with western medicine. Indeed, TCM is considered to be effective in the treatment of a wide range of medical conditions, especially for age-related diseases [1]. TCMs have also been regarded as source of antioxidant compounds [6].

From a pharmaceutical and chemical point of view, the traditional use of such a wide variety of herbs and herbal concoctions for so many medical conditions provides a rich source of potentially bioactive compounds - and much work remains to be done in characterising their composition and specific properties [7]. Therefore, an investigation of the chemical composition of herbs in terms of bioactive chemicals is of significance, not only in their clinical application, but also in the identification of novel compounds as drug leads. In this regard, there is a growing interest in the application of small molecule metallo-species in the area of medical science [8]. Whereas a number of “organic” compounds derived from natural products have been successfully developed into effective drugs for a range of disorders [8-11], the same principle has yet to be applied to a systematic search for relevant bioactive inorganic species and this remains a seriously neglected area of enquiry.

This project is aimed at establishing a research platform whereby the goal of identifying such bioactive metallo species, and also novel metal complexing ligands, for a range of different metals and across a wide range of carefully selected and categorized traditional Chinese medicinal herbs, may be facilitated. A collection of traditional Chinese herbal medicines has been established. Across these substances a quantitative analysis of the total and bioavailable levels of a range of relevant metals has been carried out. This platform has been shown to be useful for providing clues for possible leads for bioactive metallo species and metal complexing ligands, and also provides an extensive resource for other researchers.

General objectives for the establishment of the 'research platform'

- To establish a collection of traditional Chinese herbs carefully chosen from more than 300 Chinese herbal medicines reported in the authoritative literature and to characterize a sizeable (103) subset in relation to TCM symptoms related to a range of "western medical" disease states.

- To perform, in replicate, total and bioavailable elemental analyses across this subset for 24 biologically relevant metals using inductively coupled plasma - mass spectrometry (ICP-MS) and inductively coupled plasma - optical emission spectrometry (ICP-OES). This data is termed a "herbal-metallo-landscape".

- To organize and analyse the large amount of data (both total and bioavailable levels of elements for each herb) generated in this study and to demonstrate how potential leads for the discovery of metallo drugs, or novel metal complexing ligands, may be suggested.

Outline of strategy and methods

These will be described in more detail in subsequent sections but are outlined here in order to set the stage for a clear description of the project.

The study was conducted in two main stages:

1) A large number of Chinese herb samples (191) were targeted and acquired. All samples were oven dried and ground into fine powders, categorized and stored.

This stage focused on the determination of elemental concentrations in each sample of a selected subset of 103 substances. Elemental analysis was performed using ICP-MS and ICP-OES. The herbs were screened for 24 metals, including vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), lithium (Li), bismuth (Bi), arsenic (As), selenium (Se), molybdenum (Mo), cadmium (Cd), aluminium (Al), tin (Sn), antimony (Sb), lead (Pb), mercury (Hg), potassium (K), sodium (Na), calcium

(Ca), magnesium (Mg) and barium (Ba) in terms of their concentration levels relative to background. Two parallel sets of data were generated corresponding to TOTAL extractable elements and BIOAVAILABLE extractable elements. Details of the extraction methods and other experimental details are described in a subsequent section.

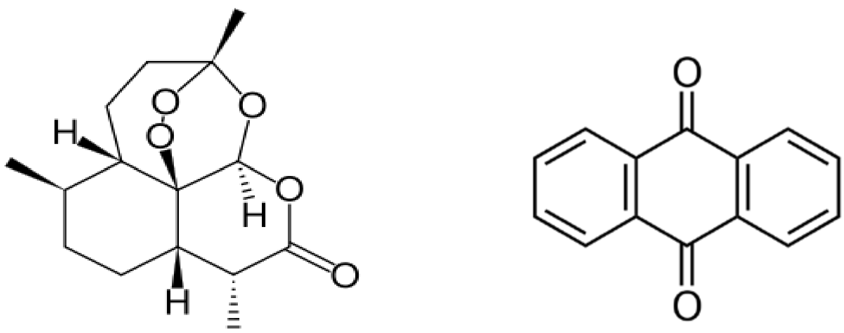
2) With such a large amount of data, potentially containing a considerable amount of useful information, a simple yet informative way of presentation and interrogation had to be devised. It must be emphasized, however, that this is not the only way of processing this database. For the purpose of this thesis, the analytical data have been organised, documented and discussed in Chapter 4 on an element-by-element basis. Thus, for each element, the total and bioavailable levels are comparatively histogrammed over the 103 herb samples. Based on a consideration of the role of a particular metal in human health and the medical application of the herb or herbs in TCM, potential leads relating to pharmaceutical applications (metallo-drug leads) and/or chemical applications (e.g. metal ion specific ligands) and/or nutritional applications and/or toxicity implications have been deduced. For example, for a given set of histograms, the features of the data that attract our attention include unusually high levels of a metal for one or more herbs and links between such herbs and existing knowledge that relates the metal to various medical conditions that such herbs might address. Other considerations include an interpretation of the presented enrichment factor analyses for each of the metals. Deductions presented in this thesis are necessarily merely representative of how this database can be mined and it is anticipated that this database will serve as a useful resource for other researchers in this area and it will be published in the international literature.

Chapter 2: Background and Literature Review

2.1 Introduction

Chinese traditional herbs are popular among the Chinese people and are reputed to restore the body's proper balance and maintain optimum health [12]. Indeed, there is evidence to suggest that such herbal remedies are beneficial in the treatment of certain diseases and herbal medicines are routinely used to treat a wide range of medical problems such as arthritis and inflammation - and are considered to be benign with minimal side effects [13]. The use of such herbal medicines and products has been increasing in popularity worldwide, particularly as "alternative medicine" in developed countries [14]. Such medicinal herbs have also been used as food supplements by many racial groups and nationalities [15].

In general, Chinese medicine contains a wide variety of herbs of different compositions [16]. Although different kinds of Chinese herbs have been used for a wide range of medical conditions, the role of naturally occurring bioactive metallo species in such substances is essentially unknown. Existing research tends to focus on identifying bioactive molecules that are organic. For example, clinically useful drugs which have been isolated from Chinese plants include the antimalarial agent artemisinin (Fig. 2.1a) [17, 18] and the laxative anthraquinone (Fig. 2.1b) [19]. The antimalarial arteminisin's mechanism of bioactivity involves its reaction with the Fe(II) in haemoglobin [18].



(a) **(b)**

Figure 2.1: **(a)** Antimalarial agent artemisinin and **(b)** laxative anthraquinone isolated from Chinese medicinal plants.

The area of medicinal inorganic chemistry is a relatively new discipline area in science [8, 20]. It is well known that trace elements act as key components of essential enzyme systems or proteins such as haemoglobin, and that they perform vital biochemical functions in our body [5, 21, 22]. Small molecule chemical species of such elements can be exquisitely bioactive, such as the cobalt complex Vitamin B12 [23]. It is therefore not surprising that small molecule bioactive metallo species have come under scrutiny as potential drug candidates [24]. Notably, even small molecule species of non-essential elements are candidates, the most famous of these being the successful platinum containing anticancer compound cisplatin [8]. In this regard, research directed towards the investigation of the content of possible small molecule bioactive metallo species in the raw materials of Chinese herbal medicines may help us to understand their roles in clinical medicine and possibly identify therapeutic lead compounds. A first step towards this goal is an accurate "landscaping" of a wide range of metals across an equally wide range of well-characterized Chinese medicinal herbs. This involves the determination of both the total and bioavailable metal content. It is anticipated that together with a detailed understanding of the basic classification and purported function of these Chinese traditional herbs, a detailed analysis of the analytical data on a metal by metal basis could lead to the identification of candidate herbs for subsequent speciation analysis. Whilst many examples of such candidates have been identified as a result of this strategy, speciation analysis for the various metals in the individual herbal substances is beyond the scope of this thesis.

The following literature review is aimed at defining the basic classification and function of Chinese traditional herbs. Following this, common techniques used for elemental analysis are outlined. The main sample preparation methods used for elemental analysis are also discussed. Finally, previous studies on the elemental analysis of Chinese traditional herbs are reviewed.

2.2 Traditional Chinese Medicine (TCM)

2.2.1 History and Development

The herbal component of the Chinese medical tradition is the product of a very long process. It has brought together folk remedies and the therapeutics of the physician-literati. It synthesised the medicine of one dynasty with another, one location with another, and one thinker with another. Each new compilation of Chinese remedies has organised, arranged and used this knowledge [25]. The first TCM textbook, "*Yellow Emperor's Inner Classic* (*Huang Di Nei Jin*)", also known as the "*Inner Classic*", was complied by unknown authors between 200 B.C. and 100 A.D. Little information is available regarding the practice of medicine prior to this event [26].

The total number of Chinese herbs has progressively increased over the last two thousand years. The use of a number of these herbs has now been further refined with respect to modern practice or by scientific research. These continue to be listed in Chinese herbal medicine textbooks and include substances such as Jiao Gu Lan (Gynostemma *pentaphyllum*), Hong Jing Tian (*Rhodiola kirilowii*), and Yue Jian Cao (Evening primrose oil). Currently, there are more than 450 substances commonly used in Chinese herbal medicine - most are of plant origin [27].

2.2.2 Main Chinese Herbal Medicine Theory

In TCM, nearly all symptomatic diagnoses are based on the philosophy of *yin* and *yang*, the two forces which the Chinese believe control the working of the universe [28]. Associated with *yin* and *yang* are the terms cold and hot, which are generally believed to stem from types of food that affect the body in different ways. According to Bensky and Gamble [25] and Yan [29], there are a number of different theories that have been developed throughout history. These are summarised as follows:

According to TCM the concept of herbs' five flavours or tastes refer to acrid (pungent), sweet, bitter, sour and salty. Herbs that have no specific flavours are said to be bland.

There are five major properties that refer to temperature characteristics: hot, cold, warm, cool and neutral. This provides a preliminary clue in relation to the application of herbs to treat different human constitutions, e.g. "hot" diseases need "cold" herbs to treat.

The properties of taste and temperature also have specific effects. The taste of a herb partly determines its therapeutic function. Acrid herbs are said to disperse and move; sweet herbs tonify, harmonise and are sometimes moistened; bitter herbs drain and dry; sour herbs are astringent; salty herbs purge and soft or bland herbs leech out dampness and promote urination.

The therapeutic actions of herbs can be described by linking their taste and temperature qualities, e.g. an acrid and warm herb can be used for releasing exterior cold; an arid and cool herb for exterior heat pattern. This led to eight TCM-based treatment principles: to promote sweating, induce vomiting, purge, harmonise, warm, clear, tonify and reduce. In clinical practice, two or more herbs are selected to form a formula for different applications.

There are three major types of contraindications or prohibitions in the use of these herbs: prohibited combinations, prohibitions during pregnancy and dietary incompatibilities.

To cultivate each herb it is necessary to have a certain amount of sunlight, proper climate and moisture, optimal soil conditions and adequate fertilization. Due to differences in climate and topography, there are areas of China that are most suited for the cultivation of specific forms of vegetation; the highest quality herbs come from these areas. Herbs should be harvested at a time when their active ingredients are most plentiful.

Herbs are always processed before ingestion or application. The main reasons are:

- To increase the herb's potency, e.g. using alcohol to extract volatile oils from Radix *Angelicae Sinensis* (Dang Gui)
- To minimize side effects, e.g. treating *Rhizoma Pinelliae Ternatae* (Ban Xia) with ginger removes its tongue-numbing side effects.
- To increase or alter an herb's properties, thereby adapting it to the needs of a particular clinical situation, e.g. *Radix Rehmanniac Glutinosae* (Sheng Di Huang), which is the dried form of the herb and used for clearing heat. After cooking in wine and drying it becomes Radix *Rehmanniae Glutinousae Conquitae* (Shu Di Huang), which is warming and used as a tonic.

The dosage is a topic of extreme importance in composing a prescription. It is with dosage that specific aspects of a therapeutic strategy can be emphasized or de-emphasized. Potentially, the character of a prescription can be radically changed. The common dosage for most of the available herbs is 3 - 10 grams. Hard, heavy, moderate and bland herbs are used in larger doses, e.g. roots, fruits, minerals and shells. Light, potentially toxic and strongly-flavoured herbs are used in smaller dose, e.g. flowers, leaves and aromatic herbs. Dosage also depends on the severity of the dysfunction, with the more severe problems usually requiring a larger dose. Very weak patients, the aged, and infants usually cannot tolerate too strong a medication or too large a dose. In TCM, Stomach Qi (function) is a very important consideration in relation to whether a patient can accept and absorb the herbs selected for the treatment. A relatively weak prescription may be used initially if the stomach Qi is considered weak.

2.2.3 Classification of Chinese Herbs

For the purpose of this study, 300 Chinese herbs were selected and are listed in Appendix A. These herbs are traditionally classified into eighteen categories according to their functions [30, 31]. This is summarised in Table 2.1. Note that the functions and indications have also been interpreted in terms of Western medicine [30, 31].

Table 2.1: The eighteen commonly used herbal categories

Category	Characteristics	Functions and Indications
Herbs that Release the Exterior **See Table I of Appendix A**	• Have thin, pungent, dispersing quality to dispel pathogens from the surface. • Light in quality (e.g. aerial parts) to lift and float pathogens out through pores of the upper body. • Act on long channel and the body's superficies.	• Dispel pathogens factors from the exterior and prevent them from penetrating body's interior. • Treat exterior patterns-e.g., symptoms such as fever, chills, possible sweating, headache and a floating pulse.
Herbs that Clear Heat **See Table II of Appendix A**	• Generally bitter, cool or cold herbs with draining, sinking qualities. • Many are sweet, salty or pungent by nature.	• Treat many different types of internal heat, whose common symptoms are a feeling of internal heat, irritability, thirst, a red tongue body and a rapid pulse.

		• In western terms, these herbs treat triad fever, inflammation and infection.
Downward-Draining Herbs **See Table III of Appendix A**	• Are bitter, cold and sinking, or salty, neutral and moist in nature.	• Eliminate pathogenic accumulations of food matter and heat from Intestines. • Address internal full conditions presenting the keynote symptom of constipation.
Herbs that Drain Dampness **See Table IV of Appendix A**	• Mostly sweet/bland and neutral, or bitter and cold in quality, and generally possess a draining and sinking action. • Act variously on the spleen, bladder and kidney channels. • Quite a few are seeds.	• Regulate the water passages and drain water accumulation through the Bladder to reduce edema, and to promote and ease urination. • In western terms, these herbs address nephritic and other disorders of water metabolism.
Herbs that Dispel Wind-Dampness **See Table V of Appendix A**	• Mostly pungent, warm, dry and activating in quality and tend to invigorate the Qi and Blood in the channels and collaterals. • Many are stems and vines of plants. • Majority enter the liver and kidney channels.	• Eliminate primarily wind and damp, and secondarily cold and heat pathogens from the muscles, channels, tendons and bones. • Address painful obstruction syndromes characterized by rheumatic pain and stiffness in the limbs, joints, muscles and

		tendons.
Herbs that Transform Phlegm and Stop Coughing **See Table VI of Appendix A**	• Generally have pungent, bitter qualities to activate and dispel phlegm and diffuse or descend Lung Qi. • A few have salty qualities. • Enter the lung channel and often the spleen and stomach channels.	• Treat both visible and invisible phlegm by either expelling or dissolving the phlegm. • Many also treat coughing and wheezing. These conditions are often seen in bronchial disorders.
Aromatic Herbs that Transform Dampness **See Table VII of Appendix A**	• Aromatic, pungent, warm and dry in nature. • Act primarily on the spleen and stomach channels. • A large number are aromatic fruits and seeds.	• Transform turbid damp in the middle warmer. • Employed for the accumulation of damp in the Spleen or middle-warmer. • Treat symptoms identified in Western conditions such as acute indigestion, food poisoning and gastroenteritis.
Herbs that Relieve Food Stagnation **See Table VIII of Appendix A**	• Neutral or somewhat warm and sweet in nature. • Consist of digestible parts of plants, for example, fruit, sprout or seed.	• Promote digestion and remove food stagnation in the Stomach and Intestines • Promote and regulate Stomach functions, while a few also enhance the Spleen's transportation functions. • Symptoms include: aversion to food, nausea and vomiting, irregular bowel movements,

		indigestion and a feeling of distension and oppression in the epigastric and/or abdominal region.
Herbs that Regulate the Qi **See Table IX of Appendix A**	• Mostly pungent, bitter and warm, with activating, dispersing and descending properties. • Many are aromatic fruits which act on the spleen and stomach. Others address liver and lung functions.	• Restore normal Qi flow in conditions of Qi stagnation. • First, activate Qi that has become stagnant, second, descend Qi. • In Western terms, address various neurological and neuromuscular disorders.
Herbs that Regulate the Blood **See Table X of Appendix A**	• Mostly pungent, warm and often aromatic in nature, although a few are bitter and cold. • Majority enter the heart and liver channels.	• Invigorate the blood and reduce blood stagnation, treating symptoms associated with blood stagnation (e.g. sharp, fixed pain). • In Western terms, they address gynaecological, neurological and traumatic disorders.
Herbs that Warm the Interior and Expel Cold **See Table XI of Appendix A**	• Mostly pungent, warm, aromatic and dry in nature. • Act mainly by warming the middle warmer and the Kidney. • Many are aromatic fruits and/or spices also used in cooking.	• Warm the body's Yang and expel an accumulation of cold. • Treat internal cold conditions arising from either Yang deficiency or from invasion of pathogenic cold. • In Western terms, they address metabolic and organ deficiencies.

Tonifying Herbs **See Table XII of Appendix A**	• Generally sweet, nourishing and thick by nature. • Vary in characteristics depending on what they tonify. • Herbs that tonify the Qi, blood and Yin consist mostly of roots.	• Supplement, replenish or tonify in deficiency conditions of the body's radicals- Qi, Blood, Yin, Yang, fluids and Essence. • In Western terms, they address a variety of functional disorders such as metabolic disorders. • Generally possess restorative properties.
Herbs that Stabilise and Bind **See Table XIII of Appendix A**	• Tend to be sour and astringent in nature. • Mostly comprised of fruits and seeds.	• Halt abnormal loss of body fluids • Treat conditions of leakage and discharge. • The herbs stabilize, brace and astringe any tendency to fluid leakage and discharge.
Herbs that Calm the Spirit **See Table XIV of Appendix A**	• Mostly enter through the Heart channel, although a few enter the Liver channel. • Their nature depends on which of the two categories they belong to.	• Calm the mind in the treatment of various mind disharmony conditions. • Common symptoms associated with it include anxiety and agitation, insomnia, frequent dreams and palpitations.
Aromatic Substances that Open the Orifices **See Table**	• Tend to be Pungent, aromatic, activating and dispersing by nature. • All enter the Heart channel. • Divided between aromatic	• Open the Heart's pure orifices, i.e. to awaken the mind or restore consciousness in conditions of full-type collapse.

XV of Appendix A	resins and animal parts.	• Symptoms include: stupor, delirium, spasm and cramping of the limbs, coma and a forceful pulse. • In western terms, these herbs address collapse and seizure disorders.
Herbs that Extinguish Wind and Stop Tremors **See Table XVI of Appendix A**	• Tend to be cold and salty in nature. • All enter the liver channel. • Majority of them are animal products rich in minerals and trace elements.	• Calm the liver on one hand and extinguish internal wind on the other. • Used to treat conditions of either liver Yang rising or liver wind stirring. • In western terms, these herbs address various neurological and neuromuscular disorders.
Herbs that Expel Parasites **See Table XVII of Appendix A**	• Have no common qualities, except that several are bitter, cold, and a few are toxic by nature. • Mostly enter the large intestine, stomach and liver channels. • Many are fruits or seeds.	• Eliminate or destroy parasites. • Typically used for treating various kinds of parasitic and fungal infestations. • Common symptoms include: umbilical pain, itching at the rectum, nose and ears, unusually large or small appetite, vomiting or spitting of saliva, emaciation, stool changes that may correlate with the moon phases and unusual appetites. • In terms of Western

		pharmacology, these herbs generally possess a variety of different antiparasitic actions.
Substances for Topical Application **See Table XVIII of Appendix A**	• Tend to be toxic by nature as they are mostly minerals. • Majority enter the Liver channel.	• Designed primarily for topical application in the treatment of various local skin and mucosal conditions.

2.2.4 Studies of the Efficacy of Chinese Herbs

There is a growing body of evidence that demonstrates the clinical effectiveness of Chinese herbal medicine [13, 32]. It should be noted that more than half of the medically important pharmaceutical drugs are either natural products or derivatives of natural products [33, 34]. Bensky and Gamble [25] stated that, "*the traditional Chinese medical system, including the herbal tradition, is not a monolithic body of 'truth', but a group of ideas developed over more than two thousand years which attempt to deal effectively with the problems of health and disease*".

The "medicinal meal" was commonly used in ancient times in Asia, and has become popular again in recent years. Recipes based on dietary therapy including herbs, are used more extensively nowadays in health prevention and the treatment of diseases [35]. Chinese herbs (natural medicinal plants) are rich natural resources containing active therapeutic components, often with low toxicity, and have been demonstrated to be beneficial in disease prevention and treatment. Chinese people have traditionally preferred to use herbs instead of drugs for health care. Many Chinese herbs are described as both food and medicines by the Chinese Ministry of Health, such as *Gan Cao* (*Radix Glycyrrhizae*), Jue Ming Zi (*Semen Cassias*), *Fu Ling* (*Poria*), *Hong Hu*a (*Flos Cathami*), *Kun Bu* (Thallus *Laminariae seu Eckloniae*), *Jiao Gu Lan* (*Gynosiemma Pentaphyllum*) and *Ma Chi Xian* (*Portulaca Oleracea*) [36, 37].

There is a large amount of research being conducted into efficacy and safety of Chinese herbs [38-43]. Chinese herbal medicines have been used to treat a variety of diseases including arthritis, cancer, asthma and allergies [44-47]. For example, Lingzhi (Ganoderma lucidum) is a very popular Chinese medicinal fungus, which has long been used as a sedative [47]. It possesses anti-tumour, anti-hepatotoxic, antinociceptive and immunomodulatory properties and has been used in the treatment of hypercholesterolaemia and hypertension [48-52]. Moreover, its immunomodulatory properties have been exploited in the development of possible treatments for both allergic asthma and food allergy [53, 54]. Xue's group [43, 55] has successfully conducted a number of clinical trials to evaluate the efficacy and safety of Chinese herbal medicines for chronic respiratory diseases and pain management. Xu and Yan [56] conducted studies on both human (boxing athletes) and mice using the Chinese herb preparation Fu Ling (Poris Cocos) for reducing body fat and weight, the results were reported as being significantly positive. It is reported [57] that Chinese Kiwi fruit drink reduces the side effects of the chemotherapy drug cyclophosphamide, which is also a DNA damaging agent. Thus appropriately used Chinese herbs/therapeutic food may assist the treatment and recovery of cancer patients. Studies by Xu, et al. [42] revealed that after four months of herbal treatment of common menopausal symptoms, there were significant changes in some symptoms i.e. lower back pain, tiredness, hot flushes, night sweats, insomnia, headache and thirst. Bone structure and function changes were also positive in general [42]. Chen and Xu [58] have investigated the health effects of seven Chinese herbs and have concluded that Shan Zha (Hawthorn fruit, *Crataegi Fructus*) is effective in lowering blood lipid levels. The Chinese generally consider Shan Zha useful in reducing ‘food stagnancy’ and ‘blood stasis’. As a medicine, it is used to treat hypercholesterolemia, angina pectoris and hypertension, because its components may increase the coronary artery blood flow rate, reducing blood cholesterol concentration and decreasing myocardial oxygen consumption [59]. Xu, et al. [12] have also studied potentially bioactive elements in twenty-two Chinese therapeutic foods and herbs, resourced from the traditional high therapeutic quality areas or provinces. Bioactive analysis focused on Lanthanum (La), Strontium (Sr), Zinc (Zn) and Selenium (Se), especially in the prevention and treatment of hyperlipidemia and its associate disorders. They found that *Rhizoma Gastrodiae Elatae*, *Fructus Crataegi* and *Herba Hedyotidis Diffusae* had a higher elevated concentration of La. Similarly, *Radix Puerariae* and *Folium Ginkgo Biloba* were rich in Sr, *Flos Carthami Tinctorii* and *Fructus Crataegi* showed a high level of Zn and *Flos Lonicerae Japonicae* and *Portulaca Oleracea* contained elevated levels of Se. The results suggested that Chinese herbs which are also therapeutic foods may be used as nutritional supplements for preventing and treating elemental deficiency, e.g., hyperlipidemia. It was suggested that more attention in this regard should be paid to herbs that contain La and are traditionally used for regulating cardiovascular disorders. The knowledge of the effects and concentrations of bioactive elements in foods and herbs could guide the selection of Chinese herbs in clinical practice in conjunction with traditional Chinese medicine theories. Further studies should also be considered in relation to Sr, Zn and blood regulating herbs, which could prove to be beneficial.

2.3 Elemental Analysis of Traditional Chinese Herbs

At least fifty elements are known to be vital for the well being of humans [60] and there is a growing interest in small molecule metallo species in the area of medical science [8, 20, 21]. According to present knowledge, the group of essential elements include: arsenic (As), bromine (Br), chromium (Cr), tin (Sn), zinc (Zn), fluorine (F), iodine (I), cobalt (Co), silicon (Si), lithium (Li), magnesium (Mg), copper (Cu), molybdenum (Mo), nickel (Ni), selenium (Se), vanadium (V), iron (Fe), and probably barium (Ba) and titanium (Ti) [61]. In addition to many of the essential substances to human health presented in Chinese herbs, some toxic elements such as arsenic (As), Cadmium (Cd), Mercury (Hg), lead (Pb), etc. may also be taken up from the environment. These elements are toxic for the human bio-system even at very low levels of intake and are usually present in plants due to increasing industrialisation and associated pollution of the biosphere, uptake from the soil, fertiliser, pesticide treatment and other industrial and anthropogenic operations [7, 62]. From the clinical point of view, it is therefore necessary to apply reliable multi-element analytical techniques for the determination of the concentration of the major, minor and trace elements in Chinese herbs.

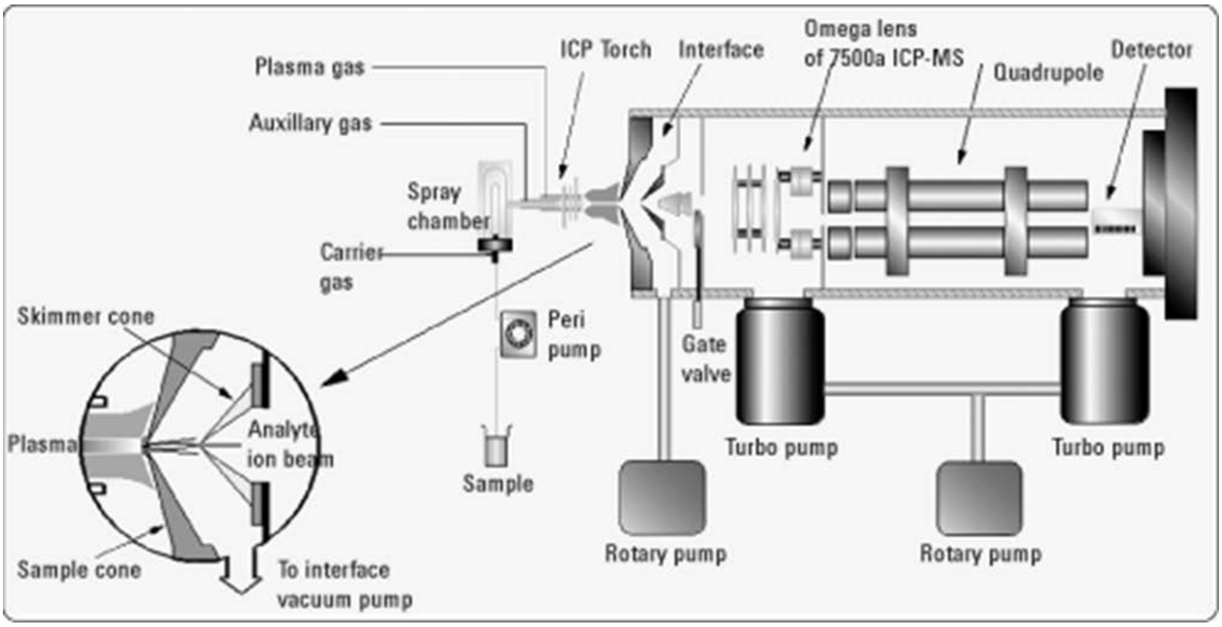
There has been some previous work carried out on the elemental content of Chinese herbs and its possible implications. For example, Xiao et al. [63] used neutron activation to analyse essential trace elements for several ‘tonics’ and ‘non-tonics’ of traditional Chinese medicines. These workers have also carried out X-ray emission (PIXE) studies on the elemental contents of thirty specific traditional Chinese medicines [64]. Zhang et al. [65] have used multivariate statistical analysis to treat the PIXE data of the 24 ‘tonics’ and 6 ‘non-tonics’ in an attempt to establish a elemental basis for this division.

Atomic absorption spectrometry (AAS) has been commonly used for the determination of the metal element contents in herbal medicines [61, 66, 67]. Generally, flame-atomic absorption spectrometry (FAAS) allows the quantification of elements only at ppm levels, requiring a relatively elevated analyte level. However, the sensitivity of FAAS can be improved when combined with preconcentration and other techniques. Graphite furnace-atomic absorption spectrometry (GF-AAS) is used for lower analyte levels. Wang et al. [7] determined the concentration of seventeen essential and toxic elements in ten raw materials of Chinese herbs, including Ginseng, Fuling, and Danggui, using combined AAS and instrumental neutron activation analysis (INAA). The results showed that the ranges of elemental concentrations varied from 10^4 to 10^{-1} mg/kg for different kinds of herbs. All herbs were reported as exhibiting enrichment capabilities from the environment for elements such as Mn, Zn, Ca, K, Mg, Cd, Cu, Pb and As. The INAA technique was also employed by Hamzah et al. [2] for the analysis of sixteen trace and major elements of ten popular Chinese herbs used in Malaysia. The concentration of elements was found to vary depending on the origin of the herb.

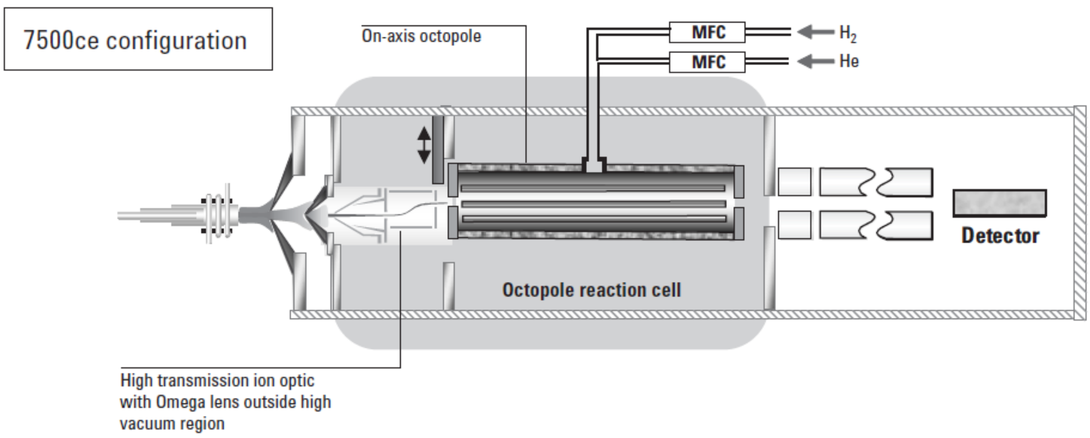
In additional to the above analytic methods, inductively coupled plasma-optical emission spectrometry (ICP-OES) (also known as inductively coupled plasma-atomic emission spectrometry or ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS) have been widely used for the elemental analysis of herbal medicines [61, 68-71]. ICP-OES is usually the method of choice for the determination of trace metals because of its speed and wide availability. However, for the determination of elements in complex matrices as in TCM, ICP-OES often lacks the high sensitivity needed. The fast developing ICP-MS technique is becoming an attractive method since it offers the advantages of high sensitivity and simultaneous multi-element analysis capability [70, 71]. ICP-MS accepts almost any sample type and also provides isotopic information. It is one of the most sensitive analytical techniques for fast multi element determination at trace and ultra trace concentrations in different sample matrices. It has recently emerged as a powerful technique and is also considered to be the most suitable technique for the determination of trace elements in bulk drugs and pharmaceuticals [72]. ICP-MS and ICP-OES techniques are routinely used by the National Measurement Institute (NMI) for the determination of trace elements and heavy metals in a wide variety of food samples. However, for many pharmaceutical products, it also has some limitations such as high capital investment and the non-availability of certified reference standards.

Figure 2.2a shows a schematic diagram of a typical commercial ICP-MS instrument from Aglient Technologies [73]. The main components of the ICP-MS spectrometer include: (1) sample introduction system, (2) plasma, (3) interface, (4) vacuum system, (5) ion lenses, (6) mass analyser (quadrupole, magnetic sector, or time-of-flight (TOF)) and (7) electron multiplier detector. The plasma is usually generated in argon at atmospheric pressure, sustained by a high frequency (30 MHz) energy field of 1000 – 2000 W. The temperature (6 – 10 × 10^3 K) in the plasma is suitable for atom excitation and ionisation of elemental species. The quartz torch consists of three concentric tubes into which different argon flows are introduced. The most commonly used method for introducing liquid samples is conventional pneumatic nebulisation. When samples are introduced into the plasma, they go through desolvation, vaporisation, atomisation and ionisation before entering the mass analyser. The ions emerging from the ICP are extracted into the low-pressure mass spectrometer interface through the sample and skimmer cones, and then focused on to the mass analyser by ion lenses. The positively charged ions are then separated according to their mass to charge ratio. The quadrupole is the most widely used mass analyser in ICP-MS. Double focusing sector field mass analysers are also used to obtain higher resolutions and thereby reduce isobaric interferences. While the TOF mass analyser has a great potential for speciation analysis. The ions are typically detected by an electron multiplier detector.

To remove interfering ions, a collision reaction cell known by the trade name, dynamic reaction cell (DRC), was introduced into the ICP-MS instrumentation. The DRC is a chamber that can be filled-up with reaction (or collision) gases (e.g. ammonia, methane, oxygen or hydrogen), either one gas type at a time or a mixture of two gases, which reacts with the introduced sample, eliminating some of the interferences [74, 75]. Aglient Technologies uses an octopole reaction cell (Fig. 2.2b) [76] in its 7500 series ICP-MS systems for interference removal. This reaction cell is placed before the traditional quadrupole chamber (Fig. 2.2a) of an ICP-MS device, and uses only the smaller molecule gases of helium or hydrogen to physically collide with the large, unwanted polyatomic ions in order to remove polyatomic interferences.



(a)



(b)

Figure 2.2: (a) Schematic diagram of a typical commercial ICP-MS spectrometer from Aglient Technologies [73] and (b) octopole reaction cell used in Aglient's 7500 series ICP-MS systems [76].

A preliminary study using ICP-MS for the determination of trace metals in TCM was carried out by Wang and co-workers in 1999 [70]. In their study, an ICP-MS-based analytical protocol was developed to determine trace metals in several TCM samples prepared by three different methods: wet digestion by $HNO_3/HClO_4$, wet digestion using HNO_3/H_2O_2, and microwave oven digestion with HNO_3/H_2O_2. It was found that ICP-MS analysis on the samples prepared by the above three different methods gave comparable metal results with satisfactory recovery of herbal standards. Chan, et al. [71] used dynamic reaction cell (DRC) ICP-MS and ICP-AES for analysis of major (Al, Ba, Ca, Fe, Na, Mg, K and P) and trace (V, As, Se, Cd, Hg and Pb) elements in Ling Zhi after microwave digestion. As mentioned earlier in Section 2.2.4, Ling Zhi is a type of fungi that is widely used as Chinese medicine in anti-cancer and immunomodulatory therapy. Chen, et al. [77] developed an ICP-MS-based method to fully characterise the inorganic content of TCM samples. The reliability of both the ICP-MS hardware and the developed method was evaluated using an Agilent 7500a ICP-MS instrument. Wang and co-workers [78] recently employed ICP-MS to analyse eleven trace elements (Cr, Mn, Ni, Co, Cu, Zn, As, Se, Mo, Cd and Pb) in root-like and rootstalk-like medicinal herb samples digested by a microwave digestion method. The detection limits of their method for all elements ranged from 0.001 – 0.260 µg/g. Most recently, ICP-MS was used by Xu et al. [12] to determine the concentrations of various potential bioactive elements such as La, Sr, Zn and Se in twenty-two Chinese therapeutic foods and herbs. These elements in their appropriate dosage range are considered to be beneficial to health.

Attention has also been focused on the determination of the concentration of some toxic elements in Chinese herbs such as Hg, Cd, Pb and As [2, 7, 71, 79-81]. Wang et al. [7] observed higher contents of Cd, Pb and As in herbs, which was possibly due to the uptake of these elements from soil polluted by industrial and anthropogenic activities. The studies conducted by Hamzah and co-workers [2], however, showed that the toxic elements in the tested herb samples were below the levels prescribed by health regulations. Several techniques have been used for the determination of Pb in herbal samples, including GFAAS [82], ICP-MS [70, 83] and ICP-OES [3]. Sun, etc. [81] developed a simple and sensitive method, named as derivative atom trapping flame atomic absorption spectrometry (D-AT-FAAS), for the determination of the lower level of lead in Chinese herbs.

It is well known that the immediate source of nutrients and metal ions available for plant uptake is from the soil solution [84-86] and the percentage of any metal occurring in the soil solution is usually smaller than the total metal pool in the soil [87-89]. Soil factors such as the total metal present in the soil, pH, clay and hydrous oxide content, organic matter and redox conditions have an impact on the concentration and speciation of metals in the soil solution. These factors will therefore affect the availability of metals for plant uptake [90]. Different parameters have been used to investigate the metal uptake by plants. For example, a biconcentration factor (BF) (ratio of metal concentration in plant to metal concentration in soil) was used to investigate heavy metal uptake by plants [90-92]. Ngole [93] used a mobility factor (MF) (ratio of an element in the mobile fractions to that in the inert fraction) as a possible indicator to determine potential uptake of heavy metals by vegetables. The most commonly used parameter for evaluation of the uptake ability of plants is the Enrichment Factor (EF) but the calculation of this as used by various researchers differ [7, 93-97].

For essential or toxic elements in traditional Chinese herbs to extert an effect, they must be bioavailable. In other words, they must be available, or potential available, both for absorption by the gut and for subsequent utilisation by the body [98]. It is well know that the bioavailability, toxicity and transport properties of an element strongly depend on its chemical form [99]. Therefore, in order to understand the way particular elements affect the functions of traditional Chinese herbs, it is necessary to conduct speciation analysis. However, only limited work has been carried out on this topic [70, 100].

This research aims to establish a database of traditional Chinese herbs by performing elemental analysis on a large number of samples using ICP-MS and ICP-OES. This database contains a selection of herbs that are considered to be effective in treating specific human disease states, such as diabetes. Such herbs will be screened for the concentration levels of 24 chemical elements. A focus will be placed on those metals that are known to be active as biomimetic against certain diseases, e.g. manganese species as anti-inflammatories (Fig. 2.3) [101, 102] and vanadium species as insulin mimetics (Fig. 2.4) [103].

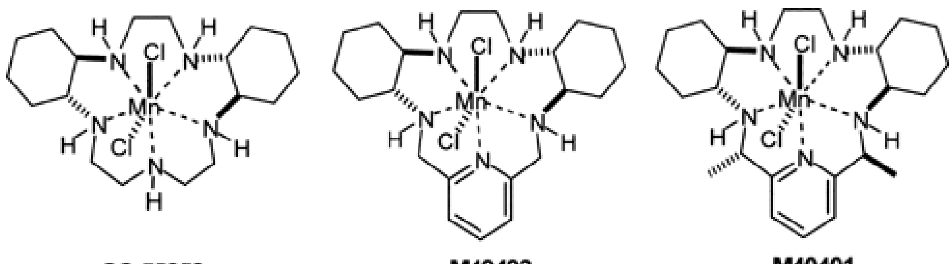


Figure 2.3: Manganese species – SOD (superoxide dismutase) mimetics for inflammation treatment [101].

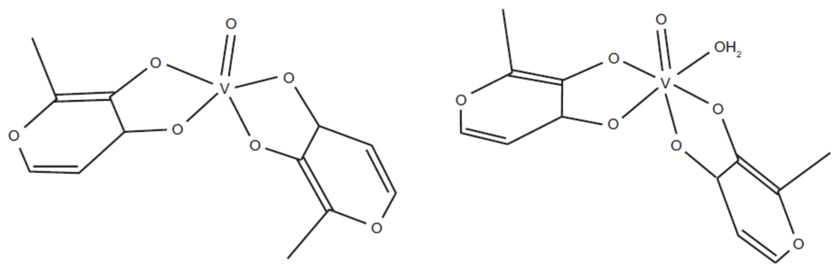


Figure 2.4: Vanadium species as insulin mimetic agents – bis-(maltolato) oxovanadium (IV) (BMOV) [103].

This project also aims to suggest possible metal complexing ligands based on a quantitative analysis of the total extractable and bioavailable contents of a range of relevant metals across the selected Chinese herb samples. This will be very helpful for other researchers to identify novel metal complexing ligands using some modern techniques such as targeted liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS), which has recently successfully been used to identify a new Ni(II) ligand in the latex of a Ni-hyperaccumulating tree *Sebertia acuminata* [104].

To the best of our knowledge, there has been no Chemical Reference Tables (CRTs) that have been previously reported in the literature, on the elemental speciation of the Chinese herbs used in this study. Since TCM is 'traditional', it does not specifically address elemental imbalances/deficiencies, but does address the potential symptoms of these. Also, to the best of our knowledge, there have been no previous GC/MS or LC/MS studies relating to the types of organic molecules that might act as ligands and be present in the herbs studied in this project.

Chapter 3: Materials and Methods

3.1 Materials

An integral part of this study was the establishment of an extensive collection of traditional Chinese herbs which are distributed over eighteen commonly used medicinal categories. These categories are described in Table 2.1. Thus from a selection of 300 candidate herbs, 191 commonly used samples were selected and sourced from University-based sources and from local pharmaceutical groceries. These are listed in Appendix A with the 191 selected herbs highlighted. In general, such raw herbal material originates from different regions of China. For this project, a subset of 103 herbal materials was carefully selected for elemental analyses. These are the most commonly used in Chinese medicine for common medical conditions (e.g. diabetes, cancer, infections, etc.) in society, and are listed and coded in Table 3.1. Note that the term "herb" refers to either the whole plant or various plant components (e.g. root, stem, fruit, seed, bark, leaf, and flower), Fig. 3.1, or combinations of such components. All raw herbs were oven dried and ground consistently into fine powders prior to sample preparation for elemental analysis.

HNO_3 (65 % WR ARISTAR England) and HCl (36 % Suprapur, Merck) were used for the digestion of each herb. Standard solutions of V, Mn, Co, Li, Bi, Se, Mo, Sb, Sn, As, Cd, Cr, Pb, Hg, Cu, Ni, Zn, Ba (each at 1000 mg/L) and Fe, Al, K, Na, Ca, Mg (each at10,000 mg/L) were purchased from AccuStandard, U.S.A. Standard Reference Materials (SRM) used for this study were AGAL-6 (Cabbage Leaf), AGAL-10 (Hawkesbury river sediment) and NMIA MX008 (Pharmaceutical Tablet) supplied by the National Measurement Institute (NMI), Australia. NCS DC 78302 (Tibetan soil) was obtained from the China National Analysis Center for Iron & Steel. High-purity water (18.2 MΩ) from a Milli-Q system (Milipore, Australia) was used in the preparation of all solutions.

Table 3.1: The 103 herbal materials tested in this study

Code	Herb name	Code	Herb name	Code	Herb name
1	Dang Gui	36	Tu Si Zi	71	Xin Ren
2	Bai Shao	37	Sang Zhi	72	Bai Jie Zi
3	Fu Ling	38	Dan Shen	73	Jie Geng
4	Chuan Xiong	39	Hu Zhang	74	Bai Zhi
5	Shu Di	40	Yu Jin	75	Shan Yao
6	Gan Cao	41	Huang Lian	76	Lian Zi
7	Long Yan Rou	42	Huang Jin	77	Sang ji Sheng
8	E Jiao	43	Jiao Gu Lan	78	Ge Gen
9	Gui Zhi	44	Guo Qi Zi	79	Chuan Niu Xi
10	Da Zhao	45	Ju Hua	80	Qiu Gua Lou
11	Ren Sheng	46	Luo Bu Ma	81	He ye
12	Qi Pi	47	Tian Ma	82	Hong Qu Mi
13	Jin Yin Hua	48	Sang Ye	83	Ji Xu Teng
14	Ku Shen	49	Sheng Di	84	Lu Hui
15	Lian Qiao	50	Dan Zhu Ye	85	Mu Dan Pi
16	Xia Ku Cao	51	Chuan Lian Zi	86	Shi Chang Pu
17	Hong Teng	52	Shen Mai Ya	87	Si Gua Rou
18	Chi Shao	53	Yin Chen	88	Yi Mu Cao
19	Pu Gong YI	54	Huang Qin	89	Yi Zhi Ren
20	Bai Jiang Cao	55	Du Zhong	90	Zhu Ru
21	Ma Chi Xian	56	Hui Niu Xi	91	Zi Su Ye
22	Bai Xian Pi	57	Pei Lan	92	Ling Zhi
23	Bai Hua She She Cao	58	Shen Jiang	93	Lou Han Guo
24	Da Huang	59	Suan Zao Ren	94	Jiang Huang
25	Tao Ren	60	Chai Hu	95	Cang Zhu
26	Hei Zi Ma	61	Wu Jia Pi	96	Chen Pi
27	Hu Ma Ren	62	Hai Zao	97	Ban Xia
28	Shan Zha	63	Mai Dong	98	Huo Xiang
29	Zhi Shi	64	Bu Gu Zhi	99	Yi Yi Ren
30	Duo Huo	65	Mo Yao	100	Huang Bai
31	He Shou Wu	66	Zi Cao(H)	101	Ze Xie
32	Lai Fu Zi	67	Zi Cao(B)	102	Fang Ji
33	Hong Hua	68	Qiang Huo	103	Sang Bai Pi
34	Shan Qi	69	Bo He		
35	Huang Qi	70	Niu Bang Zi		

Ren Sheng (Root)

Pi Pa Ye (leaf)

Ze Xie (stem)

Chuan Lian Zi (fruit)

Pan Da Hai (seed)

Ju Hua (flower)

Figure 3.1: Representative examples of different plant components used in traditional Chinese herbal medicine.

3.2 Sample preparation and analysis

Elemental analysis by ICP-MS and ICP-OES was conducted on the subset of 103 samples (Table 3.1) selected from the collection of 191 Chinese herbs (Appendix A). The 24 elements under consideration were vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), lithium (Li), bismuth (Bi), arsenic (As), selenium (Se), molybdenum (Mo), cadmium (Cd), aluminium (Al), tin (Sn), antimony (Sb), lead (Pb), mercury (Hg), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg) and Barium (Ba).

Two parallel sets of data were generated corresponding to TOTAL and BIOAVAILABLE extractable elements. Total extractable elements - digestion was carried out in a microwave digestion system, as described in more detail below. This method is preferred over the traditional hot plate method [72, 105] since it is fast, it requires minimal volume of reagents - thus reducing interference by reagent contamination, it has better recovery of volatile elements and it has superior reproducibility. Bioavailable extractable elements - a simulated stomach digestion method [100, 106] was employed as described in more detail below.

An enrichment factor (EF) analysis was carried out to systematise the relationship between the soil and the herbs and to estimate relative enrichment abilities [7, 94]. Since all the raw herb materials investigated in this study are imported from mainland China, a reference standard material of unpolluted Tibetan soil (NCS DC 78302), obtained from the China National Analysis Center for Iron & Steel, was used as the reference soil source. Al was taken as the reference element and the EF values were then calculated according to the following equation [7]:

$$EF = (C_x/C_{Al})_{herb} \,/\, (C_x/C_{Al})_{soil} \qquad (3.1)$$

where C_x and C_{Al} are the total concentrations of element X and Al in the herb and soil samples, respectively. The experimental details for the digestion of the soil sample are described below.

Total Extractable Elements

Digestion was conducted in a microwave digestion oven (Milestone MLS 1200, John Morris Scientific Pty Ltd, Australia) using a method (Method No. VL 247) developed by the NMI.

Thus a herbal sample (0.2 g ± 0.02 g) was mixed with 3 mL HNO_3 (65 %) and 0.5 mL HCl (36 %) in a 50 mL graduated disposable plastic digestion tube and placed in a DigiPrep digestion block (Digi PREP MS, SCP Science, Canada) for approximately 60 min at 95 °C. The sample was then removed from the Digiprep block, and further heated in a microwave oven for 35 min at 300 Watts. After cooling down to room temperature, the sample was filtered through a 0.45 µm cellulose acetate membrane filter (Sartorius Biotech GmbH, Germany) into a 15 mL centrifuge tube, and Milli-Q water added to a total volume of 14 mL. One mL of this solution was withdrawn and diluted to 10 mL with 5 % HNO_3 using a Gilson 402 dilutor-dispenser (Trace s/n 640c513143, John Morris Scientific Pty Ltd, Australia) prior to the ICP analysis of total extractable content of metals. The same procedures were used for the preparation of blank and SRM samples AGAL-6 (Cabbage Leaf) and NMIA MX008 (Pharmaceutical Tablet).

Bioavailable extractable elements

Samples for the measurement of the bioavailability of elements were prepared by a simulated stomach digestion method (Australian/New Zealand standard, Method No. AS/NZS ISO 8124.3:2003). Thus a homogenised sample (0.5 g ± 0.02 g) was weighed in a 50 mL plastic tube, followed by 25 mL 0.07 M HCl (pH = 1.35). The tube was sealed and the mixture was agitated continuously for 1 h with a lab tumbler (Labquip Technologies, Victoria, Australia) at 37 ± 2 °C and then allowed to stand for another 1 h at the same temperature. After digestion, the leachate was centrifuged at 5000 rpm for 5 min using a GS-6 centrifuge (Beckman U.S.A). The extract was removed and filtered through a 0.45 µm membrane filter, and 1 mL of the filtered extract was diluted to 10 mL with 0.07 M HCl prior to ICP analysis. Analogous procedures were used for the preparation of blank samples.

Enrichment Factor Analysis

The Tibetan soil samples (NCS DC 78302) used for determination of the EFs were analysed using an acid leaching method (Method No. VL 239) developed by the NMI. Thus a 50 mL plastic digestion tube with a mixture of 1.0 g of soil sample, 6 mL HNO_3 and 2 mL HCl was placed in a DigiPrep block for 1 h at 95 °C. The tube was removed from the block and Milli-Q water added up to 15 mL mark, and then shaken by a Vortex Mixer (Ratek Instrument VM1) for up to 30 seconds. After shaking, the plastic tube was heated in the DigiPrep block for a further 2 h, and more Milli-Q water was added up to 20 mL mark. The mixture was shaken and centrifuged for 10 min at 1500 rpm. The blank and SRM samples (Hawkesbury river sediment, AGAL-10) were prepared through the same procedures as above.

The elemental concentrations in the prepared herb and soil samples were analysed by ICP-MS (Agilent 7500C, Agilent Technologies, Inc Japan) and ICP-OES (PE Optima 4300 DV, Perkin Elmer instruments, USA). The levels of trace elements V, Cr, Mn, Co, Ni, Cu, Li, Bi, As, Se, Mo, Cd, Al, Sn, Sb, Pb, Hg and Zn were measured by ICP-MS which offers the advantage of high sensitivity, whilst major elements K, Ca, Na, Mg and Fe were determined by ICP-OES as these elements are usually at the percentage level [71]. Based on the results obtained from the preliminary qualitative scanning, the following working standards were used in this study: (1) 5 ppb, 10 ppb and 20 ppb for V, Cr, Mn, Co, Ni, Cu, Li, Bi, As, Se, Mo, Cd, Al, Sn, Sb, Pb, Ba and Hg; (2) 50 ppb, 100 ppb and 200 ppb for Zn; (3) 10 ppm, 20 ppm, and 50 ppm for K, Ca, Na and Mg; and (4) 0.1 ppm, 0.5 ppm and 1.0 ppm for Fe. The highest check standards used in this were 1000 ppb for V, Cr, Mn, Co, Ni, Cu, Li, Bi, As, Se, Mo, Cd, Al, Sn, Sb, Pb, Hg and Zn, 100 ppm for K, Ca, Na and Mg, and 10 ppm for Fe. The Agilent 7500C instrument used for the current work is equipped with a reaction cell which allows operating in "reaction mode". As and Cr were measured in "reaction mode" using helium as the reaction/collision gas to remove polyatomic interferences. Other elements which are known to potentially suffer from interferences (including Se, Cu, Ni, and Zn) were also analysed in "reaction mode" using either helium or hydrogen pressurised cells to remove matrix or plasma based interferences. The operating conditions for ICP-MS and ICP-OES analyses are listed in Tables 3.2 and 3.3 respectively. In order to correct for instrumental drift, a mixed internal standard solution was used. A mixture of 100 μg/L indium/rhodium & 500 μg/L germanium/scandium (diluted from SPEX Plasma Standard or equivalent) in 5 % HNO_3 were used for ICP-MS analysis and 2 mg/L lutetium (from Sigma AR grade or equivalent) in 5 % HNO_3 was used for ICP-OES analysis. All internal standards were added on-line.

Table 3.2: ICP-MS operating conditions

Rf power (W)	1500
Carrier gas flow rates (L/min)	0.9
Make up gas flow rates (L/min)	0.3
Integration time	0.1 sec/per point, 0.3 sec/per mass
Uptake time	30 sec
Uptake speed	0.5 rps
Stabilization time	30 sec

Table 3.3: ICP-OES operating conditions

Rf power (W)	1500
Sample uptake rate (mL/min)	0.9
Plasma (L/min)	17.0
Auxiliary (L/min)	0.5
Nebulizer (L/min)	0.65
View height	15 mm above load coil
Integration Time (sec)	1-5

To validate the digestion methods (microwave and simulated stomach acid) for both total and bioavailable concentrations of the twenty-four elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Li, Bi, As, Se, Mo, Cd, Al, Sn, Sb, Pb, Hg, K, Na, Ca, Mg and Ba) in the herb samples, a recovery study was carried out. A synthetic solution containing these twenty-four elements was prepared for the performance of the recovery test (Table 3.4 and 3.5). Portions of 0.2 g (for microwave digestion) and 0.5 g (for simulated stomach acid digestion) of the herbs were spiked with the synthetic solution, and then the elements were determined following the same procedures used above for the digested herb samples. The results were considered satisfactory, with recoveries being within the range of 84 – 110 % [107] except for Hg (10 %) (Table 3.5). The accuracy and precision of the data has also been checked by analysis of the

SRM: Cabbage Leaf (AGAL-6) and Pharmaceutical Tablet, and the results are shown in Table 3.6 and 3.7, respectively. These results demonstrated that there was no significant loss of analyte with both the microwave and simulated stomach acid digestion methods.

