EFFECT OF PROCESSING ON THE DISTRIBUTION OF CADMIUM IN WHEATEN PRODUCTS

A thesis submitted for the degree of Doctor of Philosophy

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Declaration

I hereby declare that all of the work within this thesis was carried out at the Academy of Grain Technology (renamed Agrifood Technology), at OMIC Australia and at Victoria University during my candidature as a PhD student. To the best of my knowledge, no part of this thesis has been submitted in part or in full for any other degree or diploma at any other university. Moreover, I declare that no material contained within this thesis has been written or published by any other person, excepting where due reference has been made to individuals in the text.

[redacted]

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Abstract

An understanding of the cadmium levels in the Australian wheat crop and areas of elevated levels that may be of concern to health has great potential for both the export market and the domestic consumer in Australia. This understanding is most effective if the sources of the cadmium in the wheat, the fate of the cadmium during the milling process and how this affects the levels in bread products is also known. The objectives of this study were to investigate cadmium levels in wheat from Australian silo sites, determine if a correlation existed between cadmium level and soil type, pH and fertiliser usage and determine the reasons for any correlation. In addition, to determine if the cadmium was partitioned during the normal commercial milling process into the milling fractions and how this affected cadmium levels in bread. These studies would provide the wheat growing, milling and bread production industries with tools for minimising cadmium levels in wheat based foods and assist in the marketing of Australian produce, both in Australia and overseas, as healthy and safe.

To analyse the large number of samples required in this study, it was essential that an accurate, timely and cost effective method for the analysis of cadmium in wheat and wheaten products be developed and validated. After initial investigations, a method using a nitric acid digestion to destroy the majority of the organic matter in the sample and atomic absorption spectrometry with a graphite furnace as the quantitative step was chosen. Analytical parameters of the digestion and quantitative steps were determined and extensive validation using a CRM wheat flour, spikes and duplicates was performed. Method uncertainty was calculated and control charts were used to monitor the performance of the analysis during the study. Involvement in a number of collaborative trials with external laboratories, both in Australia and overseas, was undertaken during the study to further monitor analytical performance. All of these quality assurance parameters showed that the method was robust and produced valid data that was suitable for the analysis required by the study.

A large number of wheat samples from silo sites within Australia were sourced from the Australian Wheat Board. These samples consisted of either, samples taken from individual loads of wheat delivered to a silo site by a grower or composite samples of each 500 tonnes of wheat delivered to a silo site. The samples were analysed for cadmium and each set of results was plotted onto a map of the Australian grain using GIS software that allowed graduated colours to represent cadmium levels. It was found that there were elevated levels of cadmium in a north-south band in the middle of the West Australian grain belt, up to 0.05 and 0.04 mg/kg for grower loads and site composite

samples respectively. In addition, areas around Port Pirie in South Australia had levels of 0.03 and 0.02 mg/kg respectively. The remainder of the Australian grain belt had low levels of between 0.01 and < 0.01 mg/kg of cadmium. These elevated levels could be correlated, to some extent, with sandy acid soils in Western Australia and the zinc smelter at Port Pirie in South Australia. A lesser correlation could be found with fertiliser usage although, it could be seen that soil type and pH had a greater effect. It was also found that cadmium levels in samples taken from grower loads on delivery to the silo were statistically higher than those taken from silo composite samples.

To determine the cadmium levels in wheat delivered to commercial flourmills, wheat samples were taken by BHA staff from rail and/or truck loads delivered to each flourmill. The samples were analysed for cadmium, then the results compared to find if differences existed between wheat delivered in each state. It could be seen that wheat delivered to commercial flourmills in Western Australia had the highest mean cadmium levels (0.03 mg/kg), South Australia was next but had higher maximum levels (0.10 mg/kg) whereas the other three states had low to not detected levels. Wheat samples that had been labelled with grade were sourced from the Australian Wheat Board quality monitoring program. These samples were analysed for cadmium and showed that samples from Western Australia had statistically higher cadmium levels than the other states.

A field trial was organised to evaluate the effect of wheat genotype and planting site on a number of quality parameters including cadmium level. Fourteen wheat genotypes were planted at three sites in New South Wales, South Australia and Western Australia as well as two sites in Queensland and Victoria. Sufficient seed was sown at each site to produce 100 kg of wheat at harvest. The wheat was analysed for cadmium and the results showed that the levels were statistically different when viewed by site and state but not when viewed by genotype. Wheat grown at the three sites in Western Australia had the highest cadmium levels (0.02 to 0.04 mg/kg), South Australia had the next highest (0.01 to 0.015 mg/kg) and the other three states had low results (generally < 0.01 mg/kg). Wheat samples were sourced from the United States of America, Canada, Europe and other wheat producing countries. The samples were analysed for cadmium and the results showed that most wheat samples from the United States of America, Canada and Europe had results of 0.05 mg/kg or higher, whereas samples from other wheat producing countries had results of 0.02 mg/kg or lower. Wheat samples from the United States of America, Canada and Europe were statistically different from the other wheat producing countries listed.

To determine the effect of milling on the distribution of cadmium between wheat and subsequent mill fractions, a preliminary trial was organised on a Buhler laboratory mill. Two ASW wheats, two Hard wheats and a Soft wheat were sourced from the Australian Wheat Board quality monitoring

program for Victoria and Western Australia. These wheats were milled on the laboratory mill, then the wheat and subsequent mill fractions were analysed for cadmium and ash. The results showed that for all wheat samples from both states, cadmium levels were increased in the bran and germ fractions but correspondingly reduced in the break and reduction roll fractions. In addition, the cadmium in each fraction was linearly correlated with the amount of ash. A larger trial was organised on the pilot mill at the Bread Research Institute at North Ryde, Sydney. Approximately 2 to 2.5 tonnes of a number of wheat grades and genotypes were milled on the pilot mill and the wheat and all subsequent fractions were analysed for cadmium and ash. The results showed that for all wheat grades and genotypes from all states, cadmium levels did not markedly alter from the wheat in the break and reduction roll fractions, but increased strongly over the pollard and other offal fractions. Results for all wheats and corresponding mill fractions showed a strong linear correlation between cadmium level and ash content.

To further examine the effects of milling on distribution of cadmium in mill fractions at a commercial level, samples of ASW and Hard wheat together with corresponding mill fractions were taken from a commercial flourmill in Victoria and a commercial flourmill in Western Australia. All wheat and corresponding mill fraction samples were analysed for cadmium and ash. The results for both ASW and Hard wheat milled in Victoria and Western Australia showed a reduction in cadmium levels for fractions from the break and reduction roll sections and an increase in cadmium for the pollard and germ fractions. For all four cases, the germ had a higher cadmium level than the pollard and results for all wheats and corresponding mill fractions showed a strong linear correlation between cadmium level and ash content.

To determine the effect of high levels of cadmium in the bran and germ fractions after milling, samples of white, wholemeal loaf and white, wholemeal flat breads were purchased from commercial outlets in Werribee, Victoria and Fremantle, Western Australia. Breads from Victoria and Western Australia were selected to establish if a relationship existed between the cadmium in the bread and the difference in mean cadmium levels in wheat from the two states. All samples were analysed for cadmium and dietary fibre to also establish if a relationship existed between the cadmium content and the amount of fibre in the bread from the bran. The overall results for both loaf and flat bread samples showed that breads from Western Australia had statistically higher cadmium content than breads from Victoria and that wholemeal breads from both states had higher cadmium content than white breads. The results also showed that cadmium content generally had a linear relationship with dietary fibre with r values ranging from 0.354 for white loaf breads from Victoria to 0.980 for wholemeal flat breads from Western Australia. This was much stronger with the higher cadmium

and dietary fibre content in the wholemeal breads. There was no statistical difference between the results for loaf and flat breads at the 95% confidence level.

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7.2.3.1 Box and whisker plot for white and wholemeal flat breads sourced from Werribee (Victoria) and Fremantle (West Australia). 195

List of Abbreviations

AAS	Atomic absorption spectrophotometer
AFT	Agrifood Technology, Werribee, Victoria
AGAL	Australian Government Analytical Laboratory
AGT	Academy of Grain Technology, Werribee, Victoria
ANOVA	One-way analysis of variance
ANZFA	Australian and New Zealand Food Authority
APW	Australian Premium White wheat
ARfD	Acute oral reference dose, this is the estimate of the amount of substance in food, expressed on a body weight basis (FSANZ, 2004a)
ASW	Australian Standard White wheat
AWB	Australian Wheat Board (now AWB Pty. Ltd.)
B1	1st break roller in a Buhler laboratory flour roller mill
B2	2nd break roller in a Buhler laboratory flour roller mill
B3	3rd break roller in a Buhler laboratory flour roller mill
BCE	Before Common Era (before the birth of Christ, BC)
BHA	Bulk Handling Authority
BRI	Bread Research Institute, North Ryde, Sydney, Australia
CCFAC	Codex Committee on Food Additives and Contaminants
CL (95%)	Confidence limit at the 95% level
CSIRO	Commonwealth Scientific and Industrial Research Organization, Australia
DF	Degrees of Freedom
EPA	Environment Protection Authority, Australia
FSANZ	Food Safety Australia and New Zealand
GF	Graphite furnace
GIS	Geographical Information System
IARC	International Agency for Research on Cancer
ICP	Inductively coupled plasma spectrophotometer
JEFCA	FAO/WHO Joint Expert Committee on Food Additives
LD_{50}	Lethal dose that kills 50% of the population
LOD	Limit of detection
LOQ	Limit of quantitation
ML	Maximum level
MPC	Maximum permitted concentration

MSDS	Material Safety Data Sheet
ND	Not determined
NFA	National Food Authority
NHMRC	National Health and Medical Research Council
NOAEL	No observable adverse effect level
NRS	National Residue Survey
NSW	New South Wales
OMIC	Overseas Merchandise Inspection Company Limited
P	Probability level in an ANOVA table
PTWI	Permissible tolerable daily intake
Qld	Queensland
QDPI	Queensland Department of Primary Industry, Queensland, Australia
r	Pearson product-moment correlation coefficient
R^2	Coefficient of determination
R1	1st reduction roller in a Buhler laboratory flour roller mill
R2	2nd reduction roller in a Buhler laboratory flour roller mill
R3	3rd reduction roller in a Buhler laboratory flour roller mill
SA	South Australia
SARDI	South Australia Research and Development Institute, South Australia
SCL	State Chemistry Laboratory, Werribee, Victoria
SD	Standard deviation
TDF	Total dietary fibre
Vic	Victoria
VIDA	Victorian Institute of Dryland Agriculture, Horsham, Victoria
WA	Western Australia
WACBH	West Australian Cooperative Bulk Handling

chapter 1 Introduction

The rural sector has an important place in the Australian economy through its direct contribution to economic output, to exports and to the balance of trade. This contribution is particularly important for a small open economy such as in Australia. The rural sector also supports a network of dependent rural communities and agriculturally oriented, manufacturing, distribution and service industries. Over the last five years, Australian rural exports have averaged $22.7 billion and this represents about 29.3% of the total value of exports (ABARE, 2000; Hooper *et al.*, 2003). The grain industry is a major contributor to the output of the rural sector. The five-year average annual value of grain production is $9.3 billion (ABARE, 2003a).

By far the largest enterprise in the Australian grain industry is wheat production, with the annual gross value of production averaging about $5.0 billion over the four years 1996/99 (McMaster, 2000; Wheat CRC, 2001; ABARE, 2003b; Sewell, 2003). This amounts to around 58% of the total value of grain production. The wheat industry supports 70,000 direct employees and 155,000 farming family members and affects over 550,000 people in rural and regional Australia (Wheat CRC, 2001). Australia is the ninth largest wheat producer in the world and is the third largest exporter, exporting about 80% of total production each year worth about $4.0 billion, against subsidy pressure from the European Community and the USA (GAFTA, 2003). Markets in the Middle East are becoming more important with up to 50% of wheat exported to these countries prior to the Gulf war. More recently, the specialty markets of South East Asia, North Asia and China have showed increasing interest in the purchase of Australian wheat, reflecting the increased Westernisation in food consumption patterns (McMaster and Gould, 1995).

The majority of the wheat utilised in Australia is used for the production of flour for human consumption or for the production of starch and gluten. Approximately 1.8 to 2.0 million tons of wheat is processed in Australian flourmills annually at a total income of $1,644 million (IBIS, 2004) and a value added component of approximately $348 million in 2001 (ABARE, 2004). The flour milling industry in Australia is currently dominated by two major players. They are, the Manildra Group which is a family owned business that was acquired by Jack Honam in 1951 and is presently operated by his son Dick Honam and the other is Milling Australia, which was formed by a joint partnership of Grain Corp and Cargill Australia after the purchase of Allied Mills from Goodman Fielder in 2002 (Ries, 2002). The sale of Allied Mills by Goodman Fielder was very hotly contested, with Milling Australia, the AWB and the Manildra Group bidding for the twelve flour mills.

Wholesale bakeries are large, automated industries that account for 65% of the total bread production. The companies that own these bakeries are vertically integrated with interests in flour milling, bakery ingredients production and related industries (Farrer, 2005). Until 1998, the four major companies were Quality Bakers Australia (Goodman Fielders), Tip Top (George Western Foods), Sunicrust (Bunge) and Regal/Cobbity Farm (Defiance Mills). Since Goodman Fielders bought Bunge and Defiance Mills, they have become the major player in this industry with twelve brands of bread products and they bake over seven million loaves and rolls a week (Goodman Fielder, 2002). In addition to the major bakeries, hot bread shops and in-store bakeries are playing an increasing role, as consumers demand fresher and more specialised products (McMaster and Gould, 1995), Bakers Delight is the largest franchise with an estimated income of in excess of $300 million (DAF 2004). With a total income of $5.1 billion from the baking industry in 2002 of which $1.63 billion was from bread (DAFF, 2004), the value added component of the domestic market is worth about $4.0 billion (Wheat CRC, 2001; ABARE, 2003b).

Cadmium is an extremely toxic element that has no or very little requirement in the maintenance of human life. It can affect most organs of the body, especially the bones and kidney, and is accumulated in the body over a normal lifetime. The levels of this metal in food are strictly regulated by a number of countries, including Australia, the USA and most countries of the European Union. It occurs naturally in soils at very low levels but can reach elevated levels by the addition of fertilisers made from cadmium rich phosphate rock or other anthropogenic processes, including the use of sewage. These increased levels in the soil can produce elevated levels in the wheat and downstream products, including bread, which may exceed the maximum permitted concentration (MPC), both in Australia and overseas.

To maintain our current market share, both in the domestic and export markets, it is essential that the Australian grain industry be seen as a supplier of clean grain and products that are free of or very low in contaminants. The increasing concern over cadmium and its effect on human health makes it essential to know levels in Australian wheat as well as the effect that processing has on downstream products. This knowledge will assist the industry to develop strategies to ensure that levels do not exceed the current Australian maximum permitted concentration of 0.1 mg/kg. An analytical procedure that is inexpensive, can analyse large numbers of samples in a robust and time efficient manner and that can produce results with a high degree of accuracy is essential if these strategies are to be effective.

Chapter 2 Review of Literature

2.1 Cadmium

2.1.1 Chemistry

Cadmium was discovered almost simultaneously by Stromeyer and Hermann in Germany in 1817 (Encarta, 2001). Certain samples of zinc carbonate were found to yield a brown oxide and a yellow sulphide. The metal was isolated from the sulphide by roasting and reduction. It was somewhat confusingly named cadmium from *Kadmia*, the ancient Greek name for zinc carbonate in which it often occurs (Aylett, 1975; Page *et al.*, 1986). Cadmium occurs in group IIb of Mendeleev's periodic table of the elements. Group Ib (copper, silver and gold) and group IIb (zinc, cadmium and mercury) follow the transition elements. These are elements in which the inner electron orbitals are becoming stable and are filled in a stepwise manner as the atomic number is increased. The number of outer (valence) electrons remains more or less constant.

The Ib elements may lose one or two electrons from these inner orbitals in order to form ions or complexes in which these elements have a valence higher than their group valence of one. Such a loss of inner electrons is not possible for the group IIb elements. These elements therefore exhibit a valence no higher than their group valence of two. In contrast, mercury can exhibit a valence of one or two, whereas zinc and cadmium exhibit two as their only stable valence (Baes, 1973). In this respect, zinc and cadmium are similar to the elements of group IIa, especially magnesium and calcium, which respectively form divalent ions of similar size. This similarity is only important in compounds with elements that are strongly electronegative, such as fluorine and oxygen and therefore involve chemical ionic bonds.

In other chemical situations however, cadmium resembles zinc and is quite unlike calcium. This change is possibly due to the tendency of post-transition elements to form stronger bonds with the less electronegative elements where sharing of electrons occurs (covalent bonds). This tendency to form covalent bonds increases in the order : zinc, cadmium, mercury and for a given metal, bonds formed with chlorine, bromine, iodine, nitrogen and sulphur become increasingly covalent in approximately the same order (Baes, 1973).

Some physical properties of elemental cadmium that have an effect on the toxicology to biological systems are shown in Table 2.1.1.1.

Table 2.1.1.1 Some properties of elemental cadmium

Property	**Value**
Relative Atomic Mass	112.41
Atomic Number	48
Specific Gravity (25^{0}C)	8.642
Atomic Radius (A, 25^{0}C)	1.489
Melting Point (^{0}C)	320.9
Boiling Point (^{0}C)	765
Vapour Pressure (mm Hg)	
394^{0}C	1
484^{0}C	10
578^{0}C	60
711^{0}C	400
767^{0}C	760
Major Stable Isotopes (%)	
^{106}Cd	1.2
^{108}Cd	0.9
^{110}Cd	12.4
^{111}Cd	12.8
^{112}Cd	24.1
^{113}Cd	12.3
^{114}Cd	28.9
^{116}Cd	7.6

(Eriberg *et al.*, 1985; Dobson, 1992; Aylett, 1975; OSHA, 1992a).

2.1.2 Occurrence

2.1.2.1 Native

Cadmium is widely distributed throughout the earth's crust and is nearly unique among the elements, in that it is always found in nature in association with zinc. The background concentrations of cadmium in uncontaminated soils are strongly dependent on the parent rocks from which the soil is derived. Soils of similar age from igneous rocks are lowest in cadmium, soils from metamorphic rocks are intermediate and soils from sedimentary rocks contain the highest concentrations of cadmium. Results from a number of authors are shown in Table 2.1.2.1.

Table 2.1 significantly significantly.2.1 Cadmium content of rocks and sediments

Rock Type	**Cadmium (mg/kg)**		
	Mean	**Minimum**	**Maximum**
Igneous			
Granitic	0.09	0.001	0.60
Basalt	0.13	0.006	0.60
Ultramafic	0.03	0.001	0.03
Metamorphic			
Gneisses	0.04	0.007	0.26
Schists	0.02	0.005	0.87
Eclogite	0.11	0.040	0.26
Sedimentary			
Limestone	0.07	0.001	0.50
Sandstone	0.02	0.01	0.41
Shale, clay	0.03	0.02	11
Red clay	0.56		
Organic mud	0.39		
Deep Ocean Sediments	0.5	0.05	17
Oceanic Manganese Oxides	8	3	21
Phosphorites	25	10	500
Recent Sediments			
Lake sediments	0.91	0.02	6.2
Stream sediments	0.16	0.03	0.4

(Horn and Adams, 1966; Waketa and Schmitt, 1970; Marowsky and Wedepohl, 1971; Fleischer *et al.*, 1974; Gong *et al.*, 1977),

In a review of cadmium analysis of soils from different parts of the world, Ure and Berrow (1982) found that the mean cadmium content of 1642 soils was 0.62 mg/kg with a range of 0.013 - 10 mg/kg. Other authors give lower results such as: 0.1 mg/kg (Friberg *et al.*, 1985; Friberg *et al.*, 1992a; Heinrichs *et al.*, 1980), 0.18 mg/kg (Goeller, *et al.*, 1973), 0.2 mg/kg (Cook and Freney, 1988; Ashton and Laura, 1992) and 0.3 mg/kg (NRCC, 1979; Page *et al.*, 1986).

Table 2.1.2.2 Cadmium content of soils in countries other than Australia (Page *et al.*, 1986)

Country	**Soil**	**No of Samples**	**Cadmium (mg/kg)**
Japan	Rice paddy	2,746	0.4
	Farmland	722	0.3
	Orchard	268	0.3
Sweden	Cultivated	186	0.22
	Uncultivated	175	0.22
Norway	Uncultivated	500	0.42
United States			
California	Agricultural	177	1.24
Michigan	Agricultural	91	0.57
	Residential	70	0.41
Minnesota	Agricultural	16	0.17
North Central	Agricultural	937	0.37
North East	Agricultural	293	0.17
Ohio	Agricultural	235	0.2
Southern	Agricultural	1,230	0.15
Western	Agricultural	742	0.33
Denmark	25 locations	51	0.26
USSR	-	44	0.24
England	Garden soils	235	0.8
Canada	Chernozems	12	0.23
	Luvisols	6	0.09
	Humic glysols	18	0.34
	Solonetz	18	0.15

Very little data is available on the background levels of cadmium in Australian soils. Williams *et al.* (1972) analysed nineteen soils from a range of sites and found the cadmium concentration varied from 0.02 to 0.38 mg/kg. In a related study by Williams and David (1976), the cadmium concentration varied from 0.016 to 0.114 mg/kg. The results from Williams and David are shown in the table below:

Table 2.1.2.3 Cadmium content of soils in Australia

Soil Type	**Cadmium (mg/kg)**
Red brown earth	0.055
Red podzolic	0.024
Krasnozem	0.030
Alluvial	0.114
Podzol	0.033
Podzol	0.016

Tiller (1992) reported total background concentrations of cadmium in 180 rural surface soils of < 1 mg/kg and Rayment (1988) reported a survey that showed 120 rural soils (0-15 cm. horizon) had cadmium concentrations of < 0.5 mg/kg dry weight. No reasons were given for these relatively high reporting limits.

2.1.2.2 Industrial Processes

For the first 60 years after its discovery, cadmium was used in small quantities, primarily as cadmium sulphide in paint pigments. The production and use of cadmium was given substantial impetus in 1919 when M.J. Udy developed a commercially applicable cadmium electro-plating process. From this time, the United States of America (USA) has been the leading producer and consumer of cadmium in the world. USA production began in 1907 when the Grasselli Chemical Company produced 14,000 lb. In 1910, the American Smelting and Refining Company also began producing cadmium at its Globe, Colorado plant. In 1962, the USA produced a record output of 11.1 million lb, this was 41% of the world total. Between 1907 and 1963, cadmium production in the USA has totalled nearly 250 million lb (Kelly, 2000).

Cadmium can also enter the environment from the wear of motor vehicle tyres. It comes from impurities in the zinc oxide used in the vulcanisation of the rubber. Contamination of soil is greatest near a highway and decreases rapidly with increasing distance from it (David and Williams, 1975; Massadeh *et al.*, 2004). Increases in excess of 1.5 mg/kg of cadmium have been recorded in the surface soil near to a busy highway (Lagerwerff and Specht, 1970a and b). David and Williams (1975) analysed a range of tyres from different countries and the results are shown in the following table.

Table 2.1.2.4 Cadmium content of tyres

Country	Make	Cadmium (mg/kg)
Japan	Bridgestone	3.4
Germany	Fulda	0.19
France	Kleber	14.1
England	Pirelli	0.75
Ireland	Semperit	0.84
Taiwan	Jane	2.1
Australia	Goodrich	0.13
Australia	Olympic	0.14

It can be seen that the cadmium content in Australian tyres is lower than most of the others shown. This was felt by the authors to be a reflection of the effectiveness of the electrolytic purification process used for the refining of the zinc oxide used in the manufacture of the tyres. In some parts of Japan, irrigation water and soil was contaminated with the wastes from zinc mines and as a result, the cadmium concentrations of the rice grain grown in these areas were increased sufficiently to cause severe health problems (Takijima and Katsumi, 1973).

2.1.2.3 Fertilizers

2.1.2.3.1 Phosphatic

The primary phosphate rocks used in Australia for fertilizers have been richly phosphatic marine sedimentary rocks. It can be seen from Table 2.1.2.3.1 that these rocks have cadmium contents with a mean of approximately 40 times that of crustal rocks. It has been shown that the cadmium can be concentrated through biogenic processes, particularly through the action of microorganisms under marine conditions (Cook and Freney, 1988). When the rock is derived from sedimentary bird guano, the cadmium content can be even higher. It is bio-accumulated by the birds from the seawater, through the fish eaten and concentrated in the droppings. Rankama and Sahama (1950) record average contents of 100 mg/kg for guano derived phosphorites. The cadmium in sulphatic fertilizers since 1940 has been derived almost exclusively from the cadmium in the primary phosphate rock (Williams and David, 1973; Hutton, 1996). This is shown by the relationship between cadmium and phosphorus in some Australian fertilizers together with the rock phosphates used for their manufacture (Williams, 1977; David *et al.*, 1978).

Table 2.1.2.3.1 Cadmium content of phosphate rocks

Origin	Cook and Freney (1998)	Baechle and Wolstein (1984)	Williams (1974)
Duchess (Qld)	0.5 - 4 (8)		7
Chile/Peru (Offshore)	3.0 (3)		
Florida	4 - 20.3 (2)	7	14
North Carolina	20.8 (5)	36	
Jordan	2.7 - 10 (2)		
Egypt	2 - 3 (2)		
Israel	15 - 28 (4)	12 (2)	
Venezuela	2.7 (1)		
Colombia	7.2 - 12 (4)		
Saudi Arabia	0.7 (1)		
Tennessee	0.04 (1)		
Nauru*	70 (16)		89 (6)
Morocco	10 (1)	8 (2)	47

Table 2.1.2.3.1 Cadmium content of phosphate rocks (continued)

Senegal	74 (1)	84	
Peru	36 (1)		27
Algeria		23	
Sahara		43	
Tunisia		56	
Togo		53	
USSR**		0.3	
Lady Annie			7
Phalaborua			4
Christmas Island*			42 (5)
Ocean Island*			99

* Guano derived phosphate, ** Igneous apatite, all other rocks are phosphorites.

(the number of samples analysed is given in brackets)

Prior to 1940, some superphosphates contained much higher levels of cadmium, up to three times the present levels, because some of the sulphuric acid used in their manufacture contained appreciable levels of cadmium. This cadmium was derived from the raw materials, especially zinc sulphate, used to manufacture the acid. Cadmium is strongly adsorbed by soils and most of it applied in fertilizers is retained in the top 10 cm of surface soil (Williams and David, 1976). The average cadmium content of Australian fertilizers is approximately 0.00045 kg of cadmium per kg of phosphorus. The application of 1000 kg of superphosphate per hectare could increase the cadmium content in the top 10 cm of soil by 0.03 - 0.04 mg/kg (Williams and David, 1973). Normal commercial applications of superphosphate to crops over 50 - 60 years have increased soil levels by 0.1 - 0.2 mg/kg cadmium, this is equivalent to from 2 to 10 times that of the initial soil content (Hutton, 1996). In contrast, the annual rate of cadmium input to arable land from phosphate fertilizers has been estimated at five g/ha for the countries of the EEC (Hutton, 1982; Lorenz *et al.*, 1994). Lind *et al.* (1998) reported that use of fertilisers with high cadmium content has lead to an increased cadmium level in the soil of many parts of Sweden. Jiao *et al.* (2004) reported that addition of commercial grade mono-ammonium phosphate (MAP) or triple superphosphate to the soil produced higher seed cadmium concentrations in flax but not in durum wheat.

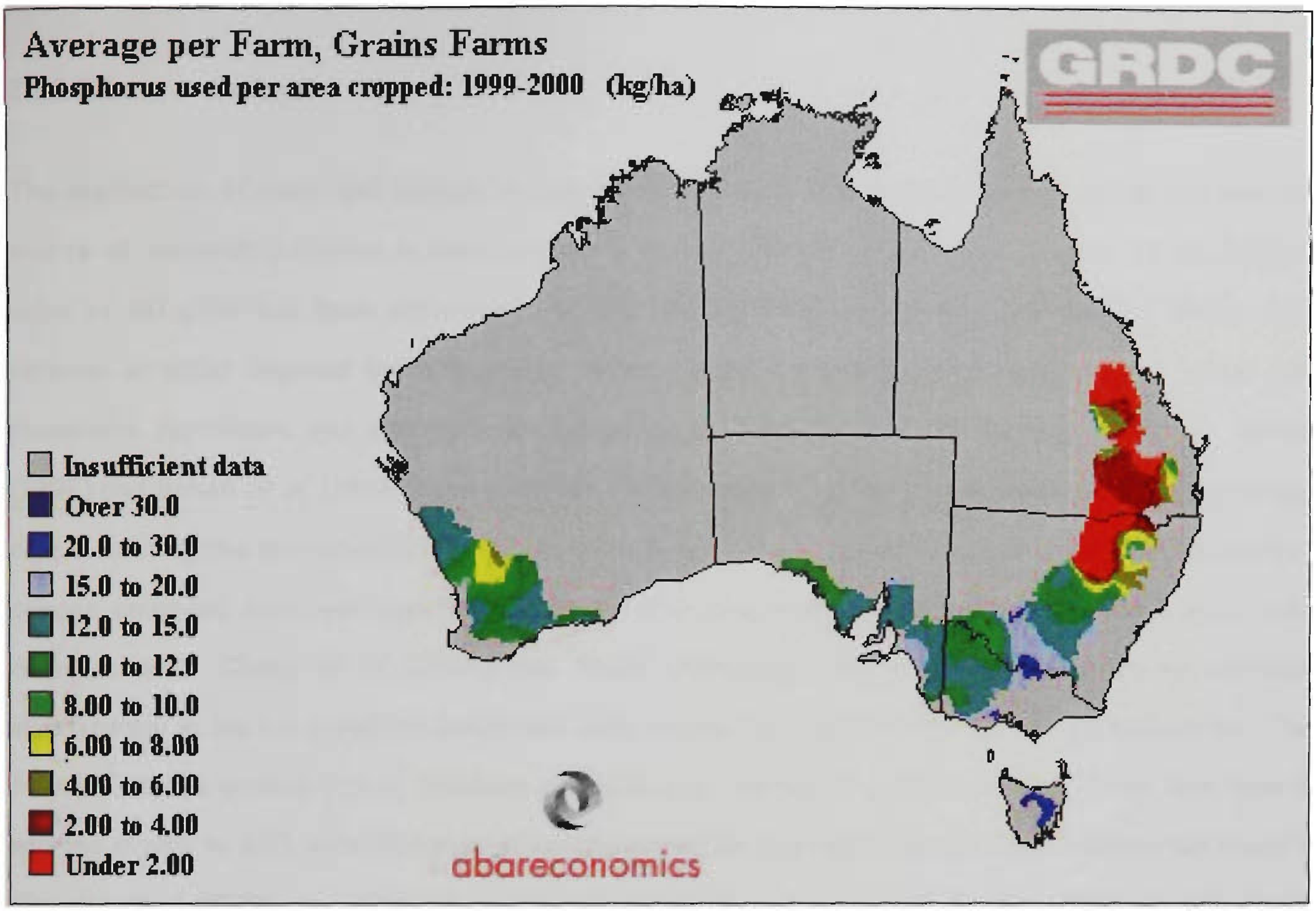


Figure 2.1.2.3.1 Map of phosphorus added as fertiliser/farm/year to the Australian grain belt (ABARE, 2002)

The map of fertiliser input to farms in the Australian grain belt over the year 1999 to 2000 was sourced from ABARE as total phosphorus input since specific data for superphosphate was not available. Superphosphate, however, is the most common form of phosphate fertiliser used on grain farms over the last 20 years (ABARE, *per comm*). It can be seen that fertiliser input in Western Australia ranges from 4.00 to 15.0 kg/ha with a band of 6.0 to 10.0 kg/ha in the centre of the grain belt. South Australian fertilizer levels are mainly 10.0 to 12.0 kg/ha with an area of 15.0 to 20.0 kg/ha around Port Pirie. Queensland is low overall ranging from < 2.0 to 4.00 kg/ha with a few very localised areas of 10.0 to 12.0 kg/ha. New South Wales can be split into three areas with the north of the state having a reasonably low input of < 2.0 kg/ha with very localised areas of 6.0 to 8.0 kg/ha, the centre of the state having inputs of 8.0 to 15.0 kg/ha. The intensive agriculture areas in the Riverina have high inputs of 15.0 to 30.0 kg/ha phosphorus fertiliser. Victoria has moderate phosphate inputs in the north-west of the state of 4.0 to 6.0 kg/ha and higher around Mildura of 12.0 to 15.0 kg/ha.

2.1.2.3.2 Sewage

The application of municipal sewage sludge to agricultural soils as a fertilizer can be a significant source of cadmium pollution in areas where it is used (Smith *et al.*, 1996; Stacey *et al.*, 2001). A value of 80 g/ha has been estimated for the United Kingdom (Hytton and Symon, 1986). On a national or wider regional basis, however, these inputs are usually much smaller than those from phosphate fertilizers and atmospheric deposition or wastes from the cadmium industry. Dobson (1992) and Brown *et al.* (1996) reported that the inorganic fraction of the sewage plays a significant role in limiting the phytoavailability of added cadmium. They found that cadmium uptake by plants in sewage amended soils was significantly lower than cadmium uptake by plants in soils with added cadmium salts. Chang *et al.* (1983) also found that cadmium and zinc concentrations increased significantly in barley grown on sandy soil with increasing rates of sewage sludge application. They found that the probability of cadmium exceeding 0.1 mg/kg of grain increased from less than 1% with no sludge to 60% with 90 tonne of composed sludge/ha/year. Similar results were also found by Chaudri *et al.* (2001) in a study of cadmium in wheat from a long-term field experiment with sewage sludge. Lindqvist-Ostblom and Eklund (2001) found in a survey of cadmium sources to municipal wastewater treatment plants in Sweden that about 30% of the cadmium came from households and the remainder from industry.

2.1.2.4 Wheat

2.1.2.4.1 Worldwide

A number of workers have published levels of cadmium in wheat worldwide and these are summarised in Table 2.1.3.4.1. As can be seen, America, Canada, European countries and New Zealand have mean values near or in excess of half the Codex limit of 0.10 mg/kg with maximum values well in excess of this limit. Argentina, USSR, Pakistan and China have much lower mean and maximum values.

Table 2.1.2.4.1 Cadmium content of wheat in countries other than Australia

Samples	**Cadmium (mg/kg)**		**Country**	**Reference**
	Mean	**Range**		
52	0.003	0.001 - 0.008	Argentina	Hack and Cocker (1983)
-	0.021	< 0.01 - 0.041	Argentina	AWB (1993)
113	0.056	0.006 - 0.126	Austria	Schindler (1983)
3	0.064	0.057 - 0.075	Belgium	Vos *et al.* (1988)
-	1.05	0.38 - 1.93	Bulgaria	Stefanov *et al.* (1995)
126	0.059	0.030 - 0.120	Canada	Dudas and Pawlik (1977)
68	0.083	0.400 - 0.020	Czechoslovakia	Polacek *et al.* (1991)
-	0.017	0.009 - 0.030	China	Chen and Gao (1993)
85	0.050	0.017 - 0.085	Finland	Varo *et al.* (1980)
34	0.050	0.026 - 0.111	France	Vos *et al.* (1988)
37	0.030	0.015 - 0.08	Germany	Horner and Kurfurst (1987)
73	0.050	0.041 - 0.063	Italy	Sillanpaa and Jansson (1992a)
9	0.088	0.049 - 0.139	Japan	AWB (1993)
-	0.04	-	New Zealand	Roberts *et al.* (1995)
-	0.054	-	New Zealand	Gray *et al.* (2001)
-	0.28	0.18 - 0.30	Pakistan	Ahmad *et al.* (1994)
3098	0.07	-	Poland	Jansson (2002)
58	0.066	0.028 - 0.132	Central Sweden	Andersson and Pettersson (1981)
49	0.096	0.053 - 0.171	Southern Sweden	Andersson and Pettersson (1981)
125	0.053	0.012 - 0.127	Sweden	Eriksson (1990)
84	0.07	0.02 - 0.35	The Netherlands	Wiersma *et al.* (1986)
188	0.067	0.017 - 0.256	The Netherlands	Vos *et al.* (1988)
250	0.063	0.053 - 0.074	United Kingdom	Adams *et al.* (2001)
9	0.08	0.07 - 0.10	USA	Morris and Greene (1970)
38	0.081	0.042 - 0.210	USA	Erdman and Moul (1982)
288	0.043	< 0.017 - 0.207	USA	Wolnik (1983)
100	0.01	< 0.01 - 0.04	USSR	Shcheglova and Golenkov (1984)
211	0.043	0 - 0.25	West Germany	Seibel and Ocker (1979)
1082	0.057	0.008 - 0.85	West Germany	Ocker and Seibel (1980)

2.1.2.4.2 Australia

Very little work has been published on levels of cadmium in Australian wheat, apart from localised surveys on polluted soil or small areas of specific interest. (Tiller, 1975; Tiller *et al.*, 1975; Merry and Tiller, 1977; Merry and Tiller, 1978; Merry *et al.*, 1981; McEwen, 2004). Mugford and Steele (1980) analysed wheat grain from 62 flourmills from six states of Australia and reported a mean of 0.09 mg/kg and a range of 0.06 - 0.15 mg/kg, although these high results have since been disputed. Oliver *et al.* (1993) reported in a Australia wide survey of wheat grain and milled products that the maximum mean cadmium values in wheat were found in the Eyre Peninsula region of South Australia and the next highest in the Fremantle zone in Western Australia. Zarcinas *et al.* (2004) analysed wheat from the 2001, 2002 and 2003 seasons grown in the Port Pirie region of South Australia for cadmium and found that 33% of samples in 2001 exceeded the MPC for cadmium of 0.1 mg/kg. The Department of Primary Industry and Energy has monitored the level of cadmium in export wheat as part of the yearly Market Basket Survey (DPIE, 2001), although this survey is now known as the Australian Total Diet Survey (ANZFA, 2003). Actual results are not shown but a summary of mean estimated daily dietary exposure for male and female adults, children and infants is given. In addition, supplementary information from the National Residue Survey Annual Report 2002 - 2003 (DAFF, 2003) lists the number of analyses, the number of positive residues found and the number over the Australian standard. A series of Australia-wide surveys have been undertaken by the Australian Wheat Board (not published) and the results are summarised below:

Table 2.1.2.4.2 Mean cadmium levels in Australian wheat found in surveys from 1982 to 1992

Year	**Mean cadmium level (mg/kg)**						**Samples**	**Laboratory**
	NSW	**Qld**	**SA**	**Vic**	**WA**	**Australia**		
1982	0.013	0.021	0.020	0.020	0.033	0.021	193	SCL
1987	0.027	0.022	0.028	0.022	0.022	0.023	62	SCL
1987	0.034	0.023	0.045	0.020	0.070	0.035	44	AMDEL
1989	0.011	0.010	0.026	0.011	0.026	0.020	507	AGAL
1990/91	0.011	0.011	0.024	0.013	0.031	0.023	999	AWB
1991/92	0.010	0.011	0.028	0.011	0.036	0.025	2367	AWB

All these surveys show a consistent pattern of low cadmium levels in New South Wales, Queensland, Victoria and most of Western Australia and South Australia with elevated levels in central Western Australia and in the York Peninsula around Port Pirie and Wallaroo in South Australia. The elevated levels at localised areas in South Australia may be due to cadmium depositing from zinc and lead smelting at Port Pirie.

2.1.3 Toxicity

Cadmium has been reported by many workers to be harmful to all living systems with no known biological requirement (Yamagata and Shigematsu, 1970; OSHA, 1992b; Brzoska and Moniuszko-Jakoniuk, 1997; Ritz, 1998; Roane and Pepper, 1999; House *et al.*, 2003), although work by Von Zglinicki *et al.,* (1992) has postulated that very low levels of cadmium may stimulate DNA synthesis and cell growth. Sillanpaa and Jansson (1992a) also reported that cadmium in very small concentrations may be beneficial to animals. A relationship between the chemistry and the toxicity of cadmium was shown by Bienvenu *et al.* (1963) when he determined the dose of various soluble metal compounds given to mice that resulted in the death of 50% of the animals within 30 days (LD_{50}). He noted regularities between the relative toxicity and the position of the element in the periodic table.

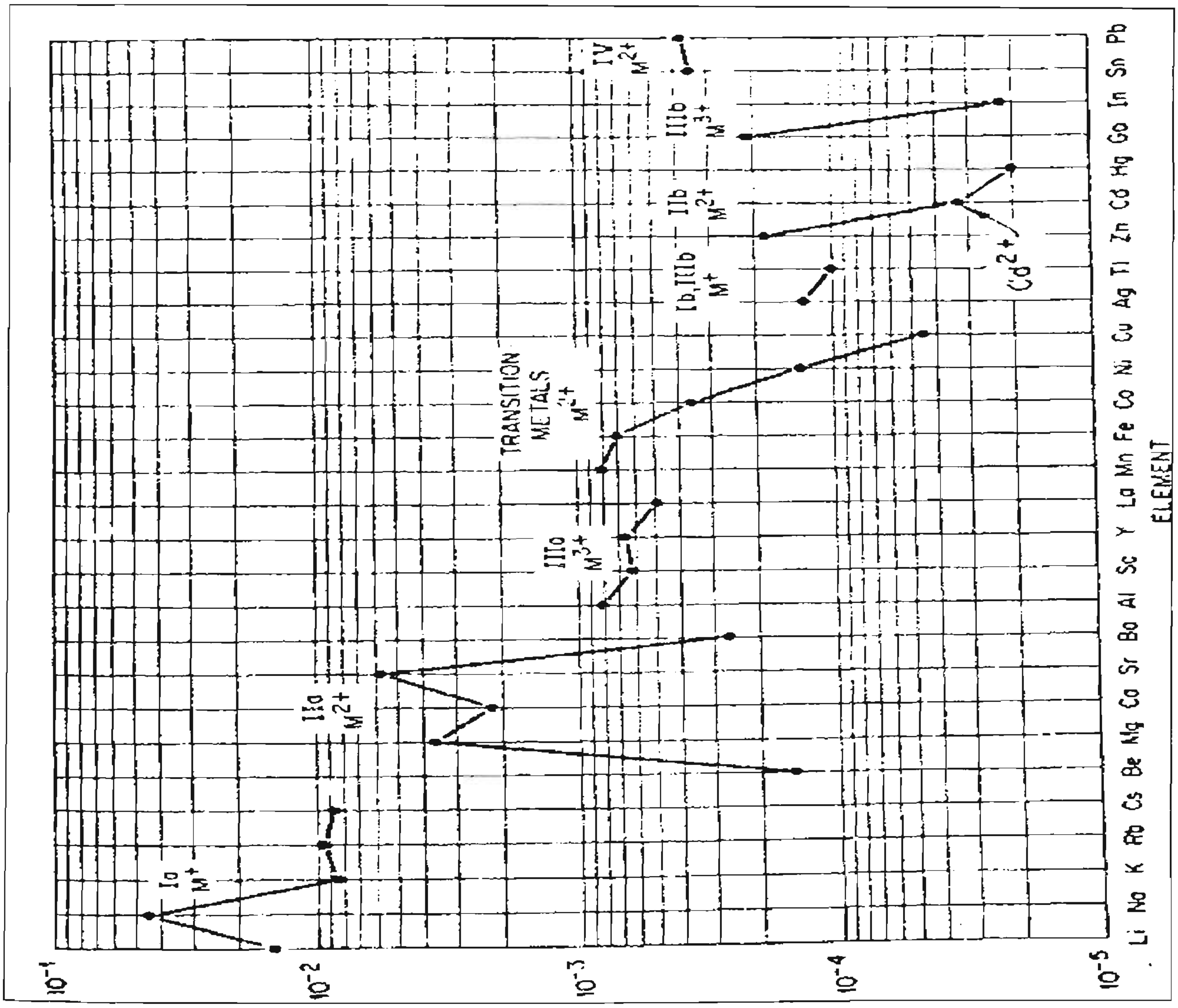


Figure 2.1.3.1 Comparison of the lethal dose of various metal ions according to their position in the periodic table and their charge.

The strongest trend appears to be the general decrease in the LD_{50} in the order : M^+ ions of group Ia, M^{2+} ions of group IIa, M^{3+} ions of group IIIa, M^{2+} ions of the first transition series and then M^+, M^{2+} and M^{3+} ions of the post transition element groups Ib - IIIb. A second trend appears in the M^{2+} transition metal cations, this is a decrease in the LD_{50} with increasing atomic number. The greater toxicity of the heavy metals is also apparent within groups IIb and IIIb. The graph highlights cadmium, a divalent, post transition metal, as one of the most toxic elements with a LD_{50} of 0.000033 g-moles/kg body weight. Only indium (as In^{3+}) and mercury (as Hg^{2+}) were found to be more toxic (Bienvenu, 1963).

All heavy metals can form a wide variety of coordination compounds and ions that bind to various polydentate organic ligands. The binding of such metal ions appears to link enzymes to substrates and may be partly responsible for the power of enzymes to catalyse biochemical reactions. The most important donor atoms that link metal ions to biological molecules are oxygen (O), nitrogen (N) and sulphur (S). The tendency for cadmium to bind to these atoms and displace other metals is shown in Figure 2.1.3.2 (Baes, 1973). This shows a series of bidentate ligands which are attached to the metal ion y two donor atoms to form a chelate complex : oxalate (OO), glycine (ON), ethylene diamine (NN), mercaptyl acetate (SO) and mercaptyl amine (SN) .

It can be seen that cadmium should not displace any of the metals shown from an oxygen donating ligand but will displace Mn^{2+} and Fe^{2+} from nitrogen donors. Cadmium is bound to sulphur more strongly than all the metals shown except Cu^{2+} and Hg^{2+}. In addition, the very extended capability of cadmium to bind to coordination sites of macromolecules, (proteins, DNA and RNA), accounts for the multiplicity of toxic effects observed *in vitro* and *in vivo* (Boisset and Narbonne, 1996).

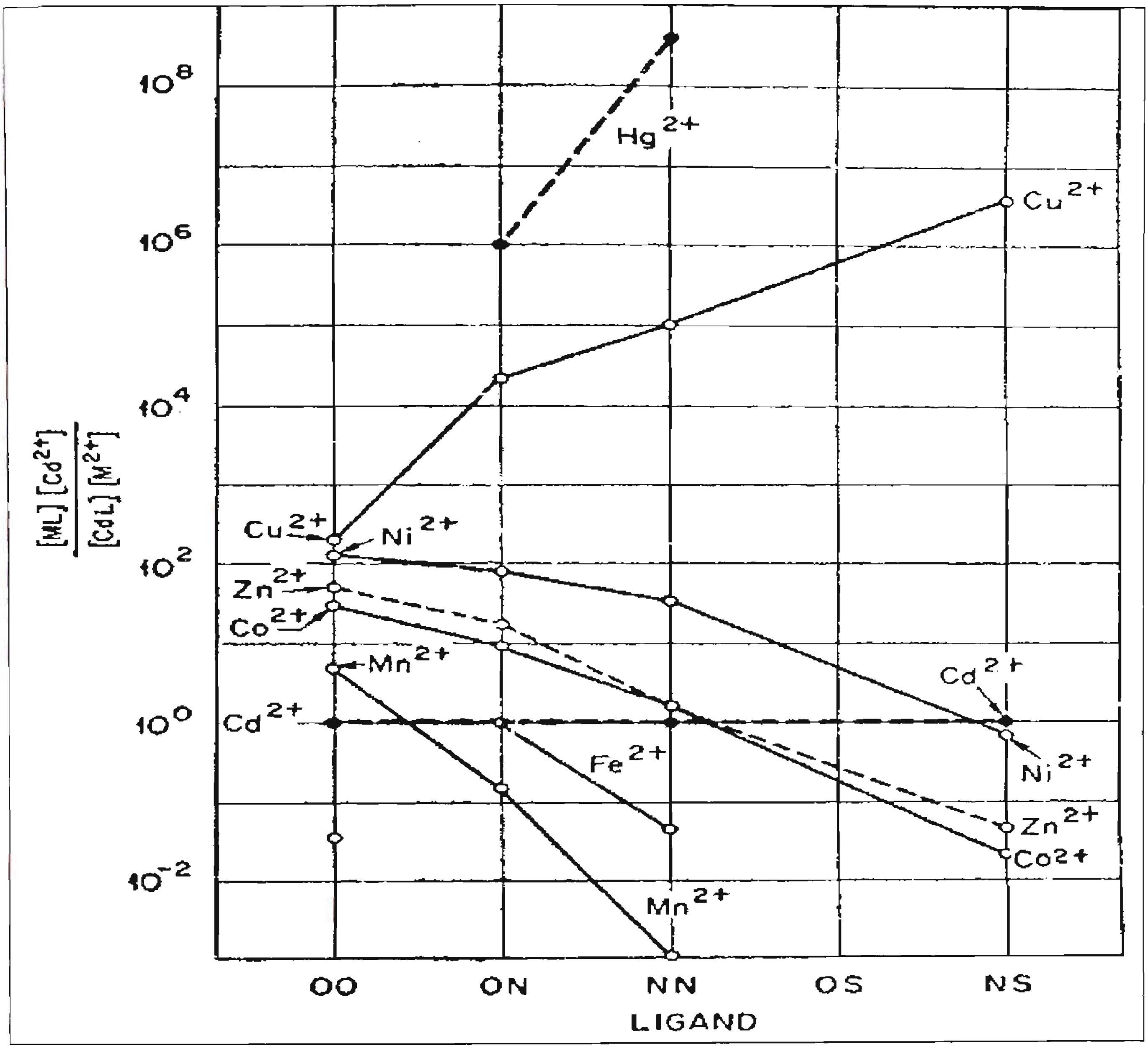


Figure 2.1.3.2 Tendency of cadmium to displace other metal ions from bidentate ligands (Baes, 1973).

These chemical reactions and the relatively strong bonding of cadmium to sulphur donating enzymes may partly explain the effect of small amounts of cadmium on living systems.

2.1.3.1 Absorption

2.1.3.1.1 Inhalation

Cadmium has been reported as far more toxic by inhalation than by ingestion (OSHA, 1992b) due to increased absorption across the respiratory membranes (Friburg *et al.*, 1976). High exposures to cadmium, however, that may be dangerous to life or health in the short term only occur in industries where workers handle large amounts of cadmium dust or fume. Severe exposure may occur before symptoms occur and these may include irritation of the upper respiratory tract, constriction of the throat, a metallic taste and a cough. Smoking has also been reported as a significant source of cadmium in the body. Cigarettes can contain up to 2 mg of cadmium each and smokers can have up to twice the cadmium in their bodies as non-smokers (Telisman *et al.*, 1997). Adams *et al.* (1993) reported that about 20% to 50% of the cadmium from inhaled smoke is absorbed and that cadmium exposure from smoking cigarettes is approximately 10 µg/day. Jarup *et al.* (1998) reported that smokers have about 4 - 5 times higher blood cadmium concentration (about 1.5 µg/L) and twice as high kidney cortex cadmium (about 20- 30 µg/g) as non-smokers. Satarug *et al.* (2004) found that the cadmium burden increased with age and the number of cigarettes smoked per day in a group of 36 healthy Thai men.

2.1.3.1.2 Gastrointestinal tract

As cadmium has been reported as a non-essential element (Von Zglinicki *et al.*, 1992) without a positive absorption mechanism, ionic cadmium is almost certainly absorbed passively through the gut wall, although Batten and Marr (1995) reported that increased phytic acid in the diet has a negative effect on mineral bioavailability. Extensive studies of this passive trans-intestinal movement by Foulkes and McMullen (1987) and Foulkes (1988) support a model of several steps:

(1a) Non-specific electrostatic binding of cationic cadmium to anionic sites on the outside of the mucosal membrane.

(1b) Temperature-sensitive internalisation into epithelial cytoplasm by movement due to membrane fluidity.

(2) Completion of the absorptive process by transport across the basolateral membrane into serosal fluid.

The rate of step 2 was reported to be 2% or less than the rate of the combined step 1 at cadmium concentrations between 2 and 200 µmole (Foulkes, 1986). Lindberg *et al.* (2204) found that cadmium absorption is by channels that are also permeable to calcium and potassium and is dependent on membrane potential. During studies on rats fed diets containing wheat, House *et al.* (2003) found that the initial cadmium absorption was only 3.8 to 2.6% of the dose and that after 15 days, 32 to 44% of the cadmium retained in the body was still in the gastrointestinal tract. In addition, they found that increased intake of dietary zinc lowered cadmium absorption and retention; they also found that retention of cadmium in the body at 15 days postprandial from diets containing adequate zinc was less than 1.3%.

2.1.3.2 Toxicology

Nephrotoxicity

Long-term exposure to cadmium results in an irreversible tubular nephropathy that may develop into renal insufficiency (Horiguchi *et al.*, 1996). The cadmium-metallothionein complex is taken up almost quantitatively from the glomerular filtrate by epithelial cells of the proximal tubule and rapidly degraded by lysosomes. Although biosynthesis of metallothionein, in response to cadmium ions liberated by proteolysis, also occurs in these cells, it has been reported by Ikeda *et al.* (2000) that part of the cadmium ions escapes this cytosolic binding system and reaches other subcellular targets (such as the mitochondria and the nucleus). As a result, the reabsorption of low molecular weight proteins (including β2-microglobulin), ions (calcium and phosphate) and small solutes (glucose and aminoacids) is irreversibly impaired. The relationship between the critical concentration of cadmium in renal cortex and the early signs of tubular dysfunction in humans has been the subject of studies of groups of exposed workers and populations living in polluted areas (Jung *et al.*, 1993; Roels *et al.*, 1993; Ikeda *et al.*, 2000; Shimbo *et al.*, 2000). A limit of urinary cadmium excretion of approximately 4 µg/g of creatinine has been derived from surveys conducted by Watanabe *et al.* (1996 and 2000) in the Ishikawa prefecture of Japan (Itai-Itai disease). Beyond this level the excretion of ß2-metallothionein-cadmium complex becomes pathological.

Belgian studies (Lauwerys *et al.*, 1990) have found evidence that the threshold of urinary cadmium for increased excretion of low molecular weight proteins, aminoacids and calcium is approximately 2 µg/g. This is reached in 10% of subjects in the general population and increases to 16% of the population over the age of 60 (Buchet, 1990). Roels (1981) in a retrospective study of the causes of mortality, from 1969 to 1976, indicates a very significant increase in mortality from nephrosis and nephritis in women over 60 and living in the Liege conurbation. This area has been markedly polluted by cadmium emissions from zinc smelters. Nishijo *et al.* (2004) reviewed research conducted in Japan, Thailand, Australia, Poland, Belgium and Sweden and identified six risk factors for cadmium related health effects in women. They were, (1) more serious renal tubular dysfunction, (2) difference in calcium metabolism, (3) kidney sensitivity, (4) Pregnancy, (5) body iron store status and (6) genetic factors.