Table 3.4: Recovery test results for the microwave digestion method

Analyte	Base value (mg/kg)	Quantity add (mg/kg)	Quantity found (mg/kg)	Recovery (%)*
V	0.0	2.50	2.70	108
Cr	0.120	2.50	2.72	101
Co	0.0514	2.50	2.69	106
Ni	0.148	2.50	2.49	94
Mo	0.00199	2.50	2.28	91
Mn	9.63	22.5	32.1	100
Cu	3.85	22.5	24.5	92
Zn	4.23	22.5	25.7	95
Fe	18.7	1000	1032	101
Al	5.40	22.5	27.0	96
As	0	2.50	2.33	93
Ba	1.34	2.50	3.75	96
Bi	0	2.50	2.25	90
Cd	0.00614	2.50	2.63	105
Pb	0.0166	2.50	2.58	103
Sb	0	2.50	2.18	87
Se	0.00292	2.50	2.71	108
Hg	0	2.50	2.12	85
Li	0.0175	2.50	2.41	96
Sn	0.124	2.50	2.38	90
Ca	9.69	1000	910	90
Mg	38.7	1000	1100	106
K	430	1000	1500	107
Na	10	1000	920	91

*[(Found-base) / added] × 100

Table 3.5: Recovery test results for the simulated stomach acid digestion method

Analyte	Base value (mg/kg)	Quantity add (mg/kg)	Quantity found (mg/kg)	Recovery (%)*
V	0.0	2.00	2.17	109
Cr	0.130	2.00	2.22	105
Co	0.0367	2.00	1.87	92
Ni	0.137	2.00	2.15	100
Mo	0.00	2.00	1.85	93
Mn	9.91	22.00	32.9	104
Cu	3.57	22.00	25.1	98
Zn	4.50	22.00	26.8	101
Fe	12.7	800	796	98
Al	4.57	22.0	28.1	107
As	0	2.00	1.85	92
Ba	1.23	2.00	3.04	91
Bi	0	2.00	1.77	88
Cd	0.00686	2.00	2.21	110
Pb	0.0166	2.00	2.11	105
Sb	0	2.00	1.67	84
Se	0	2.00	1.82	91
Hg	0	2.00	0.210	10.5
Li	0.0184	2.00	1.80	89
Sn	0.0279	2.00	1.84	91
Ca	10.6	800	780	96
Mg	41.9	800	800	95
K	450	800	1200	94
Na	10	800	760	94

*[(Found-base) / added] × 100

Table 3.6: Results of the determination of chemical elements in the SRM (AGAL-6)

Analyte	Certified (mg/kg)	Experimental (m/kg)	Recovery (%)
V	0.49	0.52	106
Cr	1.40	1.36	97
Co	0.23	0.25	109
Ni	0.67	0.71	106
Mo	2.14	2.32	108
Mn	48	44	92
Cu	54	51	94
Zn	37.7	37.6	100
Fe	348	346	99
Al	229	215	94
As	0.25	0.225	90
Ba	47.1	44.7	95
Bi	<0.01	<0.01	N.A
Cd	0.1	0.093	93
Pb	4.32	4.01	93
Sb	0.02	0.023	115
Se	0.52	0.468	90
Hg	0.0200	0.0167	84
Li	0.19	0.188	99
Sn	0.13	0.12	92
Ca	3500	3200	91
Mg	6000	5500	92
K	21000	20000	95
Na	7000	6500	108

Table 3.7: Results of the determination of chemical elements in the SRM (Pharmaceutical Tablet)

Analyte	Certified (mg/kg)	Experimental (mg/kg)	Recovery (%)
Mo	5.80	5.21	90
Mn	515	517	101
Cu	86.5	88.3	102
Cr	13.1	12.8	98
Zn	3050	2950	97
Fe	960	900	94
Al	30.9	32.5	105
Ba	4.10	3.86	94
Se	32.1	29.3	91
Ca	39200	37600	96
K	7579	7352	97
Mg	12000	11000	92
Na	3580	3710	104

Validity of the acid leaching method (Method No. VL 239) used for the soil samples was also carried out in the current work. A synthetic solution (Table 3.8) containing the above twenty-four elements was prepared for the performance of the recovery test for the Tibetan soil samples (NCS DC 78302). 1.0 g of the soil sample was spiked with the synthetic solution (Table 3.8), and the elements were determined following the same procedures used above for the digested soil samples. The results shown in Table 3.8 were considered satisfactory, with the recoveries being within the range of 90 – 110 % [107]. The accuracy and precision of the data was checked by analysis of the SRM AGAL-10, and the results are shown in Table 3.9. These results demonstrate that there was no significant loss of analyte with the acid leaching of soil sample method.

Table 3.8: Recovery test results for the the acid leaching method

Analyte	**Base value (mg/kg)**	**Quantity add (mg/kg)**	**Quantity found (mg/kg)**	**Recovery (%)** *
Al	15000	10000	26000	110
As	3.14	50	49.2	92
Ba	83.2	50	128	90
Bi	0.271	50	46.3	92
Cd	0.0490	50	45.5	91
Co	8.55	50	63.2	109
Cr	32.9	50	80.8	96
Cu	22.2	250	252	92
Hg	0.0392	50	48.3	97
Li	11.3	50	56.8	91
Mn	409	100	513	104
Mo	0.444	50	46.6	92
Ni	16.8	50	70.8	108
Pb	6.07	50	53.7	95
Sb	0.0590	50	46.4	93
Se	0.100	50	46.1	92
Sn	1.64	100	94.7	93
V	33.3	250	276	97
Fe	22000	10000	33000	110
K	5300	10000	15000	97
Ca	5900	10000	16000	101
Na	480	10000	10000	95
Mg	8400	10000	18000	96

*[(Found-base) / added] × 100

Table 3.9: Results of the determination of chemical elements in the SRM (AGAL-10)

Analyte	Certified (mg/kg)	Experimental (m/kg)	Recovery (%)
V	25.3	27.6	109
Cr	82	86	105
Co	9.16	9.18	100
Ni	17.8	17.9	101
Mo	8.6	8.4	98
Mn	241	233	97
Cu	23.2	22.8	98
Zn	57	56	99
Fe	19950	19551	98
Al	8950	8771	95
As	17.2	16.0	93
Ba	28.4	26.5	93
Bi	8.0	7.7	96
Cd	9.33	8.92	96
Pb	40.4	40.9	101
Sb	-	-	-
Se	11.0	10.6	96
Hg	11.6	10.7	92
Li	9.9	9.3	94
Sn	1.72	1.66	97
Ca	2060	2037	99
Mg	2460	2357	96
K	1480	1572	106
Na	5970	5749	96

All the ICP data presented in this thesis were treated according to the NMI's guidelines for quality assurance (QA) - acceptable ranges for replicate ICP/OES and ICP/MS analysis [107]. For triplicates, if the results have a relative percentage difference (RPD) of <= 29 % then the average of the results can be reported. If the RPD for any triplicates is > 29 %, then calculate the RPD for the closest two replicates. If this calculated RPD is <= 24 % then the average of these closest two replicates is within the acceptable ranges for report. Otherwise, non-compliance with QA needs to be investigated and a new set of samples (triplicates) have to be prepared and analysed.

Chapter 4: Results and Discussion

Total Extractable Content, Bioavailability and Enrichment Capability of Twenty-four Chemical Elements in Chinese Herbs

4.1 Introduction

Chinese medicine generally contains a wide variety of herbs and can be used for a wide range of medical conditions. As shown in Table 2.1 (Chapter 2), traditionally, Chinese herbs are classified into eighteen categories according to their functions. In this study, a library of 191 commonly used traditional Chinese herbs was established (Appendix A). A total of 103 representative samples (Table 3.1) was chosen for analysis in triplicate by ICP/OES and ICP/MS with respect to twenty-four chemical elements (V, Cr, Mn, Fe, Co. Ni, Cu, Zn, Li, Bi, As, Se, Mo, Cd, Al, Sn, Sb, Pb. Hg, K, Na, Ca, Mg. and Ba). A global database containing the total and bioavailable levels of twenty-four chemical elements in each of these 103 samples was thus generated (Appendix B). The total and bioavailable levels (in triplicate) of some representative examples, selected from the global database (Appendix B), are shown in Tables 4.1and Table 4.2, respectively.

The measured twenty-four chemical elements in the global database include the essential elements that are components of our diets and of our bodies in a medical context, and alien substances that are foreign to the organism and may represent a danger to health. According to the level of daily requirements within the medical context, three main groups of minerals: macrominerals (K, Na, Ca, and Mg), microminerals (Fe, Cu, Zn, Mn, Mo, Se, Cr, and Co) and trace elements (Sn, Ni, V, Li and Bi) were created [22]. A toxicity investigation for the alien substances (Pb, As, Cd, Hg, Al, Sb and Ba) was also conducted by specifically examining the data across the 103 herbs in the global database.

For these elements to extert an effect, they must be bioavailable for absorption by the gut and for subsequent utilization by the body [98]. Therefore, to analyse and interrogate the large amount of data produced in this study, a two-dimensional plot across the 103 herbs was produced for each of the elements. These plots compared both total and bioavailable levels of elements for each herb and allowed different levels of elements to be readily compared between the different herbs. A similar approach has been taken with respect to the analysis of data relating to possible toxic levels of the alien substances. It is acknowleged that this is not the only method that could be used to analyse this data.

Furthermore, enrichment factors were calculated by Equation 3.1 for all the twenty-four chemical elements (Al was taken as the reference element) across the global database. Generally, if the EF value for an element is close to unity, the soil should be considered to be a major contributing source to that element in the plant. When the EF value is more than 10 for a specific element, it either suggests that the plant has a high enrichment capability for that element or implies that the soil is not the only contributing source for that element [7]. Figure 4.1 shows a general representation for an enrichment factor (EF) analysis of a number of herb samples for a given chemical element. The figure is divided (by the median value of the total extractable content of element and a nominal EF of 10) into four parts: I – herbs with high values in both EF and elemental concentration; II – herbs with a higher value of EF but low level of elemental concentration; III – herbs with low values in both EF and elemental concentration; and IV– herbs with a lower value of EF but high elemental concentration.

In this study, the concentration distribution of twenty-four chemical elements was also discussed with respect to various plant components (e.g. root, stem, fruit, seed, bark, leaf, and flower).

For reference, quantitative data on recommended dietary allowance, exposure/intake and daily adverse intake levels, for each of the twenty-four chemical elements under investigation, are documented in a Table in Appendix C.

Table 4.1: Total extractable content of twenty-four chemical elements for selected herbs from the global database (Appendix B)

Herb name	Al	As	Ba	Bi	Cd	Co	Cr	Cu	Hg	Li	Mn	Mo
Dang Gui rep 1	130	0.2087	4.3435	0.00328	0.01432	0.13763	2.34345	8.217	N.D.	0.28721	17.783	0.4405
Dang Gui rep 2	130	0.1746	4.2885	0.00362	0.01384	0.13944	2.50445	8.394	N.D.	0.2983	18.433	0.4446
Dang Gui rep 3	130	0.2019	4.2045	0.00317	0.01366	0.13344	2.38324	8.344	N.D.	0.2808	17.803	0.4365
Dang Gui mean	130	0.195	4.28	0.00336	0.0139	0.137	2.41	8.32	N.D.	0.289	18.0	0.441
SD	0	0.01804	0.0700	0.00023	0.000344	0.00307	0.08386	0.09124	N.A.	0.00885	0.3696	0.00405
SE	0.000	0.010	0.040	0.00014	0.00020	0.002	0.048	0.053	N.A.	0.005	0.213	0.002
Dang Gui	130±0	0.195±0.010	4.28±0.04	0.00336±0.00014	0.0139±0.002	0.137±0.002	2.41±0.05	8.32±0.05	N.D.	0.289±0.005	18.0±0.2	0.441±0.002
Bai Shao rep 1	13.61	0.06325	6.671	N.D.	0.03104	0.03913	0.8712	8.652	N.D.	0.1829	8.5	0.06196
Bai Shao rep 2	12.97	0.0572	6.704	N.D.	0.03044	0.03845	0.8056	8.759	N.D.	0.1812	8.4	0.05542
Bai Shao rep 3	11.45	0.06165	6.886	N.D.	0.02992	0.03788	0.8163	7.355	N.D.	0.1836	8.3	0.05499
Bai Shao mean	12.7	0.0607	6.75	N.D.	0.0305	0.0385	0.831	8.71	N.D.	0.183	8.40	0.0575
SD	1.109	0.003	0.116	N.A.	0.001	0.001	0.035	0.076	N.A.	0.001	0.100	0.004
SE	0.641	0.0018	0.067	N.A.	0.0003	0.0004	0.020	0.044	N.A.	0.001	0.058	0.0023
Bai Shao	12.7±0.6	0.0607±0.0018	6.75±0.07	N.D.	0.0305±0.0003	0.0385±0.0004	0.831±0.020	8.71±0.04	N.D.	0.183±0.001	8.40±0.06	0.0575±0.0023

Table 4.1: (continued)

Herb name	Ni	Pb	Sb	Se	Sn	V	Zn	Ca	Fe	Mg	K	Na
Dang Gui rep 1	0.5293	0.2024	0.02765	0.1856	0.01199	0.4392	20.30	2200	270	2000	11000	220
Dang Gui rep 2	0.5685	0.2020	0.02334	0.1636	0.01274	0.4472	19.97	2200	270	2000	11000	220
Dang Gui rep 3	0.5241	0.1849	0.02408	0.1593	0.000219	0.4448	20.84	2200	260	2000	11000	220
Dang Gui mean	0.541	0.197	0.0250	0.170	0.0124	0.444	20.4	2200	267	2000	11000	220
SD	0.0242	0.00999	0.002304	0.01410	0.0005303	0.004105	0.4392	0	5.773	0	0	0
SE	0.014	0.006	0.0013	0.008	0.0004	0.002	0.254	0.000	3.333	0.000	0.000	0.000
Dang Gui	0.541±0.014	0.197±0.006	0.0250±0.0013	0.170±0.008	0.0124±0.0004	0.444±0.002	20.4±0.3	2200±0	267±3	2000±0	11000±0	220±0
Bai Shao rep 1	0.6240	0.06854	N.D.	0.05390	0.45397	0.05632	14.45	7100	33.67	810	3300	270
Bai Shao rep 2	0.5980	0.07870	N.D.	0.05894	0.46097	0.05712	14.36	7100	26.59	810	3300	270
Bai Shao rep 3	0.5963	0.04779	N.D.	0.06554	0.32497	0.04797	13.9	7300	24.26	820	3300	270
Bai Shao mean	0.606	0.0736	N.D.	0.0595	0.457	0.0567	14.2	7167	25.4	813	3300	270
SD	0.016	0.007	N.A.	0.006	0.005	0.001	0.295	115.470	1.648	5.774	0.000	0.000
SE	0.009	0.0051	N.A.	0.0034	0.004	0.0004	0.170	66.667	1.165	3.333	0.000	0.000
Bai Shao	0.606±0.009	0.0736±0.0051	N.D.	0.0595±0.0034	0.457±0.004	0.0567±0.0004	14.2±0.2	7167±67	25.4±1.2	813±3	3300±0	270±0

N.D. = Not detected; N.A. = Not applied

Table 4.2: Bioavailabe content of twenty-four chemical elements for selected herbs from the global database (Appendix B)

Herb name	Al	As	Ba	Bi	Cd	Co	Cr	Cu	Hg	Li	Mn	Mo
Dang Gui rep 1	20.25	0.1002	4.505	0.000889	0.01565	0.06082	1.057	7.850	N.D.	0.07224	16.35	0.1021
Dang Gui rep 2	19.43	0.08941	4.541	0.0008649	0.01598	0.06379	0.9603	7.966	N.D.	0.07445	16.39	0.0973
Dang Gui rep 3	20.11	0.09213	4.64	0.000939	0.01598	0.06572	1.079	7.956	N.D.	0.07725	16.42	0.1151
Dang Gui mean	19.9	0.0939	4.56	0.000898	0.0159	0.0634	1.03	7.92	N.D.	0.0746	16.39	0.105
SD	0.439	0.006	0.070	0.00004	0.00019	0.0025	0.0631	0.064	N.A.	0.0025	0.0351	0.0092
SE	0.253	0.0032	0.040	0.000022	0.00011	0.0014	0.036	0.037	N.A.	0.0014	0.020	0.005
Dang Gui	19.9±0.3	0.0939±0.0032	4.56±0.04	0.000898±0.000022	0.0159±0.0001	0.0634±0.0014	1.03±0.04	7.92±0.04	N.D.	0.0746±0.0014	16.4±0.02	0.105±0.005
Bai Shao rep 1	5.395	0.02496	7.159	N.D.	0.03364	0.03351	0.8252	7.158	N.D.	0.1783	8.560	0.02820
Bai Shao rep 2	4.961	0.02775	7.122	N.D.	0.03313	0.03912	0.7981	7.122	N.D.	0.1870	8.472	0.02279
Bai Shao rep 3	4.578	0.01475	7.430	N.D.	0.03461	0.03748	0.8038	7.430	N.D.	0.1889	8.790	0.02825
Bai Shao mean	4.98	0.0264	7.24	N.D.	0.0338	0.0367	0.809	7.24	N.D.	0.185	8.61	0.0264
SD	0.409	0.002	0.168	N.A.	0.001	0.003	0.014	0.168	N.A.	0.006	0.164	0.003
SE	0.236	0.0014	0.097	N.A.	0.0004	0.002	0.008	0.097	N.A.	0.003	0.095	0.0018
Bai Shao	4.98±0.24	0.0264±0.0024	7.24±0.10	N.D.	0.0338±0.0004	0.0367±0.002	0.809±0.008	7.24±0.10	N.D.	0.185±0.003	8.61±0.09	0.0264±0.0018

Table 4.2: (continued)

Herb name	Ni	Pb	Sb	Se	Sn	V	Zn	Ca	Fe	Mg	K	Na
Dang Gui rep 1	0.5035	0.1412	0.01141	0.02321	N.D.	0.215	19.35	2200	53.57	2000	12000	220
Dang Gui rep 2	0.4896	0.1398	0.0116	0.02613	N.D.	0.1866	19.25	2200	51.51	2000	12000	220
Dang Gui rep 3	0.5102	0.1449	0.01113	0.02842	N.D.	0.2218	19.09	2200	52.2	2000	12000	220
Dang Gui mean	0.501	0.142	0.0114	0.0259	N.D.	0.208	19.2	2200	52.4	2000	12000	220
SD	0.011	0.0026	0.00024	0.0026	N.A.	0.019	0.1	0.0	1.05	0.0	0.0	0.0
SE	0.006	0.002	0.0001	0.0015	N.A.	0.011	0.076	0.000	0.605	0.000	0.000	0.000
Dang Gui	0.501±0.006	0.142±0.002	0.0114±0.0001	0.0259±0.0015	N.D.	0.208±0.011	19.2±0.1	2200±0	52.4±0.6	2000±0	12000±0	220±0
Bai Shao rep 1	0.8438	0.04427	N.D.	N.D.	N.D.	0.03316	14.74	5900	16.36	800	3400	270
Bai Shao rep 2	0.6829	0.04016	N.D.	N.D.	N.D.	0.03936	14.08	5900	14.27	780	3300	270
Bai Shao rep 3	0.6906	0.05006	N.D.	N.D.	N.D.	0.02730	14.42	6200	15.19	840	3500	280
Bai Shao mean	0.6868	0.0448	N.D.	N.D.	N.D.	0.0333	14.4	6000	15.3	807	3400	273
SD	0.005	0.005	N.A.	N.A.	N.A.	0.006	0.330	173.205	1.047	30.551	100.000	5.774
SE	0.004	0.0029	N.A.	N.A.	N.A.	0.00348	0.191	100.000	0.605	17.638	57.735	3.333
Bai Shao	0.687±0.004	0.0448±0.0029	N.D.	N.D.	N.D.	0.0333±0.0035	14.4±0.2	6000±100	15.3±0.6	807±18	3400±58	273±3

N.D. = Not detected; N.A. = Not applied

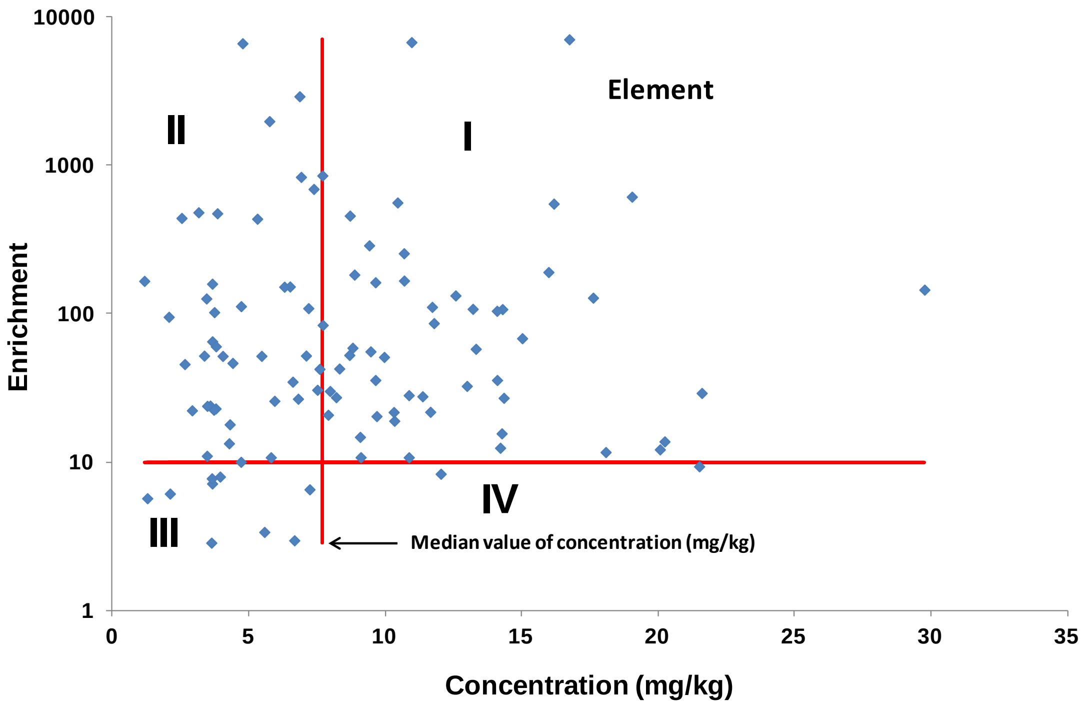


Figure 4.1: Enrichment Factor analysis from the total extractable concentration of a particular element over a number of herb samples.

4.2 Selenium, Se

4.2.1 The Role of Se in Human Health

Se is an essential element within the human body and has been associated with a number of roles in human health and disease. The pharmacology, biology and biochemistry of selenium metabolism have attracted much attention over the last decade. At the molecular level, selenium is an essential component of the enzymes glutathione peroxidise and thioredoxin reductase. Both glutathione peroxidase and thioredoxin reductase help prevent cellular rancidification (lipid peroxidation) and cell damage from free radicals [60, 108]. Free radicals are natural by-products of oxygen metabolism that may contribute to the development of chronic diseases such as cancer and heart disease. Se plays a role in the preventing blood clots by inhibiting platelet aggregation and it is reported to be involved in bolstering the immune response to viral and bacterial infection [109]. It is also associated with the inhibition of chromosome damage and mutations and is reported to have anticancer properties [110, 111]. It also plays a role in counteracting the adverse effects of heavy metals and other toxic substances in the body [109]. As a consequence of the growing recognition of the important biological role of selenium, a number of novel selenium-based pharmaceutical agents, such as antihypertensive agents, anticancer, antiviral, immunosuppressive and antimicrobial agents are being developed [108, 112]. With an increased understanding of the basic biology and biochemistry of selenium, it can be anticipated that future efforts will uncover even more sophisticated approaches for the rational development of new selenium-based pharmaceutical agents.

4.2.2 The Se Landscape for the Herb Collection

General Comments

Across the 103 medicinal herbs investigated (Table 3.1), the distribution of total and bioavailable levels of Se is mapped in Figure 4.2.1. An enrichment factor analysis for Se is represented in Figure 4.2.2 and the concentration distribution with respect to plant part is depicted in Figure 4.2.3.

From Figure 4.2.1 it can be seen that the total extractable level of Se is generally low and varies over a wide range between 0.00123 mg/kg in *Jie Geng* (herb number 73) and 0.844 mg/kg in *Jin Yin Hua* (herb number 13) (up to 686-fold), with 57 herbs having a total extractable level of less than 0.0641 mg/kg (Median). In a number of cases the level of Se is barely measurable or below the detection limit. This is reasonable as the solubility of Se in soils is low and other elements such as nitrogen, phosphorus, sulphur and cadmium can also have an impact on Se uptake and accumulation in plants [113]. The bioavailability of Se ranges from 2 % to 100 % but was generally low. Only eight herbs achieved a bioavailability of more than 80 %.

Enrichment Factor Analysis

From Figure 4.2.2, it can be seen that the majority of the herb samples tested have a high enrichment capability for Se, as indicted by an Enrichment Factor (EF) > 10 (I and II). The herbs located in part I of Figure 4.2.2 had Se concentration above the media value of 0.0641 mg/kg, indicating that these herbs had enhanced enrichment ability for Se and/or the environment had high available of Se to these herbs. The herbs in part II of Figure 4.2.2 showed a total extractable content of Se less the median value 0.0641 mg/kg even though they had EF > 10, suggesting that the environment had low available of Se to these herbs. Five herbs in part III of Figure 4.2.2 had relatively low concentration of Se (< median value of 0.0641 mg/kg), possibly due to their low enrichment ability for Se (EF < 10) and/or poorly available of Se in the environment. Only one herb showed an EF < 10 but a Se concentration of more than 0.0641 mg/kg (median value) (IV in Fig. 4.2.2). This herb might grow in an environment which is relatively rich in available Se.

Plant Part Analysis

The flower, leaf-derived and grass samples generally had a higher level of Se as shown in Figure 4.2.3. This suggests that the soil is not the only contributing source of Se for these herb samples [7], the higher level of Se might also derive from other sources such as atmospheric pollution.

4.2.3 Suggested Leads from Relating Se Content to Medicinal Properties

In the context of the data presented, it is of interest to reflect on the uses of these substances in traditional Chinese medicine. For example, *Sheng Di* (0.376 mg/kg, herb number 49 in Fig. 4.2.1) and *Ji Yin Hua* (0.276 mg/kg, herb number 13 in Fig.

4.2.1) had relatively higher bioavailable contents of Se and are commonly used to treat many different types of “internal heat” in TCM. This symptom is associated with fever, inflammation and infection [30, 31]. Another example is *Suan Zao Ren*, which also showed a relatively higher bioavailable content of Se (0.276 mg/kg, herb number 59 in Fig. 4.2.1). This herb is commonly used in the treatment of various ‘mind disharmony’ conditions in TCM. The common symptoms associated with these conditions include anxiety and agitation, insomnia, frequent dreams and palpitations [30, 31]. It was reported [114] that intake of only small amounts of Se may help with the sleeping abnormalities.

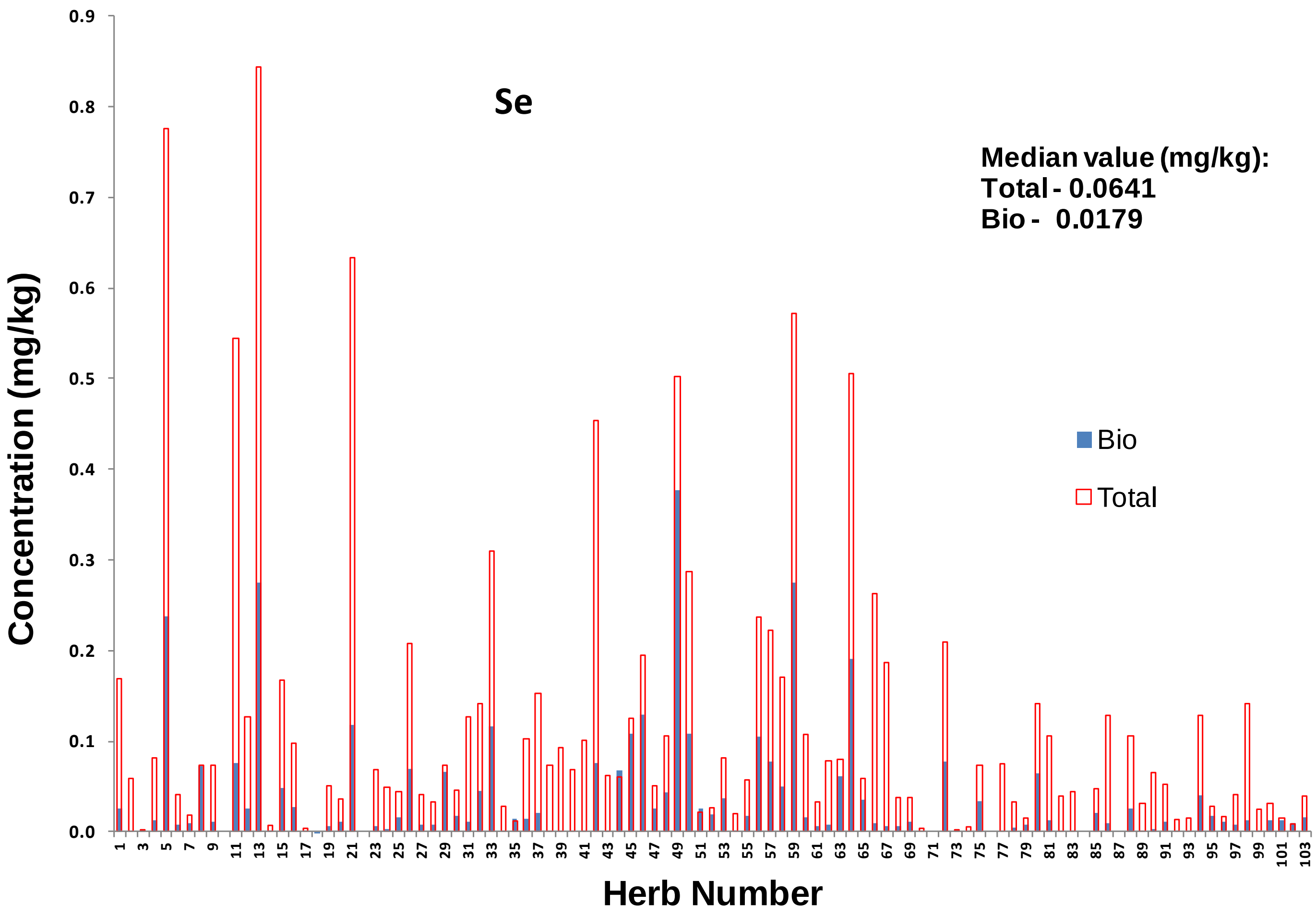


Figure 4.2.1: Total and bioavailable concentration of Se in herb samples.

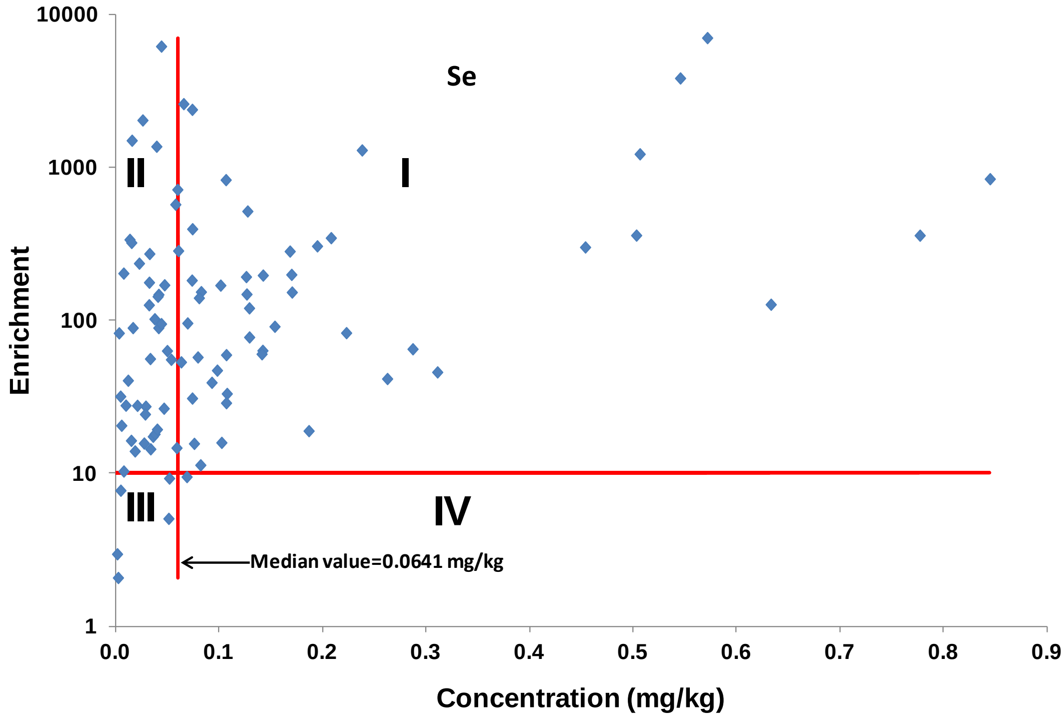


Figure 4.2.2: Enrichment Factor analysis of herb samples for Se.

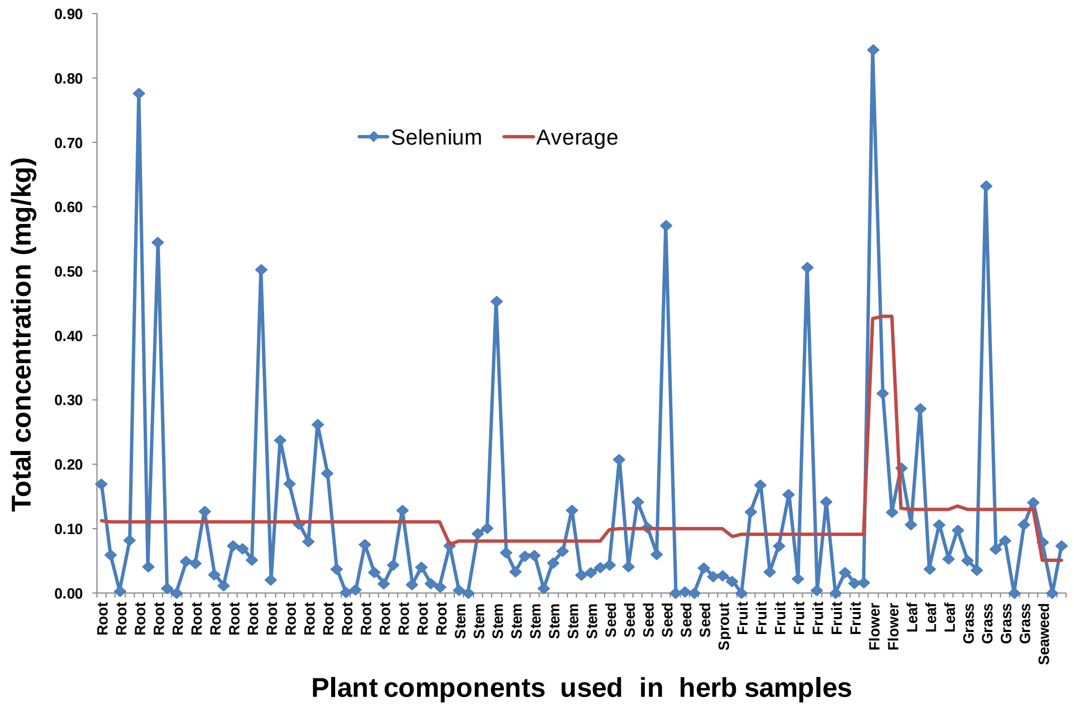


Figure 4.2.3: Variation of total concentration of Se with different plant components used in herb samples.

4.3 Zinc, Zn

4.3.1 The Role of Zn in Human Health

Zn is an essential element of "exceptional biologic and public health importance" [115] - especially for growing children, pregnant women, the elderly and people with allergies and chronic diseases [116]. Its role includes protecting the skin and improving resistance to infectious diseases, inflammations and allergies [116]. Zn deficiency affects up to two billion people in the developing world and is associated with many diseases [117]. Notably, Zn is an integral component of nearly 100 specific enzymes (e.g. alcohol dehydrogenase) [118], serves as a structural ion in transcription factors and is stored and transferred in metallothioneins [119]. Zn plays "ubiquitous biological roles" in humans. It interacts with "a wide range of organic ligands" [115], and has roles in the metabolism of RNA and DNA, signal transduction and gene expression. It is also an important participant in the Cu-Zn superoxide dismutase (CuZnSOD) enzyme which is involved in antioxidant processes [116].

Zn-based supplements and drugs have attracted considerable attention in the medical world. For example, it has been proposed that Zn lozenges (e.g. Zn acetate and Zn gluconate) can shorten the duration of the common cold, although this has yet to be substantiated [120]. Zn, in various forms, may also be a valuable adjunct therapy for cancer patients and has been reported to have an anti-metastatic effect [121]. Although not yet tested as a therapy in humans, a growing body of evidence indicates that Zn may preferentially kill prostate cancer cells [121, 122]. Because Zn naturally accumulates in the prostate [122] and because the prostate is accessible with relatively non-invasive procedures, its potential as a chemotherapeutic agent in this type of cancer has shown promise [121]. New heteroleptic complexes containing Zn(II) ion have been tested in vitro against prostate cancer cells and have shown promising anticancer activity [123]. Such Zn complexes could present is alternatives to platinum-based antitumour drugs that are associated with undesirable side effects. Other Zn(II) complexes are reported to have high anti-diabetic effects in obesity-linked type 2 diabetes [124].

4.3.2 The Zn Landscape for the Herb Collection

General Comments

In this study, the total and bioavailable levels of Zn were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.3.1. The enrichment ability of the herbs for Zn was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.3.2 and the concentration distribution with respect to plant part is depicted in Figure 4.3.3.

It was found from Figure 4.3.1 that the herbs tested were generally rich in Zn. The total extractable content of Zn ranged between 1.65 mg/kg in *Mo Yao* (herb number 65) and 134 mg/kg in *Huang Lian* (herb number 41), on a dry weight basis. The median value of the total extractable level of Zn was 19.6 mg/kg as shown in Figure 4.3.2. The overall bioavailability of Zn was also relatively high, varying from 48 % to 100 %, with a median value of 19.2 mg/kg. 100 out of 103 herbs achieved a more than 80 % bioavailability of Zn, indicating that the Zn content in these herbs is almost fully bioavailable (Figure 4.3.1).

Enrichment Factor Analysis

The herbs also demonstrated a high enrichment ability for Zn, with around 90 % of herbal samples tested having an EF > 10 (I and II in Figure 4.3.2). About half (I in Figure 4.3.2) had Zn concentrations above the median value of 19.6 mg/kg, which is attributed not only to an enhanced enrichment ability for Zn, but also a high availability of Zn in the environment where they grown. The herbs in part II of Figure 4.3.2 also had an enhanced enrichment ability for Zn but showed a total extractable content of Zn less the median value 19.6 mg/kg, suggesting that these herbs have a low environmental availability of Zn. Seven herbs located in part III of Figure 4.3.2 showed a concentration of Zn less than the median value of 19.6 mg/kg, possibly due to a low enrichment ability for Zn (EF < 10) and/or poorly availability of Zn in the environment. Two herb samples showed an EF < 10 but a Zn concentration > 19.6 mg/kg (median value) (IV in Fig. 4.3.2), suggesting that they may originate from a Zn-rich environment.

Plant Part Analysis

The concentration distribution of Zn varied with respect to plant part (Figure 4.3.3). The leaf-derived herb samples were found to have the highest average level of Zn. In this regard, it has been reported that Zn has a tendency to accumulate in older leaves [113]. It is also interesting to note that apart from a generally high level of Zn in the leaf-derived, grass and flower samples, the seed-based samples also showed relatively enhanced levels of Zn.

4.3.3 Suggested Leads from Relating Zn Content to Medicinal Properties

From the data presented in Fig. 4.3.1, *Huang Lian* (herb number 41) had the highest Zn concentration (134 mg/kg) - and this Zn content is fully bioavailabable, within experimental error. This herb is widely used in TCM to clear "internal heat" which is a condition associated with fever, inflammation and infection [30, 31]. Notably, *Huang Lian* is also used to treat diabetes [125] and has been reported to possess anticancer activities, implicating it as a promising herbal candidate for chemoprevention and chemotherapy of certain cancers [126]. It may also provide leads for small molecule anticancer Zn species. *Jiang Huang*, *Ze Xie* and *Dan Zhu Ye* respectively (numbers 94, 101, and 50 in Fig. 4.3.1) also show relatively high levels of Zn (both total and bioavailable). *Jiang Huang* is commonly used in TCM to invigorate the blood and reduce blood stagnation (e.g. sharp, fixed pain). In western terms, these symptoms address gynaecological, neurological and traumatic disorders [30, 31]. In TCM, *Ze Xie* is used as a diuretic and is also to have anti-diabetic effects [125]. *Dan Zhu Ye* is classified in the same group of *Huang Lian* in TCM, and is considered to have the ability to clear "internal heat" [30, 31] as described above.

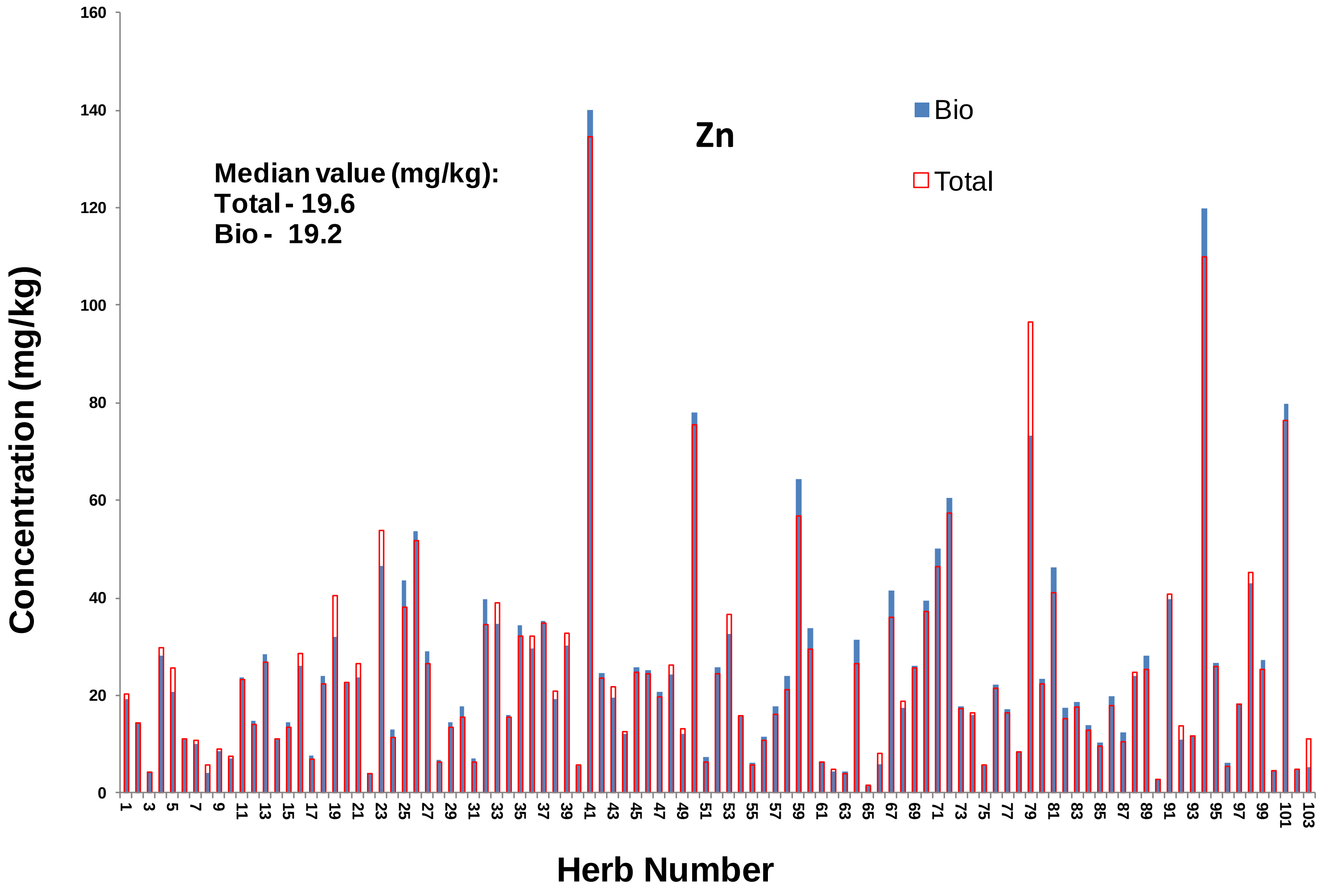


Figure 4.3.1: Total and bioavailable concentration of Zn in herb samples.

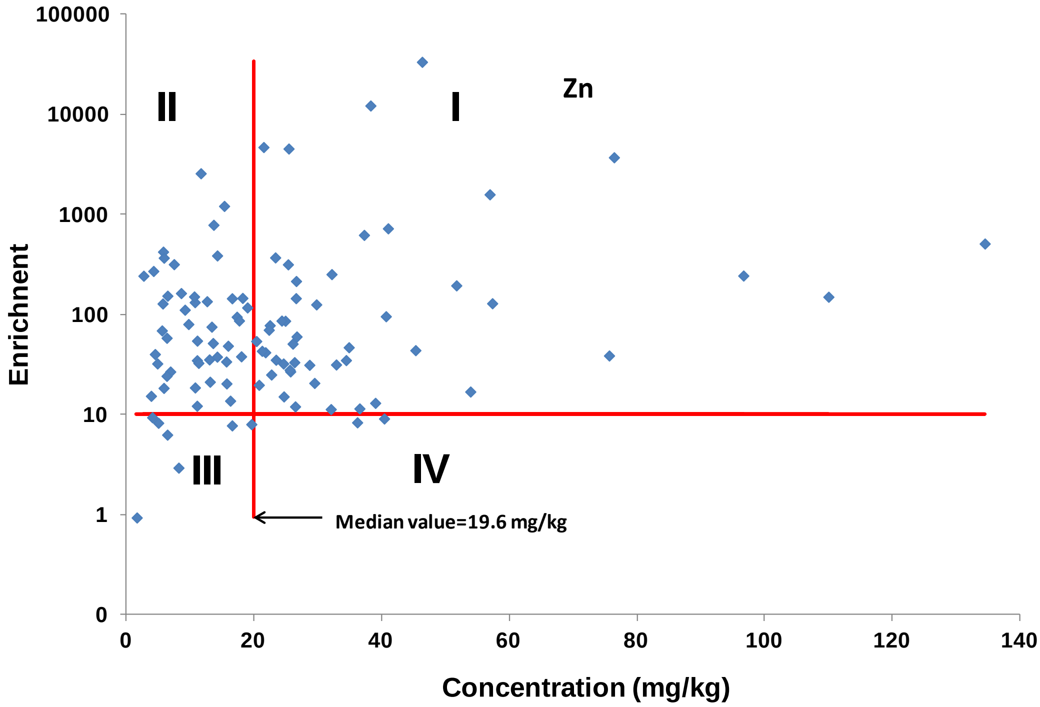


Figure 4.3.2: Enrichment Factor analysis of herb samples for Zn.

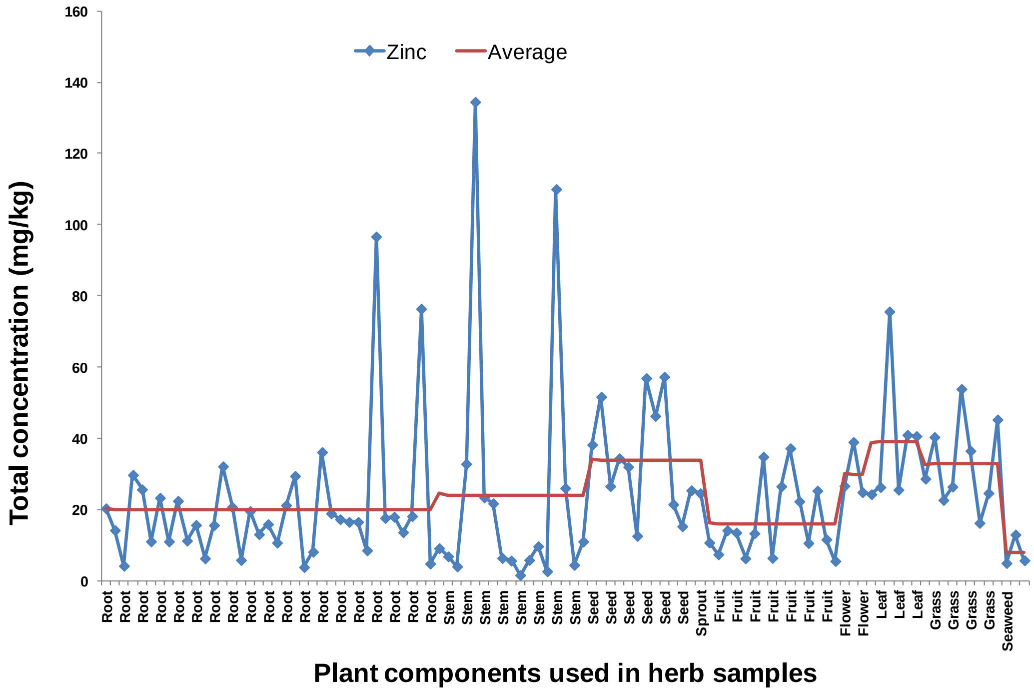


Figure 4.3.3: Variation of total concentration of Zn with different plant components used in herb samples.

4.4 Manganese, Mn

4.4.1 The Role of Mn in Human Health

Mn is an essential trace nutrient that is required for all living organisms [127]. In humans, Mn is primarily intracellular and is required for the formation of bones, for metabolism of fats and carbohydrates and for fertility [22]. Mn(II) ions function as cofactors for a large variety of enzymes with many functions. These enzymes include oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, lectins, and integrin. Manganese enzymes are particularly essential for the detoxification of superoxide free radicals in aerobic organisms. The reverse transcriptases of many retroviruses (though not lentiviruses such as HIV) contain manganese. Well known Mn-containing polypeptides include arginase, the diphtheria toxin, and Mn-containing superoxide dismutase (Mn-SOD) [128]. Mn-SOD enzyme is ubiquitous to most aerobic organisms that need to deal with the toxic effects of superoxide - formed from the 1-electron reduction of dioxygen. Mn is also important in the oxygen-evolving complex (OEC) of photosynthetic plants.

4.4.2 The Mn Landscape for the Herb Collection

General Comments

Figure 4.4.1 shows the total and bioavailable contents of Mn measured across the 103 medicinal herbs (Appendix Table 4.1). Figure 4.4.2 compares the enrichment ability of the herbs for Mn. Figure 4.4.3 presents the concentration distribution of Mn with respect to plant part. Total extractable content of Mn in the herbs tested varied from 1.61 mg/kg in *Shan Yao* (herb number 75) to 833 mg/kg in *Jiang Huang* (herb number 94), on a dry weight basis. Mn is essentially fully bioavailable (bioavailability > 80 %) in most of the analysed samples and only eight tested herb samples had a bioavailability of Mn of less than 80 %. The median value of the total and bioavailable contents of Mn was found to be 27.4 mg/kg and 24.6 mg/kg, respectively (Fig. 4.4.2).

Enrichment factor Analysis

Figure 4.4.2 represents the herbs distributed over four zones (I, II, III and IV) defined by the median value line for the total extractable content of Mn (27.4 mg/kg) and the accepted EF cut-off of 10 [7]. Around half of herbs tested showed high enrichment ability (EF > 10) for Mn (I and II in Figure 4.4.2). 30 % of the herbs measured had both a total extractable content of Mn above the median value 27.4 mg/kg and an EF value > 10 (I in Figure 4.4.2), indicating that these herbs had enhanced enrichment ability for Mn and/or the environment had a high level of available Mn. 24 % of herbs tested had an EF > 10 but showed a total extractable content of Mn less the median value 27.4 mg/kg (II in Figure 4.4.2) - suggesting that the environment had a low availability of Mn. The herbs (28 % of total herbs analysed) located in part III of Figure 4.4.2 had a relatively low level of Mn (< median value 27.4 mg/kg), possibly due to both low enrichment ability for Mn (EF < 10) as well as a low availability of Mn in the environment where they were grown. The remaining herbs, located in part IV of Figure 4.4.2, had a low enrichment ability (EF < 10) for Mn but were relatively rich in Mn - suggesting that the environment had high availability of Mn to these herbs. Such a distribution of data as depicted in Figure 4.4.2 may be attributed to an uneven distribution of Mn in the soil profile [113].

Plant Part Analysis

From Figure 4.4.3, it may be seen that the concentration distribution of Mn varied with respect to plant part. Notably, the leaf-derived, grass and stem samples were generally rich in Mn.

4.4.3 Suggested Leads from Relating Mn Content to Medicinal Properties

The results showed that *Jiang Huang* (herb number 94 in Fig. 4.4.1) is the richest in Mn (concentration 833 mg/kg) among the herbs tested, and this Mn content is fully bioavailabable. This herb is commonly used in TCM to invigorate the blood and reduce blood stagnation (e.g. sharp, fixed pain). These symptoms, in western terms, address gynaecological, neurological and traumatic disorders [30, 31]. *He Ye* (herb number 81 in Fig. 4.4.1) and *Dan Zhu Ye* (herb number 50 in Fig. 4.4.1) also had relatively high levels for both total and bioavailable content of Mn. They are generally used in TCM to clear “internal heat” which is associated with fever, inflammation and infection [30, 31]. In this regard, it is known that Mn-SOD is active as a biomimetic against inflammation [101, 102]. Low molecular weight mimics of SOD have significant pharmaceutical potential [129] and have been undergoing development over the last decade. These include complex 13 (SC-52608) [130], Manganese 5,10,15, 20-tetrakis (4-benzoic acid) porphyrin (MnTBAP) [131] Mn(III) metalloporphyrins [132], and other Mn(II) macrocycles [133]. Fridovich, Batinic-Haberle and co-workers have recently developed Mn-porphyrins as SOD-mimics [134]. Further studies are suggested to identify possible Mn species in these herbs that could also provide leads for the development of metallo anti-inflammatories. *Sang Ji Sheng* (herb number 77, Fig. 4.4.1) also showed relatively high levels for both total and bioavailable contents of Mn. This herb has general functions to eliminate primarily wind and damp, and secondarily cold and heat pathogens from the muscles, channels, tendons and bones. In western terms, these symptoms are association with painful obstruction syndromes characterised by rheumatic pain and stiffness in the limbs, joints, muscles and tendons [30, 31]. Hence the same arguments apply for the search for small molecule Mn species in this herb that could provide leads for anti-inflammatory drugs.

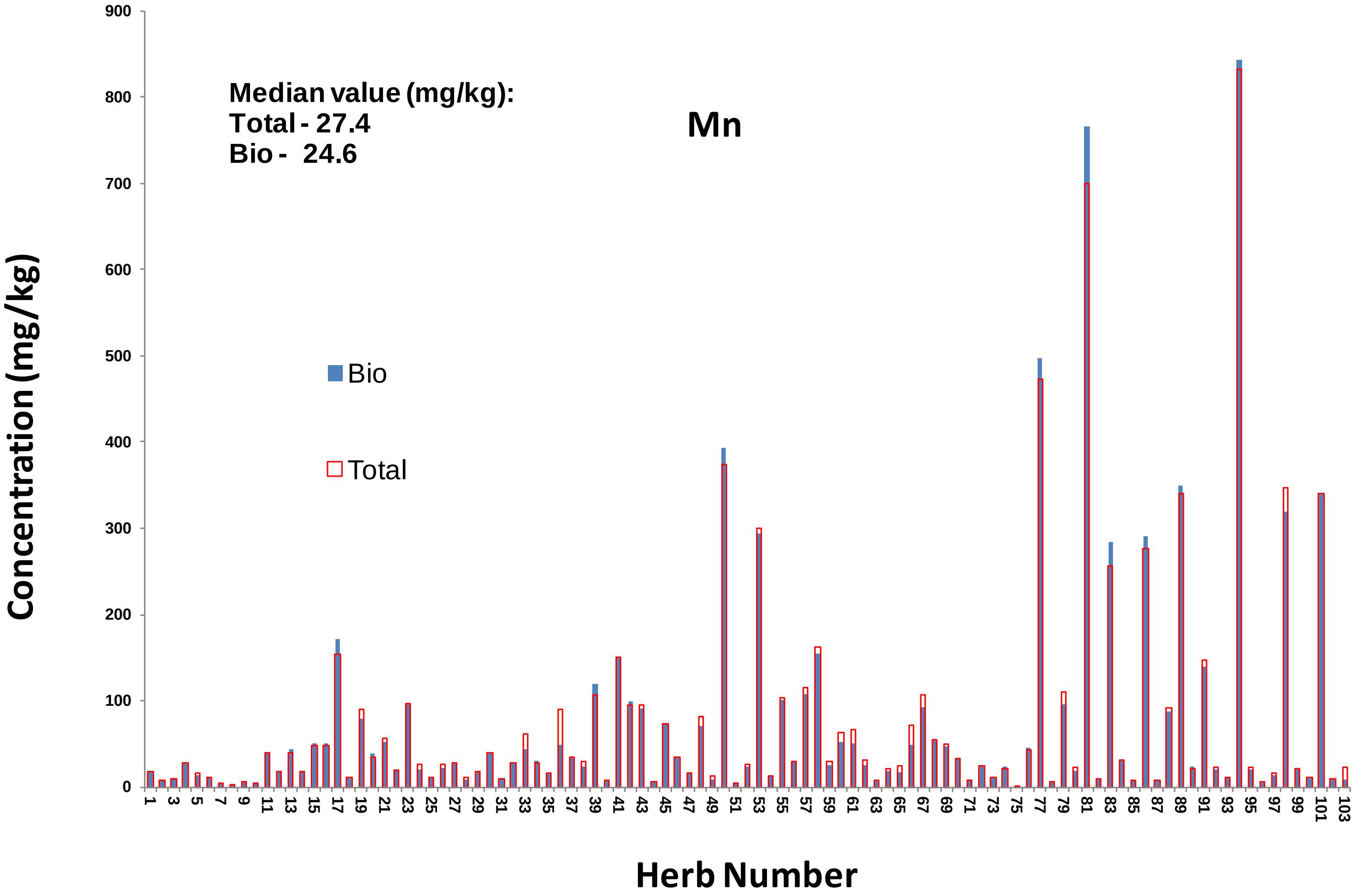


Figure 4.4.1: Total and bioavailabe concentration of Mn in herb samples.

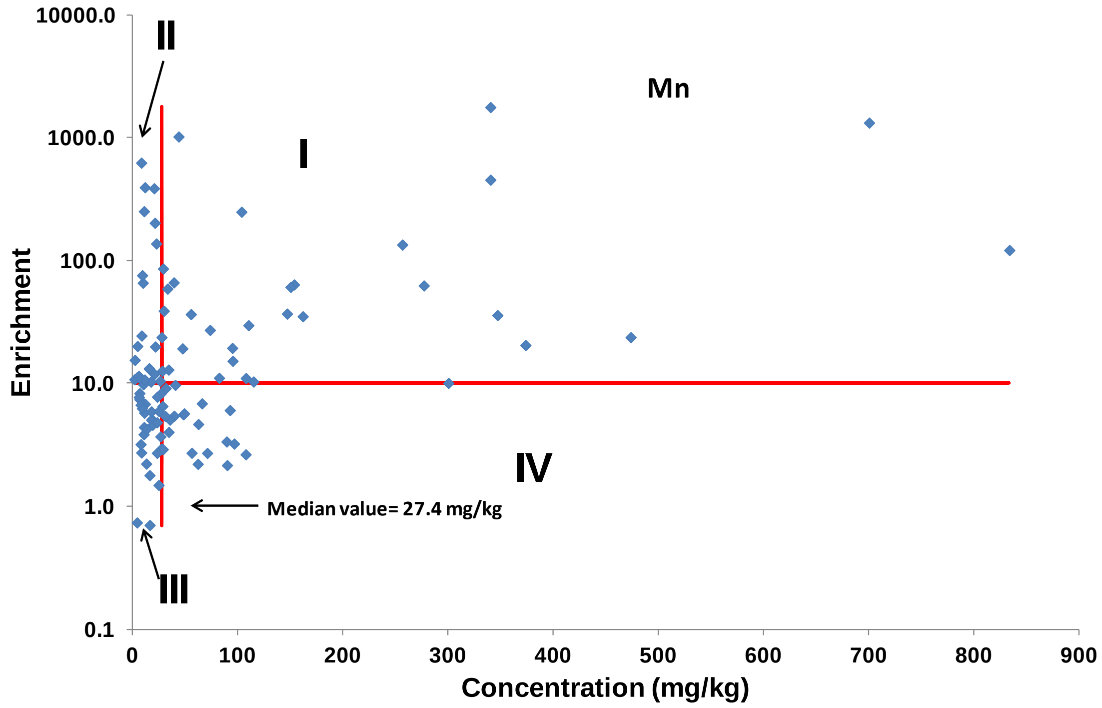


Figure 4.4.2: Enrichment Factor analysis of herb samples for Mn.

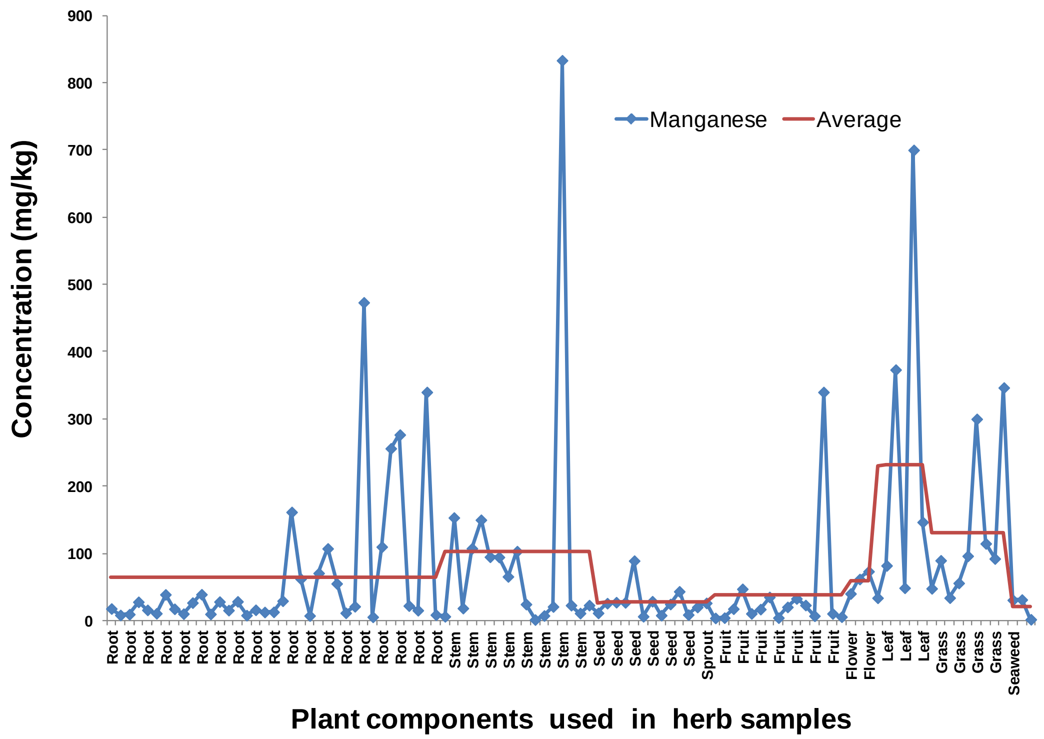


Figure 4.4.3: Variation of total concentration of Mn with different plant components used in herb samples.

4.5 Copper, Cu

4.5.1 The Role of Cu in Human Health

Cu is an essential trace element in plants and animals. It is an important cell-protective mineral in the human body [22]. Copper deficiency in the human body can produce anemia-like symptoms, neutropenia, bone abnormalities, hypopigmentation, impaired growth, increased incidence of infections, osteoporosis and abnormalities in glucose and cholesterol metabolism [135]. Cu may also play a very important role in treating and preventing arthritis, infections and cancer [22]. Cu participates in many enzyme systems and its active role in the SOD enzyme is well known. For example, 60 % of the copper in blood cells is tightly bound to Cu-Zn superoxide dismutase (CuZnSOD) which is the fifth most common protein in the human organism and protects the cells from the damage caused by free radicals and peroxides [22, 136]. Cu-Zn SOD contains two copper and two zinc atoms in immediate proximity to each other and is able to function only when both copper and zinc are present [22]. This enzyme has been tested to have positive effects in treating different types of inflamed joints associated with arthritis and osteoarthrosis [22, 137].

4.5.2 The Cu Landscape for the Herb Collection

General Comments

The total and bioavailable content of Cu measured across the 103 medicinal herbs (Appendix Table 4.1) in this study are shown in Figure 4.5.1. The total Cu level in the investigated herb samples ranged from 1.17 mg/kg in to 29.8 mg/kg, with most of herbs having a total extractable Cu content of less than 20 mg/kg. It is known that plant species vary in their tolerance to Cu and the sufficiency range of Cu in a plant species as an essential micronutrient is between 5 mg/kg and 30 mg/kg dry weight of tissue [113]. The results obtained in the current work are close to this reported Cu range. Figure 4.5.1 shows that the bioavailability of Cu in the herbs studied is relatively high. More than half of herbs have more than 80 % bioavailability of Cu, indicating that the Cu content in these herbs are almost fully bioavailable.

Enrichment Factor Analysis

The enrichment analysis (Figure 4.5.2) demonstrated a generally high enrichment ability of the herbs for Cu, with nearly 90 % of tested herbs having EF > 10 with a wide spread of concentration (I and II in Figure 4.5.2). The herbs in part I of Figure 4.5.2 had Cu concentrations above the median value of 7.71 mg/kg, indicating that these herbs had enhanced enrichment ability for Cu and/or the environment had high available of Cu to these herbs. While the herbs located in part II of Figure 4.5.2 showed a total extractable content of Cu less the median value 7.71 mg/kg even though they had EF > 10, suggesting that the environment had low available of Cu to these herbs.

Plant Part Analysis

Figure 4.5.3 showed the variation of the concentration distribution of Cu with respect to plant part. Similar to Zn, a generally high level of Cu was found in not only the leaf-derived, grass and flower samples, but also the seed samples. As mentioned above, Cu-Zn SOD contains two copper and two zinc atoms in immediate proximity to each other and both copper and zinc must be present for it to be able to function. In this regard, the seed samples were measured to be generally rich in both Cu and Zn, suggesting that these samples might have a relatively high level of Cu-Zn SOD. This needs to be further investigated.

4.5.3 Suggested Leads from Relating Cu Content to Medicinal Properties

Among the herbs tested, *Chang Niu Xi* (herb number 79 in Fig. 4.5.1) had the highest total extractable content of Cu (29.8 mg/kg) but only 34 % of its Cu content was bioavailable. *Pu Gong Yi, Yin Chen* and *Chai Hu* (herb number 19, 53 and 60 in Fig. 4.5.1, respectively) also showed a generally high level of Cu but a relatively low bioavailability for Cu. The chemical implications of Cu in these herbs might contribute the low bioavailability of Cu and are interesting to be identified in future work. It is reported [113] that toxicity will occur when Cu tissue levels exceed 20 mg/kg to 30 mg/kg, especially with respect to roots. The root sample *Chang Niu Xi* (herb number 79 in Fig. 4.5.1) had a Cu level (29.8 mg/kg) that is close to this toxic level. This suggests that this herb plant might have a high level of tolerance to Cu thus has the ability to protect itself from Cu toxicity.

Other herb samples such as *Qiang Huo, Niu Bang Zi, Tu Si Zi, He Zi Ma* and *Lian Zi* (herb number 68, 70, 36, 26 and 76 in Fig. 4.5.1 respectively) were measured to have a relatively high level in both total and bioavailable contents of Cu, but have different functions in treating or preventing diseases. *Qiang Huo* and *Niu Bang Zi* (herb number 68 and 70 in Fig. 4.5.1 respectively) are commonly used in TCM to dispel pathogens factors from the exterior and prevent them from penetrating body's interior, which treat exterior patterns such as fever, chills, possible sweating, headache and a floating pulse [30, 31]. *Tu Si Zi* and *He Zi Ma* (herb number 36 and 26 in Fig. 4.5.1 respectively) are classified as tonifying herbs in TCM, which are used in treating patterns of deficiency of the qi, blood, yin and yang [30, 31]. Clinically, they are used to strengthen the various physiological processes of the body to assist in the recovery from illness with chronic or degenerative disorders [31]. *Lian Zi* (herb number 76 in Fig. 4.5.1) has the ability to halt the abnormal loss of body fluids and is therefore generally used to treat conditions of leakage and discharge such as diarrhea and insomnia [30, 31]. *Chang Niu Xi* (herb number 79) and *Qiang Huo* (herb number 68) have also been tried in treating osteoarthrosis [138].

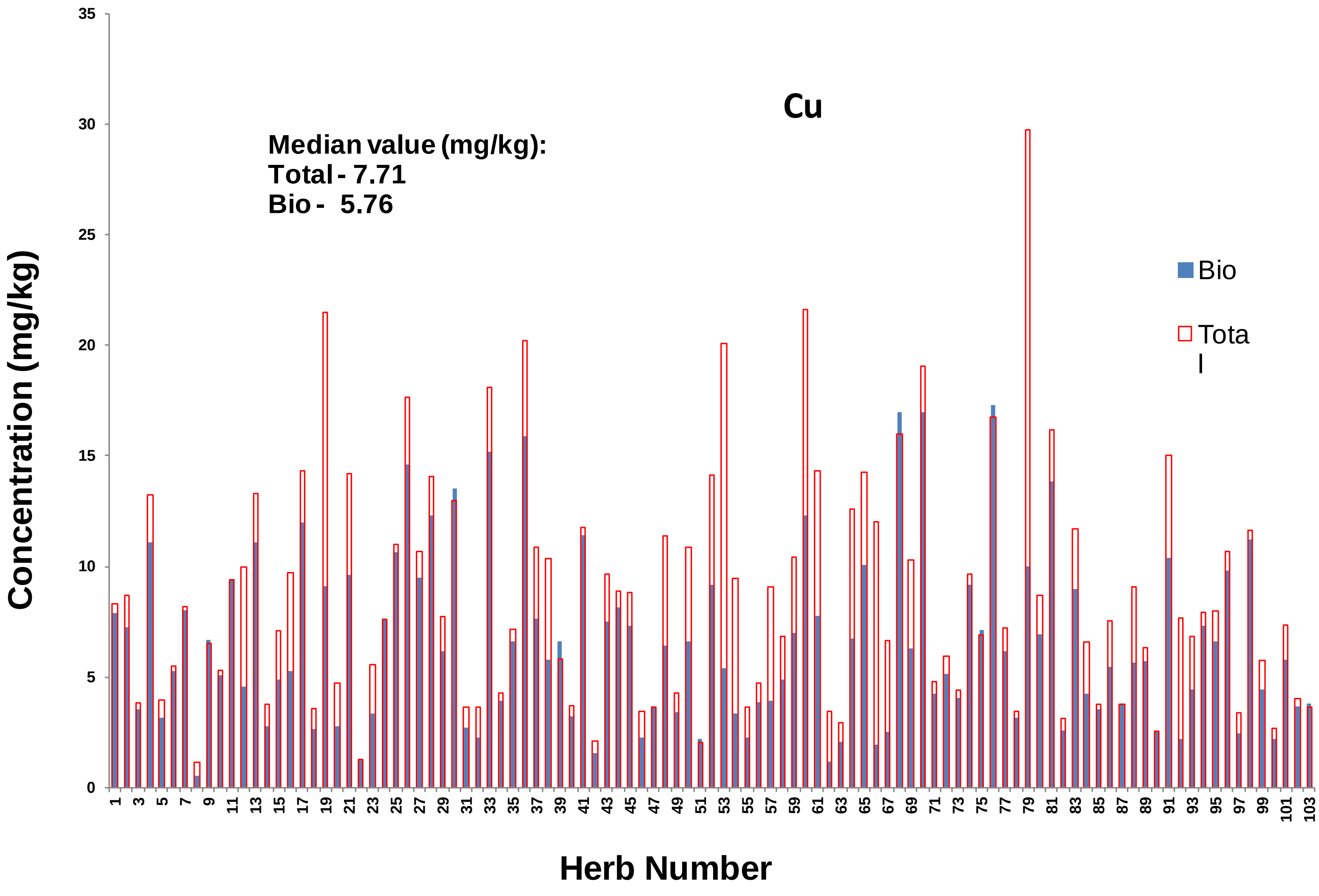


Figure 4.5.1: Total and bioavailabe concentration of Cu in herb samples.

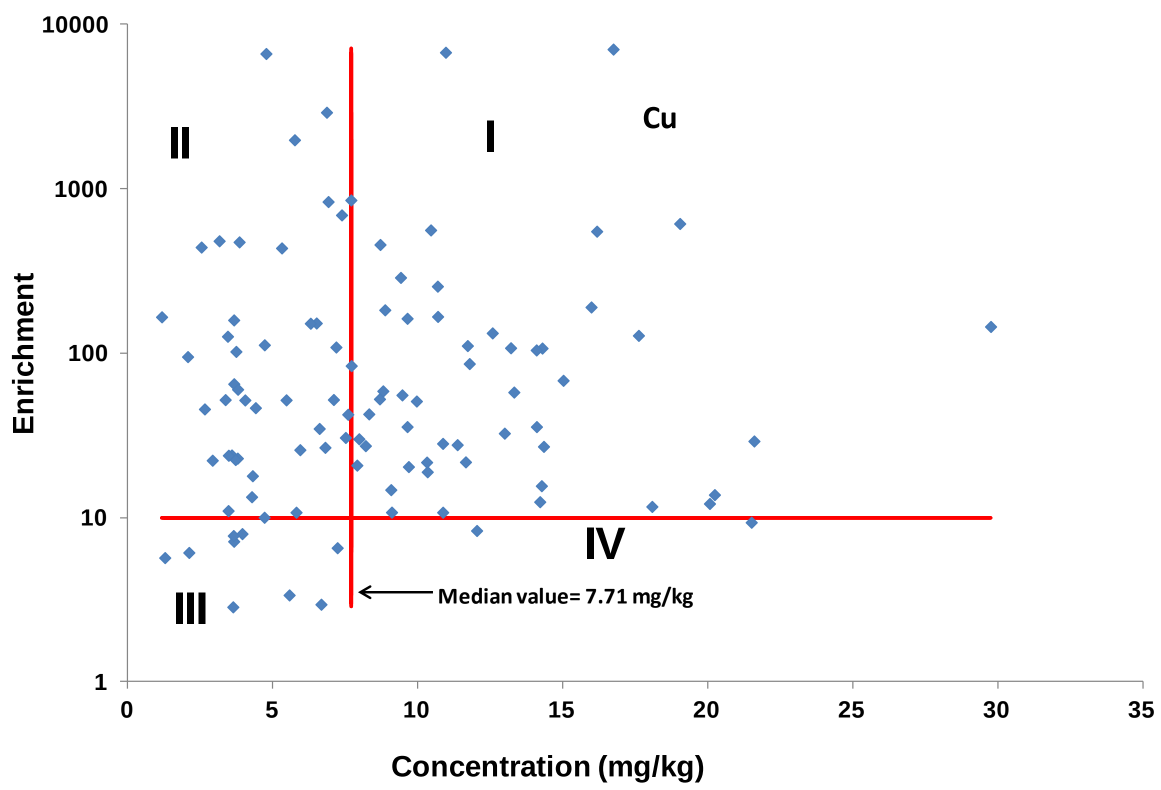


Figure 4.5.2: Enrichment Factor analysis of herb samples for Cu.

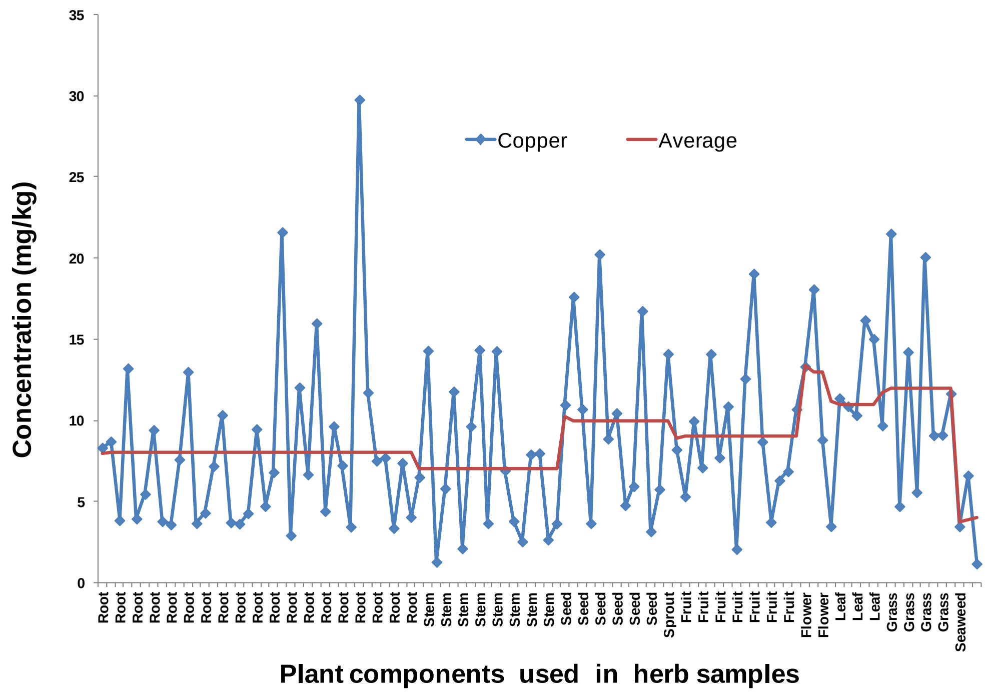


Figure 4.5.3: Variation of total concentration of Cu with different plant components used in herb samples.

4.6 Cobalt, Co

4.6.1 The Role of Co in Human Health

Co is essential to all animals, including humans, but it is not clear if Co is essential for plants [113]. In a medical context, Co is classified into the group of microminerals according to the level of daily requirements by humans [22]. Co is a key constituent of cobalamin, also known as vitamin B12 (Fig. 4.6.1) [22, 113, 139], which is the primary biological reservoir of this trace element. The cobalamin-based proteins use corrin to hold the cobalt. Coenzyme B12 features a reactive C-Co bond, which participates in its reactions [140]. In humans, B12 exists with two types of alkyl ligand: methyl and adenosyl. Methyl B12 promotes methyl (-CH_3) group transfers, while the adenosyl B12 catalyses rearrangements of atoms in the structure. An enzyme called methylmalonyl Coenzyme A mutase, also known as MCM, can catalyse the isomerization of methylmalonyl-CoA to succinyl-CoA. This is an important step in the extraction of energy from proteins and fats and requires a vitamin B12-derived prosthetic group, adenosylcobalamin, in order to function [141]. Some cobaltoproteins are known although they are far less common than other metalloproteins (e.g. those of zinc and iron). For example, methionine aminopeptidase 2 is an enzyme that occurs in humans and other mammals that binds cobalt directly without using the corrin ring of B12. Another non-corrin cobalt enzyme is nitrile hydratase, an enzyme in bacteria that is able to metabolise nitriles [142].

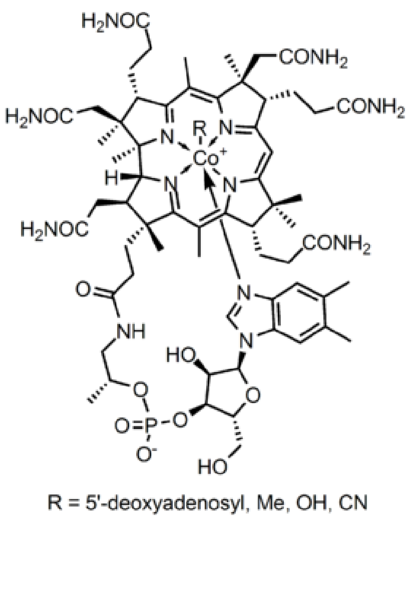


Figure 4.6.1: Structure of cobalamin, also known as vitamin B12 [139].

The total mass of Co in an average (70 kg) person is estimated to be 1.5 mg [113]. For human beings, if the daily intake of Co exceeds 20 – 30 mg, some side effects such as thyroid failure and weakening of cardiac functions might occur [22]. There has never been a reported case of cobalt deficiency, as so little of it is required in the diet. However, a deficiency of cobalt may result in a deficiency of vitamin B12 and thus lead to pernicious anaemia [139]. It is known that Co can stimulate the production of red blood cells [143, 144] and it has been tried in treating anaemia [22, 144]. Attempts have also been made to use Co to reduce high blood pressure [22]. The use of cobalt species as metallodrugs remains largely unexplored.

4.6.2 The Co Landscape for the Herb Collection

General Comments

Figure 4.6.2 shows the total and bioavailable levels of Co for the 103 medicinal herbs listed in Table 3.1. It was found that the herb samples investigated contained relatively low levels of Co (median values for both total and bioavailable contents) compared to other elements (Fe, Cu, Zn, Mn, and Cr) in the group of microminerals. Most of the tested samples had a total extractable content of Co less than 1.0 mg/kg, varying over a wide range between 0.00225 mg/kg in *Xin Ren* (herb number 71 in Fig. 4.6.2) and 1.75 mg/kg in *Huo Xiang* (herb number 98 in Fig. 4.6.2) (up to 777-fold). Notably, *Huo Xiang* (herb number 98 in Fig. 4.6.2), *Ji Xue Teng* (herb number 83 in Fig. 4.6.2), *Huang Jin* (herb number 42 in Fig. 4.6.2) and *Lei Fu Zi* (herb number 32 in Fig. 4.6.2) had higher levels in both total and bioavailable contents of Co, whereas *Zi Cao B* (herb number 67 in Fig. 4.6.2), *Tu Si Zi* (herb number 36 in Fig. 4.6.2) and *Pu Gong Yin* (herb number 19 in Fig. 4.6.2) were relatively rich in Co but showed a lower bioavailabilities.

Enrichment Factor Analysis

The analysis of enrichment factor (Fig. 4.6.3) showed that the enrichment capability of the herb samples for Co was generally low, with the majority of the herb samples tested (~ 90 %) having an EF < 10 (III and IV in Fig. 4.6.3). Only two herbs (I in Fig. 4.6.3) showed high values in both EF (> 10) and total content (> the median value of 0.159 mg/kg) for Co, indicating that these eight herbs had enhanced enrichment ability for Co and/or the environment had high available of Co to these herbs. Although the herbs in part II of Figure 4.6.3 achieved an EF > 10, the total extractable content of Co in these herbs was less than the median value of 0.159 mg/kg, suggesting that the environment had low available of Co to these herbs. Around 70 % of herb samples tested (III of Figure 4.6.3) had relatively low concentration of Co (< median value of 0.159 mg/kg), possibly due to their low enrichment ability for Co (EF < 10) and/or the environment had poorly available of Co. The herbs located in part IV of Figure 4.6.3 were rich in Co (> the media value of 0.159 mg/kg) even though they showed a relatively low enrichment ability for Co (EF < 10), suggesting that they may have grown in a Co-rich environment.

Plant Part Analysis

The grass samples showed the highest value for the average Co content (Figure 4.6.4). This is reasonable as TCM usually uses the whole plant with respect to grass as a herbal medicine and it is known that the root of a plant can easily take Co and then translocate it primarily in the transpiration stream [113].

4.6.3 Suggested Leads from Relating Co Content to Medicinal Properties

In the context of the data presented, it is of interest to reflect on the uses of these herbs in TCM. For example, *Huo Xiang* (herb number 98 in Fig. 4.6.2) is notable for having the highest level for both total and bioavailable content of Co. In TCM, this herb generally has several functions including "transforming damp and harmonising the stomach" and for stopping vomiting, "releasing the exterior and dispelling wind-cold, and activating the Qi" and for relieving pain. In Western terms, such symptoms are associated with headache, fear of cold, fever, vomiting, and pulse floating [30]. *Ji Xue Teng* (herb number 83 in Fig. 4.6.2) also showed a relatively high level of Co with essentially full bioavailability. This herb is generally used to "invigorate and nourish the blood" in TCM [30]. As mentioned above, in Western terms Co can stimulate the production of red blood cells [143, 144] and may be helpful in treating anaemia [22, 144]. Another interesting herb is *Huang Jin* (herb number 42 in Fig. 4.6.2) which had a relative higher level in both total and bioavailable contents of Co, and notably also showed the highest level of Fe - as described in Section 4.6. In TCM, *Huang Jin* is a tonifying herb which is commonly used to replenish or tonify in deficiency conditions of the body - Qi, Blood, Yin, Yang, fluids and Essence. In Western terms, the common symptoms associated with these conditions include a variety of functional disorders such as metabolic disorders (e.g. anaemia) [30].

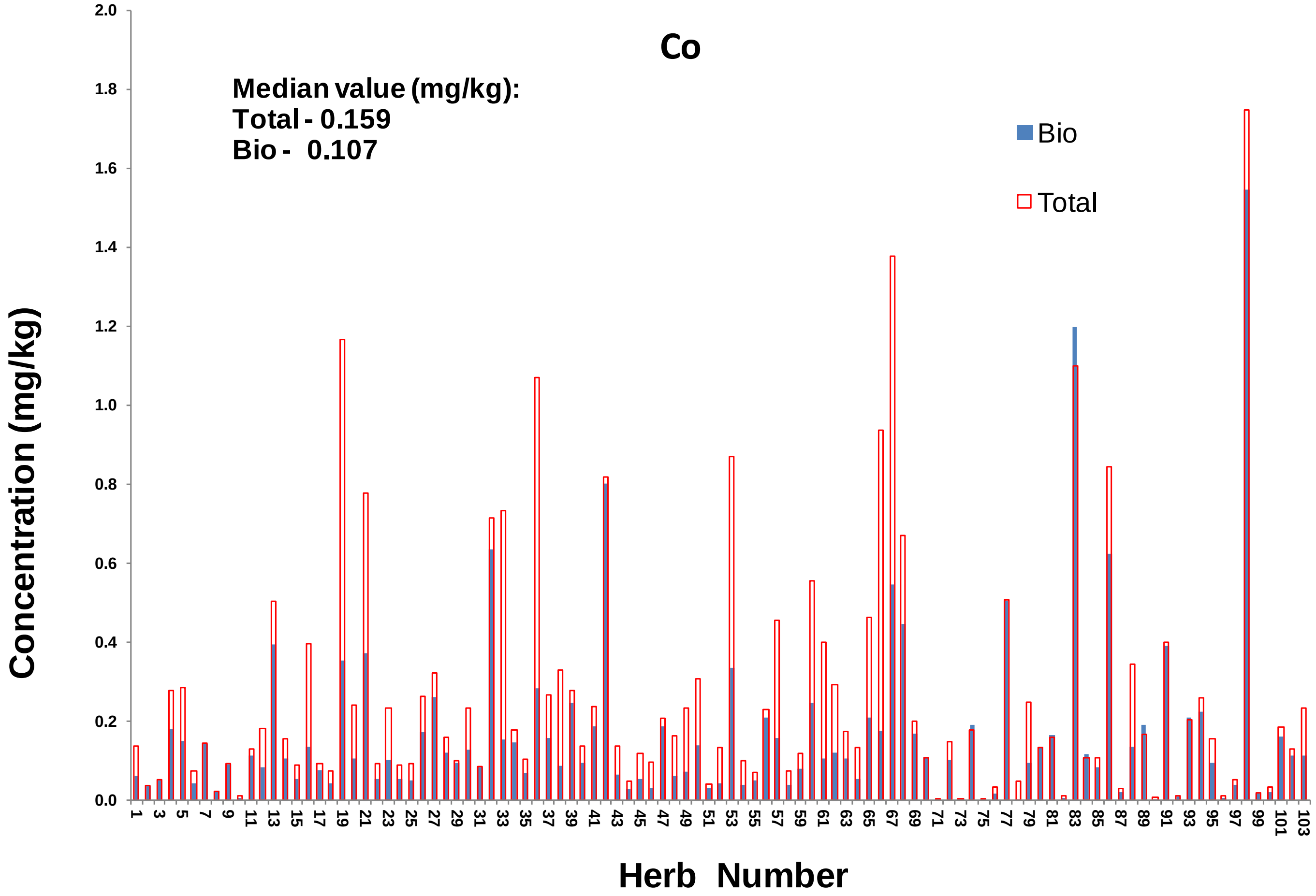


Figure 4.6.2: Total and bioavailabe concentration of Co in herb samples.

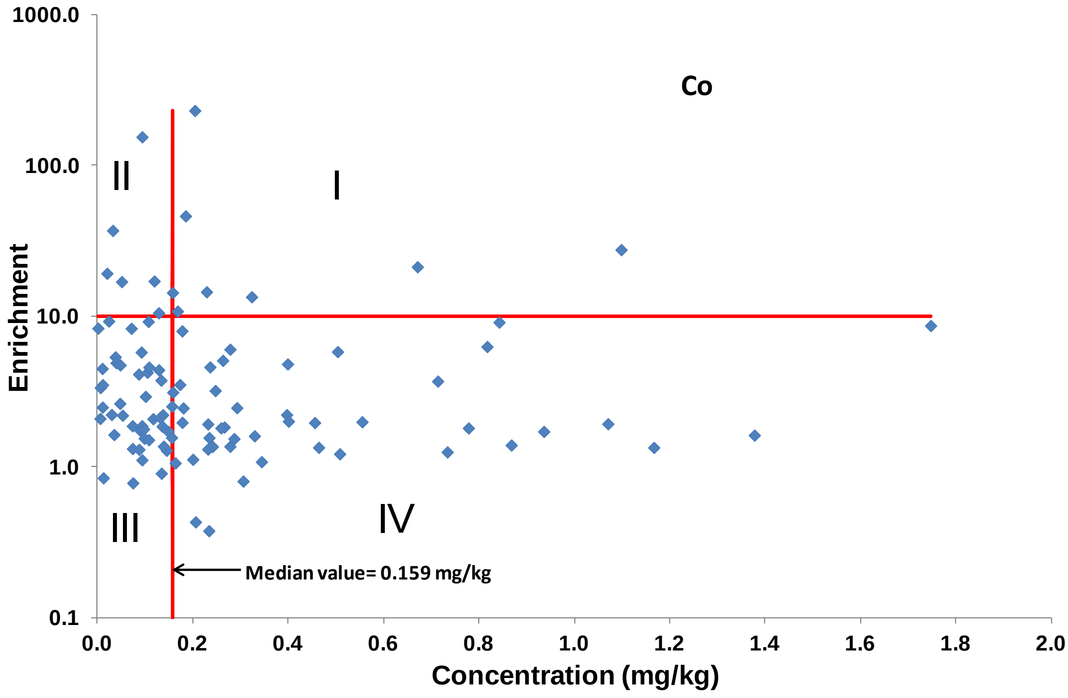


Figure 4.6.3: Enrichment Factor analysis of herb samples for Co.

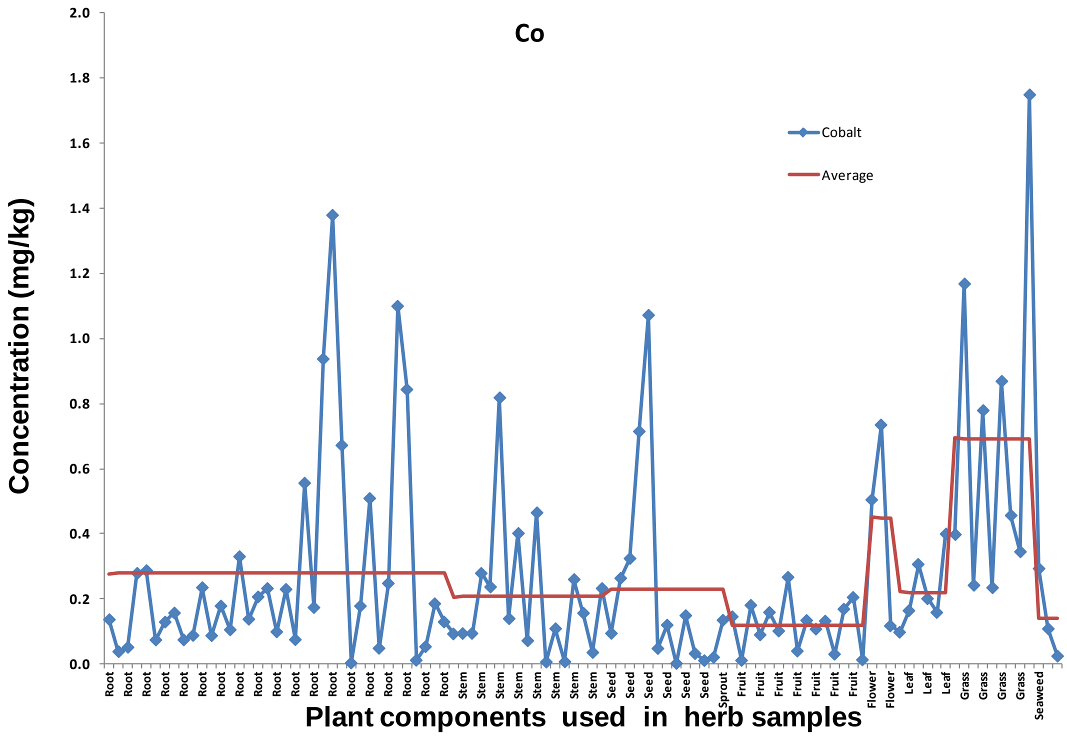


Figure 4.6.4: Variation of total concentration of Co with different plant components used in herb samples.

4.7 Iron, Fe

4.7.1 The Role of Fe in Human Health

Fe is an essential element found in nearly all living organisms and is abundant in biology. Thus Fe-proteins are found in nearly all living organisms, ranging from the evolutionarily primitive archaea to humans, often speciated as heme prosthetic groups (Fig. 4.7.1) [145]. Examples of proteins found in higher organisms include haemoglobin, cytochrome and catalase [146]. These Fe-containing proteins can transport gases, build enzymes and be used in transferring electrons. One of the reasons that Fe is such an essential element to human life is due to its redox characteristics. Amongst other functions, the human body as an aerobic organism requires iron to facilitate the transport of oxygen which is required for the production and survival of all cells in our bodies. A balance of Fe improves general health and resistance to disease [22]. Most of the human body's Fe is contained in the red blood cells. Anaemia (Fe deficiency in the blood) is the commonest nutritionally induced deficiency disease in the world [22]. Fe deficiency can also increase the risk of contracting infectious diseases as the defence mechanisms of the white blood corpuscles deteriorate [22]. Fe is most available to the body when chelated to amino acids [147] which is exploited for use as a common iron supplement. Often the amino acid chosen for this purpose is the cheapest and most common amino acid, glycine, leading to "iron glycinate" supplements [148].

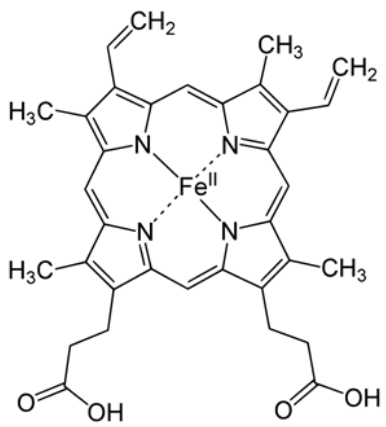


Figure 4.7.1: Structure of Heme b [145].

4.7.2 The Fe Landscape for the Herb Collection

General Comments

In this study, total and bioavailable contents of Fe were analysed by ICP and the distribution of Fe concentration across the 103 medicinal herbs (Table 3.1) is presented in Figure 4.7.2. The enrichment ability of the herbs for Fe was also investigated and the results of enrichment factor analysis are shown in Figure 4.7.3. The concentration distribution with respect to plant part is shown in Figure 4.7.4. It was found from Figure 4.7.2 that the herbs tested had very high levels of Fe compared to the other elements in the same group (microminerals). The total extractable content of Fe varied from 12.1 mg/kg to 6500 mg/kg, with a median value of 200 mg/kg. The bioavailability of Fe, however, was relatively low (< 80 %) for most of the herb samples (97 of 103).

Enrichment Factor Analysis

The herbs showed a generally low enrichment ability for Fe, with more than 90 % of herbal samples tested having an EF < 10 (III and IV in Figure 4.7.3). Two herbs (I in Figure 4.7.3) had Fe concentration above the media value of 200 mg/kg, possibly due to their enhanced enrichment ability (EF > 10) for Fe and/or the high available of Fe in the environment. The herbs in part II of Figure 4.7.3 also showed an enhanced enrichment ability (EF > 10) for Fe but their total extractable contents of Fe were less the median value 200 mg/kg, suggesting that the environment had low available of Fe to these herbs. The majority of herbs tested are located in part III of Figure 4.7.3, showing a concentration of Fe less than the median value of 200 mg/kg, possibly due to their low enrichment ability for Fe (EF < 10) and/or poorly available of Fe in the environment. Nine herb samples showed an EF < 10 but a Fe concentration > 200 mg/kg (median value) (IV in Fig. 4.7.3), suggesting that they may grow in a Fe-rich environment.

Plant Part Analysis

The concentration distribution of Fe varied with respect to plant part (Figure 4.7.4). The grass and flower herb samples showed a relatively high of average level of Fe.

4.7.3 Suggested Leads from Relating Fe Content to Medicinal Properties

From the data presented, *Huang Jin* (herb number 42 in Fig. 4.7.2) had the richest content of Fe and this content was almost fully bioavailable (bioavailability 91 %). This herb sample has been cooked when received, therefore there is a possibility that some of its Fe content was introduced during the cooking process (e.g. iron vessels). As mentioned in Section 4.5, *Huang Jin* is a tonifying herb which is commonly used in TCM to replenish or tonify in deficiency conditions of the body's radicals - Qi, Blood, Yin, Yang, fluids and Essence. The common symptoms associated with these conditions in Western terms include a variety of functional disorders such as metabolic disorders (e.g. anaemia) [30, 31]. TCM sees symptoms of anaemia as indicative of blood that has become too thick, too poor in quality or produced in too little amount (no matter what type of Anaemia is presenting). This is seen to be due to an insufficient flow of 'qi' energy, and resolved by stimulating individual organs to produce better quality blood hence an improved flow of Qi. Other herbs such as *Pu Gong Yi* (herb number 19 in Fig. 4.7.2, 3200 mg/kg), *Hong Hua* (herb number 33 in Fig. 4.7.2, 2200 mg/kg), *Zi Cao (B)* (herb number 67 in Fig. 4.7.2, 2200 mg/kg) and *Tu Si Zi* (herb number 36 in Fig. 4.7.2, 2000 mg/kg) also showed a relatively high level of Fe but had very low bioavailability (< 10) for Fe. While *Zhi Shi* (herb number 29) and *Lu Hui* (herb number 84) had less total extractable content of Fe but showed a relatively high bioavailability for Fe when compared to the herbs mentioned above. It is reported [149] that Fe deficiency and Fe excess could be related to some neurological disorders such as Alzheimer disease and Parkinson disease. *Hong Hua*, *Tu Si Zi*, *Zhi Shi*, *Tian Ma* (herb number 47 in Fig. 4.7.2) and *Si Chang Pu* (herb number 86 in Fig. 4.7.2) have been tried for the treatment of neurodegenerative disorders such as Alzheimer disease and Parkinson disease [138, 150]. Herbs that sequester relatively large amounts of Fe could have very interesting iron chelating (small-molecule) ligands present. There is a lot of interest these days in the development of chelating agents that can cross the brain blood barrier (BBB) and sequester metals (e.g. Fe) and that are non-toxic in themselves [151]. Further investigation is needed to identify novel chelating (small-molecule) ligands possibly present in those herbs that sequester disproportionally large amounts of certain metals (e.g. Fe).

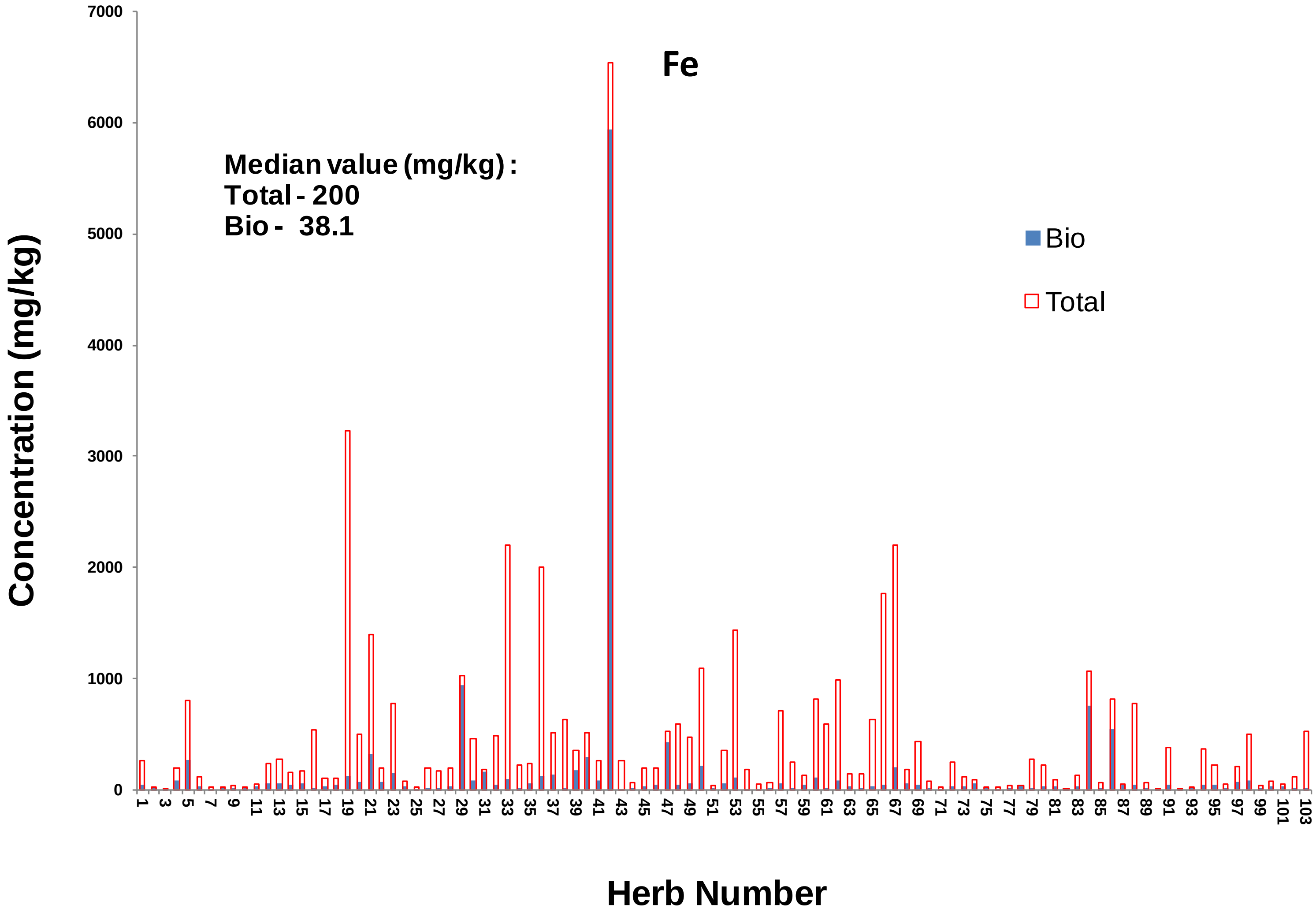


Figure 4.7.2: Total and bioavailable concentration of Fe in herb samples.

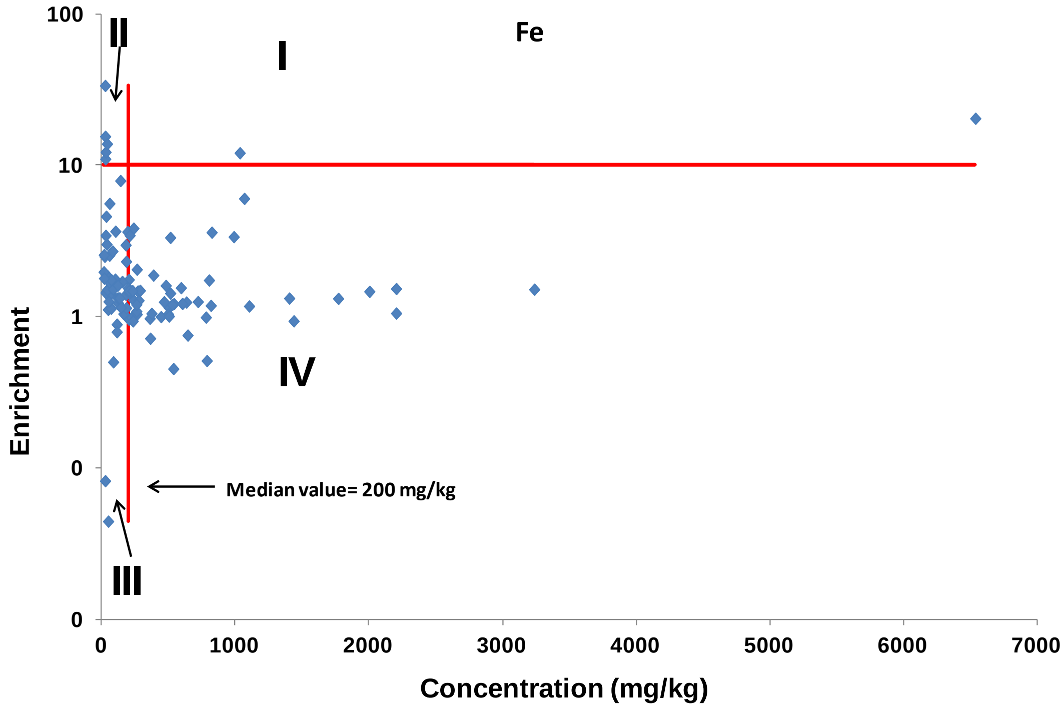


Figure 4.7.3: Enrichment Factor analysis of herb samples for Fe.

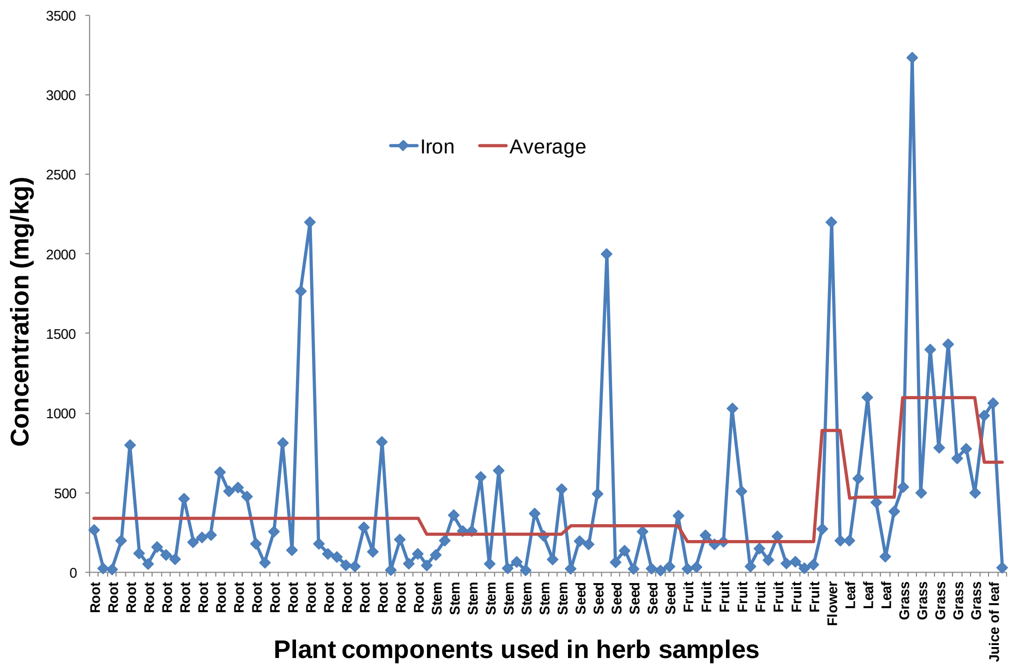


Figure 4.7.4: Variation of total concentration of Fe with different plant components used in herb samples.

4.8 Chromium, Cr

4.8.1 The Role of Cr in Human Health

Although recent studies suggest that Cr has no verified biological role and is not essential for mammals [152], this element was proposed to be an essential trace element over 50 years ago and has been accepted as an essential element for over 30 years. It is reported that Cr is required for normal carbohydrate and lipid metabolism in the body, and Cr deficiency could have an impact on many biological functions [113, 153]. Studies carried out on animals have shown that Cr deficiency reduces protein production, shortens the lifespan, results in eye damage, influences fertility and decreases glucose tolerance [22]. Although there are no commonly accepted grounds for the use of Cr in treating and preventing from illness, studies [22, 153, 154] have shown that Cr may play an important role in diabetes and insulin resistance. There are some dietary supplements for chromium available, including chromium(III) picolinate, chromium(III) polynicotinate, and related materials. The use of chromium-containing dietary supplements remains controversial and the benefit of such supplements requires further investigation [154-156].