Osteotoxocity

Although the Itai-Itai syndrome, in which renal insufficiency is associated with osteoporosis and osteomalacia, has been studied for more than twenty years (Bellinger *et al.*, 2000; Watanabe *et al.*, 2000), the precise pathogenic mechanism of this disease has not been fully established. It is not known whether bone deterioration is secondary to nephrotoxic damage or results from a direct effect on bone tissue. Studies conducted in Japan strongly suggest that osteopathies and mortality, at least in women, are closely related to the kidney damage brought on by the urinary excretion of the ß2-metallothionein-cadmium complex (Kido *et al.*, 1990; Horiguchi *et al.*, 1994). Three hypotheses have been formulated for osteotoxicity and partially confirmed by experiments on animals, human tissue and cells in culture (Boisset and Narbonne, 1996). The first involves an inhibition of the formation of the dihydroxylated metabolite of vitamin D_3 leading to an increase in the incorporation of cadmium in the bone and ultimately to osteomalacia. The production of this active metabolite of vitamin D_3 is dependant on cyclic AMP, adenylate cyclase, parathyroid hormone and cytochrome(s) P-450, all of which are factors adversely affected by exposure to cadmium.

The second hypothesis implies that cadmium counteracts the absorption of calcium in the small intestine, decreasing the bioavailability of this element and producing a decalcification of bone that is the primary characteristic of osteoporosis. The third proposes a disturbance of collagen in bone by inhibition of lysyl-oxidase. Nutritional deficiencies (calcium and vitamin D), multiparity and hormonal factors are key factors in the development of cadmium-induced bone pathology. It has been reported by Boisset and Narbonne (1996) that cadmium causes bone lesions in rodents when associated with a hypocalcic diet but this is at a lesser magnitude than that found in Itai-Itai patients. In Japanese women with renal dysfunction that was associated with bone damage or not, the isozyme of alkaline phosphatase originating from bone tissue was elevated by cadmium exposure. This indicates a direct effect of cadmium on bone mineralisation (Tsuritani *et al.*, 1994). Huogo *et al.* (2005) examined 1380 female Japanese farmers and found that environmental exposure to cadmium at levels insufficient to induce renal dysfunction did not increase the risk of osteoporosis and postulated that bone injury from calcium was a secondary effect.

Cardiovascular Toxicity

Studies, mostly carried out more than 15 years ago, have demonstrated that chronic oral administration of low levels of cadmium to rats, via food or drinking water, causes a rise of arterial pressure (ATSDR, 1989; Friberg *et al.*, 1992b). These studies indicate that the pressor effect of cadmium can only be observed in the presence of a range of experimental conditions (strain, composition of the diet, exposure time, etc). None of these studies has shown the mechanism(s) of the hypertensive effect of cadmium. This is not surprising as blood pressure is regulated by many systems that may be mutually compensating. The pressor effect of cadmium may be regarded as a direct effect on vasculature, functional damage to the kidneys and an altered liberation of neuromediators.

Some surveys, on small groups of humans with low-level environmental exposure, have found a positive association between the accumulation of cadmium and an increase in blood pressure. Other surveys have failed to confirm it or even have revealed negative correlations between the two variables (Friberg *et al.*, 1992b). None of the studies conducted in several cadmium-polluted districts in Japan, the United Kingdom, Belgium or in the United States have shown evidence of a positive relationship between blood pressure, the prevalence of cardiovascular diseases and urinary cadmium (Staessen *et al.*, 1991; Whittemore *et al.*, 1991; Bernard *et al.*, 1992). In addition, the significant increase in mortality in Japanese suffering from tubular proteinuria is not associated with a greater prevalence of hypertension and cardiovascular disease.

Reproductive Toxicity

In rodents, acute intratesticular administration of cadmium has long been shown to produce vascular lesions and interstitial oedema provoking a reduction in the production of androgens and a failure of spermiation (Friberg *et al.*, 1992b). The effect of low-level exposure on male reproductive organs is less obvious. Data relating to man is very limited and indicates that moderate long-term exposure has little effect on male sterility. Pre- and post-natal exposure of female rats to cadmium produced a reduction of oestrogen production and development of the uteri-ovarian function. This function is also affected in adult females, particularly with regard to the implantation and growth of the embryo (EPA, 1994). Exposure to cadmium during successive pregnancies increases the amount of metal stored in the kidneys of mice and it has been suggested that in mammals, including man, maternal exposure produces a placental accumulation of the metal with a subsequent loss of zinc (Goyer, 1991). He suggested that the decrease in the placental Zn/Cd ratio observed in mothers that smoke tobacco might be associated with a reduced fetal growth and consequently a decreased birth weight. Cadmium does not cross the placenta-fetal barrier and is not present in newborn children.

Genotoxicity

Cadmium appears to be both a direct and indirect mutagenic agent in both bacteria and mammalian cells. The most frequent chromosomal aberrations in mammalian cells are ruptures and lacunae (gaps) rather than exchanges. Aneuploidy and blockade of meiotic division have also been found in yeasts and oocytes, demonstrating that cadmium acts as a cytoskeleton poison. Studies of lymphocyte chromosomes from human subjects exposed to cadmium in the workplace or suffering from Itai-Itai have produced conflicting results (Boisset and Narbonne, 1995). Epidemiological data on groups of exposed workers in the USA and Europe has been collected from the beginning of 1960 and updated periodically. This shows a very clear relationship between long-term exposure to cadmium oxide dust and fumes and the prevalence of cancers of the respiratory tract and a weaker relationship to prostate cancers (IARC, 1993; Waalkes *et al.*, 1992). Cadmium has been placed in group I of the IARC classification of carcinogens (Rossman *et al.*, 1992).

No clear relationship between the prevalence of internal cancers and the dietary intake of cadmium has been shown in countries such as Japan (Arisawa *et al.*, 2001) and Belgium where environmental contamination is high. Limited data from Canada and the USA suggest that there may be a positive link between the overall level of environmental pollution by cadmium and prostrate cancer (Waalkes and Rehm, 1994). Recent work by Gordenin *et al.* (2003) has reported that cadmium disturbs a DNA repair system important in preventing cancer. Gordenin stated 'cells must replicate their DNA in order to increase their numbers enough to replace dying cells. In duplicating the DNA, however, mistakes are made with a frequency that organisms would not be able to tolerate unless corrected. Most organisms correct these mistakes by a mechanism known as post-replication mismatch repair.' He found that cadmium exposure to living cells blocks this repair of natural errors and increases mutations dramatically. He also found that the amount of cadmium needed to inhibit repair and increase mutations was very low and may be environmentally relevant to cadmium-related industry workers and smokers. The USEPA (1987) found that a two-fold increase in the risk of lung cancer was observed in 602 cadmium smelter workers that could not be correlated with exposure to arsenic or smoking.

2.1.4 Maximum Permitted Concentration

2.1.4.1 Worldwide

The Codex Alimentarius was created in the early 1960's at the joint conference of the FAO and WHO on food standards, which established the joint FAO/WHO Food Standards Program (Navarro and Wood, 2001). Although the aim of the conference was essentially economic, it was also to address, at an international level, the removal of non-tariff barriers to trade in foods caused by differing national food standards and to facilitate international trade. Today, the main purpose of the Codex Alimentarius is to ensure the protection of public health and fair trade practices and to facilitate international trade in foodstuffs via harmonised international standards. Codex standards contain requirements for foods aimed at ensuring a sound wholesome food product.

The FAO/WHO Joint Expert Committee on Food Additives (JECFA) recommended in 1980 that a provisional weekly tolerable intake (PWTI) be established for cadmium of 7 μg/kg body weight. In 1992, the International Program on Chemical Safety (IPCS) undertook a review of cadmium and produced an Environmental Health Criteria document. The IPCS conclusions were essentially that a lifetime exposure to 140-260 ug/day might lead to low molecular weight proteinuria. JECFA again considered cadmium in 1993 and had available the IPCS review. They confirmed that the Permissible Total Weekly Intake (PTWI) of 7 μg/kg of body weight should stand pending further research and reasserted 'there is only a relatively small safety margin between exposure in the normal diet and exposure that produces deleterious effects'. (ANZFA, 1997)

The joint FAO/WHO Food Standard Program Codex Committee on Food Additives and Contaminants (CCFAC or often known as the Codex Committee in this context) is the committee that sets international limits for contaminants in food in accordance with a procedure laid down in the Codex general standard for contaminants and toxins in foods. The Codex Committee met in March 1995 and a position paper prepared by France recommended that the Committee should propose international limits for cadmium in foods. Limits were not set by the committee because it was felt that sufficient results were not available and that rather than change limits piecemeal it would be better to wait until enough results were available to set limits for all foods. The European Community suggested at that time that a maximum permitted concentration (MPC) of 0.05 mg/kg would be appropriate for cereals (WHO, 2002).

In March 1999, the committee met again and agreed to return the draft guideline level for cereals, pulses and legumes to step 6 and to circulate the other maximum levels at step 3 for comments and consideration at the next session. The committee last met in March 2001 and the following comments relevant to cereals were received from Canada, Japan and the European Community (EU) (WHO, 2001a) :

Canada stated that in regard to the proposed tolerance of 0.1 mg/kg for cereals, pulses and legumes, previous monitoring of bakery goods and cereals had shown a mean level of 0.016 mg/kg and a range of < 0.005 to 0.071 mg/kg. Monitoring of dry rice cereal showed a mean value of 0.024 mg/kg and a range of 0.015 to 0.029 mg/kg. They agreed that the proposed MPC's were reasonable but they would continue to submit new data when it became available.

At the 32nd CCFAC, Japan had shown its commitment to submit the results of chronic toxicological studies on rats to the 55th JECFA. Subsequently, the Japanese government submitted these studies to JECFA along with epidemiological study data from Sweden. JECFA subsequently decided to maintain the provisional tolerable weekly intake (PTWI) for cadmium at 7 µg/kgbw/wk and to request further toxicokinetic and dietary studies to 'improve confidence in estimates of predicted excess prevalence of renal tubular dysfunction' (WHO, 2001b). The European Community stated that the proposed levels were generally in line with the proposed draft levels in the EU and they would support them with the exception that a maximum level of 0.1 mg/kg for cereals excluding bran, germ, wheat grain and rice is supported. They requested that the separate level of 0.2 mg/kg for wheat grain and rice should also include bran and germ.

At the 36th meeting of CCFAC in March 2004, it was decided to retain the draft ML for cadmium in wheat of 0.1 mg/kg at step 5 and that bran and germ would be excluded (CCFAC, 2004a). The next meeting of CCFAC will be in April 2005 and discussions of the new draft maximum levels for cadmium are item 17 (d) on the agenda (Codex, 2004a). These draft maximum levels were adopted by the 27th session of the codex Alimentarius Commission in July 2004 for cadmium in wheat grain, potato, stem and root vegetables, leafy vegetables and other vegetables (CCFAC, 2004b).

2.1.4.2 Australia

The Australian National Health and Medicine Research Council (NHMRC) at its 77th session in November 1973 first adopted a recommendation setting a maximum permitted level of cadmium in fish, crustaceans, mollusks, the fish content of fish products and the fish content of canned fish products of 2 mg/kg. In 1980, the Food Standards Committee of the NHMRC reviewed the relevant information on cadmium in foods and recommended that the following revised standards for cadmium to be included in the Standard for Metals in Foods :

Table 2.1.4.1 Australian Food Standards Code (1980) : Metals in Foods

Food	**MPC of cadmium (mg/kg)**
All other foods	0.05
Beverages and liquids	0.05
Bran and wheatgerm	0.2
Edible offal	2.5
Fish	0.2
Shellfish (including mollusks)	1.00

The recommendation was based on a JECFA provisional tolerable weekly intake for cadmium of 0.0067 to 0.0083 mg/kg body weight. The new standard was considered to be acceptable to the States and Territories, except for the maximum permitted concentration in mollusks which was raised to 2 mg/kg in accord with the recommendation of the 77th session of JECTA. These levels were set based on the 1975 and 1976 market basket surveys and some results from the NSW Government Analytical Laboratory. The recommendations were arrived as a result of consideration of the toxicology and the range of cadmium levels found in the food together with professional judgment by the advisory committee. The results were considered inadequate to make even reasonable estimates of the likely intake of cadmium.

In 1985, the National Food Authority (NFA, renamed from the NHMRC) received a number of proposals to review and to generally increase the MPC of cadmium in various food commodities. These requests were as a result of surveys that had shown that some products did not comply with the current standards. The first formal request to the NFA was an application (A208) to increase the MPC for cadmium in rice crackers form 0.05 to 0.1 mg/kg. The NFA replied that it had problems proceeding with commodity-by-commodity reviews as applications were submitted. It was felt that if a disproportionate amount of the PTWI is allocated to the first commodity reviewed, this might impact on the outcome of a review of a second commodity. They also felt that industry should not suffer the uncertainty that would result if the MPC for a commodity was increased with the possibility of being subsequently decreased as a result of the standard-by-standard review. The NFA expanded the application for a change in the MPC for rice crackers to include a number of other food groups. But they stated that :

'It is apparent that the piecemeal development of the cadmium standards and an individual commodity review will make it difficult to assess whether the consumption of cadmium by individuals is close to the PTWI. All national survey and food consumption data need to be considered as well as individual commodity data to assess the likely intakes for the overall population'.

Table 2.1.4.2 Australian Food Standards Code (1985) : Metals in Foods

Food	**MPC of cadmium (mg/kg)**	
	Current (mg/kg)	**Proposed (mg/kg)**
Crustaceans	0.05	0.1
Pasta products	0.05*	0.08
Poppy seeds	0.05	2.5
Potatoes and potato chips	0.05*	0.1

* included in 'all other foods'

It was stated at the 1995 Cadmium Workshop in Canberra that 'the NFA's stated intention to undertake a full review of the MPC for cadmium in all foods should be carried out as a matter of urgency to address the needs of many groups within the food industry, the agricultural community and Regulatory authorities' (Connolly, 1988). The NFA published the following limits for cadmium in the Australian Food Standards Code as Standard A12 (NFA, 1995) :

Table 2.1.4.3 Australian Food Standards Code (1995) : Standard A12

Food	**MPC of cadmium (mg/kg)**
Beverages and other liquid foods	0.05
Bran	0.02
Cocoa	0.5
Cocoa paste	0.35
Chocolate	0.25
Drinking chocolate, powder	0.25
Crustaceans	0.2
Fish	0.2
Edible offal other than liver	2.5
Liver	1.25
Meat Muscle	0.2
Molluscs	2.0
Seaweed	0.2
Water	0.005
Wheat germ	0.2
Foods not containing a food otherwise specified	0.05

The NFA was renamed the Australian and New Zealand Food Authority (ANZFA) in 1998 and published the following revised MPC's for cadmium in the Australian Food Standards Code also as Standard A12.

Table 2.1.4.4 Australian Food Standards Code (1998) : Standard A12

Food	**MPC of cadmium (mg/kg)**
Chocolate and cocoa products	0.5
Kidney of cattle, sheep and pig	2.5
Leafy vegetables	0.1
Liver of cattle, sheep and pig	1.25
Meat of cattle, sheep and pig (excluding offal)	0.05
Mollusks (excluding dredge/bluff oysters and queen scallops)	2
Peanuts	0.1
Rice	0.1
Root and tuber vegetables	0.1
Wheat	0.1

A level for 'all other foods' was not set.

ANZFA was renamed Food Safety Australia and New Zealand (FSANZ) in 2001 and reconfirmed the MPCs for cadmium in Table 2.1.5.4 as maximum levels (MLs) in the Australian Food Standards Code, Volume 2 Standard 1.4.1. FSANZ also stated 'it is well known that cadmium is ubiquitous in the environment and occurs at low levels in foods other than those listed in the Standard. Therefore, in order to assist with the enforcement of ML's in mixed foods that may contain this contaminant, the calculation requires the inclusion of a representative contaminant level for those foods that do not have an allocated ML'.

In the past, a ML was set for 'all other foods'. As the category for 'all other foods' has been discontinued, a representative level has been set at the limit of quantification (LOQ) for cadmium. This is currently 0.005 mg/kg and is consistent with the LOQ published in the 1966 Australian Market Basket Survey (NRS, 1966). FSANZ stated that 'It is acknowledged that the LOQ may change with time as analytical techniques become more sensitive. The Standard will be reviewed periodically in respect with this issue' (FSANZ, 2002). FSANZ has maintained the levels set by FSANZ in 1998 (Table 2.1.4.4) and has not suggested a change in the near future (FSANZ, 2004b).

2.1.4.3 USA

The acute oral reference dose (ARfD) of the US EPA is based on the assumption that a threshold exists for toxic effects such as cellular necrosis and is expressed as mg of the toxic substance consumed per kg of body weight per day. The ARfD is an estimate of a daily exposure even to sensitive sub-groups, which is likely to be without appreciable risk over a lifetime of exposure (US EPA, 2002, FSANZ, 2003). Hazard Quotients are used to quantify heavy metal exposures. The total daily intake of cadmium is calculated and divided by the ARfD. When the resultant hazard quotient value is less than one, no harm is expected. If the value is greater than one, the threshold has been exceeded and toxicity is likely to occur (Oosthuizen and Boyce, 2002). The US EPA ARfD for cadmium is 0.001 mg cadmium/kg body weight/day. No significant proteinuria was used as the benchmark for establishing the ARfD.

In determining the oral (food ingested) ARfD, consideration was given to the fact that cadmium would also be ingested with drinking water. Based on a no observable adverse effect level (NOAEL) of 0.005 mg/kg/day and an uncertainty factor of 10, an acute oral (food) ARfD of 0.001 mg/kg/day was determined for cadmium exposure (US EPA, 2002)

2.2 WHEAT

2.2.1 Domestication

Man has used wheat as a food since prehistoric times. It is generally believed that wheat evolved from wild grasses somewhere in the Near East. These grasses were adapted to the steppes or semi-arid areas, characterised by winter rains and dry summers and developing with available autumn-winter moisture. Depending on the elevation of these areas, the grasses reached maturity in late spring or summer (O'Dea, 1987). Humans probably first gathered wheat from the wild as long as 12,000 to 17,000 years ago (Mattern, 1991; Abdel-Aal *et al.*, 1998). Types collected may have been *Triticum monococcum* (einkorn) and later *T. dicoccum* (emmer) (Atwell, 2001).

It is certain that wheat was one of the first cereal grains to be cultivated around 10,000 to 12,000 BCE in the area known as the Fertile Crescent (Quail, 1996; Abdel-Aal *et al.,* 1998; Guglhupf, 2004). This is a mountainous region in the upper reaches of the Tigris-Euphrates drainage basin bounded on the east by the Zagros mountain system and on the north by the Taurus Mountains. This was in the area known as Mesopotamia and is now part of Iraq (Fabriani and Lintas, 1988). Domestication of wheat and barley, as well as that of other wild grain legumes, resulted in the settling of local people and determining the transition of humans from the 'shepherd-hunter-gatherer' phase to the 'farmer' phase (O'Dea, 1987).

The potential of having a cereal, a food of high energy and quality all year round, made possible the first human settlements. Consequently, higher levels of social life evolved, including agricultural activities and the establishment of new types of artisans and tradesmen. Starch and plant proteins represented a source of food that was easy to store without continuous care and reduced the need to search for the forage required by domestic animals. The first basic step towards civilization was made when humans started sowing seeds that had been gathered from the previous season, adopting the first measures for soil preparation and tillage and discovering the best time for sowing.

Several varieties of emmer have been found in the rubbish heaps of the lake dwellers of Wangen and Robenhausen (Matz, 1991a). These deposits date to the end of the Neolithic epoch, a little before the Bronze Age. Carbonised remains of wheat grains and imprints of grains in baked clay have been found in the Neolithic site of Jarno in northern Iraq having an estimated radiocarbon date of 6700 BCE (Inglett, 1974). Both emmer and durum (*T. durum*) wheat have been found in Egyptian tombs of the first dynasty, indicating cultivation along the Nile at least 6000 years ago (Shellenberger, 1969).

Mention of wheat is made by the Assyrians and Babylonians in stone ruins dating from 3000 BCE (AWB, 1997). The Chinese were growing wheat in 2700 BCE and had developed elaborate rituals to honour it. It has been cultivated around the Eastern Mediterranean for at least 5000 - 6000 years. Radiocarbon dating studies have been applied to the elucidation of a pattern of the spread of wheat cultivation. These place the beginning of the cultivation of wheat approximately 6000 years ago. Cultivation began in the Syria-Palestine area and spread west and south into Egypt and east into Iran. From Iran, wheat spread into India, China, Russia and Turkistan. From Egypt, Palestine and Syria, it moved into South and Central Europe (Matz, 1991a).

The first wheats to reach Europe were emmer and einkorn about 3000 BC. Bread wheat (*T. aestivum*), in many respects similar to that used today, began to spread over Europe from southern Russia about 2000 BCE (Atwell, 2001). The Spaniards first brought wheat to the North American continent in 1520 to the area now known as Mexico. The first wheat cultivated in the region now encompassed by the USA was sown by a man named Gosnold on the Elizabeth Islands of the southern coast of Massachusetts (Matz, 1991a). The First Fleet under Governor Phillip brought the first wheat to Australia in 1788. This was planted by the Governor's servant, Henry Dodd, in a small plot of 8 acres at Farm Cove, east of Sydney (Eckersley, 1994; Simmonds, 1989).

2.2.2 Structure

Wheat, like all cereal grains, produces dry, one seeded fruit that develop within floral envelopes called glumes. These envelopes are formed from modified leaves and do not split open to shed the seed at maturity (Lasztity, 1999). The seed consists of germ or embryo and endosperm enclosed by a nucellar epidermis and a seed coat. A fruit coat or pericarp surrounds the seed and adheres closely to the seed coat. This type of fruit, usually called a kernel or grain, is more properly described as a caryopsis (Lasztity, 1999). It is characteristic of the members of the grass family Gramineae (Pomeranz, 1971).

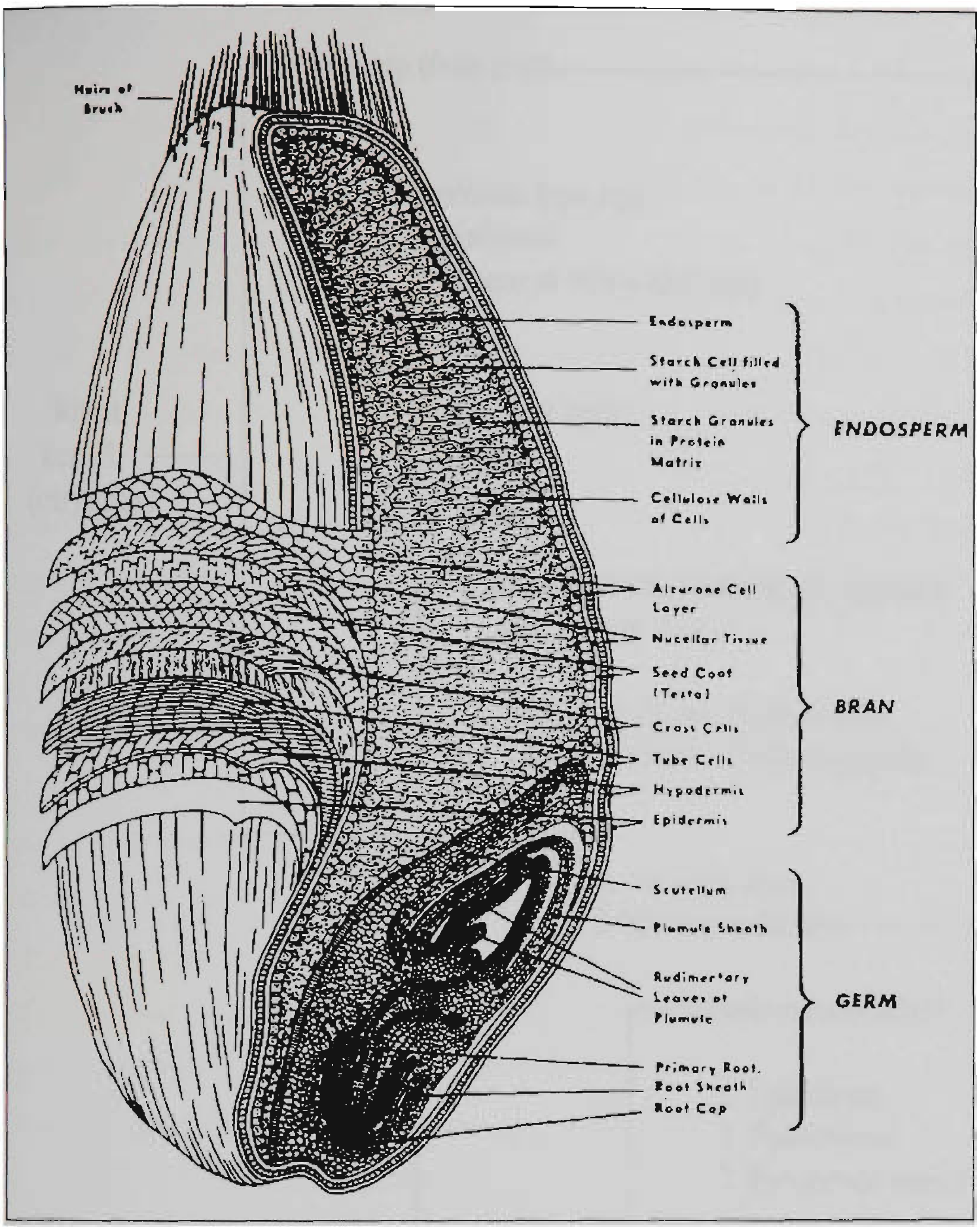


Figure 2.2.1 Longitudinal section of a wheat kernel (Pyler, 1973)

There are several features of the endosperm that affect milling end-use and quality. The endosperm actually consists of two major cell types, the starchy endosperm that is the main consistent of flour and the aleurone layer. Aleurone cells in most wheats are one cell thick at maturity and form the outermost layer of endosperm tissue. They almost completely surround the kernel over the starchy endosperm and germ. The aleurone layer is removed from the starchy endosperm during milling and is considered a part of the bran by the miller. The bran is botanically a combination of the pericarp, seed coats, nucellus and aleurone layer (Hoseney , 1986).

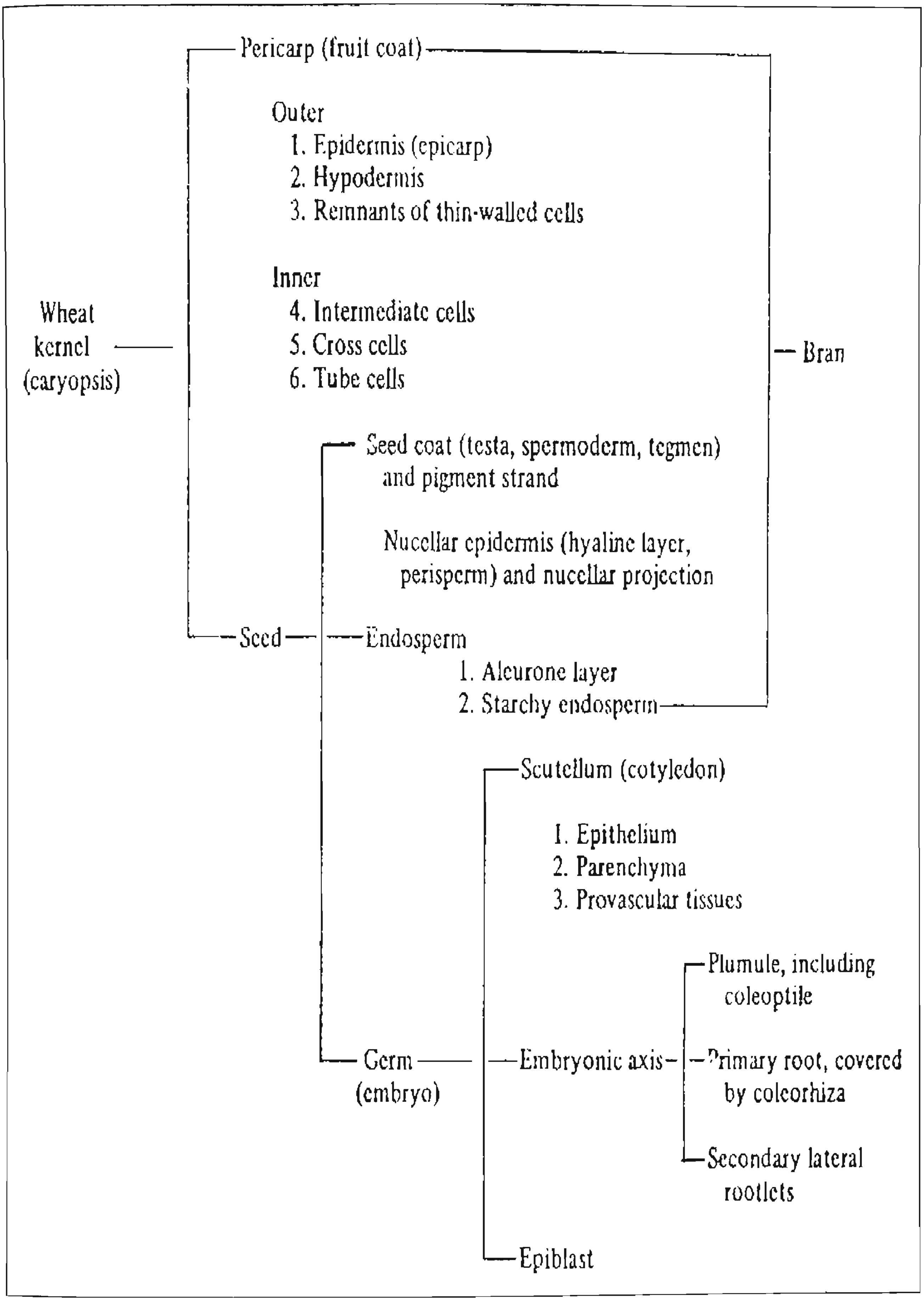


Figure 2.2.2 Structure of a wheat kernel (MacMasters *et al.*, 1971)

2.2.2.1 Pericarp

The pericarp surrounds the entire seed and acts as a protective covering. It is composed of several layers and these are, from the outside towards the center of the kernel, epidermis, hypodermis (these two layers together are known as the outer pericarp and are called beeswing by millers). In addition, remnants of thin-walled cells, intermediate cells, cross-cells and tube cells form layers known as the inner pericarp. The epidermis consists of a single layer of cells that forms the outer layer of the kernel except in the area where the harvested kernel was attached to the parent plant. On the outer walls of the epidermal cells is a thin, relatively water-impervious cuticle that is especially delicate. This extends over the germ and for a short distance from it towards the brush. Many epidermal cells at the apex of the kernel are modified to form the hairs that constitute the brush (Mattern, 1991).

The hypodermis is usually composed of one, but occasionally two, layers of thickened cells. Remnants of thin-walled cells lie just inward from the hypodermis and may represent the innermost portion of the hypodermis. This area forms a natural plan of cleavage between the outer and inner pericarp. Movement of water and fungal hyphae are also facilitated by the lack of continuous cellular structure in this area. The irregularly shaped intermediate cells are absent over at least some parts of the kernel. They always occur at the brush end and in the vicinity of the germ. Here they are somewhat flattened and their projections join them to one another and to the cross cells so that numerous intercellular result in these regions (Henry and Kettlewell, 1996).

Over most of the kernel, a layer of elongate cross-cells lies directly interior to the remnants of thin-walled cells. These cells are so called because their long axis lie perpendicular to the length of the kernel. Over the germ, the irregularly shaped cross-cells have knoblike projections on the outer walls. Tube cells of long and more or less cylindrical shape constitute the inner epidermis of the pericarp. They are found in a narrow band along the back of the kernel and lie with their long axis parallel to the length. They gradually spread towards the sides near each end and cover both the protruding tip of the germ and the apical end of the kernel under the brush (Matz, 1991b).

2.2.2.2 Seed coat

The seed coat joins the pigment strand in the crease and together they form a complete coat around the endosperm and the germ. This seed coat is firmly joined to either the cross or tube cells on the outside and to the nucellar epidermis on the inside. At maturity, it becomes filled with a cork-like material. The seed coat can be divided into three layers: a thick outer cuticle, a colour layer that contains pigment and a very thin inner cuticle. A fourth, extremely thin, layer of hyaline material has been reported. This is formed from the swollen pectin-containing walls of the outer cell layer of the seed coat (Pomeranz, 1971).

2.2.2.3 Nucellar epidermis

The nucellar epidermis, commonly called the hyaline layer, is a compressed cellar layer lying between the seed coat and the aleurone layer. In the crease, this is joined to a band of cells which runs parallel to the to the pigment strand and is called the nucellar projection, The nucellar epidermis usually covers the entire kernel with the exception of the germ.

2.2.2.4 Aleurone layer

Botanically, the aleurone layer is the outer layer of the endosperm, but millers commonly regard it as part of the bran. It is usually one cell thick and almost completely surrounds the kernel over the starchy endosperm and the germ. The aleurone cells are approximately square or slightly oblong with the longer axis most commonly perpendicular to the surface of the kernel. The aleurone cell walls are largely cellulosic in composition and adjacent cells are cemented together by non-cellulosic material (Bradbury *et al.*, 1956). Each cell of the aleurone layer contains a large nucleus and a large number of aleurone granules embedded in a matrix.

The aleurone layer is modified over part of the kernel as described by Bradbury *et al.*, (1956). Where it borders the endosperm cavity near the crease, the walls between adjacent cells of the layer and between them and the starchy endosperm cells contain pits. They suggested that the layer in this region has not functioned meristematically during kernel development as it differentiates at an early stage. The modified aleurone layer extends over the edge of the scutellum and continues as an interrupted layer over much of the embryonic axis. The aleurone layer extends over the plumule of the embryo but does not contain aleurone granules in this area, neither does it extend over the projecting embryo tip (Pomeranz, 1971).

2.2.2.5 Starchy endosperm

The starchy endosperm is composed of three types of cells, peripheral, prismatic and central, which differ in shape size and location within the kernel. The peripheral cells are just inside the aleurone layer and are either approximately spherical or elongated towards the center of the endosperm. Several rows of radically elongated prismatic cells lie inward from the peripheral cells and extend from the back of the kernel to the crease. The central cells lie inward from the prismatic cells and are irregular in size and shape. The source of flour is the starchy endosperm obtained when wheat is milled. Its cells are packed with starch granules which lie embedded in a largely protein matrix. In each kernel, there is usually an outer vitreous portion of the starchy endosperm and an inner floury portion (Henry and Kettlewell, 1996).

The starch occurs as large lens-shaped granules and smaller spherical granules without a sharp separation in size between the two groups. Pomeranz (1971) reported that small starch granules in vitreous endosperm are rounded and are separated by considerable proteinaceous material while those in floury endosperm are closely packed, polygonal and surrounded by relatively little proteinaceous material. Two types of endosperm cell protein have been reported (MacMasters *et al.*, 1971). The first, consisting of salt-soluble albumins and globulins, is largely functional cytoplasmic and membrane protein. The other, the gluten-forming gliadin and glutenin, is storage protein produced in discrete particles by protein-forming proteoplasts similar to starch-forming amyloplasts. In the mature kernel, the identity of these protein bodies is largely lost due to compression by the developing starch granules (Cornell and Hoveling, 1998).

2.2.2.6 Germ

The germ is composed of two major parts, the embryonic axis which is made up of rudimentary root and shoot and the scutellum that functions as a storage, digestive and absorbing organ. The upper part of the embryonic axis, the plumule, forms the shoot of the plant when the seed germinates. The plumule consists of several embryonic foliage leaves, attached to a very short rudimentary stem and the coleoptile which curves over the stem apex. The lower part of the embryonic axis is composed of the primary root, with a root cap on its tip and the enclosing coleorhiza. Two pairs of lateral roots are also present.

Below the plumule, the scutellum is attached to the embryonic axis on the side toward the endosperm. The scutellum spreads and extends like a shield over that side of the embryonic axis. A slight projection near the upper tip of the scutellum is known as the central scale. The epiblast, a small, scalelike structure, arises from the embryonic axis opposite the point of attachment of the scutellum. The scutellum contains food that is stored during maturation. At germination the scutellum not only supplies the food that has previously been stored in it but also becomes a digesting and absorbing organ for the transference of food from the endosperm to the growing parts of the embryonic axis. The outer cell layer of epidermis, of the portion of the scutellum adjacent to the endosperm is modified to form a layer of secreting cells, the scutellar epithelium.

Partially developed conducting tissues form a provascular bundle that may be observed extending into the scutellum. The provascular bundle extends upward and divides into many branches that spread through much of the scutellum. The greatest portion of the scutellum is composed of unspecialised (parenchyma) cells that contain considerable amounts of oil and protein, but little starch. The embryo is a separate and distinct part of the seed. Hence, there are natural lines of cleavage between embryo and endosperm and embryo and bran, although there appears to be a "cementing layer" between the bran and germ and the endosperm. Treatment (conditioning) with moisture helps to weaken the bond between these structures by affecting this layer and facilitates the separation of the bran and germ during milling (Pomeranz, 1971).

Table 2.2.2.1 Percentage by weight of each component of the wheat kernel (Pomeranz, 1988).

Component	**Percentage by weight (%)**
Outer pericarp	4.35
Cross-cell layer	1.44
Nucellar layer	2.21
Aleurone layer	6.7 - 7.0
Starchy endosperm	81.4 - 84.1
Embryonic axis	1.0 - 1.6
Scutellum	1.4 - 2.0

2.2.3 Mineral Content

A high proportion of the minerals in the wheat kernel are associated with the bran and germ fractions and specifically the aleurone layer (Ambalamaatil *et al.*, 2002). They appear to be concentrated in structures known as aleurone bodies in the aleurone layer as irregular spherical granules called globoids (Lasztity, 1999). These granules have a high phytic acid content (25-70%) and are rich in potassium (2-20%) and magnesium (1.5-12%) due to the ability of phytic acid to chelate minerals (O'Dell *et al.*, 1972; Mugford and Steele, 1980, Batten, 1994; Lasztity, 1999; Lott *et al.*, 2000; Ahn *et al.*, 2003). Each aleurone body typically contains between one and ten phytin granules surrounded by a protein-containing envelope (Lui and Altschul, 1967). The exact composition of each granule depends on the mineral status of the plant during its growth but they represent the main source of mineral ash in the wheat grain (Simmonds, 1989; Henry and Kettlewell, 1996). This uneven distribution of minerals and subsequent ash in the grain results in their concentration in the germ and bran fractions after milling (Staudt and Ziegler, 1973; Ziegler and Greer, 1988; Butt *et al.*, 2004).

Table 2.2.2.2 Proportion of ash in wheat and wheat fractions, (Pyler, 1973).

Product	Ash (%)
Wheat and mill products	
Wheat	1.73
First patent floor	0.40
First clear flour	0.81
Second clear flour	1.34
Red dog	3.15
Bran	6.38
Shorts	4.10
Germ	4.80
Flour streams	
First break flour	0.66
Second break flour	0.56
Third break flour	0.49
Fourth break flour	0.64
Fifth break flour	1.03
First middlings flour	0.36
Third middlings flour	0.38
Fifth middlings flour	0.44
Seventh middlings flour	0.65
Ninth middlings flour	0.61
First tailings from purifier	3.67

2.2.4 Processing

2.2.4.1 Milling

The purpose of modern commercial flourmilling is to first separate the endosperm from the bran and germ in as large pieces as possible and then reduce the size of the endosperm pieces to flour-sized particles through a series of gradual grinding steps. In contrast, primitive people used stones to pound grain and release the edible seeds from their hulls. The mortar and pestle and the saddlestone were later developed to improve this process. The ground material was winnowed in the air to remove the hulls and the coarse meal was made into porridge or flat cakes. Each household owned a mortar and pestle or saddlestone and used it to prepare the days supply of crushed grain (Bass, 1988).

As methods of cultivation improved and with them the yield of harvested grain, so did the primitive milling process. By Egyptian times, the single stage mortar and pestle grinding had evolved into a multi-stage process that added sieving, further grinding and a final sieving to the grinding and winnowing process. Subsequent advances, which began about 3000 years ago, about the time leavened bread began to appear, involved larger saddlestones, grooved rubbing surfaces and hoppers to feed grain to the lower stone. Rotary stone mills, both animal, slave and hand powered, were developed by the Romans about 300 BCE and represented a major advance in the development of flourmilling. Hand powered rotary mills, known as querns, soon superseded saddlestones and were widely used until the end of the 19th century. The quern is still a popular piece of household equipment in many developing countries (Bass, 1988).

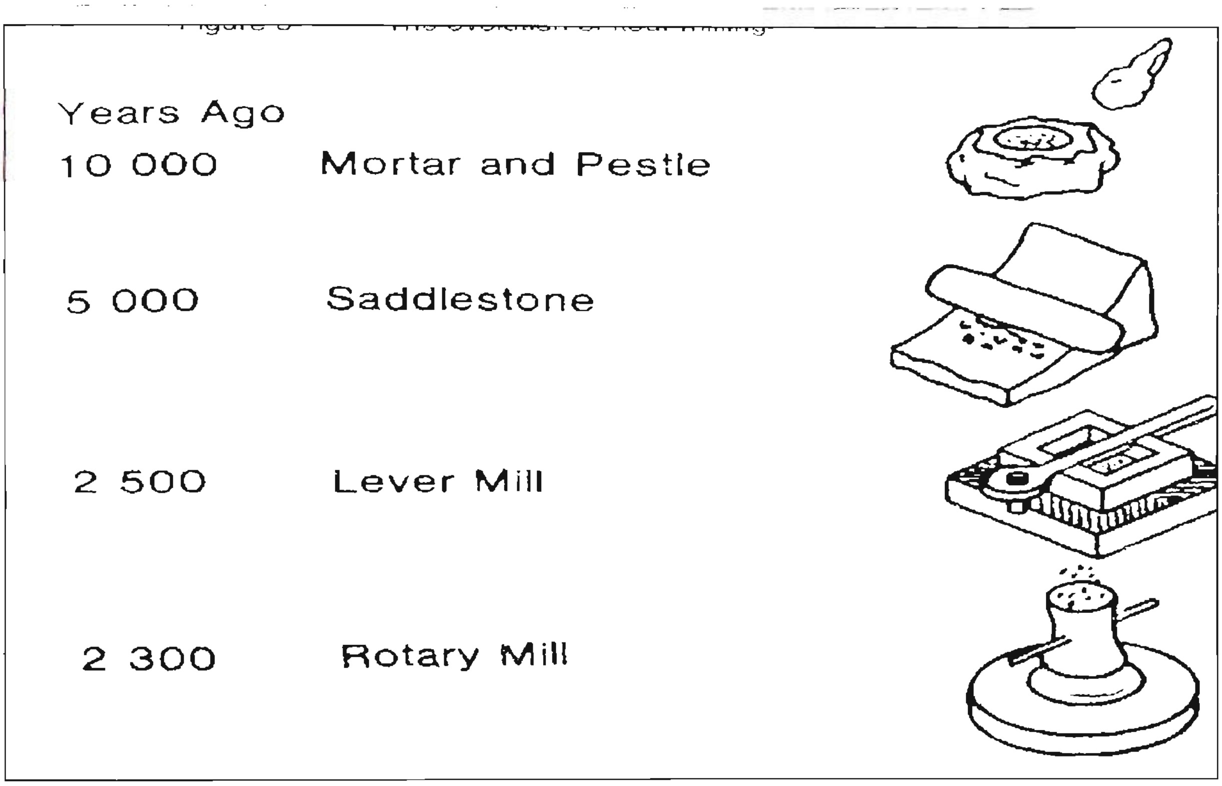


Figure 2.2.4.1.1The evolution of early flourmilling (Bass, 1988)

Animal power was replaced by waterpower about the time of Christ and the dome shaped stones gradually evolved into flatter, horizontal millstones. The first windmills were built about 1000 AD. Wind and water driven mills remained until the late 19th century and were eventually replaced by steam powered and subsequently by electrically driven mills (Saxelby and Venn-Brown, 1980; Bass, 1988).

The developments that led to the present concept of a gradual reduction process in milling occurred during the latter half of the 19th century. The middlings purifier, invented in France in 1860, was adapted for use in other European mills and in North America. This machine facilitated removal of much of the branny material that characterised flours of that time. This period also saw the introduction of the roller mill. As the advantages of this machine over millstones became apparent, there was a rapid transition to iron rolls. The gradual reduction system, adopted by most millers, needed higher capacities and a larger area of sifting surface. At this time, the plansifter, another product of European milling engineers, became widely accepted for the grading and sifting functions of the mill (Bass, 1988). The objective in modern milling is to break open the grain, scrape off as much endosperm from the bran skin as possible, consistent with obtaining flour of the required ash or color specification then gradually grind or reduce the practically pure endosperm into flour. After each reduction, the stock, material going to the sieve, is sifted to remove the flour. The material remaining in the sieve may then be classified into three types of particles larger than flour. Pure or relatively pure endosperm, composites of endosperm and bran varying in size, shape and proportion of endosperm to bran and pure or relatively pure bran (Posner and Hibbs, 1997a).

The effectiveness of the milling process depends on how well it is able to separate the composite particles, so as to recover endosperm essentially uncontaminated with bran. If the wheat has been correctly tempered, the miller may take advantage of the following three phenomena to achieve the milling objectives. Bran tends to remain in larger and coarser particles than endosperm. Bran is much lighter than endosperm, composite particles of the two constituents are separable, according to their relative composition, in a machine such as the purifier, which segregates on the basis of density differences. When a mixture of pure bran and pure endosperm particles of equal size, which could therefore not be separated by sifting, are ground with a slight shearing action through smooth rolls, bran tends to flatten. In contrast, endosperm tends to fragment into smaller particles, thereby providing a means of separation by subsequent sifting (Inglett and Anderson, 1974).

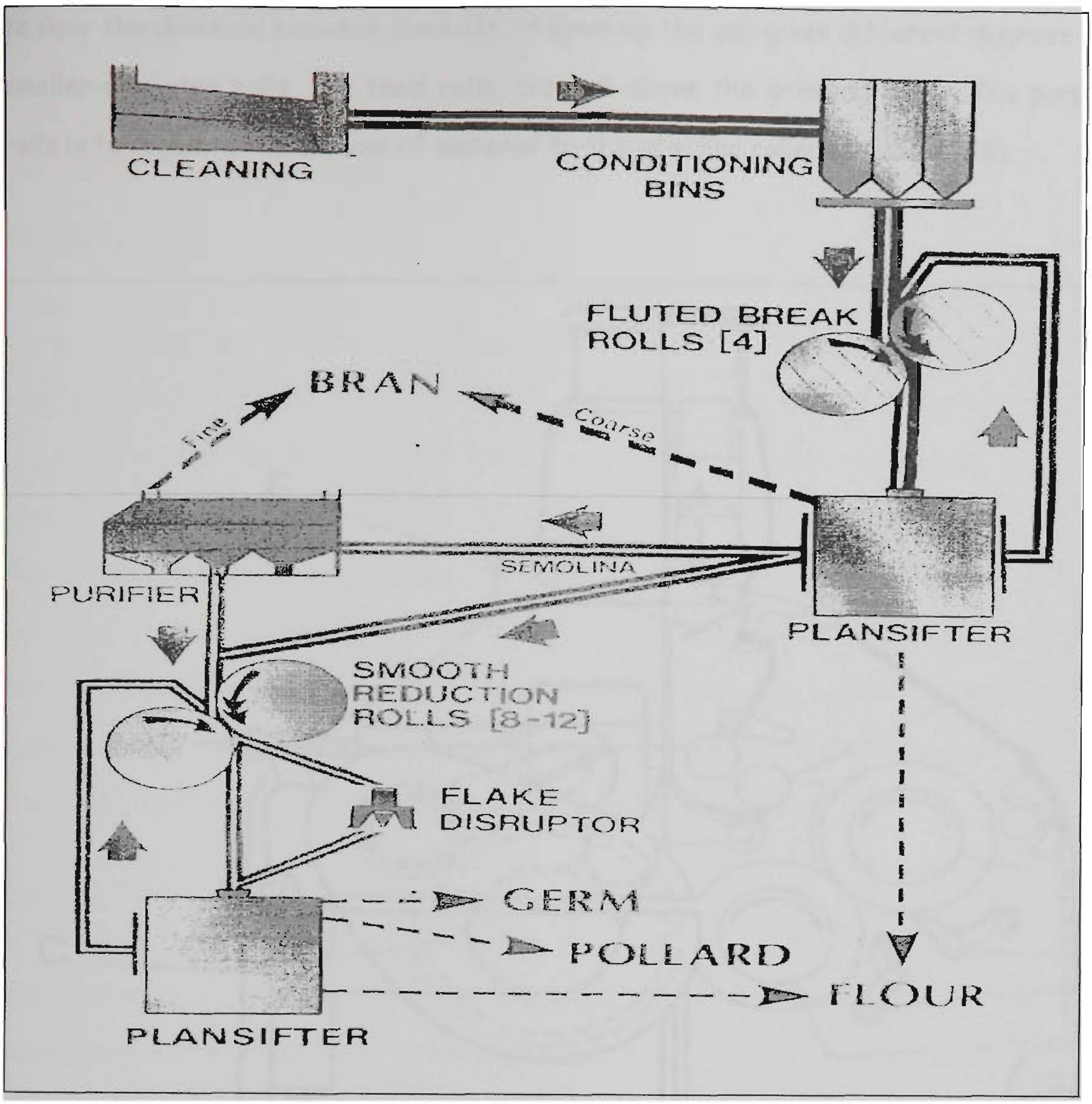
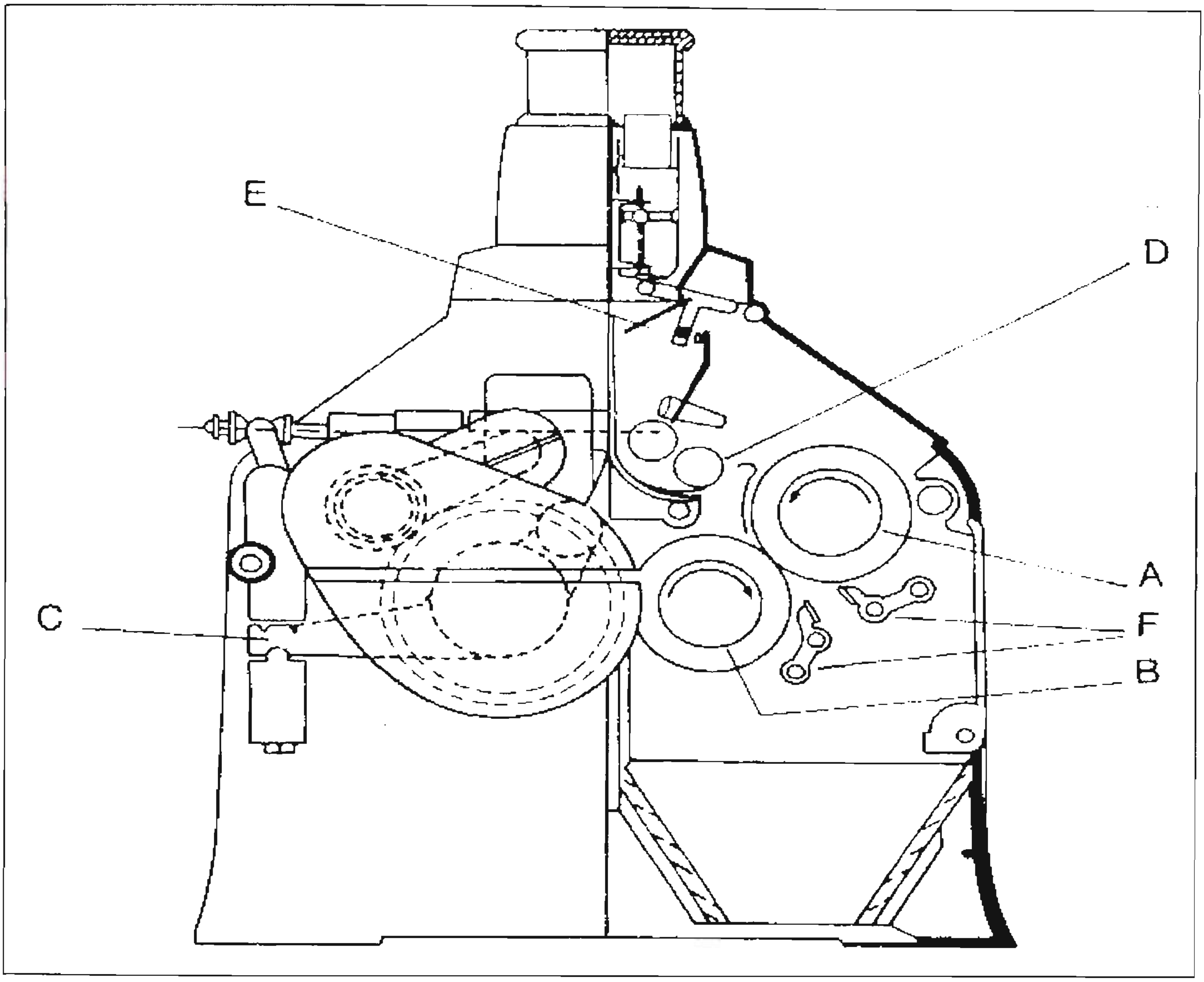


Figure 2.2.4.1.2 The flow through a flourmill (NABIM, 1979)

The wheat is "opened up" on the first break rolls, which are grooved or corrugated to impart a shearing action to the grain. The ground material leaving a break roll passes to the grading or sieving system. The largest particles consist of the tough wheat bran and adhering endosperm. These large particles proceed to the second break rolls, where they is again ground on corrugated rolls and sent to the grading system, second break sifter, for further sifting. At each successive break stage, more endosperm is scraped away from the bran and separated in the grading system, until finally only the flat bran particles remain for final processing as a by-product (Kent, 1985).

A modern roller mill is shown in Figure 2.2.4.1.3. It consists of two pairs of cast-iron rolls, chilled on the outside to reduce heating, with a hard, weather-resistant surface and mounted in a heavy cast-iron frame. Two pairs are normally mounted back to back in a single frame, each pair being driven separately and having separate feeding and adjustment mechanisms. The upper rolls of a pair are carried in fixed bearings, but the lower roll is supported by pivot arms, which may be adjusted to vary the distance between the rolls. Adjusting the gap gives different degrees of grinding. Two smaller-diameter rolls, the feed rolls, are set above the grinding rolls. The purpose of the feed rolls is to regulate the stream of material to the grinding rollers (AWB, 1986).



A -	Upper roll
B -	Lower roll
C -	Pivot arms
D -	Feed Rolls
E -	Feed gate
F -	Brushes

Figure 2.2.4.1.3. Modern roller mill (Bass, 1988)

Grading

The smaller particles are separated according to size on sieves before being passed to the purifiers. Inevitably, as the wheat is broken open in the break system, a small amount of endosperm is reduced to flour particle size. This flour is sifted out in the grading system. By far the larger proportion of endosperm passes to the purification system in the form of semolina and middlings, these are particles of endosperm that are larger than flour (Posner and Hibbs, 1997a).