4.8.2 The Cr Landscape for the Herb Collection

<u>*General Comments*</u>

In the current work, total and bioavailable contents of Cr were analysed by ICP across the 103 medicinal herbs (Table 3.1) and the distribution of Cr concentrations is shown in Figure 4.8.1. The results obtained from enrichment factor analysis for Cr are presented in Figure 4.8.2 and the concentration distribution with respect to plant part is shown in Figure 4.8.3. Figure 4.8.1 shows that the herb materials studied in this work presented a high variability (up to 910-fold) in the total content of Cr, ranging from 0.0477 mg/kg in *Long Yan Rou* (herb number 7 in Figure 4.8.1) to 43.2 mg/kg in *Dan Zhu Ye* (herb number 50 in Figure 4.8.1) and *Bai Hua She She Cao* (herb number 23 in Figure 4.8.1), with a median value of 1.80 mg/kg (Figure 4.8.2). There were also significant differences in the bioavailabe content of Cr between different herb materials (Figure 4.8.1), and most of herb samples showed a relatively low bioavailability (< 80 %). One third of the herbs tested are almost non-bioavailable, with the bioavailability being less than 10 % - therefore, perhaps less toxic.

Enrichment Factor Analysis

The enrichment factor analysis results (Figure 4.8.2) showed that the herbs had generally low enrichment ability for Cr, with 60 % of herbal samples tested having an EF < 10 (III and IV in Figure 4.8.2). The herbs in part I of Figure 4.8.2 had Cr concentration above the median value of 1.80 mg/kg, most likely due to their enhanced enrichment ability (EF > 10) for Cr and/or the high available of Cr in the environment. Although the herbs located in part II of Figure 4.8.2 also showed enhanced enrichment ability (EF > 10) for Cr, their total extractable contents of Cr were less the median value 1.80 mg/kg. This may suggest that the environment had low available of Cr to these herbs. The herbs in part III of Figure 4.8.2 showed a relatively low concentration of Cr (< the median value of 1.80 mg/kg), possibly due to their low enrichment ability for Cr (EF < 10) and/or poorly available of Cr in the environment. The herb samples in part IV of Fig. 4.8.2 showed a relatively low enrichment ability for Cr but had a Cr concentration > 1.80 mg/kg (median value), suggesting that they may grow in a Cr-rich environment.

Plant Part Analysis

The concentration distribution of Cr also varied with respect to plant part (Figure 4.8.3). The grass, leaf and flower herb samples showed higher average levels of Cr.

4.8.3 Suggested Leads from Relating Cr Content to Medicinal Properties

In TCM, the herbs showing higher levels of total Cr content (> 20 mg/kg) in Fig. 4.8.1 except for *Tu Si Zi* (herb number 36 in Fig. 4.8.1), are commonly used to clean "internal heat" which is associated with fever, inflammation and infection [30, 31]. *Tu Si Zi* is a tonifying herb which is commonly used in TCM to replenish or tonify in deficiency conditions of the body- Qi, Blood, Yin, Yang, fluids and Essence. The common symptoms associated with these conditions in Western terms include a variety of functional disorders such as metabolic disorders (e.g. anaemia) [30]. Notably, some of these herbs such as *Dan Zhu Ye* (herb number 50 in Fig. 4.8.1), which has the highest bioavailability, and *Tu Si Zi* (herb number 36 in Fig. 4.8.1), with some bioavailability, have also been tried in the treatment of diabetes [125]. Herbs such as these could be candidates for Cr speciation studies in a search for small molecule Cr compounds that are biologically active with respect to diabetes.

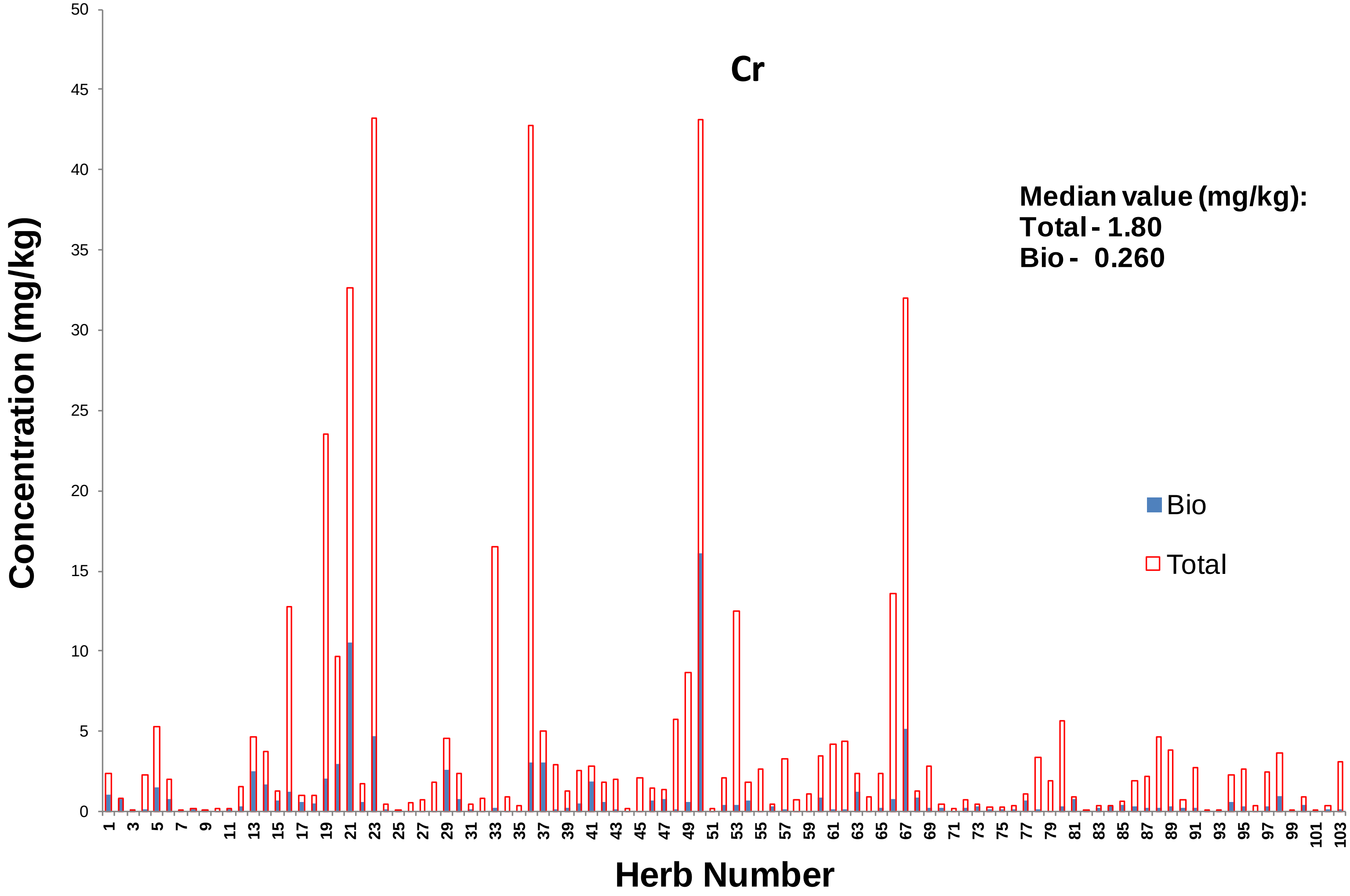


Figure 4.8.1: Total and bioavailable concentration of Cr in herb samples.

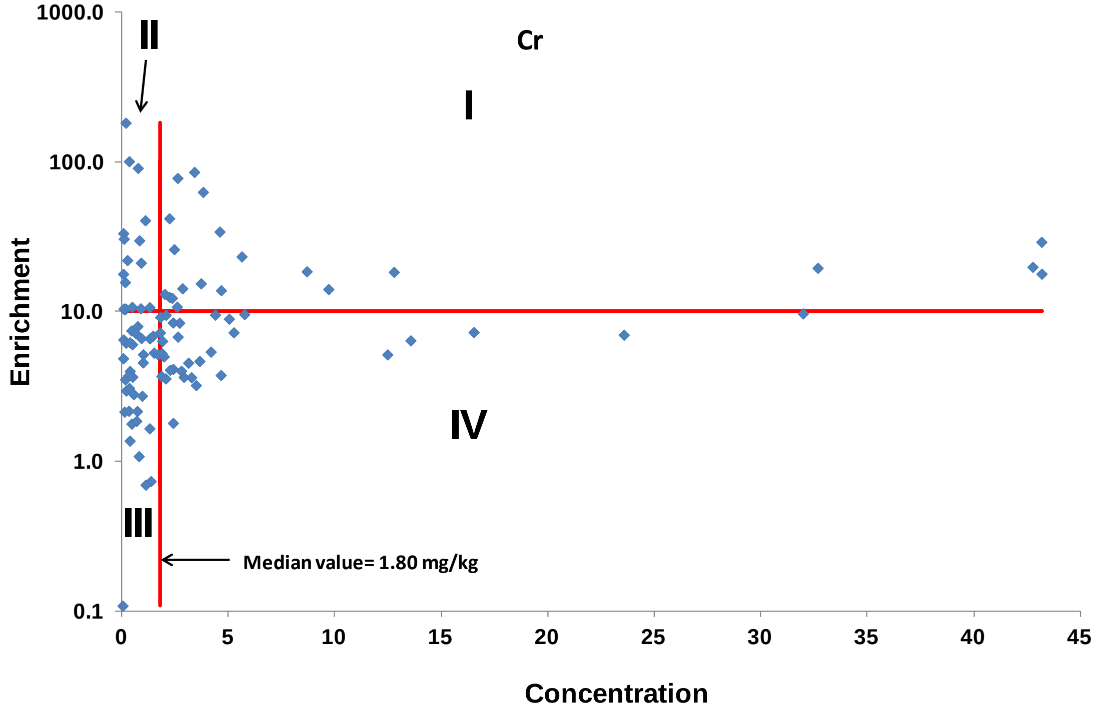


Figure 4.8.2: Enrichment Factor analysis of herb samples for Cr.

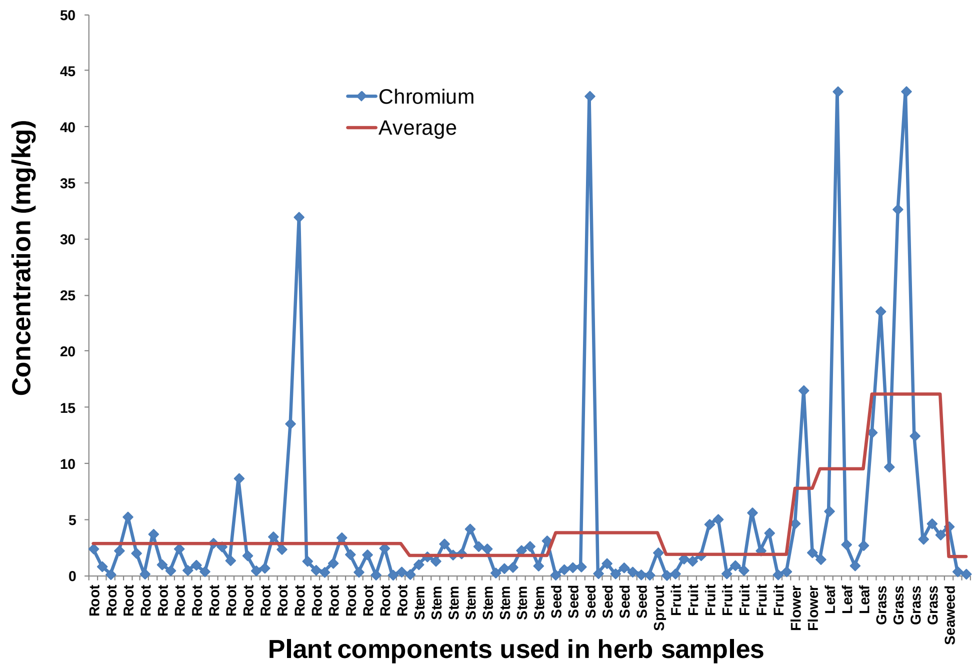


Figure 4.8.3: Variation of total concentration of Cr with different plant components used in herb samples.

4.9 Molybdenum, Mo

4.9.1 The Role of Mo in Human Health

Mo is the only second row of transition metal classified as essential for both plants and animals [22, 113]. Mo is the only classified as an essential element in the second row of transition metals and is essential for both plants and animals However, the significance of Mo for human health is not fully known [22]. The most important role of Mo in living organisms is as a metal heteroatom at the active site of certain enzymes. More than 50 Mo containing enzymes had been identified by 2002, mostly in bacteria, and this number is increasing every year [157, 158]. In animals and plants, enzymes utilize Mo at the active site as a tricyclic Mo cofactor and these enzymes catalyse the oxidation and sometimes the reduction of certain small molecules, as part of the regulation of nitrogen, sulfur and carbon cycles [159].

Human requirements for Mo are generally very low but are not known in great detail [22, 113]. The human body contains about 0.07 mg of Mo per kilogram of weight [160]. The average daily intake of Mo varies between 0.12 and 0.24 mg, but this depends on the Mo content of the food intake [161]. It is well known that there is a strong relationship between the biochemistry of Mo and Cu [113]. High levels of Mo can interfere with the body's uptake of copper, producing copper deficiency known as molybdenosis. However, low Mo intake can result in Cu toxicity [113].

4.9.2 The Mo Landscape for the Herb Collection

General Comments

In this study, the total and bioavailable levels of Mo were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.9.1. The enrichment ability of the herbs for Mo was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.9.2 and the concentration distribution with respect to plant part is depicted in Figure 4.9.3.

Figure 4.9.1 showed that the total extractable content of Mo in the herbs tested was found to be generally low compared to other elements in the microminerals group but had very high variability (~3000-fold), ranging from 0.00199 mg/kg in *Fu Ling* (herb number 3) to 7.77 mg/kg in *Huang Qi* (herb number 35), on a dry weight basis. The median value of the total extractable level of Mo was 0.222 mg/kg. The overall bioavailability of Mo was also relatively low, with a median value of the bioavailable of Mo being 0.0366 mg/kg. Only 4 out of 103 herbs (*Fu Ling*, *E Jiao*, *Jiang Huang* and *Fang Ji*, herb number 3, 8, 94 and 102 in Figure 4.9.1, respectively) achieved a more than 80 % bioavailability of Mo, indicating that the Mo content in these few herbs is almost fully bioavailable.

Enrichment Factor Analysis

The herbs demonstrated a high enrichment ability for Mo, with more than 90 % of herbal samples tested having an EF > 10 (I and II in Figure 4.9.2). About half of them (I in Figure 4.9.2) had Mo concentrations above the median value of 0.222 mg/kg, which is attributed not only to an enhanced enrichment ability for Mo, but also a relatively high availability of Mo in the environment where they grown. The herbs in part II of Figure 4.9.2 also had an enhanced enrichment ability for Mo but showed a total extractable content of Mo less the median value 0.222 mg/kg, suggesting that these herbs have a low environmental availability of Mo. Five herbs located in part III of Figure 4.9.2 showed a concentration of Mo less than the median value of 19.6 mg/kg, possibly due to a low enrichment ability for Mo (EF < 10) and/or poorly availability of Mo in the environment. Two herb samples (IV in Fig. 4.9.2) had an EF < 10 but a Mo concentration > 0.222 mg/kg (median value), suggesting that they may originate from a Mo-rich environment.

Plant Part Analysis

The concentration distribution of Mo with respect to plant part is shown in Figure 4.9.3. It was found that there were no significant differences in the total extractable content of Mo between the plant parts. The leaf-derived, seed-based, fruit and flower herb samples showed slightly enhanced levels of Mo.

4.9.3 Suggested Leads from Relating Mo Content to Medicinal Properties

From the data presented in Figure 4.9.1, *Huang Qi* (herb number 35) had the highest level in both total and bioavailable contents of Mo. *Bu Gu Zhi* and *Tu Si Zi* (herb number 64 and 36 in Figure 4.9.1, respectively) are also relatively Mo rich but the bioavailability of Mo in these two herbs is relatively low. These herbs are classified as tonifying herbs which is commonly used in TCM to replenish deficiency conditions of the body – Qi, Blood, Yin, and Yang etc., which address a range of functional disorders such as metabolic disorders (e.g. anaemia and malabsorption) [30, 31]. *Huang Qi* has also been used in treating several Western medical diseases such as diabetes, diverticulitis, and fibromyalgia syndrome [138].

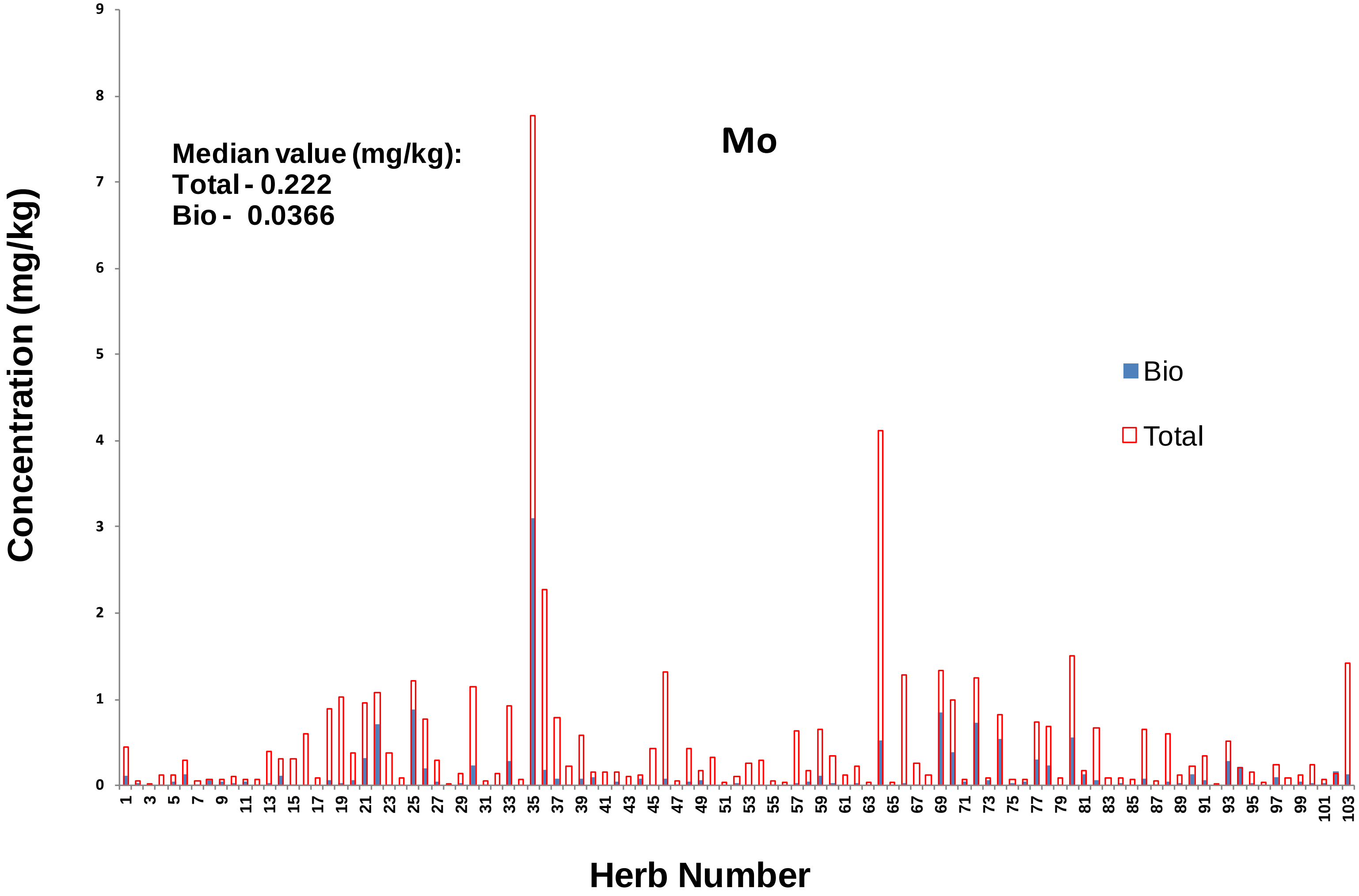


Figure 4.9.1: Total and bioavailable concentration of Mo in herb samples.

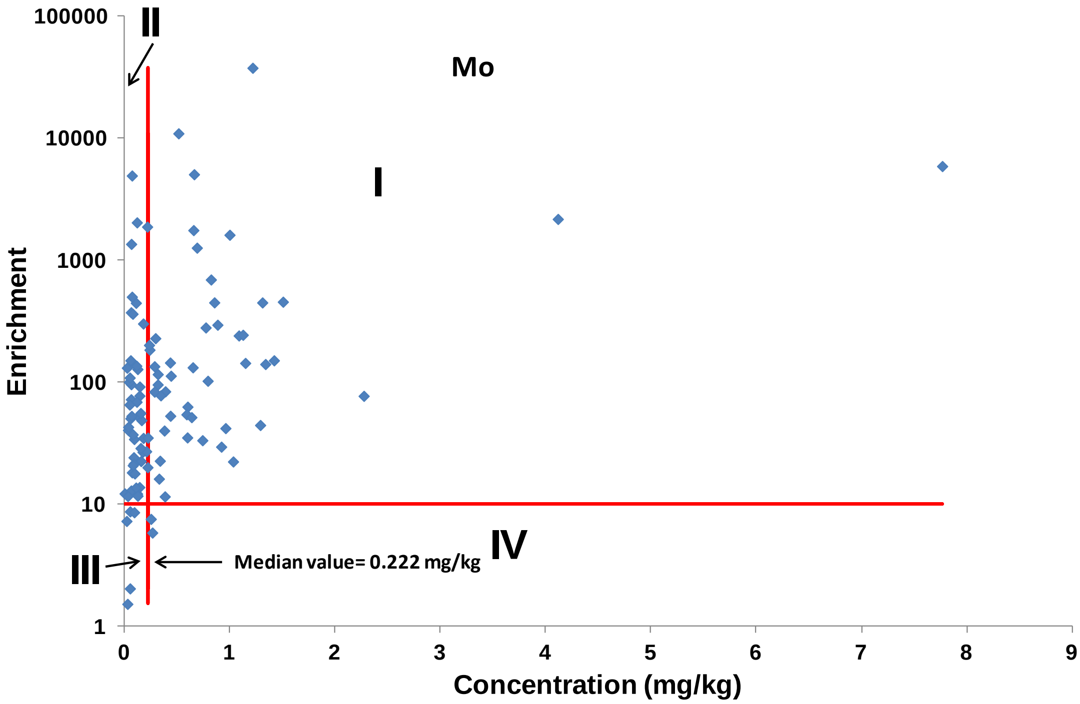


Figure 4.9.2: Enrichment Factor analysis of herb samples for Mo.

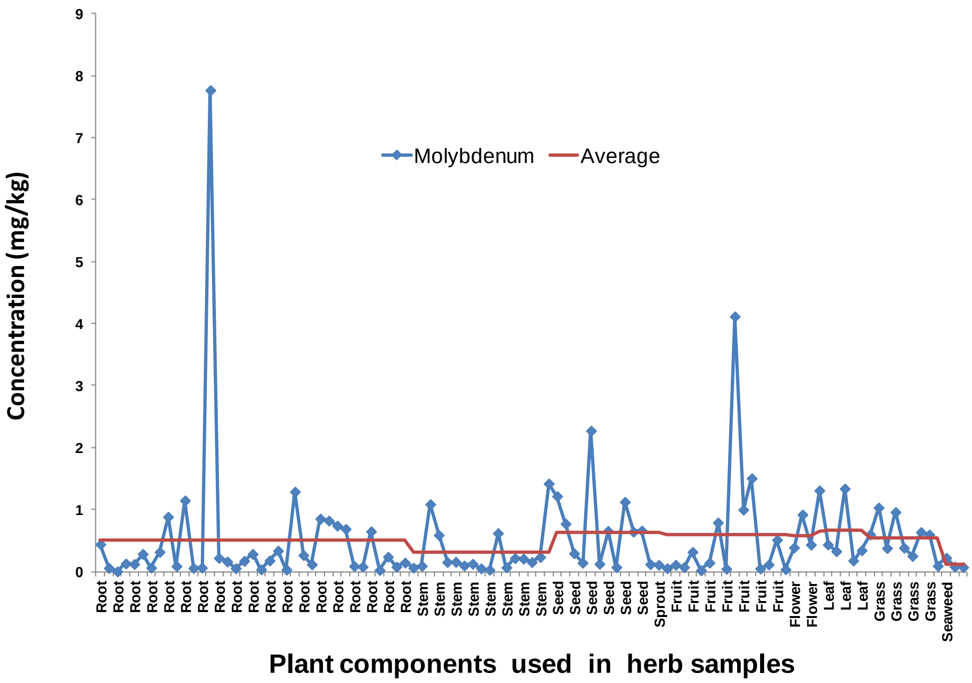


Figure 4.9.3: Variation of total concentration of Mo with different plant components used in herb samples.

4.10 Vanadium, V

4.10.1 The Role of V in Human Health

V is one of the essential trace elements that scientists know least about [22, 162]. V appears to play a very limited role in biology, and is more important in ocean than in land environments [163]. The concentration of V in the human body is very low (0.01 – 0.6 µg/g wet weight) [113] and scientists are not sure exactly what effects V may have, or what amount might be helpful. However, it is known that high doses of V may be deleterious [164]. Although the significance for human health of V as an essential element is still unknown, V may have a role to play in the treatment of diabetes and cancer [22, 162, 165, 166]. V complexes such as bis-(maltolato) oxovanadium (IV) (BMOV) can be used as an insulin-mimetic agent for the treatment of diabetes [8]. However, V is not currently recommended for any disease or condition in humans as most of the studies using V have been conducted on animals [164]. V is a relatively controversial dietary supplement, primarily recommended for increasing insulin sensitivity [167] and to enhance body-building. Whether it works for the latter purpose has not been proven, and there is some evidence to suggest that athletes who take it are merely experiencing a placebo effect [164, 168].

4.10.2 The V Landscape for the Herb Collection

General Comments

In this study, the total and bioavailable levels of V were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.10.1. The enrichment ability of the herbs for V was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.10.2 and the concentration distribution with respect to plant part is depicted in Figure 4.10.3.

It can be seen from Figure 4.10.1 that the herbs tested had a generally low level of V. The total extractable content of V ranged between 0.0278 mg/kg in *Tao Ren* (herb number 25) and 4.43 mg/kg in *Pu Gong Yin* (herb number 19), on a dry weight basis. The median value of the total extractable level of V was 0.273 mg/kg as shown in Figure 4.10.1. The overall bioavailability of V was also relatively low, with a median value of 0.0924 mg/kg (Figure 4.10.1). The bioavailability of V varied from 2 % to 100 %. Only around 10 % of herbs tested achieved a more than 80 % bioavailability of V, indicating that the V content in these herbs is almost fully bioavailable.

Enrichment Factor Analysis

The herbs also showed a low enrichment ability for V, with all the herbal samples tested having an EF < 10 except 2 herbs (Figure 4.10.2). Only one herb (I in Figure 4.10.2) had V concentrations above the median value of 0.273 mg/kg, which is attributed not only to an enhanced enrichment ability for V, but also a high availability of V in the environment where they grown. The herb in part II of Figure 4.10.2 also had an enhanced enrichment ability for V but showed a total extractable content of V less the median value 0.273 mg/kg, suggesting that these herbs have a low environmental availability of V. The herbs located in part III of Figure 4.10.2 showed a concentration of V less than the median value of 0.273 mg/kg, possibly due to low enrichment ability for V (EF < 10) and/or poorly availability of V in the environment. The herb samples in part IV in Fig. 4.10.2 showed an EF < 10 but a V concentration > 0.273 mg/kg (median value), suggesting that they may originate from a V-rich environment.

Plant Part Analysis

The concentration distribution of V varied with respect to plant part (Figure 4.10.3). The grass and flower samples showed a relatively high average level of V.

4.10.3 Suggested Leads from Relating V Content to Medicinal Properties

Among the 103 herbs tested, *Pu Gong Yin*, *Zi Cao (B)* and *Ma Chi Xian* (herb number 19, 67 and 21 in Fig. 4.10.1, respectively) showed a relatively high level in both total and bioavailable contents of V. The total content of V in *Hai Zao* and *He Ye* (herb number 62 and 81 in Fig. 4.10.1, respectively) was not as high as that in the three herbs listed above but they also showed a relatively high bioavailable content of V. These herbs are traditionally used in TCM to clear "internal heat" which is a condition associated with fever, inflammation and infection [30, 31]. *Pu Gong Yin* has also been used orally for diabetes and cancer [169]. This herb may need to be further investigated for the possible development of insulin mimetics for diabetes. Notably, *Hai Zao* is also used for treatment of cancer [170].

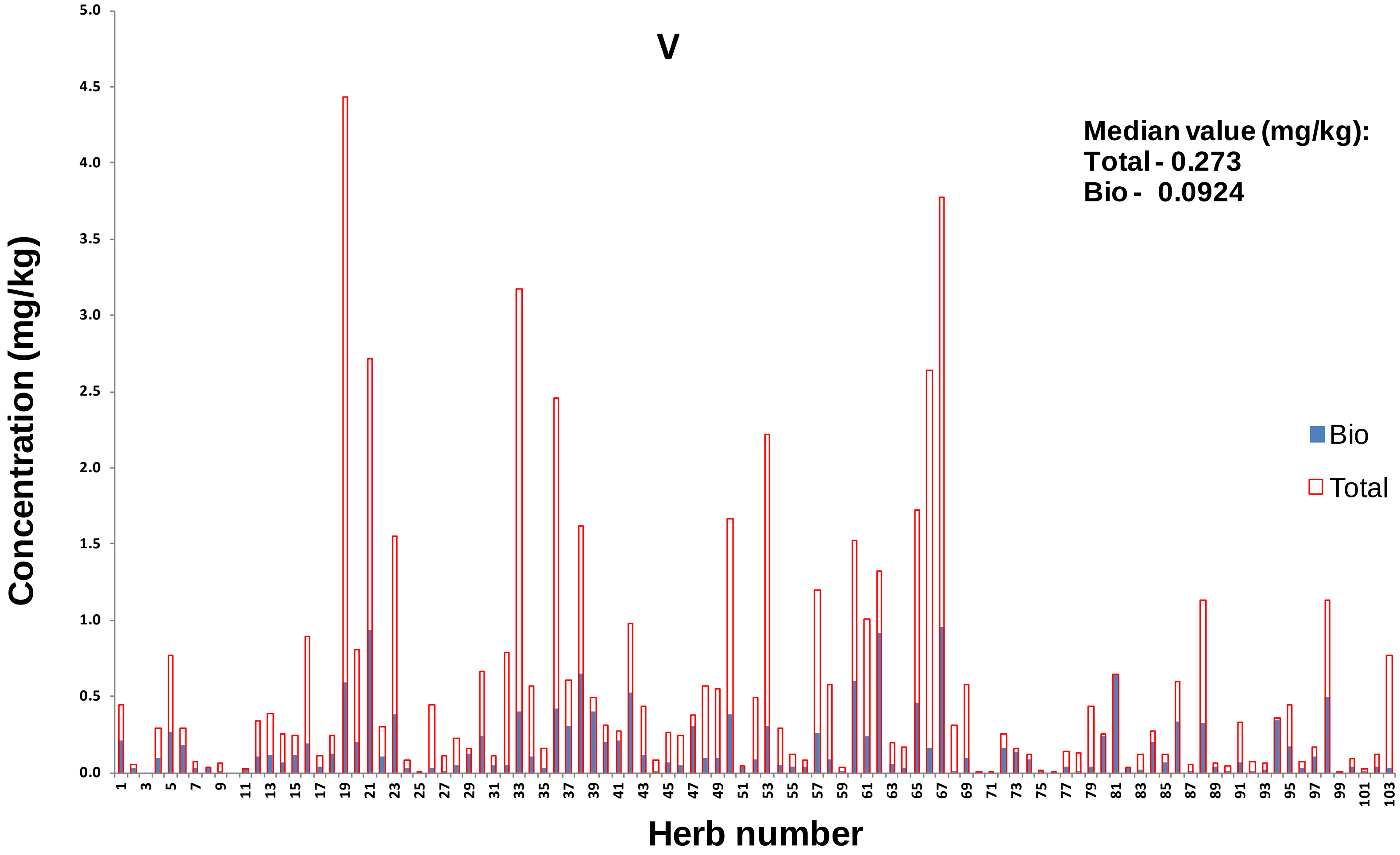


Figure 4.10.1: Total and bioavailable concentration of V in herb samples.

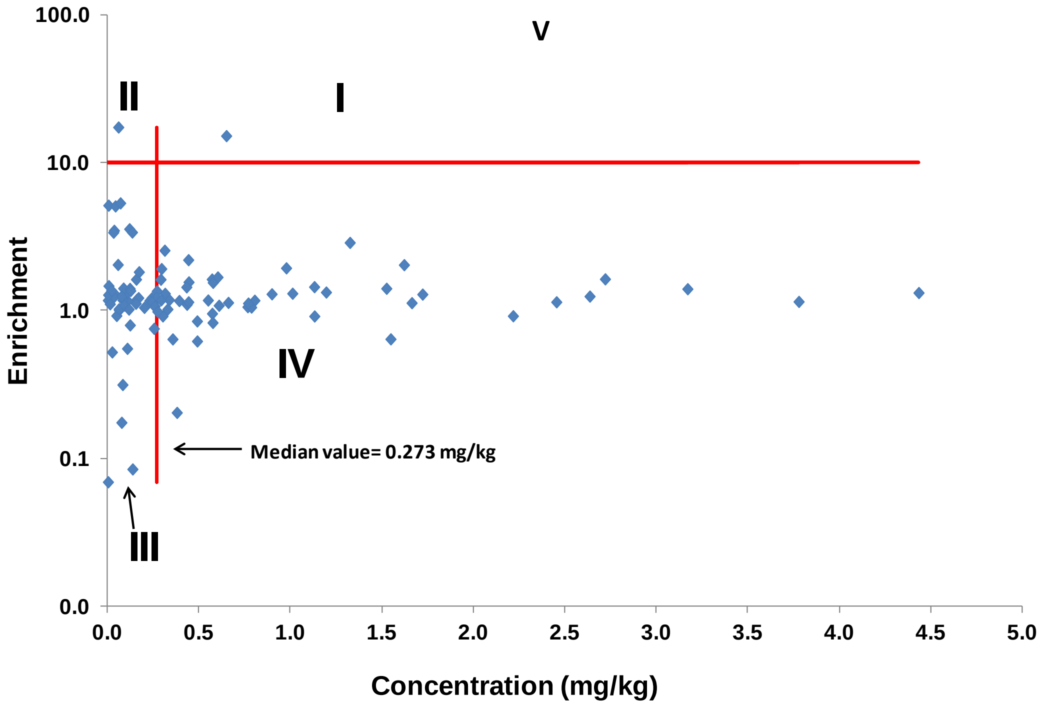


Figure 4.10.2: Enrichment Factor analysis of herb samples for V.

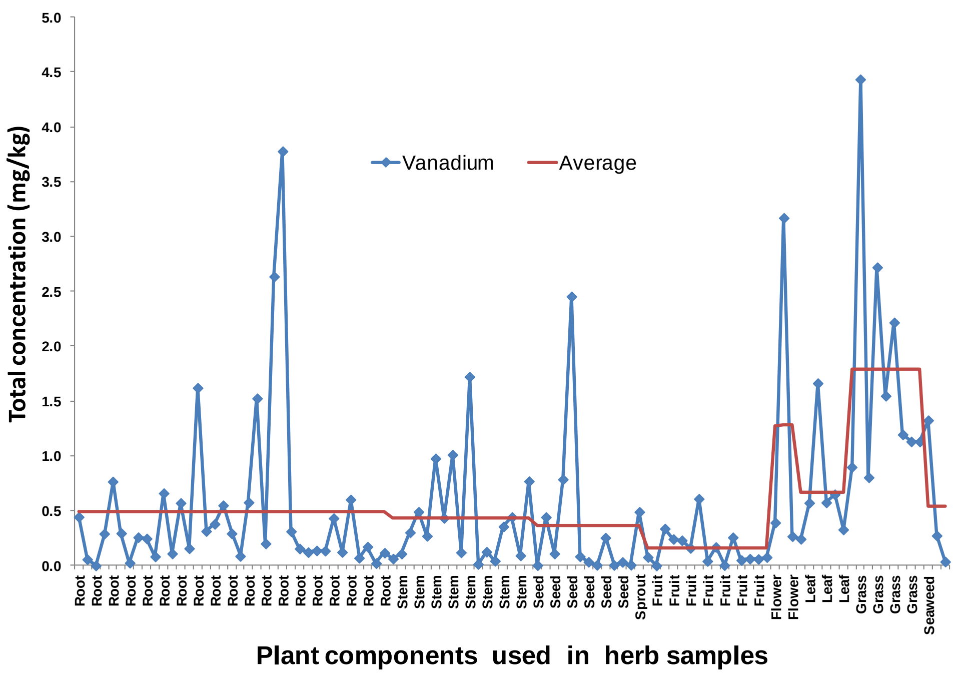


Figure 4.10.3: Variation of total concentration of V with different plant components used in herb samples.

4.11 Nickel, Ni

4.11.1 The Role of Ni in Human Health

Although Ni was regarded as a potentially toxic element for many decades, it was first suggested as essential in the early 1970s [113]. Ni is now classified an essential element and plays important roles in the biology of microorganisms and plants [22, 113, 171]. Ni is apparently involved in the protection of the cell membranes but actual requirements for human beings are not known [22]. Ni deficiency could occur under certain circumstances and has been found to result in a range of illness in animals [22]. High levels of Ni can be toxic and can also cause contact allergies and cancer [22, 113, 172, 173].

4.11.2 The Ni Landscape for the Herb Collection

General Comments

This study measured the total and bioavailable levels of Ni in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis, and the results are presented in Figure 4.11.1. The enrichment ability of the herbs for Ni was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.11.2 and the concentration distribution with respect to plant part is depicted in Figure 4.11.3.

Figure 4.11.1 showed that the total extractable content of Ni in the herbs tested ranged between 0.0822 mg/kg in *E Jiao* (herb number 8) and 7.22 mg/kg in *Bu Gu Zhi* (herb number 64), on a dry weight basis. The median value of the total extractable level of Ni was 0.914 mg/kg as shown in Figure 4.11.1. The bioavailability of Ni in the herbs tested was estimated to be between 4 % and 100 %, with around 40 % of herbs having achieved a more than 80 % bioavailability of Ni, indicating that the Ni content in these herbs is almost fully bioavailable. The median value of the bioavailable content of Ni was 0.591 mg/kg (Figure 4.11.1).

Enrichment Factor Analysis

The enrichment factor analysis showed that around half of herbal samples tested achieved an EF > 10 (I and II in Figure 4.11.2). About half of them (I in Figure 4.11.2) had Ni concentrations >= 0.914 mg/kg (median value), which is attributed not only to an enhanced enrichment ability for Ni, but also a high availability of Ni in the environment where they were grown. The herbs in part II of Figure 4.11.2 also had an enhanced enrichment ability for Ni but showed a total extractable content of Ni less the median value of 0.914 mg/kg, suggesting that these herbs have a low environmental availability of Ni. The herbs located in part III of Figure 4.11.2 showed a concentration of Ni less than the median value of 0.914 mg/kg, possibly due to low enrichment ability for Ni (EF < 10) and/or poor availability of Ni in the environment. The herb samples in IV of Fig. 4.11.2 showed an EF < 10 but a Ni concentration > 0.914 mg/kg (median value), suggesting that they may originate from a Ni-rich environment.

Plant Part Analysis

The concentration distribution of Ni varied with respect to plant part (Figure 4.11.3); the grass and flower samples showing relatively enhanced levels of Ni.

4.11.3 Suggested Leads from Relating Ni Content to Medicinal Properties

It can be seen from the data presented in Fig. 4.11.1 that *Bu Gu Zhi*, *Suan Zao Ren*, *Yin Chen* and *Niu Bang Zi* (herb number 64, 59, 53 and 70, respectively) had relatively high levels in both total extractable and bioavailable contents of Ni. These herbs have different functions in TCM. *Bu Gu Zhi* is commonly used to replenish deficiency conditions of the body – Qi, Blood, Yin, and Yang etc., which address a range of functional disorders such as metabolic disorders (e.g. anaemia and malabsorption) [30, 31]. *Suan Zao Ren* is traditionally used to calm the mind in the treatment of various mind disharmony conditions which have common symptoms including anxiety and agitation, insomnia, frequent dreams and palpitations [30]. *Yin Chen* is widely used to clear “internal heat” which is a condition associated with fever, inflammation and infection [30, 31]. *Niu Bang Zi* is commonly used to treat exterior patterns, marked by such symptoms as fever, chills, possible sweating, headache and floating pulse [30]. As mentioned earlier in this section, Ni can be toxic at high concentration. It was reported [113] that the average intake of Ni for human is 0.3 – 0.5 mg/day. Therefore, from the clinical point of view, it is necessary to evaluate the safety of using the herbs discussed above by the determination of Ni concentration. To the best of our knowledge, there has been no work carried out in the search for small molecule bioactive nickel species that might have a role to play in the treatment of disorders such as those associated with the herbs described above (64, 59, 53, 70) - which are associated conditions such as anxiety, insomnia or inflammation. It would be of interest to pursue these clues in this regard and this demonstrates how this strategy can initiate research in new areas.

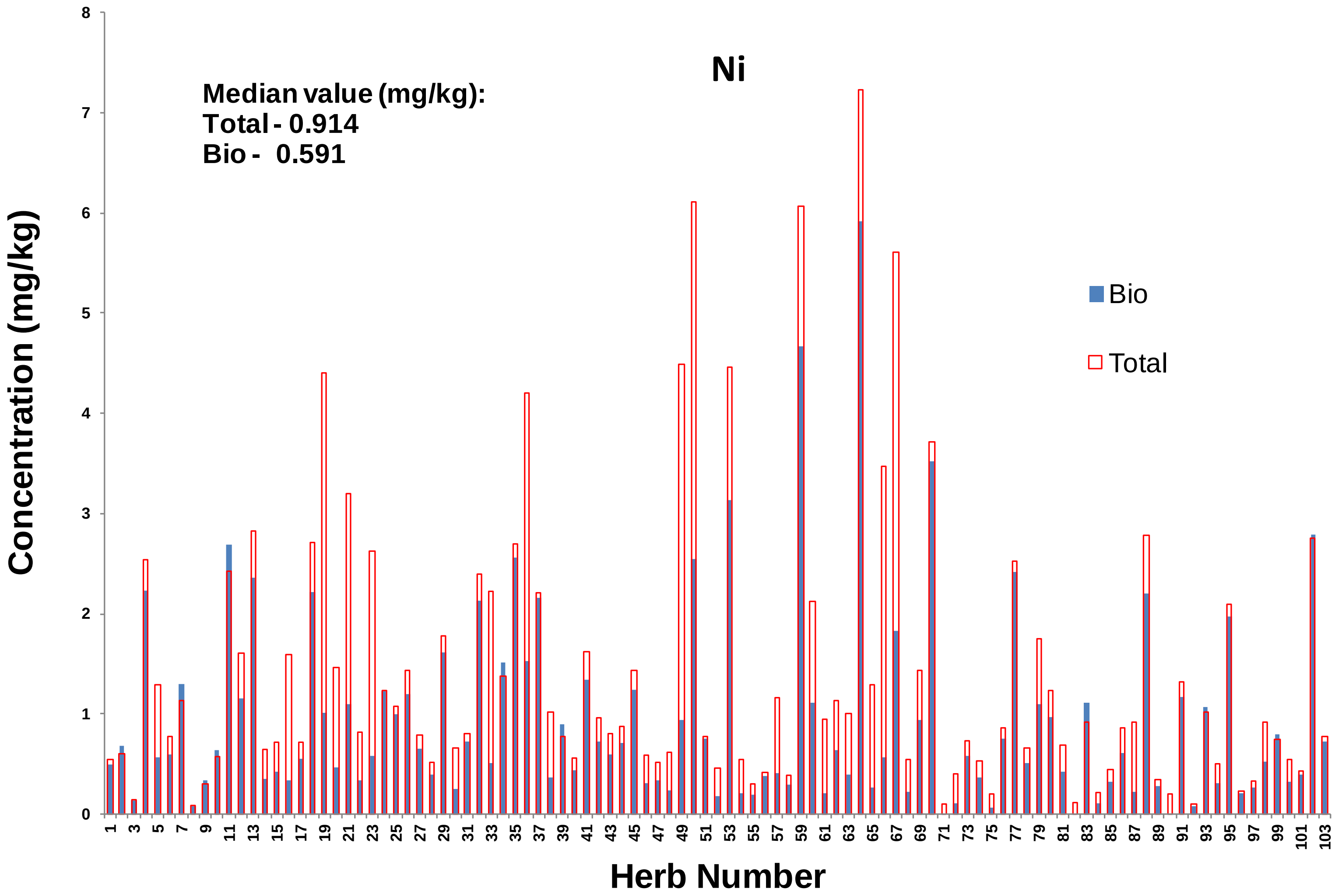


Figure 4.11.1: Total and bioavailable concentration of Ni in herb samples.

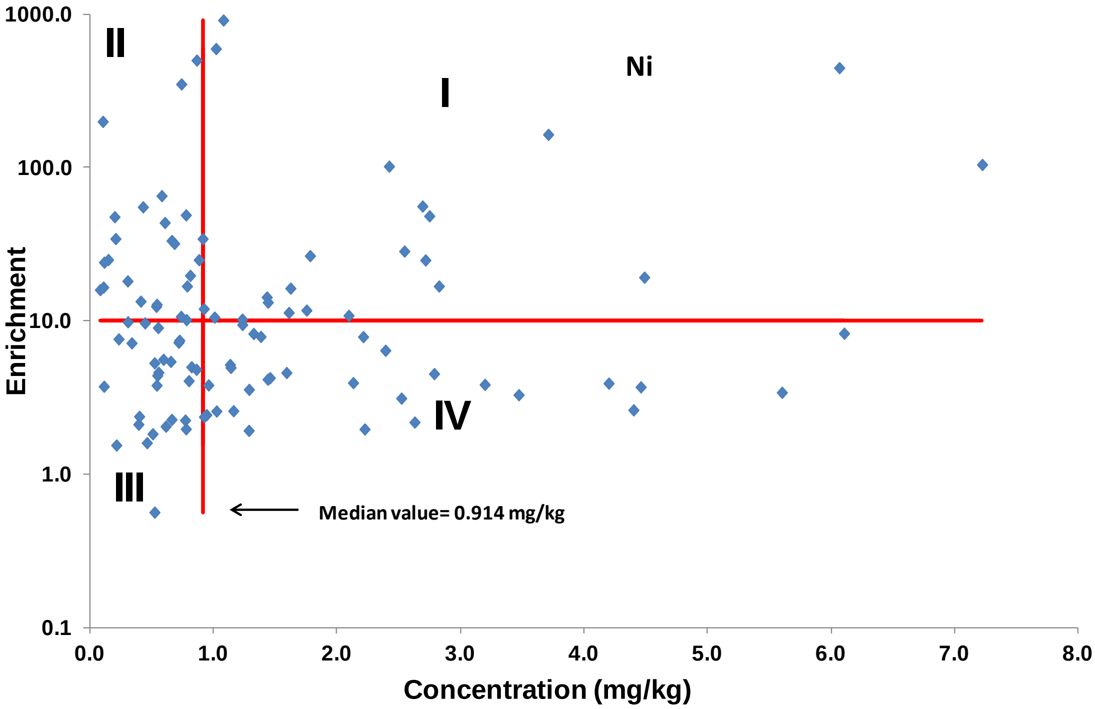


Figure 4.11.2: Enrichment Factor analysis of herb samples for Ni.

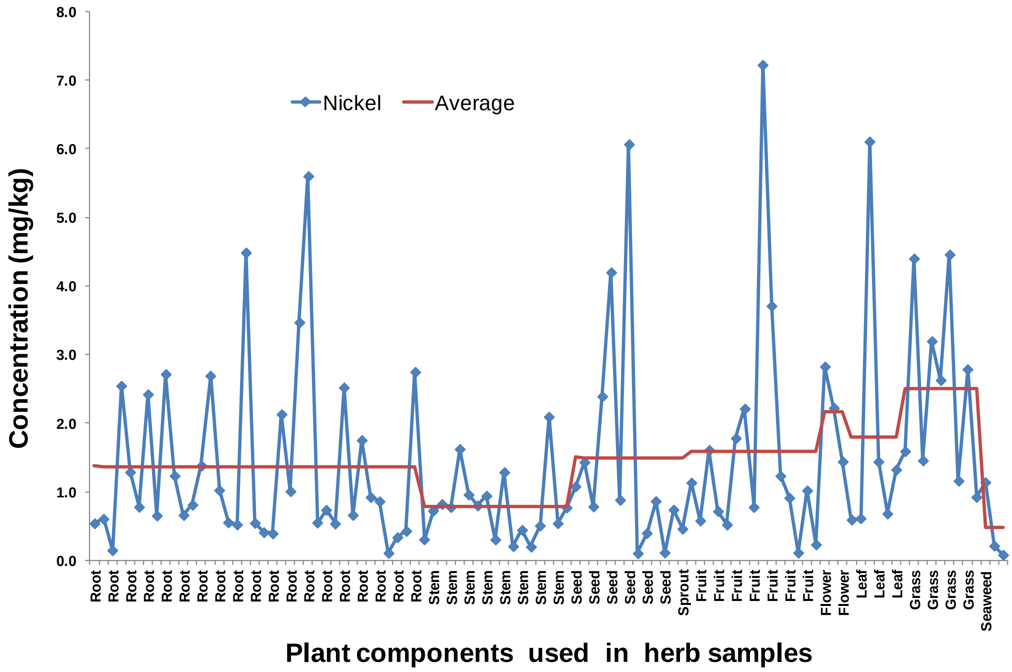


Figure 4.11.3: Variation of total concentration of Ni with different plant components used in herb samples.

4.12 Lithium, Li

4.12.1 The Role of Li in Human Health

Li is reported to have a physiological role in animals and is considered to be an essential element in humans [113, 174]. Li compounds have been used in the treatment of a number of neurological disorders including, bipolar disorder, Huntington's chorea, tardive dyskinesia, spasmodic torticollis, Tourette's syndrome, L-dopa induced hyperkinesias, Parkinsonism, organic brain disorders, drug induced delusional disorders, migraine and cluster headaches, periodic hypersomnolence, epilepsy, Meniere's disease and periodic hypokalemic paralysis [175]. A proposed mechanism of action for neurological disorders involves the inhibition by Li compounds of the scavenging pathway for capturing inositol in the resynthesis of polyphosphoinositides in the brain [176]. For the treatment of bipolar disorder, Li compounds are the most widely used and studied medication [177] and can reduce the severity and frequency of mania [113, 177]. They continue to be the standard against which newer medications are measured [174]. Encouragingly, it has been demonstrated that these drugs can significantly reduce suicide risk [177]. Li treatment also helps prevent future manic episodes, and therefore it may be prescribed for long periods of time (even between episodes) as a maintenance therapy [177]. Notably, it was reported [178] that naturally occurring Li in drinking water may increase the human lifespan.

4.12.2 The Li Landscape for the Herb Collection

General Comments

In this study, the total and bioavailable levels of Li were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.12.1. The enrichment ability of the herbs for Li was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.12.2 and the concentration distribution with respect to plant part is depicted in Figure 4.12.3.

It was found from Figure 4.12.1 that the total extractable content of Li in the herbs tested was generally low (0.00105 mg/kg – 5.42 mg/kg on a dry weight basis), except *Wu Jia Pi* (61.0 mg/kg herb number 61), which is extraordinarily high. The median value of the total extractable level of Li was 0.198 mg/kg as shown in Figure 4.12.1. The overall bioavailability of Li was also relatively low, varying from 3 % to 100 %. However, 21 out of 103 herbs achieved a more than 80 % bioavailability of Li, indicating that the Li content in these herbs is almost fully bioavailable. In particular, it should be noted that the outstanding herb, number 61, has its elevated level of Li that is effectively fully bioavailable.

Enrichment Factor Analysis

The herbs demonstrated a high enrichment ability for Li, with nearly 80 % of herbal samples tested having an EF > 10 (I and II in Figure 4.12.2). The herb samples located in part I of Figure 4.12.2 including *Wu Jia Pi* (not shown in Figure 4.12.2, herb number 61 in Figure 4.12.1) had Li concentrations above the median value of 0.198 mg/kg, possibly due to their enhanced enrichment ability for Li and/or a high availability of Li in the environment where they grown. The herbs in part II of Figure 4.12.2 also had an enhanced enrichment ability for Li but showed a total extractable content of Li less the median value 0.198 mg/kg, suggesting that these herbs have a low environmental availability of Li. The herbs located in part III of Figure 4.12.2 showed a concentration of Li less than the median value of 0.198 mg/kg, possibly due to low enrichment ability for Li (EF < 10) and/or poorly availability of Li in the environment. The herb samples showing an EF < 10 but a Li concentration > 0.198 mg/kg (median value) (IV in Fig. 4.12.2) might originate from a Li-rich environment.

Plant Part Analysis

Figure 4.12.3 showed that the concentration distribution of Li varied with respect to plant part. The leaf-derived, grass and flower herb samples showed relatively enhanced levels of Li if *Wu Jia Pi* (herb number 61 in Figure 4.12.1) was not included in the calculation (Fig. 4.12.3b inset).

4.12.3 Suggested Leads from Relating Li Content to Medicinal Properties

From the data presented in Fig. 4.12.1, *Wu Jia Pi* (herb number 61) had the highest level of Li (61.0 mg/kg) - and this Li content is almost fully bioavailable (bioavailability 88 %), within experimental error. *Lu Bu Ma* (herb number 46) was found to be the second richest in Li (5.42 mg/kg) and its Li content is also fully bioavailable.

Wu Jia Pi is widely used in TCM to dispel wind-damp which addresses painful obstruction syndromes characterised by rheumatic pain and stiffness in the limbs, joints, muscles and tendons [30]. *Lu Bu Ma* is used in TCM to nourish the heat and calm the spirit treat which are conditions associated with insomnia, palpitations and post-concussion syndromes, etc [31].

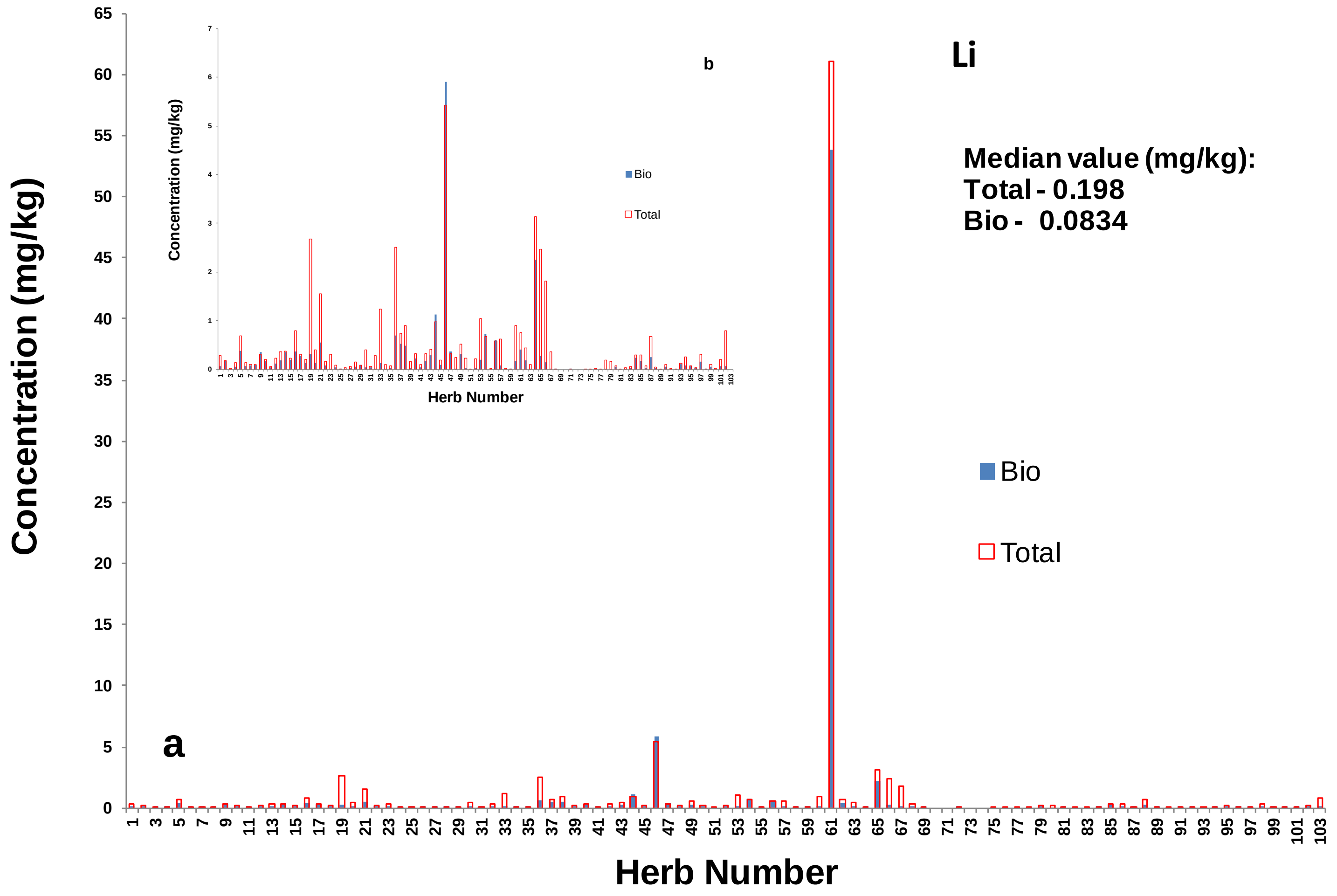


Figure 4.12.1: Total and bioavailable concentration (mean values of triplicates) of Li in herb samples with **(a)** and without *Wu Jia Pi* (herb number 61) **(b)**.

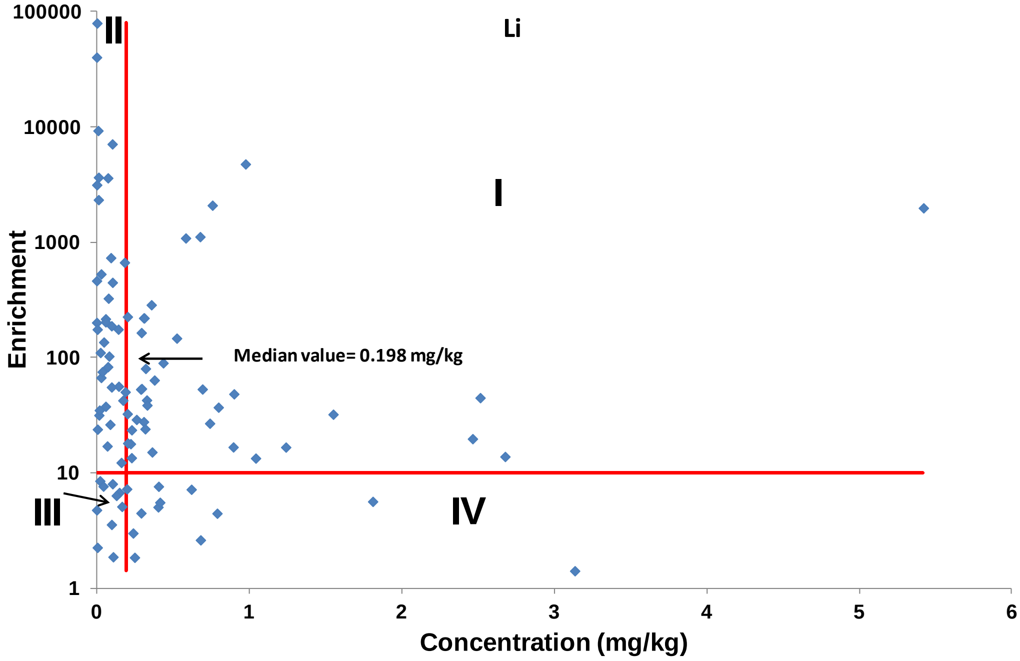


Figure 4.12.2: Enrichment Factor analysis of herb samples for Li.

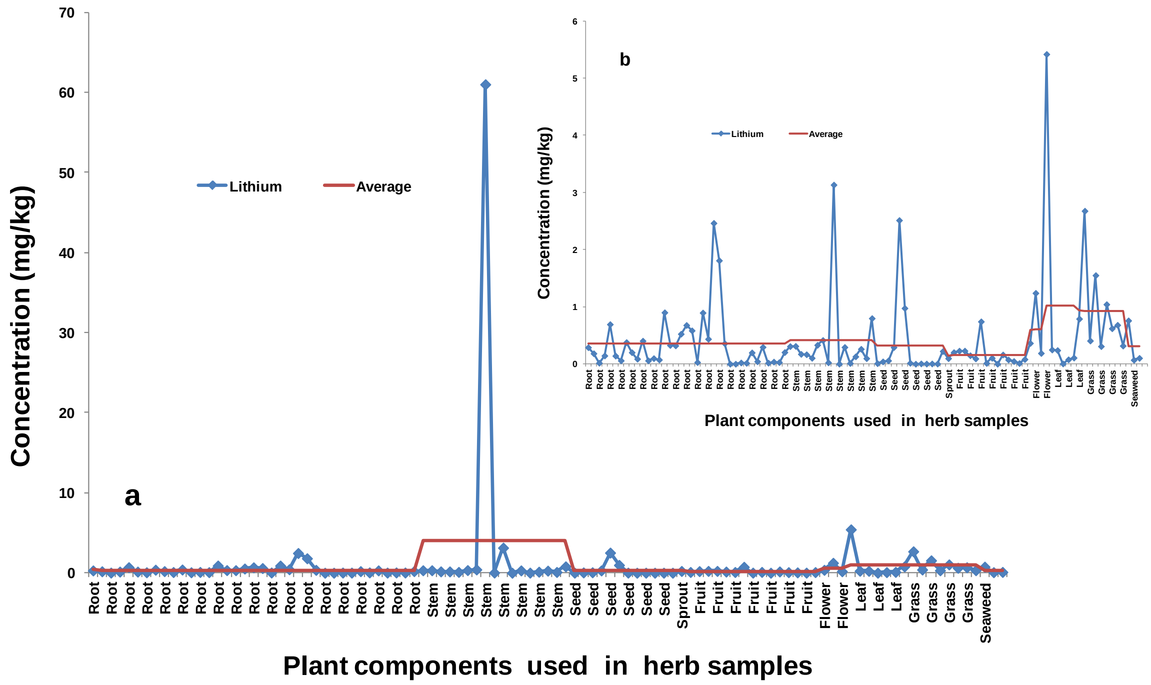


Figure 4.12.3: Variation of total concentration of Li with different plant components used in herb samples: **(a)** with *Wu Jia Pi* (herb number 61 in Figure 4.12.1); **(b)** without *Wu Jia Pi*.

4.13 Arsenic, As

4.13.1 The Role of As in Human Health

As is one of the most toxic of the elements [179]. However, the toxicity of As varies dramatically with its speciation. For example, As(III) species are generally considered to be more toxic than As(V) species [180]. Some As containing compounds such as arsenocholine (AsC) and arsenobetaine (AsB) are innoxious, and certain species such as monomethylarsonic acid (MMA), dimethyarsinic acid (DMA) and trimethylarsenic oxide (TMAO) are also reported to have a low toxicity [181]. Despite its potential toxicity, As is also considered an essential element [22, 179, 182], e.g. a level of 0.00001 % is needed for growth and for a healthy nervous system [182]. However, exposure to significant level of inorganic As can cause various health effects, such as irritation of the stomach and intestines, decreased production of red and white blood cells, skin changes and lung irritation, even increasing the chances of cancer development (e.g. skin cancer, lung cancer and liver cancer) [179]. Safe exposure level of inorganic As was set at 15 µg by Food and Agricultural Organisation (FAO)/World Health Organisation (WHO) in terms of Provisional Tolerable Weekly Intake (PTWI) values for kg body weight [183-185]. Exposure to high doses of organic As may also cause certain effects to human health such as nerve injury and stomach aches [179]. As-containing compounds had been widely used as pesticides, herbicides and soil sterilants in the past, so some agricultural soils could have fairly high level of As [113]. As is fairly easily absorbed by plants [179]. Therefore, from the clinical point of view, it is necessary to evaluate the safety of Chinese herbs by the determination of the concentration of As.

4.13.2 The As Landscape for the Herb Collection

General Comments

The current work analysed the total and bioavailable contents of As in triplicate (Appendix B) across the 103 Chinese medicinal herbs and also investigated the enrichment ability of the herbs for As. The data obtained from concentration measurements are presented in Figure 4.13.1. The results from the enrichment factor analysis are shown in Figure 4.13.2. The concentration distribution with respect to plant part is depicted in Figure 4.13.3.

It may be seen from Figure 4.13.1 that all of the herb samples analysed contained detectable levels of As, except *Fu Ling* (herb number 3), *Da Zhao* (herb number 10) and *Lou Han Guo* (herb number 93). The detected total extractable content of As ranged from 0.00139 mg/kg in *Xin Ren* (herb number 71) to 4.27 mg/kg in *Hong Hua* (herb number 33), on a dry weight basis, except unwashed *Hai Zao* (herb number 62, not presented in Figure 4.13.1). The unwashed *Hai Zao* sample (seaweed) was found to have the highest total extractable content of As (160 mg/kg) and this As content is almost fully bioavailable (bioavailability > 80 %). However, the As level in the *Hai Zao* sample was significantly reduced (around 70 % reduction) after the sample was washed by tap water. It is known that seaweed generally contains higher concentration of As than terrestrial plants as marine plants have the ability to concentrate As from seawater [186]. It was also reported [180] that the total concentration of As in seaweeds can be reduced by soaking them in water, but the level of inorganic As still remained significantly high. Therefore, caution should be exercised with respect to the consumption of seaweed-based herbal medicine.

Figure 4.13.1 also shows that *Hong Hua* (herb number 33) and *Yin Chen* (herb number 53) have similar levels in the total extractable content of As, but their bioavailability for As was significantly different. *Yin Chen* achieved 98 % bioavailability of As, whereas *Hong Hua* only managed 19 % of its As content to be bioavailable. The bioavailable content of As in *Hong Hua* was estimated to be around 0.8 mg/kg which is just under the limit of 1 mg/kg as recommended by World Health Organisation (WHO) for medicinal plants [187] thus it is barely safe for consumption. Although 7 herb samples, including *Hai Zao*, had bioavailable contents of As exceeded the limit of 1 mg/kg as recommended by WHO for medicinal plants [187], this does not necessarily indicate that these would be toxic because the toxicity of As varies dramatically with its speciation as mentioned earlier in this section.

Enrichment Factor Analysis

The enrichment factor analysis showed that around half of the herbs tested demonstrated high enrichment ability for As (EF > 10) (I and II in Figure 4.13.2, note: *Hai Zao* is not included in the figure). The herb samples presented in part I in Figure

4.13.2 had As concentrations above the median value of 0.190 mg/kg, which is attributed not only to an enhanced enrichment ability for As, but also a high availability of As in the environment where they grown. The herbs in part II of Figure 4.13.2 also had an enhanced enrichment ability for As but showed a total extractable content of As less the median value 0.190 mg/kg, suggesting that these herbs have a low environmental availability of As. Those herbs located in part III of Figure 4.13.2 showed a concentration of As less than the median value of 0.190 mg/kg, possibly due to a low enrichment ability for As (EF < 10) and/or poorly availability of As in the environment. The herb samples having an EF < 10 but a As concentration > 0.190 mg/kg (median value) (IV in Fig. 4.13.2) may originate from an As-rich environment.

Plant Part Analysis

The concentration distribution of As varied with respect to plant part (Figure 4.13.3, *Hai Zao* is not known in the figure). The flower, grass and leaf-derived herb samples were found to have significantly higher level of As compared to other plant components used in herbs.

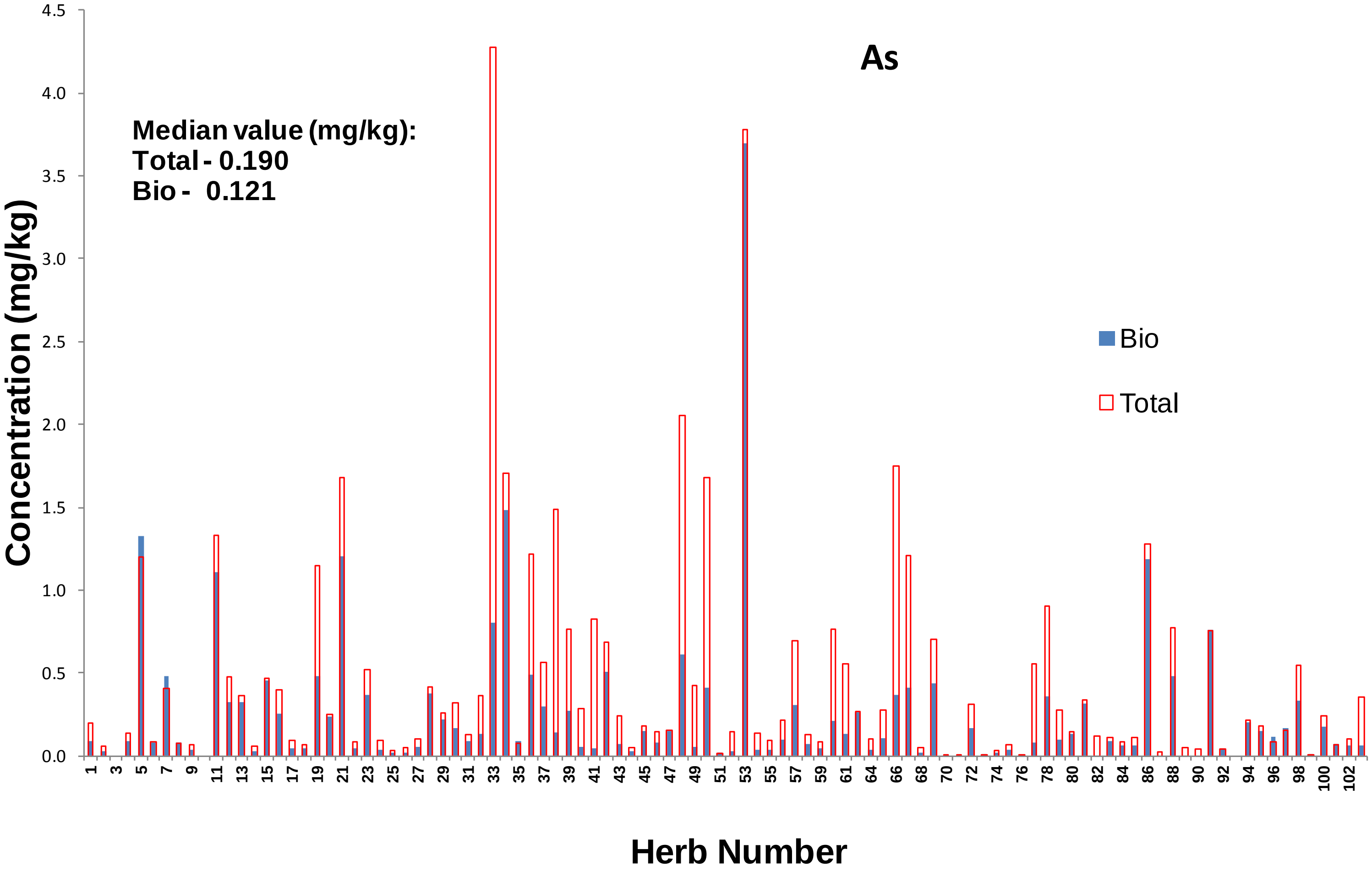


Figure 4.13.1: Total and bioavailable concentration of As in herb samples.

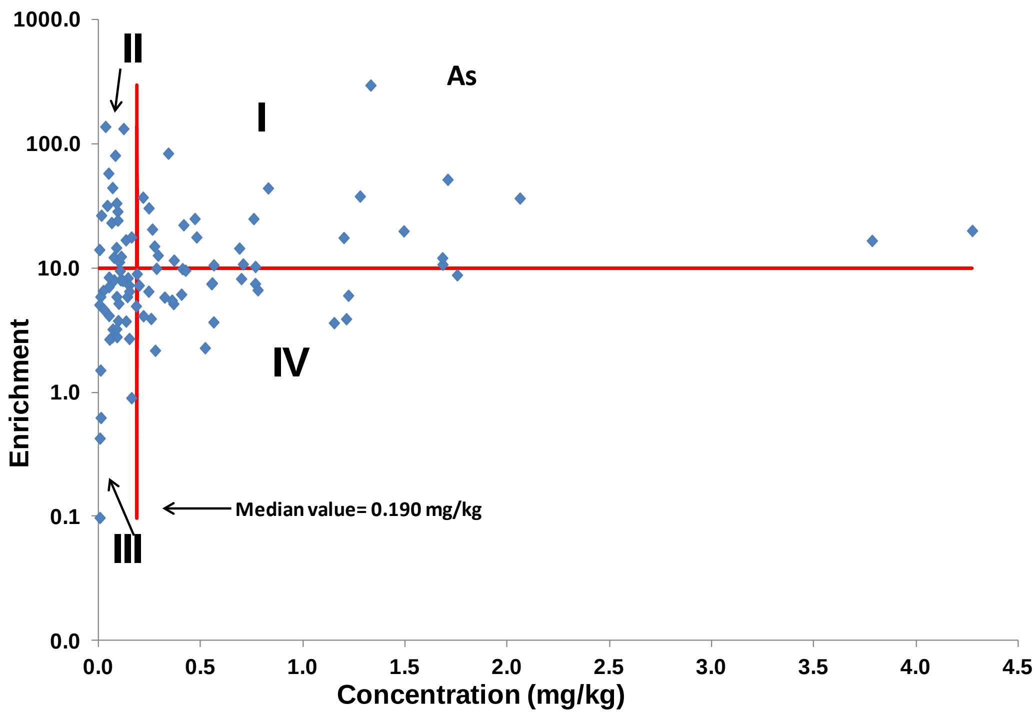


Figure 4.13.2: Enrichment Factor analysis of herb samples for As.

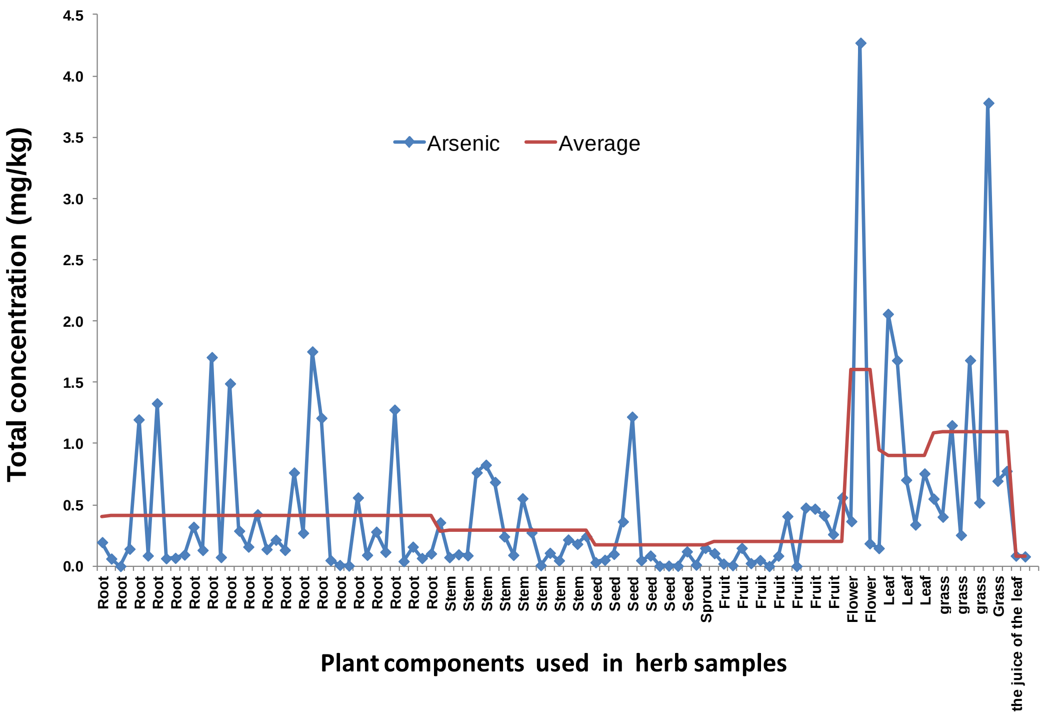


Figure 4.13.3: Variation of total concentration of As with different plant components used in herb samples.

4.14 Lead, Pb

4.14.1 The Role of Pb in Human Health

Pb was regarded as quite safe to use for most applications in earlier days. Since the middle of the 20th century, the health effects of Pb have become much better understood [188]. Now it is well known that Pb is a highly poisonous metal to animals as well as for human beings, and causes both immediate and long-term health problems at certain exposure levels [113, 188], such as damaging the nervous system and causing brain disorders [189]. Safe exposure level of Pb was set at 25 µg by the FAO/WHO in terms of PTWI values for kg body weight [183-185].

Pb is also a major pollutant [113]. Pb exposure is a global issue as Pb mining and Pb smelting are common in many countries [189]. Soil is contaminated through particulate accumulation from Pb in pipes, Pb paint and residual emissions from leaded gasoline [113]. Pb can be ingested through the food chain, for example, from crop plants and fruits and vegetables contaminated by high levels of Pb in the soils where they were grown [113, 189]. Therefore, from the clinical point of view, it is necessary to evaluate the safety of Chinese herbs by the determination of the concentration of Pb.

4.14.2 The Pb Landscape for the Herb Collection

<u>*General comments*</u>

In this study, the total and bioavailable levels of Pb were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.14.1. The enrichment ability of the herbs for Pb was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.14.2 and the concentration distribution with respect to plant part is depicted in Figure 4.14.3.

The tested herb materials showed a high level of variability (up to 8400-fold) in the total extractable content of Pb, ranging from 0.00268 mg/kg in *Xin Ren* (herb number 71 in Figure 4.14.1) to 22.5 mg/kg in *Hong Hua* (herb number 33 in Figure 4.14.1).

The median value of the total extractable level of Pb was 0.441 mg/kg as shown in Figure 4.14.2. There were also some differences in the bioavailability of Pb between different herb materials. The bioavailable content of Pb in *Xin Ren* was below the minimum detection limit. While all the other herb samples showed bioavailability of Pb between 2 % to 100 %, three (*Huang Lian*, *Hai Zao* and *Chuan Niu Xi*, herb number 41, 62 and 79 in Figure 4.14.1, respectively) were almost non-bioavailable, with the bioavailability being less than 10 %. A similar level of total content of Pb was observed in *Jin Yi Hua* (13.7 mg/kg, herb number 13 in Figure 4.14.1) and *Chuan Niu Xi* (10.1 mg/kg, herb number 79 in Figure 4.14.1) but there were significant differences in bioavailability (95 % for *Jin Yi Hua* and 9 % for *Chuan Niu Xi*). These results are very interesting and further studies on the chemical and biological implications for these two herb samples are needed. It can also be seen from Figure 4.14.1 that *Hong Hua* (herb number 33), *Jin Yi Hua* (herb number 13) and *Chuan Niu Xi* (herb number 79) showed a relatively high level in the total extractable Pb content, but only *Jin Yi Hua* had a bioavailable Pb content of more than 10 mg/kg. The limit of Pb content in the final dosage form of the medicinal plant material is prescribed by WHO to be 10 mg/kg [187]. Both total (13.7 mg/kg) and bioavailable (13.0 mg/kg) levels measured in *Jin Yin Hua* exceeded this limit and therefore this herb needs to be carefully evaluated for safety prior to consumption.

<u>*Enrichment Factor Analysis*</u>

The enrichment factor analysis results (Figure 4.14.2) showed that about half of herbs (I and II in Figure 4.14.2) tested demonstrated high enrichment ability for Pb (EF > 10). The herbs in part I of Figure 4.14.2 had Pb concentrations above the median value of 0.441 mg/kg, which is attributed not only to an enhanced enrichment ability for Pb, but also a high availability of Pb in the environment where they grown. The herbs located in part II of Figure 4.14.2 also had enhanced enrichment ability for Pb but showed a total extractable content of Pb less the median value of 0.441 mg/kg, suggesting that these herbs have a low environmental availability of Pb. The herbs presented in part III of Figure 4.14.2 showed a concentration of Pb less than the median value of 0.441 mg/kg, possibly due to low enrichment ability for Pb (EF < 10) and/or poorly availability of Pb in the environment. The herb samples shown in IV of

Fig. 4.14.2 had an EF < 10 but a Pb concentration > 0.441 mg/kg (median value), suggesting that they may originate from a Pb-rich environment.

Plant Part Analysis

The concentration distribution of Pb varied with respect to plant part (Figure 4.14.3). The flower and leaf-derived samples were found to have significantly higher levels of Pb. This might derive mainly from atmospheric pollution, since plants are reported to have a relatively low Pb uptake capacity via their roots [190].

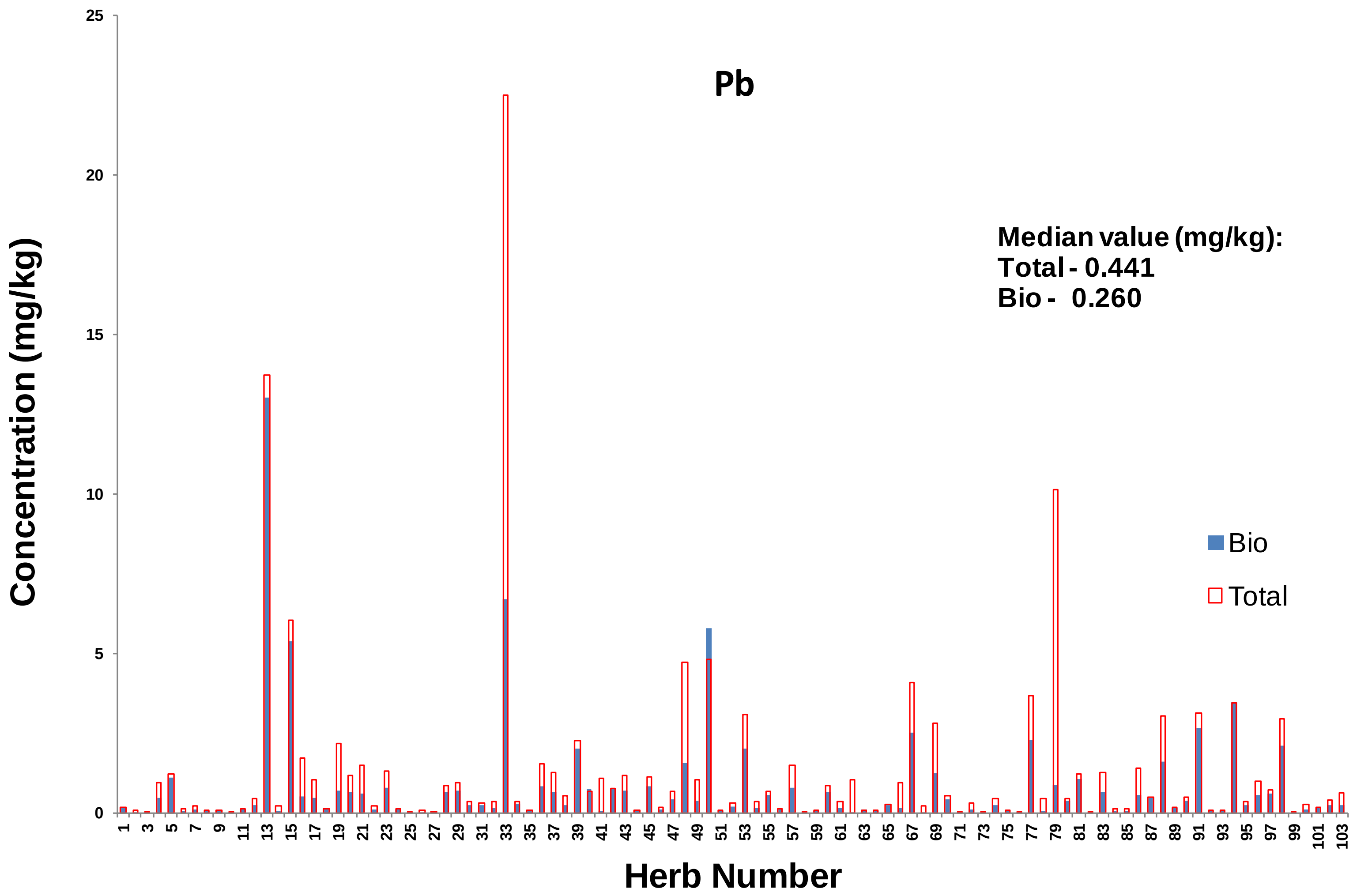


Figure 4.14.1: Total and bioavailable concentration of Pb in herb samples.

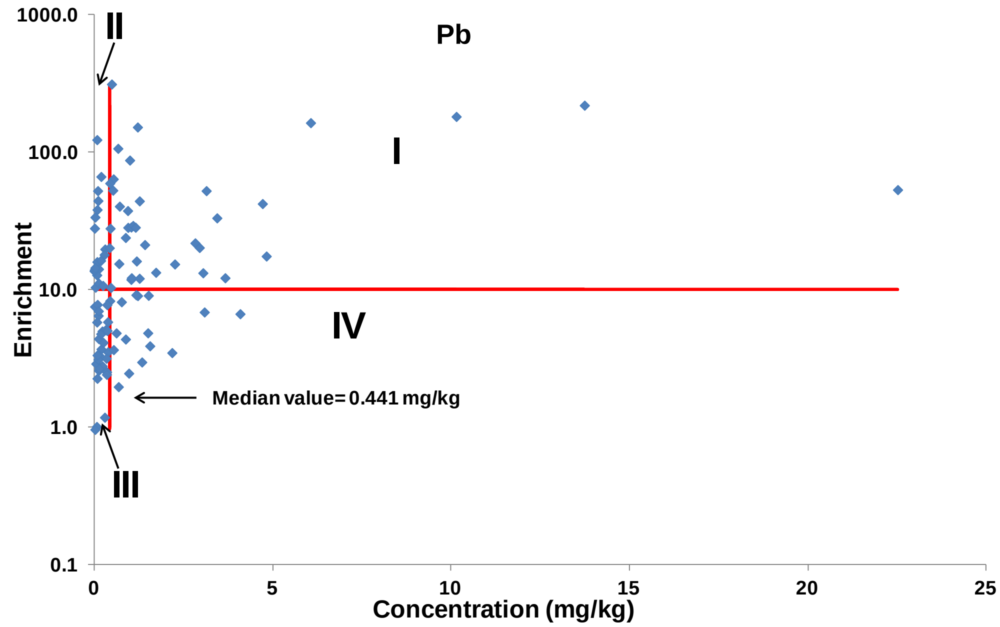


Figure 4.14.2: Enrichment Factor analysis of herb samples for Pb.

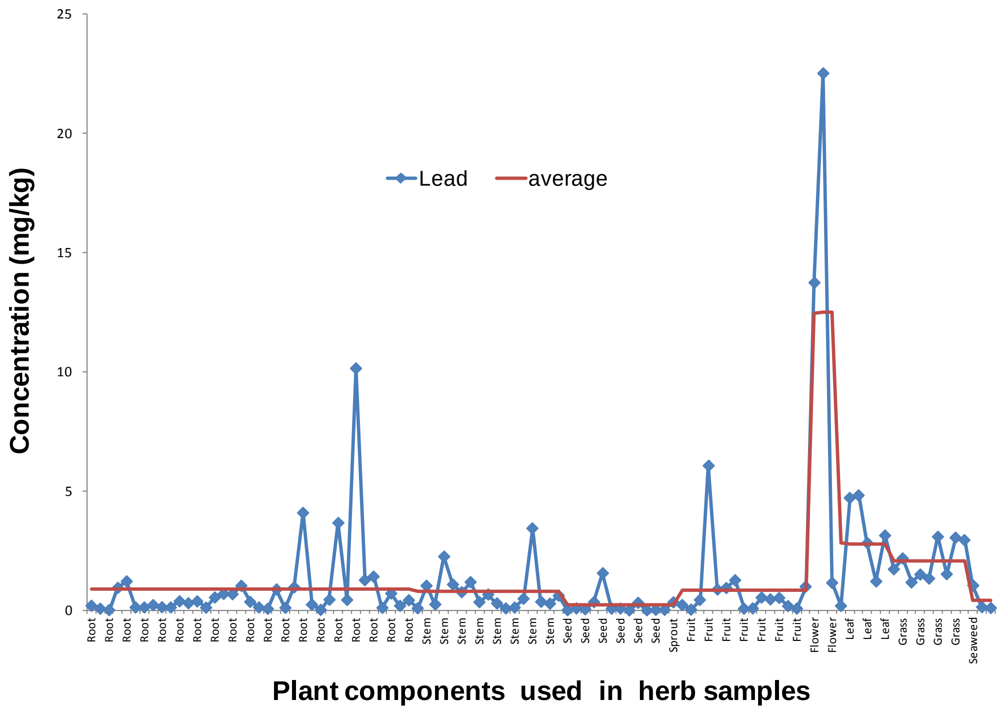


Figure 4.14.3: Variation of total concentration of Pb with different plant components used in herb samples.

4.15 Cadmium, Cd

4.15.1 The Role of Cd in Human Health

Cd is a non-essential heavy metal that can be toxic to the human bio-system even at very low levels of intake. For example, Cd exposure is a risk factor associated with early atheroslcerosis and hypertension, which can both lead to cardiovascular disease [191]. The safe exposure level of Cd has been set at 7 μg/kg of body weight by FAO/WHO in terms of PTWI values [183-185]. Cd is also an environmental hazard due to its presence in waste products such as sewage sludge and landfill [113]. Human activities have a significant impact on the Cd content of surface soils, and the increase in soil Cd content can be reflected in a similar increase in crop plant tissues [113].

4.15.2 The Cd Landscape for the Herb Collection

General Comments

In the current work, the total and bioavailable levels of Cd across the 103 medicinal herbs were measured in triplicate (Appendix B) and the results are presented in Figure 4.15.1. The enrichment ability of the herbs for Cd was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.15.2 and the concentration distribution with respect to plant part is depicted in Figure 4.15.3.

It was found from Figure 4.15.1 that the herbs tested had a median value of the total extractable Cd content of 0.0476 mg/kg. The overall bioavailability of Cd was extremely high. The Cd content was essentially fully bioavailable in all cases except *Da Zhao* (herb number 10) which had both total extractable and bioavailable contents of Cd being below the minimum detection limit. It should be noted that WHO has prescribed, for various medicinal plants, a limit of no more than 0.3 mg/kg for Cd in the ‘final dosage form’ of the plant material [187]. In this study, around 10 % of the herb samples were measured to have both total extractable and bioavailable contents of Cd above this limit, therefore it is advised that these herbs needs to be carefully evaluated for safety prior to consumption. It was also noticed that *Yin Chen* (herb number 53) had the highest Cd concentration (1.34 mg/kg) - and this Cd content is fully bioavailabable, within experimental error. This herb might be used as soil decontamination candidate.

Enrichment Factor Anaylsis

The herbs demonstrated a high enrichment ability for Cd, with 99 out of 103 herbal samples tested having an EF > 10 (I and II in Figure 4.15.2). Those shown in part I of Figure 4.15.2 had Cd concentrations above the median value of 0.0476 mg/kg, possibly due to their enhanced enrichment ability for Cd and/or a high availability of Cd in the environment where they grown. The herbs in part II of Figure 4.15.2 also had an enhanced enrichment ability for Cd but showed a total extractable content of Cd less the median value 0.0476 mg/kg, suggesting that these herbs have a low environmental availability of Cd. The herbs located in part III of Figure 4.15.2 showed a concentration of Cd less than the median value of 0.0476 mg/kg, which may be attributed to low enrichment ability for Cd (EF < 10) and/or poor availability of Cd in the environment. It is interesting to note that no herb samples were found in part IV in Fig. 4.15.2 which is designed to accommodate the herbs having an EF < 10 but a Cd concentration > 0.0476 mg/kg (median value).

Plant Part Analysis

The concentration distribution of Cd varied with respect to plant part (Figure 4.15.3). The grass herb samples showed a generally high level of Cd. While the seed-based and flower samples had a relatively low content of Cd. It has been reported [113] that Cd is not translocated to the developing seed in some seed crops even when they are exposed to high levels of available Cd in the rooting medium.

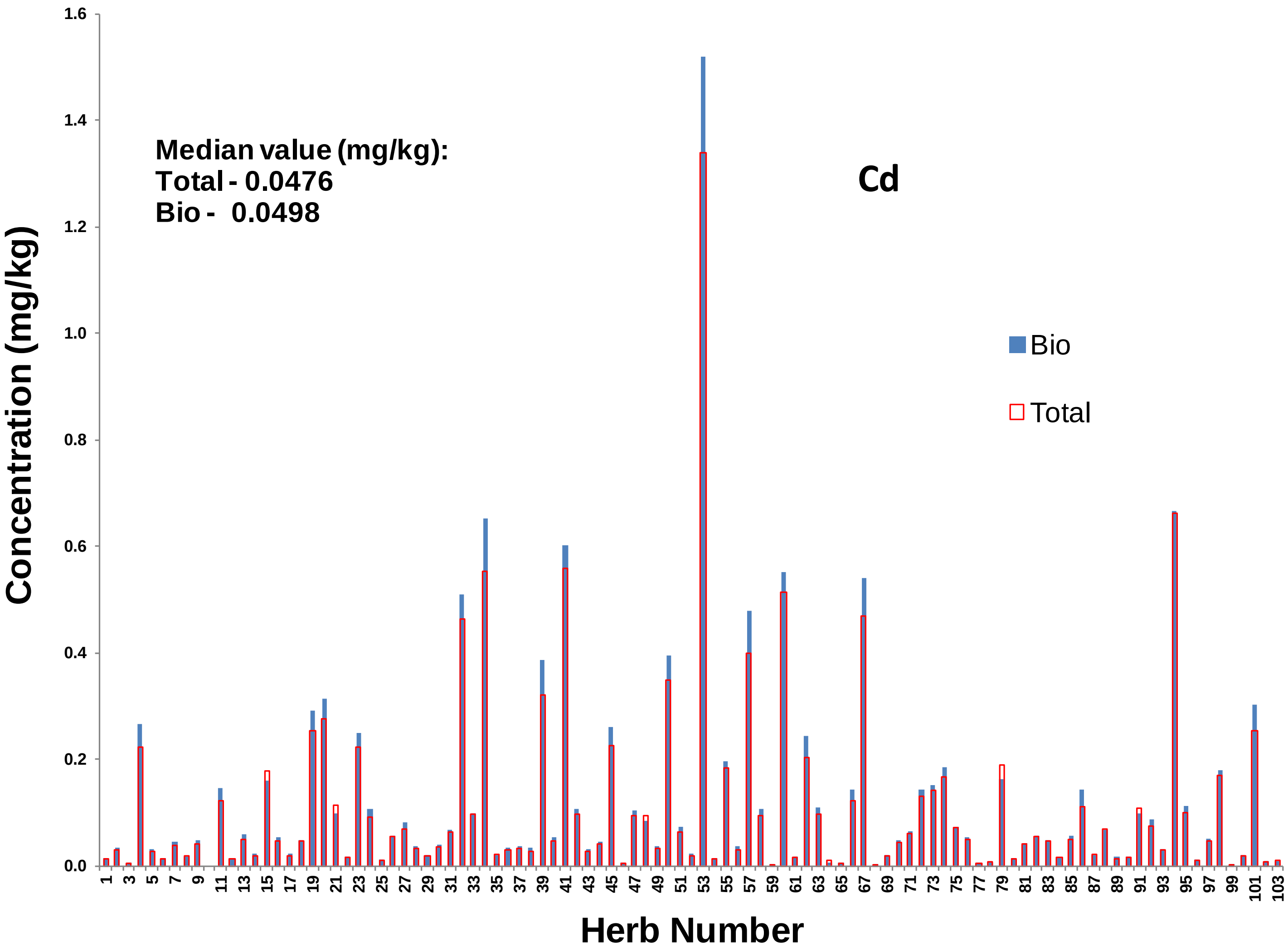


Figure 4.15.1: Total and bioavailable concentration of Cd in herb samples.

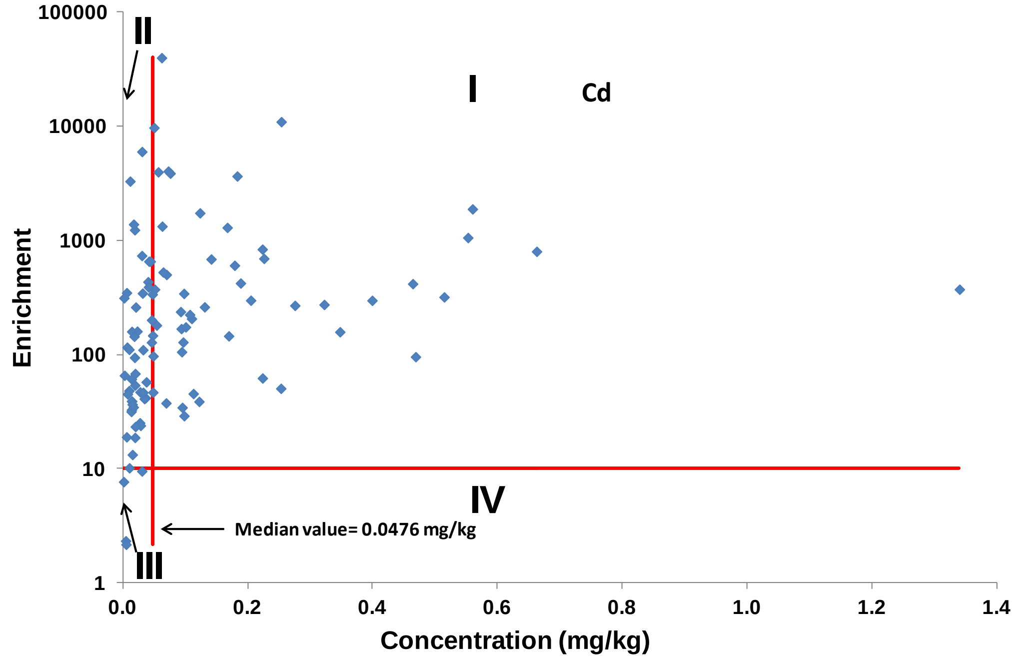


Figure 4.15.2: Enrichment Factor analysis of herb samples for Cd.

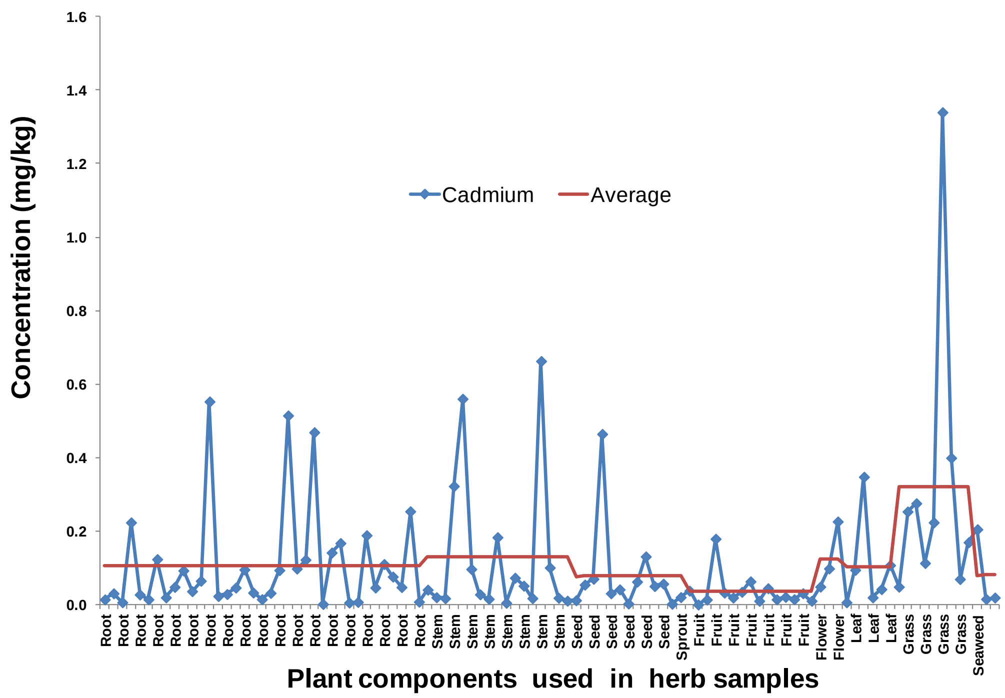


Figure 4.15.3: Variation of total concentration of Cd with different plant components used in herb samples.

4.16 Mercury, Hg

4.16.1 The Role of Hg in Human Health

Hg is a heavy metal that can occur in several forms. Hg and most of its compounds are extremely toxic. It can be toxic to the human bio-system even at very low levels of intake. Hg poisoning can cause damage to the brain, kidney, and lungs [192] and result in several diseases including acrodynia (pink disease) [193], Hunter-Russell syndrome and Minamata disease [194]. WHO has set an upper limit of 1 μg/L for Hg in drinking water and a PTWI value for a 60 kg man is set at 43 μg by FAO/WHO [113]. Since Hg can be easily taken up by plants [113], Chinese herbs that are grown in a Hg-rich or Hg-contaminated environment may contain Hg at dangerously high levels. Therefore, from the clinical point of view, it is necessary to evaluate the safety of herbal medicines by the determination of the level of Hg.

4.16.2 The Hg Landscape for the Herb Collection

General comments

In this study, the total and bioavailable levels of Hg (Figure 4.16.1) were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. The total extractable content of the toxic heavy metal Hg in approximately half of the investigated herb samples was too low to be detected by the methods used in this study. The detected levels of Hg in herbal samples were also very low, with the highest value being 0.0958 mg/kg in *Ma Chi Xian* (herb number 21 in Figure 4.16.1). The median value of the total extractable level of Hg was 0.0116 mg/kg. This suggested that the herb samples tested in this study might not originate from a Hg-rich or Hg-contaminated environment. The data obtained for the bioavailable content of Hg were not considered reliable since the simulated stomach acid digestion method used in the study only achieved 10.5 % recovery for Hg (Table 3.5 in Chapter 3). However, this does not necessarily guarantee the non-existence of bioavailable Hg in these studied herb materials.

Enrichment Factor Analysis

The enrichment ability of the herbs for Hg was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.16.2. It was found that most of the herbs with detectable Hg had high enrichment ability for Hg (EF > 10) (I and II in Figure 4.16.2). Around half of them (I of Figure 4.16.2) showed Hg concentrations above the median value of 0.0116 mg/kg, which might be attributed not only to an enhanced enrichment ability for Hg, but also a relatively high availability of Hg in the environment where they grown. The herbs located in part II of Figure 4.16.2 also had enhanced enrichment ability for Hg but showed a total extractable content of Hg less the median value of 0.0116 mg/kg, suggesting that these herbs have a low environmental availability of Hg. The herbs presented in part III of Figure 4.16.2 showed a concentration of Hg less than the median value of 0.0116 mg/kg, possibly due to their low enrichment ability for Hg (EF < 10) and/or poorly availability of Hg in the environment. The herb samples shown in IV of Fig. 4.16.2 had an EF < 10 but an Hg concentration > 0.0116 mg/kg (median value), suggesting that they may originate from an environment with a relatively high available of Hg.

Plant Part Analysis

Figure 4.16.3 shows the concentration distribution with respect to plant part. The concentration distribution of Hg varied with respect to plant part. The grass, flower and leaf-derived samples were found to have relatively higher levels of Hg.

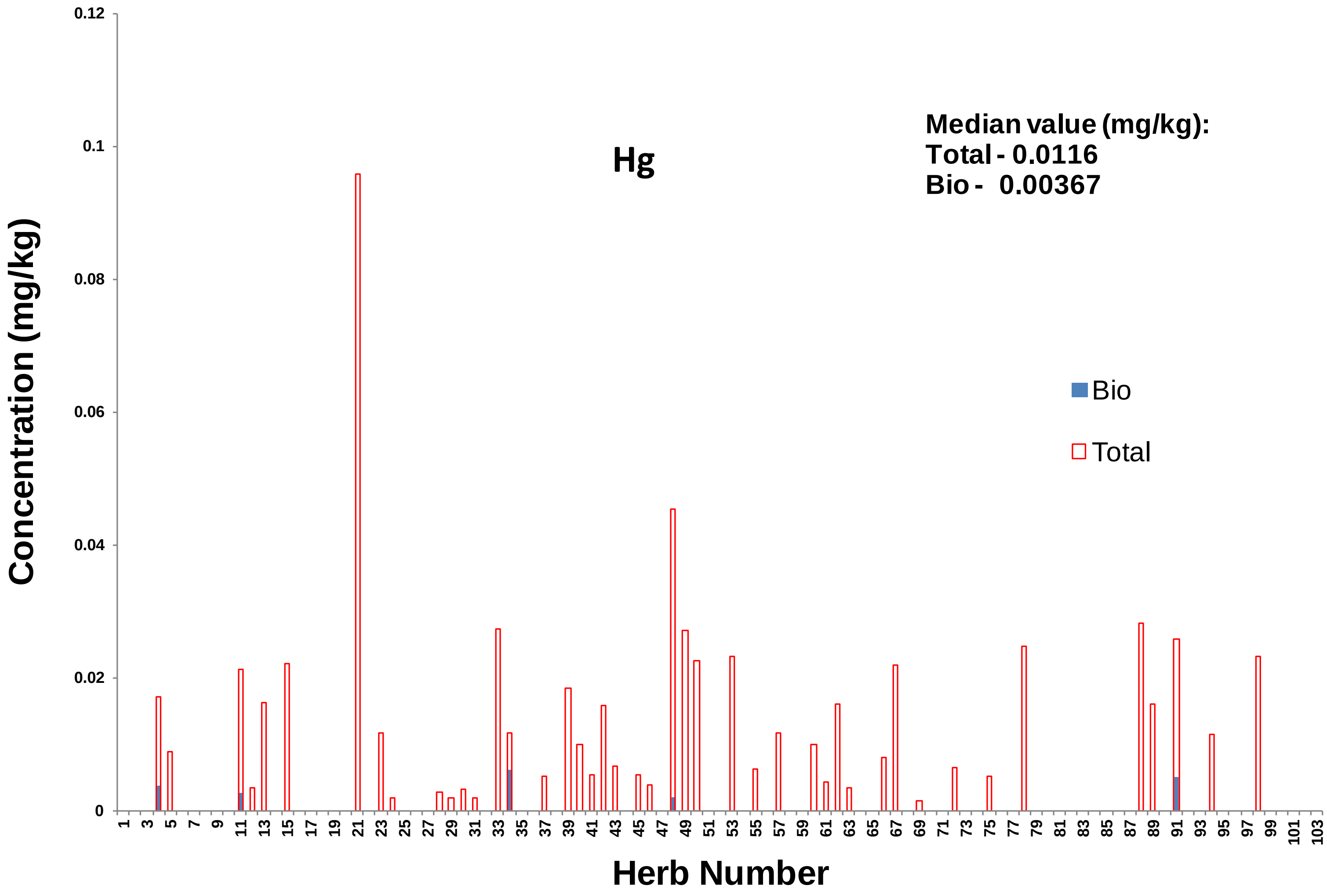


Figure 4.16.1: Total and bioavailable concentration of Hg in herb samples.

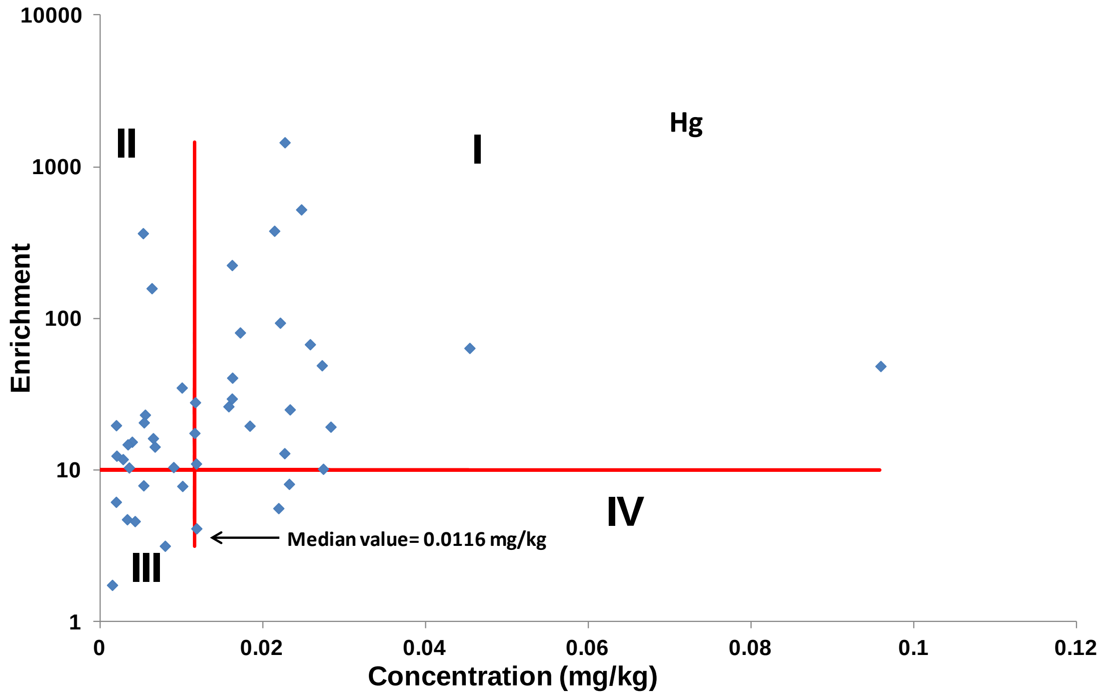


Figure 4.16.2: Enrichment Factor analysis of herb samples for Hg.