Purification

Break middlings are a mixture of pure endosperm, endosperm with particles of bran attached and small particles of bran. The objective of purification is to separate this mixture into homogeneous fractions so that as much of the branny material as possible is removed before the endosperm particles, which at this stage are referred to as "farina" or "semolina", are further reduced to flour. The purifier separates bran particles and pieces of endosperm with adhering bran from particles of pure endosperm. The feed to a purifier comes from the grading system and is a mixture of particles of similar size but of varying density. By subjecting the mixture to a controlled flow of air as it passes over a sieve, the purifier can separate the essentially pure endosperm from the branny particles (Posner and Hibbs, 1997a; Leonard, 1999).

Sizing

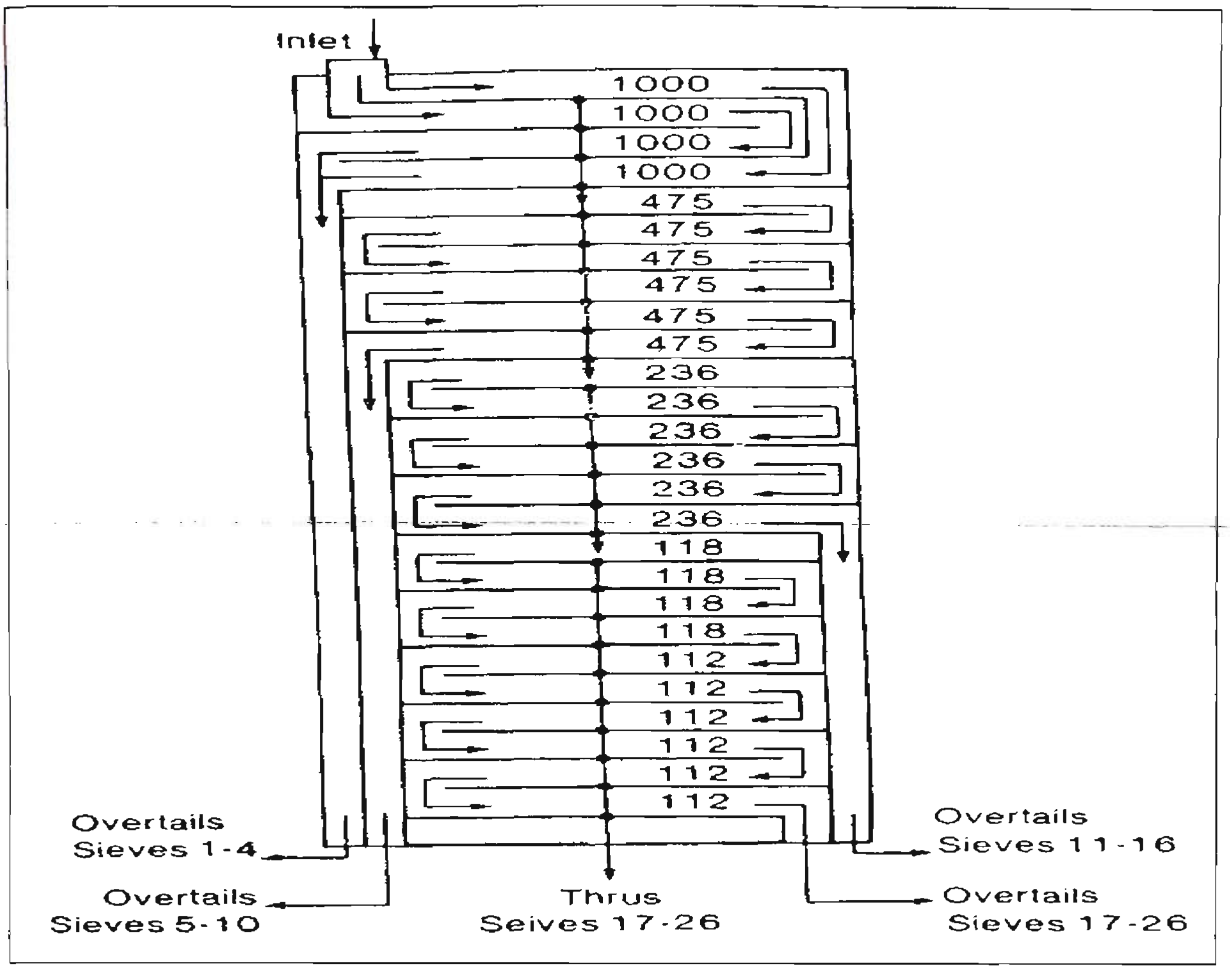
This system is a re-break process for the larger granules of semolina with adhering bran that come from the purification system. The main objective is to scrape this stock to release endosperm from adhering bran with minimal production of flour. This objective is achieved by grinding the branny stock lightly in corrugated rolls to facilitate separation of bran from endosperm. The ground stock is then sifted and the larger bran particles are separated first. The endosperm fraction may still contain smaller bran particles, which can then be removed by purification. If the ground stock is sufficiently pure, it may be directed to the reduction system or returned to the sizings system (Posner and Hibbs, 1997a; Posner and Hibbs, 2004a).

Reduction

Purified semolina and middlings are gradually ground through smooth rolls to flour-particle size in six to eight successive stages of grinding and sifting. Flour is removed after each grinding stage. A small proportion of fine material remains on the sieve after the last reduction passage. This fine material passes to the by-products system along with branny material from the purifiers. The coarse by-product from the break system is called bran and the finer branny material from the break, purification and reduction systems is known as shorts (Posner and Hibbs, 2004b).

Some flour is produced at each grinding stage. In the simple flow diagram shown in Figure 2.2.4.1.2, 12 different "machine" flours are produced. Four from the break passages and eight from the reduction passages and each machine flour has different characteristics. All machine flours may be combined to produce "straight run flour" or blended in various combinations to produce "split run flours". All machine flours are sifted, mainly in very finely woven silk or nylon sieves that are systematically stacked by sections in sifters. Any material sufficiently small in particle size to pass through the very fine apertures of these sieves is by definition flour. Any material that is not fine enough to sift through as flour is graded by size and returned to the most appropriate point in the system for further processing (AWB, 1986).

A typical sifter consists of several layers of sieves, rotating in a horizontal plane. The sieves are arranged in sections, each section containing as many as 30 layers of sieves. The complete machine may have two, four, or eight sieve sections. A sifter provides a much larger area of sifting surface in much less space than was provided by the reels and centrifugals of yesterday's mills. The sieves of a sifter are covered with coarse wire mesh for coarse separations, or fine wire mesh, nylon, or silk for finer separations of flour and middlings. Sieve covers are drawn tightly over the frames, which fit into flat metal trays. The sifted material, or 'throughs', of the sieve fall into the tray to be directed out of the machine or to a finer sieve lower down in the sifter for further separation. Overtails leave the machine through an internal channel or may be directed to another sieve in the same section for further sifting (AWB, 1986).



Numbers show sieve sizes in microns

Figure 2.2.4.1.4 Flour sifter (Bass, 1988)

The whitest flours are made from the purest stocks of the early passages of the reduction system. At each succeeding grinding stage, stocks become less and less pure as the white flour has already been extracted. Consequently, on lower or "tail-end" passages of break and reduction systems, the flours become progressively darker, higher in nonfunctional protein, and therefore inferior in baking performance. This suggests that the milling process tends to extract endosperm progressively from the center of the wheat kernel toward the bran layer as flour is produced from the head toward the tail end of the mill (Posner and Hibbs, 1997c).

Mill Feed

The by-products of wheat flour milling, known as mill feeds, are bran, shorts, and germ. Bran is the coarse, flaky product that has been passed through the break system after adhering endosperm has been removed. After it emerges from the final break passage sifter, it is passed through a finisher, or bran duster, to recover the final vestiges of adhering endosperm. Shorts are the finer branny material emerging from the last break sifter, along with the final overtails of the lower passages of purification, and possibly also from the final reduction sifter. Shorts are also passed through a finisher, or shorts duster, to strip the remaining endosperm. The bran and shorts dusters are similar in design, consisting of a brush or beaters rotating vertically or horizontally inside a cylinder of fine screen, through which the endosperm removed from the mill feed is passed (Bass, 1988; NABIM, 1988),

2.2.4.2 Baking

The development of bread and bread baking is linked with the development of civilisation itself and there is evidence that some type of bread has been a staple food in all known civilisations (BRI, 1997a and 1998). Meagher (2003) suggests that bread making can be traced as far back as 8,000 years and recent work by Weiss (2005) provides strong evidence that wheat and barley were ground and baked in southwest Asia at least 20,000 years ago. Stone age man ate whole or cracked grains raw or after being dried, like nuts or seeds are eaten today. This led to porridges of stone-crushed grains mixed and cooked with water and flat cakes of dough baked directly on coals, or on heated stones and tiles (Bailey, 1975). It is usually accepted that the Egyptians developed the first true breads around 3000 to 2500 BCE (Bread, 2000; Oakenfull, 2001; Bothams, 2004a), when fermented dough was added to flour to make flat cakes rise when baked. This was the origin of sourdough bread and is a process that is still used today. Bailey (1975) places the first bread in Mesopotamia in about 5000 BCE because of the wheat and barley that grew in the irrigated river valley on small plots of land. Yeast was isolated from moldy bread around 1000 BCE and could be introduced directly into the flour. In addition, at this time, a new strain of wheat was developed that allowed the production of white bread because it did not require heating to be threshed (Oakenfull, 2001). Up to thirty varieties of bread were popular in ancient Egypt (Breadinfo, 2004).

An early improvement to baking bread on top of the coals or griddle stones was the addition of a clay bell-shaped dome that enveloped the baking dough. This insulation technique improved the rising action and cooked the dough more evenly. The Egyptians baked bread in stacked moulds placed in an oven and the Assyrians placed dough in heated pots that were sealed and buried in the ground (Oakenfull, 2001). A bakers' guild was formed in Rome around about the year 168 BCE and from then on, the industry began to form into a separate profession. The guild, called 'Collegium Pistorum', did not allow the members or their children to leave and take up another profession. In addition, the members were forbidden to mix with 'comedians and gladiators' and from attending performances at the amphitheatre, so that they would not be contaminated with the vices of the ordinary people (Holloway, 2002).

A number of workers (Breadinfo, 2004; Holloway, 2002; Bothams, 2004b) have suggested that the Greeks developed bread baking into a culinary art form. Front-loaded bread ovens that are similar to those used today, were developed in ancient Greece. The Greeks liked their bread white and created a wide variety of dough types, loaf shapes and styles of serving bread with other foods. Baking developed as a trade and profession as bread increasingly was prepared outside of the family home by specially trained workers to be sold to the public. By 300 AD, the Greeks had developed over seventy different kinds of bread (Bailey, 1975). Greek settlements in Gaul predated Roman control of the area and they brought the tradition of baking with them. Greek bakers in Gaul began to use beer yeast to improve the rising action of certain breads and began to dominate the bread trade in Rome. Most of their bread was made of dough produced from nine pounds of leaven to twelve bushels of flour. It was baked in ovens, under ashes, over charcoal or in the manner of waffles, using a hinged iron pan or grill. During the expansion of the Roman Empire, Greek baking traditions influenced a vast area of the world, especially Western Europe.

Leavened bread became the staple food of the Roman masses, although flat cakes and especially porridges were still widely consumed. Workers took hunks of bread into the fields to sustain themselves during the day. Eventually, bread became so important to Rome that public bakeries were working continuously to supply the increasing demand. A bakery might be required to produce between 50,000 and 100,000 loaves daily with slaves working the mills in shifts throughout the day. Pompeii had twenty public bakeries suppling the 20,000 inhabitants. In 123 BCE, the Roman government sold grain to the people at half price and by 58 BCE, it began to distribute the grain free (Bailey, 1975).

In the centuries following the fall of the Roman Empire, rye, a weed of the wheat fields, became the dominant crop over much of the northern half of Europe. Rye was almost unknown in the Roman Empire and was introduced by the Teutonic tribes that colonised northern Europe. By the Middle Ages, the everyday brown bread of the common people was made from rye, often mixed with barley or even pea flour. At the table, thick slices of bread known as trenchers were served with a variety of dishes, often forming the centrepiece or base of a meal. Long before John Montagu, the fourth earl of Sandwich, made his famous innovation of adding a second slice on top in the 1700's, bread was commonly served as a base for meats and sauces (Gughupf, 2004). Certain soups and stews were poured on top of bread placed in a bowl or dish.

During this time and until well after the Middle Ages, leavened bread, unlike porridge and flat cakes, was usually prepared outside of the family home. The process of making bread required equipment and specialised skills beyond the reach of the average household. In addition, bread was not usually prepared in establishments that sold and distributed bread. This was due to the flammable nature of the structure of the bake house. They were often constructed near rivers to provide a supply of water to put out fires, prepare the dough and for cleaning equipment. In addition, bake houses were frequently located next to water-powered mills where flour was produced (Oakenfull, 2001). This reduced the time and expense of transporting flour.

Ovens and related baking equipment were also expensive. Wealthy feudal lords or businessmen often owned bake houses. They allowed the bakery owners of the region to use their facilities in a communal fashion on a fee-basis or in exchange for provision of bread (Breadinfo, 2004). This widespread practice made the cost of producing bread lower for the individual bakery owners. As construction standards improved in cities over time, officials allowed bake ovens to be relocated adjacent to bakeries. Although, like most food related businesses, such sites were restricted to the outer regions of urban areas to minimise rat infestations. As the food staple, bread was the item most commonly sold on credit and was frequently bartered.

The baking trade was highly regulated through rulers, officials and guilds, known as corporations in France. There were laws governing and penalties enforcing the cleanliness of baking establishments, the ingredients used in dough, the quality of the finished product, the training of the bakers (History, 2004) and the cost of the finished product. In 1202, King John passed a law that fixed the price of a loaf of bread and allocated the price between the cost of material and the profit of the baker (Holloway, 2002). As in other crafts and trades at the time, bakers began their training as unpaid apprentices who worked for room and board for seven years. After several years, they progressed to the title of journeyman, which allowed them to train apprentices. Accomplished journeymen could later become certified masters. The corporations, especially the regulation of competition, were gradually abolished in the 1700s and 1800s (BRI, 1992). The apprentice-journeyman-master training and certification system however, still exists today in many European countries and strict laws governing the ingredients and additives permitted in bread are still in force in France and Germany.

Loaf bread

The different commercial processes in use throughout the world today for making yeast leavened pan bread can be loosely grouped into the following categories, although these are based somewhat on historical order (BRI, 1997b). The following descriptions are taken substantially from BRI (1997b), Meagher (2003) and discussions with baking staff at the Bread Research Institute.

Straight dough :

This process is probably the oldest and still most widely used around the world in smaller bakeries and for variety breads due to its simplicity and minimum use of equipment, although it is rarely used in Australia. It has a number of variations, combining the advantages of a long fermentation with the simplicity of a single mixing stage using conventional low energy mixers. All the ingredients are mixed together at the one time and the dough undergoes bulk fermentation for usually about 2.5 hours. The process is simpler and has less fermentation losses that the sponge and dough process but is less forgiving towards changes in operating parameters and ingredient variations.

Sponge and Dough :

This process is still used exclusively for pan bread production throughout the USA and less widely in other countries. From 50 to 70% of the flour, salt and water are mixed into a soft sponge with all the other ingredients. The sponge is permitted to ferment for about 4.5 hours and the remaining flour, water and salt is then added during the remix stage. The long fermentation stage often requires the addition of malt flour and sugars, more labour and equipment and results in a fermentation loss of up to 4%. All of these restrictions are usually balanced against a claim of better flavour and texture, lower yeast use and reduced sensitivity to process variables.

Short or no-time :

This includes a number of processes that are especially applicable to lean dough that contain much less sugar and shortening than the two long fermentation formulas mentioned above, also they were developed to reduce time, labour and baking losses. They are sometimes called mechanical dough development (MDD) or chemical dough development (CDD) processes. The MDD uses a high-speed mixer to put energy into the dough in a short time, reduce fermentation losses and hence reduce the time required for bread production. It is common practice to add oxidizing agents to assist the mechanical development; these usually include ascorbic acid, enzymes and atmospheric oxygen.

The Chorleywood bread process (CBP), developed in the UK in the early 1960s, is now widely used by plant bakeries in Europe, Canada, New Zealand and especially Australia. The dough is mixed for only about three minutes and then immediately sent to the divider and proofer for a brief rest period before moulding and panning. The Australian rapid dough process was developed at the Bread Research Institute to permit smaller bakeries and hot bread shops to utilise many of the advantages of the MDD while using medium energy mixers. Mixing times of ten to twelve minutes are relatively long compared to the three minutes used in the CBP, but various improvers are added to assist the process.

Continuous bread making :

This is an extension of the sponge process in which the fermenting dough is pumped to a continuous mixer which mechanically and rapidly develops the dough, often injecting air to control the volume. The resulting bread has a fine, almost cake like texture and most plant bakeries that once used this process have reverted to the more automated sponge and dough method.

Production of loaf bread in Australia :

All plant bakeries in Australia use slight variations of the Chorleywood bread process to produce packaged white and wholemeal loaf bread. Smaller specialist bakeries and hot bread shops use a variation of the Australian rapid dough process.

Flat bread :

Flat breads are quite different to loaf breads with low specific volume, high crust to crumb ratios and a firm cohesive mouth feel (Quail, 1996). Qarooni (1990) has classified flat breads into two groups according to their cross section :

1. Single layered flat bread such as tanoor from the Middle East and chapatti from India.
2. Double layered flat bread such as Arabic (pita or pocket) bread and baladi bread from Egypt.

The main difference between the production of these two types of flat bread is the final proof period. For single layered bread, the final proof is less than two minutes and the sheeted dough is docked before it enters the oven to avoid the separation of the two layers that would occur during baking at high temperature. For double-layered bread, the final proof usually exceeds fifteen minutes and during this time, changes take place within the dough that allows the even separation of the two layers during baking.

Table 2.2.4.2 Major countries where flat breads are consumed and their main flat bread types (Badt, 1995; Quail, 1996)

Country	**Type of Flat Bread**
Egypt	Baladi (Balady), Shami (Arabic) and Shamsey
Gulf Countries	Arabic, Rogag (Suage) and Tanoor
India	Chapatti, Naan, Pappadam and Puri
Iran	Barbari, Lavash, Sangak, Suage and Taftoon
Iraq	Gubbiz (Tanoor), Sang-gak and Suage
Israel	Matzo and Pita (pocket)
Jorden	Armany, Ka'ik, Kmage, Markook and Suage
Lebanon	Arabic and Baladi (Balady)
Saudi Arabia	Burr, Korsan, Mafrood (Arabic), Tamees and Tanoor
Sudan	Shamsi
Syria	Kmage, Kubz (Arabic) and Tanoor
Turkey	Bazlama, Gomme, Pisili and Yufka

The naming of these breads can be difficult because they are often consumed in a number of different countries each using a different name. In addition, the English spelling varies as this is usually derived from the phonetic spelling.

Production of flat bread in Australia :

The major flat breads consumed in Australia by a considerable margin are one of two Arabic styles with pita or pocket bread being a thick, double layered Arabic bread and Lebanese being a thin, double layered Arabic bread. Formation of the two layers occurs when the bread forms pockets during baking at 350^0 to 600^0C. Most commonly produced with white flour of 72 to 78% extraction, it is also made with higher extraction flours and wholemeal but usually without the addition of other whole or kibbled grains. The bread can range from quite thin with 4 mm walls to quite thick with 25 mm walls. Low baking water absorption flour is used for production and the dryness of the dough has allowed successful automated processing. Individual flat breads may vary markedly in their size, shape and textural characteristics. Due to their popularity and availability in Australia, only Arabic (mainly Lebanese) style flat breads were used in this study.

2.3 Australian soils

Australia's low relief, tectonic stability and virtual absence of glaciation have preserved extensive areas of deeply weathered mantle that covered the continent during the Tertiary period. Deep weathering has strongly influenced soil patterns in this country and is a key factor in the intrinsically low fertility levels in many regions. Weathering and leaching are often so complete that the mineral component is comprised of only a small residue of kaolinite, gibbsite, iron oxides and variable amounts of quartz (McKenzie *et al.*, 2004).

2.3.1 Soil Type

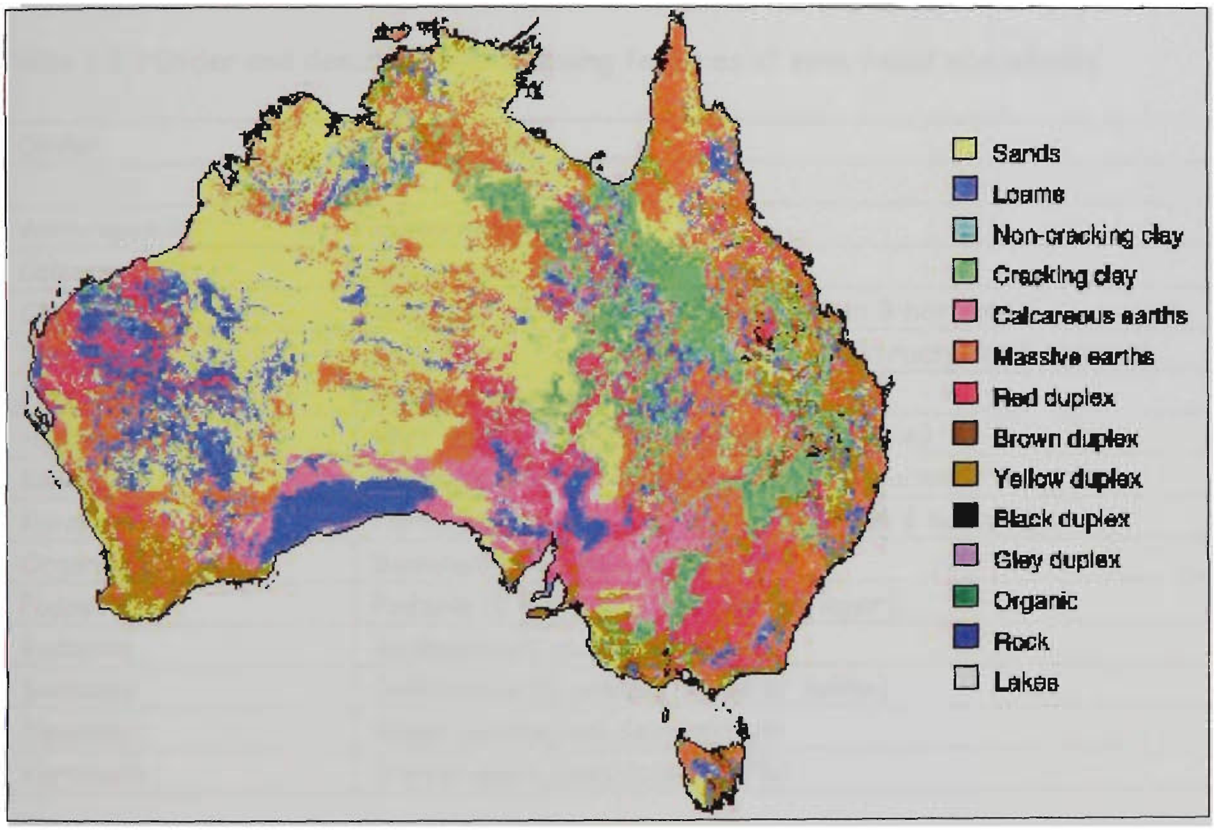


Figure 2.3.1 Atlas of Australian soil types (BRS, 1998)

The atlas of Australian soils shown in Figure 2.3.1 is a digital version of the set of ten maps compiled by Northcote *et al.* over the period 1960 to 1968 and published by Melbourne University Press as 'Atlas of Australian Soils, Sheets 1 to 10'. The digital atlas was created by the National Resource Information Centre, Bureau of Rural Sciences in 1991 from scanned tracings of the published hardcopy maps. The figure shows large areas of yellow duplex sandy loams throughout the wheat belt of Western Australia, throughout much of the wheat belt in New South Wales and to a lesser extent in Victoria. The South Australian wheat belt has areas of loam tending to duplex sandy loam around St Vincent's Gulf. The Queensland wheat belt shows more complex soils without a large area of definite soil type.

The revision of the soil classifications used in Figure 2.3.1 was started in 1981 when the Soil and Land Resources sub-committee of the Standing Committee on Soil Conservation recommended the formation of a working party to research the options for improving soil classification in Australia (Isbell, 1984). The selected option for the new Australian classification was for a multi category scheme with classes defined on the basis of diagnostic horizons or materials and their arrangement in vertical sequence as seen in an exposed soil profile. This meant that soil profiles and laboratory data rather than geographic attributes were to be used and this allows the inclusion of all changes in soil horizons. This work was reported by Isbell (1992) and further developed in Isbell (1998), the descriptions of the soil orders are summarised in Table 2.3.1.

Table 2.3.1 Order and description of defining features of soils found in Australia

Order	**Description**
Anthroposols	Human-made soils
Calcarosols	Calcareous throughout
Chromosols	Often brightly coloured (pH > 5.5 in B horizon)
Dermosols	Often with clay skins on ped faces (structured B horizon)
Ferrosols	High iron content (B horizon)
Hydrosols	Wet soils (prolonged seasonal saturation)
Kandosols	Strongly weathered soils (massive B horizon)
Kurosols	Pertaining to clay increase (pH < 5.5 in B horizon)
Organosols	Dominantly organic materials
Podosols	Podzols (B horizon, under an ash layer)
Rudosols	Rudimentary soil development
Sodosols	Influenced by sodium (sodic or saline)
Tenosols	Weak pedological development
Vertosols	Shrink-swell clays (clay > 35%)

2.3.1.1 Anthroposols

These soils are formed from human activities that have caused a profound modification, mixing, truncation or burial of the original soil horizons. They have some pedogenic features such as weakly developed A1 horizons or the presence of identifiable pre-existing soil material. They have a very limited distribution but are associated with mining activities, particularly in the Hunter Valley of New South Wales and Central Queensland as well as other areas of open-cut mining where the soil has been replaced after mining.

2.3.1.2 Calcarosols

The key feature of calcarosol soils is the presence of variable amounts and forms of calcium carbonate usually throughout the profile or from 0.2 m down. The second key feature is the absence of a clear or abrupt textural B horizon due to increasingly fine calcium carbonate. Calcarosols have a very distinct distribution pattern, shown as calcareous earths in Figure 2.3.1, they are almost absent over much of eastern Australia, Tasmania and most of the Northern Territory. They occur in a crescent pattern around the Great Australian Bight from northwestern Victoria and southwestern New South Wales, through southern South Australia and into southeastern West Australia. They vary between slightly to reasonably alkaline with a pH range of 7.4 to 9.9 (McKenzie *et al.*, 2004) also they are widely used for cereal cultivation and irrigated horticulture.

2.3.1.3 Chromosols

The essential feature of chromosols is their colour and the strong texture contrast between the A and B horizons. Figure 2.3.1 shows the distinctive subcoastal distribution pattern of chromosols around much of the continent. They are slightly acidic to fairly alkaline with a pH range of 5.3 to 9.7 as well as being prominent in the wheat belt of southern New South Wales and northern Victoria where they are used for cereal and oil-seed growing. They are similarly important throughout southwestern Australia and parts of South Australia.

2.3.1.4 Dermosols

These soils lack a strong texture contrast between the A and B horizons, are not calcareous throughout, but have moderately to strongly structured B2 horizons. These B2 horizons usually contain a considerable amount of clay so do not have a free iron oxide content greater than five per cent. Dermosols are dominantly soils of the wetter, east Australian coastal and subcoastal areas (Figure 2.3.1) where rainfall may range up to 4000 mm per year. Soil pH ranges from strongly acidic at 3.5 in areas of high rainfall to alkaline at 8.7 in areas with calcareous subsoils. Cereal crops, especially wheat, are commonly grown on dermosols in central New South Wales.

2.3.1.5 Ferrosols

These soils are defined in a way similar to dermosols but their B2 horizons have a free iron oxide content greater than five per cent in the fine earth fraction; this is the main cause of their characteristic physical and chemical properties. Their distribution pattern is similar to the dermosols (Figure 2.3.1) but much less prevalent, hence it is determined by the occurrence of basalt in the underlying subsoil. Their pH range is from 4.7 to 7.1 (McKenzie *et al.*, 2004) but the more acidic ferrosols were traditionally known as Krasnozems although other red soils have been included in this category. Land use on ferrosols is very diverse although extensive agriculture is not common due to climatic, topographic or soil constants. It mainly consists of a range of horticultural crops and beef cattle grazing.

2.3.1.6 Hydrosols

Hydrosols are defined on the basis of seasonal or permanent wetness and the greater part of the soil profile must be saturated for two to three months or more in most years, shown as grey duplex soils in Figure 2.3.1. In addition, the Figure shows two very different areas of hydrosols. The occurrences in the central arid and semi-arid region of Western Australia are in ancient drainage systems with saline flats. The pH of these soils can range from acidic at 3.9 to reasonably alkaline at 9.2 (McKenzie *et al.*, 2004). In the semi-arid areas of the West Australian wheat belt, secondary salinisation caused by rising water tables as a result of vegetation clearing has produced saline hydrosols in many drainage lines. Most non-saline hydrosols require some form of drainage before they can support a land use other than nature conservation or sparse grazing of native pastures. Many small areas have been cleared and sown to improved pastures.

2.3.1.7 Kandosols

These soils do not have a clear or abrupt textural B horizon, are not calcareous throughout and the clay content of the usually weakly structured B2 horizon exceeds fifteen per cent. A feature of many kandosols are their considerable depth, this may be up to 3 m or more but in deep soils, the clay content can increase to 35 to 50 per cent. In Australia, the kandosols have been previously known as red and yellow earths, although brown forms are also widespread (Figure 2.3.1). The pH of these soils can range from acidic at 4.5 to very slightly alkaline at 7.5 (McKenzie *et al.*, 2004). Kandosols are the second most widespread of the order, they occupy 17 per cent of the continent and are common in all states and territories except Victoria and Tasmania. Only small areas of kandosols are used for extensive agriculture. Red kandosols are used for cereals in the wheat belt of southern New South Wales, while mainly yellow or brown forms are used for cereals in southwest Western Australia.

2.3.1.8 Kurosols

Kurosols have a clear or abrupt textural B horizon with an upper 0.2 m layer that is strongly acidic with a pH that can be as low as 3.9 (McKenzie *et al.*, 2004). A feature of many kurosols is the high sub-soil content of exchangeable magnesium, with or without sodium, and a very low exchangeable Ca:Mg ratio of less than 0.1. The distribution of the kurosols extends from southern Queensland through coastal and sub-coastal New South Wales to Tasmania. Small areas of kurosols are common but not extensive in southwest Western Australia. Some areas of kurosols have been cleared and used for dairying on improved pastures and small areas in Western Australia are used for cereal growing.

2.3.1.9 Organosols

Organic materials of specified thickness and organic carbon content, which varies according to clay content, dominate these soils. Most organosols are wet soils, many of these have long been known as peat. The Figure 2.3.1, shown as organics, almost certainly underestimates the occurrence of organosols because the generally small areas of individual occurrence are not large enough to be shown, they are very rare in Western Australia. The pH can range from 3.8 to 8.0 (McKenzie *et al.*, 2004) and some of the less acidic organosols in Southern Australia have been drained in order to sow pasture for dairying or intensive agriculture.

2.3.1.10 Podosols

These soils have B horizons dominated by the accumulation of compounds of organic matter and luminium, with or without iron. They are usually sand textured to depth with very little clay and have long been known in Australia as Podzols. Australian Podosols can be very deep with a bleached A2 horizon of considerable thickness where the B horizon may be 20 m or more. The pH can range from 3.8 to 6.5 (McKenzie *et al.*, 2004) with the Podosols being largely confined to well-defined parts of the coastal zone as well as some offshore islands in southern Queensland and Bass Strait (Figure 2.3.1). Agricultural use of Podosols is limited due to low fertility, poor water retention with seasonal waterlogging. Some of these areas have been drained and used for sugar cane, irrigated vegetables, dairying and sheep grazing on improved pastures.

2.3.1.11 Rudosols

These soils consist of materials that have not been greatly affected by pedological weathering processes. As a result, they have little or no soil development, apart from minimal development of A1 horizon material in cracks or fissures in the parent rock. The clay content of rudosols is largely governed by the mineralogical composition of the soil parent materials, rudosols often contain large amounts of clay-sized minerals not usually associated with the clay fraction of more developed soils. Figure 2.3.1 shows that the largest areas of rudosols occur in arid or semi-arid regions of central and northwest Australia where they occupy the Great Sandy, Gibson, Tanami, Great Victorian and Simpson deserts. Due to the nature of the parent materials, the pH of these soils is relatively neutral, ranging from 6.4 to 7.1 (McKenzie *et al.*, 2004). Large areas of these soils are used for sparse cattle and sheep grazing of native pastures. A few small areas are used for crops, sugar cane together with dairying on improved pastures.

2.3.1.12 Sodosols

The key feature of these soils is the presence of a clear and abrupt textural B horizon, the upper 0.2 m of which has a pH of greater than 5.5 (Northcote and Skene, 1972). These soils tend to be neutral to strongly alkaline due to carbonate accumulations throughout the B horizon. The pH can range from 5.5 in southwestern New South Wales to 9.6 in eastern South Australia (McKenzie *et al.*, 2004). Sodosols are widely distributed throughout the eastern half of Australia and the west of Western Australia (Figure 2.3.1) and largely dominate the Australian agriculture landscape. They are widely used for grazing and both dryland and irrigated agriculture. Cereal growing is widely practiced on these soils in the winter-dominant rainfall zones, although water logging can be a problem in wet years.

2.3.1.13 Tenosols

These are the most widespread soils in Australia, occupying more than a quarter of the continent. Tenosols have only weak pedological development and B horizons that are only weakly defined in terms of colour, texture and structure. They are particularly widespread in the northwestern half of the continent, shown as sands in Figure 2.3.1, where vast areas occur as red and yellow soil on sandplains. There are also large areas in Western Australia of red loamy soils with a red-brown hardpan at shallow depths (0.3 to 1.0 m). Like rudosols, these soils tend to be fairly neutral to slightly alkaline, ranging from a pH of 6.3 in western West Australia to 8.3 in eastern South Australia. The largest areas of tenosols are used for sparse sheep and cattle grazing of nature pastures. This is because of their low and erratic rainfall, poor water retention and almost universal low fertility. Tenosols are used for a limited extent for cereal growing in the southwestern coastal areas of Western Australia.

2.3.1.14 Vertosols

Vertosols are shrink-swell, clay textured soils containing 35 per cent or more of clay, although they may have very thin crusty surface horizons. When dry, these soils often crack to considerable depths and have long been known as black earths and grey, brown or red cracking clays. They occur extensively in Queensland, New South Wales and the Northern Territory, with much smaller occurrences in the other states (Figure 2.3.1). The pH of these soils varies widely with 3.5 in southeastern Queensland to 9.2 in northwestern Victoria (McKenzie *et al.*, 2004). Vertosols are used in Australia for the grazing of sheep and cattle on native and improved pasture and for extensive dryland wheat, cotton and oilseeds farming where rainfall is adequate. They are also extensively irrigated for a variety of crops including cotton, rice and sugar cane.

2.3.2 Soil Acidity

The acidification of soils is a natural process that begins when rocks are colonised by algae and lichens. Acids produced from the carbon and nitrogen cycles begin to dissolve the rocks and soil minerals to form the parent soil. In natural ecosystems, soils gradually become more acidic with time so that older and more weathered soils are usually more acidic than younger ones. Nitrate leaching from weathering is also considered to be a major cause of soil acidification in all ecosystems (Chartres and Geeves, 1992). Australia's soils are old and highly weathered (Hamblin and Kyneur, 1993), although some will obviously be more acidic than others, the natural acidity can also exist deeper down in the soil profile.

Soil acidity can also be affected by farming practices involving high levels of fertiliser application and crop-pasture rotations (Coventry, 1992). The pioneer work of Williams and Donald (1957) and Williams (1980) showed that under continuous pasture systems, the soils gradually became more acidic, this has occurred with time across a range of duplex soil types and crop farming systems in southern Australia. This decrease in pH value shows a common trend where crops are grown in rotation with pasture or grain legumes together with the effect of ammonium forming nitrogen fertilisers in lowering soil pH has been reported by a number of workers (Helyar, 1976; Mason, 1980; Coventry, 1992).

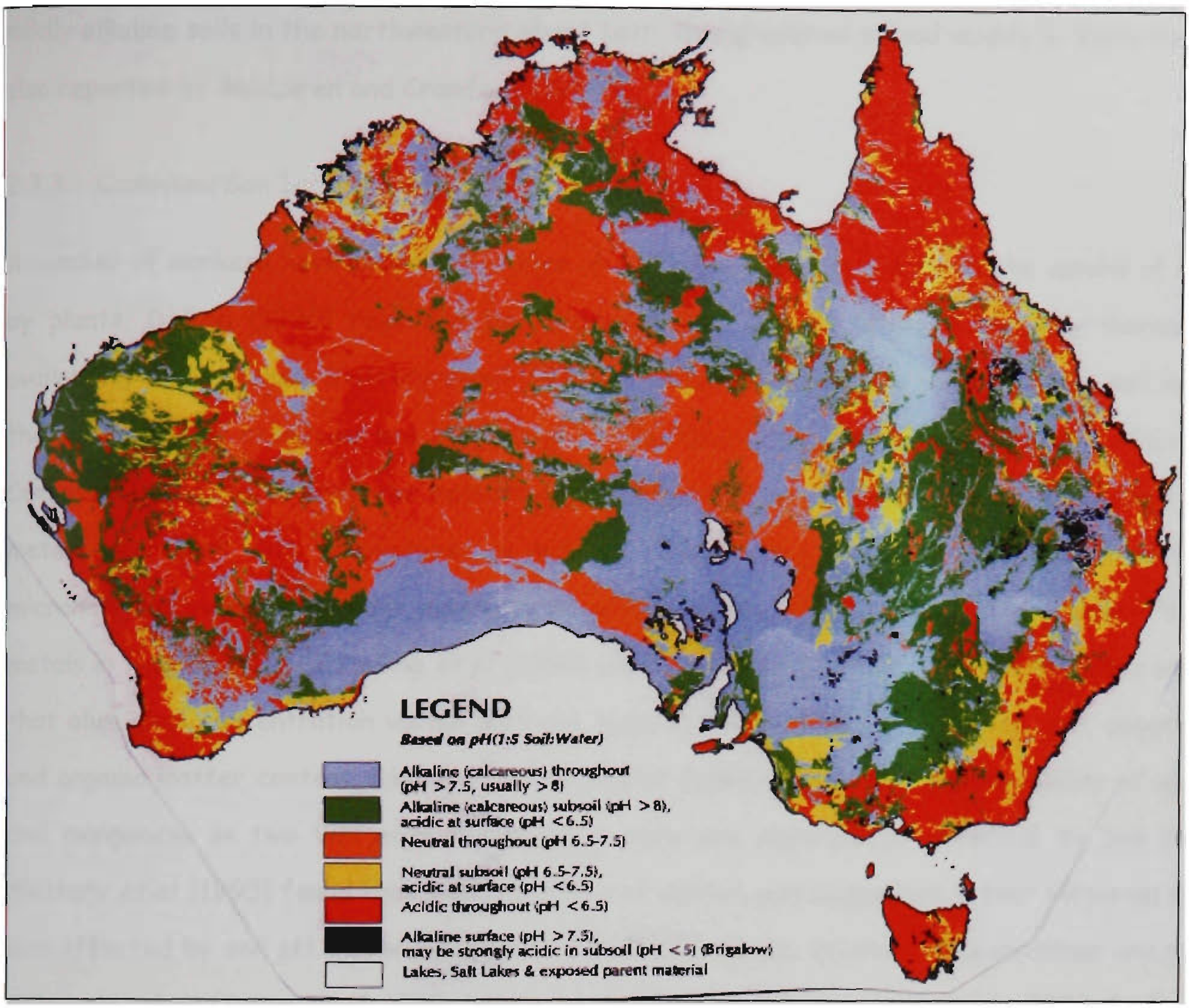


Figure 2.3.2 Distribution of soil pH within the topsoil and first horizon sub-soil across Australia (Spouncer *et al.*, 1996)

Table 2.3.2 Definition of soil pH (Australian Agricultural Assessment, 2001)

pH range	**Description**
< 4.3	Extremely acidic
4.3 to 4.8	Highly acidic
4.9 to 5.5	Moderately acidic
5.6 to 7.0	Mildly acidic
7.1 to 7.7	Mildly alkaline
7.8 to 8.5	Moderately alkaline
> 8.5	Highly alkaline

In Figure 2.3.2, it can be seen that the Western Australian wheat belt is dominated by areas of moderately acidic to neutral soils with a thin crescent of highly acidic soils along the southwestern coast, also shown by Curnow and McLaughlin (1988). The Queensland and New South Wales wheat belts show large areas of mildly acidic soils with a crescent of highly acidic soils on the New South Wales Coast. Victoria has areas of highly acidic soils in the southern wheat belt and mildly acidic to mildly alkaline soils in the northwestern wheat belt. This gradation of soil acidity in Victorian soils is also reported by MacLaren and Crawford (1996).

2.3.3 Cadmium/Soil Interaction

A number of workers have shown the effect of soil type as well as soil pH on the uptake of minerals by plants. Dolling (1990) reported that reducing the soil pH below 4.5 rapidly increases the availability of aluminium. Slattery *et al.* (1995) reported that reducing the pH of the soil increases the availability of aluminium and manganese but decreases the availability of calcium. McLaren and Cameron (1996) stated 'with the exception of molybdenum and selenium, the plant availability of all metals decreases with an increase in soil pH'. They found that the influence of soil pH on micronutrient availability is due mainly to its effect on the reactions controlling concentrations of metals in the soil solution. Dolling *et al.* (1990) surveyed 38 acid soils in Western Australia and found that aluminium concentration varied with pH, type and amount of clay minerals, salt concentration and organic matter content. Slattery and Ronnfeldt (1992) found that the availability of aluminium and manganese in two Victorian red podsolic soils was significantly affected by soil pH while Slattery *et al.* (1995) found that the availability of calcium and magnesium in four Victorian soils was also affected by soil pH. For many metals, including cadmium, solution concentrations are greatest at low pH values (Sauve *et al.*, 1999; Hough *et al.*, 2003; Krishnamurti and Naidu, 2003; Rachou *et al.*, 2004; Renella, 2004).

A mechanism for this effect has been given by Garcia-Miragaya and Page (1978) where pH dramatically affects the metal binding capacity of the soil by limiting the available exchange sites at low pH, this effect was also reported by Naidu *et al.* (1994) and Nan *et al.* (2002). In soils, H^+ ions bind to negatively charged particles more tightly than other cations, so that any metal bound to a soil particle will be displaced in the presence of excess H^+. This interaction becomes effective at a soil pH of less than 6.0. Bingham *et al.* (1980) reported optimum cadmium mobility at a soil pH of between 4.5 and 5.5, however Mustafa *et al.* (2004) reported a maximum cadmium desorption at a pH of 5.0. Solution concentrations of many metals are also controlled by adsorption/desorption reactions at the surface of soil colloids. The presence in the soil of these colloids enables the soil to adsorb metals and therefore will have a major influence in controlling micronutrient availability to plants. In general, the greater the number of potential micronutrient adsorption sites, the lower will be solution micronutrient concentrations and mineral availability to plants (McLaren R.G. and Cameron K.C., 1996). This effect is especially shown with the greater adsorption of metals and thus lesser availability to plants, in soils of high clay content (Wu *et al.*, 2004). Metals have a corresponding lower adsorption in sandy soils, therefore, a greater availability to plants (Eriksson and Andersson, 1990; Mann and Richie, 1995; Lee *et al.*, 1996).

Soil organic matter, has a large capacity to adsorb or complex metals and produce micronutrient deficiencies, particularly of copper and manganese, that are common in soils with high organic matter levels (Collins *et al.*, 2003). Some metals, for example, cobalt and zinc, are more strongly adsorbed hence they are retained by colloidal iron and manganese oxide minerals. Soils with high levels of manganese oxides are known to give rise to cobalt deficiencies (McLaren and Cameron, 1996). Conversely, the solubilisation of iron and manganese oxides in soils due to low pH also releases into solution other metals that have been adsorbed or occluded by these minerals. Minamisawa *et al.* (2004) found that high adsorptive capabilities were shown for cadmium on a range of biomaterials, including chitosan, coffee, green tea, tea, yuzu, aloe and Japanese tea, at pH 4.0 and 6.7.

In an American study of durum wheat grain together with soil from a field in North Dakota, Wendell *et al.* (1999) found that the cadmium uptake in grain was related to soil salinity, including the concentrations of chloride, sulphate and sodium while zinc was related to soil pH. They concluded that the greater uptake of cadmium in the presence of chlorides was related to the formation of cadmium chloride complexes that increase the solubility and mobility of cadmium. This relationship was also reported by Smolders and Peijnenburg (2002), where they found that in grain and potato tubers, soil-plant transfer of cadmium is high in saline soils but in zinc deficient soils, the increased uptake of cadmium with decreased pH was not found consistently.

In a study by Chen (2000), of rice grown on industrial waste contaminated soils in Taiwan, he found that there was a significant correlation between the concentration of cadmium in wheat grain and the concentration in soil solution. He also found that cadmium uptake was influenced by soil properties, particularly clay content and pH. These relationships were also found by Chen (1991), Liu *et al.* (1998) and Kukier and Chany (2001), who reported that the uptake of cadmium by rice is affected by locality and soil type. Singh *et al.* (1995) found that the concentration of cadmium, nickel and zinc in wheat, carrot and lettuce decreased with increasing soil pH in both sandy loam and clay loam soils. They also found that the effect of soil pH was more pronounced in the sandy loam soil.

2.3.4 Conclusion

Soil types vary across Australia with a central area of yellow duplex soil throughout the Western Australian wheat belt surrounded by large areas of sands. The topsoil pH of areas of intensive agriculture in Australia varies from extremely acidic in the coastal regions of southern Queensland, New South Wales and southwestern Western Australia to highly alkaline in central South Australia. These variations are due to the chemical composition of the material from which the soil has developed and by how much the soil has been leached. Soils derived from rocks rich in quartz tend to be acidic and soils derived from limestone or basalts can be quite alkaline. In addition, acid sulphate soils, with very low pH values, occur in coastal marine environments as well as saline discharge areas. Acid soils can also develop in areas of high rainfall as water washes away soluble bases containing calcium where long term cropping together with the addition of fertilisers can increase soil acidity.

2.4 Method of Cadmium Analysis

2.4.1 Digestion

In most of the early literature (Williams *et al.*, 1972; Kinrade and van Loon, 1974; Kjellstrom *et al.*, 1974; Korityohann and Hopkins, 1976; Capar, 1977), wheat samples are digested and/or completely destructed in order to separate cadmium from the organic matrix before analysis. The two most common methods used to oxidise and destroy organic matrixes before cadmium analysis are wet and dry ashing. In dry ashing, the sample is oxidised in air or oxygen at a relatively high temperature, usually between 400^{0}C to 600^{0}C, in a muffle furnace for more than twelve hours, usually overnight (Feinberg and Ducauze, 1980; Alegria *et al.*, 1988). Vos *et al.* (1986) used a combination of dry ashing at 450^{0}C with the addition of nitric acid. Dry ashing normally results in the complete destruction of the organic material in the sample and the ash is subsequently dissolved in nitric acid, hydrochloric acid or some other suitable solvent (Skurikhin, 1993). Loss of cadmium during ashing at temperatures up to 600^{0}C has been reported to be negligible after 109 hours by Koirtyohaun and Hopkins (1976) and Knowles (1987) used an ash temperature of 650^{0}C in the graphite furnace for the determination of cadmium in blood. Although Jorhem (1993), reported losses of cadmium in specific sample tissues when ashed at 750^{0}C and suggested an ashing temperature of 450^{0}C.

In wet ashing, concentrated acids such as nitric acid, sulphuric acid, perchloric acid or mixtures of these with or without hydrogen peroxide are used to digest the sample (Ahmad *et al.*, 1994; Yizai *et al.*, 1994). The digestion is usually performed on a heating plate or an aluminium digestion block so there is no risk of loss through volatilisation, as long as the samples do not boil dry. Digestion in a closed system with acids in a teflon vessel under high pressure can be used for samples containing volatile elements or matrices that are difficult to destroy (Holak, 1972; Plessi and Monzani, 1990). Gawalko *et al.* (1997) and Chiy *et al.* (1998) reported an extensive survey of this technique for a number of metals, including cadmium, in wheat and wheaten products. De Vries *et al.* (1975) reported that wet ashing with a nitric/perchloric acid mixture gave higher results than dry ashing although the ash temperature was not given.

When low cadmium concentrations are involved, it is usually necessary to concentrate the sample before analysis. Chelating agents such as dithizone, ammonium pyrrolidine dithiocarbamate (APDC) or sodium diethyl dithiocarbamate (Na-DDC) may be used in order to complex and extract the cadmium from the aqueous phase then concentrate it in an organic solvent, usually methyl iso-butyl ketone (MIBK). The use of chelating agents to extract cadmium from aqueous solutions was reviewed by Chakravarty *et al.* (1993). This series of chelate extraction and concentration steps selectively removes the cadmium from the other metals in the digest in order to improve the detection limit and precision of the analysis by concentration in the organic phase (Brooks *et al.*, 1967; Williams *et al.*, 1972; Kinrade and van Loon, 1973; Childs and Gaffke, 1974). A further purification and concentration step can be used by back extraction of the cadmium from the organic phase that destroys the chelate complex using dilute aqueous acid (Shelly, 1990).

All the above procedures attempt to achieve the complete destruction of the organic matrix. It was shown by Premi and Cornfield (1968), Schachter and Boyer (1980) and Morales-Rubio *et al.* (1993) that trace amounts of a number of elements are quantitatively extracted from plant material and organic residues simply by boiling with hydrochloric or nitric acid, hence complete destruction of the organic matter is not necessary. Robards and Worsfold (1991) reported work on the extraction of cadmium from biological samples with a nitric acid/hydrogen peroxide mixture. A number of analysts, including Farre and Lagarda, (1986a) and (1986b), Alegria *et al.* (1988), Knutti *et al.* (1988), Barbera *et al.* (1989), Pina and Amy (1989), Versieck (1989), Alegria *et al.* (1990), Rincon-Leon *et al.* (1990), Sillanpaa and Jansson (1992b) and Batten *et al.* (2000) have since used this single acid technique with nitric acid to analyse a wide variety of elements including cadmium. The technique was comprehensively reviewed by Knight, (1980). Cartwright and Spouncer (1987) reported work on the conditions for the nitric acid digestion of barley for a survey of mineral nutrient concentration in South Australia. This work was further developed by Spouncer *et al.* (1991). Another technique that has been used by a number of workers is the introduction of samples, either as a liquid, a dry powder or as a slurry, into the spectrophotometer using a direct sampling device (Hoenig and van Hoeyweghen, 1987; Horner and Kurfurst, 1987; Vesterberg and Engqvist, 1988; Boonen *et al.*, 1993; Vinas *et al.*, 1994). This technique uses the destruction of the organic material at the ashing stage of the graphite furnace, removing the need for any pre-digestion. A spectrometric technique using a diode array together with dithizone to produce a coloured complex has been suggested by van Staden and Taijaard (2004) but a detection limit is not given. Gonzalez *et al.* (2000) use a concentration and clean-up step involving a C18 solid phase extraction column with ethanol as eluent for the determination of cobalt in flour and flour by-products. Versieck (1989) gives an extensive review of sample digestion and quality control and Cocchi *et al.* (2004) reviewed a number of different sample treatments for metal determination by atomic absorption spectroscopy.

Plastic and not glass laboratory ware must be used for the storage of solutions containing cadmium during all steps of the analysis, including storage of standards. This is due to the absorption of cadmium from aqueous solutions onto glass containers, especially in dilute solutions (King *et al.*, 1974). They found that this absorption onto glass increased markedly at a pH greater than 7.0 and that Teflon, polyethylene and polypropylene have the lowest adsorptive properties relative to aqueous species of cadmium. They suggest that all solutions should be kept in suitable plastic containers at a pH of 1.0 or lower, especially for long term storage of cadmium standards. All reagents must be of a suitable standard for the determination of trace levels of cadmium in biological material. It has also been reported by Shelly (1990) that rubber is a major potential source of contamination. Rubber gloves should not be used for sample preparation or handling of vessels or equipment, the use of vinyl gloves is suggested. Vinas *et al.* (1994) reported contamination from micropipette tips and other plastic and glassware so they suggested soaking all volumetric material in a mixture of 10% hydrochloric and 20% nitric acid overnight followed by rinsing with ultrapure water.

2.4.2 Atomic Absorption Spectrophotometer

A wide range of methods has been used for the analysis of cadmium in wheat. These include colorimetry, polarography, x-ray fluorescence, neutron activation, isotope dilution, inductively coupled plasma spectroscopy (ICP), inductively coupled plasma /mass spectroscopy (ICP/MS) and atomic absorption spectroscopy (AAS) with flame, hydride formation or graphite furnace (GF) (O'Laughlin *et al.*, 1976; Stoeppler, 1982 and 1983; Voth, 1983a; Satzger, 1988; Versieck *et al.*, 1988; Matusiewicz *et al.*, 1997). Due to its high volatility, by far the most common method for trace analysis of cadmium in biological materials is AAS with graphite furnace. This is also due to the high sample throughput, low comparable cost, low limit of detection (0.01 to 0.005 mg/kg of sample), as well as the relatively simple sample preparation for this method (Price, 1983; Welz, 1985; Jorhein, 1993). The use of atomic absorption spectroscopy with graphite furnace for the analysis of cadmium was reviewed by Ihnat (1997) for the AOAC committee on residues and related topics. Although a number of workers have suggested the use of a furnace lined with tungsten (Gine *et al.*, 1993; Yizai *et al.*, 1994).

Table 2.4.2 Detection limits and dynamic ranges of various analytical instruments for the determination of cadmium (Matsumoto and Fuwa, 1986; Ebdon *et al.*, 1993; Szlyk and Szydlowska-Czerniak, 2004)

Instrument	**Method**	**Detection limit**	**Dynamic range**
Atomic absorption spectrometry	Flame	1 ppb	0.01 - 0.5 ppm
	Graphite furnace	0.008 ppb	0.008 - 0.2 ppb
Atomic fluorescence spectrometry	Hydride formation	0.002 ppb	0.002 - 0.1 ppb
Polarography	Direct current	0.6 ppm	0.6 - 10^3 ppm
	Alternating current	1 ppm	1 - 10^3 ppm
	Square wave	0.01 ppm	0.01 - 10^3 ppm
X-ray fluorescence	Wavelength dispersive	5 ppm	5 - 10^4 ppm
	Energy dispersive	50 ppm	50 - 10^4 ppm
Neutron activation		1 ppb	1 - 10^5 ppm
Isotope dilution		10 ppb	10 - 10^5 ppm
Inductively coupled plasma emission spectroscopy		1 ppb	1 - 10^6 ppm

It can be seen from Table 2.4.2 that atomic absorption spectroscopy with graphite furnace is the most sensitive instrument for the determination of cadmium that is commonly available and was used for all analyses throughout this study.