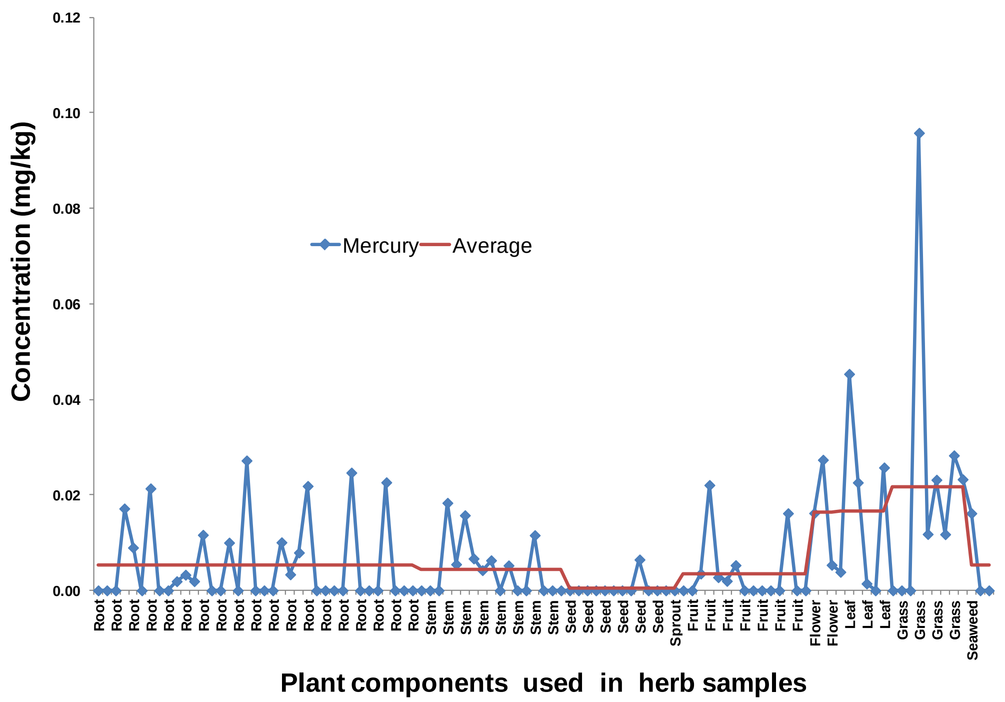


Figure 4.16.3: Variation of total concentration of Hg with different plant components used in herb samples.

4.17 Tin, Sn

4.17.1 The Role of Sn in Human Health

Sn is known to be an essential trace element for some animals [22, 195]. For example, rats need 1 – 2 mg Sn per kg bodyweight for their normal growth and development [22]. Rats with Sn deficiency showed poor growth, reduced feeding efficiency, hearing loss and bilateral (male pattern) hair loss [22, 195]. However, the role of Sn in human health and nutrition is not yet established [195]. It was proposed that Sn supports the adrenal glands, with both Sn and iodine subsequently affecting cardiac output: i.e Sn + adrenals control the left side of the cardiac output, and iodine + thyroid control the right side of the cardiac output [196]. Sn-deficiency is a common nutritional cause of low adrenals, which can lead to left-sided cardiac insufficiency [195]. Although inorganic Sn compounds are not easily absorbed by the digestive system, organic Sn compounds could present toxicity problems [22, 113].

4.17.2 The Sn Landscape for the Herb Collection

General Comments

In this study, the total and bioavailable levels of Sn were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.17.1. The enrichment ability of the herbs for Sn was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.17.2 and the concentration distribution with respect to plant part is depicted in Figure 4.17.3.

It was found from Figure 4.17.1 that the studied herb samples presented a generally low level of Sn for both total extractable and bioavailable contents when compared to other trace elements (Li, V and Ni) in the same group. Sn concentrations for around half of the herb samples were below the minimum detection limit. The detected total extractable content of Sn ranged from 0.00125 mg/kg in *Bai Zhi* (herb number 74 in Figure 4.17.1) to 1.57 mg/kg in *Chuan Niu Xi* (herb number 79 in Figure 4.17.1), on a dry weight basis. The median value of the total extractable level of Sn was 0.0664 mg/kg as shown in Figure 4.17.1. The overall bioavailability of Sn was also generally low. None of the herbs tested achieved a more than 80 % bioavailability of Sn except *Lu Hui* (99 %, herb number 84 in Figure 4.17.1).

Enrichment Factor Analysis

The enrichment ability of the herbs for Sn was also relatively low. Only one third of the herbs with detectable Sn achieved an EF > 10 (I and II in Figure 4.17.2). The herbs in part I of Figure 4.17.2 had Sn concentrations above the median value of 0.0664 mg/kg, which is attributed to their enhanced enrichment ability for Sn and/or a high availability of Sn in the environment where they grown. The herbs presented in part II of Figure 4.17.2 also had an enhanced enrichment ability for Sn but showed a total extractable content of Sn less the median value 0.0664 mg/kg, suggesting that these herbs have a low environmental availability of Sn. The herbs located in part III of Figure 4.17.2 showed a concentration of Sn less than the median value of 0.0664 mg/kg, possibly due to low enrichment ability for Sn (EF < 10) and/or poorly availability of Sn in the environment. Only 4 herb samples showed an EF < 10 but a Sn concentration > 0.0664 mg/kg (median value) (IV in Fig. 4.17.2), suggesting that they may originate from a relatively Sn-rich environment.

Plant Part Analysis

The concentration distribution of Sn varied with respect to plant part (Figure 4.17.3). The stem and flower herb samples showed a relatively low level of Sn.

4.17.3 Suggested Leads from Relating Sn Content to Medicinal Properties

From the data presented in Fig. 4.17.1, *Chuan Niu Xi* (herb number 79 in Figure 4.17.1) had the highest Sn concentration (1.57 mg/kg) but this Sn content is not readily bioavailable (bioavailability 15 %), while *Lu Hui* and *Ji Xu Teng* (herb number 84 and 83 in Figure 4.17.1, respectively) demonstrated relatively high bioavailabilities. *Chuan Niu Xi* and *Ji Xu Teng* are commonly used in TCM to invigorate the blood and reduce blood stagnation, thereby treating symptoms associated with blood stagnation, especially sharp, fixed pain of any kind [30]. In western terms these herbs address a range of different gynaecological, neurological and traumatic disorders [30]. *Lu Hui* is traditionally used to eliminate pathogenic accumulations of food matter and heat from the intestines by promoting bowel movement which is associated with a main symptoms of constipation and abdominal distension [30]. Therefore these herbs are candidates for possible Sn species that might have some therapeutic value.

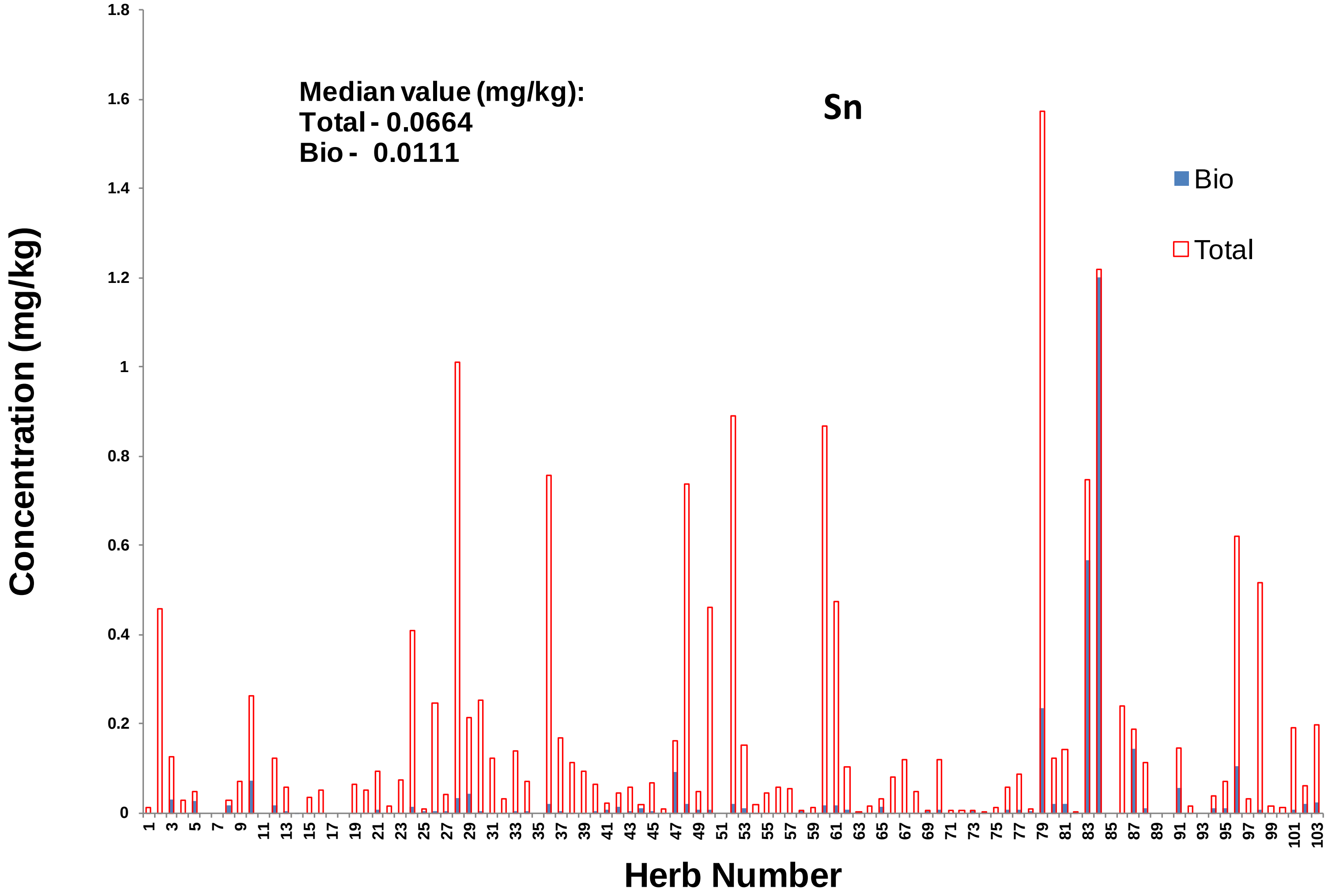


Figure 4.17.1: Total and bioavailable concentration of Sn in herb samples.

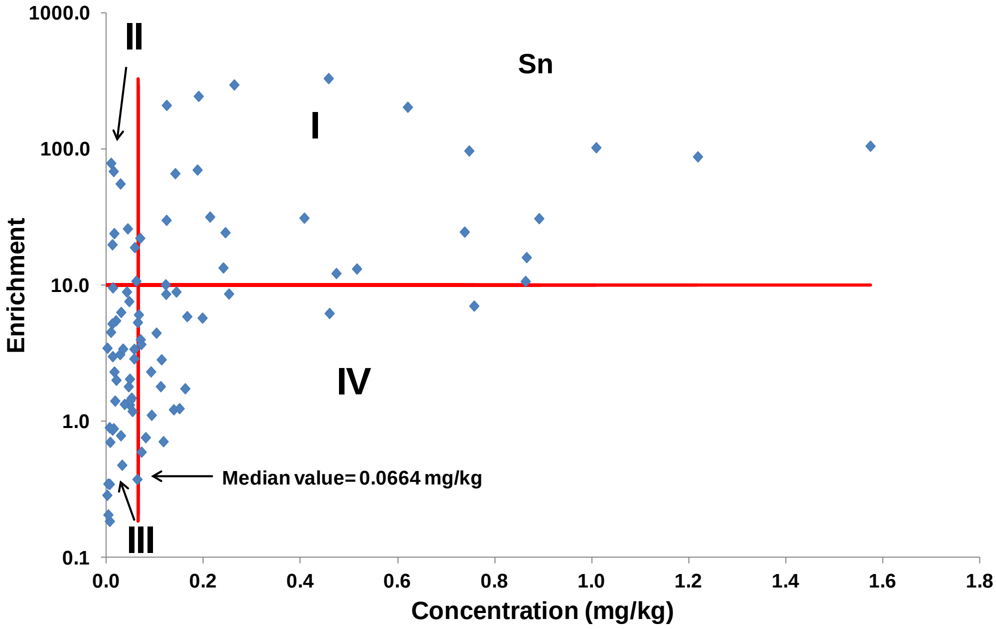


Figure 4.17.2: Enrichment Factor analysis of herb samples for Sn.

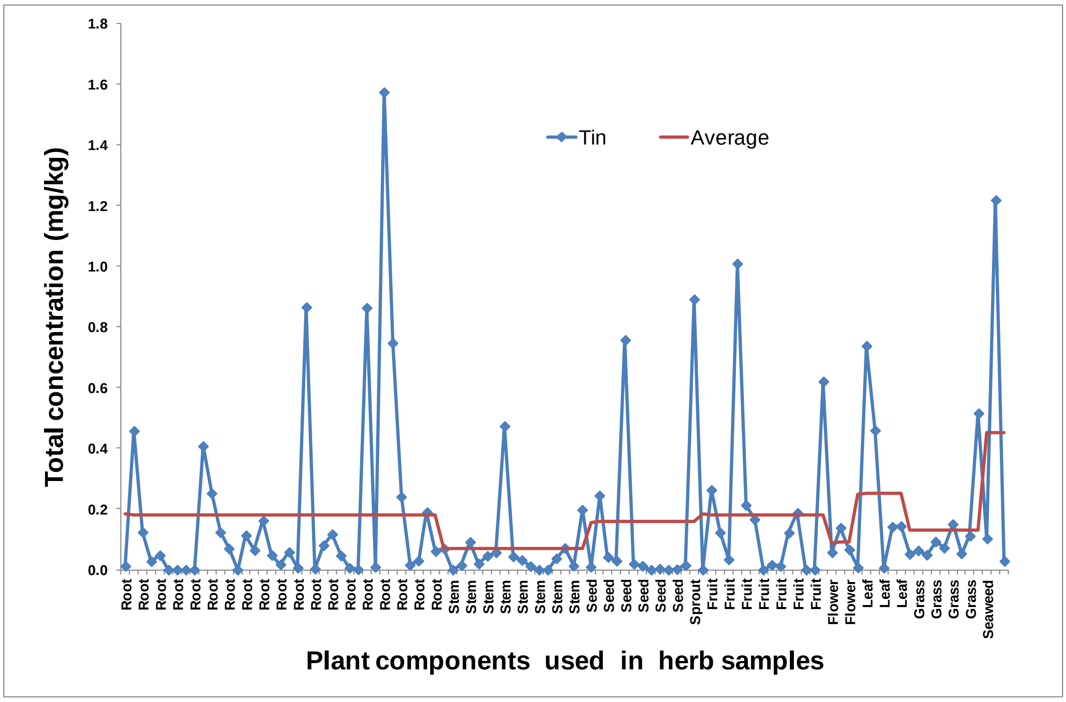


Figure 4.17.3: Variation of total concentration of Sn with different plant components used in herb samples.

4.18 Antimony, Sb

4.18.1 The Role of Sb in Human Health

Sb and many of its compounds are toxic [197], and Sb(V) forms are more toxic than Sb(III) forms [113]. The effects of Sb poisoning are similar to As poisoning, but the toxicity of Sb is much lower than that of As [197]. Toxic intake of Sb for animals/man is 100 mg [113].

4.18.2 The Sb Landscape for the Herb Collection

General Comments

In this study, the total and bioavailable levels of Sb were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.18.1. The enrichment ability of the herbs for Sb was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.18.2 and the concentration distribution with respect to plant part is depicted in Figure 4.18.3.

It was found that 43 out of 103 herb samples studied had a total content of Sb below the minimum detection limit. The detected total extractable content of Sb was less than 0.3 mg/kg for all herbs except *Hong Hua* (1.33 mg/kg, herb number 33), on a dry weight basis. The median value of the detected total extractable level of Sb was 0.0269 mg/kg as shown in Figure 4.18.2. The overall bioavailability of Sb was also relatively low. The bioavailable content of Sb in around half of herb samples was below the minimum detection limit. The detected bioavailable content of Sb was less than 0.1 mg/kg for all herbs, with a median value of 0.0106 mg/kg. *Hong Hua* (herb number 33 in Figure 4.18.1) had the highest level of Sb (1.33 mg/kg) but showed the lowest bioavailability of Sb (3 %) among the tested herb samples. Therefore, it is perhaps less toxic.

Enrichment Factor Analysis

The herbs with detectable Sb content demonstrated generally high enrichment ability for Sb (EF > 10) (I and II in Figure 4.18.2). About half of them (I in Figure 4.18.2) had total extractable Sb concentrations above the median value of 0.0269 mg/kg, which is attributed their enhanced enrichment ability for Sb and/or a relatively high availability of Sb in the environment where they grown. The herbs in part II of Figure 4.18.2 also had an enhanced enrichment ability for Sb but showed a total extractable content of Sb less the median value 0.0269 mg/kg, suggesting that these herbs have a low environmental availability of Sb. The herbs located in part III of Figure 4.18.2 showed a concentration of Sb less than the median value of 0.0269 mg/kg, possibly due to low enrichment ability for Sb (EF < 10) and/or poorly availability of Sb in the environment. The herb samples showed an EF < 10 but a Sb concentration > 0.0269 mg/kg (median value) (IV in Fig. 4.18.2), suggesting that they may originate from a Sb-poor environment.

Plant Part Analysis

The concentration distribution of Sb varied with respect to plant part (Figure 4.18.3). The flower herb samples were found to have the highest average level of Sb.

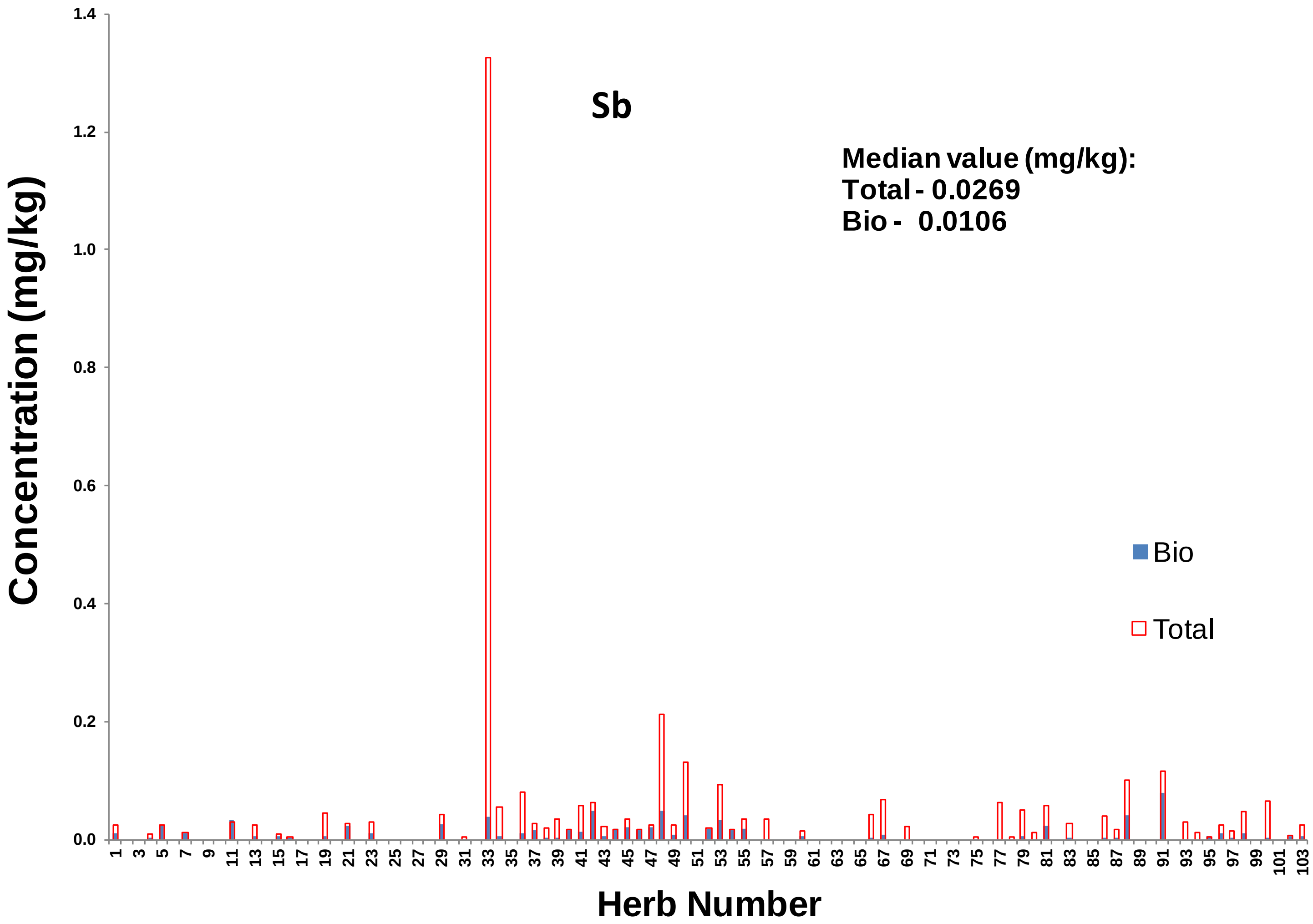


Figure 4.18.1: Total and bioavailable concentration of Sb in herb samples.

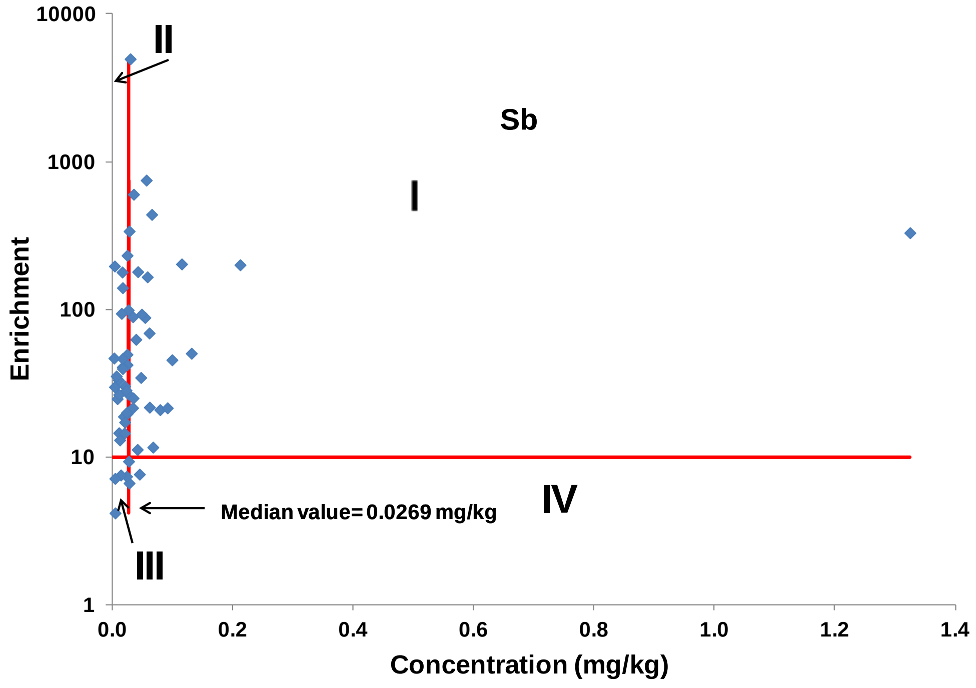


Figure 4.18.2: Enrichment Factor analysis of herb samples for Sb.

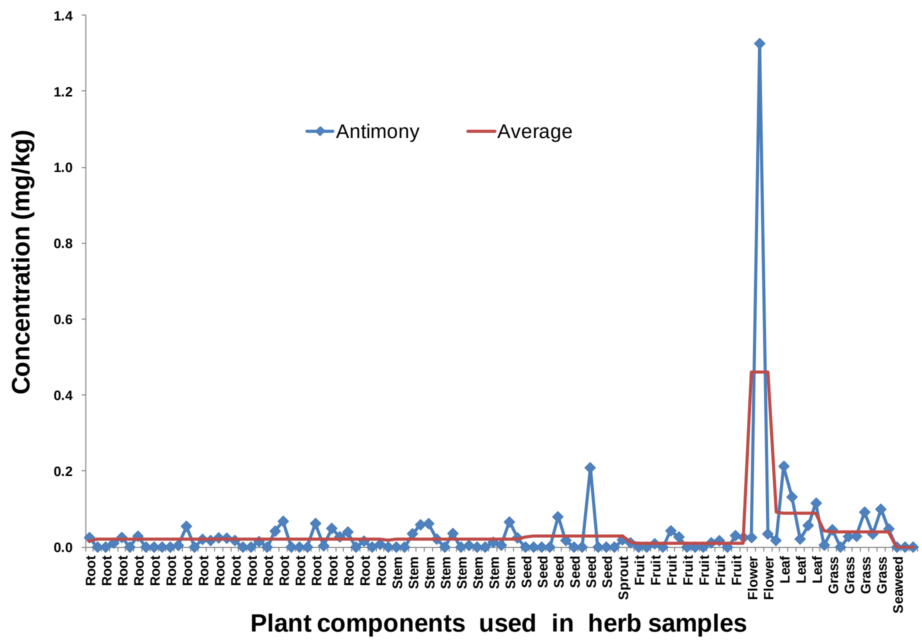


Figure 4.18.3: Variation of total concentration of Sb with different plant components used in herb samples.

4.19 Bismuth, Bi

4.19.1 The Role of Bi in Human Health

Bi is neither essential nor stimulatory in mammals [198]. Bi and its compounds are less toxic than lead and its other periodic table neighbours (e.g. Sn, Sb) [7] and it is not bio-accumulative [199]. Bi is used in the preparation of a number of pharmaceutical products [8, 198] relating to antibiotic applications. For example, Bi compounds including nitrate, salicylate salts and colloidal Bi subcitrate have a long history of use in the treatment of gastrointestinal disorders [200] and ranitidine Bi citrate was marked as a novel antiulcer drug in the 1990s [201]. Bi compounds (Bi oxychloride, BiOCl) were also used as an ingredient in cosmetics [202, 203] and as a pigment in paint [204]. However, the use of some of these substances is declining [205]. Although it is considered as one of the least toxic heavy metals, Bi poisoning does exist and mostly affects the kidney and liver [199].

4.19.2 The Bi Landscape for the Herb Collection

General Comments

In this study, the total and bioavailable levels of Bi were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.19.1. The enrichment ability of the herbs for Bi was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.19.2 and the concentration distribution with respect to plant part is depicted in Figure 4.19.3.

It is evident from Figure 4.19.1 that the herbs studied present a generally low level of Bi for both total extractable and bioavailable contents. The detected total extractable content of Bi ranged between 0.000352 mg/kg in *Da Huang* (herb number 24) and 0.365 mg/kg in *Sang Ye* (herb number 48), on a dry weight basis. The median values of total extractable and bioavailable contents Bi were 0.00799 mg/kg and 0.00281 mg/kg, respectively, as shown in Figure 4.19.1. None of herbs achieved a more than 80 % bioavailability of Bi except Long Yan Rou (84 %, herb number 7 in Figure 4.19.1).

Enrichment Factor Analysis

The herbs also demonstrated a low enrichment ability for Bi, with only around 10 % of herbal samples tested having an EF > 10 (I and II in Figure 4.19.2). The herbs located in part I of Figure 4.19.2 had Bi concentrations above the median value of 0.00799 mg/kg. Four herbs (II in Figure 4.19.2) also had an enhanced enrichment ability for Bi but showed a total extractable content of Bi less the median value of 0.00799 mg/kg, suggesting that these herbs might have a low environmental availability of Bi. The herbs presented in part III of Figure 4.19.2 showed a concentration of Bi less than the median value of 0.00799 mg/kg, possibly due to a low enrichment ability for Bi (EF < 10) and/or poorly availability of Bi in the environment. The herb samples showed an EF < 10 but a Bi concentration > 0.00799 mg/kg (median value) (IV in Fig. 4.19.2), suggesting that they may originate from a relatively Bi-rich environment.

Plant Part Analysis

The concentration distribution of Bi varied with respect to plant part, as shown in Figure 4.19.3. The root samples showed a relatively low level of Bi, which may be because the Bi cation (Bi^{3+}) is poorly absorbed in soils [113]. The leaf-derived herb samples were found to have the highest average level of Bi. Grass and flower samples were also relatively rich in Bi.

4.19.3 Suggested Leads from Relating Bi Content to Medicinal Properties

From the data presented in Fig. 4.19.1, *Sang Ye* (herb number 48) had the highest total extractable content of Bi (0.365 mg/kg). This herb was known to have pharmacological properties such as antibacterial, antidiabetic and lowering cholesterol [206] and is commonly used in TCM to treat exterior patterns, marked by such symptoms as fever, chills, possible sweating, headache and floating pulse [30]. *Dan Zhu Ye* and *Zi Su Ye* (herbs 50 and 91, respectively) also showed a relatively high level in total extractable content of Bi. *Zi Su Ye* has the same functions as *Sang Ye*, while *Dan Zhu Ye* is traditionally used in TCM to clear “internal heat” which is a condition associated with fever, inflammation and infection [30, 31]. It was also reported that *Dan Zhu Ye* and *Zi Su Ye* can help resolve stomach upheaval [207]. They might have similar functions to the Bi containing drugs used in the treatment of gastrointestinal disorders and antiulcer as mentioned above.

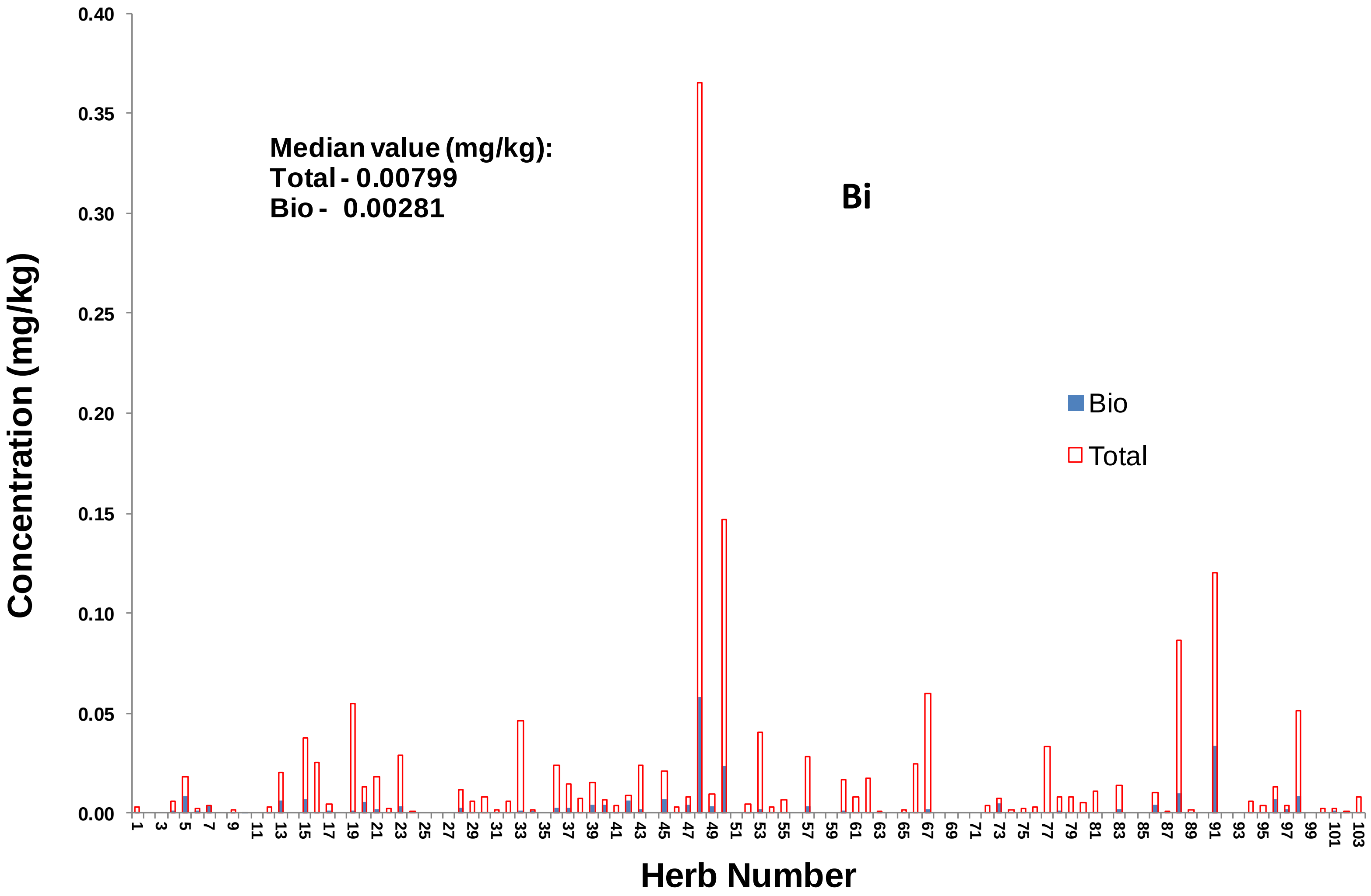


Figure 4.19.1: Total and bioavailable concentration of Bi in herb samples.

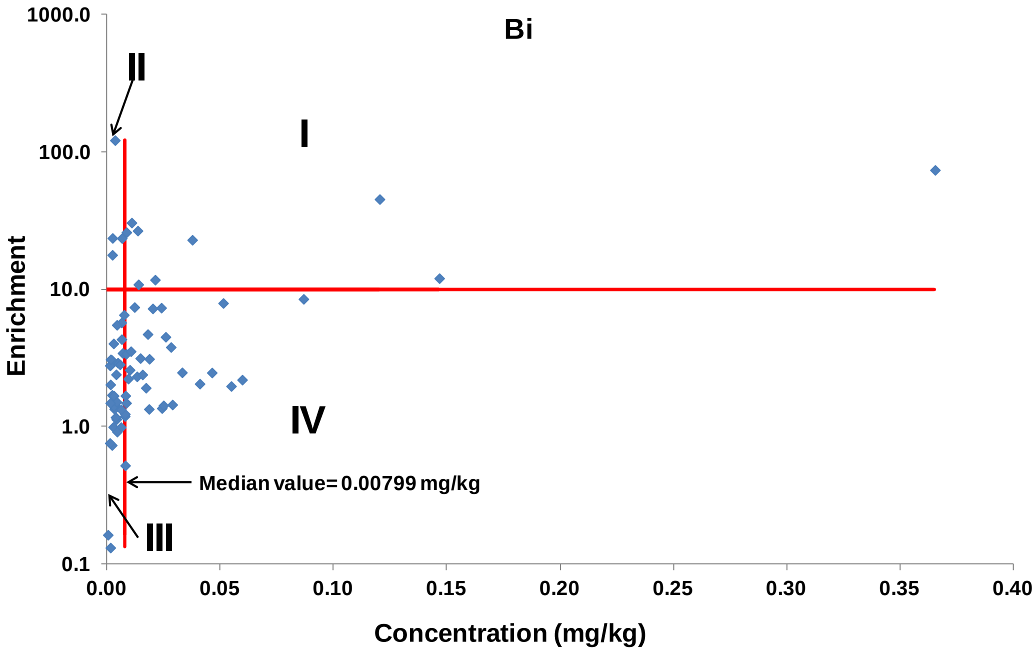


Figure 4.19.2: Enrichment Factor analysis of herb samples for Bi.

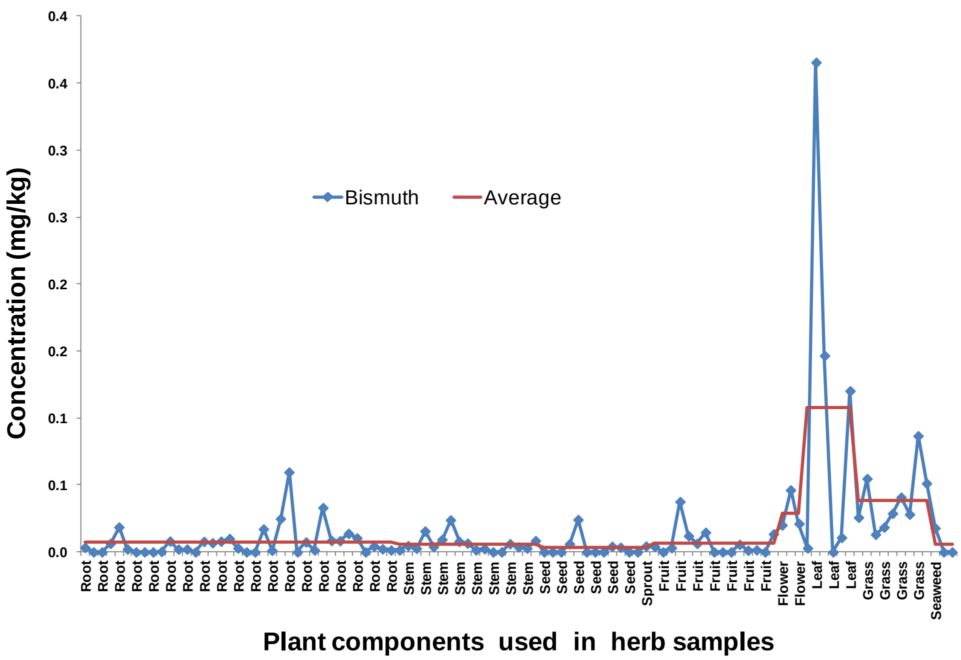


Figure 4.19.3: Variation of total concentration of Bi with different plant components used in herb samples.

4.20 Aluminium, Al

4.20.1 The Role of Al in Human Health

Al is the third most abundant element in the lithosphere [113]. Despite its natural abundance, Al has no known function in biology and it is debatable whether it is of any benefit to human biology [22]. Most naturally occurring Al compounds are insoluble [113], therefore it is remarkably nontoxic. However, there are potential health risks arising from human exposure to bioavailable Al as a result of the widespread occurrence of the element in the environment and in industry [22]. The effects of Al on Alzheimer's disease are of particularly interest as some studies have suggested that Al exposure may be a major risk factor for Alzheimer's disease [22, 113, 208]. However, research in this area has been inconclusive and the scientific consensus does not yet exist about whether Al exposure could increase the risk of Alzheimer's disease [209].

4.20.2 The Al Landscape for the Herb Collection

General Comments

The current work measured the total and bioavailable levels of Al in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.20.1. In this study, Al was also taken as the reference element to calculate the EF values for other elements. Consequently, no enrichment factor analysis was carried out on Al itself. The concentration distribution with respect to plant part was also investigated and the results are shown in Figure 4.20.2.

It was found from Figure 4.20.1 that the total extractable content of Al varied between 0.479 mg/kg in *Xin Ren* (herb number 71) and 1500 mg/kg in *Pu Gong Yin* (herb number 19), on a dry weight basis. The median value of the total extractable level of Al was 110 mg/kg as shown in Figure 4.20.1. Only 5 herb samples (*Fu Ling*, *Long Yan Rou*, *Zhi Shi*, *Tian Ma* and *Sang Ji Sheng*, herb number 3, 7, 29, 47, 77, respectively) achieved a bioavailability of Al > 80 %. Eleven herbs can be considered as exhibiting relatively high levels of total Al exhibiting, namely (*Pu Gong Yi*, *Ma Chi Xian*, *Bai Hua She She Cao*, *Hong Hua*, *Tu Si Zi*, *Tian Ma*, *Dan Zhu Ye*, *Yin Chen*, *Zi Cao (H)*, *Zi Cao (B)* and *Sang Ji Sheng* (herb number 19, 21, 23, 33, 36, 47, 50, 53, 66, 67, and 77, respectively). Nine of these showed low bioavailabilities except *Tia Ma* and *Shan Ji* (herb number 47 and 77, respectively in Figure 4.20.1), which showed a relatively high level in both total extractable and bioavailable contents of Al.

Plant Part Analysis

The concentration distribution of Al varied with respect to plant part (Figure 4.20.2). The grass and leaf-derived herb samples were found to have relatively enhanced levels of of Al.

4.20.3 Suggested Leads for Investigating Al sequestration Ligand Systems and Possible Toxicity Implications

In this study, *Tian Ma* (herb number 47, Figure 4.20.1) and *Sang Ji Sheng* (herb number 77, Figure 4.20.1) showed a relatively higher level in both total and bioavailable contents of Al. Herbs that sequester relatively large amounts of Al could have very interesting Al chelating ligands and possible toxicity implications, and this needs to be further investigated.

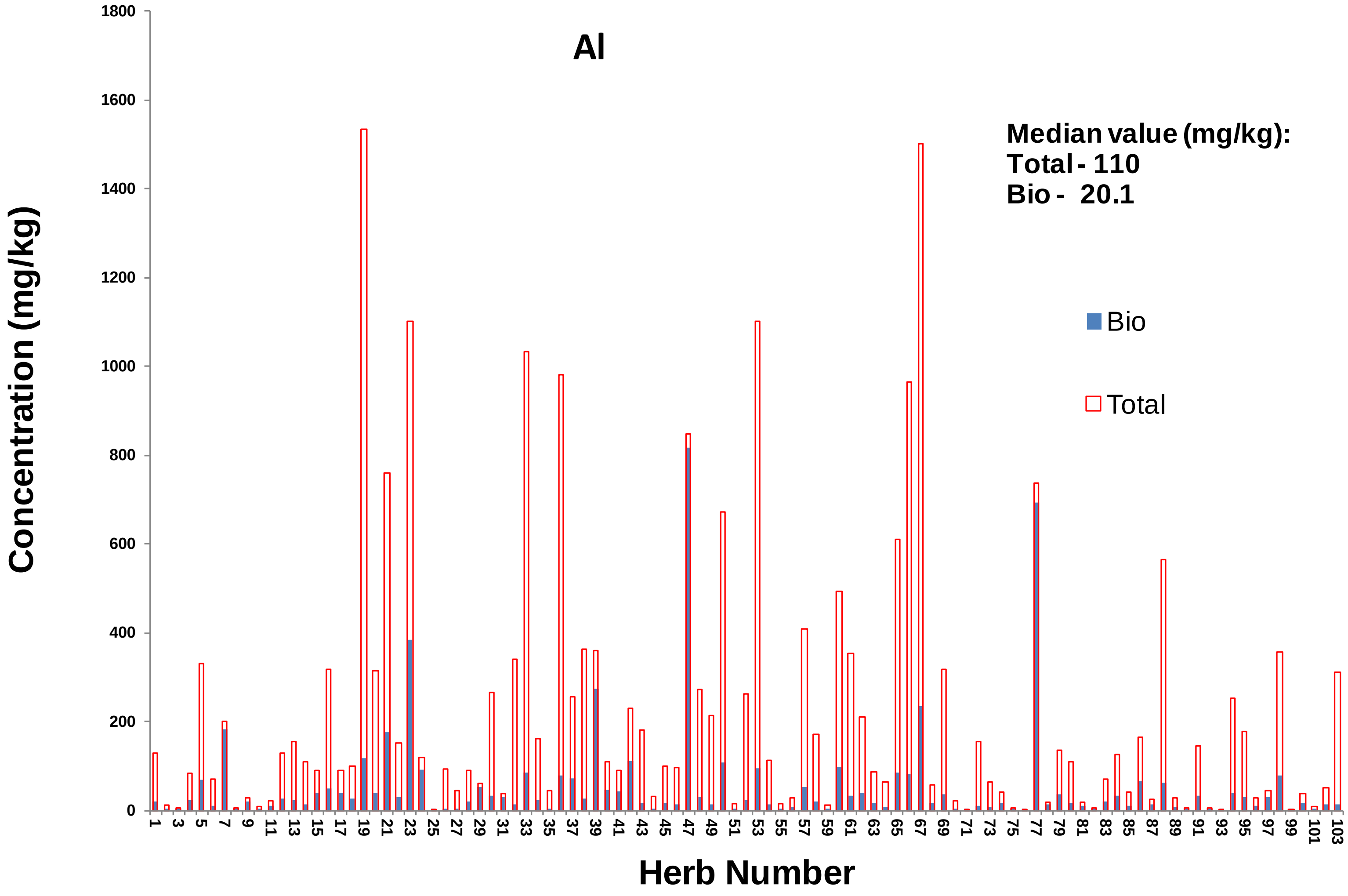


Figure 4.20.1: Total and bioavailable concentration of Al in herb samples.

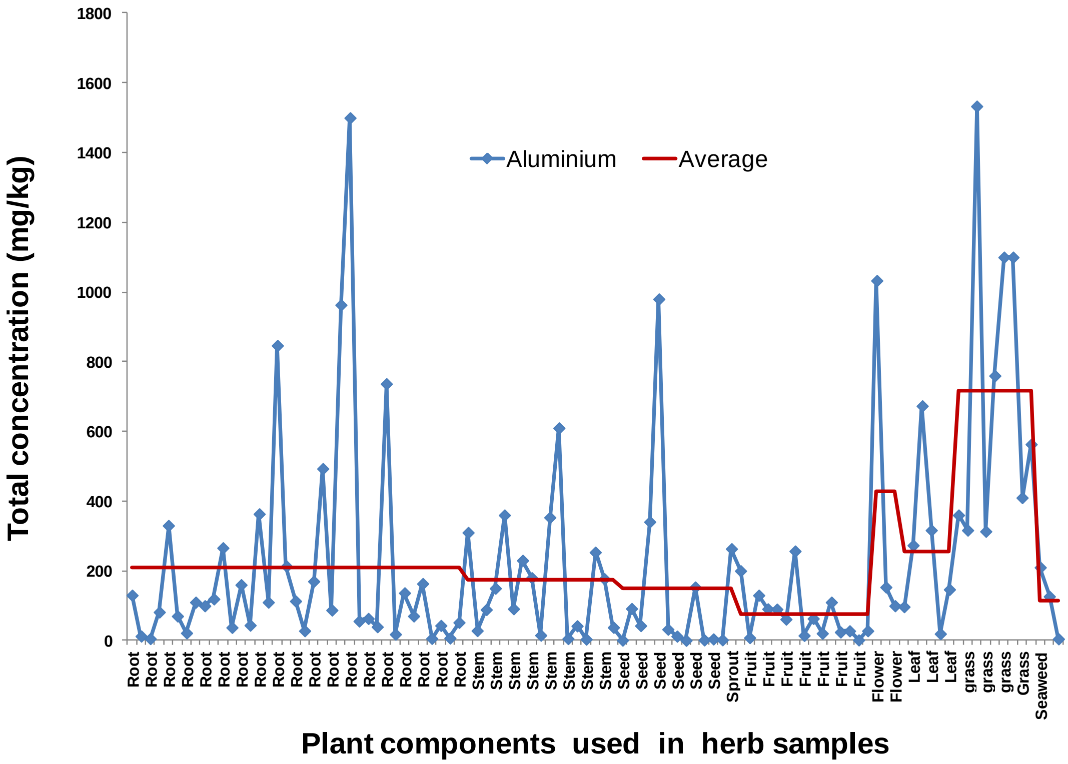


Figure 4.20.2: Variation of total concentration of Al with different plant components used in herb samples.

4.21 Barium, Ba

4.21.1 The Role of Ba in Human Health

Ba is physiologically inactive in plants under normal circumstances [113]. In the human body the presence of Ba may cause damage [22], especially in soluble form (notably chloride, nitrate and hydroxide) [113, 210]. Barium sulfate ($BaSO_4$) is highly insoluble and is therefore generally nontoxic to humans, which has made it practical for use in medical applications as a radiation-absorbent contrast material for X-ray examination of the gastrointestinal tract, the "barium meal" [113, 210]. However, under some circumstances, $BaSO_4$ and other insoluble barium compounds may also be potentially toxic [210].

4.21.2 The Ba Landscape for the Herb Collection

General Comments

In this study, the total and bioavailable levels of Ba were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.21.1. The enrichment ability of the herbs for Ba was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.21.2 and the concentration distribution with respect to plant part is depicted in Figure 4.21.3.

Excluding herbs 33 and 41, total extractable levels of Ba ranged from 0.330 mg/kg in *Yi Yi Ren* (herb number 99) to 160 mg/kg in *Hong Teng* (herb number 17), Figure 4.21.1b. The median values of the total extractable and bioavailable levels of Ba are 12.4 mg/kg and 10.5 mg/kg respectively. Notably the two "excluded" herbs showed extraordinarily high levels of 3000 mg/kg in *Hong Hua* (herb number 33) and 6600 mg/kg in *Huang Lian* (herb number 41) as depicted in Figure 4.21.1 (b). The overall bioavailability of Ba was generally high, with most of the herbs having achieved a bioavailability of Ba more than 80 %, - indicating that the Ba content in these herbs is almost fully bioavailable. However, *Hong Hua and Huang Lian* (herb numbers 33 and 41 in Figure 4.21.1b) had very low Ba bioavailability (1 % – 2 %). This indicates that the Ba is tightly bound into these materials and could suggest some interesting "complexing" chemistry for this ion that warrants further chemical enquiry.

Enrichment Factor Analysis

The herbs also demonstrated a generally high enrichment ability for Ba, with around 70 % of herbal samples tested having an EF > 10 (I and II in Figure 4.21.2). The herb shown in part I of Figure 4.21.2 had Ba concentrations above the median value of 12.4 mg/kg, which is attributed not only to an enhanced enrichment ability for Ba, but also a high availability of Ba in the environment where they grown. The herbs presented in part II of Figure 4.21.2 also had an enhanced enrichment ability for Ba but showed a total extractable content of Ba less the median value 12.4 mg/kg, suggesting that these herbs have a low environmental availability of Ba. The herbs located in part III of Figure 4.21.2 showed a concentration of Ba less than the median value of 12.4 mg/kg, possibly due to low enrichment ability for Ba (EF < 10) and/or poorly availability of Ba in the environment. Five herb samples showed an EF < 10 but a Ba concentration > 12.4 mg/kg (median value) (IV in Fig. 4.21.2), suggesting that they may originate from a Ba-rich environment. What about herbs

Plant Part Analysis

The concentration distribution of Ba varied with respect to plant part (Figure 4.21.3). The root, stem and grass herb samples showed relatively enhanced levels of Ba. This may be reasonable as Ba occurs in relatively high concentrations in the earth's crust [113].

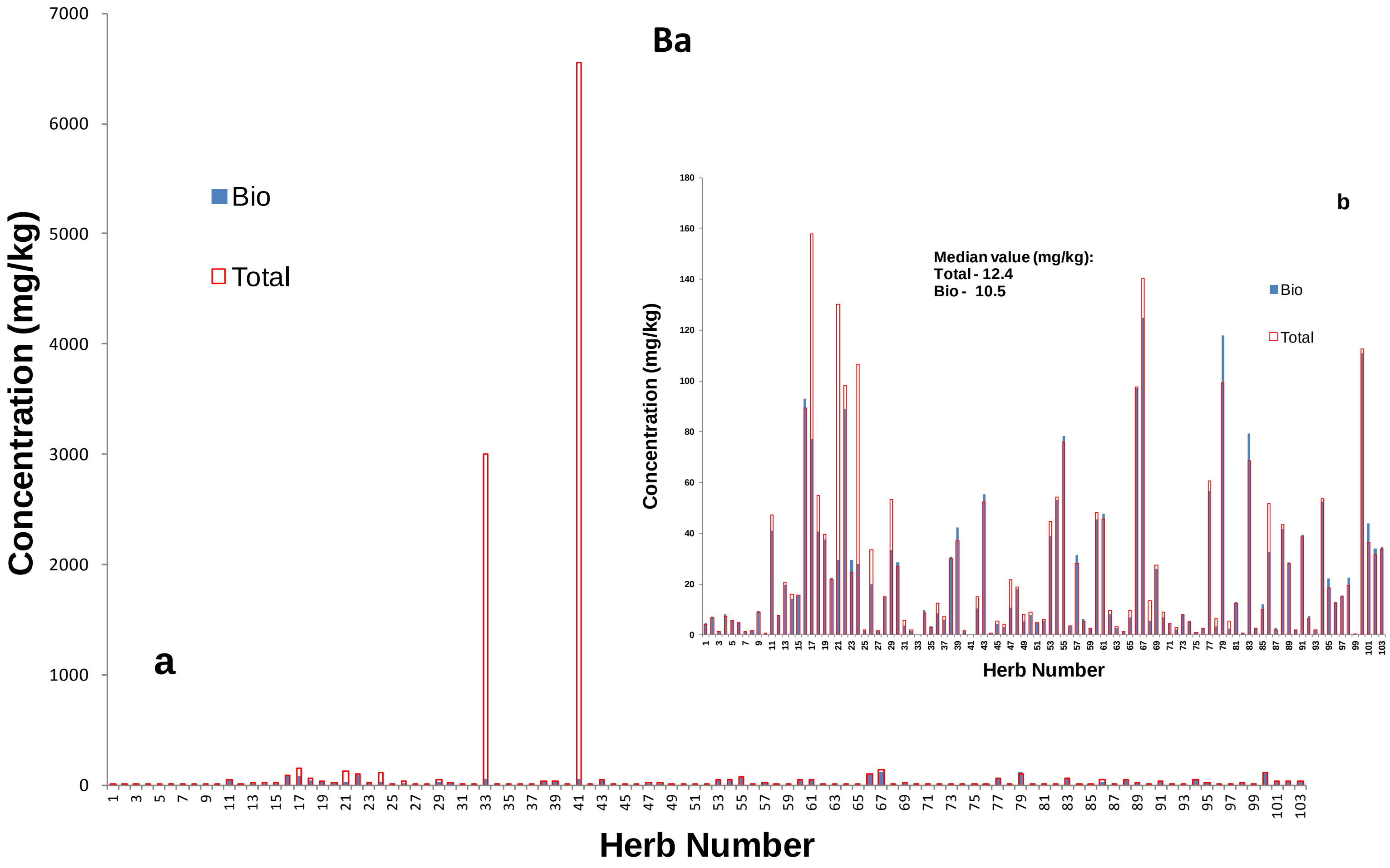


Figure 4.21.1: Total and bioavailable concentrations of Ba: **(a)** in *Hong Hua* (herb number 33) and *Huang Lian* (herb number 41) in relation to all the other herbs; **(b)** in herb samples, excluding *Hong Hua* (herb number 33) and *Huang Lian* (herb number 41).

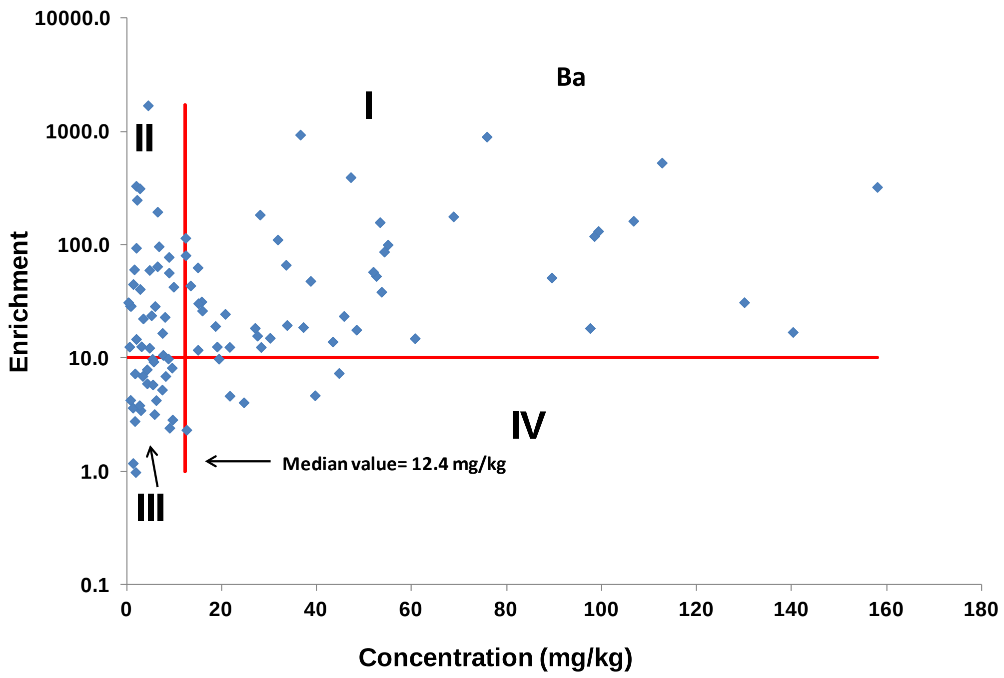


Figure 4.21.2: Enrichment Factors analysis for Ba in herb samples; excluding *Hong Hua* (herb number 33) and *Huang Lian* (herb number 41) in part I due to their extraordinarily high levels of Ba.

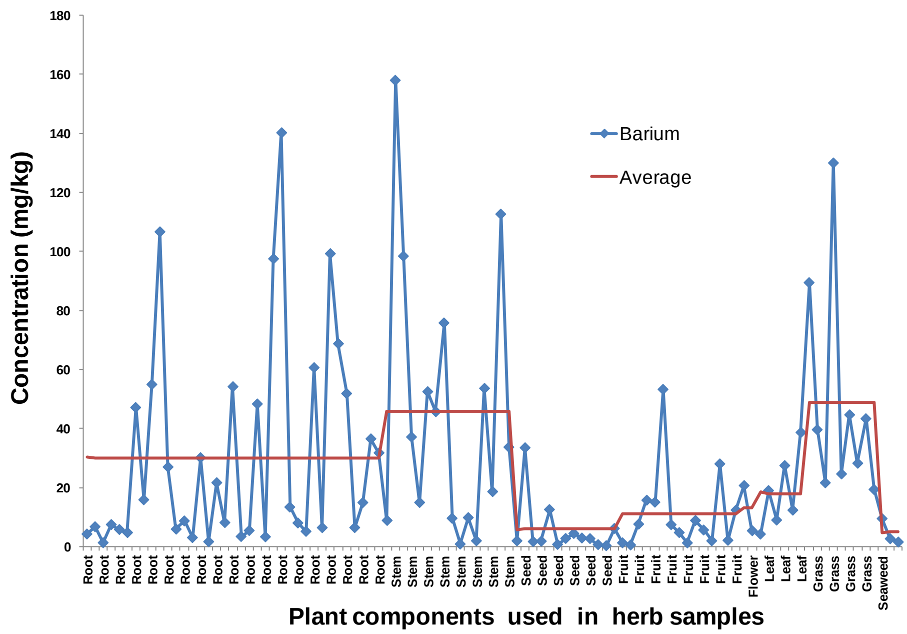


Figure 4.21.3: Variation of total concentration of Ba with different plant components used in herb samples.

4.22 Potassium, K

4.22.1 The Role of K in Human Health

K is an essential mineral micronutrient in human nutrition and is important in maintaining fluid and electrolyte balance in the body. K ions play a vital role for the normal functioning of the nervous system, the muscles, the heart and the brain [22]. A shortage of K in body fluids may cause a potentially fatal condition known as hypokalemia, typically resulting from vomiting, diarrhea, and/or increased dieresis [211]. K deficiency can lead to fatigue, muscle weakness, cardiac arrhythmia etc [212]. High K concentration in the blood, on the other hand, can also cause weakness, drowsiness and arrhythmia etc [22].

4.22.2 The K Landscape for the Herb Collection

General Comments

In this study, the total and bioavailable levels of K were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.22.1. The enrichment ability of the herbs for K was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.22.2 and the concentration distribution with respect to plant part is depicted in Figure 4.22.3.

It was found from Figure 4.22.1 that the herbs tested showed significant high level of both total extractable and bioavailable contents of K. The total extractable content of K was measured to be between 430 mg/kg in *Fu Ling* (herb number 3) and 51,500 mg/kg in *Hai Zao* (herb number 62), on a dry weight basis. The median value of the total extractable level of K was 7,900 mg/kg as shown in Figure 4.22.1. All the herbs tested achieved a more than 80 % bioavailability of K, indicating that the K content in the herbs is almost fully bioavailable.

Enrichment Factor Analysis

The herbs also demonstrated high enrichment ability for K. Almost all the herbal samples tested achieved an EF > 10 (I and II in Figure 4.22.2). About half (I in Figure 4.22.2) had K concentrations above the median value of 7,900 mg/kg, which is attributed not only to an enhanced enrichment ability for K, but also a high availability of K in the environment where they were grown. The herbs in part II of Figure 4.22.2 also had an enhanced enrichment ability for K but showed a total extractable content of K less the median value 7,900 mg/kg, suggesting that these herbs have a low environmental availability of K. Two herbs located in part III of Figure 4.22.2 showed a concentration of K less than the median value of 7,900 mg/kg, possibly due to a low enrichment ability for K (EF < 10) and/or relatively low availability of K in the environment. None of herb samples showed an EF < 10 with a K concentration > 7,900 mg/kg (median value) (IV in Fig. 4.22.2).

Plant Part Analysis

The concentration distribution of K varied with respect to plant part (Figure 4.22.3). The grass, flower, leaf-derived and flower herb samples were found to have a relatively higher level of K compared to other plant components used.

4.22.3 Suggested Leads from Relating K Content to Nutritional Properties

In this study, *Hai Zao* (herb number 62, Figure 4.22.1) and *Ma Chi Xian* (herbs number 21, Figure 4.22.1) showed a relatively higher level in both total and bioavailable contents of K (> 45,000 mg/kg). It was reported [22] that most people have a K intake of 2 – 4 g per day on average. As mentioned in Section 4.22.1, K deficiency could potentially cause some fatal medical conditions. Therefore, K deficiency (e.g. caused by diuretics) should be treated with K supplements (e.g. in the form of mineral salt) of 2 – 4 g per day [22]. *Hai Zao* and *Ma Chi Xian* may be used as K supplements for the treatment of K deficiency. There are also possible leads for K ion specific ligands with respect to the higher values. Also it is well-known that many naturally occurring antibiotic ionophores exhibit high affinities for K^+ and Na^+ [213, 214]. *Hai Zao* and *Ma Chi Xian* might have those K and Na complexes of antibiotics [215] and this needs to be further investigated.

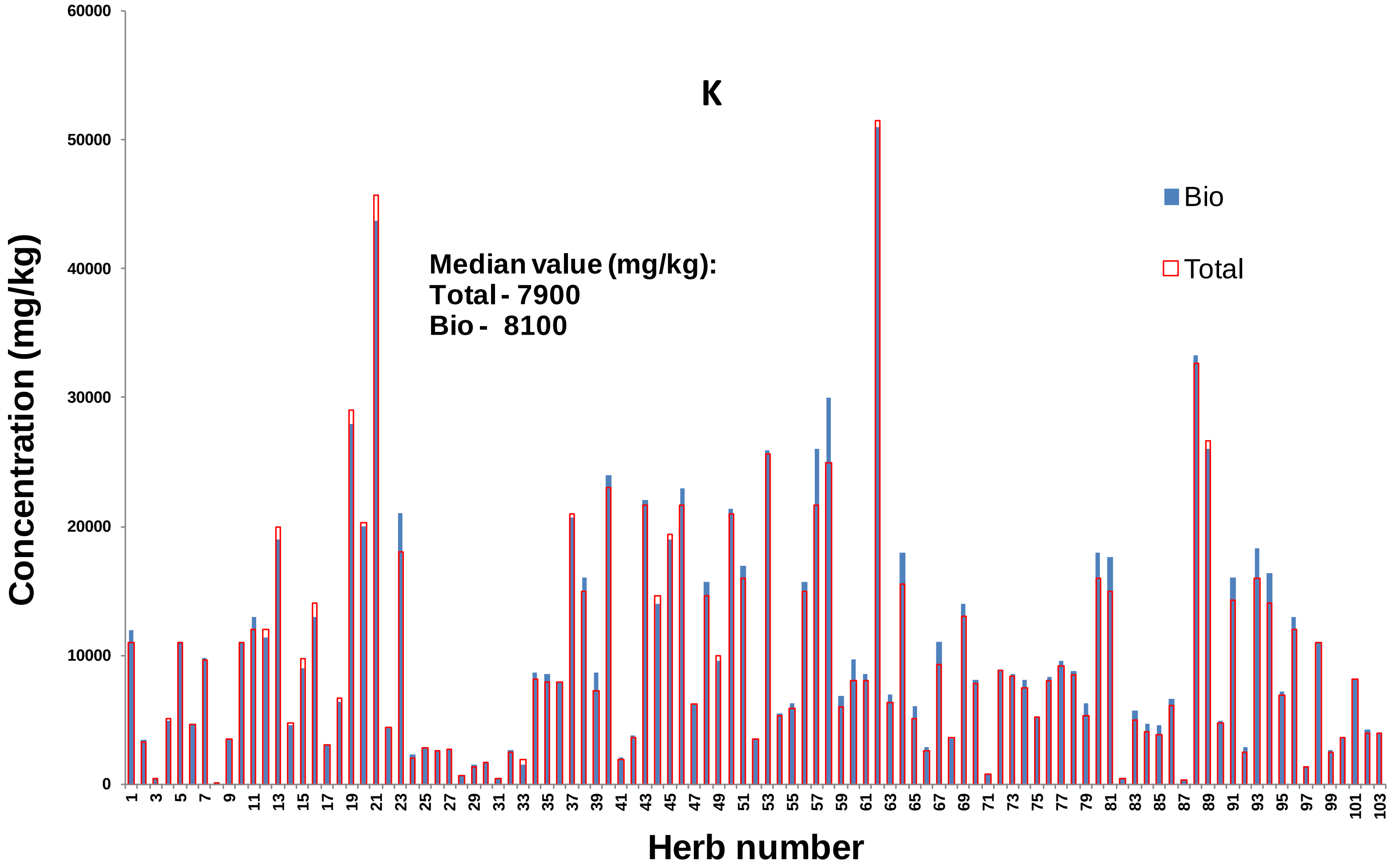


Figure 4.22.1: Total and bioavailable concentration of K in herb samples.

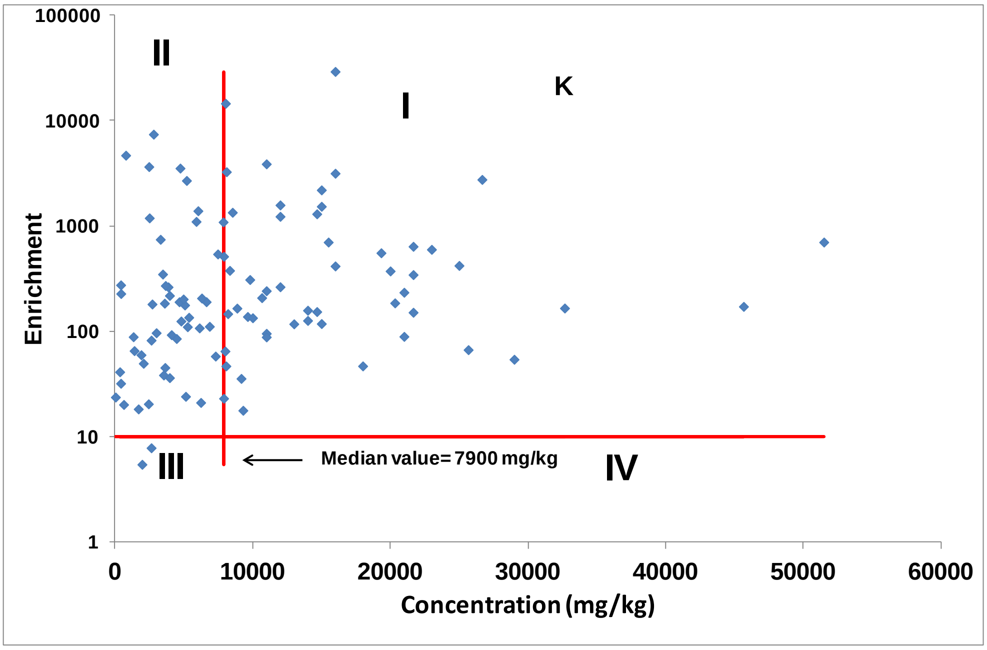


Figure 4.22.2: Enrichment Factor analysis of herb samples for K.

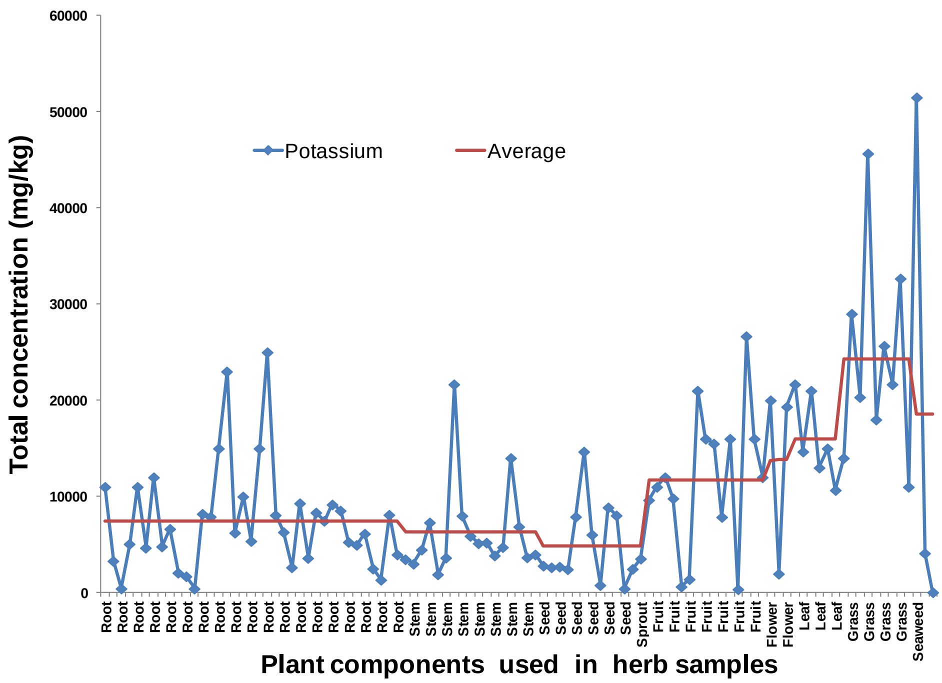


Figure 4.22.3: Variation of total concentration of K with different plant components used in herb samples.

4.23 Calcium, Ca

4.23.1 The Role of Ca in Human Health

Ca is essential for living organisms, particularly in cell physiology, where movement of Ca^{2+} into and out of the cytoplasm functions as a signal for many cellular processes. Ca is required for vascular contraction and vasodilation, muscle function, nerve transmission, intracellular signaling and hormonal secretion [22, 216]. Ca is the most abundant metal by mass in the body [216]. 99 % of the body's Ca supply is stored in the bones and teeth where it supports their structure and function [22, 216]. The body uses bone tissue as a reservoir for, and source of Ca, to maintain constant concentrations of Ca in blood, muscle, and intercellular fluids [216]. Daily needs or the world's daily average up-take of Ca by a 70 kg adult is around 500 mg [68]. Ca deficiency over the long term causes osteopenia which, if untreated, can lead to osteoporosis [22, 216]. However, excessively high levels of Ca in the blood, known as hypercalcemia, can also cause renal insufficiency, vascular and soft tissue calcification, hypercalciuria (high levels of Ca in the urine) and kidney stones [216-218]. Several clinical trials have demonstrated a relationship between increased Ca intakes and both low blood pressure and risk of hypertension [219-221]. However, other studies have found no association between Ca intake and incidence of hypertension [222]. Data from observational and experimental studies on the potential role of Ca in preventing colorectal cancer, though somewhat inconsistent, are highly suggestive of a protective effect [216]. Several studies have found that higher intakes of Ca from foods (low-fat dairy sources) and/or supplements are associated with a decreased risk of colon cancer [223, 224]. But other observational studies have found the associations to be inconclusive [225, 226].

4.23.2 The Ca Landscape for the Herb Collection

General Comments

In this study, the total and bioavailable levels of Ca were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.23.1. The enrichment ability of the herbs for Ca was also investigated. The results obtained from the enrichment factor analysis are shown in

Figure 4.23.2 and the concentration distribution with respect to plant part is depicted in Figure 4.23.3.

It is evident from Figure 4.23.1 that the herbs tested were generally rich in Ca. The total extractable content of Ca ranged between 9.69 mg/kg in *Fu Ling* (herb number 3) and 55,333 mg/kg in *Chuan Niu Xi* (herb number 79), on a dry weight basis. The median value of the total extractable level of Ca was 4200 mg/kg, as shown in Figure 4.23.1. The overall bioavailability of Ca was also relatively high, with about three quarters of the herbs showing more than 80 % bioavailability.

Enrichment Factor Analysis

The herbs also demonstrated high enrichment ability for Ca. 98 of the 103 herbal samples tested had an EF > 10 (I and II in Figure 4.23.2). The herbs shown in part I of Figure 4.23.2 had Ca concentrations above the median value of 4200 mg/kg, which is attributed not only to an enhanced enrichment ability for Ca, but also a high availability of Ca in the environment where they grown. More than half of herbs (II in Figure 4.23.2) also showed an enhanced enrichment ability for Ca but had a total extractable content of Ca less the median value 4200 mg/kg, suggesting that these herbs have a low environmental availability of Ca. Five herbs (III in Figure 4.23.2) showed a concentration of Ca less than the median value of 4200 mg/kg, possibly due to a low enrichment ability for Ca (EF < 10) and/or poor availability of Ca in the environment. No herb samples were found to have an EF < 10 but a Ca concentration > 4200 mg/kg (median value) (IV in Fig. 4.23.2).

Plant Part Analysis

The concentration distribution of Ca varied with respect to plant part (Figure 4.23.3). The leaf-derived, grass and stem herb samples showed relatively enhanced levels of Ca.

4.23.3 Suggested Leads from Relating Ca Content to Nutritional Properties

According to the United States Department of Agriculture National Nutrient Database [227], parmesan cheese has the Ca content around 11,000 mg/kg (55 mg Ca per common measure of 5 g cheese). In the present study, *Chuan Niu Xi* (herb number 79, Figure 4.23.1, around 60,000 mg/kg) and *Zi Cao* (H) (herb number 66, Figure 4.23.1, around 35,000 mg/kg) had much higher bioavailable content of Ca (within experimental error) than parmesan cheese. Therefore, it is proposed that these herbs may be useful as a Ca source for Ca deficient persons who are allergic to Ca-rich foods such as dairy products. In TCM, *Chuan Niu Xi* is famous for clearing Wind and Dampness, invigorating the blood and unblocking the channels [30]. This herb is also used for the treatment of osteoporosis [138].

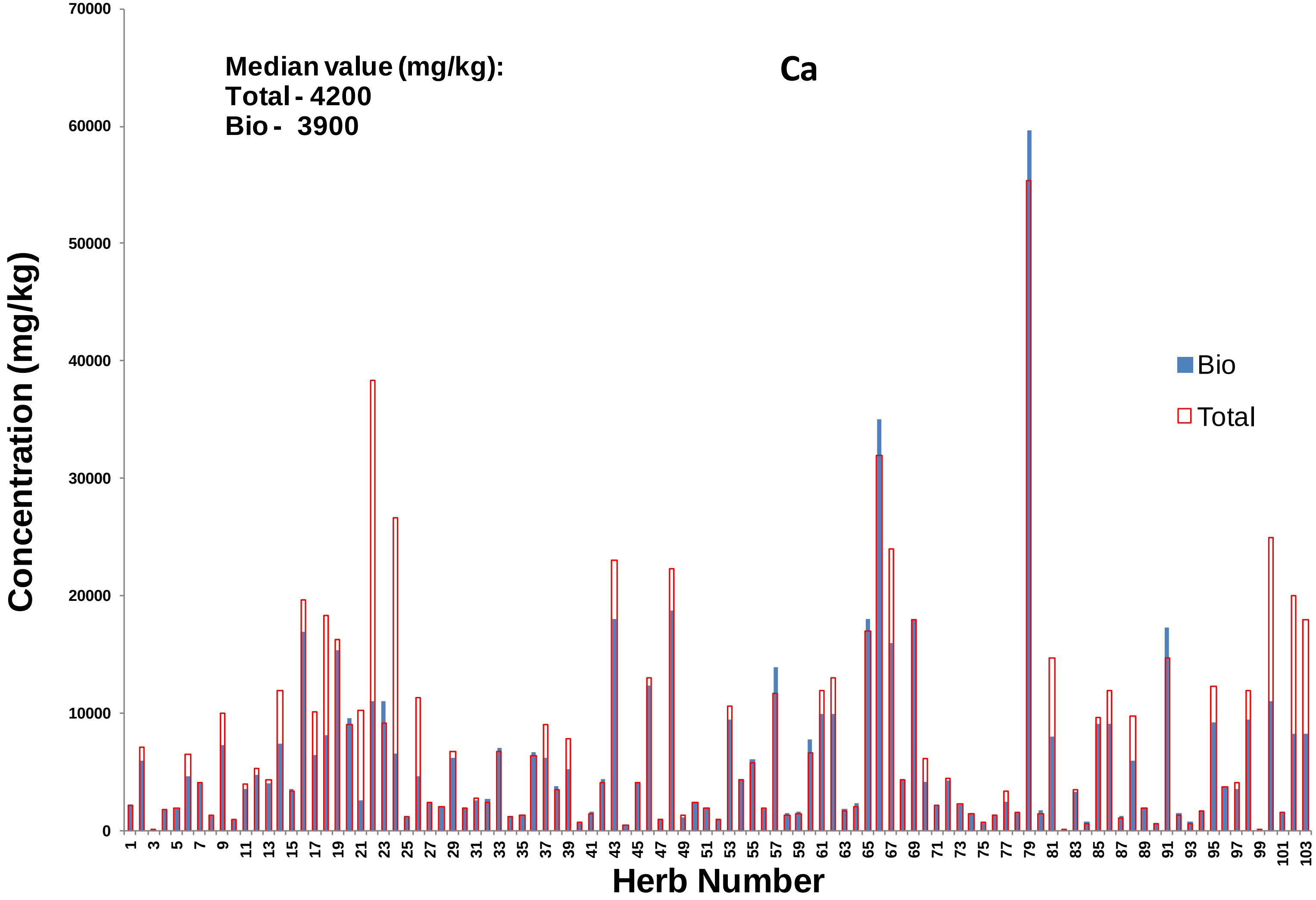


Figure 4.23.1: Total and bioavailable concentration of Ca in herb samples.

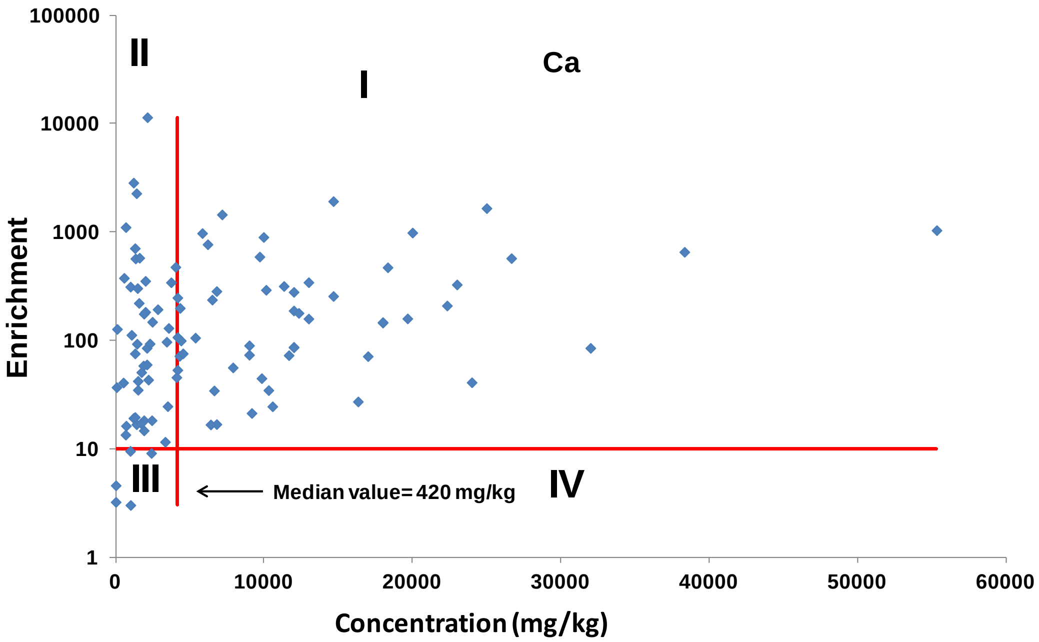


Figure 4.23.2: Enrichment Factor analysis of herb samples for Ca.

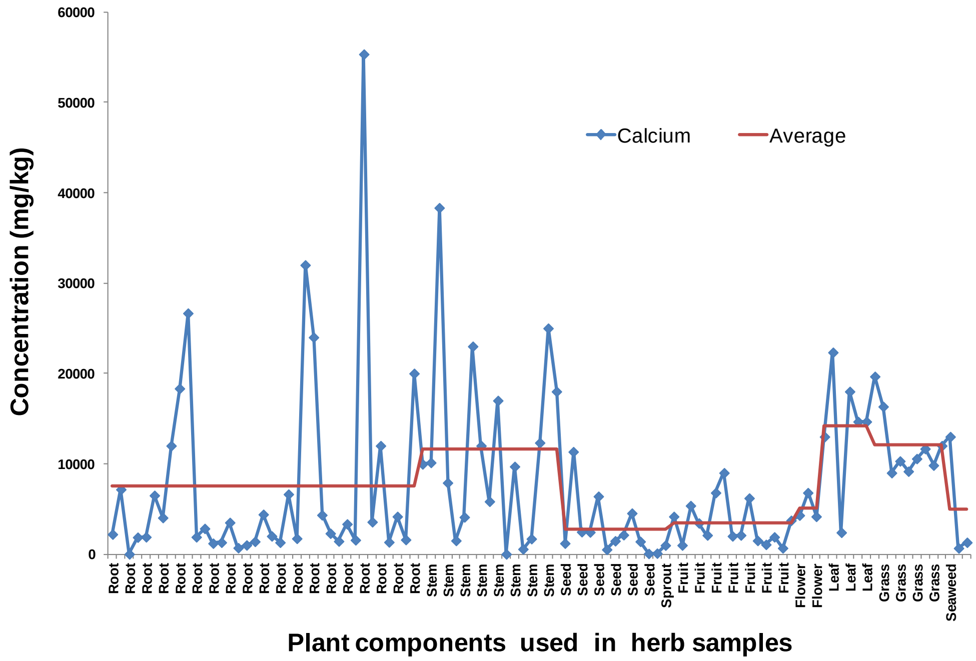


Figure 4.23.3: Variation of total concentration of Ca with different plant components used in herb samples.

4.24 Sodium, Na

4.24.1 The Role of Na in Human Health

Na is an essential element for all animals and some plants. In the human body, Na regulates the electrolyte and acid-alkali balances, the conductive capacity of nerves, muscle contractions, and the production of adrenaline and amino acids [22]. Na deficiency is extremely rare since the minimum physiological requirement for Na is only 500 mg daily [22, 228]. However, a malfunction in the kidneys and heavy sweat secretion could lead to Na deficiency which is associated with the symptoms such as indisposition, dizziness, weakness in the muscles, loss of weight, etc. [22]. Excessive consumption of Na on a regular basis can raise blood pressure, lead to hypertension and oedema [22, 228]. High Na intake can also lead to osteoporosis as Na can increase urinary Ca losses [228].

4.24.2 The Na Landscape for the Herb Collection

General Comments

In this study, the total and bioavailable levels of Na were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is presented in Figure 4.24.1. The enrichment ability of the herbs for Na was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.24.2 and the concentration distribution with respect to plant part is depicted in Figure 4.24.3.

It was found from Figure 4.24.1 that the herbs tested also had a generally high level in Na, with a median value of the total extractable content of 160 mg/kg as shown in Figure 4.24.1. *Hai Zao* (herb number 62 in Figure 4.24.1) had the highest Na concentration and this Na content is fully bioavailabable, within experimental error. The overall bioavailability of Na was also relatively high, with most of herbs achieved a more than 80 % bioavailability of Na, indicating that the Na content in these herbs is almost fully bioavailable.

Enrichment Factor Analysis

The herbs also demonstrated a high enrichment ability for Na, with around 70 % of herbal samples tested having an EF > 10 (I and II in Figure 4.24.2). The herbs presented in part I of Figure 4.24.2 had Na concentrations above the median value of 160 mg/kg, which is attributed not only to an enhanced enrichment ability for Na, but also a high availability of Na in the environment where they grown. The herbs in part II of Figure 4.24.2 also had an enhanced enrichment ability for Na but showed a total extractable content of Na less the median value 160 mg/kg, suggesting that these herbs have a low environmental availability of Na. The herbs located in part III of Figure 4.24.2 showed a concentration of Na less than the median value of 160 mg/kg, possibly due to low enrichment ability for Na (EF < 10) and/or poorly availability of Na in the environment. Three herb samples showed an EF < 10 but a Na concentration > 160 mg/kg (median value) (IV in Fig. 4.24.2), suggesting that they may originate from a Na-rich environment.

Plant Part Analysis

The concentration distribution of Na varied with respect to plant part (Figure 4.24.3). Apart from *Hai Zao* (herb number 62 in Figure 4.24.1) which had the highest Na concentration, the seed-based and leaf-derived herb samples showed relatively enhanced levels of Na.

4.24.3 Suggested Leads from Relating Na Content to Nutritional Properties

As mentioned above, Na deficiency could lead to indisposition, dizziness, weakness in the muscles, loss of weight, etc. [22]. Although Na deficiency is extremely rare, it could still happen due to a malfunction in the kidneys and heavy sweat secretion. *Hai Zao* (herb number 62 in Figure 4.24.1) was found in this study to have the highest Na concentration and this Na content is fully bioavailable. This herb is traditionally used in TCM to clear “internal heat” (associated with fever, inflammation and infection) [30, 31], and for treatment of cancer [170]. It was also reported [229] that *Hai Zao* benefits the kidneys, lungs, liver and stomach meridians, and can promote urination and decrease body and leg oedema. Therefore, this herb may be useful as a Na source for Na deficiency in persons with kidney problems. As mentioned in Section 4.21, this herb also had the highest concentration for K. Many naturally occurring antibiotic ionophores exhibit high affinities for K^+ and Na^+ [213, 214], but the selectivity of K^+/Na^+ varies widely among the naturally occurring ionophores [230]. *Hai Zao* has shown the highest level for both K and Na. This herb might contain some naturally occurring antibiotic ionophores with a lower K^+/Na^+ selectivity and this needs to be further investigated.

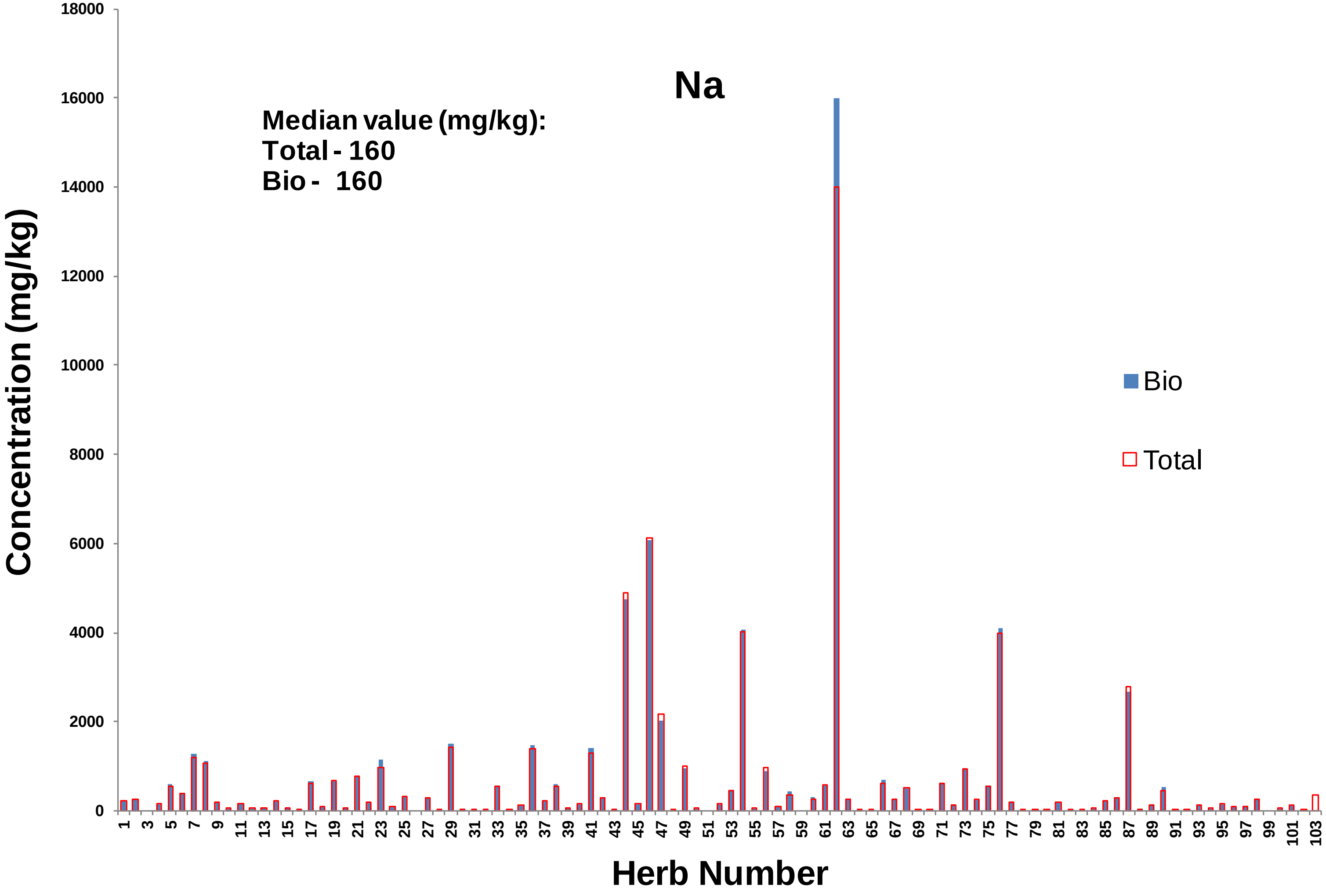


Figure 4.24.1: Total and bioavailable concentration of Na in herb samples.

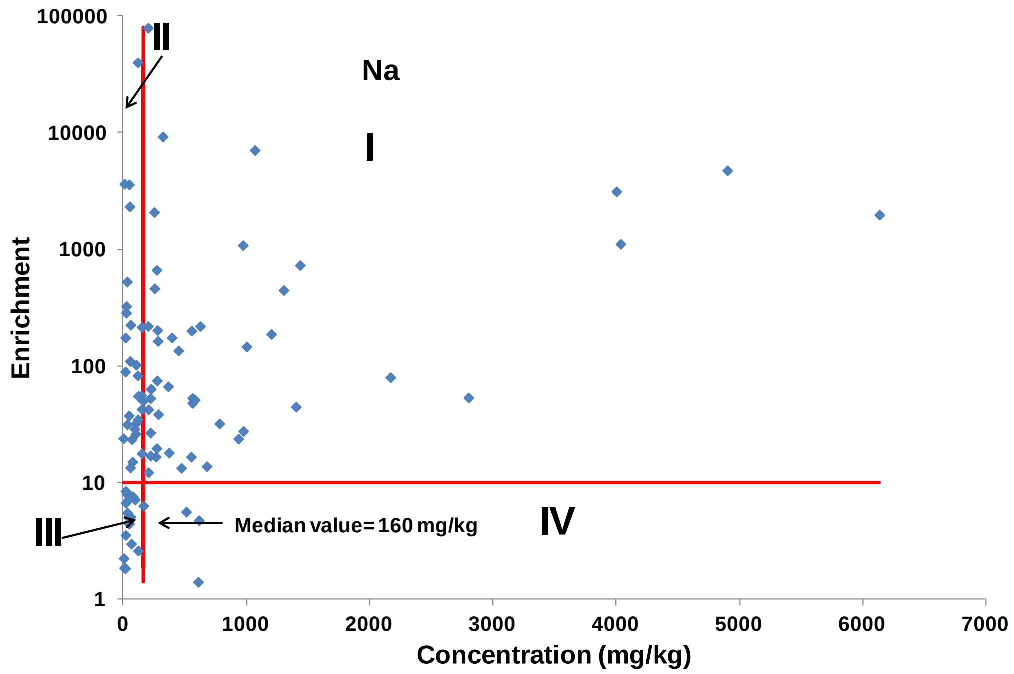


Figure 4.24.2: Enrichment Factor analysis of herb samples for Na.

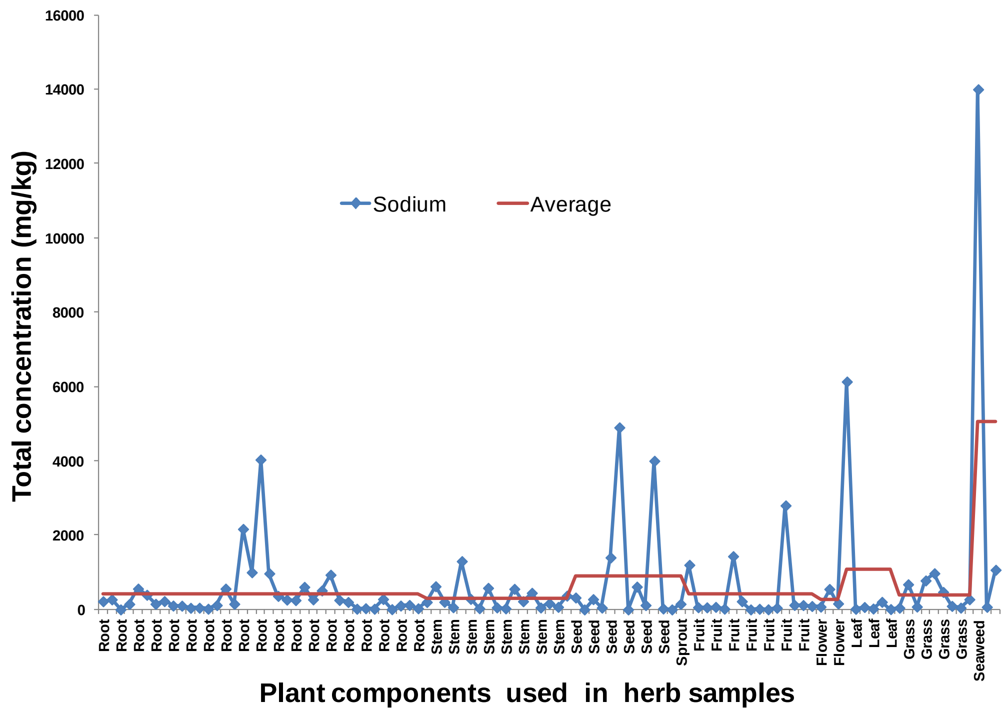


Figure 4.24.3: Variation of total concentration of Na with different plant components used in herb samples.

4.25 Magnesium, Mg

4.25.1 The Role of Mg in Human Health

Mg is an essential element and plays an important role in SOD enzyme system, as do Cu, Zn and Mn [22, 128]. Over 300 enzymes require the presence of Mg ions for their catalytic action [231], including all enzymes utilising or synthesising adenosine triphosphate (ATP), or those that use other nucleotides to synthesise DNA and RNA [232]. ATP exists in cells normally as a chelate between ATP and a Mg ion [233]. Mg compounds are used medicinally as common laxatives, antacids (e.g. milk of magnesia) and in a number of situations where stabilisation of abnormal nerve excitation and blood vessel spasm is required (e.g. to treat eclampsia) [233]. Although human Mg deficiency (including conditions that show few overt symptoms) is relatively rare [234], low levels of Mg in the body have been associated with the development of a number of human illnesses such as asthma, diabetes, and osteoporosis [235]. Mg deficiency may also be related to depression and Mg treatment has been reported to be effective in the treatment of this disease [236, 237].

4.25.2 The Mg Landscape for the Herb Collection

General Comments

In this study, total and bioavailable levels of Mg were measured in triplicate (Appendix B) across the 103 medicinal herbs chosen for analysis. This data is represented in Figure 4.25.1. The enrichment ability of the herbs for Mg was also investigated. The results obtained from the enrichment factor analysis are shown in Figure 4.25.2 and the concentration distribution with respect to plant part is depicted in Figure 4.25.3.

It may be seen from Figure 4.25.1 that the herbs tested were generally rich in Mg. The total extractable content of Mg was measured to be between 38.7 mg/kg in *Fu Ling* (herb number 3) and 18,000 mg/kg in *Duo Huo* (herb number 30), on a dry weight basis. The median value of the total extractable level of Mg was 1,800 mg/kg, as shown in Figure 4.25.1. The overall bioavailability of Mg was also relatively high. All the herbs studied achieved a more than 80 % bioavailability of Mg, indicating that the Mg content in the tested herbs is almost fully bioavailable.

Enrichment Factor Analysis

The herbs also demonstrated a high enrichment ability for Mg. Around 80 % of herbal samples tested having an EF > 10 (I and II in Figure 4.25.2) and half of them (I in Figure 4.25.2) had Mg concentrations above the median value of 1,800 mg/kg. This may be attributed not only to an enhanced enrichment ability for Mg, but also a high availability of Mg in the environment where they grown. The herbs presented in part II of Figure 4.25.2 also had an enhanced enrichment ability for Mg but showed a total extractable content of Mg less the median value 1,800 mg/kg, suggesting that these herbs have a low environmental availability of Mg. The herbs located in part III of Figure 4.25.2 showed a concentration of Mg less than the median value of 1,800 mg/kg, possibly due to low enrichment ability for Mg (EF < 10) and/or poorly availability of Mg in the environment. Eight herb samples showed an EF < 10 with a Mg concentration > 1,800 mg/kg (median value) (IV in Fig. 4.25.2), suggesting that they may originate from a Mg-rich environment.

Plant Part Analysis

The concentration distribution of Mg varied with respect to plant part (Figure 4.25.3). The flower, grass and leaf-derived herb samples were found to have relatively enhanced levels of Mg.

4.25.3 Suggested Leads from Relating Mg Content to Nutritional Properties

The recommended daily requirement of Mg is 400 mg for adult males and 310 mg for adult females [238]. To prevent Mg deficiency, people need to include Mg-rich foods in their diet as much as possible. In this study, *Zhi Shi*, *Duo Huo* and *Hong Hua* (herbs number 29, 30 and 33, respectively, Figure 4.25.1) showed a relatively higher bioavailable content of Mg (> 15,000 mg/kg). In TCM, *Zhi Shi* has functions to restore normal Qi flow in conditions of Qi stagnation. In Western terms, this is interpreted to mean that it addresses various neurological and neuromuscular disorders involving digestive, respiratory or reproductive functions [30]. *Duo Huo* is commonly used to eliminate wind and damp, and addresses painful obstruction syndromes characterised by rheumatic pain and stiffness in the limbs, joints, muscles and tendons [30]. It was also reported [138] that this herb can be used for treatment of osteoarthritis. There is a possible connection with respect to its use for inflammatory conditions and the role of Mg in the biochemistry of SOD. *Hong Hua* is traditionally used to invigorate the blood. In Western terms, it would address a variety of different gynaecological, neurological and traumatic disorders [30]. It was also used for treatment of diabetes and osteoporosis [138]. Notably, these herbs had a much higher bioavailable content of Mg than foods considered to be Mg-rich such as oysters (around 570 mg/kg) and pumpkin seeds (around 5,000 mg/kg) [238]. Therefore, these herbs may be useful as an Mg source for Mg deficient persons who are allergic to Mg-rich foods such as seafood.

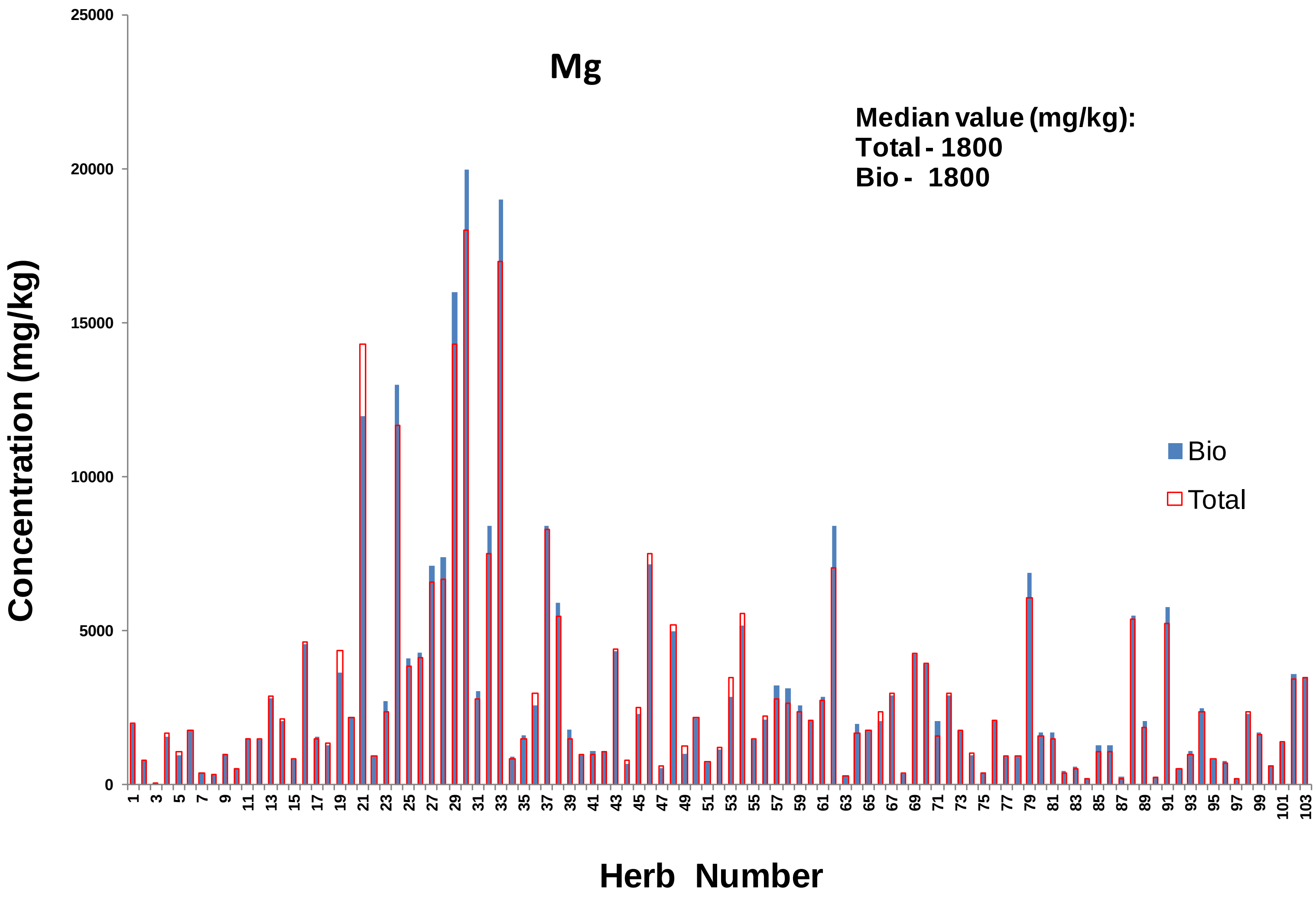


Figure 4.25.1: Total and bioavailable concentrations of Mg in herb samples.

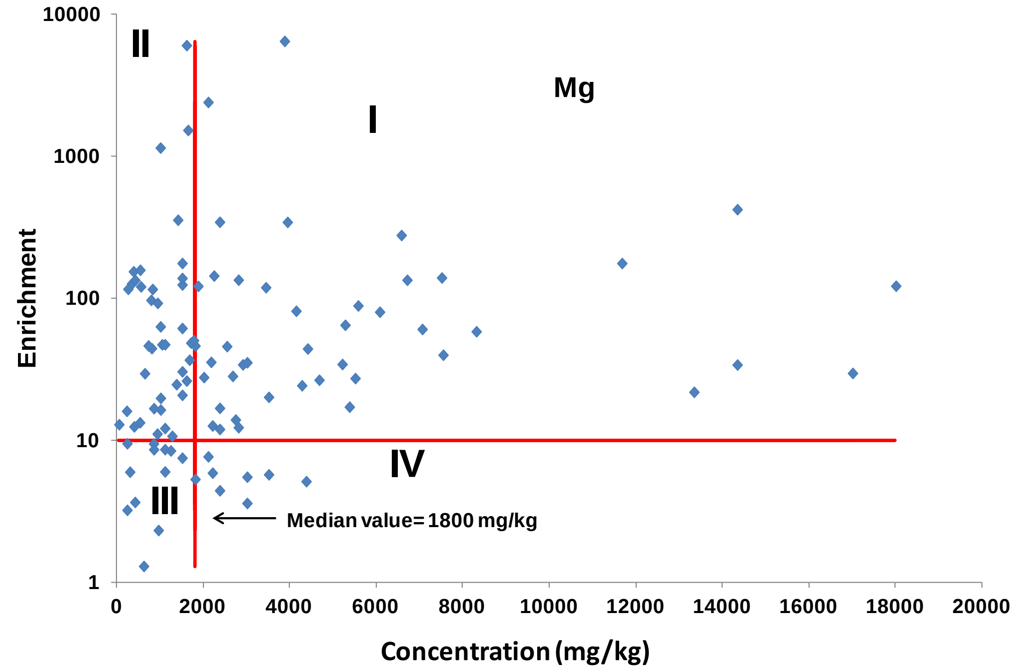


Figure 4.25.2: Enrichment Factor analysis of herb samples for Mg.

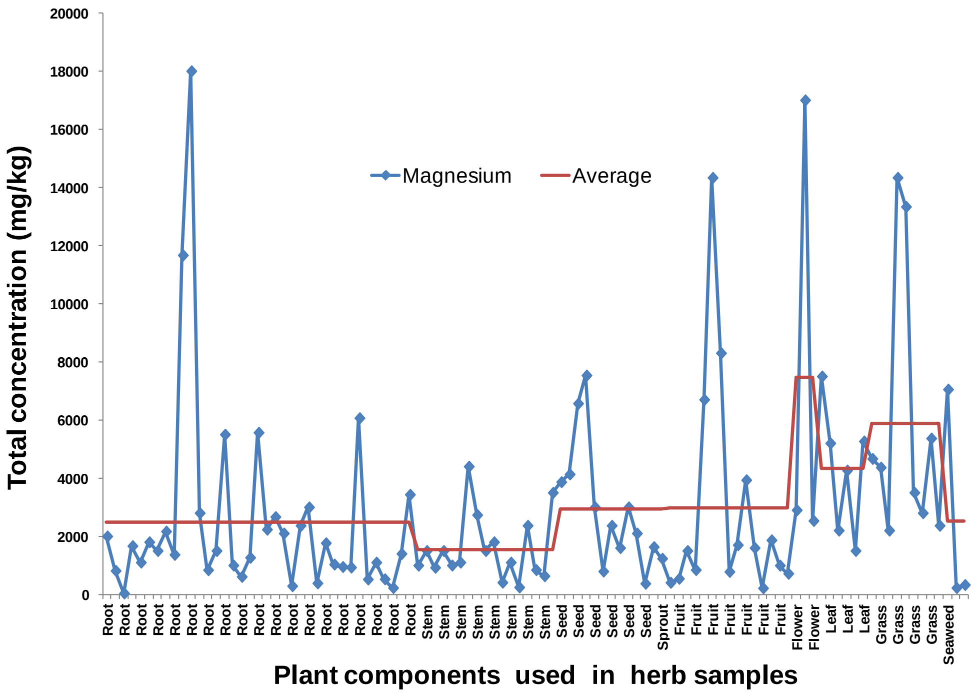


Figure 4.25.3: Variation of total concentration of Mg with different plant components used in herb samples.

Chapter 5: Conclusions and Recommendations

5.1 Conclusions

This study was driven by the need for a better understanding of the nature of potentially bioactive metallic elements in traditional Chinese herbs in relation to their use for specific medical conditions. A strategy has been devised and implemented to develop this novel area of bioinorganic chemistry. Therefore, to examine possible bioactive metallo species in different Chinese herbs, a library of traditional Chinese herbs (191 herbs) was established (Chapter 3) and a global database containing the total and bioavailable levels of twenty-four chemical elements in each of 103 herb samples (Table 3.1) selected from the herb library was thus generated (Appendix B). The measured twenty-four chemical elements in the global database include the essential elements: macrominerals (K, Na, Ca, and Mg), microminerals (Fe, Cu, Zn, Mn, Mo, Se, Cr, and Co) and trace elements (Sn, Ni, V, Bi and Li), as well as “alien” substances (Pb, As, Cd, Hg, Al, Sb and Ba) [22, 113, 174].