2.4.2.1 Graphite Furnace

An electrically heated furnace for atomisation of the elements was first developed by L'vov and Massman in 1970 (Matsumoto and Fuwa, 1986). It was made of carbon, or in rare cases, of a heat resistant metal such as tungsten or tantalum and electrical heating of the furnace carries out the atomisation of the sample. The maximum temperature obtained with the furnace is 2,800^{0}C, although this is slightly lower than that of an N_2O_5-acetylene flame, it can atomise most metals with the aid of the reducing power of carbon. The carbon tube is shielded by a flow of argon in order to prevent air oxidation of the tube. The tube is cooled with water and then a 10 - 100 μL sample solution is injected into the furnace from an entrance port located in the centre of the tube. The temperature of the furnace is dependent on the voltage applied across the two end caps. This temperature is first gradually elevated to around 100^{0}C to dry the sample. After drying is completed, the furnace is gradually and continuously heated to the temperature required to remove all organic material by ashing. Finally, the temperature is quickly elevated to 2,300^{0}C, this atomises the sample and at the same time the absorption peak is observed (Benzo *et al.*, 1992). The peak height is usually used for the determination, although Jorhein (1993) suggests the use of the peak area but does not give a reason.

2.4.2.2 Pyrolytic Graphite Platforms

Chemical interferences from the matrix can be effectively reduced by the use of a graphite platform placed inside the carbon tube of the furnace. In normal graphite tube atomisers, the sample is deposited directly onto the furnace wall and then is vaporised from the surface of the graphite during a time of rapidly increasing wall temperature. The sample is not heated to the maximum atomisation temperature before vaporisation occurs so it can leave the furnace at a temperature that is lower than required for maximum absorbance. This is due to the time lag between the temperature reached by the furnace wall and the vapour temperature and the cooler vapour temperatures increase the probability of interfering reactions (Hoenig, 1985). L'vov (1978) reported that these interferences can be substantially reduced by vaporising sample solutions in a furnace that has reached an appropriate maximum temperature. The work by L'vov involved the use of a graphite platform that is inserted into the graphite furnace tube. The sample solution is deposited on to the platform instead of the furnace wall and during atomisation, the platform temperature lags that of the furnace wall by several hundred degrees. Under these conditions, the analyte compounds are not vaporised until the furnace wall and gaseous environment has approached a steady-state maximum temperature, minimising vapour phase interferences (Doidge, 1982; Voth, 1983b).

Hoenig (1985) reported problems with the injection of sample volumes approaching 20 μL into the cold graphite furnace due to sample migration from the platform. For this reason, Matusiewicz *et al.* (1997) recommended a series of smaller additions into a hot graphite tube at 120^0 to 150^{0}C be used to introduce the sample to the graphite furnace so that each solution aliquot was dried before the next addition. Beach (1990a and 1990b) reported the use of a forked platform that fitted into groves or 'notches' in the graphite tube. It was reported that this provides minimal thermal contact between the platform and the tube wall, thus allowing simple but accurate positioning of the platform within the tube with easy alignment of the sample dispensing capillary. A notched pyrolytic graphite tube with a forked pyrolytic graphite platform with hot injection was used for all analyses throughout this study.

2.4.2.3 Matrix Modifier

Due to the volatility of cadmium and the need to fully destroy the organic material remaining in the sample digest during the ash phase, a matrix modifier is usually added to the graphite furnace (Voth, 1983b; Hoeing, 1985).

Table 2.4.2.3 Chemical modifiers used in the electrothermal atomic absorption spectrometry of cadmium

Modifier	**Reference**
Palladium/tartaric acid	Yizai *et al.* (1994)
Ascorbic acid	Varian (1998)
Ammonium dihydrogen orthophosphate	Beach (1989)
Palladium nitrate/magnesium nitrate	Welz *et al.* (1988)
Lanthanum/nitric acid	Welz *et al.* (1988)
Palladium/ammonium nitrate	Smeyers-Verbeke *et al.* (1990)
Ammonium sulphate	APHA (1989)
Phosphoric acid	Frary (1986)
Rhodium	Pereira *et al.* 2004

The matrix modifier used throughout this study was 1.0% ammonium dihydrogen orthophosphate (Beach, 1989). The addition of ammonium phosphate to the furnace allowed the formation of cadmium phosphates during the initial heating cycle. These phosphate salts are much less volatile than elemental cadmium and allow the use of an ash temperature high enough to destroy the majority (if not all) of the organic material.

2.5 Aims

2.5.1 Overall aim

To establish that a difference exists in cadmium levels between wheat grown in the different states of Australia, that this difference is due to environmental factors and is fractionated during milling into the offal products which is reflected in milled and baked products.

2.5.2 Specific aims

- That the nitric acid digestion method for cadmium in wheat and wheat products produces valid and reproducible data.
- Soil type, soil acidity and superphosphate usage correlate with elevated cadmium levels in WA (and to a lesser extent in SA).
- Cadmium is deposited in the aleurone layer of the wheat grain and is fractionated during milling.
- Differences in cadmium levels exist between white and wholemeal breads and between breads from Western Australia and Victoria.

Chapter 3 Materials and Methods

3.1 Wheat

All samples of wheat, mill fractions and bread used in this study were analysed on an 'as is' basis and cadmium, ash and dietary fibre results were not adjusted for moisture content. Wheat used for milling was conditioned as per Table 3.2.1.3 for the Buhler mill, Table 3.2.2.1 for the BRI pilot mill and the guidelines for normal milling practice for the two commercial flourmills.

3.1.1 Site Silo Samples

3.1.1.1 Grower load wheat samples

Grower load wheat samples were taken from individual random truckloads of wheat as they were delivered to the silo receival point during the 92/93 harvest. Three samples were taken from each truck by silo staff using a sequential grain sampler (supplied by WACBH), transferred to a plastic bucket and mixed by hand in the sampling stand (ICC, 2004a and b). A 400 g sub-sample was transferred to a 500 mL plastic jar and labelled with the monitoring program, state, site and date. The grade and genotype of the wheat was also recorded if this information was available from the grower.

After analysis for cadmium, all results generated were entered into a relational database using the dBase IV software (dBase, 1995). The results were then sorted into silo sites or flourmills with the number of samples, means and maximums calculated. The number of grower load samples taken together with the mean and maximum cadmium levels at each site were plotted onto a map of the Australian grain belt using the geographical information system (GIS) facilities of the Arcview 3.1 suite of software (White *et al.*, 1997; ERSI, 1998; Cattle *et al.*, 2002). Arcview plots each datum point at the site of the relevant silo as a coloured circle with the colour dependant on the value of the data. The colours were selected during the study to range smoothly from green for the lowest value, or not detected, to red for the highest value. Silo sites that were not sampled were represented by a small yellow asterisk. The map of Australia, Australian grain belt and GIS coordinates for each silo site were provided by the Australian Wheat Board. Statistical analysis for determination of ANOVA values, plotting of box and whisker plots and dendograms was performed using the SYSTAT 9.0 suite of software (Kaplan, 1987; SPSS, 1999; Bett-Garber *et al.*, 2001)

3.1.1.2 Site composite wheat samples

A sample was taken from each 500 tonnes of wheat by grade supplied to an individual silo storage site during the 92/93 harvest season. All samples for a particular silo site were mixed together at the site and then the composite sample was labelled with the state, silo site, date and grade by silo staff. After analysis for cadmium, the results were plotted onto a map of the Australian grain belt and statistical analysis was then performed as detailed in Section 3.2.1.1.

3.1.2 Commercial flourmill wheat samples

Samples of wheat supplied to Australian commercial flourmills were sourced from the Australian Wheat Board flourmill monitoring program. Each rail wagon and/or truckload of wheat supplied to a domestic commercial flourmill was sampled by the relevant bulk handling company as it was out loaded at the site for outturn to the flourmill (ICC, 2004a and b). The total samples representing wheat supplied to the mill for one month were mixed at the mill and then a 1 kg sub-sample was taken by mill staff to be submitted for analysis. After analysis for cadmium, the results were plotted onto a map of the Australian grain belt and statistical analysis performed as in Section 3.1.1.1.

3.1.3 Commercial grade wheat samples

Samples of wheat where the grower had provided the grade were sourced from the AWB Australian wheat monitoring program from 1992 to 1996 and were used for commercial grade samples. The number of these samples that was available from each state was somewhat opportunistic. Far more samples labelled with the grade were available from Western Australia. This is due to the ability of each silo in that state to separate each grade at inloading. The following grade descriptions are recognised by the Australian Wheat Board and were used throughout the study (AWB, 1998; Schulze, 2003) :

Australian Prime Hard (APH) is an important, high protein milling grade wheat. The grade is principally for export due to the key features of high milling extraction as well as dough characteristics. It is grown in Queensland and New South Wales. It is ideally suited to bread making and noodle production. This grade competes in the international market against high protein grades such as Canadian western red spring (CWRS) and US dark northern spring (DNS).

Australian Hard (AH) comprises hard grain wheat varieties selected on the basis of superior milling and baking qualities. This grade segregation is grown in all states producing dough of medium to high strength that is used in a wide range of breads and noodles. It competes against low protein Canadian red spring (CRS) and US hard winter red (HWR) wheats.

Australia Premium White (APW) is a blend of white hard grain varieties selected to ensure high milling performance, consistent quality with a minimum protein of 10.0%. This grade produces clean, bright flour at high extraction rates. It is used for a wide range of end products including Middle Eastern/Indian flat breads, instant/fresh Asian noodles and Chinese steamed bread.

Australian Standard White (ASW) is a multi-purpose wheat grade in the low to medium protein range. Due to its versatile nature, it is a popular option for international as well as domestic flourmillers and is grown in all states except Queensland. This grade produces a wide range of bread products including Middle Eastern flat bread and European style loaf bread, it is also used by stock feeders.

Australian Standard White Noodle (ASWN) is restricted to a small number of specific soft-grained, medium protein varieties, with high paste viscosity and creamy flour colour. It is perfect for noodle manufacture, however, it is only grown in Western Australia. It can be blended with other wheat grades to meet customer specifications for white salted noodles and soft Japanese Udon-style noodles.

Australian Soft (Soft) is used to produce sweet biscuits, cakes, cookies and pastries. For cake production, low protein is important and levels of around 9.5% are recommended. The soft flour market is much smaller than the market for hard wheat flour, hence it is only sown in Western Australia.

Australian General Purpose (AGP) accepts lower quality milling wheats that have failed to meet the AWB receival standards for ASW. Millers use this grade to blend and extend higher quality milling wheats.

Australian Feed wheat has lower receival standards than the general purpose grade. It is made up of downgraded receivals of milling wheat with some special purpose wheat such as the Lawson genotype.

3.1.4 Australian genotype field trial wheat samples

Wheat samples were sourced from the Australian wheat genotype field trial. This trial was set up in March 1995 using collaborators in five states to plant 14 wheat varieties at three sites in New South Wales, South Australia and Western Australia as well as at two sites in Queensland and Victoria. The object of the trial was to determine the effect of planting site and wheat genotype on a range of wheat quality parameters including cadmium levels. Sufficient seed was sown at each site to produce a minimum of 100 kg of wheat at harvest. The sites used are shown in Figure 3.1.4.1 and listed in Table 3.1.4.1.

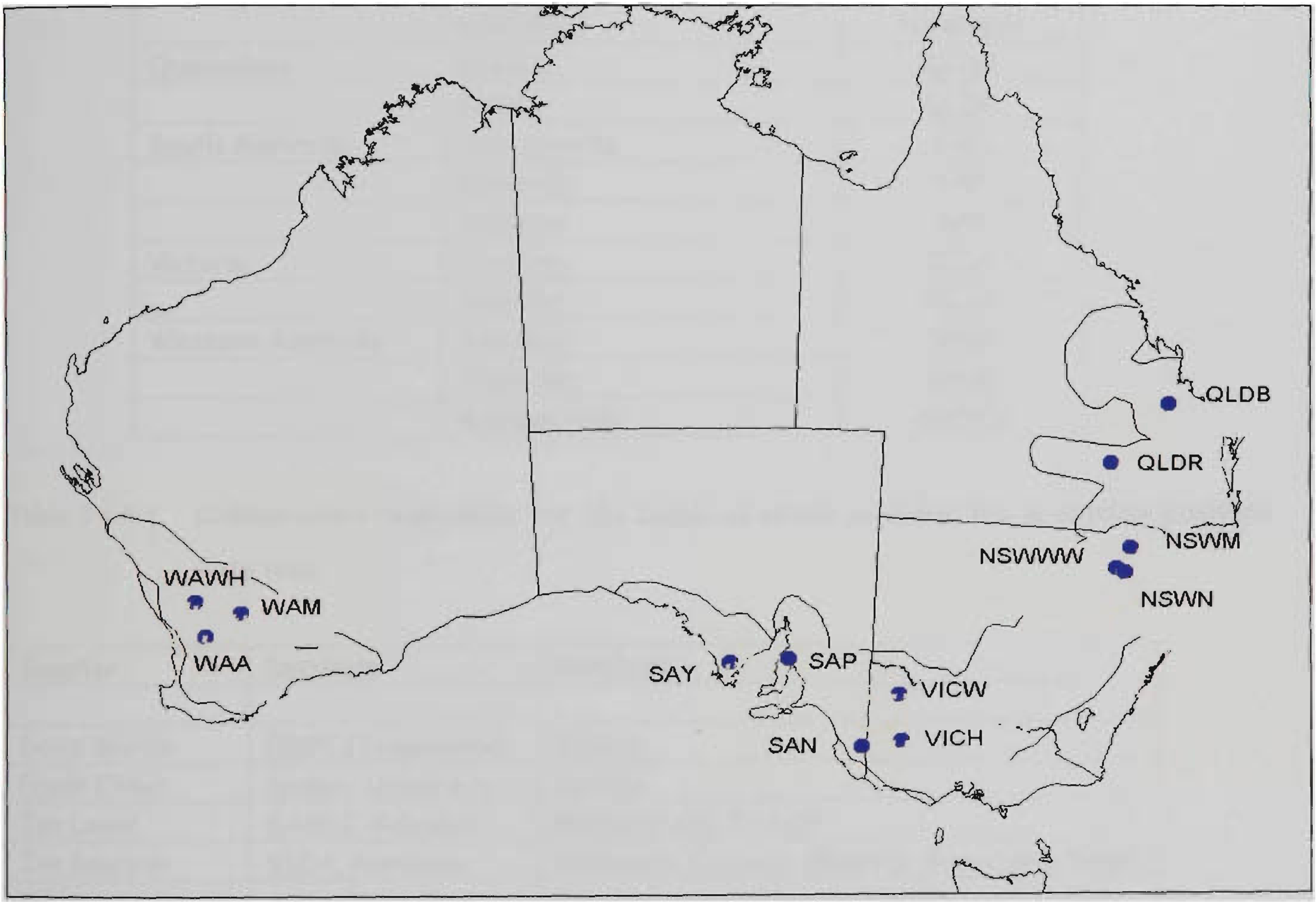


Figure 3.1.4.1 Map of Australian grain belt and planting sites for the Australian genotype field trial

It can be seen from Figure 3.1.4.1 that the sites selected in Western Australia are concentrated in the middle of the grain belt, from pervious studies it was determined that this area had wheat with the highest cadmium levels. Paskeville is on acid sandy soils, wheat from this area had the highest cadmium levels in South Australia. The other sampling sites are spread randomly throughout the remaining grain belt.

Table 3.1.4.1 Summary of states, sites and abbreviations used in the Australian genotype field trial

State	Site	Abbreviation
New South Wales	Moree	NSWM
	Narrabri	NSWN
	Wee Waa	NSWWW
Queensland	Bileoela	QLDB
	Roma	QLDR
South Australia	Narracoorte	SAN
	Paskeville	SAP
	Yeelanna	SAY
Victoria	Horsham	VICH
	Walpeup	VICW
Western Australia	Avondale	WAA
	Merriden	WAM
	Wongan Hills	WAWH

Table 3.1.4.2 Collaborators responsible for the supply of wheat seed for the Australian genotype field trial

Supplier	Institute	Genotype
David Martin	QDPI (Toowoomba)	Batavia
Frank Elison	Sydney University	Suneca
Jim Lewis	SARDI, Adelaide	Machete and Trident
Joe Panozzo	VIDA, Horsham	Goldmark, Katunga, Meering, Vectis and Yanec
Walter Anderson	Agriculture (WA)	Bodallin, Cadoux, Eradu, Halberd and Tammin

Table 3.1.4.3 Details of the wheat genotypes used in the Australian genotype field trial and the BRI pilot milling trial (AWB, 2000, 2002 and 2005)

Genotype	Description
Batavia	An Australian prime hard late-maturing wheat grown in Queensland and Southern New South Wales, used for domestic milling and Ramen noodle production. Released by the Department of Primary Industry, Queensland in 1991.
Bodallin	An older spring wheat released by Agriculture (WA) in 1981. This wheat is not widely grown in any state, thus it is usually limited to the general purpose grade.

Table 3.1.4.3 Details of the wheat genotypes used in the Australian genotype field trial and the BRI pilot milling trial (continued)

Cadoux	A preferred soft grained white salted noodle (ASWN) spring wheat grown in Western Australia, released by Agriculture (WA) in 1992.
Eradu	A preferred white salted noodle (ASWN) spring wheat grown in Western Australia, released by Agriculture (WA) in 1982.
Goldmark	A hard spring wheat grown as AH in Victoria but has been superseded in New South Wales by higher yielding genotypes. Released by Agriculture Victoria in 1996.
Halberd	An older Australian premier white spring wheat grown widely in South Australia and Western Australia as well as in some selected areas of low moisture in Victoria. Released by the University of Adelaide at Roseworthy in 1969.
Katunga	A soft spring wheat released by Agriculture Victoria in 1992. This wheat is not widely grown in any state, it is usually limited to the general purpose grade.
Krichauff	A mid-season maturing spring wheat released by the University of Adelaide at Waite in 1997 for the production of yellow alkaline noodles, grown as ASW in all states. It is not suitable for the production of loaf or flat breads or steamed buns due to the yellow pigmentation.
Machete	An older hard spring wheat widely grown as AH in South Australia and Western Australia but less in other states, used for domestic milling. Released by the University of Adelaide at Roseworthy in 1985.
Meering	An older hard spring wheat grown as AH in Victoria, however, grown as APW in all other states. It is mainly used for domestic milling and it was released by Agriculture Victoria in 1984
Rosella	A late-maturing winter wheat released by the New South Wales Department of Agriculture in 1985. It is grown for noodle production in Victoria and New South Wales and for ASW or AGP in the other states.
Suneca	An older Australian noodle winter wheat grown in northern New South Wales and used for Ramen noodle production. Released by the University of Sydney at Roseworthy in 1982
Sunlin	A mid-season maturing spring wheat released by the University of Sydney at Narrabri in 1996. It produces hard grain that is excellent for bread production and is grown as PH in New South Wales and Queensland, but as APW in the other states.
Tammin	A soft spring wheat with fair milling qualities, grown in states usually as a feed wheat. Released by Agriculture (WA) in 1994.
Trident	An Australian standard white wheat grown in South Australia. Released by the University of Adelaide at Roseworthy in 1993.
Vectis	Released by Agriculture Victoria/University of Adelaide in 1993 as a soft spring wheat replacement for Wyuna. It is grown in all states for biscuit manufacture and as a general purpose milling wheat.
Yanec	Released by Agriculture Victoria in 1996 as a rust resistant ASW quality spring wheat for the special purpose noodle segregation. It is grown in all states.

3.1.5 Wheat samples from countries other than Australia

Wheat samples for USA, Canada and Europe were mainly sourced from the AWB fungal identification trial. This trial was organised by David Webley of the AWB in 1994 and 1995 to determine levels of fungal and mycotoxin contamination in wheat from silo sites in the three major competitor countries. Other wheat samples from these and the remaining countries were taken by AWB staff on marketing or technical missions overseas or from samples sent to the AWB for analysis of competitor wheat quality parameters.

3.2 Flourmill streams

3.2.1 Buhler Laboratory mill

Two Australian standard white (ASW) wheat samples, two hard wheat samples and a soft wheat sample were sourced from the AWB quality monitoring program for Victoria and for Western Australia. Five kilos of each sample was taken and mixed by passing three times through a Borner divider. After conditioning and tempering (AACC, 1995a), 1 kg of each sample was milled on the model MLU-202 Buhler laboratory mill at AgriFood Technology, formerly the Academy of Grain Technology (AGT), Werribee, Victoria, Australia.

The Buhler laboratory mill at the Academy includes six grinding stages and their respective sifting sections. The three breaks are arranged on one pair of rolls, sections of which are machined to different diameters in order to accommodate a different gap for each grinding stage. Another pair of similar rolls is used for the three reduction stages, although the break rolls are corrugated, reduction rolls have smooth surfaces. All products are pneumatically conveyed throughout the mill. The total sieving area is 0.841 m^2 where the average feed rate is approximately 1 kg/hour. Figure 3.2.1 shows the material flow path through the mill with the sampling points for the various flours, bran and shorts (B1, B2, B3, R1, R2, R3, bran and shorts).

The roll gaps were adjusted according to AACC approved methods 26-21A, (AACC, 1995b), 26-30A, (AACC, 1995c) and 26-31A (AACC, 1995d). In these methods, the gap is initially set on each side of the rolls, and then the dials are turned to accommodate the required gaps for the grinding stages. The mill was set up with the following settings suggested in the manufacturers handbook (Buhler, 1984). These settings were routinely used at the AGT laboratory for the test milling of wheat in the quality assurance program for the Australian Wheat Board.

Table 3.2.1.1 Roll Settings for the Buhler laboratory mill

Roll Gap Settings	
Break Roll number 2	9.0 mm
Break Roll number 3	5.8 mm
Reduction Roll number 1	5.2 mm
Reduction Roll number 3	4.3 mm
Spring Tension	
Break Rolls	53 and 53 mm
Reduction Rolls	60 and 60 mm

Table 3.2.1.2 Sieve details for the Buhler laboratory mill

Break sieves	**B1**	**B2**	**B3**
Top	30 *GG*	36 *GG*	40 *GG*
Bottom	9 N	9 N	9 N
Reduction sieves	**R1**	**R2**	**R3**
Top	9 N	9 N	10 N
Bottom	9 N	9 N	10 N

GG = *Grit grade* N = Nylon

Table 3.2.1.3 Flour extraction rates used in the Buhler laboratory mill

Wheat	**ASW**	**Hard**	**Soft**
Extraction Rate (%)	74.5	75.0	77.5
Conditioning (%)	15.0	16.0	14.5

Milling staff at the laboratory do not routinely use scalper units on roll 1 or roll 2 and they were removed during this study. Samples of the six flour streams were taken from the respective flour outlet, coarse bran samples were taken from the top of the sieves of the last break (B3), finally shorts samples were taken from the top of the sieves of the last reduction stage (R3).

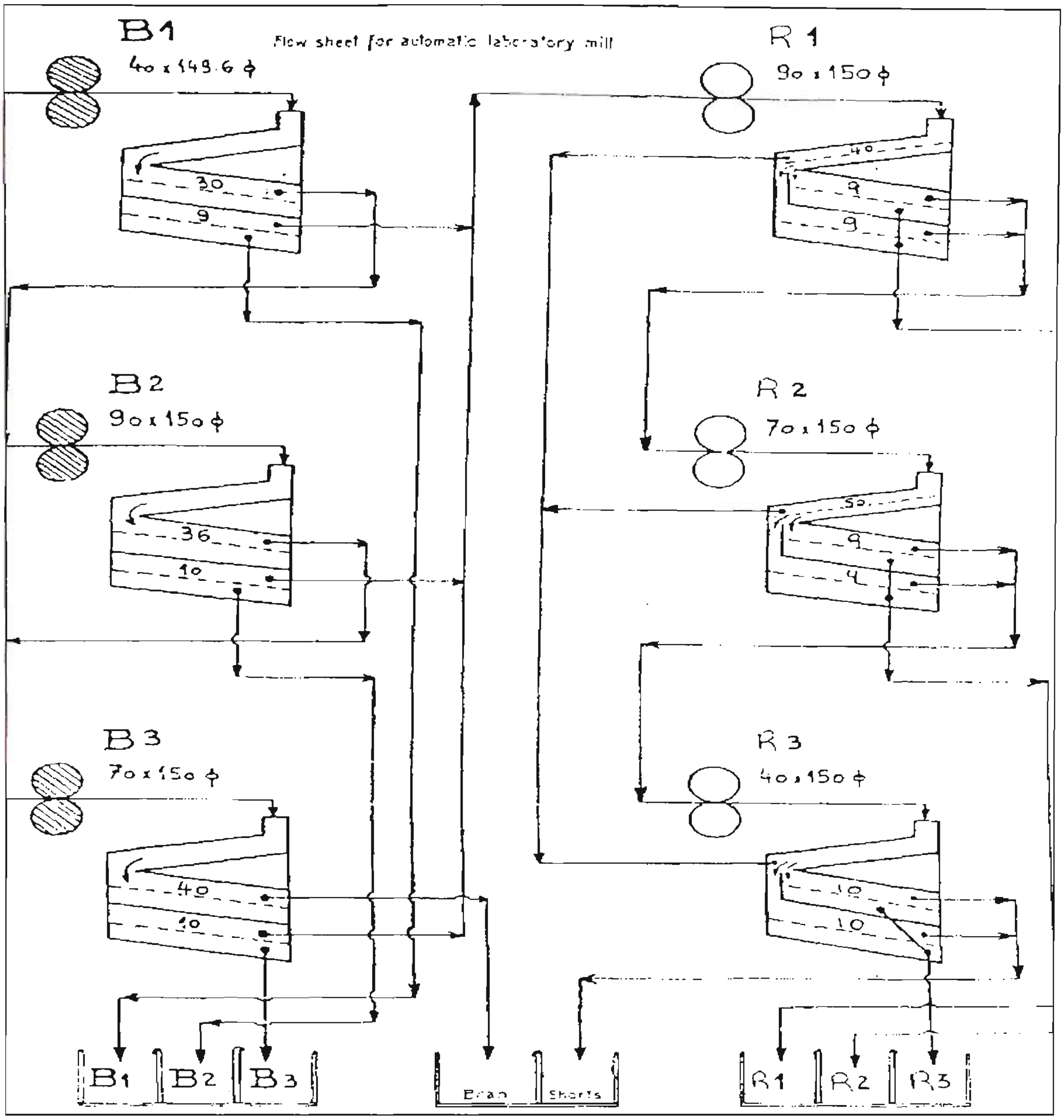


Figure 3.2.1 Material flow diagram and sampling points for the Buhler laboratory flourmill (Posner and Hibbs, 1997c)

3.2.2 Pilot mill

This trial was organised by the Australian Wheat Board to determine the differences after milling in a number of quality parameters, including cadmium, between the wheat genotype, grade and where the wheat was grown. A range of wheat samples were collected from the AWB quality monitoring program and sent to the Bread Research Institute at North Ryde, New South Wales (BRI) for milling on the pilot mill.

Table 3.2.2.1 Wheat samples collected by the AWB for the BRI pilot milling trial

State	**Zone**	**Grade/Genotype**	**Tonnes**	**Conditioning**		**Extraction (%)**
				Water (%)	**Hours**	
NSW	Newcastle	APW	2.5	16.0	18	60
		Sunlin	2.5	16.5	18	82
Qld		Leichhardt	2	16.5	18	60
SA	Port Adelaide	AH	2.5	16.5	18	82
		APW	2.5	16.5	18	82
		Kirchauff	2.5	16.5	18	60
Vic	Geelong	APW	2.5	16.5	18	60
		Rosella	2	15.0	12	60
		Sunlin	2	16.5	18	82
		Yanac	2	15.0	12	60
WA	Esperance	AH	2.5	16.5	18	60
		Cadoux	2.5	15.0	12	60
		Eradu	2.5	15.0	12	60
	Fremantle	Halberd	2.5	16.0	18	60
	Albany	Soft	2.5	14.5	8	60

Table 3.2.2.2 Details of further wheat genotypes used in the BRI pilot mill trial

Genotype	**Description**
Krichauff	An early to midseason maturing spring wheat released for the production of yellow alkaline noodles by the University of Adelaide at Waite in 1997. It is grown as ASW grade in all states.
Leichhardt	An early maturing spring wheat grown as an Australian hard grade in Queensland and New South Wales, used for domestic milling. Released by the Department of Primary Industry, Queensland in 1995.
Rosella	A late maturing winter wheat released by the New South Wales Department of Agriculture in 1985 as speciality wheat for noodle production.
Sunlin	A midseason maturing spring wheat released by the University of Sydney in 1996, grown in northern New South Wales. It produces hard grain, of good milling and dough quality, used extensively for bread making.

The pilot mill at the BRI is a small scale commercial flourmill which has the capability of performing trial millings using a number of flow options. It can receive wheat from sacks or Bulker bags, screening it at a rate of three tonnes per hour. The screen room incorporates a fully automatic dampening system to provide accurate moisture addition to any required level of conditioning, for any required length of time. The roller floor has six Robinson VE Matic Rollermills with four break rolls, a scratch or sizing system with seven reduction rolls. The rolls are 250 mm diameter and 400 mm in length. The mill has access points that allow samples to be taken of the wheat and any fraction in the complete milling process.

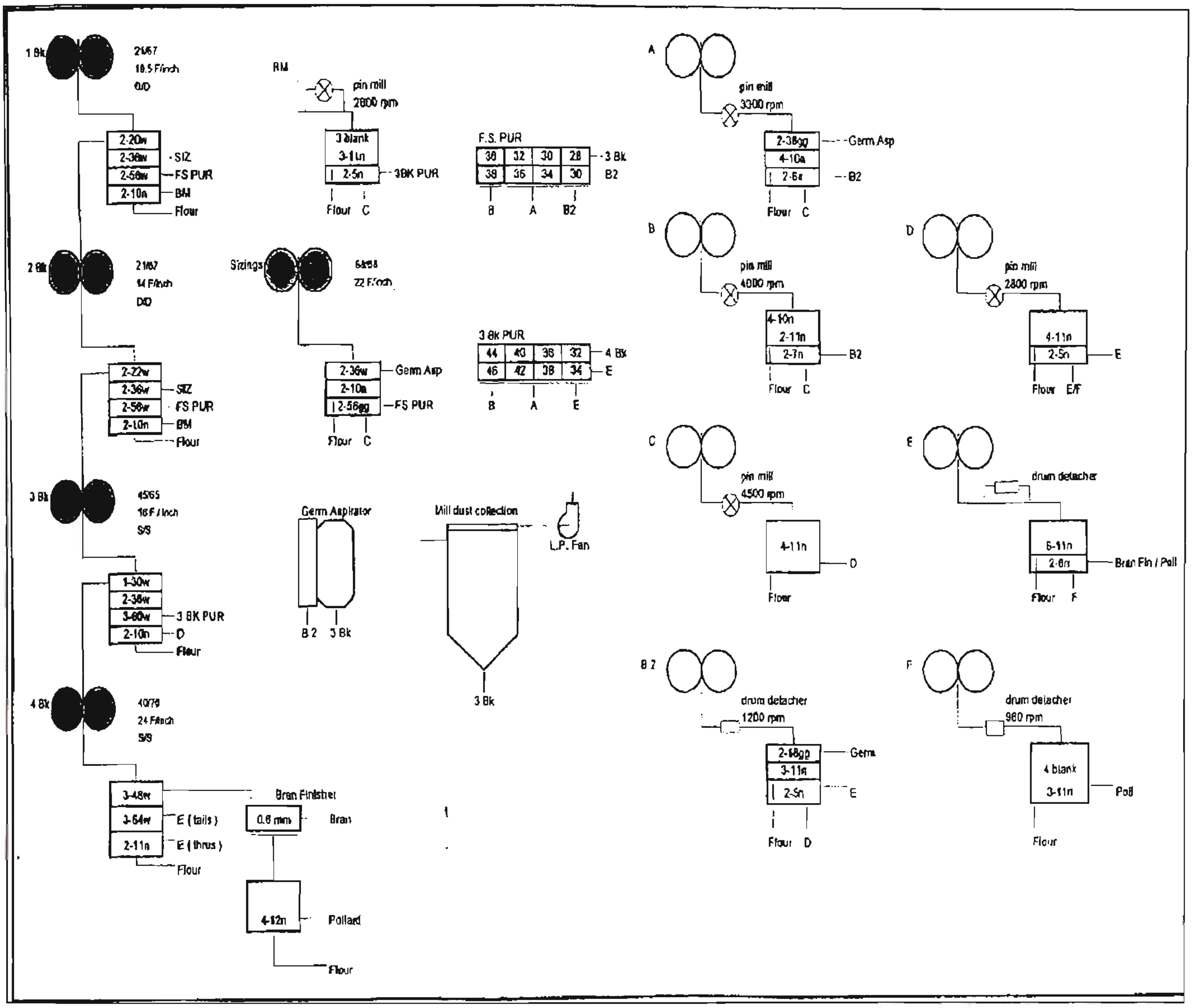


Figure 3.2.2 Flow diagram of the pilot mill at the Bread Research Institute

Table 3.2.3.3 Details of milling fractions taken from the BRI pilot mill

	Break section	**Reduction section**	**Offal**
Wheat	1st Break	B2	Pollard Finisher
	2nd Break	A	Bran
	3rd Break	B	Pollard
	4th Break	C	Germ
	Break Middlings	D	
	Sizer	E	
		F	

Samples of each wheat and the corresponding mill fractions detailed in Table 3.3.2.3 were taken from the sampling points shown in Figure 3.3.2 by BRI staff then sent to the Academy of Grain

Technology (AGT) at Werribee, Victoria. At the AGT, 1 kg representative sub-samples were taken, mixed by passing three times through a Borner divider then analysed for cadmium and ash.

3.2.3 Commercial flourmill

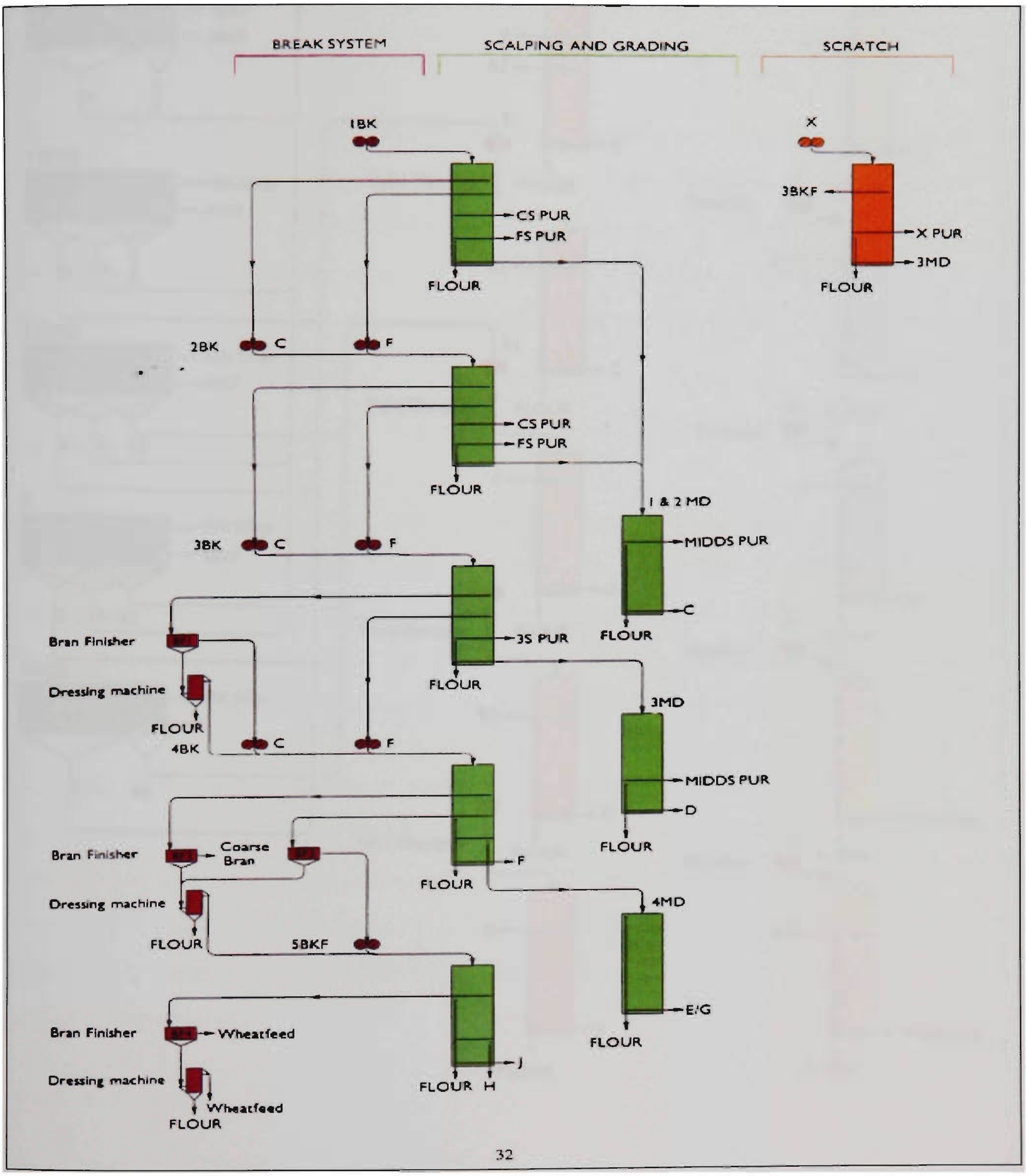


Figure 3.2.3.1 Flow diagram and sampling points of the break roll section of a commercial flourmill

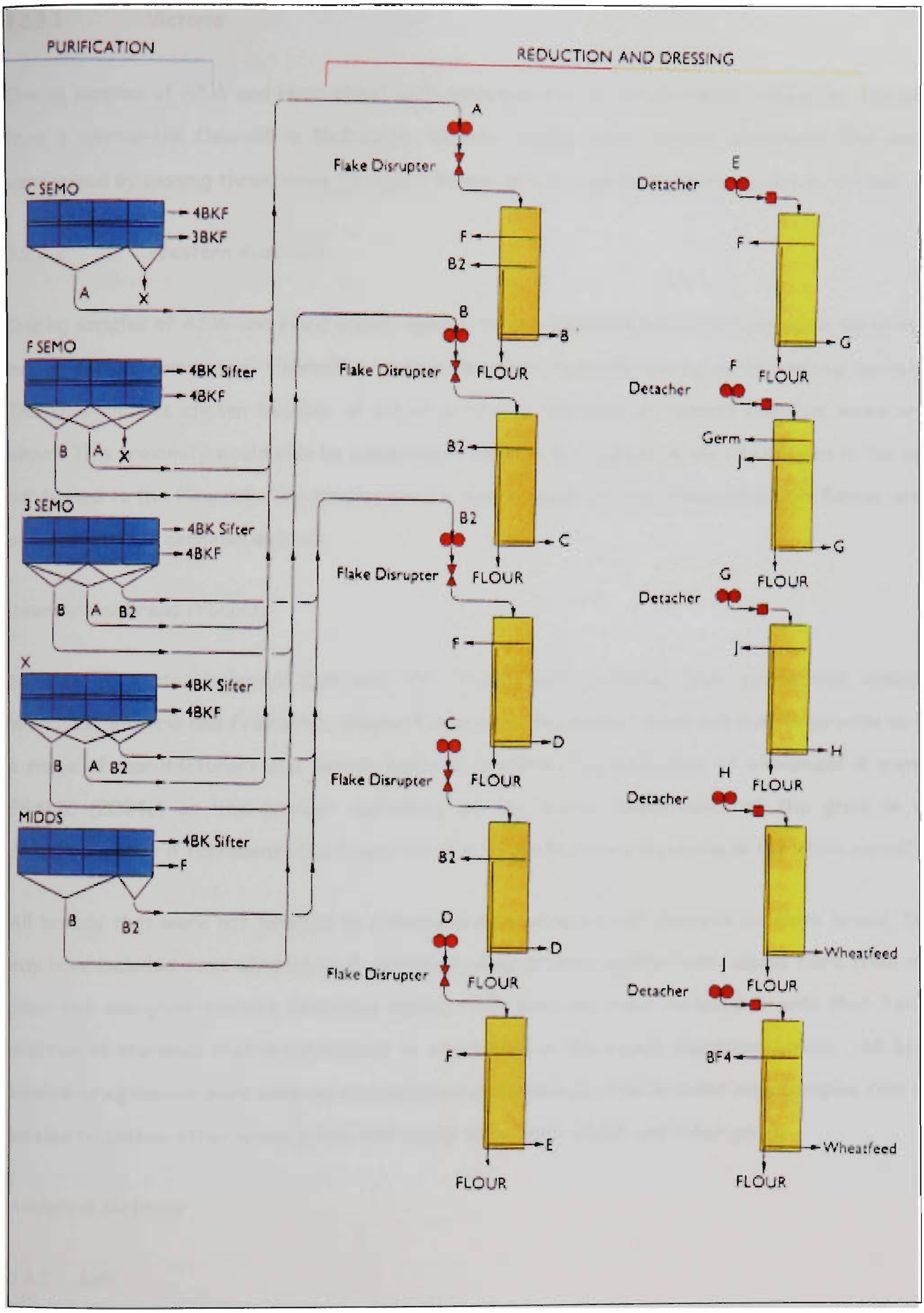


Figure 3.2.3.2 Flow diagram and sampling points of the reduction roll section of a commercial flourmill

3.2.3.1 Victoria

One kg samples of ASW and Hard wheat with corresponding mill fractions were taken by the author from a commercial flourmill in Melbourne, Victoria during normal milling operations. The samples were mixed by passing three times through a Borner divider and analysed for cadmium and ash.

3.2.3.2 Western Australia

One kg samples of ASW and Hard wheat, again with corresponding mill fractions, were taken by the author from a commercial flourmill in central Western Australia during normal milling operations. The flourmill was chosen because of its proximity to the area of highest cadmium levels in the wheat. This proximity would also be expected to provide the highest levels of cadmium in the wheat out turned to the flourmill. The samples were mixed by passing three times through a Borner divider and analysed for cadmium and ash.

3.3 Downstream Bread Products

Samples of white/wholemeal loaf and flat breads were sourced from commercial outlets in Werribee, Victoria and Fremantle, Western Australia. The breads were selected to provide as wide a range of manufacturers and sample types as possible. The definition of wholemeal is given by FSANZ (2004c) as 'the product containing all the milled constituents of the grain in such proportions that it represents the typical ratio of those fractions occurring in the whole cereal'.

All breads that were not labelled as wholemeal were sampled and analysed as white bread. These may have included some samples that contained other grains together with added fibre from other grain and non-grain sources (including lupins). This also may have included breads that had the addition of starches that are resistant to breakdown in the human digestive system. All breads labeled as wholemeal were sampled and analysed as wholemeal. This included some samples that were labeled to contain other whole grains and added fibre from wheat and other grains.

3.4 Analytical Methods

3.4.1 Ash

The most common methods for ash determination in flour and other milling products involve burning or incinerating a known mass of the sample to be analysed then weighing the residue (Evers *et al.*, 2001). As this incineration destroys everything but the mineral components, the mass of the residue provides an indication of the contribution that the minerals made to the original flour. The method used for the determination of the ash content of flourmill streams in this study was taken directly from the AOAC (2002a) and ICC (2004e). Both these methods are substantially identical, involving accurately weighing approximately five grams of sample into a preweighed porcelain crucible that has been dried at 550°C in a muffle furnace then cooled in a desiccator; ashing overnight at 550°C, cooling in a desiccator and weighing to constant weight. The ash is calculated as a percentage of the original sample weight to two significant figures. More recent work by LECO (2003) describes an automated system (Leco Corporation, model number TGA-601) that allows the determination of ash in flour with minimal involvement by an operator.

3.4.2 Dietary fibre

The dietary fibre in food was defined by the Australian representative to the Codex Alimentarius committee on nutrition and foods for special dietary uses (Codex, 2004) as 'fibre that is neither digested nor absorbed in the small intestine, consisting of either edible carbohydrate polymers, naturally occurring in the food as consumed, carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means or synthetic carbohydrate polymers'. The representative proposed that dietary fibre must have at least one of the following properties, increase laxative properties and stool bulk, stimulate colonic fermentation, reduce blood total and/or LDL cholesterol levels or reduce post-prandial blood glucose levels. The method used for the determination of dietary fibre in this study was taken from Prosky (1988), Lee *et al.* (1992), Cho *et al.* (1997), AOAC (2002b) and ICC (2004f), it determines total dietary fibre in foods and food products, including vegetables, fruits and cereal grains. This method is used throughout Australia for the determination of dietary fibre in bread.

Two duplicate one gram samples were accurately weighed into a cellulose soxhlet thimble, dried and defatted using soxhlet extraction (soxhlet extraction apparatus, Labquip Technologies, Melbourne, Australia) with petroleum ether (boiling point 40 to 60°C, Merck Chemicals, Australia). They were then gelatinised by boiling with thermostable α-amylase (Megazyme International, from *Bacillus licheniformis*, 3,000 units/mL, Ceralpha method) in phosphate buffer, 0.08M, pH =6.0 (dissolve 1.400 g of anhydrous disodium monohydrogen orthophosphate and 9.68 g of sodium dihydrogen orthophosphate monohydrate in 700 mL deionised water. Dilute to 1L with deionised water and pH to 6.0 with either 0.275 N sodium hydroxide (dissolve 11.0 g of sodium hydroxide in 700 mL deionised water, cool and dilute to 1L with deionised water) or 0.325 N hydrochloric acid (dilute 27.0 mL of 12 N hydrochloric acid to 1L with deionised water) using a pH meter.

The remaining material was then enzymatically digested with protease (Megazyme International, from *Bacillus licheniformis* , Subtilisin A, 350 tyrosine units/mL) and amyloglucosidase (Megazyme International, from *Aspergillus niger*, 200 units/mL, AMG assay) to remove the protein and starch. Ethanol (95.0%, Merck Chemicals, Australia) was added to precipitate the soluble dietary fibre and the total residue was filtered, sequentially washed with ethanol and acetone (Merck Chemicals, Australia) and then dried at 105^{0}C in an air oven overnight, cooled in a desiccator and weighed. One of the duplicate samples was analysed for protein by the method of Kjeldahl (AACC, 1990; AOAC, 2002c) and the other was analysed for ash (AOAC, 2002a). The dietary fibre is the weight of the residue minus the weight of the protein and the ash and is calculated with the following formula:

Total dietary fibre (TDF) = ((WR - ((Protein + Ash) * WR) – Blank) * 100)/WS

Where WR = Average weight of the sample residue in mg
Protein = Average protein in the sample residue
Ash = Average ash in the sample residue
Blank = Average weight of the blank residue in mg
WS = Weight of the sample in mg

3.4.3 Cadmium

A standard solution of 1000 mg/L cadmium in 0.5 M nitric acid was purchased from BDH Laboratory Supplies, United Kingdom, catalogue number 141354H. This standard was used throughout the study to quantify the cadmium content levels in all samples. A one kg blank wheat sample with a reported cadmium content of < 0.005 mg/kg and a 1 kg wheat sample with a reported cadmium content of 0.060 mg/kg (± 0.002, n = 125) were sourced from Danni Oliver of the CSIRO Division of Land and Water, Adelaide, South Australia. These samples were mixed by passing each sample three times through a Borner divider. A white loaf bread sample was purchased from a supermarket in Werribee, Victoria and the whole sample was blended in a commercial food processor until reduced to a fine uniform crumb structure.

A standard wheat flour, certified reference material (CRM) 1567a, was sourced from the United States National Bureau of Standards. The certified cadmium concentration was reported as 0.026 ± 0.002 mg/kg and this was used as a reference check sample during the study and for the construction of the CRM control chart in section 4.2.1. The procedure used for the determination of the level of cadmium in all products was developed and validated during this study and is detailed in Chapter 4.

Chapter 4 Development and Validation of a Single Acid Procedure for the Analysis of Cadmium in Wheat and Wheaten Products

4.1 Method Development

All laboratory work in this study was carried out under the guidelines detailed in the Australian standard for good laboratory practice in chemical analysis (Standards Australia, 1985). The single acid digestion method was developed and validated using the procedures outlined in Varian (1988) and ICH (1994 and 1996). For atomic absorption spectroscopy, these state that methods should be developed in a sequential manner, developing the instrument parameters, evaluation of the analytical standard, digestion conditions for blank and spiked samples as well as calculation of the minimum detection level (MDL). In addition, they state that the method uncertainty should be calculated and method validation should be carried out by analysis of check samples, followed by comparison of results with other laboratories with experience in this field.

4.1.1 Optimisation of instrument parameters

Instrument parameters for the atomic absorption spectrophotometer and graphite furnace used in this work were chosen after discussion with staff at Varian Australia, recommendations in the Varian analytical methods handbook (Varian, 1998), Australian standard AS 2134.2 - 1989 (Standards Australia, 1989) and preliminary work carried by the author. Matusiewicz *et al.* (1997) reported that substantial optimisation of analytical parameters for cadmium detection by atomic absorption spectrometry with graphite furnace was not required as this information was readily available from the literature. The graphite furnace temperatures were chosen to maximize the destruction of the extraneous organic material without the volatilisation and loss of cadmium. An ammonium phosphate modifier was added to convert the cadmium to refractory cadmium phosphate, thus allowing the use of a higher ash temperature. The instrument operating conditions listed in Tables 4.1.1.1 and 4.1.1.2 were used in the method development.

Table 4.1.1.1 Instrument parameters for the Varian 8000 atomic absorption spectrometer.

Lamp current (mA)	4	Background correction	On
Slit width (mm)	0.5	Hot inject	Yes
Slit height	Normal	Temperature (^0C)	120
Wavelength (nm)	228.8	Inject rate	5
Sample introduction	Sampler automixing	Replicates	3
Measurement time (sec)	1		

Table 4.1.1.2 Furnace conditions for the Varian GFA graphite furnace attachment

Step Number	Temperature (^0C)	Time (Sec)	Gas Flow (L/min)	Gas	Read
1	250	20.0	3.0	Argon	No
2	300	20.0	3.0	Argon	No
3	550	22.0	3.0	Argon	No
4	2200	1.2	0	Argon	Yes
5	2300	3.0	0	Argon	Yes
6	2300	3.0	3.0	Argon	No

4.1.2 Linearity of Standard Curve

A 1000 mg/L cadmium solution was serially diluted with 40% nitric acid to give a 5 µg/L standard. This standard solution was analysed 10 times with the instrument parameters given in Tables 4.1.1.1 and 4.1.1.2 to produce the series of absorbance values tabled below.

Table 4.1.2.1 Relationship between absorbance and cadmium standard concentrations

Run	**Standard Concentration (µg/L)**					
	0	**0.5**	**1.0**	**2.0**	**3.0**	**5.0**
	Absorbance (abs units)					
1	0.0027	0.0337	0.082	0.1733	0.2493	0.3981
2	0.0025	0.0366	0.0773	0.1697	0.2569	0.4012
3	0.0036	0.0355	0.0741	0.1604	0.2372	0.3688
4	0.0018	0.0596	0.1022	0.1892	0.2762	0.4398
5	0.0031	0.0370	0.0743	0.1781	0.2704	0.4049
6	0.0016	0.0367	0.0860	0.1708	0.2509	0.3896
7	0.0028	0.0467	0.0887	0.1858	0.2736	0.4468
8	0.0033	0.0362	0.0816	0.1786	0.2695	0.4287
9	0.0060	0.0517	0.0997	0.1925	0.2778	0.4470
10	0.0019	0.0383	0.0835	0.1810	0.2831	0.4352
Mean	0.0029	0.0414	0.0848	0.1779	0.2645	0.4160
Std Dev	0.0013	0.0084	0.0097	0.0098	0.0150	0.0271
CL (95%)	0.0008	0.0052	0.0060	0.0061	0.0093	0.0168

In Table 4.1.2.1, the typical relationship between the amount of cadmium in the solution introduced into the graphite furnace and the absorbance of the radiation passing through the furnace is detailed. It can be seen that there is a steady increase in absorbance as the amount of cadmium is increased. This increase is consistent with the standard concentration and the absorbance following Beers Law (Gilmutdinov *et al.*, 1994).

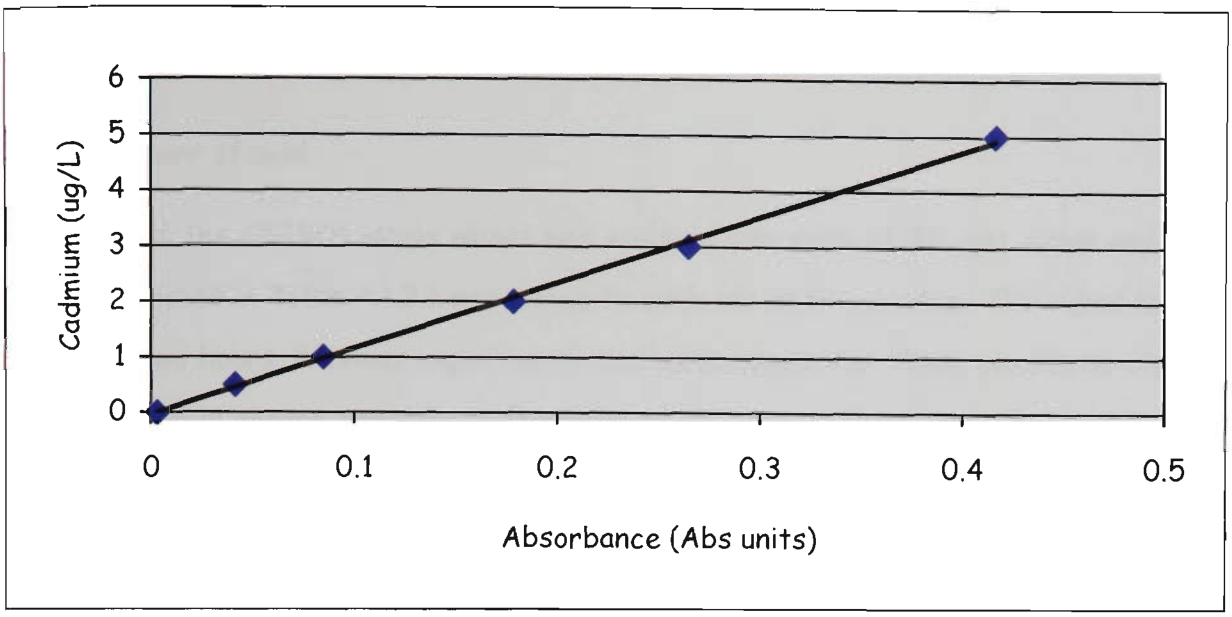


Figure 4.1.2.1 Linear regression plot of absorbance against cadmium concentration

A typical regression plot of the absorbance of the radiation beam in the graphite furnace against the amount of cadmium in the standard solution is shown in Figure 4.1.2.1.

Table 4.1.2.2 Regression parameters from plot of absorbance against cadmium concentration

Slope	**Intercept**	**Regression Coefficient (r)**
11.9	0.044	0.999

ANOVA analysis of the data in Table 4.1.2.1 gave a P value of < 0.001 at 8 DF. As P is < 0.001, it can be shown that a very highly significant linear correlation exists at the 99.9% confidence level between the cadmium concentration in the standard solution and the absorbance of the radiation beam in the graphite furnace (Zou *et al.*, 2003; Lowry, 2005). This linear relationship was used to determine the cadmium level in the wheat and wheaten products from direct calculation using the slope and the intercept given in Table 4.1.2.2 (Pandya *et al.*, 1986; Huber, 1997). With a R^2 value of 0.998, it can also be shown that 99.8% of the cadmium concentration can be predicted from the absorbance of the radiation beam in the graphite furnace.

4.1.3 Optimisation of digestion conditions

A sample of wheat was supplied by Danni Oliver, CSIRO, Division of Soils with a supplied cadmium concentration of 0.060 mg/kg (± 0.002, n = 125). The sample was labelled CSIRO1 and was used in the following experiments to determine the optimum digestion conditions for the nitric acid extraction of cadmium from wheat. It was decided to alter the amount of acid, the digestion temperature and then the extraction time in this sequence while holding the other conditions constant. By altering each parameter in a sequential manner, it was possible to determine the maximum effect of each but also keep the digestion time to a minimum.

4.1.3.1 Amount of acid

One gram of the CSIRO1 whole wheat was weighed into each of 36 test tubes and the amount of nitric acid listed in Table 4.1.3.1 was added to each set of three tubes. The tubes were digested at 100^{0}C for two hours. Previous experiments had determined that these conditions with an excess of nitric acid would ensure complete destruction of the wheat. The tubes were cooled, made up to 20 mL with water and the extract was analysed on the graphite furnace.

Table 4.1.3.1 The amount of cadmium extracted from CSIRO1 by altering the amount of acid added and holding the temperature at 100 ^{0}C and the extraction time at 2 hours.

Nitric acid added (ml)	**Cadmium (mg/kg)**
0	0
0.25	0.005
0.5	0.015
1.0	0.015
1.5	0.020
2.0	0.035
2.5	0.045
3.0	0.050
4.0	0.050
6.0	0.055
8.0	0.055
10.0	0.055

The relationship between the amount of cadmium extracted from the wheat and the amount of nitric acid added is shown in Table 4.1.3.1. It can be seen that the amount of extracted cadmium reaches a maximum of 0.055 mg/kg when 6.0mL of nitric acid has been added (both in blue) and the addition of extra nitric acid did not result in the extraction of more cadmium.

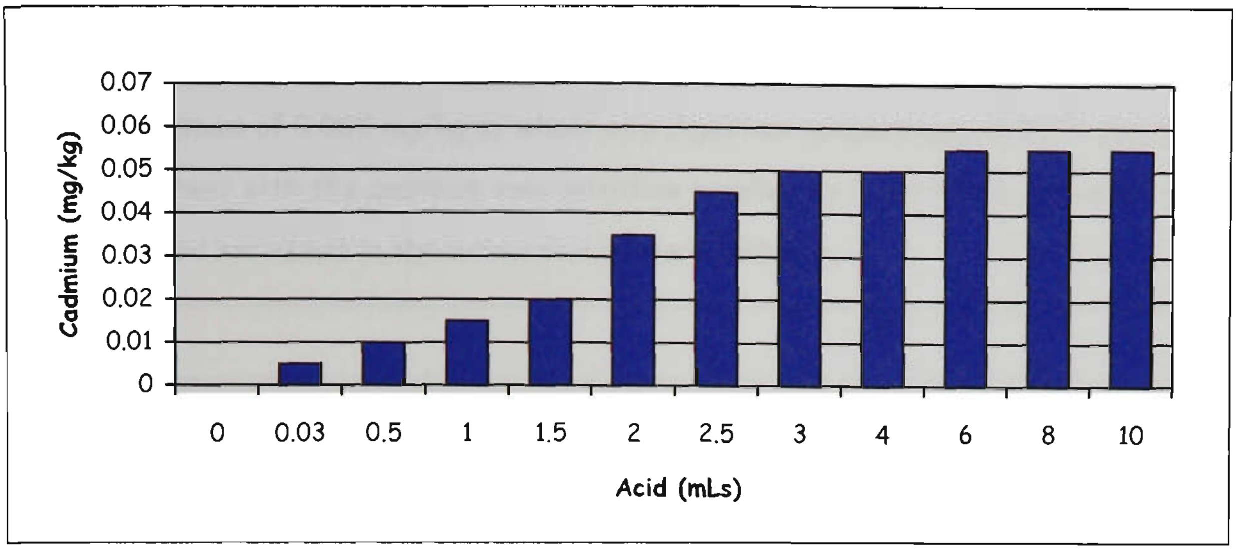


Figure 4.1.3.1 Plot of amount of cadmium extracted from CSIRO1 against the amount of acid added

It can be seen from Figure 4.1.3.1 that the amount of cadmium extracted reaches a maximum of 0.055 mg/kg of wheat after the addition of 6.0 mL of nitric acid. It was decided to use 8.0 mL of nitric acid to maximize the digestion of the organic material in the test tube and reduce the organic load during the ash cycle in the graphite tube.

4.1.3.2 Digestion temperature

One gram of CSIRO1 whole wheat was weighed into each of 27 test tubes and 8.0 mL nitric acid was added to each tube. Each set of three tubes was digested for two hours at the temperatures listed in Table 4.1.3.2. The tubes were cooled, made up to 20 mL with water and the extract was analysed on the graphite furnace.

Table 4.1.3.2 The amount of cadmium extracted from CSIRO1 by altering the digestion temperature and holding the amount of acid added at 8 mL and the extraction time at 2 hours.

Temperature (^0C)	**Cadmium (mg/kg)**
30	0.010
40	0.015
50	0.025
60	0.045
70	0.055
80	0.055
90	**0.060**
100	0.060
110	0.060

The relationship between the amount of cadmium extracted from the wheat and the digestion temperature is shown in Table 4.1.3.2. It can be seen that the amount of extracted cadmium reaches a maximum of 0.060 mg/kg of wheat at a digestion temperature of 90 ^0C (both in blue). This value is consistent with the cadmium concentration supplied by Danni Oliver, however, increasing the temperature did not result in the extraction of more cadmium.

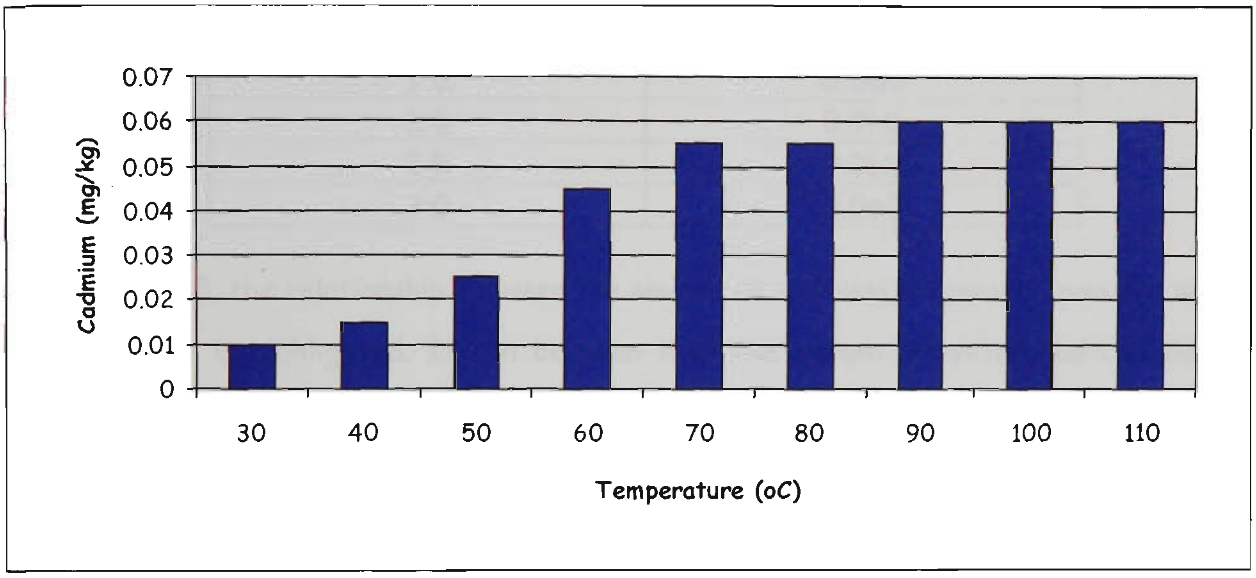


Figure 4.1.3.2 Plot of cadmium extracted from CSIRO1 against changing the digestion temperature

It can be seen from Figure 4.1.3.2 that the amount of cadmium extracted reaches a maximum at a temperature of 90 ^0C. It was decided to digest at a temperature of 105 ^0C to maximise the digestion of the organic material in the test tube and reduce the organic load during the ash cycle in the graphite tube.

4.1.3.3 Digestion Time

One gram of CSIRO1 whole wheat was weighed into each of 24 test tubes and 8.0 mL nitric acid was added to each tube. Each set of three tubes was digested for the times listed in Table 4.1.3.3 at a temperature of 105 ^0C. The tubes were cooled, made up to 20 mL with water then the extract was analysed on the graphite furnace.

Table 4.1.3.3 The amount of cadmium extracted from CSIRO1 by altering the digestion time while holding the temperature at 100^{0}C and the amount of acid at 8 mLs.

Time (hours)	Cadmium (mg/kg)
0	0
0.25	0.015
0.5	0.035
1.0	0.050
1.5	**0.060**
2.0	0.060
2.5	0.060
3.0	0.060

In Table 4.1.3.3, the relationship between the amount of cadmium extracted from the wheat and the digestion time is highlighted. It can be seen that the amount of extracted cadmium reaches a maximum of 0.060 mg/kg at a digestion time of 1.5 hours (both in blue). This value is also consistent with the cadmium concentration supplied by Danni Oliver, increasing the digestion time did not result in the extraction of more cadmium.

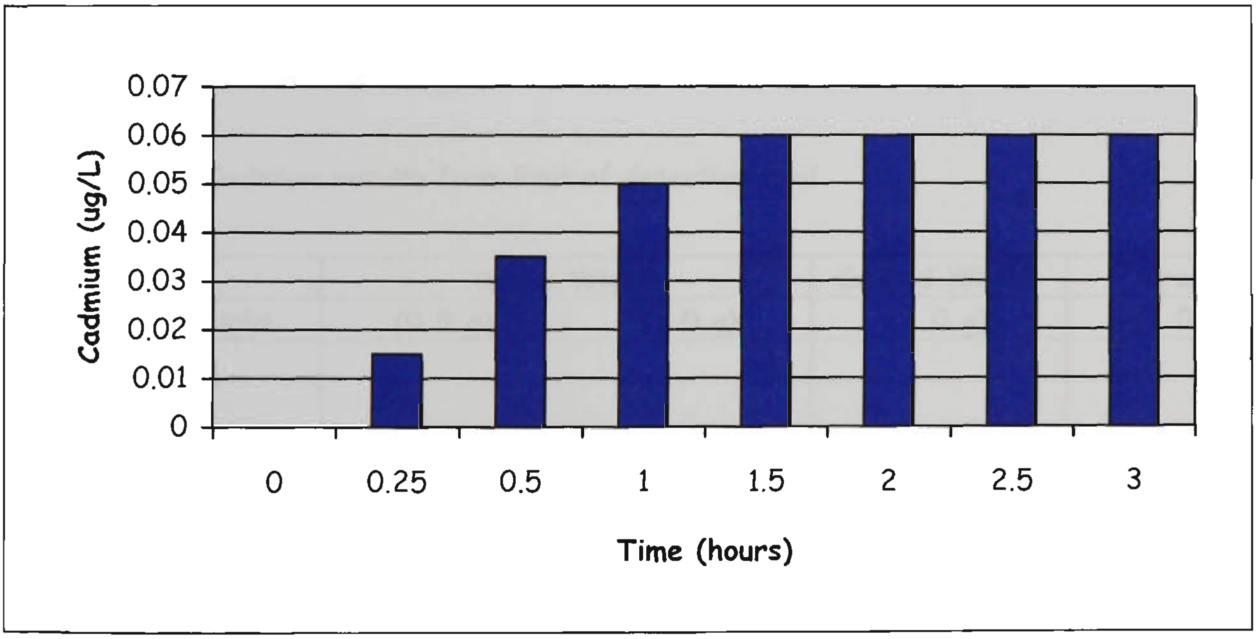


Figure 4.1.3.3 Plot of cadmium extracted from CSIRO1 against changing the digestion time

It can be seen from Figure 4.1.3.3 that the amount of cadmium extracted reaches a maximum at a digestion time of 1.5 hours. It was decided to digest for 2.0 hours to maximize the digestion of the organic material in the test tube thus reducing the organic load during the ash cycle in the graphite tube.

4.1.3 Minimum Detection Level

The minimum detection level (MDL) of an analyte in an analysis has been defined by the Analytical Methods Committee of the Royal Society of Chemistry (1987) as being the lowest concentration of the analyte that can be distinguished with reasonable concentration from a field blank, defined as a hypothetical sample containing zero concentration of the analyte. The committee reported that the MDL is defined as three times the standard deviation of a number of replicate analyses of the analyte in a blank sample. Piso and de Boer (1994) defined two detection limits, the instrumental detection limit covering only the uncertainty of measurement of the instrument (IDL) and the detection limit of the total analytical procedure (MDL). It is common throughout trace analysis to use the MDL as defined by the Royal Society of Chemistry and this was used in this study.

George (1998) further verified this concept for contaminated environmental samples where it is difficult to obtain samples with zero background levels of the analyte in question, which is often the position with cadmium. A sample of blank wheat supplied by Danni Oliver of the CSIRO Division of Soils was analysed 10 times using the method given in Section 4.1.6, both as whole wheat and after being ground to a fine meal in a coffee grinder. A white bread sample from Victoria was ground to a fine crumb structure in the coffee grinder and was also analysed 10 times. The results for these analyses are listed in Table 4.1.4.1.

Table 4.1.4.1 *Cadmium results from limit of detection trial*

	Whole Wheat		**Ground Wheat**	**Bread**
Sample weight	**(0.5 g)**	**(1.0 g)**	**(1.0 g)**	**(1.0 g)**
Run				
1	0.003	0.005	0.006	0.004
2	0.150	0.010	0.007	0.005
3	0.009	0.012	0.006	0.003
4	0.013	0.007	0.006	0.006
5	0.003	0.003	0.009	0.005
6	0.013	0.007	0.007	0.003
7	0.011	0.008	0.007	0.004
8	0.010	0.004	0.006	0.005
9	0.002	0.006	0.009	0.005
10	0.009	0.007	0.007	0.003
Mean	0.009	0.007	0.007	0.004
Std Dev (SD)	0.005	0.003	0.001	0.001
MDL (SD x 3)	0.015	0.009	0.003	0.003

Using the statistical analysis given by the Analytical Methods Committee of the Royal Society of Chemistry (1987) and by Gibbons (1997), George *et al.* (1998), Zorn *et al.* (1999) and Buzoianu (2000), the following detection limits (LOD) were calculated and used in the method.

Table 4.1.4.2 Limit of detection for whole wheat, ground grain and milling fractions and bread

Sample (1.0 g)	**Limit of detection (mg/kg)**
Whole grain	0.01
Ground grain and milling fractions	0.005
Ground bread	0.005

4.1.5 Uncertainty

The definition of uncertainty given in the Eurachem/Citac guide (Eurachem, 2000) is 'a parameter associated with the results of a measurement that characterises the dispersion of the values that could be reasonably attributed to the measurand'. The guide further states that 'Uncertainty of measurement does not imply doubt about the validity of a measurement; on the contrary, knowledge of the uncertainty implies increased confidence in the validity of a measurement result'.

Thompson *et al.* (1999) states that 'the parameter may be a standard deviation, or a given multiple of it, or the half width of an interval having a stated level of confidence'. The measurement uncertainty statement gives a range within which the true value of the measurand is considered to lie. This range is centred on the measurement value and while the true value is likely to be close to the measurement value, it may lie anywhere within the uncertainty range (Cook, 1999).

In estimating the uncertainty of a measurement, it is usual to take each source of uncertainty and treat it separately to obtain the contribution from that source (ISO, 1993). The following calculations to determine the uncertainty of the single acid method for the determination of cadmium were derived from the National Analytical Reference Laboratory handbook on the measurement of uncertainty (NARL, 2001) and the cause and effect model used is taken from Eurachem/Citac guide (Eurachem, 2000)

Mass of sample (m) = 1 g $\delta_m = 0.00005$ g

Volume (V) = 10 mL $\delta_V = 0.0816$ mL

From the table of spikes in Table 4.2.1.2

Mean of the spike = 117% One SD = 21.5%

Number of repeats = 100

δ_{spike} = $21.5/\sqrt{(100)}$

= 2.15

Reproducibility (from Eurachem, 2000)

SD = 0.0648 µg/kg ($\delta_{precision}$)

Working standard concentration = 5 µg/L and cadmium concentration = 2.5 µg/kg

Hence $\delta_{working\ cadmium\ concentration}$ = 0.0296 µg/L

Primary standard concentration = 1000 mg/L with a supplied uncertainty of ± 5 mg/L.

Volume of standard flask = 100 mL δ_V = 0.0578 mL

Volume of pipette = 10 mL δ_V = 0.0115 mL

$$\delta_{Cadmium} = \text{Cadmium} \times \sqrt{(0.00005/1)^2 + (0.0816/10)^2 + (5.7/117)^2 + (0.0648/2.5)^2 + (0.0296/5)^2}$$

$$= \text{Cadmium} \times 0.0528$$

$$\sim 5.3\%$$

Given a coverage factor of 2

$\delta_{cadmium}$ = 10.6% (~ 11%)

This is in line with the value of 10% given by Horiwitz (1988) for the determination of metals at this level of concentration. In this method, uncertainty was further refined by the use of a standard reference material and control charts.

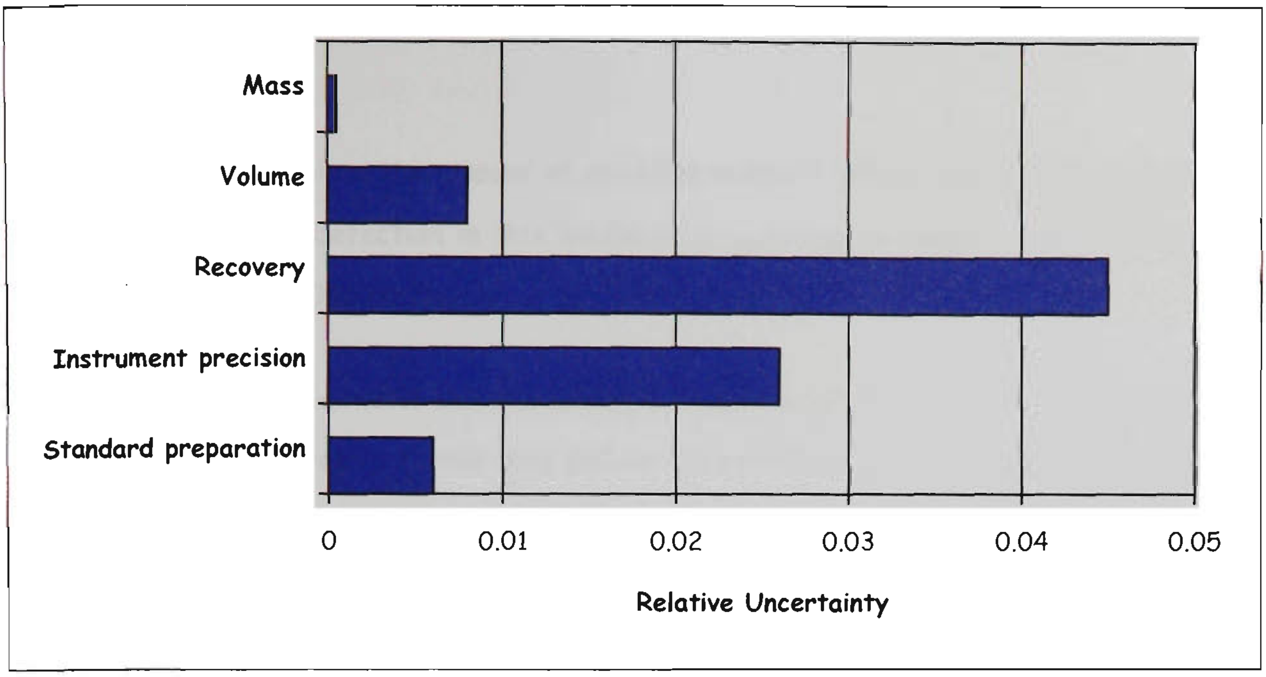


Figure 4.1.5.1 Contribution of the method parameters to the total uncertainty

In Figure 4.1.5.1, it can be seen that the recovery of cadmium from a spiked sample and the instrument precision contribute the major effects on the uncertainty of the analytical result.

4.1.6 Method

A method for the analysis of cadmium in wheat and wheaten products was compiled from the results in sections 4.1.1 to 4.1.5 using the guidelines described in the Australian standard AS 2929 (Standards Australia, 1990a), Australian Standard AS 17025 (Standards Australia, 1999) and NATA technical note 17 (NATA, 2004). This method complies with the guidelines in ICC (2004g) and was used for all cadmium analysis in this study. The method is detailed below.

4.1.6.1 Procedure for the analysis of cadmium in wheat and wheaten products

1. Preface

This procedure for the determination of cadmium in wheat, wheat meal, wheat milled fractions and breads was developed by Lindsay Swinden to determine the levels of cadmium in these products for his PhD thesis.

1. Foreword

Cadmium is an elemental metal that has little or no known role in the maintenance of life. It is ubiquitous throughout nature and occurs at low to very low background levels in most soils and plants. It may occur at elevated levels in grains due to additions of cadmium to the soil by man.

2. Scope

This procedure determines the amount of cadmium in wheat, wheat meal, wheat milled fractions and breads. The limit of detection in this method for cadmium in wheat is 0.01 mg/kg and in other wheaten products is 0.005 mg/kg.

Cadmium is a very toxic metal and the primary standard should be handled with care. Nitric acid, 69% is extremely corrosive to the skin and all operations with this reagent should be carried out whilst wearing gloves, face protection and always in a fume cupboard.

The relevant material safety data sheets should be referred to and the relevant hazards well understood before undertaking this procedure.

4. Reference Documents

Not applicable

5. Records

Not applicable

6. Definitions

6.1 AAS is the abbreviation for an atomic absorption spectrophotometer

6.2 MSDS is the abbreviation for material safety data sheet.

6.3 GF is the abbreviation for a graphite furnace

7. Principle

The sample is weighed, either whole or ground, and digested with nitric acid. The cadmium in the digest is detected and quantified with an AAS equipped with a graphite furnace.

8. Reagents

8.1 Pure Reagents

Unless otherwise specified, all reagents shall be of an analytical reagent grade suitable for the trace analysis of metals by AAS with graphite furnace.

8.1.1 Nitric acid, 69%

8.1.2 Ammonium dihydrogen orthophosphate

8.1.3 Detergent, Tween 80^R or equivalent

8.1.4 Water

8.1.5 Wheat Flour, certified standard reference (CRM), NBS #1567a or equivalent

8.2 Solutions

The following instructions provide a guide to the preparation of solutions. The amounts required should be adjusted to suit a particular number of samples.

8.2.1 Blank solution (40% nitric acid)

Add 400 mL of nitric acid, 69% to 400 mL of water, allow to cool and make up to 1000 mL with water.

8.2.2 Modifier (0.5% ammonium dihydrogen orthophosphate)

Weigh 0.50 g of ammonium dihydrogen orthophosphate and make up to 100 mL with water.

8.2.3 Wash solution

Add 0.100 mL of nitric acid 69% and 2 mL of Tween 80^R to 800 mL of water and make up to 1000 mL with water.

8.3 Standards

8.3.1 Primary standard

Cadmium, 1000 mg/L

Store the primary standard at less than 10^0C.

8.3.2 Secondary standard, 1.0 mg/L

Transfer 0.5 mL of primary standard to a 500 volumetric flask and make up to the mark with blank solution.

Enter all information into the primary and secondary standards register. F/008

Store the secondary standard at less than 10^{0}C in a polyethylene bottle.

8.3.3 Tertiary standard, 0.005 mg/L

Transfer 1.0 mL of secondary standard to a 200 volumetric flask and make up to the mark with blank solution.

Make up on the day of analysis and store in a polyethylene bottle.

9 Apparatus

9.1 Glassware, including A grade volumetric glassware.

9.2 Divider, Borner or equivalent

9.3 Laboratory mill, capable of reducing the size of the sample to pass through a 0.5 mm sieve

9.4 Analytical balance, capable of weighing any mass up to and including the combined mass of the digestion tube and the sample to an accuracy of +/- 0.0005 g

9.5 Heating block, capable of sustaining a temperature of 90^{0}C for 2 hours

9.6 Glass digestion tubes, 150 x 20 mm O.D., calibrated at 10 and 20 mL

9.7 Vortex mixer

9.8 Partition tube, graphite, notched, Varian^{TM} # 63-100023-00

9.9 Platform, graphite, forked, Varian^{TM} # 63-100024-00

9.10 Atomic absorption spectrophotometer

The instrument shall be equipped with a graphite furnace. The AAS and the graphite furnace shall be set up according to the manufacturer. The atomic absorption spectrophotometer shall be capable of the following operating conditions:

9.10.1 AAS program

Lamp current (mA)	4
Slit width (mm)	0.5
Slit height	Normal
Wavelength (nm)	228.8
Sample introduction	Sampler automixing
Measurement time (sec)	1
Replicates	3
Background correction	On
Hot inject	Yes
Temperature (^0C)	120
Inject rate	5

9.10.2 Furnace parameters

Step Number	Temperature (^0C)	Time (Sec)	Gas Flow (L/min)	Gas	Read
1	250	20.0	3.00	Argon	No
2	300	20.0	3.0	Argon	No
3	550	22.0	3.0	Argon	No
4	2200	1.2	0	Argon	Yes
5	2300	3.0	0	Argon	Yes
6	2300	3.0	3.0	Argon	No

9.10.1 Autosampler parameters

	Tertiary standard (μL)	Blank solution (μL)	Modifier (μL)
Reagent blank	0	17	5
Standard 1 (0.5 mg/L)	2	15	5
Standard 2 (1.0 mg/L)	4	13	5
Standard 3 (2.0 mg/L)	8	9	5
Standard 4 (3.0 mg/L)	12	5	5
Sample	10	5	5

10. Sampling and Sample Preparation

Samples shall be prepared for analysis either blended by passing through a Borner divider 3 times or by grinding to pass through a 0.5 mm sieve and blending thoroughly by hand.

11. Procedure

11.1 Initialise the AAS with a suitable selection of standards.

11.2 Weigh 1.000 g of a representative sub-sample of the sample to the nearest 0.005 g into a digestion tube.

11.3 Quality assurance shall include an NBS CRM wheat sample and a reagent blank at the beginning of each rack of 24 samples. The 10th sample shall be weighed in duplicate and the 19th in triplicate with the last of the triplicate being spiked at a level of 0.03 mg/kg (30 uL of 1.0 mg/L)

11.4 Add 8 mL of nitric acid, 69% and leave for 22 +/- 4 hours. Place on the heating block and heat at 90°C for 2.0 +/- 0.5 hours. Allow to cool and make up to 20 mL with water.

11.5 Vortex and allow to settle. Transfer approximately 1 mL of the digest into an auto sampler cup and run the auto sampler.

12. Calculation

The concentration of cadmium in the test sample is calculated to two significant figures using the following equations:

Cadmium concentration ($\mu g/L$) = 11.913 x Absorbance (Abs units) + 0.044

Cadmium level in product (mg/kg) = Cadmium concentration ($\mu g/L$) x 20

13. Precision

Plot the recoveries of spiked samples using the software Control Chart ! Pro+. Recoveries must lie between two standard deviations of the current mean recovery.

If the above condition is not achieved then repeat the analysis and investigate any problems before accepting results.

4.2 Method Validation

The single acid method for the analysis of cadmium in cereal products was validated using the procedures outlined in the Eurachem guide (Eurachem, 1998), NATA technical note 17 (NATA, 1998), Australian standard AS 17025 (Standards Australia, 1999), King and Lawn (1999), Ellison *et al.* (2000) and Eurachem/Citac guide QUAM (Eurachem, 2000). These procedures include the use of standard reference materials, control charts and the participation in collaborative trials with laboratories of demonstrated competence in the relevant field.

4.2.1 Control Charts

The use of Shewhart control charts to validate analytical analysis has been reported by many workers (Grant and Leavenworth, 1988; Bissell, 1994; Stenson, 1999 and others). They allow the immediate identification of any cause of variation in the analysis so that it can be dealt with by correction or elimination before the process is out of control. It is common practice in trace metal analysis to use the results from the analysis of a certified reference material (CRM) in a control chart to monitor the validity and statistical control of the results. It is also good analytical practice to monitor the results of the duplicates and spiked samples (CITAC, 1995; NATA, 1995). This also allows the direct monitoring of the performance of the atomic absorption spectrophotometer.

A standard reference material (CRM 1567a) wheat flour with a certified cadmium concentration of 0.026 ± 0.002 mg/kg was sourced from the National Institute of Standards and Technology (NIST), USA (Standards Australia, 1991). The wheat flour SRM, a reagent blank, duplicates and a 0.05 mg/kg spike added to the last sample in the batch, were analysed with each batch of 20 samples. Typical results from the SRM and the spike quality assurance samples are shown in Tables 4.2.1.1 and 4.2.1.2.

Table 4.2.1.1 Typical cadmium results from the analysis of the NIST CRM wheat flour for 1993 to 1997

Cadmium (mg/kg)						
0.025	0.025	0.023	0.023	0.026	0.024	0.030
0.028	0.026	0.028	0.028	0.030	0.023	0.026
0.025	0.024	0.025	0.028	0.025	0.022	0.022
0.027	0.025	0.031	0.025	0.030	0.026	0.028
0.030	0.027	0.030	0.024	0.024	0.032	0.028
0.026	0.027	0.031	0.030	0.030	0.028	0.028
0.032	0.035	0.029	0.029	0.023	0.023	0.024
0.028	0.028	0.029	0.029	0.027	0.031	0.025
0.020	0.031	**0.020**	0.023	0.025	0.026	0.028
0.027	0.026	0.028	0.029	0.033	0.024	0.028
0.030	0.027	0.024	**0.038**	0.027	0.025	0.026
0.026	0.027	0.028	0.028	0.025	0.031	0.025
0.024	0.023	0.023	0.024	0.024	0.025	0.023
0.027	0.028	0.025	0.034	0.034	0.026	0.034
0.021	0.031	0.030	0.025	0.028	0.021	0.026

Table 4.2.1.1 details the variation shown in 105 typical cadmium analyses of the NIST wheat flour over the period 1993 to 1997. The table shows two low results of 0.020 mg/kg and a high result of 0.038 mg/kg (all in blue). This level of variation, with confidence limits of ± 0.001 mg/kg (0.05 probability level), is less than that expected from the method uncertainty given in section 5.1.5 (± 0.003 mg/kg). This highlights the fact that variation, or uncertainty, in the results can be more accurately determined from the analysis of actual samples than calculation from method parameters.

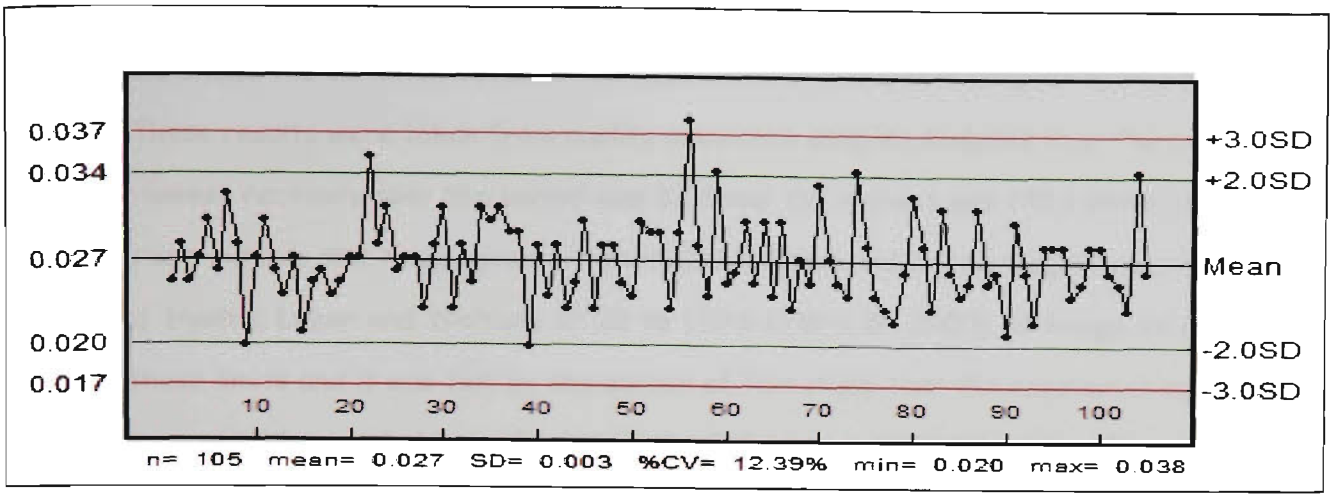


Figure 4.2.1.1 Shewhart control chart of the NIST SRM wheat flour results from Table 4.2.1.1

A Shewhart control chart of cadmium results from 105 analyses of the NIST wheat flour SRM over the period 1993 to 1997 is shown in Figure 4.2.1.1. The chart has warning lines at 2 standard deviations from the mean (0.020 mg/kg and 0.034 mg/kg) with control lines at 3 standard deviations from the mean (0.017 mg/kg and 0.037 mg/kg). The results touch or cross the warning lines at 5 points, this is expected if the results are normally, or approximately normally, distributed. The results cross the control line at one point, this is also expected from normal distribution. The chart highlights that the analysis is in statistical control over this period (Standards Australia, 1990b). The mean value of 0.027 mg/kg is in good correlation with the certified value of 0.026 mg/kg supplied by the National Institute of Standards and Technology (NIST), USA. The difference of 0.001 mg/kg is well within the confidence limits of ± 0.002, also supplied by the Institute.

Table 4.2.1.2 Typical recovery results from whole wheat samples from 1993 to 1997 spiked at 0.05 mg/kg cadmium

Recovery (%)						
86.3	78.4	93.5	99.4	72.9	88.0	112.7
117.9	120.9	93.6	85.0	116.7	95.7	59.3
83.5	85.3	83.4	89.4	115.4	87.3	88.4
122.3	104.8	116.8	73.8	101.6	85.6	97.6
71.6	75.6	105.2	62.0	56.7	89.1	81.6
87.7	78.4	89.2	82.4	83.5	103.8	90.7
83.4	86.6	**53.2**	87.2	88.9	99.6	79.1
101.1	108.2	92.6	93.7	84.7	84.7	71.4
110.4	82.7	88.1	87.9	94.6	80.1	88.0
82.7	91.3	111.4	110.3	81.1	55.0	97.8
62.6	82.7	93.0	105.0	75.6	71.9	114.3
75.2	63.4	93.7	86.7	68.7	**143**.1	100.7
106.3	97.4	115.2	76.4	79.6	102.7	109.2
78.1	117.1	93.9	79.0	78.6	95.4	94.9
93.5	119.0	129.4	84.2	93.7	113.8	87.0

Table 4.2.1.2 shows the variation between the recoveries of 105 wheat samples spiked at 0.05 mg/kg cadmium. These results were taken from quality assurance samples analysed over the period 1993 to 1997. The lowest recovery over the period was 53.2 and the highest was 143.1 (both in blue). These recoveries are outside the range given for pesticide residue method development by the Japanese Ministry of Health, Labor and Welfare of 70 to 120% (JMHLW, 2003), although no explanation is given for these limits and it was felt by the author of this study that the recoveries achieved in the study were acceptable as only 12 results out of the 105 were outside the range. The results in Table 4.2.1.2 gave an average recovery of 90.8% and following the guidelines in Thompson *et al.,* (1999), the cadmium results in this work were not corrected for recovery.

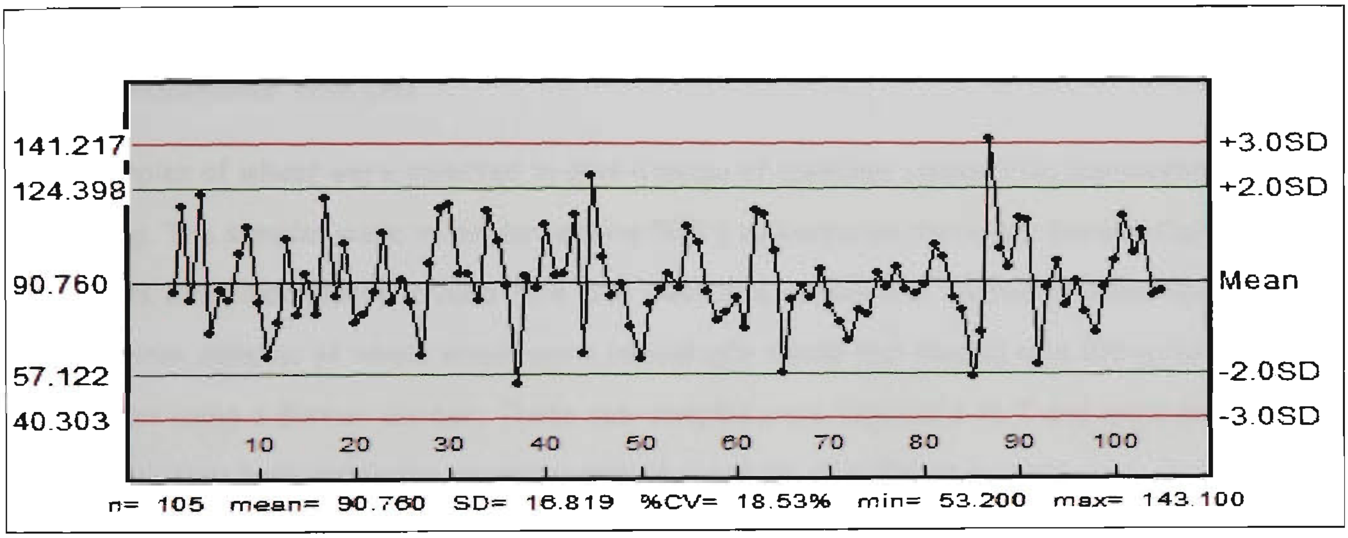


Figure 4.2.1.2 Shewhart control chart of the wheat spike recoveries from Table 4.2.1.2

A Shewhart control chart of cadmium recovery results from 105 analyses of the 0.05 mg/kg wheat spike over the period 1993 to 1997 is shown in Figure 4.2.1.2. The chart has warning lines at 2 standard deviations from the mean (57.1% and 124.4%) with control lines at 3 standard deviations from the mean (40.3% and 141.2%). The results touch or cross the warning lines at 5 points which is expected if the results are normally, or approximately normally, distributed. The results cross the control line at one point as is also expected from normal distribution. The chart highlights that the analysis is in statistical control over this period.

The 95% confidence limits for the recoveries were calculated from the equation given in Youden and Steiner (1975) :

Standard deviation = 16.82 x Number of results = 105

95% confidence limits = $1.96 \times 16.82/(\sqrt{105})$

= 3.22%

Hence, it is expected that 95% of the recovery results will fall between 87.6% and 94.0% and that the uncertainty of the method is approximately 6% (coverage of 2). This again highlights the fact that variation or uncertainty in the results can be more accurately determined from the analysis of actual samples than calculation from method parameters.

4.2.2 Collaborative trials

It is well recognised in analytical analysis that participation in collaborative trials with laboratories of known expertise in the area of interest provides an important method of validating analytical technique and results (CITAC, 1995).

4.2.2.1 Collaborative Trial One

Seven samples of wheat were selected to give a range of cadmium values from approximately 0.02 to 0.15 mg/kg. The samples were mixed by passing 500 g sub-samples through a Borner divider. Four of the wheats were individually ground to a fine meal in a commercial coffee grinder. These samples and the three samples of whole wheat were individually mixed and divided into 100 g representative sub-samples using a Borner divider. These sub-samples were labelled 1 to 7 and were mailed to two laboratories that have extensive experience in the analysis of cadmium in grain.

The laboratories were : CSIRO Division of Soils, South Australia (CSIRO)
Victorian State Chemistry Laboratory, Werribee, Victoria (SCL)

The laboratories were requested to analyse the samples with their normal procedure and provide the results with details of the digestion method and instrument used.

Table 4.2.2.1.1 Digestion conditions and instruments for interlaboratory trial number one

Laboratory	**Digestion**	**Instrument**
CSIRO	Nitric acid/solvent extraction	AAS with GF
SCL	Nitric acid/microwave	ICP
This work	Nitric acid	AAS with GF

All three laboratories used a single nitric acid digestion, the CSIRO laboratory used a solvent extraction procedure to concentrate the cadmium in order to remove organic contaminants. The SCL used a microwave digestion system to maximise destruction of the organic material.

Table 4.2.2.1.2 Mean cadmium results for collaborative trial one

Sample	Treatment	Cadmium (mg/kg)			
		SCL	CSIRO	Swinden	Mean
1	Ground	0.061	0.065	0.065	0.064
2	Ground	0.023	0.025	0.025	0.024
3	Ground	0.030	0.028	0.030	0.029
4	Whole	0.060	0.061	0.065	0.062
5	Ground	**0.099**	**0.119**	0.110	0.109
6	Whole	0.030	0.029	0.030	0.030
7	Whole	**0.140**	0.147	0.150	0.146

The results from Table 4.2.2.1 show good correlation between the three laboratories over the seven samples. The only exception is the results for sample five and seven from the Victorian State Chemistry laboratory that are low (in blue) compared to the other two laboratories. In addition, the results from sample five from the CSIRO are high.

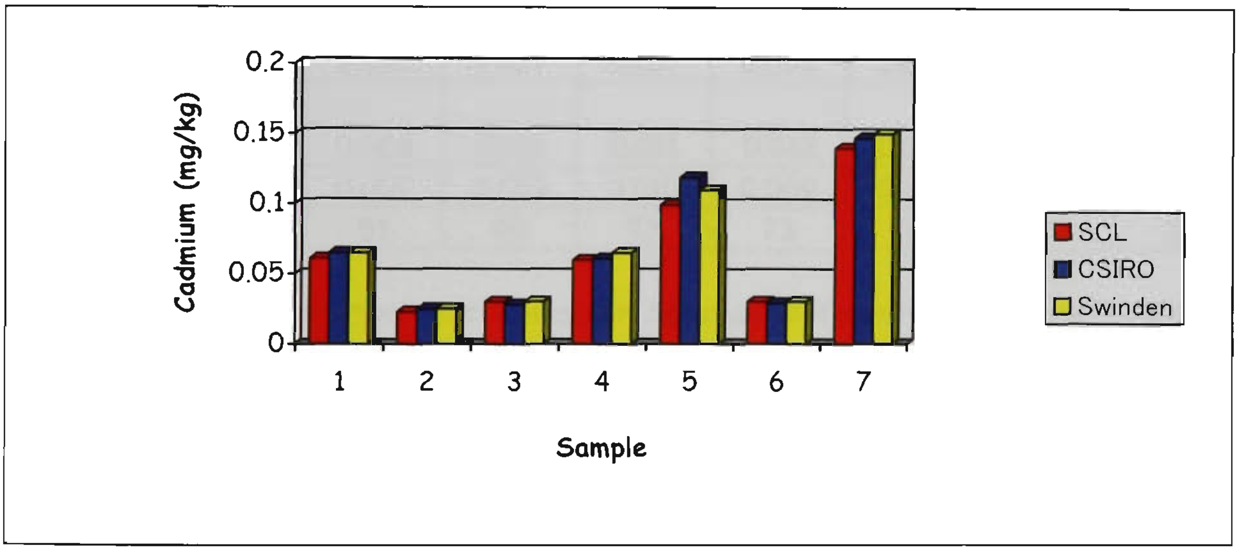


Figure 4.2.2.1 Plot of cadmium results from collaborative trial one

A good correlation between the three laboratories is also shown in Figure 4.2.2.1, but highlights that the SCL provided the lowest result for samples one, two, four, five and seven and the CSIRO was the highest for sample number five.

4.2.2.2 Collaborative Trial Two

The National Residue Survey (NRS) is an operational unit of the Australian Government Department of Agriculture, Fisheries and Forestry that monitors chemical residues as well as other contaminants in Australian agricultural produce and food commodities. As a part of this monitoring program, the NRS organises a series of biannual blind collaborative trials to select laboratories to provide analytical services. These trials include the analysis of wheat grain for pesticide residues and metals including cadmium. As a part of the validation process for the cadmium method in this study, a set of samples for the first trial in mid 1994 was sourced from the NRS, analysed, then the results submitted to the NRS. All results for the collaborative trial were sourced from the NRS and are listed in Table 4.2.2.2.

Table 4.2.2.2 Cadmium results for collaborative trial two

Laboratory	**Cadmium (mg/kg)**							
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Spike							0.030	0.032
A	**0.027**	**0.023**	**0.025**	**0.025**	**0.026**	**0.035**	**0.045**	**0.050**
B	0.003	ND	0.003	0.004	0.010	0.020	ND	ND
C	0.004	ND	0.005	ND	ND	**0.004**	0.020	0.025
D	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.020	0.028	0.037
E	0.008	0.007	0.009	0.006	0.006	0.018	0.029	0.029
F	ND	ND	ND	ND	ND	**0.005**	0.029	0.031
Swinden	0.007	0.010	0.007	0.007	0.008	0.017	0.030	0.035
Mean	0.010	0.008	0.010	0.011	0.013	0.017	0.031	0.037
SD	0.010	0.009	0.009	0.010	0.009	0.010	0.009	0.011
% CV	100	51	90	93	73	62	27	29

In Table 4.2.2.2 the variation that can occur between different laboratories in blind trials is highlighted. Laboratory 'A' (in blue) has reported results that are markedly higher than the other six laboratories where this difference is approximately consistent at 0.02 mg/kg for all samples. The other results show reasonable correlation except for laboratories 'C' and 'F' that show markedly lower results for sample six than the other four laboratories with an overall consensus mean of 0.017 mg/kg.

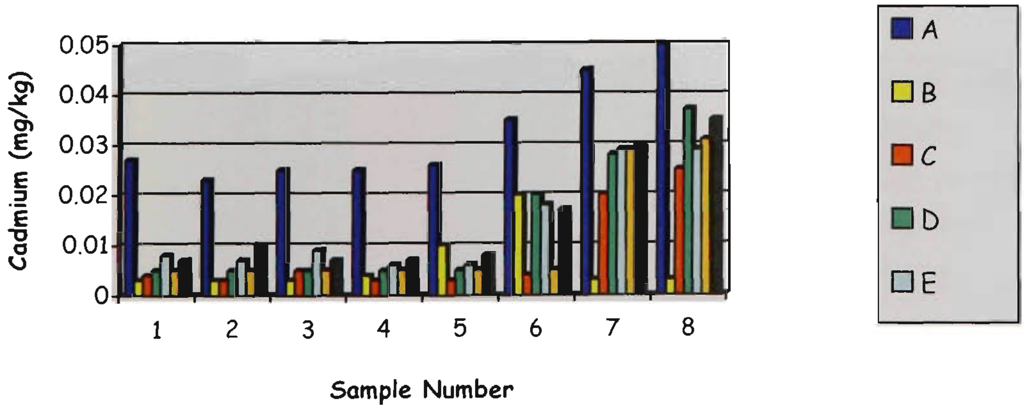


Figure 4.2.2.2 Plot of cadmium results from collaborative trial two

It can be seen from Figure 4.2.2.2 that laboratory A reported results that were consistently considerably higher than the other six laboratories. In addition, laboratory C reported a low result for sample six.

4.2.2.3 Collaborative Trial Three

A sample of wheat was sourced from the CSIRO Division of Soils with a reported cadmium level of 0.067 mg/kg. The sample was mixed by passing through a Borner divider, ground to a fine meal in a commercial coffee mill then divided into 500 g sub-samples. The samples were sent to three other laboratories with experience in the analysis of cadmium in grain.

Table 4.2.2.3 Cadmium results for collaborative trial three

Laboratory	Cadmium (mg/kg)
AGAL (NSW)	0.073
CSIRO (Division of Soils)	**0.067**
Department of Agriculture (WA)	0.071
Swinden	0.073
Varian Australia	0.071
Mean	0.071
Standard Deviation	0.003

Table 4.2.2.3 shows good correlation between the five laboratories, the CSIRO Division of Soils gave the lowest result (in blue) although this result is within two standard deviations of the mean.

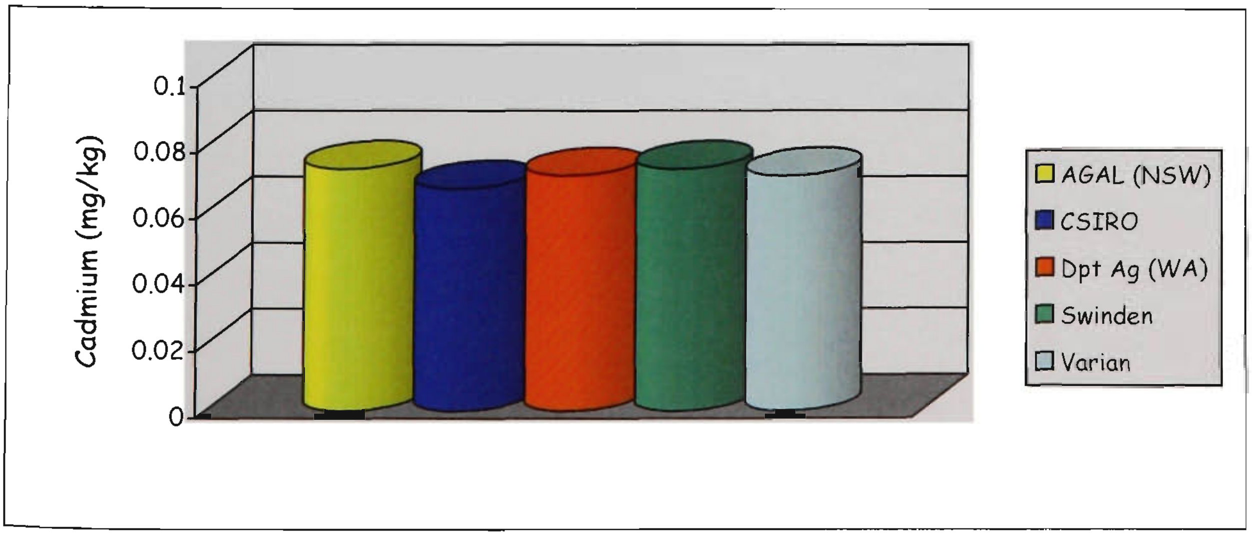


Figure 4.2.2.3 Cadmium levels reported by the five laboratories for the sample of wheat in collaborative trial three

Figure 4.2.2.3 shows the very good correlation between the five laboratories. The CSIRO (Division of Soils) gave the lowest result (in blue) but it is well within two standard deviations from the mean.

4.2.2.4 Collaborative Trial 4

A collaborative trial was organised by the Overseas Merchandise Inspection Company of Japan (OMIC). Four separate samples of whole wheat were sent to the five laboratories listed in Table 4.2.2.4. OMIC is an international inspection agency with laboratories in Tokyo, Portland, Bangkok and Melbourne. The Food Department of the Japanese Ministry of Health, Labour and Welfare monitors all foodstuffs imported into Japan for quality and contaminants.

Table 4.2.2.4 Cadmium results for collaborative trial four

Laboratory	**Cadmium (mg/kg)**			
Sample	**1**	**2**	**3**	**4**
Food Agency (Japan)	0.64	0.034	0.22	1.1
OMIC (Bangkok)	0.63	0.05	0.24	1.12
OMIC (Tokyo)	0.66	0.042	0.241	1.15
OMIC (USA)	**0.45**	0.03	**0.18**	**0.91**
Swinden	0.62	0.029	0.26	1.2
Mean	0.60	0.037	0.23	1.10
Standard deviation	0.085	0.009	0.030	0.11

A P value of > 0.05 suggests that the results from the five laboratories are not significantly different at the 95% confidence level. The laboratory at OMIC (USA) gave consistently lower results (in blue) than the other four laboratories apart from sample two, although the four results were within two standard deviations of the mean.

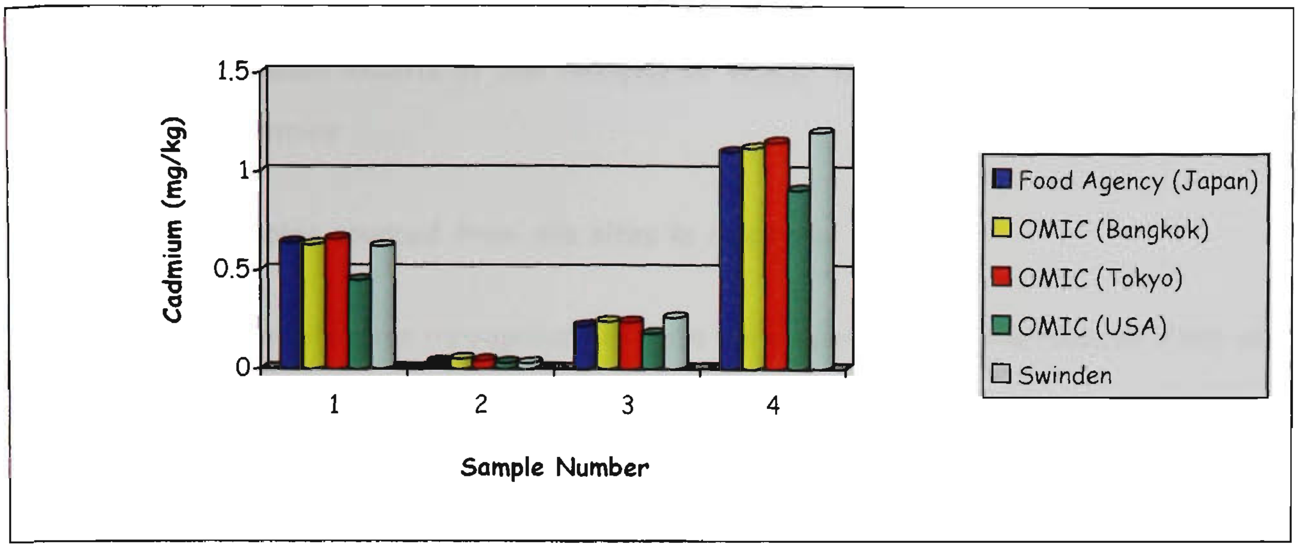


Figure 4.2.2.4 Cadmium levels reported by the five laboratories for the four samples of wheat in collaborative trial four

It can be seen from Figure 4.2.2.4 that all five laboratories had good correlation over the four samples, with the minor exception of OMIC (USA) who gave the lowest results for each sample.