After elemental mapping on a wide range of well-characterized traditional Chinese herbs by ICP-MS and ICP-OES, it was demonstrated that both total extractable and bioavailable contents of the twenty-four chemical elements varied significantly across the different herbs (Chapter 4). The detectable total extractable concentrations of twenty-four chemical elements have been found to vary from around 0.000352 mg/kg (352 ppt) (Bi in *Da Huang*, herb number 24 in Table 3.1) to 55,000 mg/kg (55,000 ppm) (Ca in *Chuan Niu Xi*, herb number 79 in Table 3.1) on a dry weight basis.

In the group of microminerals (Fe, Cu, Zn, Mn, Mo, Se, Cr, and Co), the detectable total extractable concentrations of these elements ranged from around 0.00123 mg/kg (1230 ppt) (Se in *Jie Geng*, herb number 73 in Table 3.1) to 6500 mg/kg (6,500 ppm) (Fe in *Huang Jin*, herb number 42 in Table 3.1) on a dry weight basis. The Fe content in *Huang Jin* was found to be almost fully bioavailable (bioavailability 91 %). Across 103 herb samples tested, the total extractable contents of Fe (median value 200 mg/kg), Mn (median value 27.4 mg/kg), Zn (median value 19.6 mg/kg) and Cu (median value 7.71 mg/kg) were relatively high. However, Fe showed the lowest overall bioavailability among these four elements, with only 4 of 103 herbs having bioavailability > 80 %. Zn and Mn showed a significantly higher overall bioavailability, with their contents in more than 90 % of tested herb samples being almost fully bioavailable (bioavailability > 80 %). With respect to Fe and disease states, it is known the role of Fe in the brain is related to some neurological disorders such as Alzheimer disease and Parkinson disease [149]. Indeed, certain drugs are under development that are essentially Fe chelating ligands that have the ability to pass through the blood brain barrier [151]. Herbs that are found to sequester relatively large amounts of Fe (e.g. *Hong Hua* and *Tu Si Zi*, herb number 33 and 36, respectively) could contain novel, naturally occurring, iron species that assist in the development of futher candidates for drugs of this type. Therefore, further studies that involve an identification of the Fe species present are indicated. This provides one example of how the database generated by this project can stimulate further research. In a similar vein, *Pu Gong Yin* (herb number 19) showed relatively high levels for Cu, Fe, Co and Cr – all metals with implication for the development of metallo-pharmaceuticals.

The total extractable content of the trace elements (Sn, Ni, V, Bi and Li) in the herb samples was detected to be between 0.000352 mg/kg (Bi in *Da Huang*, herb number 24 in Table 3.1) and 61.0 mg/kg (Li in *Wu Jia Pi*, herb number 61 in Table 3.1) on a dry weight basis. The herbs showed a generally low level of Bi (median value 0.00799 mg/kg), but were relatively rich in Ni (median value 0.914 mg/kg). The overall bioavailability of these trace elements was generally low. Only *Long Yan Rou* (herb number 7 in Table 3.1) and *Lu Hui* (herb number 84 in Table 3.1) achieved a more than 80 % bioavailability for Bi and Sn, respectively. Only around 10 – 40 % of herbs tested achieved a more than 80 % bioavailability for V (10 %), Li (20 %) and Ni (40 %). In this study, *Wu Jia Pi* (herb number 61 in Table 3.1) had an extraordinarily high level of Li (61.0 mg/kg, median value 0.198 mg/kg) - which is almost fully bioavailable (bioavailability 88 %), within experimental error. *Wu Jia Pi* is widely used in TCM to dispel wind-damp which addresses painful obstruction syndromes characterised by rheumatic pain and stiffness in the limbs, joints, muscles and tendons [30]. Li compounds are well known medication for the treatment of bipolar disorder [177]. *Wu Jia Pi* may be used for the treatment of this medical condition. This represents yet another example of a herb that warrants further investigation as a potential drug lead. It was also found that the herbs had the highest levels in both total extractable (median value: 0.914 mg/kg) and bioavailable (median value: 0.591 mg/kg) contents of Ni among the trace elements detected. The average intake of the essential element Ni for humans is 0.3 – 0.5 mg/day [113] and this element is toxic at high concentration. Therefore, from the clinical point of view, it is necessary to evaluate the safety of using TCM by the determination of Ni concentration. Among the 103 herbs tested, *Pu Gong Yin*, *Zi Cao (B)*, *Ma Chi Xian*, *Hai Zao* and *He Ye* (herb number 19, 67, 21, 62 and 81 in Table 3.1, respectively) showed a relatively high level in both total and bioavailable contents of V. These herbs could be candidatures for treatment of diabetes and cancer.

The elements (K, Na, Ca, and Mg) in the macrominerals group were found to be generally rich in the herb samples tested. The median value of the total extractable concentration was in an order of K (7900 mg/kg) > Ca (4200 mg/kg) > Mg (1800 mg/kg) > Na (160 mg/kg). The overall bioavailability of these elements was also relatively high. The contents of K and Mg in the herb samples studied are almost fully bioavailable (bioavailability > 80 %). Most of herbs achieved a more than 80 % bioavailability of Na and Ca. Among 103 herbs tested, *Chuan Niu Xi* (herb number 79 in Table 3.1) was found to have the highest level in both total extractable and bioavailable contents of Ca (around 55,000 mg/kg). According to the United States Department of Agriculture National Nutrient Database [227], Parmesan cheese has the Ca content around 11,000 mg/kg (55 mg Ca per common measure of 5 g cheese). *Chuan Niu Xi* had much higher bioavailable content of Ca than Parmesan cheese. This herb may be used as a Ca source for people who have Ca deficiency but are allergic to the Ca-rich foods such as dairy products.

The non-essential elements (Pb, As, Cd, Hg, Al, Sb and Ba) measured in this study can be toxic to living organisms above certain concentration levels. Among these elements, Al had the highest median value (110 mg/kg) of the total extractable content but showed an overall low bioavailability, with only 5 % of herb samples having achieved a bioavailability Al > 80 %. Among 103 herb samples tested, *Huang Lian* (herb number 41 in Table 3.1) had an extraordinarily high level for the total extractable content of Ba – suggesting significant enrichment although this Ba content is non-bioavailable (bioavailability 1 %). In spite of the low bioavailability, the speciation of Ba in this herb is certainly of great interest since it could reveal one or more novel Ba specific ligands (probably ionophoric). The determined total extractable contents of the other elements in this group were generally low. Compared to the other herb samples, *Hong Hua* (herb number 33 in Table 3.1) showed a relatively high level in the total extractable content of all the elements (Pb 22.5 mg/kg, As 4.27 mg/kg, Hg 0.0274 mg/kg, Al 1030 mg/kg, Sb 1.33 mg/kg, Ba 3010 mg/kg) in this group except Cd. However, its overall bioavailability (< 30 %) for these elements was generally low – therefore perhaps less toxic.

The results obtained from the enrichment factor analyses suggest that both plant characteristics and local environment influenced the concentrations of the elements tested in the current study. The herb samples studied in the current work demonstrated a generally strong enrichment ability (EF > 10) for K, Na, Ca, Mg, Zn, Cu, Se, Mo, Li, Hg, Cd, and Sb, but showed a relatively low enrichment capability for Fe, Co, Bi, and V. From the clinical point of view, for those herbs having strong enrichment ability for heavy metals Hg, Cd, and Sb, it is necessary to evaluate their heavy metal content prior to consumption.

The concentration distribution of elements also varied with respect to plant part used. In general, the flower, grass and leaf-derived herb samples had a relatively high level in the average of total extractable concentration of elements compared to other plant components used in herbs.

5.2 Recommendations for Future Work

The current work has outlined the total extractable and bioavailable contents of 24 elements determined by ICP-OES and ICP-MS across 103 Chinese herbal samples. This has led to some basic understanding of the trends of levels of these 24 elements for each herb sample and the enrichment capability of this herb for these elements. However, to better understand the nature of roles of each element in herbs, further studies should be focused on the chemical and biological implications of each element (speciation investigations) in Chinese herbs collected in our herb library. Table 5.1

lists the candidature herbs that would be recommended from this study for further chemical enquiry, including speciation investigations, for different applications including pharmaceutical, chemical, nutritional and toxicity applications. The candidature herbs for further investigations for chemical applications were recommended based on their total extractable contents of the elements measured, while those herbs recommended for further investigations for pharmaceutical, nutritional and toxicity applications were selected based on their bioavailable contents of the elements.

Table 5.1: Candidature herbs identified in this study for further enquiry - including speciation investigations. *Note that this is not necessarily exhaustive and the data may be interrogated in other ways.*

	Fe	Cu	Zn	M	M	Se	Cr	Co	Sn	Ni	V	Bi	Li	Pb	As	Cd	Hg	Al	Sb	Ba	K	Ca	Na	M
Huang Jin (42)																								
Pu Gong Yi (19)																								
Hong Hua (33)																								
Zi Cao(B) (67)																								
Tu Si Zi (36)																								
Tian Ma (47)																								
Si Chang Pu (86)																								
Zhi Shi (29)																								
Lu Hui (84)																								
Qiang Huo (68)																								
Niu bang Zi (70)																								
Duo Huo (30)																								
Lian Zi (76)																								
Chuan Niu Xi (79)																								
Yin Chen (53)																								
Chai Hu (60)																								
Huang Lian (41)																								
Ze Xie (101)																								
Dan Zhu Ye (50)																								
Jiang Huang (94)																								
Sang Ji Sheng (77)																								
He Ye (81)																								
Huang Qi (35)																								
Bu Gu Zi (64)																								
Sheng Di (49)																								
Shu Di (5)																								
Jin Yin hua (13)																								
Suan Zao Ren (59)																								
Bai Hua She SheCao (23)																								
Huo Xiang (98)																								
Ji Xue Teng (83)																								
Ma Chi Xian (21)																								
Hai Zao (62)																								
Sang Ye (48)																								
Zi Su Ye (91)																								
Wu Jia Pi (61)																								
Lu Bu Ma (46)																								
Lian Qiao (15)																								
Shan Qi (34)																								
Zi Cao (H)																								

Pharmaceutical applications — Chemical applications — Nutritional applications — Toxicity applications

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Appendix A

Eighteen commonly used herbal categories and over 300 Chinese herbs. The 191 herbs that have been highlighted in yellow represent the entire collection. The herbs highlighted in pink represent the subset of 103 herbs chosen for investigation. These are also numerically coded as in Table 3.1

Table I. Herbs that Release the Exterior

	Pharmaceutical Name	Family	English Name
Ma Huang	Ephedrae Herba	Ephedraceae	Ephedra Stem
Gui Zhi (9)	Cinnamomi	Lauraceae	Cinnamon twig
Zi Su Ye (91)	Perillae Folium	Labiatae	Perilla leaf
Jing Jie	Schizonepetae Herba	Labiatae	Schizonepeta stem or bud
Fang Feng	Saposhnikoviae Radix	Apiacae	Saposhnikovia root
Qiang Huo (68)	Notopterygii Rhizoma seu Radix	Apiaceae	Notopterygium root
Gao Ben	Ligustici Rhizoma	Apiaceae	Chinese lovage root
Bai Zhi (74)	Angelicate dahuricate Radix	Apiaceae	Angelica root
Xi Xin	Asari Herba	Aristolochiaceae	Chinese wild ginger
Sheng Jiang (58)	Zingberis Rhizoma recens	Zingiberaceae	Fresh ginger rhizome
Cong Bai	Allii fistulosi bulbus	Liliaceae	Spring onion
Xiang Ru	Moslae Herba	Labiatae	Mosla
Cang Er Zi	Xanthii Fructus	Asteraceae	Xanthium fruit
Cang Er Cao	Xanthii Herba	Asteraceae	Cockleburr
Xin Yi Hua	Magnoliae Flos	Magnoliaceae	Magnolia flower
E Bu Shi Cao	Centipedae Herba	Asteraceae	Small centipede herb
Xi He Liu	Tamaricis Cacumen	Tamaricaceae	Tamarisk stems and leaves
Bo He (69)	Menthae haplocalycis Herba	Lamiaceae	Field mint
Niu Bang Zi (70)	Arctii Fructus	Asteraceae	Great burdock fruit
Chan Tui	Cicadae Periostracum	Cicadidae	Cicada Molting
Sang Ye (48)	Mori Folium	Moraceae	White mulberry leaf
Ju Hua (45)	Chrysanthemi Flos	Asteraceae	Chrysanthemum flower
Man Jing Zi	Viticis Fructus	Verbenaceae	Vitex fruit
San Sou Chi	Sojae Semen Preparatum	Fabaceae	Prepared soybean
Da Dou Chi	Sojae Semen germinatum	Fabaceae	Young soybean sprout

Fu Ping	Spirodelae Herba	Lemnaceae	spirodela
Mu Zei	Equiseti hiemalis Herba	Equistetaceae	Shave grass
Ge Gen (78)	Puerariae Radix	Fabaceae	Kudzu root
Chai Hu (60)	Bupleuri Radix	Apiaceae	Thorowax root
Sheng Ma	Cimicifufae Rhizoma	Ranunculaceae	cimicifuga

Table II Herbs that Clear Heat

	Pharmaceutical Name	Family	English Name
Shi Gao	Gypsum fibrosum		gypsum
Zhi Mu	Anemarrhenae Rhizoma	Liliaceae	Anemarrhena rhizome
Zhi Zi	Gardeniae Fructus	Rubiaceae	Gardenia fruit
Dan Zhu Ye (50)	Lophateri Herba	Gramineae	Lophatherum stem and leaves
Ya Zhi Cao	Commelinaceae Herba	Commelinaceae	Common dayflower herb
Xi Gua	Citrulli Fructus	Cucurbitaceae	Watermelon fruit
Xia Ku Cao (16)	Prunellae Spica	Labiatae	Prunella
Han Shui Shi	Glauberitum		Calcitum
Lian Zi Xin	Nelumbinis Plumula	Nymphaeaceae	Lotus plumule
Lu Gen	Phragmitis Rhizoma	Gramineae	Reed rhizome
Tian Hua Fen	Trichosanthis Radix	Cucurbitaceae	Trichosanthes
Jue Ming Zi	Cassiae Semen	Fabaceae	Cassia seeds
Qing Xiang Zi	Celosiae Semen	Amaranthaceae	Celosia seeds
Mi Meng Hua	Buddlejae Flos	Loganaiaceae	Buddleia flower bud
Gu Jing Cao	Eriocauli Flos	Eriocaulaceae	Pipewort scapus
Ye Ming Sha	Vespertilionis Faeces	Vespertilionidae	Bat feces
Shui Niu Jao	Bubali Cornu	Bovidae	Water buffalo horn
Sheng Di Huang (49)	Rehmanniae Redix	Scrophulariaceae	Rehmannia root
Xuan Shen	Scrophulariae Radix	Scrophulariaceae	Scrophularia
Mu Dan Pi (85)	Moutan Cortex	Ranunculaceae	Moutan root bark

Zi Cao (66)	Arnebiae/Lithospermi	Boraginaceae	Groomwell root
Huang Qin (54)	Scutellariae Radix	Labiatae	Scutellaria
Zi Cao (67)	Arnebiae/Lithospermi	Boraginaceae	Groomwell root
Huang Lian (41)	Coptidis Rhizome	Ranunculaceae	Coptis rhizome
Huang Bai (100)	Phellodendri	Rutaceae	Phellodendron bark
Long Dan Cao	Gentianae Radix	Gentianaceae	Gentian root
Ku Shen (14)	Sophorae flavescentis Radix	Fabaceae	Sophora root
Qi Pi (12)	Fraxini Cortex	Oleaceae	Fraxinus bark
Jin Yin Hua (13)	Lonicerae Flos	Caprifoliaceae	Honeysuckle flower
Ren Dong Teng	Lonicerae Caulis	Caprifoliaceae	Honeysuckle vine
Lian Qiao (15)	Forsythiae Fructus	Olesceae	Forsythia fruit
Da Qing Ye	Isatidis Folium	Apiaceae	Isatis leaf
Nan Ban Lan Ye	Baphicacanthis Folium	Acanthaceae	Baphicacanthus leaf
Ban Lan Gen	Isatidis/baphicacanthis Radix		
Bei Ban Lan Gen	Isatidis Radix	Apiaceae	Isatis root
Nan Ban Lan Gen	Baphicacanthis cusiae Rhizoma	Acanthaceae	Baphicacanthus root
Qing Dai	Indigo naturalis	Apiaceae	Indigo
Pu Gong Ying (19)	Taraxaci Herba	Asteraceae	Dandelion
Zi Hua Di Ding	Violaceae	Violaceae	Vilet
Ye Ju Hua	Chrysanthemi indici Flos	Asteraceae	Wild chrysanthemum flower
Si Gua Luo (87)	Luffae fructus Retinervus	Cucurbitaceae	Loofah
Tian Kui Zi	Semiaquilegiae Radix	Ranunculaceae	Semiaquilegia root tuber
Hong Tian Kui	Begoniae fimbristipulatae Herba	Begoniaceae	Tea begonia
Long Kui	Solani nigri Herba	Solanaceae	Black nightshad
Wan Nian Qing	Rohdeae japonicae Radix et Rhizoma	Liliaceae	Rohdea root
Bai Jiang Cao (20)	Patriniae Herba	Valerianaceae	Patrinia
Su Bai Jiang	Thlaspi Herba	Apiaceae	Thlaspi

Yu Xing Cao	Houttuyniae Herba	Saururaceae	Houttuynia
Chong Lou	Paridis Rhizoma	Liliaceae	Pairs rhizome
Quan Shen	Bistortae Rhizoma	Polygonaceae	Bistort rhizome
Chuan Xin Lian	Andrographitis Herba	Acanthaceae	Andrographis
Bai Hua She She Cao (23)	Hedyotis diffusae Herba	Rubiaceae	Heydyotis
Shan Ci Gu	Cremastrae/Pleiones Pseudobulbus	Orchidaceae	Cremastra/Pleione
Bai Tou Weng	Pulsatillae Radix	Ranunculaceae	Chinese anemone root
Wei Ling Cai	Potentillae chinensis Herba	Roseceae	Chinese silverweed
Ya Dan Zi	Bruceae Fructus	Simarubaceae	Brucea fruit
Ma Chi Xian (21)	Portulacae Herba	Portulacaceae	Purslane
He Ye (81)	Nelumbinis Folium	Nymphaeaceae	Lotus leaf
Lu Dou	Phaseolin radiati Semen	Fabaceae	Mung bean
Hong Teng (17)	Sargentodoxae caulis	Lardizabalaceae	Asrgentodoxa vine
Bai Xian Pi (22)	Dictamni Cortex	Rutaceae	Dictamnus root bark
Tu Fu Ling	Smilacis glabrae Rhizoma	Liliaceae	Smilax
Cuan pen	Sedi Herba	Crassulaceae	Hanging stonecrop
Ji Gu Cao	Abri Herba	Fabaceae	Prayer-beads
Ji Xue Cao	Centellae Herba	Apiaceae	Centella
Ban Zhi Lian	Scutellariae barbatae Herba	Labiatae	Bearded scutellaria
Ma Bo	Lasiosphaera/calvatia	Lycoperdaceae	Puffball
Shan Dou Gen	Sophorae tonkinenesis Radix	Fabaceae	Bushy sophora
Bei Dou Gen	Menispermi Rhizoma	Menispermaceae	Asiatic moonseed rhizome
Qing Guo	Canarii Fructus	Burseraceae	Chinese white olive
She Gan	Belamcandae Rhizoma	Iridaceae	Belamcanda
Hui Niu Xi (56)	Achyranthis Radix terrena	Amaranthaceae	Local achyranthranthis root
Bai Lian	Ampelopsis Radix	Vitaceae	Ampelopsis

Lou Lu	Rhapontici Radix	Asteraceae	Rhaponticum root
Yu Zhou Lou Lu	Echinopsis Radix	Asteraceae	Echinops
Shi Shang Bai	Selaginellae doederleinii Herba	selaginellaceae	selaginella
Qing Hao	Artemisiae annuae Herba	Asteraceae	Sweet wormwood
Di Gu Pi	Lycii Cortex	Solanaceae	Lycium bark
Bai Wei	Cynanche Atrati Radix	Asclepiadaceae	Cynanche root
Yin Chai Hu	Stellariae Radix	Caryophyllaceae	Stellaria root
Hu Huang Lian	Picrorhizae rhizome	Scrophulariaceae	Picrorhiza rhizome

Table III Downward-draining Herbs

	Pharmaceutical Name	Family	English Name
Da Huang (24)	Rhei Radix et Rhizom	Polygonaceae	Rhubarb root and rhizome
Mang Xiao	Natrii Sulfas	Mineral	Mirabilite
Fan Xie Ye	Sennae Folium	Fabaceae	Senna leaf
Lu Hui (84)	Aloe	Liliaceae	Dried concentrate of the juice of the aloe leaf
Huo Ma Ren (27)	Cannabis Semen	Cannabaceaea	Hemp seeds
Yu Li Ren	Pruni Semen	Rosaceae	Bush cherry pit
Qian Niu Zi	Pharbitidis Semen	Convolvulaceae	Morning glory
Gan Sui	Kansui Radix	Euphorbiaceae	Kan-sui root
Da Ji	Knoxiae/Euphorbiae Radix		
Hong Da Ji	Knoxiae Radix	Euphorbiaceae	Knoxia root
Jing Da Ji	Euphorbiae pekinensis Radix	Euphorbiaceae	Euphorbia root
Yuan Hua	Genkwa Flos	Thymeleaceae	Genkwa flower
Ba Dou	Crotonis Frutus	Euphorbiaceae	Croton seed
Shang Lu	Phytolaccae Radix	Phytolaccaceae	Poke root

Table IV Herbs that Drain Dampness

	Pharmaceutical Name	Family	English Name
Fu Ling (3)	Poria	Polyporaceae	China root
Zhu Ling	Polyporus	Polyporaceae	Polyporus
Ze Xie (101)	Alismatis Rhizoma	Alismataceae	Alisma rhizome
Yi Yi Ren (99)	Coicis Semen	Gramineae	Coix seeds
Che Qian Zi	Plantaginis Semen	Plantaginaceae	Plantago
Hua Shi	Talcum	Mineral	Talcum
Chuan Mu Tong	Clemaitdis armandii caulis	Ranunculaceae	Clematidis caulis
Tong Cao	Tetrapanacis medulla	Araliaceae	Rice paper plant pith
Deng Xin Cao	Junci Medulla	Juncaceae	Juncus pith
Qu Mai	Dianthi Herba	Caryophyllaceae	Dianthus
Bian Xu	Polygoni avicularis Herba	Polygonaceae	Knotgrass
Shi Wei	Pyrrosiae Folium	Polypodiaceae	Pyrrosia
Di Fi Zi	Kochiae Fructus	Chenopodiaceae	Kochia fruit
Dong Gua Zi	Benincasae Semen	Cucurbitaceae	Winter melon seed
Dong Gua Pi	Benincasae Exocarpium	Cucurbitaceae	Winter melon rind
Dong Kui Guo	Malvae Fructus	Malvaceae	Mallow fruit
Qing Ma Zi	Abutili Semen	Malvaceae	India mallow seed
Bi Xie	Dioscoreae hypoglaucea Rhizoma	Dioscoreaeceae	Tokoro
Mian Bi Xie	Dioscoreae septemlobae Rhizoma	Dioscoreaeceae	Seven-lobed tam rhizome
Jin Qian Cao	Lysimachiae Herba	Primulaceae	lysimachia
Hai Jin Sha	Lygodii spora	Lygodiaceae	lygodium
Chi Xiao Dou	Phaseolin Semen	Fabaceae	Adzuki bean
Yin Chen (40)	Artemisiae scopariae Herba	Asteraceae	Virgate wormwood
Fang Ji (102)	Stephaniae tetrandrae Radix	Menispermaceae	Stephania root
Ban Bian Lian	Lobeliae chinensis Herba	Campanulaceae	Chinese lobelia
Yu Mi Xu	Maydis Stigma	Gramineae	Cornsilk

Table V Hebs that Dispel Wind-Dampness

	Pharmaceutical Name	Family	English Name
Du Huo (30)	Angelicae pubescentis Radix	Apiaceae	Pubescent angelica root
Wei Ling Xian	Clematidis Radix	Ranunculaceae	Clematis root
Hai Tong Pi	Erythrinae Cortex	Fabaceae	Erythrina bark
Mu Gua	Chaenomelis Fructus	Rosaceae	Chaenomeles
Can Sha	Bombycis faeces	Bombycidae	Silkworm feces
Sang Ji Sheng (77)	Taxilli Herba	Loranthaceae	Taxiius
Hu Ji Sheng	Viscid	Loranthaceae	Colored mistletoe
Wu Jia Pi (61)	Acanthopanacis cortex	Araliaceae	Acanthopanax root bark
Xiang Jia Pi	Perplocae Cortex	Asclepiadaceae	Chinese silkvine root bark
Lao Guan Cao	Erodii/Geranii Herba	Geraniaceae	Cranesbill
Lu xian Cao	Pyrolae Herba	Pyrolaceae	Pyrola
Shen Jin Cao	Lycopodii Herba	Lycopodiaceae	Groung pine
Xu Chang Qing	Cynanche pancullati Radix	Asclepiadaceae	Paniculate cynanchum
Chuan Shan Long	Dioscoreae nipponicae Rhizoma	Dioscoreaceae	Japanese dioacorea rhizome
Qing Feng Teng	Sinomenii Caulis	Menispermaceae	Orient vine
Liang Mian Zhen	Zanthoxyli Radix	Rutaceae	Shiny bramble
Hai Feng Teng	Piperis kadsurae Caulis	Piperaceae	Kadsura pepper stem
Qian Nian Jian	Homalomenae Rhizoma	Araceae	Homalomena rhizome
Qin Jiao	Gentianae macrophyllae Radix	Gentianaceae	Large gentian root
Sang Zhi (37)	Mori Ramulus	Moraceae	Mulberry twig
Xi Xian Cao	Siegesbeckiae Herba	Asteraceae	Siegesbeckia
Chou Wu Tong	Clerodendri Folium	Verbenaceae	Clerodendron leaf
Luo Shi Teng	Trachelospermi Caulis	Apocynaceae	Star jasmine stem
Kuan Jin Teng	Tinosporae sinensis Caulis	Menispermaceae	Chinese tinospora stem
Bai Hua She	Agkistrodon/Bungsrus		

Qi She	Agkistrodon	Viperdae	Agkistrodon
Wu Shao She	Zaocys	Colubridae	Black-striped snake
She Tui	Serpentis Periostracum	Colubridae	Snake skin slough
Qiu Gua Luo (80)	Luffae fructus Retinervus	Cucurbitaceae	Loofah
Song Jie	Pini Lignim nodi	Pinaceae	Knotty pine wood

Table VI Herbs that Transform Phlegm and Stop Coughing

	Pharmaceutical Name	Family	English Name
Qian Hu	Peucedani Radix	Apiaceae	Peucedanim root
Bei Mu	Fritillariae Bulbus		
Chuan Bei Mu	Fritillariae cirrhosea Bulbus	Liliaceae	Sichuan fritillaria bulb
Zhe Bei Mu	Fritillariae thunbergia Bulbus	Liliaceae	Zhenjiang fritillaria bulb
Gua Lou	Trichosanthis Fructus	Cucurbitaceae	Trichosanthes fruit
Gua Lou Pi	Trichosanthis Pericarpium	Cucurbitaceae	Trichosanthes peel
Gua Lou Ren	Trichosanthis Semen	Cucurbitaceae	Trichosanthes seed
Tian Zhu Huang	Bambusae Concretio silicea	Gramineae	Bamboo sugar
Zhu Li	Bambusae Succus	Gramineae	Bamboo sap
Zhu Ru (90)	Bambusae Caulis in taeniam	Gramineae	Bamboo shavings
Fu Shi	Pumex	Mineral	Pumice
Fu Hai Shi	Costaziae Os	Celloporidae	Constaziae skeleton
Qing Meng Shi	Chlorite Lapis	Mineral	Chlorite
Jin Meng Shi	Micae lapis aureus	Mineral	Vermiculite schist
Ge Qiao	Meretricis/cyslinae Concha	Veneridae	Clam shell
Kun Bu	Eckloniae Thallus	Alariaceae	Kelp
Hai Zao (62)	Sargassum	Sargassacea	Sargassum
Pang Da Hai	Sterculiae lychnophorae Semen	Sterculiaceae	Sterculia seed
Ze Qi	Euphorbiae helioscopiae Herba	Euphorbiaceae	Sun spurge
Huang Yao Zi	Dioscoreae bulbiferae	Dioscoreaceae	Dioscorea bulbiferra

	Rhizoma		tuber
Ming Dang Shen	Changii Radix	Apiaceae	Changium root
Bi Qi	Eleocharitis Rhizoma	Cyperaceae	Water chestnut
Ban Xia (97)	Pinekkiae rhizome preparetum	Araceae	Pinellia rhizome
Zhi Tian Nan Xing	Arisaematis rhizome preparatum	Araceae	Prepared arisaema rhizoma
Bai Jie Zi (72)	Sinapis Semen	Apiaceae	White mustard seed
Zao Jia	Gleditsiae Frustus	Fabaceae	Gleditsia fruit
Zao Jjiao Ci	Gleditsiae Spina	Fabaceae	Gleditisia thorn
Jie Geng (73)	Platycodi Radix	Campanulaceae	Platycodon root
Xuan Fu Hua	Inulae Flos	Asteraceae	Inula flower
Bai Qian	Cynanche stauntonii Rhizoma	Asclepiadaceae	Cynanchum root
Xing Ren (71)	Armeniacae Semen	Roseceae	Apricot seed or kernel
Zi Wan	Asteris Radix	Asteraceae	Aster root
Kuan Dong Hua	Farfarae Flos	Asteraceae	Tussilago flower
Zi Su Zi	Perillae Fructus	Labiatae	Perila fruit
Pi Pa Ye	Eriobotryae Folium	Rodaceae	Loquat laef
Bai Bu	Atemonae Raidx	Stemonaceae	Stemona root
Sang Bai Pi (103)	Mori Cortex	Moraceae	Mulberry root bark
Ting Li Zi	Lepidii/Descurainiae Semen	Apiaceae	Tingli seed
Zi Jin Niu	Ardisiae jiponicea Herba	Myrsinadeae	Japanese ardisia
Mu Hu Die	Oroxyli Semen	Bignoniaceae	Oroxylum seed
Luo Han Guo (93)	Momordicae Fructus	Cucurbitaceae	Momordica fruit
Gua Di	Melo Pedicellus	Cucurbitaceae	Melon stalk
Li Lu	Veratri nigri Radix rt Rhizoma	Liliaceae	Veratrum root and rhizoma

Table VII Aromatic Herbs that Transform Dampness

	Pharmaceutical Name	Family	English Name
Cang Zhu (95)	Atractylodis Rhizoma	Asteraceae	Atractylodes rhizome
Hou Po	Magnoliae offocinalis Cortex	Magnoliaceae	Magnolia bark
Huo Xiang (98)	Pogostemonis/Agastaches Herba	Labiatae	Agastache
Guang Huo Xiang	Pogostemonis Herba	Labiatae	Patchouli
Pei Lan (57)	Eupatorii Herba	Asteraceae	Eupatorium
Sha Ren	Amomi Fructus	Zingberaceae	Amomum fruit
Bai Dou Kou	Amomi Fructus rotundus	Zingberaceae	Round cardamon
Hong Dou Kou	Galangae Fructus	Zingberaceae	Major galangal seeds
Cao Dou Kou	Alpiniae katsumadai Semen	Zingberaceae	Katsumada's galangal seeds
Cao Guo	Tsaoko Fructus	Zingberaceae	Tsaoko fruit

Table VIII Herbs that Relieve Food Stagnation

	Pharmaceutical Name	Family	English Name
Shan Zha (28)	Crataegi Fructus	Rosaceae	Crataegus fruit
Hong Qu Mi (82)	Massa medicate fermentata	None: fermented product of several different plants	Red Yeast Rice
Mai Ya (52)	Hordei Fructus germinatus	Gramineae	Barley sprouts
Gu Ya	Setariae Fructus germinatus	Gramineae	Grain sprouts
Su Ya	Setariae Fructus germinatus	Gramineae	Millet sprouts
Dao Ya	Oryzae Fructus germinatus	Gramineae	Rice sprouts
Lai Fu Zi (32)	Raphani Semen	Apiaceae	Radish seed
Ji Nei Jin	Gigeriae galli endothelium corneum	Phasianidae	Gizzard lining

Table IX Herbs that Regulate the Qi

	Pharmaceutical Name	Family	English Name
Chen Pi (96)	Citri reticulatae Pericarpium	Rutaceae	Aged tangerine peel
Ju Hong	Citri reticulatae Exocarpium rubrum	Rutaceae	Pomelo flavedo
Qing Pi	Citri reticulatae viride Pericarpium	Rutaceae	Unripe tangerine peel
Zhi Shi (29)	Aurantii fructus immaturus	Rutaceae	Unripe bitter orange
Zhi Ke	Aurantii fructus	Rutaceae	Bitter orange
Fo Shou	Citri sarcodactylis Fructus	Rutaceae	Finger citron fruit
Gan Song	Nardostachydis Radix seu Rhizoma	Valerianaceae	Nardostachys root
Xiang Yuan	Citri Fructus	Rutaceae	Citron
Da Fu Pi	Arecae Pricarpium	Palmae	Areca husk
Xiang Fu	Cyperi Rhizoma	Cyperaceae	Cyperus
Mu Xiang	Aucklandiae Radix	Asteraceae	Aucklandia
Wu Yao	Linderae Radix	Lauraceae	Lindera root
Chen Xiang	Aquilariae lignum resinatum	Thymeleaceae	Aquilaria wood
Tan Xiang	Santali albi lignum	Santalaceae	Sandalwood
Xie Bai	Allii macrostemi Bulbus	Liliaceae	Chinese garlic
Mei Gui Hua	Rosae rugosae Flos	Rosaceae	Rosebud
Dao Dou	Canavaliae Semen	Fabaceae	Sword bean
Chuan Lian Zi (51)	Toosendan Fructus	Meliaceae	Toosendan fruit
Li Zhi He	Litchi Semen	Sapindaceae	Leechee nut
Shi Di	Kaki Calyx	Ebenaceae	Persimmon calyx
Ba Yue Zha	Akebiae Fructus	Lardizabalaceae	Akebia fruit
Suo Luo Zi	Aesculi Semen	Hippocastanaceae	Horse chestnut
Zi Su Geng	Perillae caulis	Labiatae	Perilla stem

Table X Herbs that Regulate the Blood

	Pharmaceutical Name	Family	English Name
San Qi (34)	Notoginseng Radix	Araliaceae	Nitogingseng root
Pu Huang	Typhae Pollen	Typhaceae	Pryha pollen
Qian Cao	Rubiae	Rubiaceae	Madder root
Da Ji	Cirsii japonica Herba sive Radix	Asteraceae	Large thistle
Xiao Ji	Cirsii Herba	Asteraceae	Small thistle
Di Yu	Sanguisorbae Radix	Rosaceae	Sanguisorba
Huai Mi	Sophorae Flos immaturus	Fabaceae	Sophora bud
Huai Hiao	Sophorae Fructus	Fabaceae	Sophora fruit
Ce Bai Ye	Platycladi Cacumen	Cupressaceae	Oriental arborvitae leaf twig
Bai Mao Gen	Imperatae	Gramineae	Imperata rhizome
Zhu Ma Gen	Boehmeriae Radix	Urticaceae	Ramie root
Zi Zhu	Callicarpae formosanae Folium	Verbenaceae	Beauty-berry leaf
Xian He Cao	Agrimoniae Herba	Rosaceae	Agrimony
Bai Ji	Bletillae Rhizoma	Orchidaceae	Bletilla rhizome
Zong Lu Pi	Trachycarpi Petiolus	Palmae	Trachycarpus stiple fiber
Ou Jie	Nelumbinis Nodis rhizomatis	Nymphaeaceae	Lotus rhizome node
Lian Fang	Nelumbinis recepaculum	Nymphaeaceae	Lotus receptacle
Hua Rui Shi	Ophicalcitum	Mineral	Ophicalcite
Ai Ye	Aartemisiae Argyi Folium	Asteraceae	Mugwort leaf
Zao Xin Tu	Terra flava usta	Inorganic material	Oven earth ignited yellow earth
Chuan Xiong (4)	Chuanxiong Rhizoma	Apiaceae	Chuanxiong root
Dan Shen (38)	Salviae miltiorrhizae	Laiatae	Salvia root
Ji Xue Teng (83)	Spatholobi Caulis	Fabaceae	Spatholobus root and vine
Yan Hu Suo	Corysalis Rhizoma	Papaveraceae	Corydalis rhizome

Yu Jin (40)	Curcumae Radix	Zingiberaceae	Curcuma
Jiang Huang (94)	Curcumae longae Rhizoma	Zingiberaceae	Turmeric rhizome
Yi Mu Cao (88)	Leonuri Herba	Labiatae	Chinese mothwort
Ze Lan	Lycopi Herba	Labiatae	Lycopos
Yue Ji Hua	Rosae chinensis Flos	Rosaceae	China tea rose flower
Hu Zhang (39)	Polygoni cuspidate Rhizoma	Polygonaceae	Bushy knotweed rhizome
Chi Shao (18)	Paeoniae Radix rubra	Ranunculaceae	Red peony root
Tao Ren (25)	Persicae Semen	Rosaceae	Peach kernel
Hong Hua (33)	Carthami Flos	Asteraceae	Safflower
Fan Hong Hua	Croci Stigma	Iridaceae	Saffron
E Zhu	Curcumae rhizome	Zingiberaceae	Curcuma rhizome
San Leng	Sparganii rhizome	Sparganiaceae	Sparganium
Ru Xiang	Olibanum	Burseraceae	Frankincense
Mo Yao (65)	Myrrha	Burseraceae	Myrrh
Niu Xi	Achyranthis bidentatae Radix	Amarathaceae	Achyranthes root
Chuan Niu Xi (79)	Cyathulae Radix	Amaranthaceae	Cyathula root
Wang Bu Liu Xing	Vaccariae Semen	Caryophyllaceae	Vaccaria seed
Lu Lu Tong	Liquidambaris Fructus	Hamamelidaceae	Liquidambar fruit
Lliu Ji Nu	Artemisiae anomalae Herba	Asteraceae	Anomalous artemesia
Zi Ran Tong	Pyritum	Mineral	Pyrite
Xue Jie	Daemonoropis Resina	Palmae	Dragon’s blood
Su Mu	Sappan ligum	Fabaceae	Sappan wood
Wu Ling Zhi	Trogopterori Faeces	Petauristidae	Flying squirrel feces
Jiang Xiang	Dalbergiae odoriferae Lignum	Fabaceae	Dalbergia heartwood
Wa Leng zi	Arcae Concha	Arcidae	Ark shell
Shui Zhi	Hirudo hirudinidae	Hirudinidae	Leech
Tu Bie Chong	Eupolyphaga	Blattidae	Ground beetle

	/Steleophaga		
Gan Qi	Toxicodendri Resina	Anacardiaceae	Lacquer
Ling Xiao Hua	Campsis Flos	Bignoniaceae	Campsis flower
Mao Dong Qing	Llicis pubescentis Radix	Aquifoliaceae	Hairy holly root
Ma Bian Cao	Verbenae	Herba	Verbena
Gui Jian Yu	Euonymi Ramulus	Celastraceae	Spondke tree wings

Table XI Herbs that Warm the Interior and Expel Cold

	Pharmaceutical Name	Family	English Name
Zhi Fu Zi	Aconiti Radix lateralis preparata	Ranunculaceae	Processed aconite accessory root
Zhi Chuan Wu	Aconiti Radix preparata	Ranunculaceae	Processed Sichuan aconite main root
Zhi Cao Wu	Aconiti kusenezoffii Radix preparata	Ranunculaceae	Processed wild aconite root
Gan Jiang	Zingiberis Rhizoma	Zingiberaceae	Dried ginger rhizome
Rou Gui	Cinnamomi Cortex	Lauraceae	Cinnamon bark
Wu Zhu Yu	Evodiae fructus	Rutaceae	Evodia fruit
Hua Jiao	Zanthoxyli Pericarpium	Rutaceae	Sichuan pepper
Gao Liang Jiang	Alpiniae officinarum Rhizoma	Zingiberaceae	Lesser galangal rhizome
Ding Xiang	Caryophylli Flos	Myrtaceae	Clove
Xiao Hui Xiang	Foeniculi Fructus	Apiaceae	Fennel fruit
Bi Cheng Qie	Litseae fructus	Lauraceae	Cubeb fruit
Bi Ba	Piperis longi Fructus	Piperaceae	Long pepper fruit
Hu Jiao	Piperis Fructus	Piperaceae	Pepper

Table XII Tonifying Herbs

	Pharmaceutical Name	Family	English Name
Ren Shen (11)	Ginseng Radix	Araliaceae	Ginseng root
Dang Shen	Codonopisis Radix	Campanulaceae	Codonopsis root
Tai Zi Shen	Pseudostellariae Radix	Caryophllaceae	Pseudostellaria root
Huang Qi (34)	Astragali Radix	Fabaceae	Astragalus root
Shan Yao (75)	Dioscoreae Rhizoma	Dioscoreaceae	Chinese yam
Bai Zhu	Atractylodis macrocephalae Rhizoma	Asteraceae	Ovate atractylodes
Da Zao (10)	Jujubae Fructus	Rhamnaceae	Chinese date
Gan Cao (6)	Glycyrrhizae Radix	Fabaceae	Licorice root
Ci Wu Jia	Acanthopanacis senticosi Radix et Caulis	Araliaceae	Spiny acanthopanax
Huang Jing	Polygonati Rhizoma	Liliaceae	Polygonatum
Sha Ji	Hippophae Fructus	Eleagnaceae	Swallow thorn
Yi Tang	Maltosum	None noted	Malt suger
Shu Di (5)	Rehmanniae Radix preparata	Scrophulariaceae	Cooked rehmannia root
He Shou Wu (31)	Polygoni multiflori Radix preparata	Polygonaceae	Processed fleeceflower root
Dang Gui (1)	Angeliceae sinensis Radix	Apiaceae	Chinese anglica root
Bai Shao (2)	Paeoniae Radix alba	Ranunculaceae	White pony root
E Jiao (8)	Asini Corii Colla	Equidae	Ass-hide glue
Guo Qi Zi (44)	Lycii Fructus	Solanaceae	Lycium fruit
Sang Shen	Mori Fructus	Mori fructus	Mulerry
Long Yan Rou (7)	Longan arillus	Sapindaceae	Longan
Lu Rong	Cervi Cornu pantotrichum	Cervidae	Deer velvet
Ge Jie	Gecko	Gekkonidae	Gecko

Dong Chong xia Cao	Cordyceps	Hypocreaceae	Cordyceps
Rou Cong Rong	Cistanches Herba	Orobanchaceae	Cistanche
Suo Yang	Cynomorii Herba	Cynomorisceae	Fleshy stem of cynomorium
Yin Yang Huo	Epimedii Herba	Berberidaceae	Aerial parts of epimedium
Ba Ji Tian	Morindae officinalis Radix	Rubiaceae	Morinda root
Hu Lu Ba	Trigonellae Semen	Fabaceae	Fenugreek seed
He Tao Ren	Juglandis Semen	Juglandaceae	Walnut
Bu Gu Zhi (64)	Psoraleae Fructus	Fabaceae	Psoralea fruit
Yi Zhi Ren (89)	Alpiniae oxyphyllae Fructus	Zingiberaceae	Alpinia fruit
Xian Mao	Curculiginis rhizoma	Liliaceae	Curculigo
Du Zhong (55)	Eucommiae Corteex	Eucommiaceae	Eucommia bark
Gou Ji	Cibotii Rhizoma	Dicksoniaceae	Cibotium rhizome
Xu Duan	Dipsaci Radix	Dipsacaeae	Dipsacus
Gu Sui Bu	Drynariae Rhizoma	Polypodiaseae	Drynaria rhizome
Tu Si Zi (36)	Cuscutae Semen	Convolvulaceae	Chinese dodder seed
Sha Yuan Zi	Astragali complanati Semen	Fabaceae	Complanate astragalus seed
Zi He Che	Hominis Placenta	Hominidae	Human placenta
Jiu Cai Zi	Allii tuberosi Semen	Liliaceae	Chinses leek seeds
Hai Gou Shen	Callorhini Testes et Penis	Pinnipedae	Male seal sexual organs
Yang Qi Shi	Actinolitum	mineral	Actinolite
Zhong Ru Shi	Stalactitum	Mineral	Tip of tubular stalactites
Hai Long	Syngnathus	Syngnatidae	Pipe-fish
Hai Ma	Hippocampus	Syngnatidae	Sea horse
Hai Shen	Stichopus	Stichopodidae	Sea cucumber
Sha Shen	Glehniae		Glehnia

Bei Sha Shen	Glehniae Radix	Apiaceae	Glehnia root
Nan Sha Shen	Adenophorae Radix	Campanulaceae	Adenophora root
Xi Yang Shen	Panacis quinquefolii Radix	Araliaceae	American ginseng root
Mai Dong (63)	Ophiopogonis Radix	Liliaceae	Ophiopogon tuber
Tian Men Dong	Asparagi Radix	Liliaceae	Asparagus tuber
Shi Hu	Dendrobii Herba	Orchidaceae	Dendrobium
Yu Zhu	Polygonati odorati Rhizoma	Liliaceae	Scented Solsmon's seal rhizome
Bai He	Lilii bulbus	Liliaceae	Lily bulb
Mo Han Lian	Ecliptae Herba	Asteraceae	Eclipta
Nu Zhen Zi	Ligustri lucidi Fructus	Oleaceae	Ligustrum
Hei Zi Ma (26)	Sesami Semen nigrum	Pedaliaceae	Black sesame seeds
Gui Ban	Testudinis Plastrum	Testudinidae	Fresh-water turtle plastron
Bie Jia	Tironycis Carapax	Trionychidae	Chinese soft-shelled turtle shell
Yin Er	Tremella	Tremellaceae	Tremella fruiting body
Chu Shi Zi	Broussonetiae Fructus	Moraceae	Paper mulberry fruit
Jiao Gu lan (43)	Gynostemma pentaphyllum	Cucurbitaceae	Herbaceous vine

Table XIII Herbs that Stabilize and Bind

	Pharmaceutical Name	Family	English Name
Shan Zhu Yu	Corni Fructus	Cornaceae	Cornus
Wu Wei Zi	Schisandrae Fructus	Magnoliaceae	Schisandra fruit
Wu Mei	Mume Fructus	Rosaceae	Mume fruit
He Zi	Chebulae Fructus	Combretaceae	Chebule
Rou Dou Kou	Myristicae Semen	Myristicaceae	Nutmeg seeds
Shi Liu Pi	Granati pericarpium	Punicaceae	Pomegranate husk
Chun Pi	Ailanthi Cortex	Simarubaceae	Ailanthus bark or root bark

Chi Shi Zhi	Halloysitum rubrum	Sheet silicate mineral	Halloysite
Yu Yu Liang	Limonitum	Mineral	Limonite
Lian Zi (76)	Nelumbinis Semen	Nymphaeaceae	Lotus seed
Lian Xu	Nelumbinis Stamen	Nymphaeaceae	Lotus stamen
Qian Shi	Euryales Semen	Nymphaeaceae	Euryale seeds
Jin Ying Zi	Rosae laevigatae Fructus	Rosaceae	Rose laevigata
Fu Pen Zi	Rubi Fructus	Rosaceae	Chinese raspberry
Wu Bei Zi	Galla chinensis	Anacardiaceae	Gallnut of Chinese sumac
Bai Guo	Ginkgo Semen	Ginkgoaceae	Ginkgo nut
Fu Xiao Mai	Tritici Fructus levis	Gramineae	Light wheat grain
Ma Huang Gen	Ephedrae Radix	Ephedraceae	Ephedra
Nuo Dao Gen	Oryzae glutinosea Radix	Gramineae	Glutinous rice root
Hai Piao Xiao	Sepiae Endoconcha	Sepiidae	Cuttlefish bone
Sang Piao Xiao	Mantidis Ootheca	Mantidae	Mantis egg-case
Ji Guan Hua	Celosiae cristatae Flos	Amaranthaceae	Coxcomb flower

Table XIV Substance that Calm the Spirit

	Pharmaceutical Name	Family	English Name
Long Gu	Fossilia Ossis Mastodi	Fossil	Dragon bone
Long Chi	Fossilia Dentis Mastodi	Carbonates	Fossilized teeth
Mu Li	Ostreae Concha	Ostreidae	Oyster shell
Ci Shi	Magnetitum	Oxide mineral	Magnetite
Zhen Zhu	Margarita	Pteriidae	Pearl
Zhen Zhu Mu	Margaritaiferae Concha usta	Pteriidae	Mother-of- Pearl
Zi Shi Ying	Fluoritum	Halide mineral	Fluorite
Hu Po	Succinum	Fossil	Amber
Zi Bei Chi	Mauritiae/cypraeae Concha	Cypraeidae	Cowry shell
Sheng Tie Luo	Ferri Frusta	Oxide	Oxidized iron filings
Suan Zao Ren (59)	Ziziphi spinosae Semen	Rhamnaceae	Spiny zizyphus seeds

Bai Zi Ren	Platycladi Semen	Cupressaceae	Arborvitae
Yuan Zhi	Polygalae Radix	Polygalaceae	Polygala root
Ling Zhi (92)	Ganoderma	Polyporaceae	Ganoderma
He Huan Pi	Albiziae Cortex	Fabaceae	Silktree bark
He Huan Hua	Albiziae Flos	Fabaceae	Silktree flower
Ye Jiao Teng	Polygoni muliflori Caulis	Polygonaceae	Fleeceflower caulis

Table XV Aromatic Substances that Open the Orifices

	Pharmaceutical Name	Family	English Name
She Xiang	Moschus	Cervidae	Musk
Su He Xiang	Styrax	Hamamelidaceae	Resin of rose maloes
An Xi Xiang	Benzoinum	Styraceae	Benzoin
Bing Pian	Bornelum	Dipterocarpaceae	Borneol
Shi Chang Pu (86)	Acori tatarinowii Rhizoma	Araceae	Grassleaf sweerflag rhizome
Niu Huang	Bovis Calculus	Bovidae	Cattle bezoar

Table XVI Substance that extinguish Wind and Stop Tremors

	Pharmaceutical Name	Family	English Name
Ling yang Jiao	Saigae tataricea Cornu	Bovidae	Antelope horn
Gou Teng	Uncariae ramulus cum Uncis	Rubiaceae	Uncaria vine
Tian Ma (47)	Gastrodiae Rhizome	Orchidaceae	Gastrodia rhizome
Ci Ji Li	Tribuli Fructus	Zygophyllaceae	Caltrop fruit
Lu Dou Yi	Glycinis Teata	Fabaceae	Soybean skin
Luo Bu Ma (46)	Apocyni veneti Folium	Apocynaceae	Dogbane leaf
Shi Jue Ming	Haliotidis Concha	Haliotidae	Abalone shell
Di Long	Pheretima	Megascolecidae	Earthworm
Bai Jiang Can	Bombyx batryticatus	bombycidae	Silkworm
Quan Xie	Scorpio	Buthidae	Scorpion

Wu Gong	Scolopendra	Saolopendridae	Centipede
Dai Zhe Shi	Haematitum	Oxide mineral	Hematite

Table XVII Herbs that Expel Parasites

	Pharmaceutical Name	Family	English Name
Shi Jun Zi	Quisqualis	Combretacea	Rangoon creeper fruit with seeds
Ku Lian Pi	Meliae Cortex	Meliaceae	Chinaberry bark or root bark
Fei Zi	Torreyae	Semen	Torreya seeds
He Shi	Carpesii abrotanoidis Fructus	Asterceae	Carpesium fruit
Lei Wan	Omphalia	Tricholomataceae	Fruiting body of Omphalia
Wu Yi	Ulmi macrocarpae Fructus	Ulmaceae	Elm cake
Bing Lang	Arecae Semen	Palmae	Areaca
Mian Ma Guan Zhong	Dryopteridis crassirhizomae Rhizoma	Polypodiaceae	Dryopteris
Nan Gua Zi	Cucurbitae moschatae Semen	Cucurbitaceae	Pumpkin seeds and husks
Da Suan	Allii sativi Bulbus	Liliaceae	Garlic bulb
Chang Shan	Dichroae Radix	Saxifragaceae	Dichroa root

Table XVIII Substances for Topical Application

	Pharmaceutical Name	Family	English Name
Bai Fan	Alumen	Sulfate mineral	Alum
Lu Gan Shi	Calamine	Carbonate mineral	Calamine
Liu Huang	Sulfur	Mineral	Sulfur
Qian Dian	Minium	Oxide mineral	Minium
She Chuang Zi	Cnidii Fructus	Apiaceae	Cnidium seed
Tu Jing Pi	Pseudolaricis Cortex	Pinaceae	Golden larch bark
Mu Bie Zi	Momordicea Semen	Cucurbitaceae	Momordica seeds

Da Feng Zi	Hydnocarpi Semen	Flacoutiaceae	Hydnocarpus seeds
Er Cha	Catechu	Fabaceae	Black cutch
Zhang Nao	Camphora	Lauraceae	Camphor
Ban Mao	Mylabris	Milodae	Mylabris
Chan Su	Bufonis Venenum	Bufonidae	Toad venom
Feng Fang	Vespae nidus	Vespidae	Wasp nest

Appendix B

(In attached CD)

A global database containing the total and bioavailable levels of twenty-four chemical elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Li, Bi, As, Se, Mo, Cd, Al, Sn, Sb, Pb, Hg, K, Na, Ca, Mg, and Ba) determined by ICP (in triplicate) for each of the 103 samples listed in Table 3.1.

Appendix C

Recommended human daily dietary and toxic intakes of twenty-four chemical elements (Al, Sb, As, Ba, Bi, Cd, Cr, Co, Cu, Fe, Pb, Li, Mn, Hg, Mo, Ni, Se, Sn, V, Zn, K, Ca, Na, and Mg) [113].

Element	Daily Dietary Intake (mg)	Toxic Intake (mg)
Al	2.45	5.0
Sb	-	100
As	0.04 -1.4	5 - 50
Ba	-	200
Bi	0.002 - 0.005	-
Cd	0.007 - 3.0	0.5
Cr	0.05 - 0.2	200
Co	*	500
Cu	2.0 - 5.0	250
Fe	6.0 - 40	200
Pb	0.06 - 0.5	1.0
Li	0.1 - 2	92 - 200
Mn	0.4 - 10	10 - 20
Hg	0.002 - 0.004	0.4
Mo	0.05 - 0.35	5.0
Ni	0.3 - 0.5	50
Se	0.006 - 0.2	5.0
Sn	5.8	2.0
V	0.04	-
Zn	5.0 - 40	150 - 600
K	1950 - 5460	>12000
Ca	800	-
Na	500 - 2300	>18000
Mg	320	>2000

* Recommended intakes of cobalt have not been set as the only form of cobalt required by the body is vitamin B12, of which cobalt is an integral part.