4.3 Discussion

A structured system of development and validation was used to produce a method for the analysis of cadmium in wheat and wheaten products. This involved the validation of both the single acid digestion of the samples and the analysis using atomic absorption spectrometry with graphite furnace. The validation of the procedure was further established by successful involvement in a number of collaborative trials, both internationally and in Australia. A method uncertainty was calculated and LOD's were developed for all matrices to be used in the study using standard procedures.

During the development, it was shown that the nitric acid digestion of the wheat and wheat products destroyed the samples sufficiently to allow analysis by atomic absorption spectroscopy. A number of experiments were undertaken to establish the correct amount of acid, temperature and digestion time required to ensure complete digestion of the samples. A method was produced and used for the analysis of cadmium in all samples in this study. The use of control charts of standard reference materials and spiked samples was used throughout the study to monitor statistical control of the digestion process and instrumentation used in the analysis.

Chapter 5 Cadmium Results of the Analysis of Wheat from Sites within Australia and other Countries

5.1 Wheat samples sourced from silo sites in Australia

Wheat samples from silo sites throughout Australia were sourced from the relevant AWB wheat monitoring program.

5.1.1 Grower Load samples

Grower load wheat samples were taken from individual random truckloads of wheat as they were delivered to the receival point during the 92/93 harvest.

Table 5.1.1.1 Summary of sites, sample numbers and cadmium results for grower load samples by state.

	Number		Cadmium (mg/kg)	
State	**Sites**	**Samples**	**Mean**	**Maximum**
NSW	47	150	0.01	0.04
Qld	23	61	< 0.01	0.04
SA	**19**	**638**	**0.03**	**0.12**
Vic	41	426	0.02	0.08
WA	**41**	**682**	**0.05**	**0.22**
Total	170	1,957	0.04	0.22

The results in Table 5.1.1.1 highlight the larger number of samples taken in South Australia and Western Australia. These states also gave higher mean and maximum cadmium levels found in wheat (all in blue). The sample numbers for these two states were chosen because preliminary work for this study showed that higher cadmium levels would be expected from these states. The sites, number of samples, mean and maximum values for all grower load samples in this study are listed in Appendix 1.

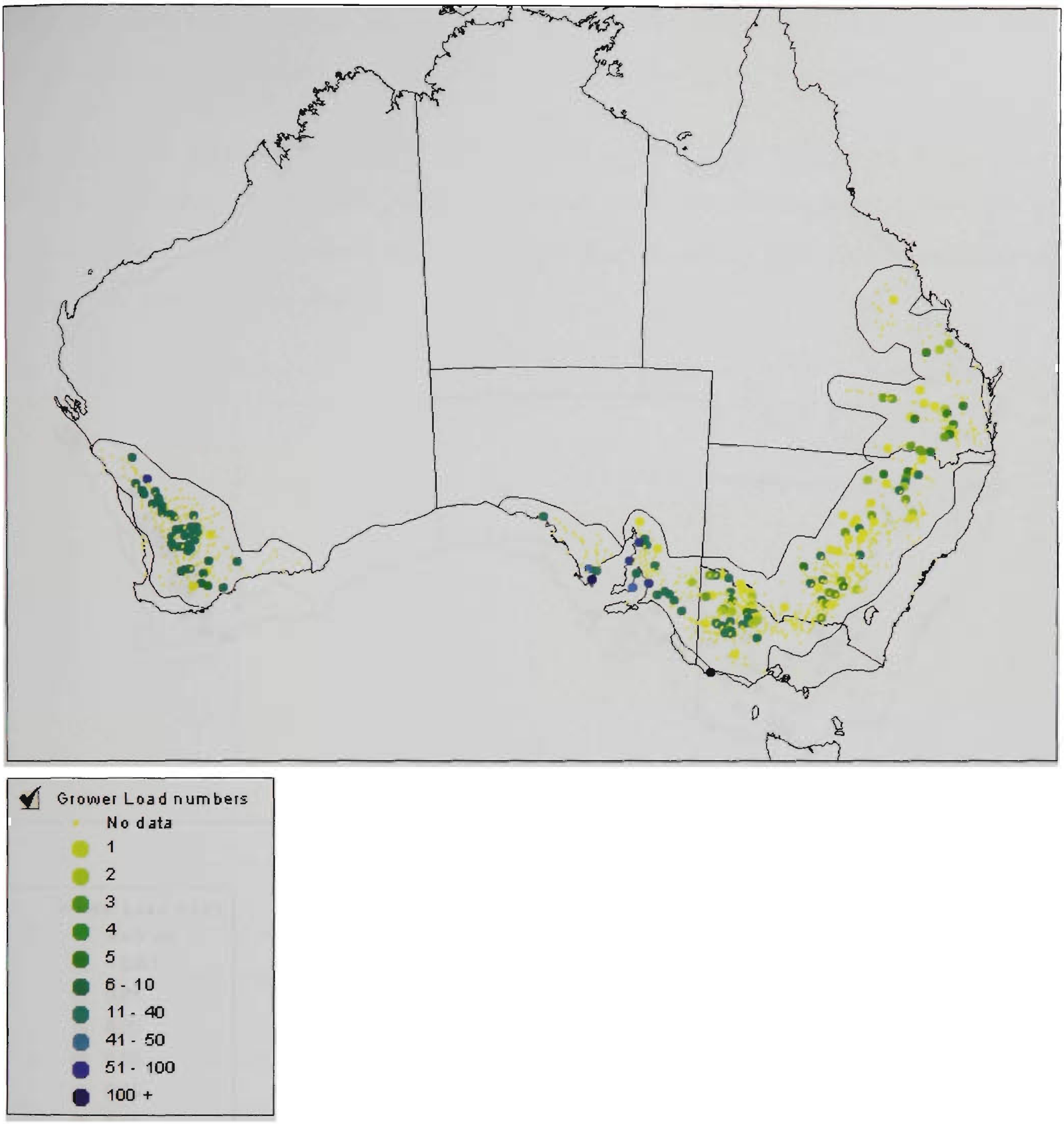


Figure 5.1.1.1 Map of the Australian grain belt, sites and related number of samples for each site for grower load wheat samples

Figure 5.1.1.1 shows a map of the Australian grain belt together with the number of grower load wheat samples taken at each site. A list of these sites is given in Appendix 1. The map shows a lesser number of sites and samples taken in Queensland with increasing numbers through New South Wales, Victoria, South Australia and highest number of sites and samples in Western Australia concentrating on an approximately North-South line through the middle of the grain belt. The high numbers in Western Australia and around Port Pirie in South Australia reflect cadmium levels found in these areas during preliminary work on this project.

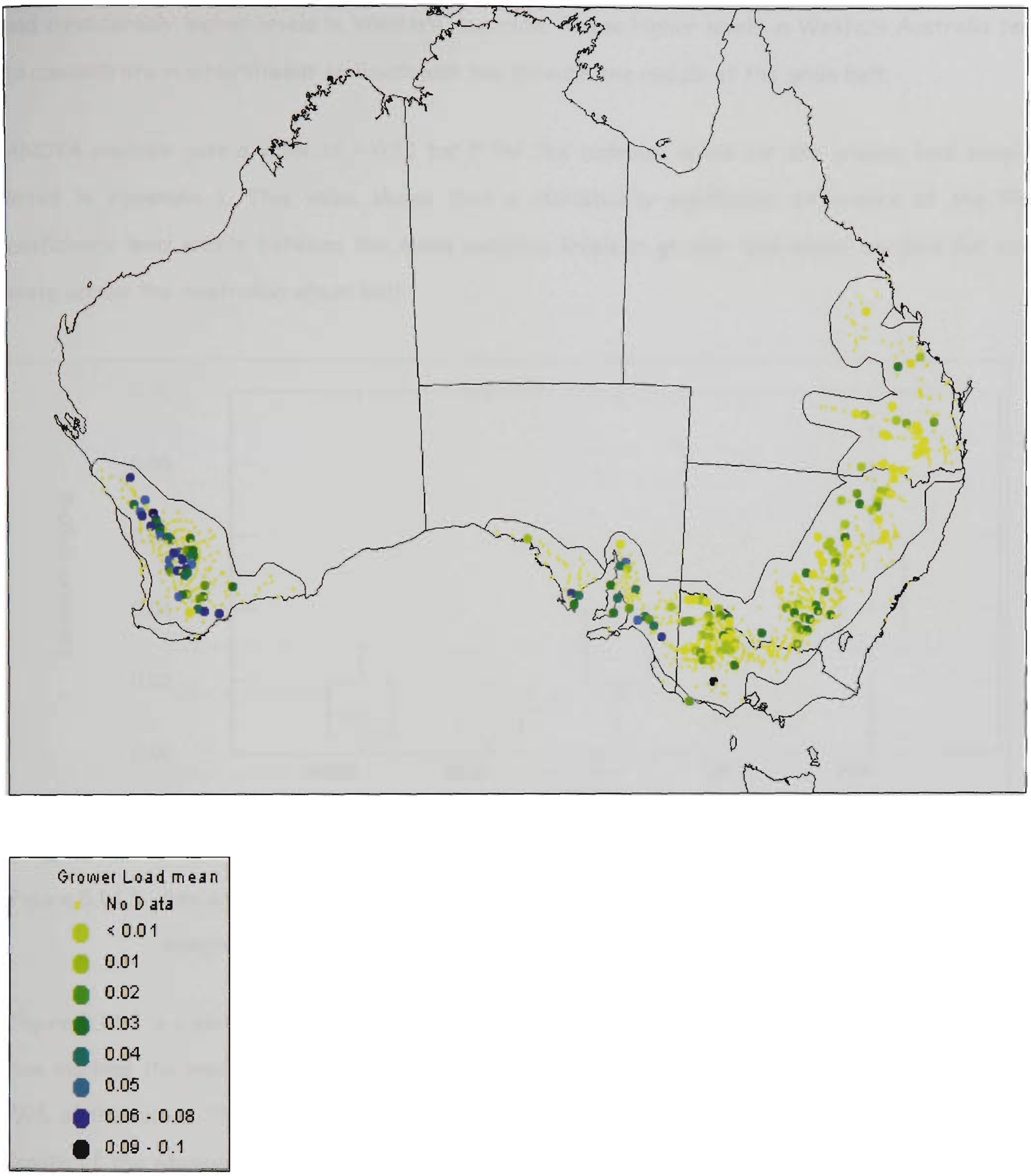


Figure 5.1.1.2 Map of the Australian grain belt, sites and related mean cadmium result for each site for grower load wheat samples

Figure 5.1.1.2 shows a map of the Australian grain belt with the mean cadmium levels found in grower load wheat samples taken at each site. The mean level at each site is represented by a coloured spot ranging from yellow for the lowest to dark blue for highest. Sites that were not sampled are shown by a small yellow asterisk. A reasonably smooth gradation of cadmium levels can be seen from low levels in Queensland, through New South Wales and Victoria, with higher levels in South Australia and considerably higher levels in Western Australia. These higher levels in Western Australia tend to concentrate in a Northwest to Southeast line through the middle of the grain belt.

ANOVA analysis gave a value of < 0.01 for P for the cadmium levels for the grower load samples listed in Appendix 1. This value shows that a statistically significant difference at the 99% confidence level exists between the mean cadmium levels in grower load wheat samples for each state across the Australian wheat belt.

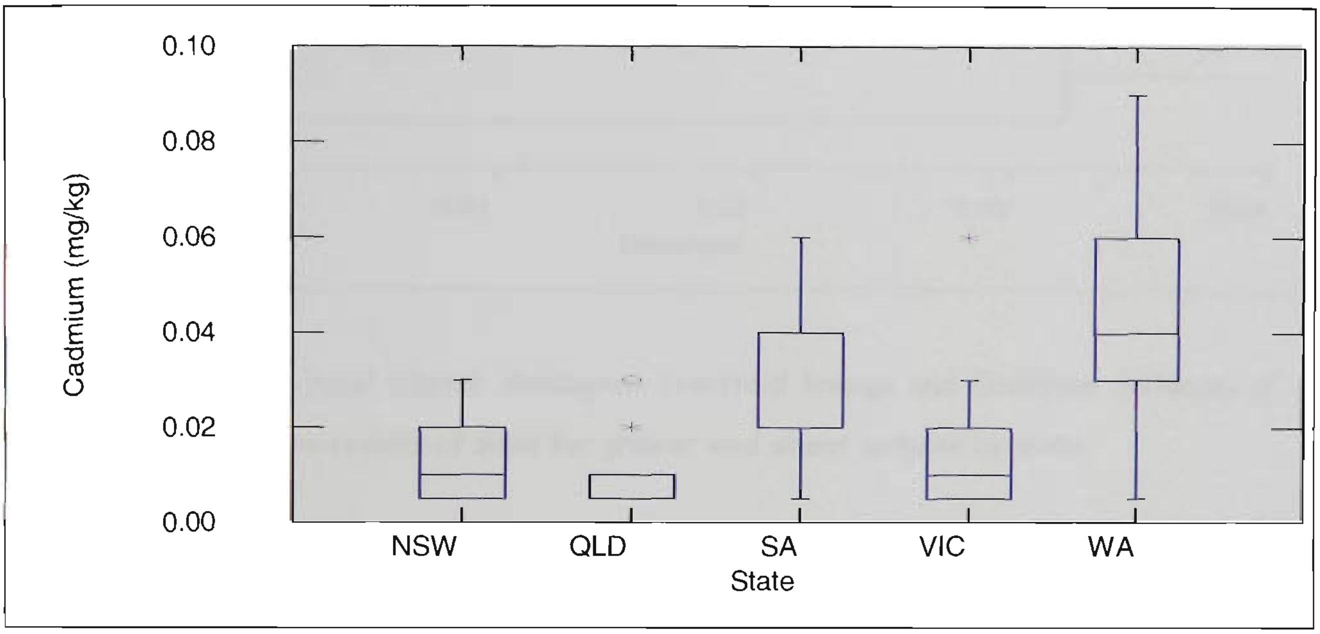


Figure 5.1.1.3 Box and whisker plot of mean cadmium results of sites for grower load wheat samples by state

Figure 5.1.1.3 is a plot of the range of cadmium levels for each state with the horizontal line in each box marking the median of the values and the length of the box showing the range of the central 50% of the values. The box edges or hinges show the first and third quartile of the results with the length of the whiskers or fences show the range of values that lie between 1.5 times the absolute value of the difference between the two hinges (Wilkinson, 1999). Values greater than the fence but less than twice the fence are plotted with asterisks. Values greater than twice the value of the fence are plotted with circles

It can be seen that bottom hinge of Western Australia and South Australia overlap with each other but not with the top hinge of New South Wales, Queensland and Victoria. This suggests that Western Australia and South Australia form one group and the other three states form another.

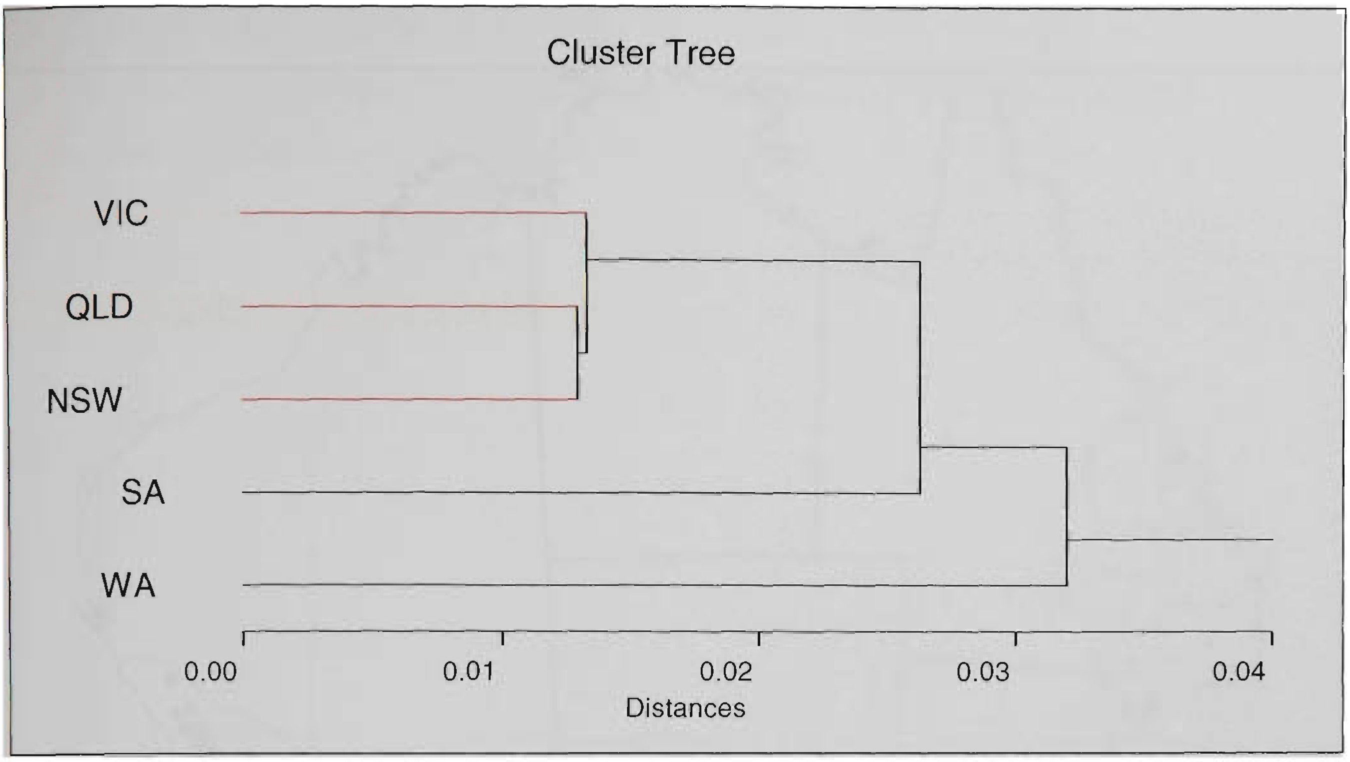


Figure 5.1.1.4 Hierarchical cluster dendogram (centroid linkage and Euclidean distance) of mean cadmium results of sites for grower load wheat samples by state

Figure 5.1.1.4 shows a hierarchical cluster tree produced by treating each state as a separate cluster and then joining the two closest states as the next cluster, continuing in a stepwise manner to join the next state until all states are combined into one cluster. Centroid linkage uses the mean of the cadmium levels for each state as the reference point for distances to other states or clusters. Clustering is computed using the normalised Euclidean distance (a root mean square distance). These parameters are often used to produce a dendogram of quantitative data (Wilkinson *et al.*, 1999).

It can be seen that the dendogram falls into two groups, with Victoria, Queensland and New South Wales in one group with South Australia and Western Australia in the other. This grouping is similar to that produced in Figure 5.1.1.2 and is expected from the mean cadmium levels shown for the grower load samples listed in Appendix 1.

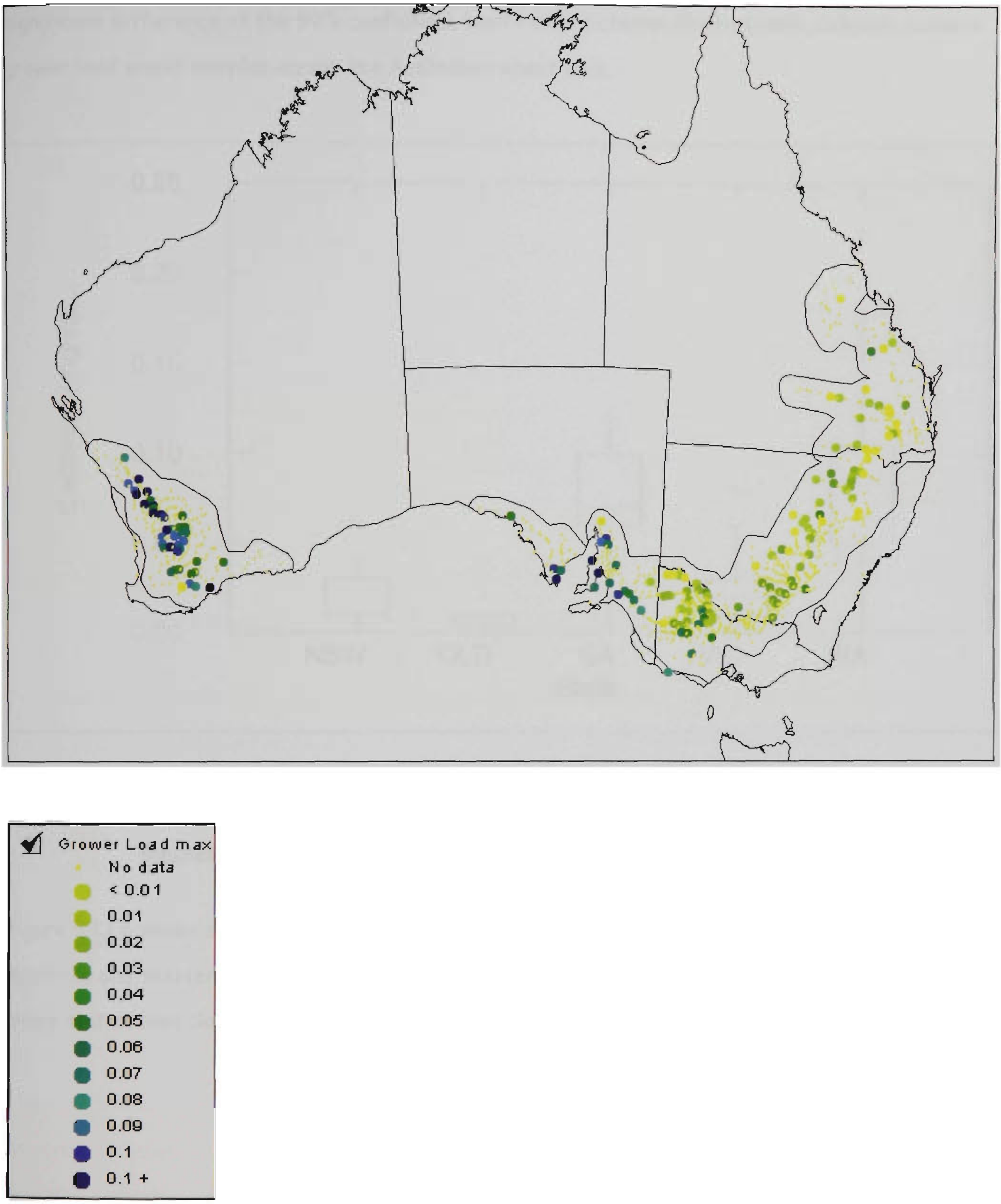


Figure 5.1.1.5 Map of the Australian grain belt, sites and related maximum cadmium result for each site for grower load wheat samples

Figure 5.1.1.5 shows a reasonably smooth gradation of cadmium levels with low levels in Queensland, through New South Wales and Victoria, with considerably higher levels in South Australia and much higher maximum levels in Western Australia. These higher levels in Western Australia concentrate in a Northwest to Southeast band through the middle of the grain belt. The value of < 0.001 for P for the maximum cadmium levels of the grower load samples listed in Appendix 1 shows that a highly significant difference at the 99% confidence level exists between the maximum cadmium levels in grower load wheat samples across the Australian wheat belt.

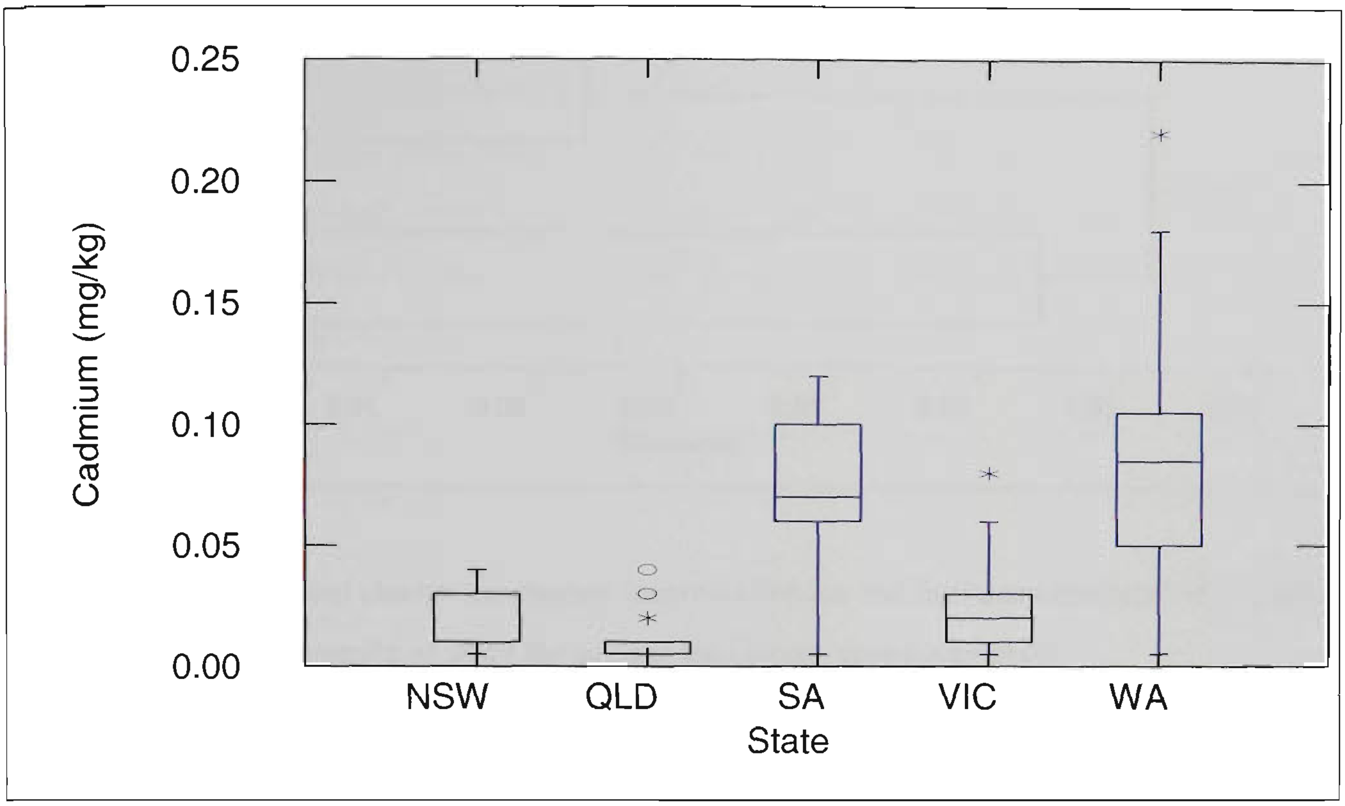


Figure 5.1.1.6 Box and whisker plot of maximum cadmium results of sites for grower load wheat samples by state

Figure 5.1.1.6 shows similar results to Figure 5.1.1.2 with two groups being seen, the boxes for South Australia and Western Australia overlap each other but show a greater separation from the other three states. New South Wales, Queensland and Victoria form the other group.

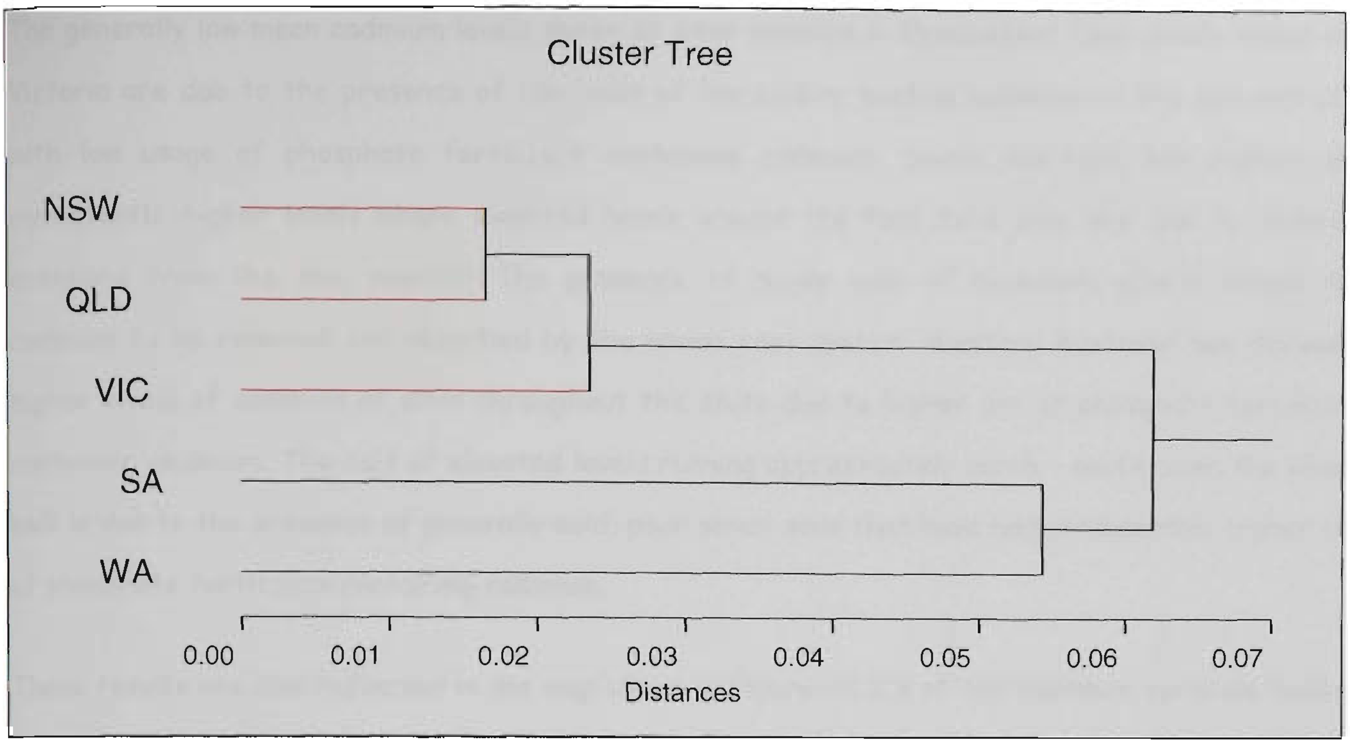


Figure 5.1.1.7 Hierarchical cluster dendogram (centroid linkage and Euclidean distance) of maximum cadmium results of sites for grower load wheat samples by state

The dendogram also shows a similar pattern to Figure 5.1.1.4 with two groups being seen. With New South Wales, Queensland and Victoria in one group while South Australia and Western Australia are in the other. A greater separation between the groups can be seen on the distance scale.

5.1.1.1 Discussion

The P value of < 0.05 in the ANOVA analysis of the results given for grower load samples in Appendix 1 shows that the mean levels of cadmium for grower load wheat samples are significantly different for each state at the 95% confidence level.

The map in Figure 5.1.2.1 displays the sites and number of samples taken at each site showing a reasonably smooth gradient from Queensland through New South Wales, Victoria, South Australia with the highest numbers in Western Australia. These numbers were developed from cadmium levels found during preliminary work in this project. The map in Figure 5.1.2.2 displays the mean cadmium level at each site, it shows a gradient with generally low levels in Queensland through New South Wales and Victoria. South Australia, however, has higher levels around the zinc smelter at Port Pirie. Western Australia is generally higher with a marked band of much higher values down the middle of the grain belt.

The generally low mean cadmium levels shown at sites sampled in Queensland, New South Wales and Victoria are due to the presence of clay soils of low acidity binding cadmium to the soil particles with low usage of phosphate fertilizers containing cadmium. South Australia has slightly but consistently higher levels where elevated levels around the Port Pirie area are due to cadmium emissions from the zinc smelter. The presence of sandy soils of moderate acidity allows the cadmium to be released and absorbed by the wheat root system. Western Australia has markedly higher levels of cadmium at sites throughout the state due to higher use of phosphate fertilizers containing cadmium. The belt of elevated levels running approximately north - south down the wheat belt is due to the presence of generally acid, poor sandy soils that have had considerably higher use of phosphate fertilizers containing cadmium.

These results are also reflected in the map shown in Figure 5.1.2.3 of the maximum cadmium level at each site. The map shows an area of higher maximum levels in SA centred on Port Pirie and much higher maximum levels generally over the WA grain belt with a central band of elevated maximum levels again running approximately in a North-South direction.

The results given for grower load samples in Appendix 1 are consistent with the limited amount of cadmium levels in wheat reported by other workers in Australia. Tiller (1975), Tiller *et al.* (1975), Merry and Tiller (1977), and McEwen (2004) have produced comparable results for South Australia from wheat grown around the zinc smelter at Port Pirie and on the acid soils of the York Peninsula. A considerable amount of unpublished data has been produced by the AWB from Australia wide surveys over the years 1982 to 1992. The Department of Agriculture in Western Australia commissioned an unpublished survey by Dr K Tiller in 1994 in response to concerns about cadmium levels in West Australian wheat. No other published data could be found linking cadmium levels in wheat with sites in Australia.

5.1.2 Site Composite samples

Composite wheat samples were taken from the AWB silo monitoring program as a composite sample from each 500 tonnes of wheat received at the silo over the 92/93 season. A 400 g sub-sample was transferred to a 500 mL plastic jar and labelled with the monitoring program, state, site and date. The grade and genotype of the wheat was also recorded if this information was available.

Table 5.1.2.1 Summary of sites, sample numbers and cadmium results for composite site samples by state.

	Number		**Cadmium (mg/kg)**	
State	Sites	Samples	Mean	Maximum
NSW	114	171	0.01	0.04
Qld	17	21	< 0.01	0.02
SA	**124**	**658**	**0.02**	**0.20**
Vic	118	184	0.01	0.06
WA	**193**	**3,767**	**0.04**	**0.13**
Total	566	4,801	0.03	0.20

The results in Table 5.1.2.1 highlight the much larger number of samples taken in South Australia and Western Australia. The higher mean and maximum cadmium levels found in wheat for these states are highlighted (all in blue). Again, the numbers of samples were chosen because preliminary work in this project showed that higher cadmium levels would be expected in these states. Considerably more sites were sampled and samples taken at each site than in the grower load survey in Section 5.1.1, except Queensland, with a far greater emphasis on South Australia and Western Australia. The sites, number of samples, mean and maximum values for all composite site samples in this study are listed in Appendix 1.

ANOVA analysis gave a value of < 0.05 for P for the cadmium levels for the site composite samples listed in Appendix 1. This value shows that a significant difference at the 95% confidence level exists between the mean cadmium levels in site composite wheat samples for each state across the Australian wheat belt.

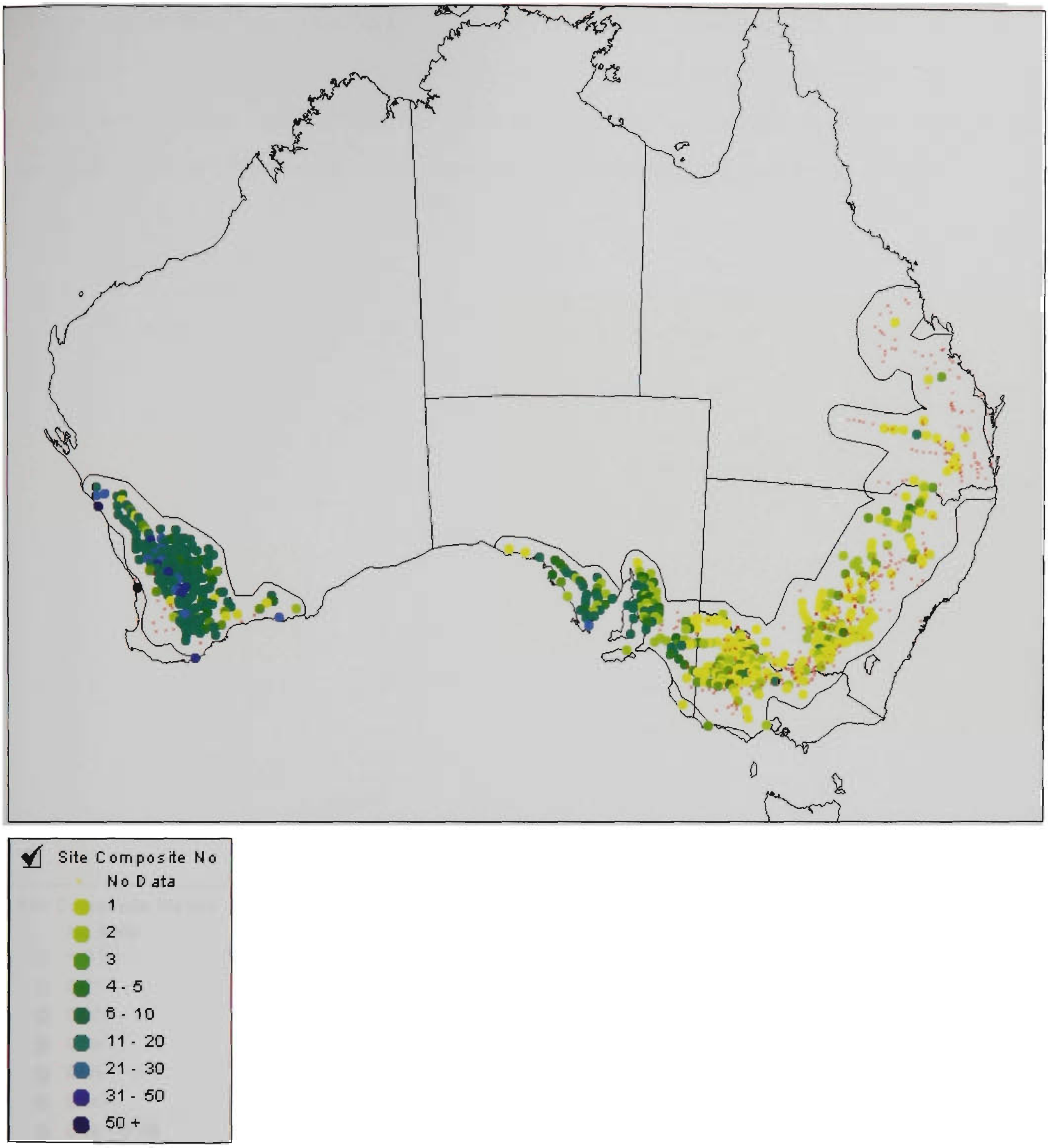


Figure 5.1.2.1 Map of sites and related number of samples for each site for site composite wheat samples in Australia

Figure 5.1.2.1 shows a map of the Australian grain belt with the number of composite site wheat samples taken at each site, a list of these sites is given in Appendix 1. The map shows that few sites and number of samples were taken in Queensland with increasing numbers of sites and samples through New South Wales, Victoria, South Australia with the highest in Western Australia. The high numbers in Western Australia and to a lessor extent in South Australia, reflect cadmium levels found in these areas during preliminary work on this project.

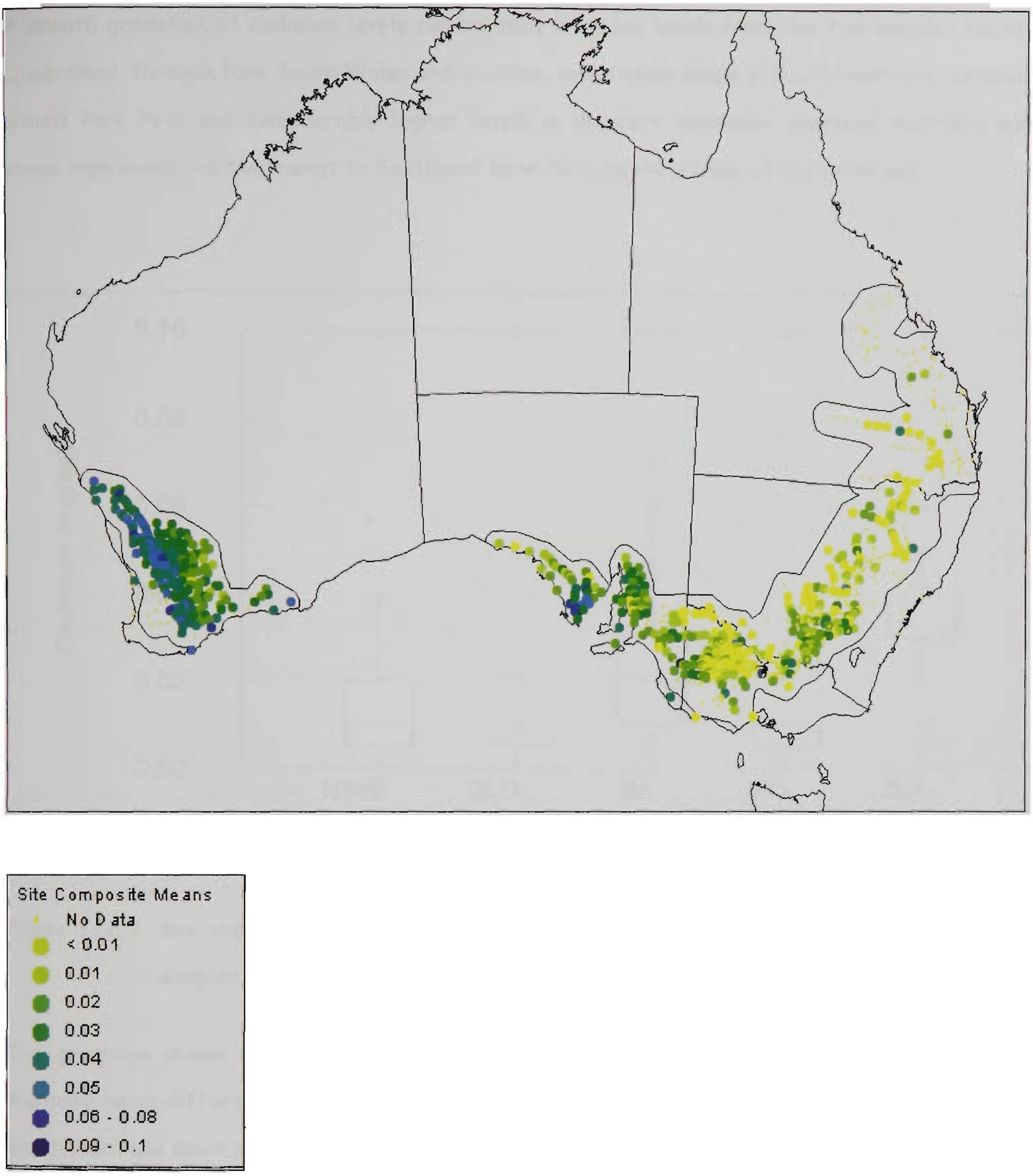


Figure 5.1.2.2 Map of sites and related mean cadmium levels for site composite wheat samples in Australia

Figure 5.1.2.2 shows a map of the Australian grain belt with the mean cadmium levels of composite site wheat samples taken at each site. The mean level at each site is represented by a coloured spot ranging from yellow for the lowest to dark blue for highest, sites that were not sampled are shown by a small yellow asterisk.

A smooth gradation of cadmium levels can be seen from low levels from the few samples taken in Queensland, through New South Wales and Victoria, with higher levels in South Australia, especially around Port Pirie and considerably higher levels in Western Australia. Western Australia again shows high levels in a Northwest to Southeast band through the middle of the grain belt.

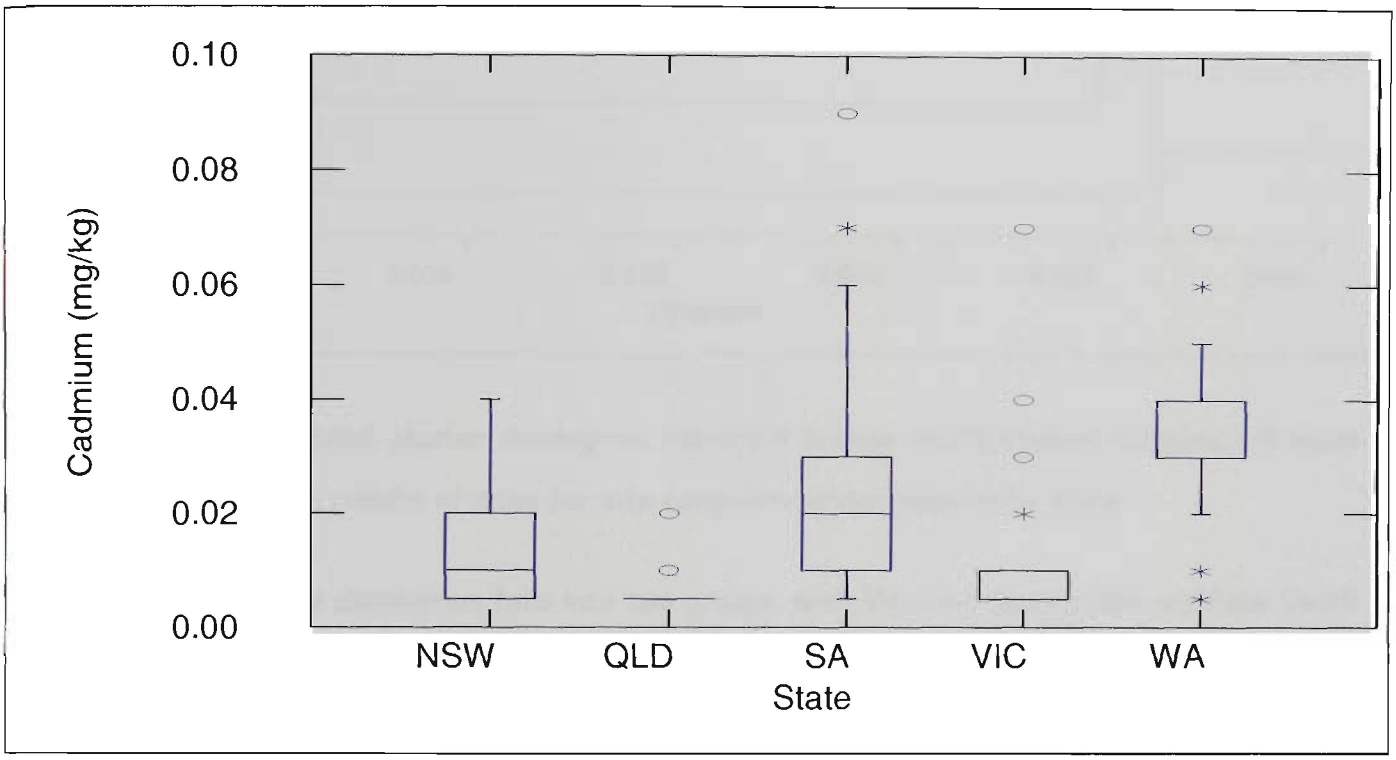


Figure 5.1.2.3 Box and whisker plot of mean cadmium results of sites for site composite wheat samples by state

The groupings shown in Figure 5.1.2.3 are not as clear as in Figure 5.1.1.2 with only Western Australia being different and the boxes of the other four states overlapping each other, although South Australia again shows a higher mean than the other three states. This is consistent with the mixing of wheat with a high cadmium level with wheat of lower cadmium levels during silo filling and the production of site composite samples.

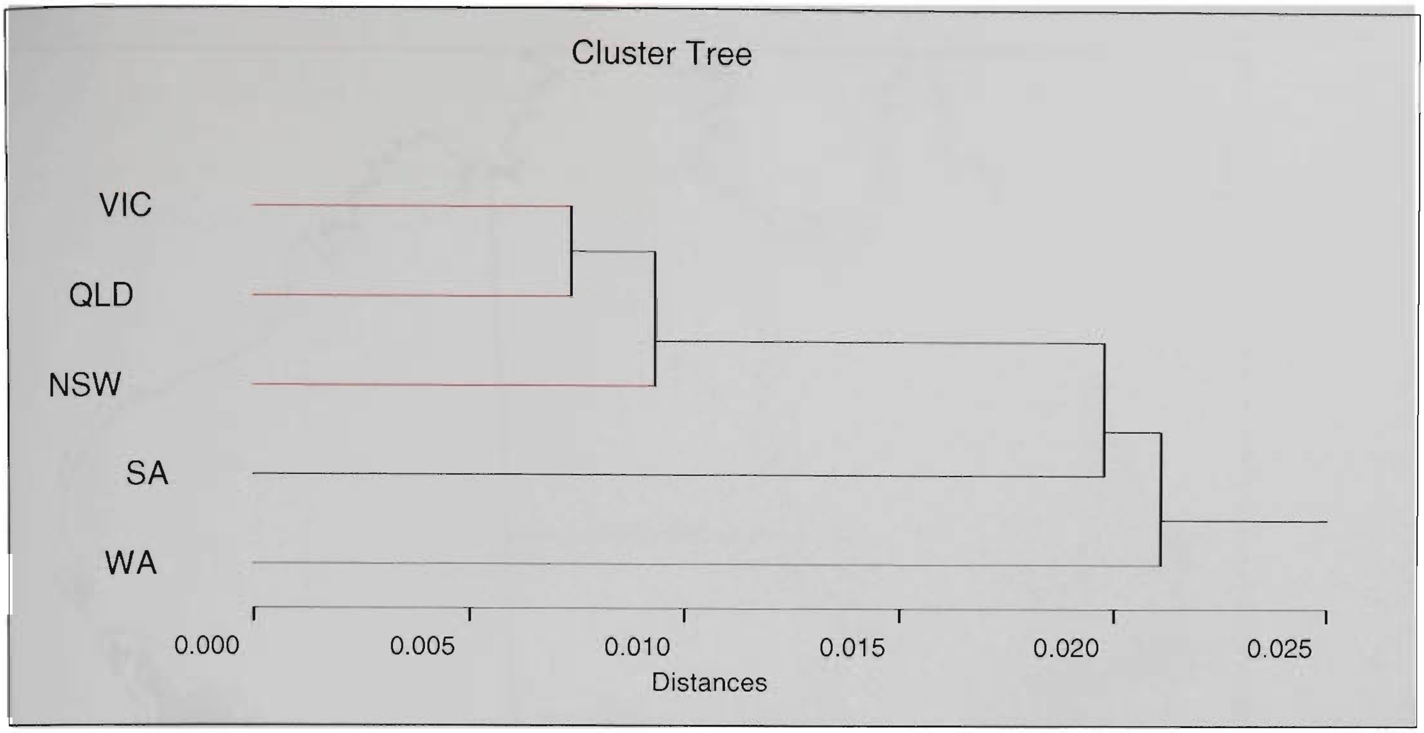


Figure 5.1.2.4 Hierarchical cluster dendogram (centroid linkage and Euclidean distance) of mean cadmium results of sites for site composite wheat samples by state

It can be seen that the dendogram falls into two groups, with Victoria, Queensland and New South Wales in one group and South Australia and Western Australia in the other. This grouping is slightly different to that produced in Figure 5.1.2.3 in that South Australia is grouped with Western Australia, not the other three states.

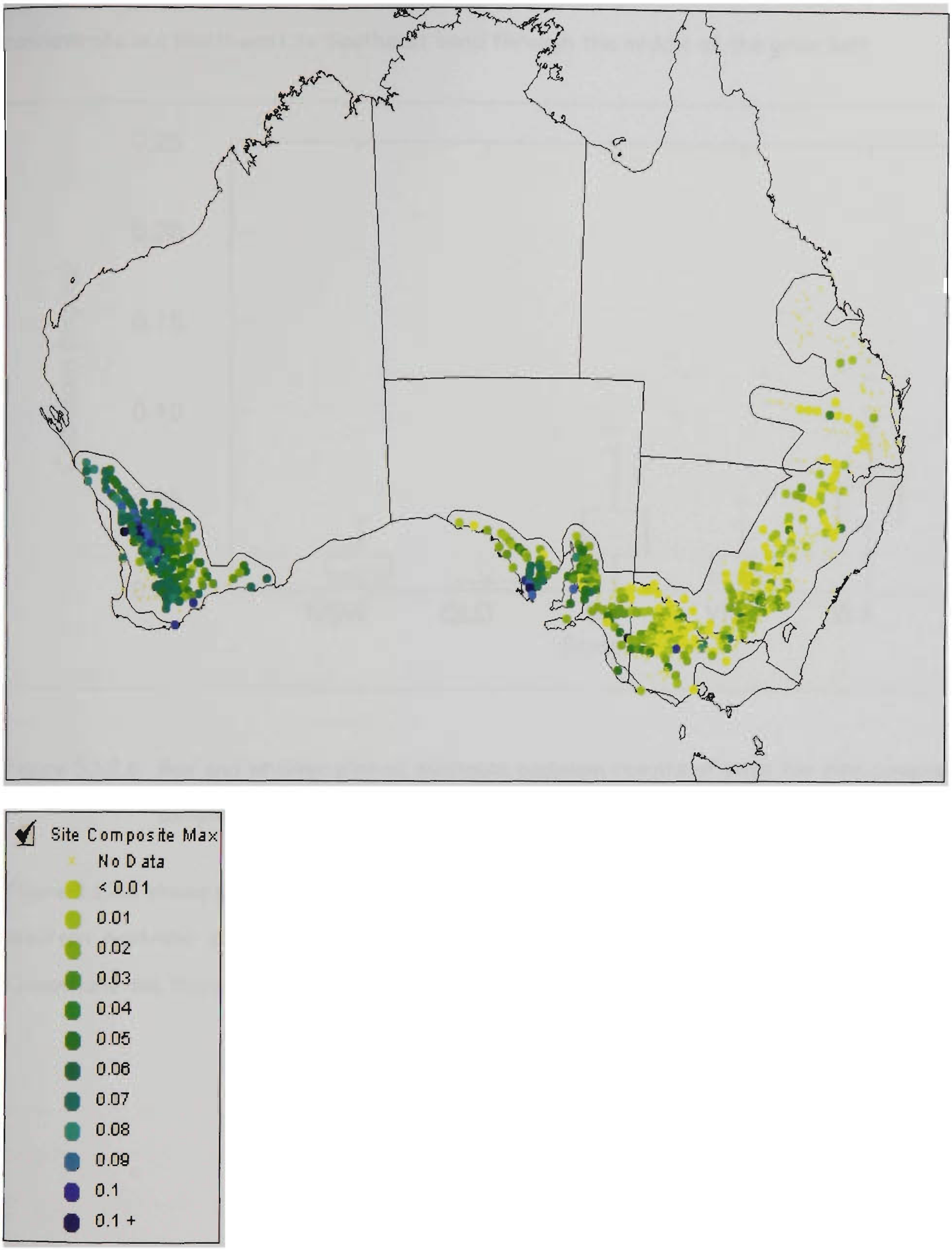


Figure 5.1.2.5 Map of sites and related maximum cadmium levels for site composite wheat samples in Australia

Figure 5.1.2.5 is similar to Figure 5.1.1.7 but with generally lower levels overall. It also shows a reasonably smooth gradation of cadmium levels that can be seen from low levels in Queensland, through New South Wales and Victoria, with considerably higher levels in South Australia and much higher maximum levels in Western Australia. These higher levels in Western Australia again concentrate in a Northwest to Southeast band through the middle of the grain belt.

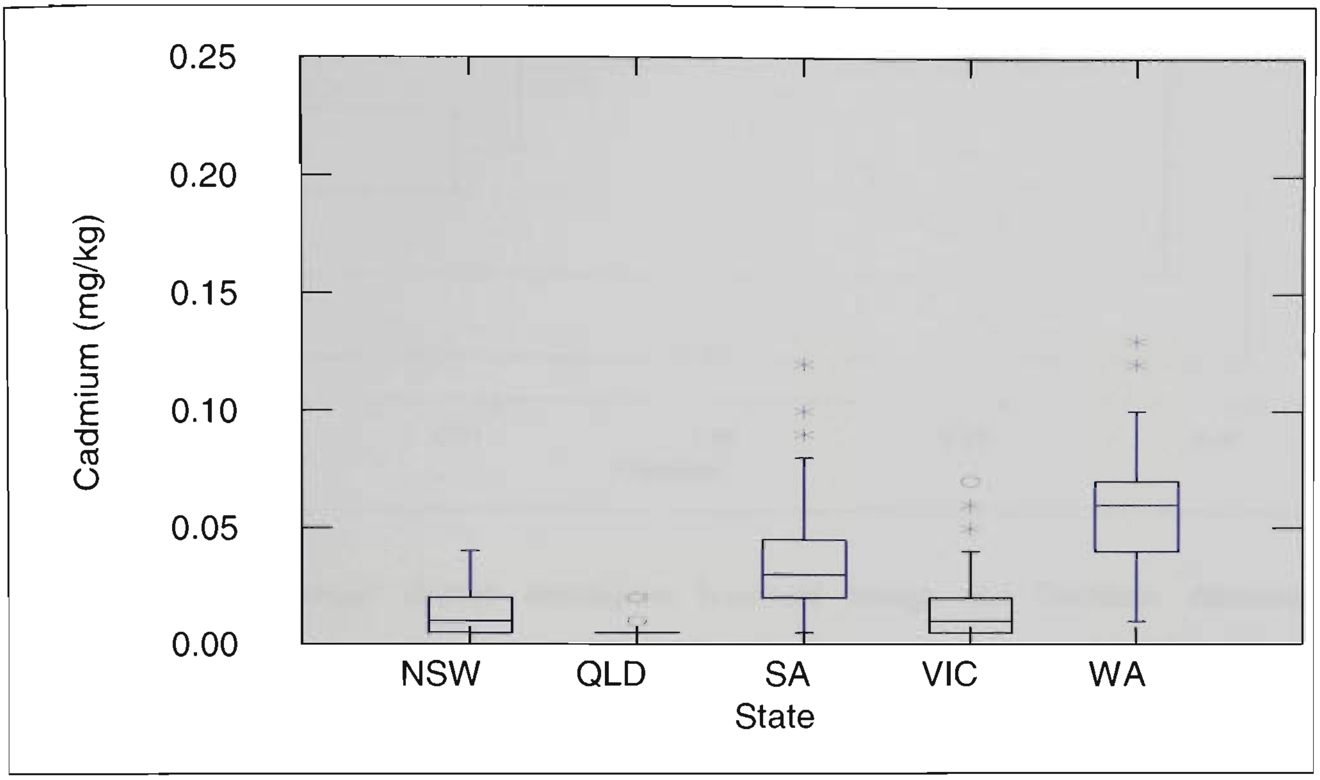


Figure 5.1.2.6 Box and whisker plot of maximum cadmium results of sites for site composite wheat samples by state

Figure 5.1.2.6 shows more structured groupings than Figure 5.1.2.3, the boxes for South Australia and Western Australia slightly overlap each other showing further separation from New South Wales, Queensland and Victoria.

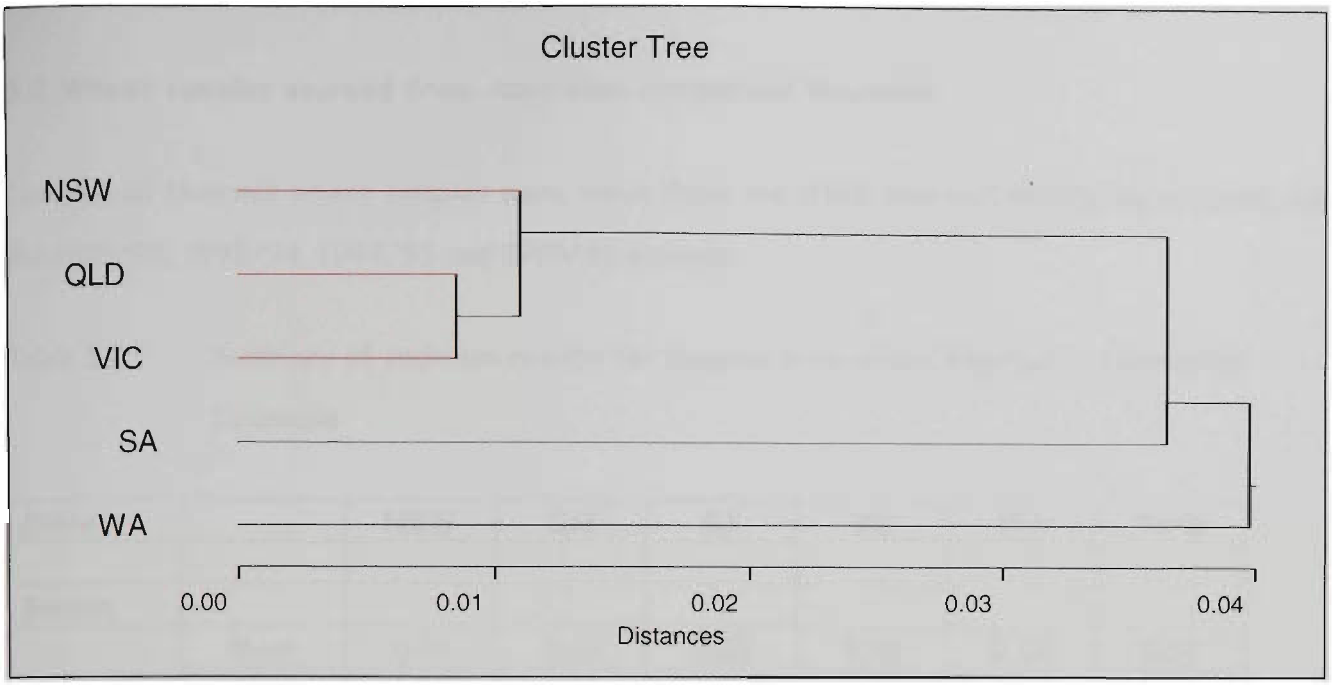


Figure 5.1.2.7 Hierarchical cluster dendogram (centroid linkage and Euclidean distance) of maximum cadmium results of sites for site composite wheat samples by state

It can be seen that the dendogram in Figure 5.1.2.7 falls into two groups, with Victoria, Queensland and New South Wales in one group and South Australia and Western Australia in the other. This grouping is similar to that shown in Figure 5.1.2.6 with South Australia again being grouped with Western Australia.

5.1.3 Discussion

The results given in Section 5.1.2 for site composite samples show a similar pattern to the results given for grower load samples in Section 5.1.1 with reduced differences between the states. This reduced difference is expected due to the dilution of the high cadmium wheats from blending at the silos. The P values of < 0.05 in the ANOVA analysis for both the grower load and site composite samples listed in Appendix 1 is consistent with the means levels of cadmium for both grower load and site composite samples being statistically different for each state at the 95% confidence level.

5.2 Wheat samples sourced from Australian commercial flourmills

Commercial flourmill wheat samples were taken from the AWB flourmill monitoring program during the 1991/92, 1993/94, 1994/95 and 1995/96 seasons.

Table 5.2.1 Summary of cadmium results for samples from wheat supplied to commercial flourmills

State		NSW	Qld	SA	Vic	WA	Total
Season							
1991/92	Mean	0.01	0.01	**0.02**	0.01	**0.03**	0.02
	Max	0.02	0.03	**0.09**	0.01	**0.08**	0.09
	Number	221	110	**226**	84	**358**	999
1993/94	Mean	0.02	0.01	**0.02**	< 0.01	**0.03**	0.02
	Max	0.03	0.03	**0.10**	0.02	**0.06**	0.10
	Number	179	163	**223**	64	**293**	897
1994/95	Mean	0.01	0.01	**0.02**	0.01	**0.03**	0.02
	Max	0.03	0.03	**0.07**	0.04	**0.06**	0.07
	Number	41	33	**77**	57	**218**	414
1995/96	Mean	0.01	0.01	**0.02**	0.01	**0.03**	0.02
	Max	0.02	0.02	**0.05**	0.03	**0.08**	0.08
	Number	107	103	**134**	81	**288**	713
Total	Mean	0.01	0.01	**0.02**	0.01	**0.03**	0.02
	Max	0.03	0.03	**0.10**	0.04	**0.08**	0.10
	Number	548	409	**660**	286	**1,157**	3,060

The data in Table 5.2.1 highlight the much larger number of samples taken in Western Australia with the higher mean and maximum cadmium levels found in wheat supplied to flourmills in this state and South Australia (all in blue). Again, the numbers of samples were chosen because preliminary work in this study showed that higher cadmium levels would be expected in these states. The mean and maximum levels do not seem to be correlated with season. The value of < 0.05 for P for the ANOVA analysis of the cadmium levels in Appendix 2, by state, shows that a statistically significant difference at the 95% confidence level exists between the cadmium levels in wheat samples supplied to commercial flourmills across Australia analysed by state.

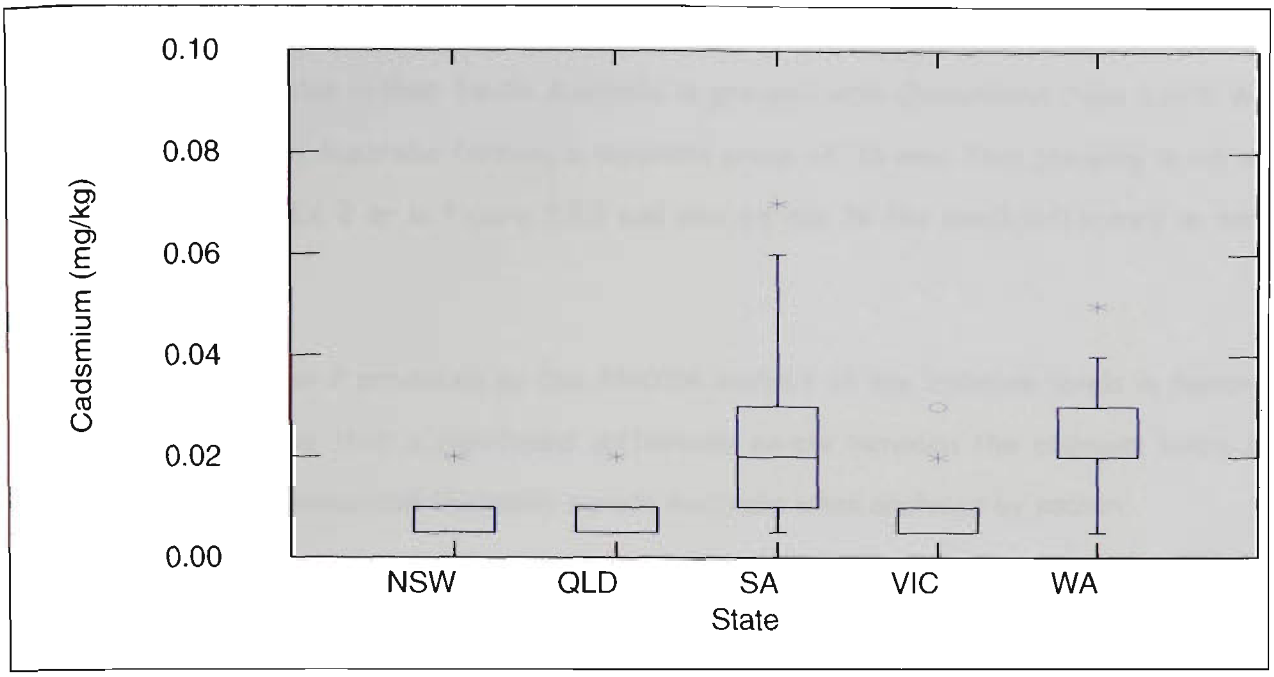


Figure 5.2.1 Box and whisker plot of cadmium results of sites for wheat samples supplied to commercial flourmills by state

Figure 5.2.1 shows similar groupings to those for grower load and site composite wheat samples with Western Australia and South Australia forming one group and New South Wales, Queensland and Victoria forming the other. The figure shows reduced differences between the means than in the box and whisker plots for grower loads and site composite wheat samples. .

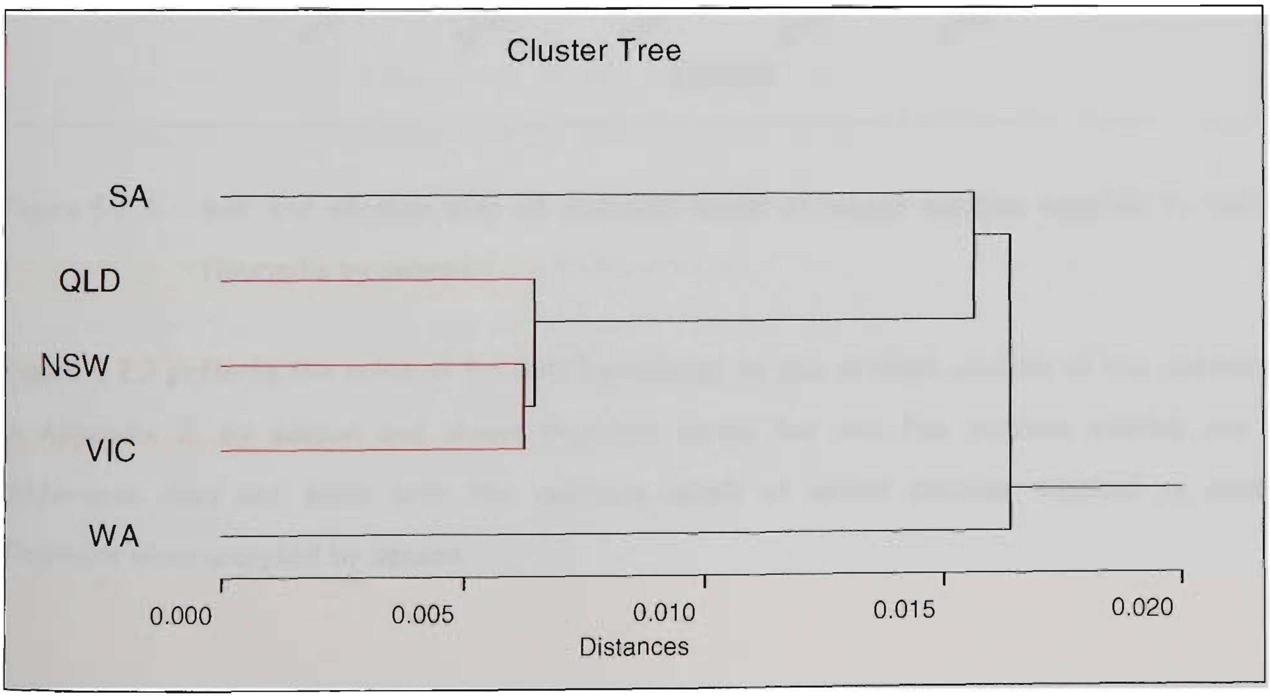


Figure 5.2.2 Hierarchical cluster dendogram (centroid linkage and Euclidean distance) of cadmium results for wheat samples supplied to commercial flourmills by state

Figure 5.2.2 indicates a slightly different dendogram to those shown for grower load and site composite wheat samples in that South Australia is grouped with Queensland, New South Wales and Victoria with Western Australia forming a separate group of its own. This grouping is not shown by the results in Appendix 2 or in Figure 5.2.1 and may be due to the small difference in means with these results.

The value of > 0.05 for P produced by the ANOVA analysis of the cadmium levels in Appendix 2 by season, does not prove that a significant difference exists between the cadmium levels in wheat samples supplied to commercial flourmills across Australia when analysed by season.

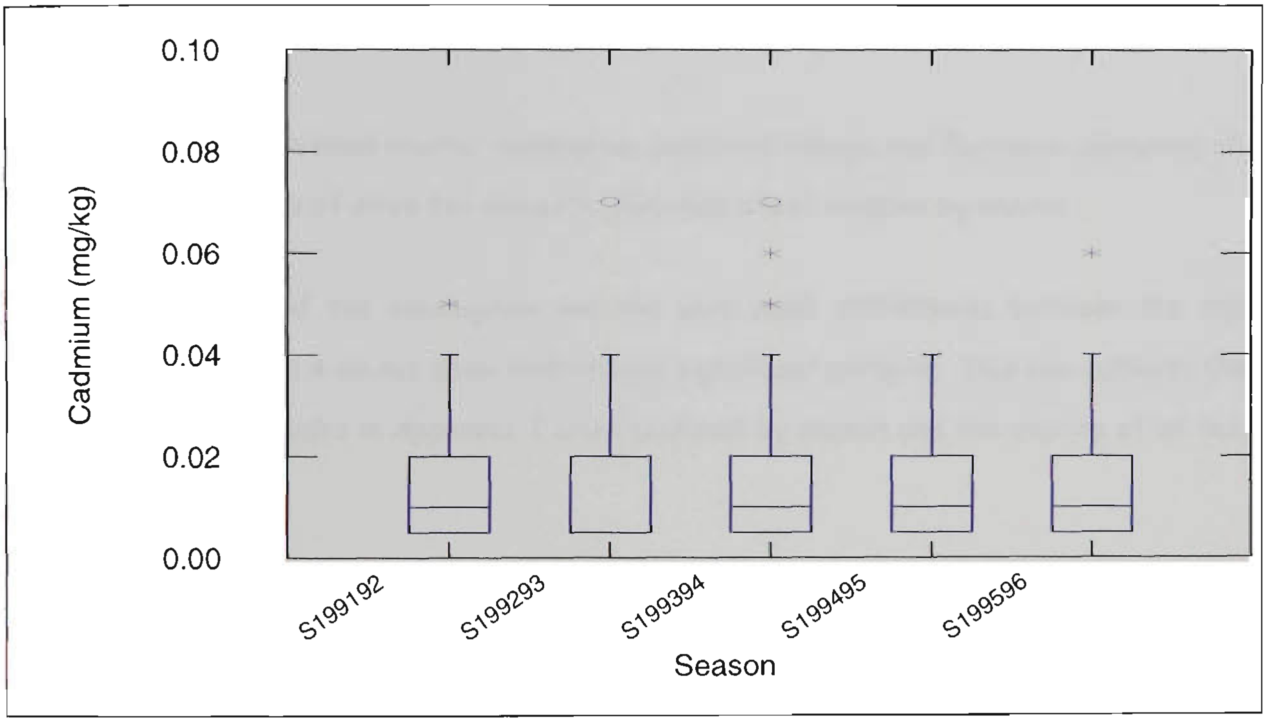


Figure 5.2.3 Box and whisker plot of cadmium levels of wheat samples supplied to commercial flourmills by season

Figure 5.2.3 reflects the value of P = 0.883 produced by the ANOVA analysis of the cadmium levels in Appendix 2, by season and shows that the boxes for the five seasons overlap and that a difference does not exist with the cadmium levels of wheat samples supplied to commercial flourmills when analysed by season.

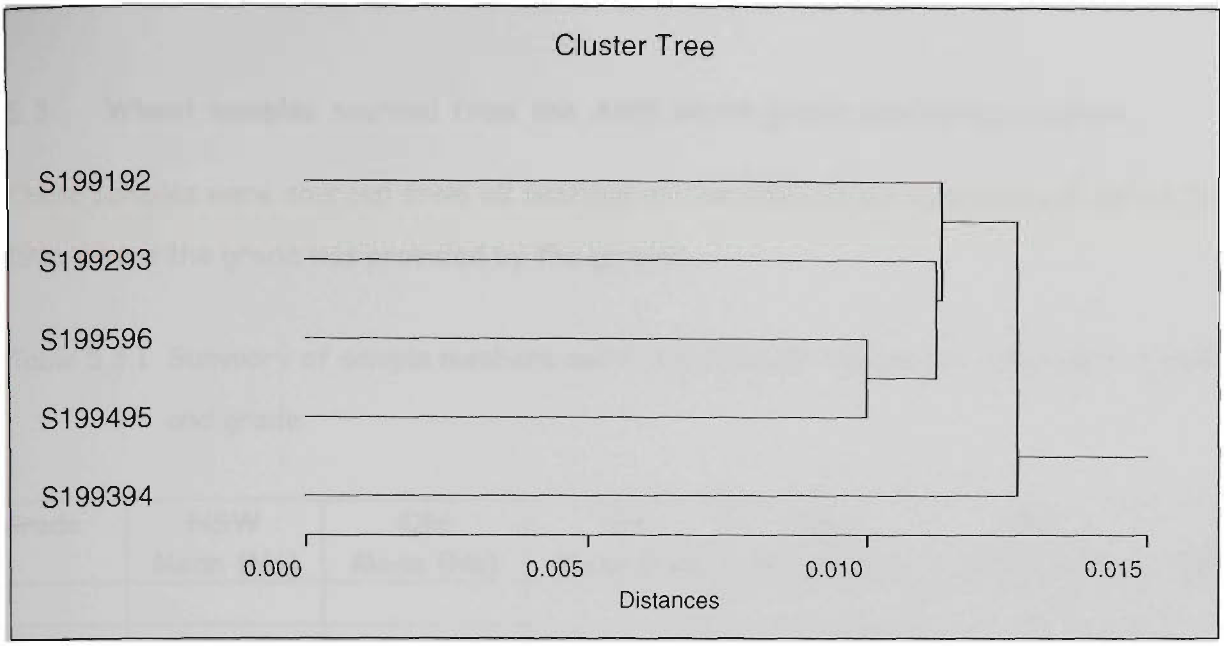


Figure 5.2.4 Hierarchical cluster dendogram (centroid linkage and Euclidean distance) of cadmium results of sites for domestic flourmill wheat samples by season

The flat structure of the dendogram and the very small differences between the tops of the branches in Figure 5.2.4 do not show statistically significant grouping. This also reflects the value of P = > 0.05 for the results in Appendix 2 when analysed by season and the overlap of all the boxes in Figure 5.2.3.

5.2.5 Discussion

The value of < 0.05 for P given by the ANOVA analysis of the cadmium levels of the wheat samples from commercial flourmills in Table 5.2.1 indicates that the levels are statistically different at the 95% confidence level when viewed by state. This is also shown in Figure 5.2.1, revealing statistical differences between the clusters of cadmium levels for New South Wales, Queensland and Victoria compared with South Australia and Western Australia. A P value of > 0.05 and overlapping box plots in Figure 5.3.3 reveal a lack of significant difference between the cadmium levels of the wheat samples from commercial flourmills in Table 5.2.1 when viewed by season.

5.3 Wheat samples sourced from the AWB wheat grade monitoring program

These samples were sourced from all sections of the AWB wheat monitoring program from 1992 to 1996 where the grade was provided by the grower.

Table 5.3.1 Summary of sample numbers and mean cadmium results for AWB wheat samples by state and grade

Grade	NSW Mean (No)	Qld Mean (No)	SA Mean (No)	Vic Mean (No)	WA Mean (No)	Total Mean (No)
ASW	0.01 (273)	0.01 (116)	**0.02 (385)**	0.01 (408)	**0.03 (4,067)**	0.03 (5,249)
Feed	0.01 (23)	-	**0.04 (129)**	0.01 (136)	**0.04 (45)**	0.03 (333)
General Purpose	0.01 (69)	0.01 (1)	**0.03 (94)**	0.02 (214)	**0.04 (1,271)**	0.04 (1,649)
Hard	0.01 (211)	0.01 (150)	**0.02 (318)**	0.01 (210)	**0.02 (736)**	0.02 (1,625)
Prime Hard	0.01 (171)	0.01 (292)	**0.01 (5)**	-	-	0.01 (468)
Soft	0.01 (16)	-	**0.03 (28)**	0.01 (7)	**0.04 (811)**	0.04 (862)
Total	0.01 (763)	0.01 (559)	**0.02 (959)**	0.01 (975)	**0.03 (6,930)**	0.03 (10,186)

The value of < 0.01 for P for the ANOVA analysis of cadmium levels in Table 5.3.1 by state shows that a statistically significant difference at the 99% confidence level exists between the mean cadmium results for AWB grade monitoring wheat samples when analysed by state. It can also be seen that Western Australia (in blue) has the largest number of samples and highest cadmium levels with South Australia showing the next highest values for these parameters (also in blue).

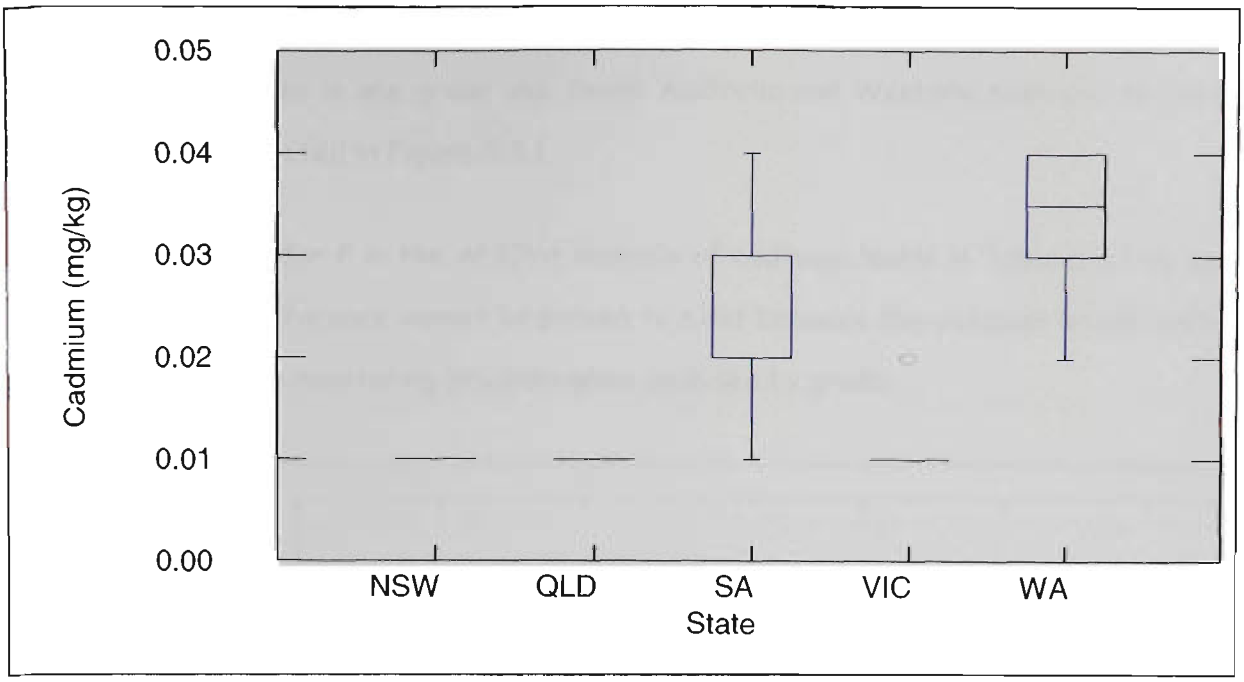


Figure 5.3.1 Box and whisker plot of mean cadmium results of wheat samples from the AWB grade monitoring program by state

Figure 5.3.1 shows three groups, the boxes for South Australia and Western Australia do not overlap each other but both show a separation from the other three states. New South Wales, Queensland and Victoria form the other group.

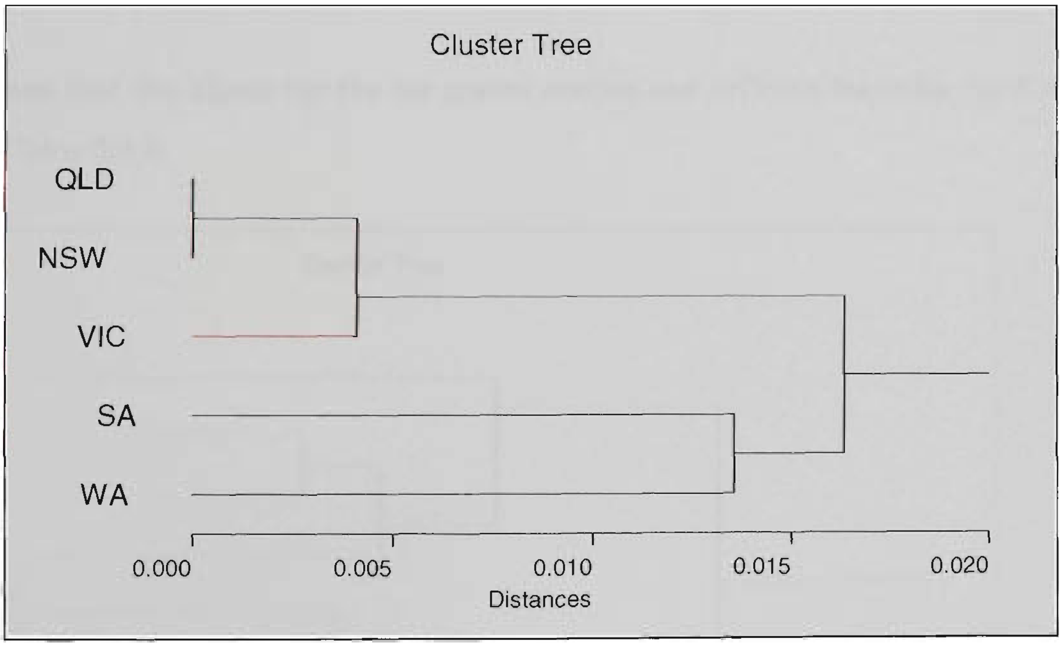


Figure 5.3.2 Hierarchical cluster dendogram (centroid linkage and Euclidean distance) of mean cadmium results of wheat samples from the AWB grade monitoring program by state

It can be seen that the dendogram in Figure 5.3.2 falls into two groups, with Victoria, Queensland and New South Wales in one group and South Australia and Western Australia in the other. This grouping is also reflected in Figure 5.3.1.

The value of > 0.05 for P in the ANOVA analysis of cadmium levels in Table 5.3.1 by season shows that a significant difference cannot be proven to exist between the cadmium levels in wheat samples from the AWB grade monitoring program when analysed by grade.

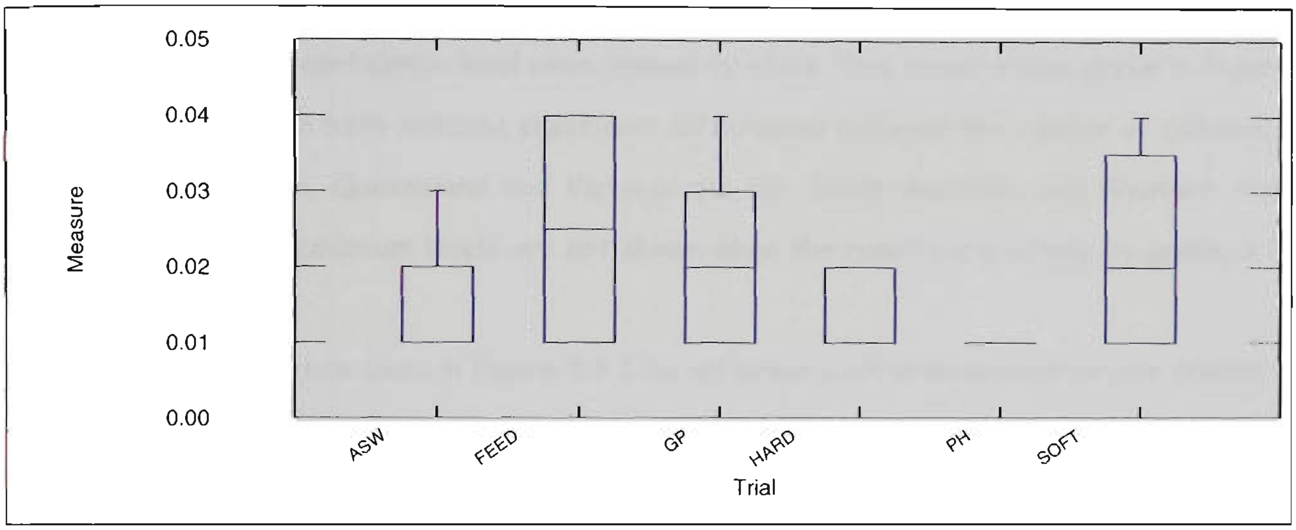


Figure 5.3.3 Box and whisker plot of cadmium results of wheat samples from the AWB grade monitoring program by grade

Figure 5.3.3 shows that the boxes for the six grades overlap and reflects the value for P of > 0.05 for the data in Table 5.3.1.

Figure 5.3.4 Hierarchical cluster dendogram (centroid linkage and Euclidean distance) of cadmium results of wheat samples from the AWB grade monitoring program by grade

The dendogram in Figure 5.3.4 does show an apparent stepped structure with the results falling into three groups but the very small differences in means does not allow statistical significance for this grouping.

5.3.1 Discussion

The value of < 0.05 for P given by the ANOVA analysis of the cadmium levels of wheat samples from the AWB grade monitoring program (Table 5.3.1) shows that these results are significantly different at the 95% confidence level when viewed by state. This trend is also shown in Figure 5.3.1 and Figure 5.3.2, which both indicate significant differences between the cluster of cadmium levels for New South Wales, Queensland and Victoria and for South Australia and Western Australia. These differences in cadmium levels are not shown when the results are viewed by grade, a P value of

> 0.05 and overlapping box plots in Figure 5.3.3 do not prove a difference between the grades.

5.4 Wheat samples sourced from the Australian wheat genotype field trial

Wheat samples were sourced from the Australian wheat genotype field trial. This trial was set up in 1996 using collaborators in five states, planting 14 wheat genotypes at three sites in New South Wales, South Australia and Western Australia and at two sites in Queensland and Victoria.

The 166 wheat samples were analysed for cadmium and the results are shown in Table 5.4.1.

Table 5.4.1 Summary of sites, genotypes and mean cadmium results for samples from the Australian wheat genotype field trial

Variety	Site													No.	Mean
	New South Wales			Queensland		South Australia			Victoria		Western Australia				
	M	N	WW	R	B	P	NA	Y	H	W	A	ME	WH		
Batava	<0.005	<0.005	0.010	<0.005	0.005	*	0.010	0.015	<0.005	<0.005	0.010	0.015	0.020	12	0.010
Cadoux	<0.005	<0.005	0.015	<0.005	0.010	0.015	0.010	*	0.005	<0.005	0.020	0.020	0.045	12	0.010
Eradu	<0.005	<0.005	*	<0.005	0.010	0.020	0.015	0.010	0.005	0.005	0.040	0.025	0.040	12	0.010
Goldmark	<0.005	*	0.010	<0.005	*	0.015	0.015	0.015	<0.005	*	0.035	0.035	0.070	10	0.020
Hartog	0.005	0.005	*	<0.005	0.010	*	0.010	0.020	<0.005	<0.005	0.020	0.020	0.035	11	0.010
Janz	<0.005	<0.005	0.010	0.005	0.010	0.005	0.010	0.010	<0.005	<0.005	0.015	0.010	0.030	13	0.010
Katunga	<0.005	<0.005	0.010	<0.005	0.010	*	*	0.010	0.005	<0.005	0.020	0.025	0.030	11	0.010
Machete	<0.005	<0.005	0.015	<0.005	0.010	0.020	0.010	0.015	0.005	<0.005	0.015	0.020	0.035	13	0.010
Meering	<0.005	<0.005	<0.005	<0.005	0.010	0.015	0.010	0.010	0.005	0.005	0.020	0.020	0.040	13	0.010
Suneca	0.005	0.005	0.010	<0.005	0.020	0.020	0.020	0.015	0.005	<0.005	0.025	0.035	0.055	13	0.015
Tammin	<0.005	<0.005	*	<0.005	0.010	0.010	0.015	*	0.005	0.005	0.020	0.020	0.030	11	0.010
Trident	0.005	<0.005	<0.005	<0.005	0.010	0.010	0.010	0.010	0.005	<0.005	0.020	0.025	0.035	13	0.010
Vectis	<0.005	<0.005	*	<0.005	<0.005	0.010	*	*	<0.005	<0.005	0.015	0.020	0.035	10	0.010
Yanec	<0.005	<0.005	<0.005	<0.005	0.005	0.010	0.010	0.010	<0.005	*	0.010	0.020	0.020	12	0.010
Number	14	13	10	14	13	11	12	11	14	12	14	14	14	166	
Mean	<0.005	<0.005	0.010	<0.005	0.010	0.015	0.010	0.010	<0.005	<0.005	0.020	0.020	0.040		0.010

Table 5.4.2 Summary of states, sites and abbreviations used in the genotype field trial

State	**Site**	**Abbreviation**
New South Wales	Moree	NSWM
	Narrabri	NSWN
	Wee Waa	NSWWW
Queensland	Bileoela	QLDB
	Roma	QLDR
South Australia	Narracoorte	SAN
	Paskeville	SAP
	Yeelanna	SAY
Victoria	Horsham	VICH
	Walpeup	VICW
Western Australia	Avondale	WAA
	Merriden	WAM
	Wongan Hills	WAWH

The results in Table 5.4.1 highlight the difference between cadmium levels for wheat genotypes grown at sites in South Australia and Western Australia (all in blue) and at sites in New South Wales, Queensland and Victoria. It does not appear to show a difference between varieties.

The value of < 0.01 for P for the ANOVA analysis of cadmium levels in Table 5.4.1 by state shows that a statistically significant difference at the 99% confidence level exists between the cadmium levels for wheat genotypes grown in each state across the Australian wheat belt.

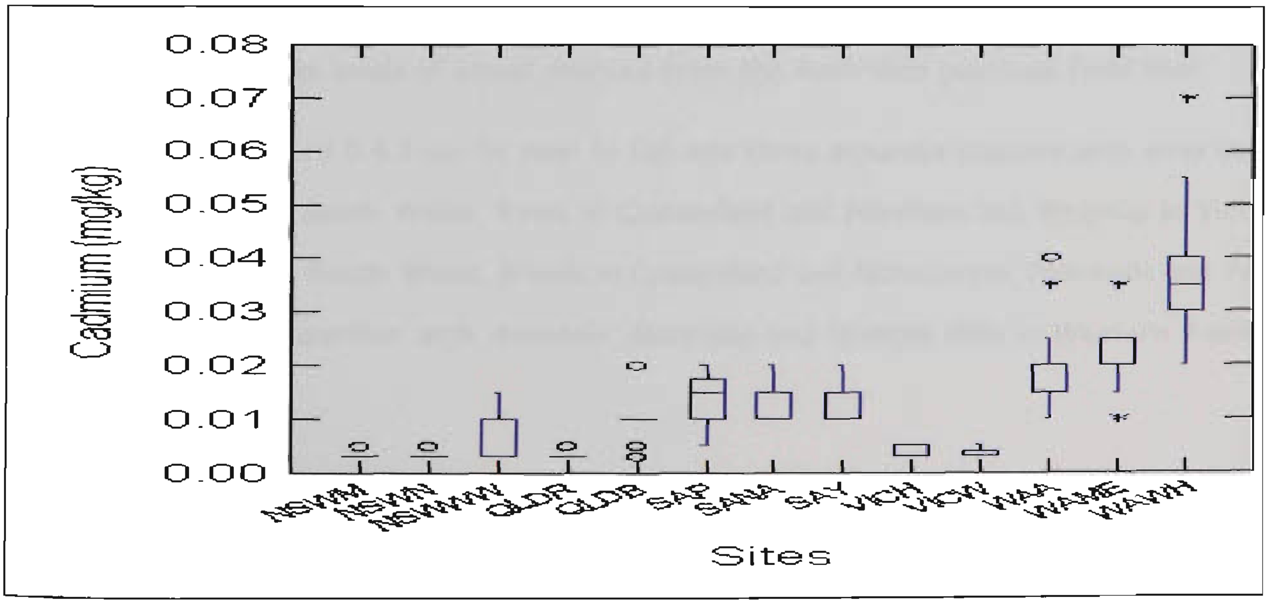


Figure 5.4.1 Box and whisker plot of cadmium results of wheat samples from the Australian genotype field trial by site

The boxes in figure 5.4.2 can be seen to fall into three separate groups with the Western Australian sites in one, the South Australian sites in another with sites in New South Wales, Queensland and Victoria in the remaining group. There is a slight overlap between Paskeville in South Australia and Avondale in Western Australia although the means for these sites are distinctly different.

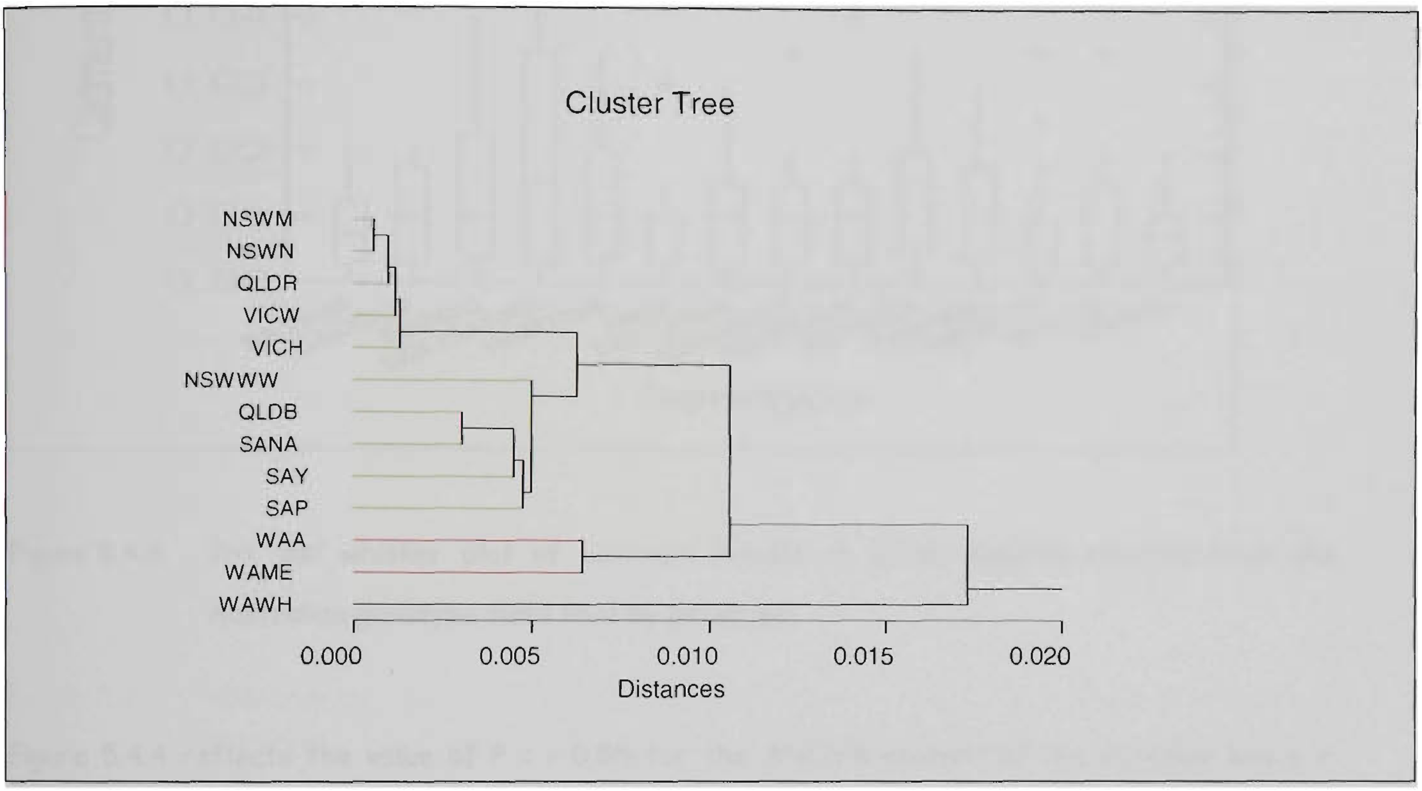


Figure 5.4.2 Hierarchical cluster dendogram (centroid linkage and Euclidean distance) by site of cadmium levels of wheat sourced from the Australian genotype field trial

The dendogram in Figure 5.4.3 can be seen to fall into three separate clusters with sites at Moree and Narrabri in New South Wales, Roma in Queensland and Horsham and Walpeup in Victoria in one, Wee Waa in New South Wales, Bileola in Queensland and Naracoorte, Paskeville and Yeelanna in South Australia in another with Avondale, Merriden and Wongan Hills in Western Australia in the final group.

The value of < 0.01 for P for the ANOVA analysis of the cadmium levels in Table 5.4.1 by site shows that a statistically significant difference at the 99% confidence level does exist between the cadmium levels in wheat samples from the Australian genotype field trial when analysed by site.

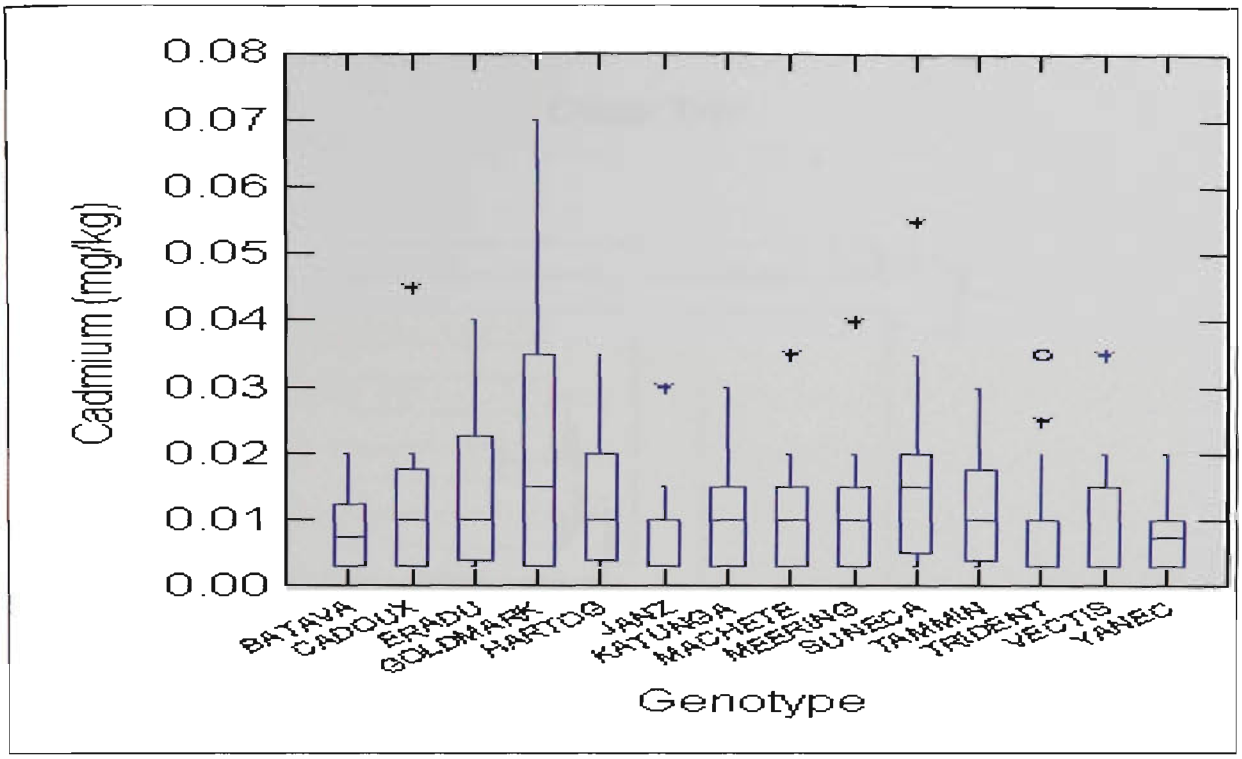


Figure 5.4.3 Box and whisker plot of cadmium results of wheat samples sourced from the Australian genotype field trial by genotype

Figure 5.4.4 reflects the value of P = > 0.05 for the ANOVA analysis of the cadmium levels in Table 5.4.1 by genotype and shows that the boxes for the fourteen genotypes overlap, hence a difference cannot be proven to exist between the cadmium levels of wheat samples from the Australian genotype field trial when analysed by genotype.

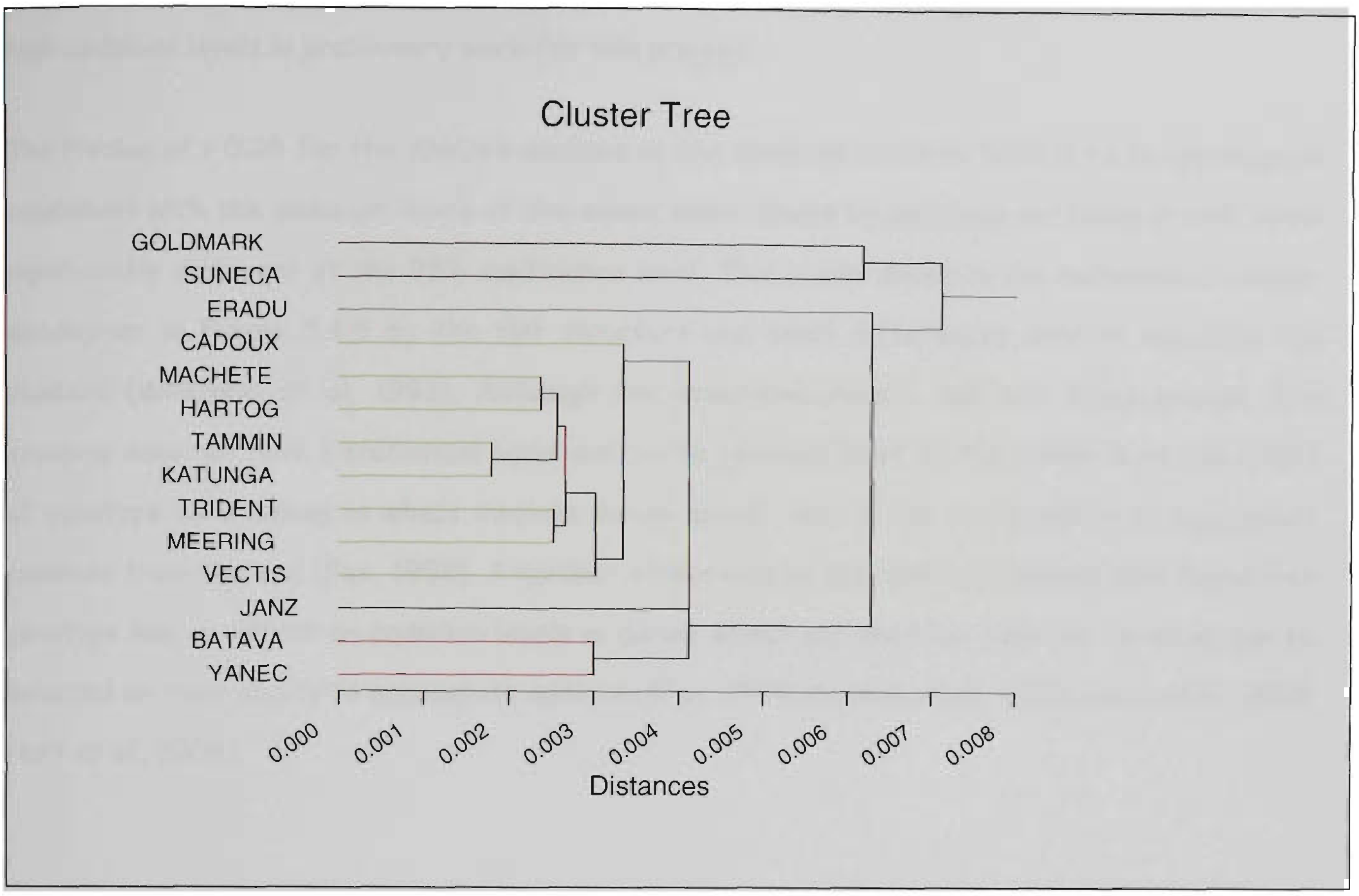


Figure 5.4.4 Hierarchical cluster dendogram (centroid linkage and Euclidean distance) by genotype of cadmium levels of wheat sourced from the Australian genotype field trial

The lack of an obvious stepped structure and the very small differences between the means in the dendogram in Figure 5.4.5 shows that a difference does not exist in the cadmium levels of wheat samples from the Australian genotype field trial when analysed by genotype.

5.4.5 Discussion

When the cadmium levels are viewed by site, the P value of < 0.05 shows that they are statistically different by site at the 95% confidence level. This is also shown by the stepped structure and differences used to calculate the clusters in the hierarchical cluster dendogram in Figure 5.4.3 (Wilkinson *et al.*, 1993). The sites fall into three groups with NSWM, NSWN, QLDR, VICW and VICH in the first group, NSWWW, QLDB, SANA, SAY and SAP in the next group and WAA, WAME and WAWH in the final group. This last group can be split into two sub-groups of WAA and WAME in one and WAWH in the other. These groups follow the trend of the grower load and composite site results with the lowest values generally for the eastern states, mid range for SA and highest for WA. Wongan Hills is in the centre of the WA grain belt and has shown high to very high cadmium levels in preliminary work for this project.

The P value of > 0.05 for the ANOVA analysis of the cadmium levels in Table 5.4.1 by genotype is consistent with the cadmium levels of the wheat when viewed by genotype not being proven to be significantly different at the 95% confidence level. This is also shown in the hierarchical cluster dendogram in Figure 5.4.5 by the flat structure and small differences used to calculate the clusters (Wilkinson et al, 1993). Although the genotypes visually fall into three groups, this grouping does not have a statistical basis and can be ignored. Most of the research on the effect of genotype on cadmium in wheat involves durum wheat, this is due to its ability to accumulate cadmium from the soil (Fox, 1998). A number of workers in the USA and Canada have found that genotype has an effect on cadmium levels in durum wheat and that low cadmium varieties can be selected on their ability to accumulate cadmium (Fox, 1998; Cakmak *et al.*, 2000; Hart *et al.*, 2000; Hart *et al.*, 2004).

5.5 Wheat samples sourced from silo sites in other wheat growing countries around the world

Wheat samples for USA, Canada and Europe were mainly sourced from the AWB fungal identification trial. All samples were analysed for cadmium using the method detailed in Chapter four.

Table 5.5.1. Summary of sample numbers, mean and maximum cadmium results for wheat samples sourced from silo sites in the United States of America

State	Cadmium (mg/kg)		Number
	Mean	Maximum	
Arizona	0.11	0.11	1
California	0.09	0.28	8
Colorado	0.07	0.10	10
Idaho	0.05	0.07	3
Illinois	0.05	0.11	35
Indiana	0.05	0.09	14
Iowa	0.06	0.06	1
Kansas	0.07	0.12	85
Louisiana	0.05	0.17	5
Michigan	0.06	0.10	30
Minnesota	0.05	0.14	34
Mississippi	0.05	0.08	2
Missouri	0.05	0.10	13
Montana	0.01	0.01	1
Nebraska	0.07	0.11	7
North Carolina	0.03	0.04	3
North Dakota	0.07	0.28	110
Ohio	0.09	0.28	27
Oklahoma	0.04	0.06	12
Oregon	0.07	0.14	6
South Dakota	0.04	0.06	15
Tennessee	0.02	0.02	1
Texas	0.06	0.12	18
Utah	0.06	0.06	1
Washington	0.03	0.05	4
Wisconsin	0.03	0.03	1
Wyoming	0.08	0.08	2
Total	0.07	0.28	463

The data in Table 5.5.1 shows generally high to very high mean cadmium levels in wheat from the West and Northwestern states (results over 0.05 mg/kg in blue) and generally lower levels in the Southeast. The maximum cadmium levels also reflect a similar trend. These mean levels are also shown in Figure 5.5.1.

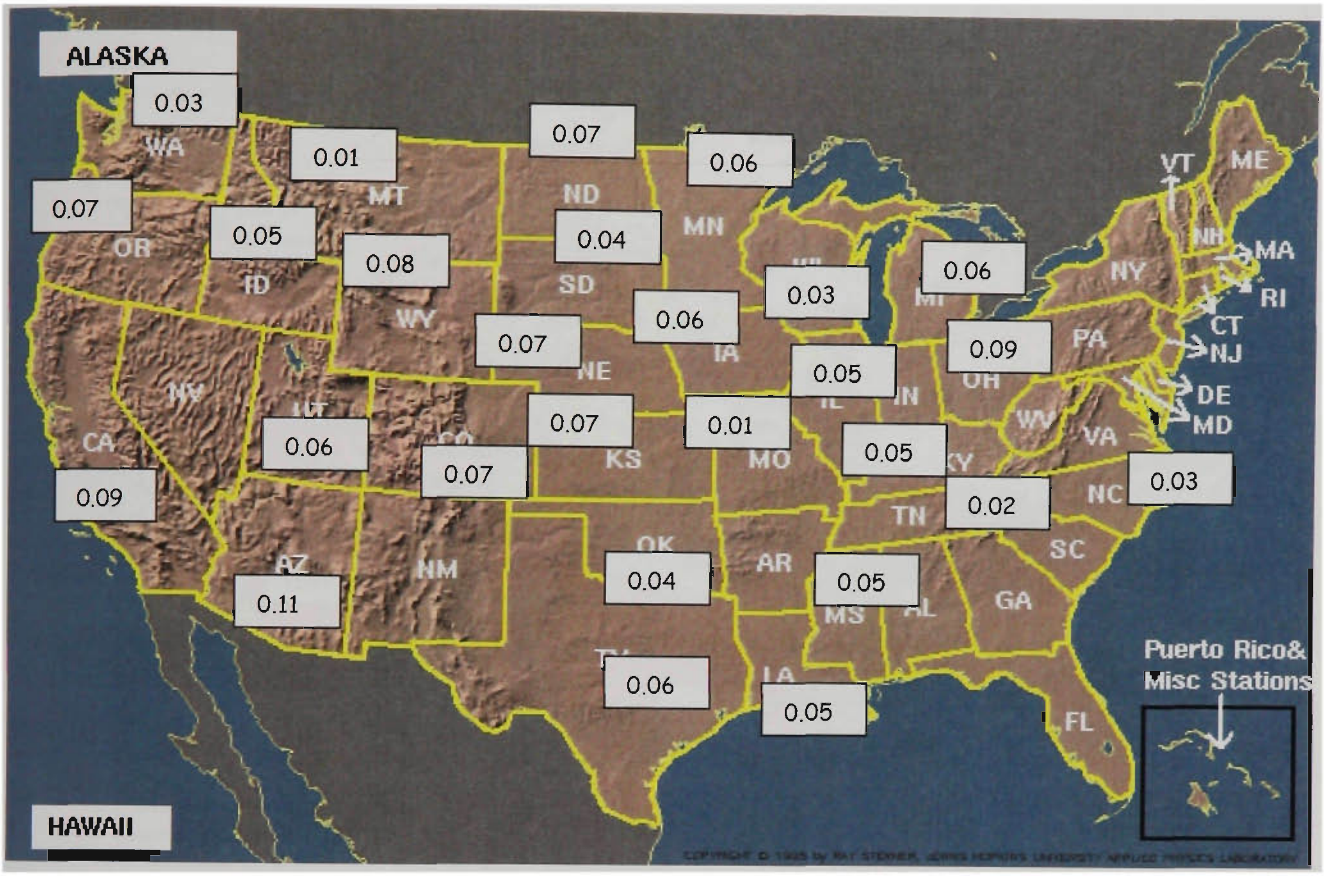


Figure 5.5.1 Map of the United States of America showing mean cadmium levels in mg/kg

Table 5.5.2 Summary of sample numbers, mean and maximum cadmium results for wheat samples sourced from silo sites in Canada

Province	Cadmium (mg/kg)		Number
	Mean	Maximum	
Alberta	0.07	0.10	31
Manitoba	0.05	0.11	24
Ontario	0.07	0.07	4
Saskatchewan	0.07	0.20	86
Unknown	0.05	0.19	10
Total	0.07	0.20	155

Table 5.5.2 shows that all wheat samples from Canadian silos had high to very high cadmium levels and corresponding high maximum levels (all in blue). These mean levels are also shown in Figure 5.5.2.

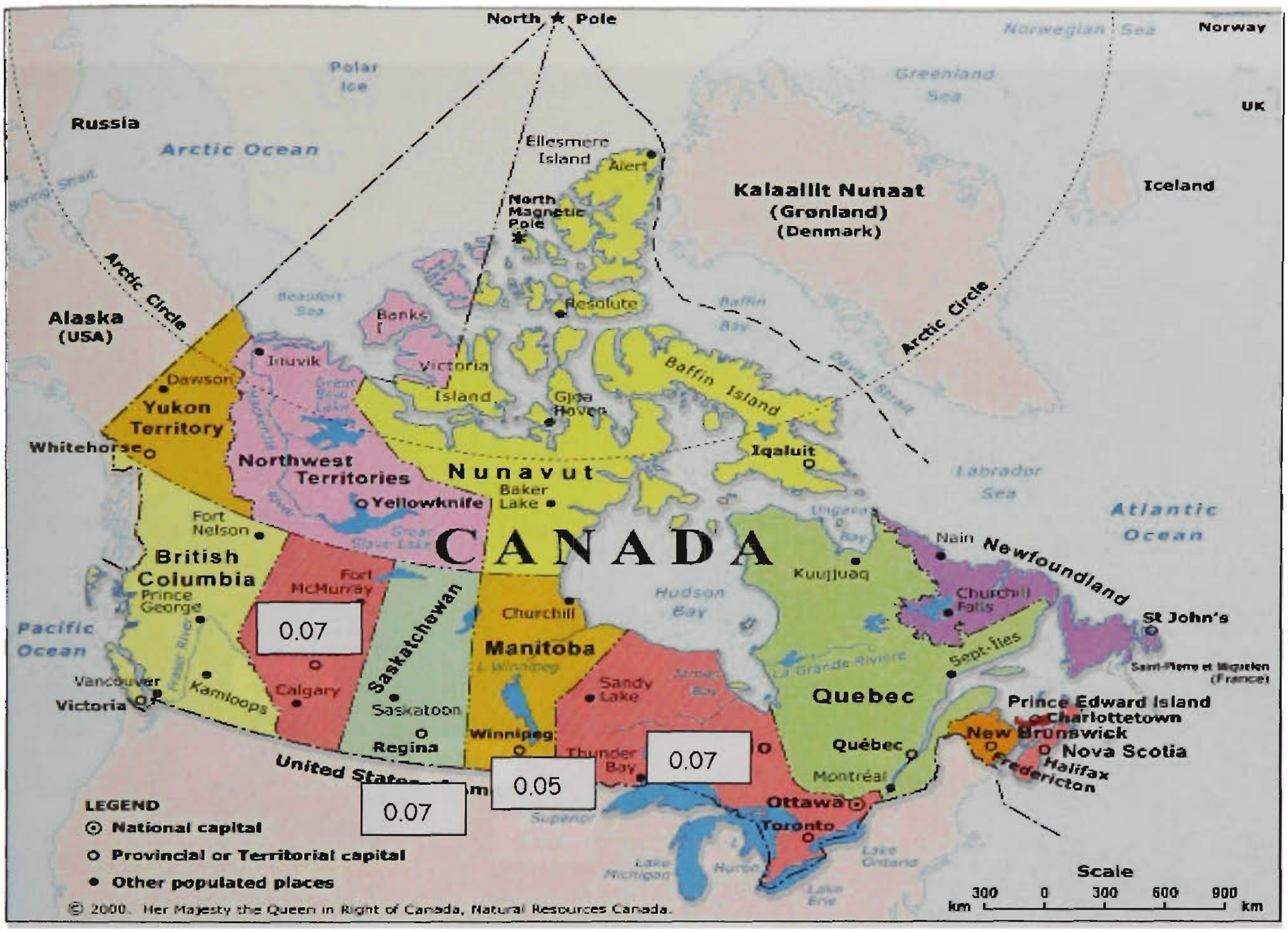


Figure 5.5.2 Map of Canada showing mean cadmium levels in mg/kg

Table 5.5.3 Summary of sample numbers, mean and maximum cadmium results for wheat samples sourced from silo sites in European Countries

Country	Cadmium (mg/kg)		Number
	Mean	Maximum	
France	0.05	0.10	38
Germany	**0.06**	0.07	3
Holland	**0.06**	**0.12**	30
Hungary	0.04	0.04	1
United Kingdom	0.04	0.10	57
Total	0.05	0.12	129

Table 5.5.3 shows that all wheat samples from silos in the European countries tested had high cadmium levels and corresponding high maximum levels with Holland and Germany having the highest mean (in blue) and Holland having the highest maximum (in blue). These mean levels are also shown in Figure 5.5.3

Figure 5.5.3 Map of Europe showing mean cadmium levels in mg/kg

Table 5.5.4 Summary of sample numbers, mean and maximum cadmium results for wheat samples sourced from silo sites in other countries

Country	Cadmium (mg/kg)		Number
	Mean	Maximum	
Argentina	0.01	0.01	1
China	0.01	0.02	21
Egypt	0.02	0.02	2
India	0.02	0.03	5
Russia	0.02	0.02	1
Saudi Arabia	0.02	0.02	1
South Africa	0.02	0.03	3
Turkey	0.01	0.01	1
Total	0.02	0.03	34

Table 5.5.4 shows that wheat samples from silos in the countries listed have low to very low mean cadmium levels with corresponding low maximum levels.

The value of < 0.01 for P for the ANOVA analysis of cadmium levels in Tables 5.5.1 to 5.5.4 shows that a statistically significant difference at the 99% confidence level exists between the cadmium levels found in wheat samples from the countries listed.

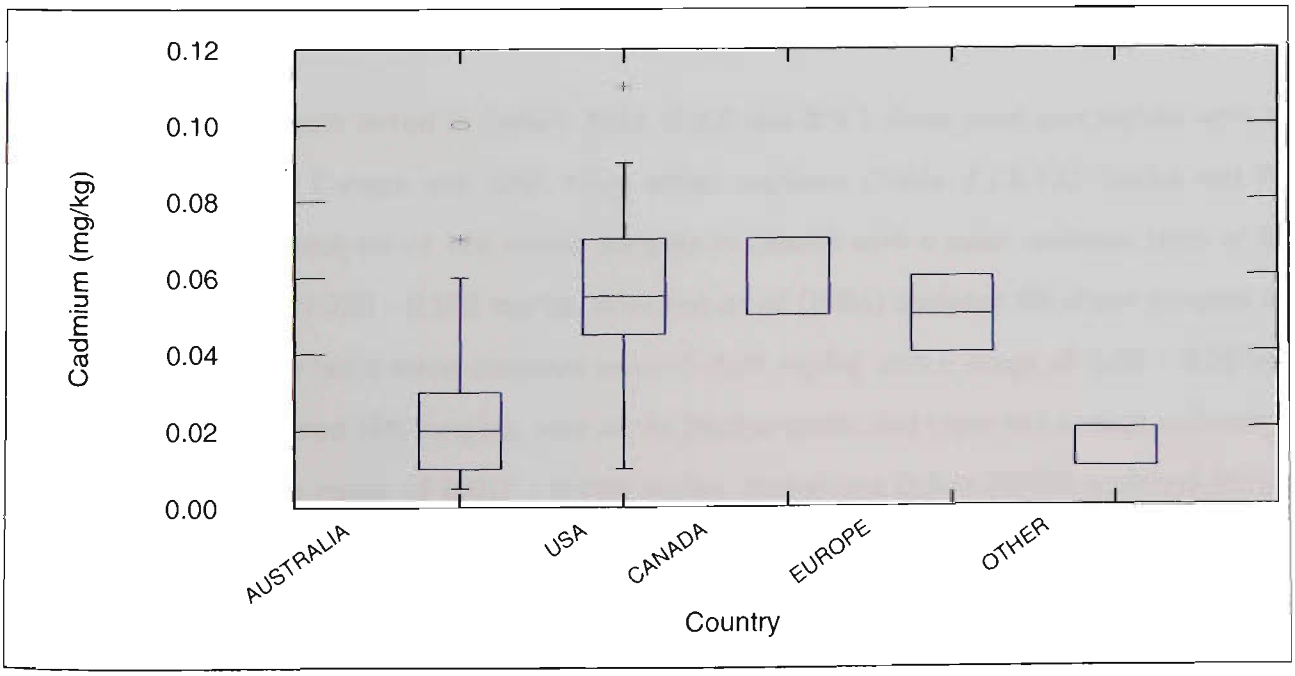


Figure 5.5.1 Box and whisker plot of cadmium results of wheat samples from silo sites in Australia and other countries

The boxes in Figure 5.5.1 can be seen to fall into two distinctive groups with Australia and other countries in one and the United States of America, Canada and Europe in the other.

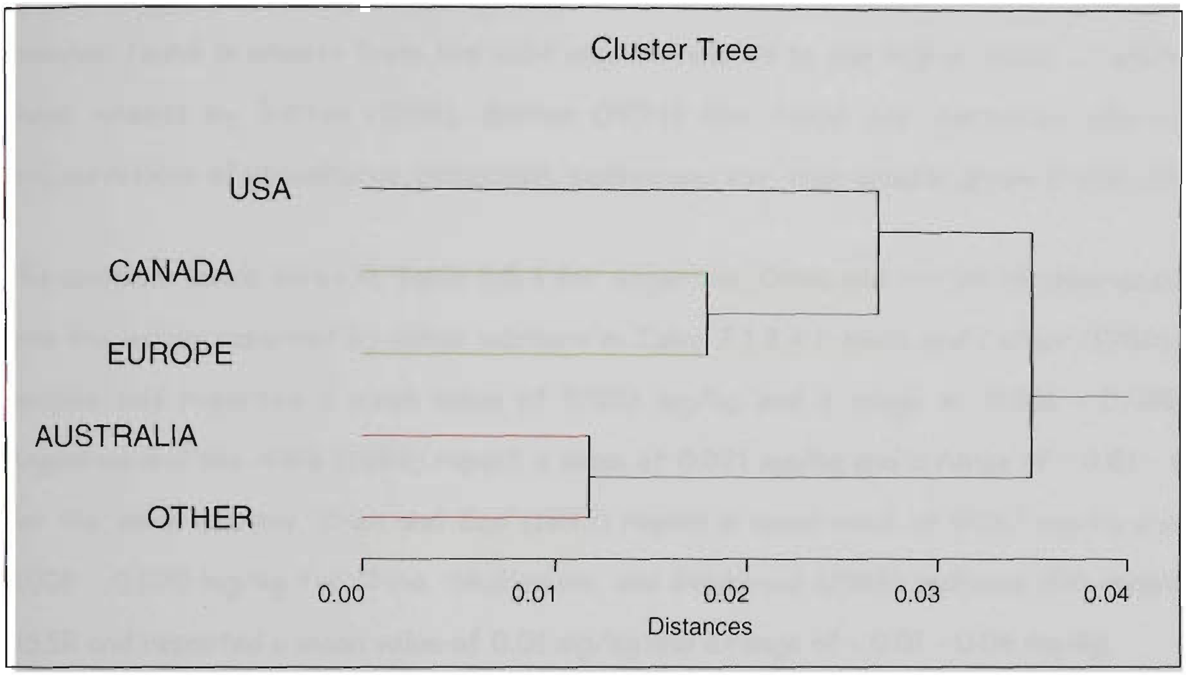


Figure 5.5.2 Hierarchical cluster dendogram (centroid linkage and Euclidean distance) of cadmium levels of wheat sourced from Australia and other countries.

The dendogram in Figure 5.5.2 can also be seen to fall into two groups with Australia and other countries in one and the United States of America, Canada and Europe in the other.

5.5.1 Discussion

The mean cadmium levels listed in Tables 5.5.1, 5.5.2 and 5.5.3 show good correlation with those reported for Canada, Europe and USA from other workers (Table 2.1.3.4.1). Dudas and Pawlik (1977) reported the analysis of 126 wheat samples in Canada with a mean cadmium level of 0.057 mg/kg and a range of 0.030 - 0.120 mg/kg. Wiersma *et al.* (1986) analysed 84 wheat samples in the Netherlands and reported a mean cadmium level of 0.07 mg/kg with a range of 0.02 - 0.35 mg/kg. Vos *et al.* (1986) analysed 188 samples, also in the Netherlands, and reported a mean cadmium level of 0.067 mg/kg with a range of 0.017 - 0.256 mg/kg. Seibel and Ocker (1979) analysed 211 wheat samples in West Germany and reported a mean cadmium level of 0.043 mg/kg with a range of 0 - 0.25 mg/kg. Ocker and Seibel (1980) analysed 1082 wheat samples also in West Germany and reported a mean of 0.057 mg/kg with a maximum of 0.008 - 0.85 mg/kg. Morris and Greene (1970) analysed 9 samples in the USA and reported a mean of 0.08 mg/kg and a range of 0.07 - 0.10 mg/kg. Erdman and Moul (1982) analysed 288 wheat samples, also in the USA, and reported a mean cadmium level of 0.081 mg/kg and a range of 0.042 - 0.210 mg/kg. Wolnik (1983) analysed 288 wheat samples, also in the USA, and reported a mean cadmium level of 0.043 mg/kg and a range of < 0.017 - 0.207 mg/kg. He found a similar spread as the results in Table 5.5.1 with high cadmium levels across the northwestern states and lower levels in the eastern states. The higher levels of cadmium found in wheats from the USA may be related to the higher levels of phytate found in these wheats by Batten (1994). Batten (1994) also found the Australian wheats had lower concentrations of phosphorus, potassium, sodium and iron than wheats grown in the USA.

The cadmium levels listed in Table 5.5.4 for Argentina, China and the USSR show good correlation with the values reported by other workers in Table 2.1.3.4.1. Hack and Cocker (1983) analysed 52 samples and reported a mean value of 0.003 mg/kg and a range of 0.001 - 0.008 mg/kg for Argentina and the AWB (1993) report a mean of 0.021 mg/kg and a range of < 0.01 - 0.041 mg/kg for the same country. Chen and Gao (1993) report a mean value of 0.017 mg/kg and a range of 0.009 - 0.030 mg/kg for China. Shcheglova and Golenkova (1984) analysed 100 samples from the USSR and reported a mean value of 0.01 mg/kg and a range of < 0.01 - 0.04 mg/kg.

The P value of > 0.05 given in the ANOVA analysis for the cadmium results listed in Table 5.5.4 is consistent with the mean levels of cadmium not being proven to be significantly different at the 95% confidence level for the range of countries tested.

Chapter 6 Cadmium and Ash Results of the Analysis of Wheat and Mill Streams

6.1 Cadmium and ash results for wheat samples sourced from the AWB quality monitoring program and milled on the Buhler laboratory mill

Two Australian standard white (ASW) wheat samples, two hard wheat samples and a soft wheat sample were sourced from the AWB wheat quality monitoring program for Victoria and Western Australia.

6.1.1 Wheat samples sourced from the AWB wheat quality monitoring program for Victoria

Two wheat samples of ASW and Hard wheat and a single sample of Soft wheat were sourced from the AWB wheat monitoring program for Victoria. The samples were labelled Victorian ASW 1, ASW 2, Hard 1, Hard 2 and Soft respectively and milled on the Buhler laboratory mill at the Academy of Grain Technology. The wheats and resulting mill fractions were analysed for cadmium and ash and these results are shown in Table 6.1.1.1.

Table 6.1.1.1 Cadmium and ash results from Victorian ASW 1, ASW 2, Hard 1, Hard 2 and Soft wheat samples sourced from the AWB wheat quality monitoring program and milled on the Buhler laboratory mill at the Academy of Grain Technology.

Wheat Grade	ASW 1		ASW 2		Hard 1		Hard 2		Soft	
Fraction	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)
Wheat	0.007	1.41	0.031	1.23	0.018	1.47	0.021	1.39	0.009	1.18
B1	0.007	0.43	0.022	0.37	0.019	0.45	0.013	0.41	0.007	0.41
B2	0.005	0.40	0.021	0.33	0.019	0.42	0.010	0.38	0.005	0.40
B3	0.007	0.59	0.020	0.45	0.014	0.63	0.016	0.48	0.009	0.47
R1	0.009	0.41	0.023	0.36	0.017	0.42	0.010	0.39	< 0.005	0.40
R2	0.008	0.38	0.021	0.33	0.018	0.46	0.010	0.36	0.005	0.38
R3	0.009	0.40	0.031	0.43	0.022	0.51	0.012	0.40	0.010	0.43
Bran	**0.022**	**3.64**	**0.053**	**3.27**	**0.045**	**5.17**	**0.030**	**3.63**	**0.023**	**3.07**
Shorts	**0.025**	**4.13**	**0.056**	**3.50**	**0.052**	**5.20**	**0.031**	**3.91**	**0.031**	**3.34**
Offal/Flour Ratio	3.133	8.931	**2.370**	8.947	3.065	12.360	2.959	10.732	**4.045**	7.521

The data in Table 6.1.1.1 highlights the relationship between wheat and mill fractions for cadmium and ash concentrations in SW, hard and soft wheat samples from Victoria. It can be seen that a far greater proportion of the cadmium in the wheat is fractionated into the bran and shorts than into the break and roll fractions. This is consistent for all three wheat grades as reflected in the ash results (both shown in blue). The fractionation of the ash into the bran and germ related fractions has been reported by many workers (Kashlan *et al.*, 1991; Peterson and Fulcher, 2002; Simmons *et al.*, 2002; Akubor and Badifu, 2004) and is related to the storage of metals in the aleurone layer of the wheat. The aleurone layer (or beeswing) is removed with the bran during milling.

The highest bran (0.053 mg/kg) and shorts (0.056 mg/kg) cadmium concentrations were from the ASW 2 wheat and the lowest bran (0.022 mg/kg and shorts (0.025 mg/kg) cadmium comcentrations were from the ASW 1 wheat. The ash results did not follow the same pattern, with the highest ash in the bran (5.17%) and shorts (5.20%) fractions from the Hard 1 wheat and the lowest in the bran (3.07%) and shorts (3.34%) fraction from the soft wheat. These levels are affected by the amount of cadmium in the original wheat grain.

It is normal commercial flour milling practice to combine all flours from the break and roller fractions to make a 'straight run' flour. This maximises the amount of flour that can be produced from the wheat and increases the profitability of the milling process. To calculate the amount of cadmium that is removed from the flour with the bran and shorts fraction (collectively known as offal in the flour milling trade), a ratio was calculated from the total cadmium in the offal divided by the total cadmium in the flour fractions and adjusted for the appropriate extraction rate given in Table 3.3.1.3. This calculation was repeated for the ash content.

The cadmium ratio is highest for the Soft wheat (4.045) and lowest for ASW 2 (2.370) (both in blue). ANOVA analysis of the Hard grades data in Table 6.1.1.1 gives of P value of < 0.05 and shows that a significant differences occurs between the Hard grades at the 95% confidence level. ANOVA analysis of the Soft grades data in Table 6.1.1.1 gives of P value of > 0.05 and shows that a significant differences cannot be proven between the Soft grades. The highest ash ratios are in the Hard wheats and this s consistent with other workers (Kashlan *et al.*, 1991; Every *et al.*, 2002; Peterson and Fulcher, 002; Mousia *et al.*, 2004).

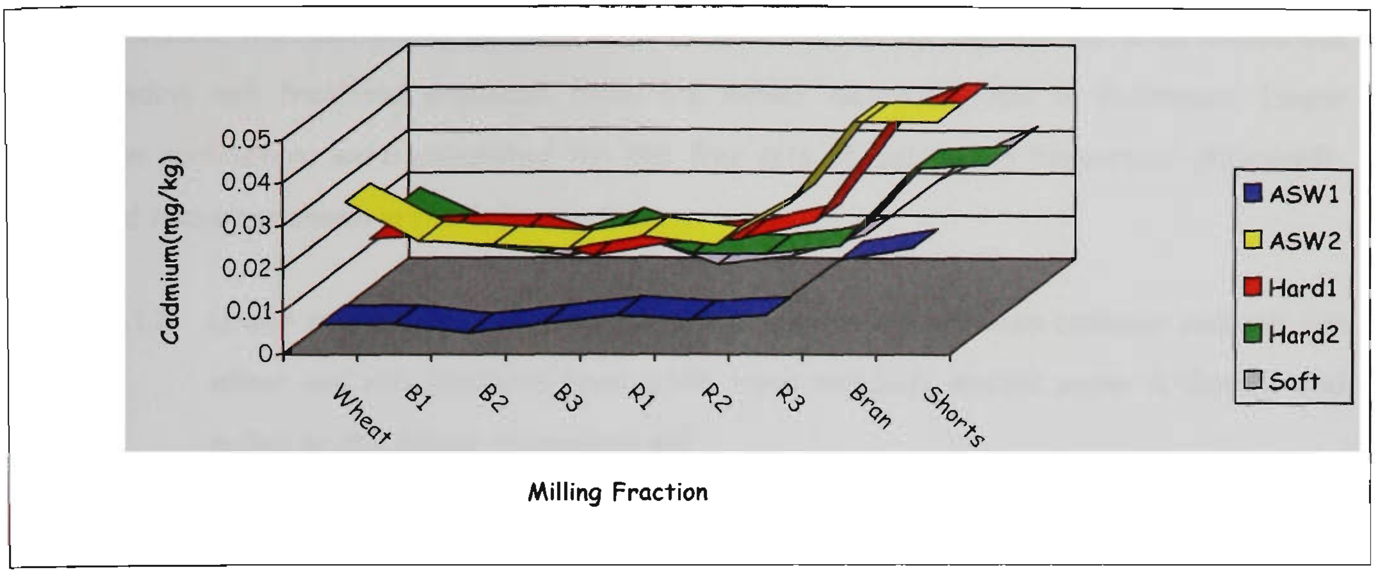


Figure 6.1.1.1 Relationship between cadmium level, wheat and corresponding mill fractions for Victorian wheat milled on the Buhler laboratory mill.

In Figure 6.1.1.1, the relationship between cadmium levels in the five Victorian wheats and corresponding mill fractions produced from the Buhler laboratory mill is shown. It can be seen that the four of the five wheats (ASW 2, Hard 1, Hard 2 and Soft) and corresponding fractions have a similar pattern with an immediate reduction in cadmium concentration in the break fractions with an increase over the R2 and R3 roll fractions and a sharp increase over the bran and shorts fractions. ASW 1 shows a steady increase in cadmium concentration from the wheat over the break and roller fractions with a larger increase over the bran and shorts.

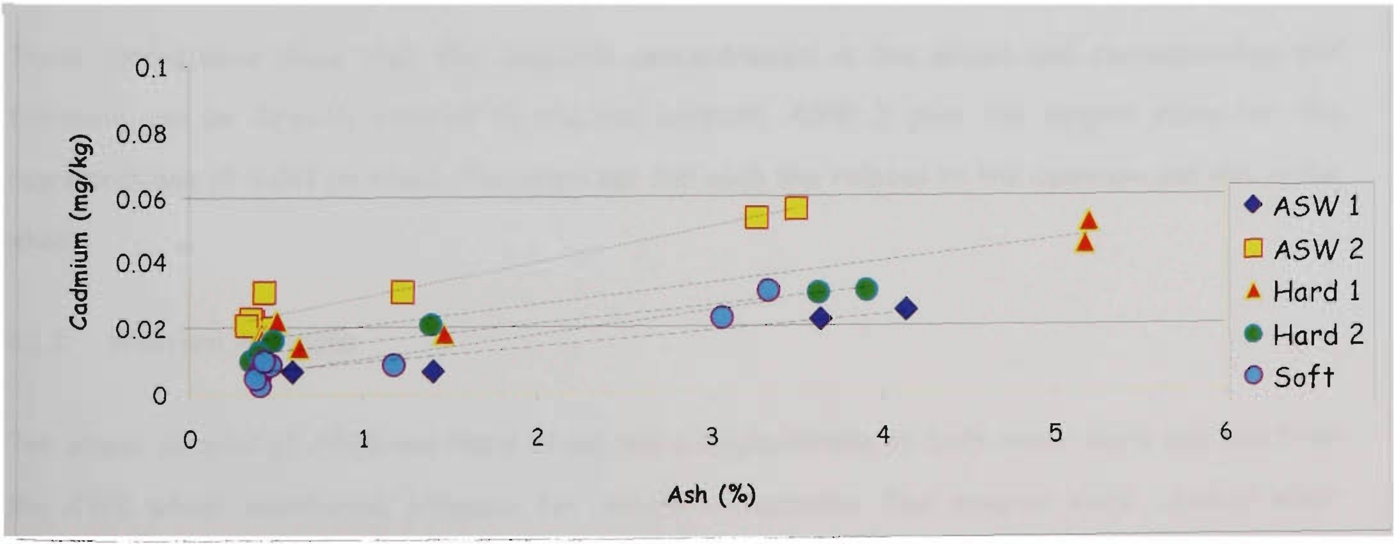


Figure 6.1.1.2 Relationship between cadmium and ash for wheat and corresponding mill fractions from ASW, Hard and Soft wheats grown in Victoria and milled on the Buhler laboratory mill

In Figure 6.1.1.2, the relationship between cadmium level and ash for the five Victorian wheats and corresponding mill fractions produced from the Buhler laboratory mill is illustrated. Linear regression parameters were calculated for the five sets of data using PowerPoint (Microsoft, 2002) and these are shown in the following table.

Table 6.1.1.2 Linear regression parameters for the relationship between cadmium and ash for wheat and mill fractions from ASW, Hard and Soft wheats grown in Victoria and milled on the Buhler laboratory mill

Wheat	**Slope**	**Intercept**	**Correlation Coefficient (r)**
ASW 1	0.005	0.005	**0.956**
ASW 2	**0.011**	0.019	**0.976**
Hard 1	0.007	0.014	0.964
Hard 2	0.006	0.010	0.968
Soft	0.007	0.003	0.958

ANOVA analysis of the data in table 6.1.1.2 gave a P value of < 0.001 at 7 DF for all five wheats. As P is < 0.001, it can be shown that a very highly significant linear correlation exists at the 99.9% confidence level between cadmium and ash. ASW 2 has the highest correlation with an r of 0.976 and ASW 1 has the lowest of the five wheat grades with an r of 0.956 (both in blue).

These correlations show that the cadmium concentration in the wheat and corresponding mill fractions can be directly related to the ash content. ASW 2 gave the largest slope for the regression line at 0.011 (in blue). The intercept for each line relates to the cadmium and ash in the wheat.

6.1.2 Western Australia

Two wheat samples of ASW and Hard wheat and a single sample of Soft wheat were sourced from the AWB wheat monitoring program for Western Australia. The samples were labelled West Australian ASW 1, ASW 2, Hard 1, Hard 2 and Soft respectively. The samples were milled on the Buhler laboratory mill and the cadmium and ash results are shown in Table 6.1.2.1.

Table 6.1.2.1 Cadmium and ash results from West Australian ASW, Hard and Soft wheat grades milled on the Buhler laboratory mill.

Fraction	ASW 1		ASW 2		Hard 1		Hard 2		Soft	
	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)
Wheat	0.042	1.43	0.050	1.25	0.027	1.13	0.038	1.26	0.044	1.40
B1	0.032	0.55	0.038	0.35	0.020	0.38	0.033	0.51	0.031	0.36
B2	0.030	0.53	0.045	0.36	0.019	0.35	0.029	0.50	0.031	0.35
B3	0.032	0.69	0.045	0.31	0.020	0.31	0.032	0.53	0.034	0.38
R1	0.030	0.51	0.031	0.33	0.017	0.36	0.034	0.48	0.032	0.35
R2	0.032	0.50	0.032	0.35	0.019	0.33	0.036	0.42	0.039	0.33
R3	0.039	0.50	0.039	0.30	0.020	0.39	0.044	0.47	0.050	0.41
Bran	**0.083**	**4.56**	**0.070**	**3.22**	**0.034**	**3.16**	**0.083**	**4.44**	**0.093**	**4.37**
Shorts	**0.082**	**4.81**	**0.070**	**3.64**	**0.040**	**3.42**	**0.081**	**4.19**	**0.098**	**4.68**
Offal/flour ratio	2.539	**8.570**	**1.826**	10.290	2.216	10.691	**2.716**	10.215	2.572	**12.129**

The data in Table 6.1.2.1 highlights the relationship between the cadmium and ash concentrations in wheat and mill fractions from ASW, hard and soft wheat grades sourced from Western Australia. Similarly for the data in Table 6.1.1.1, it can be seen that a far greater proportion of the cadmium in the wheat is fractionated into the bran and shorts (both shown in blue) than into the break and roll fractions. This is consistent for all wheat grades and this is reflected in the ash results (also both shown in blue). In addition, for the three wheat grades in the table, the cadmium content of the bran (0.093) and shorts (0.098) fractions is highest in the Soft wheat.

The cadmium offal/flour ratio is highest in the Hard 2 wheat (2.716) and lowest in the ASW 2 wheat (1.826). The ash offal/flour ratio is highest in the soft wheat (12.129) and lowest in the ASW 1 (8.570) (all shown in blue).

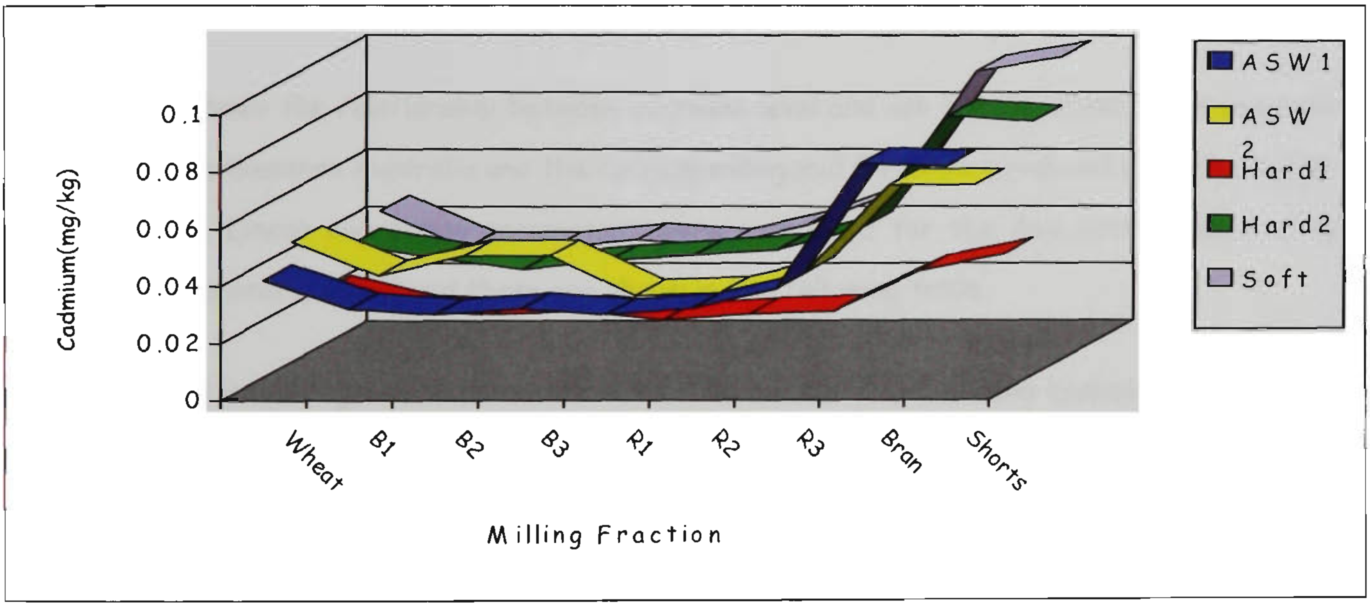


Figure 6.1.2.1 Relationship between cadmium, wheat and corresponding milling fractions for the five Western Australian wheats milled on the Buhler laboratory mill.

Figure 6.1.2.1 shows the relationship between cadmium levels in Western Australian wheat and corresponding mill fractions produced from the Buhler laboratory mill. As with the four Victorian samples, it shows that the five wheats and corresponding fractions have a similar pattern with an immediate reduction in cadmium level in the break fractions with an increase over the R2 and R3 roll fractions and a much larger increase over the bran and shorts fractions. This relationship is more marked in the West Australian wheats and corresponding fractions than Victoria due to the higher levels of cadmium in the wheat.

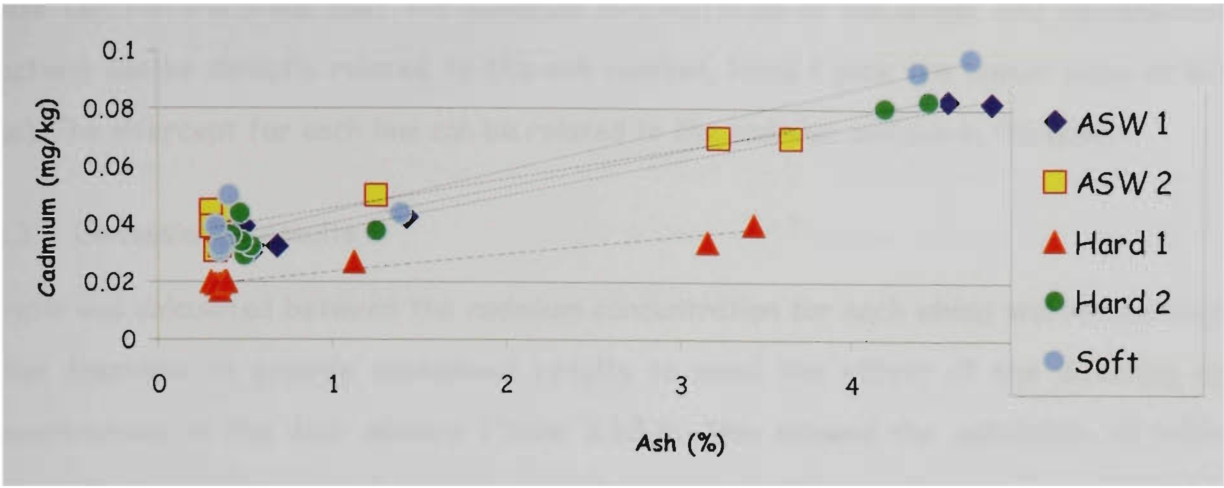


Figure 6.1.2.1 Relationship between cadmium and ash for wheat and corresponding mill fractions from ASW, Hard and Soft wheats grown in Western Australia and milled on the Buhler laboratory mill

Figure 6.1.2.1 shows the relationship between cadmium level and ash for the ASW, Hard and Soft wheats grown in Western Australia and the corresponding mill fractions produced from the Buhler laboratory mill. Linear regression parameters were calculated for the five sets of data using PowerPoint (Microsoft, 2002) and these are shown in the following table.

Table 6.1.2.2 Linear regression parameters for the relationship between cadmium and ash for wheat and mill fractions from ASW, Hard and Soft wheats grown in Western Australia and milled on the Buhler laboratory mill

Wheat	**Slope**	**Intercept**	**Correlation Coefficient (r)**
ASW 1	0.012	0.026	0.990
ASW 2	0.010	0.035	0.940
Hard 1	0.006	0.017	0.975
Hard 2	0.012	0.028	0.975
Soft	0.014	0.030	0.973

ANOVA analysis of the data in table 6.1.2.2 gave a P value of < 0.001 at 7 DF for all five wheats. As P is < 0.001, it can be shown that a very highly significant linear correlation exists at the 99.9% confidence level between cadmium and ash. ASW 1 has the highest correlation with an r of 0.990 and ASW 2 has the lowest of the five wheat grades with an r of 0.940 (both in blue).

These correlations show that the cadmium concentration in the wheat and corresponding mill fractions can be directly related to the ash content. Hard 1 gave the lowest slope at 0.006 (in blue). The intercept for each line can be related to the cadmium and ash in the wheat.

6.1.3 Discussion of results

A ratio was calculated between the cadmium concentration for each wheat and the corresponding milled fractions to provide normalised results to avoid the effect of the differing cadmium concentrations in the four wheats (Table 3.1.3.1). This allowed the calculation of analysis of variance between the results for the two grades and each state.

Table 6.1.3.1 Ratios between the cadmium concentration in the wheat and the cadmium concentration in the corresponding milling fraction from wheats grown in Victoria and Western Australia and milled on the Buhler laboratory mill

	Victoria					**Western Australia**				
	ASW 1	ASW 2	Hard 1	Hard 2	Soft	ASW 1	ASW 2	Hard 1	Hard 2	Soft
Wheat										
B1	1.000	0.710	1.056	0.619	0.778	0.762	0.760	0.741	0.868	0.705
B2	0.714	0.677	1.056	0.476	0.556	0.714	0.900	0.704	0.763	0.705
B3	1.000	0.645	0.778	0.762	1.000	0.762	0.900	0.741	0.842	0.773
R1	1.286	0.742	0.944	0.476	0.333	0.714	0.620	0.630	0.895	0.7273
R2	1.143	0.677	1.000	0.476	0.556	0.762	0.640	0.704	0.947	0.886
R3	1.286	1.000	1.222	0.571	1.111	0.929	0.780	0.741	1.158	1.136
Bran	**3.143**	1.710	2.500	1.429	2.556	1.976	1.400	**1.259**	2.184	2.114
Shorts	**3.571**	**1.057**	2.889	1.476	3.444	1.952	1.400	1.482	2.132	2.227

The highest ratios in Table 6.1.3.1 are for ASW 1 (3.143 for bran and 3.571 for shorts) from Victoria and the lowest are for Hard 1 (1.259 for bran) from Western Australia and for ASW 2 (1.057 for shorts) from Victoria (all in blue). ANOVA analysis of the data in Table 6.1.3.1 gave a P value of > 0.05 for the bran and shorts ratios for Victoria and Western Australia and a P value of < 0.05 for the two samples of ASW and Hard from each state. This shows that a significant difference occurs between the ASW and Hard ratios at the 95% confidence level but cannot be proven for the bran and shorts.

6.2 Cadmium and ash results for wheat samples sourced from the AWB quality monitoring program and milled on the BRI pilot mill

The wheat genotype and grade samples listed in Table 3.3.2.1 were milled on the pilot mill at the Bread Research Institute (BRI) at North Ryde in New South Wales. The wheat and corresponding mill fraction samples were analysed for cadmium and ash.

6.2.1 Wheat sourced from the AWB pilot milling program for New South Wales

The results from the analysis of the cadmium and ash in each wheat and the corresponding mill fractions are shown in the table below.

Table 6.2.1.1 Cadmium and ash results for wheat and corresponding mill fractions sourced from the AWB pilot milling program for New South Wales and milled on the BRI pilot mill

Sample	APW		Sunlin	
	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)
Wheat	0.005	1.43	< 0.005	1.51
1st Break	< 0.005	0.44	< 0.005	0.51
2nd Break	0.005	0.39	< 0.005	0.45
3rd Break	0.006	0.81	< 0.005	0.77
4th Break	0.005	1.67	0.005	1.39
Break Middlings	0.006	0.42	< 0.005	0.47
Sizer	0.005	0.44	0.005	0.45
B2	0.006	0.67	< 0.005	0.61
A	0.006	0.33	< 0.005	0.38
B	0.006	0.42	< 0.005	0.39
C	0.005	0.38	< 0.005	0.40
D	0.006	0.53	< 0.005	0.60
E	0.006	1.67	< 0.005	1.54
F	0.005	1.04	< 0.005	1.72
Pollard Finisher	0.010	2.66	0.006	2.24
Bran	**0.012**	**5.70**	**0.008**	**5.54**
Pollard	**0.016**	**3.66**	**0.012**	**2.89**
Germ	**0.012**	**4.18**	**0.010**	**4.27**
Offal/Flour Ratio	0.750	1.711	1.183	4.854

Table 6.2.1.1 highlights the relationship between wheat and mill fractions for cadmium level and ash in APW and Sunlin wheat grown in New South Wales. It can be seen that a greater proportion of the cadmium in the wheat is fractionated into the pollard finisher, bran, pollard and germ

fractions than into the various break and roll fractions and this is reflected in the higher ash results for these fractions (all shown in blue).

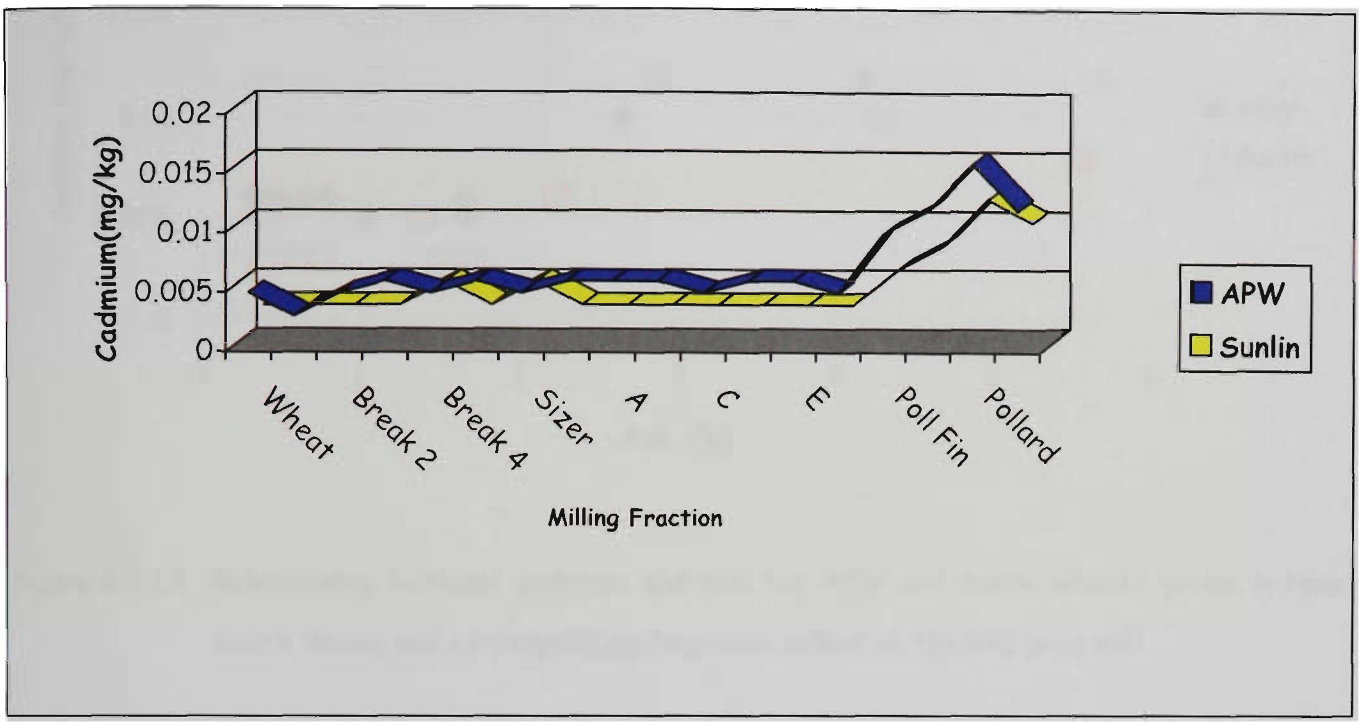


Figure 6.2.1.1 Relationship between cadmium level, wheat and corresponding mill fractions for APW and Sunlin wheats grown in New South Wales and milled on the BRI pilot mill.

In Figure 6.2.1.1 the relationship between cadmium levels in APW and Sunlin wheat grown in New South Wales and the corresponding mill fractions produced from the BRI pilot mill can be seen. The figure shows that the two wheats and corresponding fractions have a similar pattern with little or no change in cadmium level in the break and flour fractions with a marked increase over the pollard finisher, bran and pollard fractions and a slight decrease in the germ fraction.

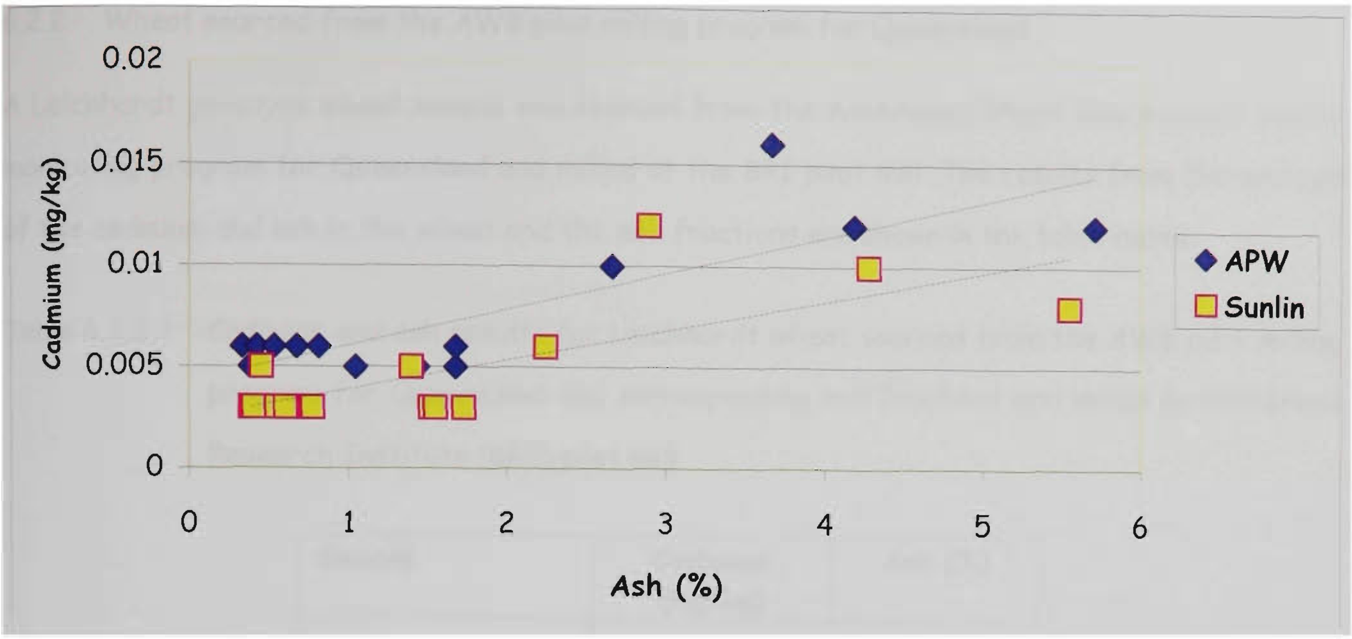


Figure 6.2.1.2 Relationship between cadmium and ash for APW and Sunlin wheats grown in New South Wales and corresponding fractions milled on the BRI pilot mill

In Figure 6.2.1.2 the linear relationship between cadmium levels and ash in APW and Sunlin wheats grown in New South Wales can be seen. Linear regression parameters were calculated for the two sets of data using PowerPoint (Microsoft, 2002) and these are shown in the following table.

Table 6.2.1.2 Linear regression parameters for the relationship between cadmium and ash for wheat and mill fractions from APW and Sunlin wheats grown in New South Wales and milled on the BRI pilot mill

Wheat	**Slope**	**Intercept**	**Correlation Coefficient (r)**
APW	0.002	0.004	**0.844**
Sunlin	0.002	0.002	0. 779

ANOVA analysis of the data in table 6.2.1.2 gave a P value of < 0.001 at 16 DF for both wheats. As P is < 0.001, it can be shown that a very highly significant linear correlation exists at the 99.9% confidence level between cadmium and ash. APW has the highest correlation with an r of 0.844 (in blue). These correlations show that the cadmium concentration in the wheat and corresponding mill fractions can be directly related to the ash content. Both slopes were identical and the intercept for each line can be related to the cadmium and ash in the wheat.

6.2.2 Wheat sourced from the AWB pilot milling program for Queensland

A Leichhardt genotype wheat sample was sourced from the Australian Wheat Board wheat quality monitoring program for Queensland and milled at the BRI pilot mill. The results from the analysis of the cadmium and ash in the wheat and the mill fractions are shown in the table below.

Table 6.2.2.1 Cadmium and ash results for Leichhardt wheat sourced from the AWB pilot milling program for Queensland and corresponding mill fractions and milled on the Bread Research Institute (BRI) pilot mill

Sample	**Cadmium (mg/kg)**	**Ash (%)**
Wheat	0.007	1.42
1st Break	0.006	0.50
2nd Break	0.005	0.47
3rd Break	0.012	0.74
4th Break	0.010	1.49
Break Middlings	0.008	0.46
Sizer	0.006	0.39
B2	0.008	0.50
A	0.008	0.31
B	< 0.005	0.33
C	0.006	0.37
D	0.006	0.45
E	< 0.005	0.82
F	0.006	1.18
Pollard Finisher	0.007	2.31
Bran	**0.018**	**5.44**
Pollard	**0.022**	**3.52**
Germ	**0.011**	**4.45**
Offal/Flour Ratio	**0.814**	**1.949**

Table 6.2.2.1 highlights the relationship between wheat and mill fractions for cadmium level and ash in Leichhardt wheat grown in Queensland. It can be seen that a greater proportion of the cadmium in the wheat is fractionated into the bran, pollard and germ fractions than into the various break and roll fractions and this is reflected in the higher ash results for these fractions. The highest cadmium level of the offal fractions is in the pollard (0.022 mg/kg) and the lowest is in the germ (0.011 mg/kg) (all shown in blue).

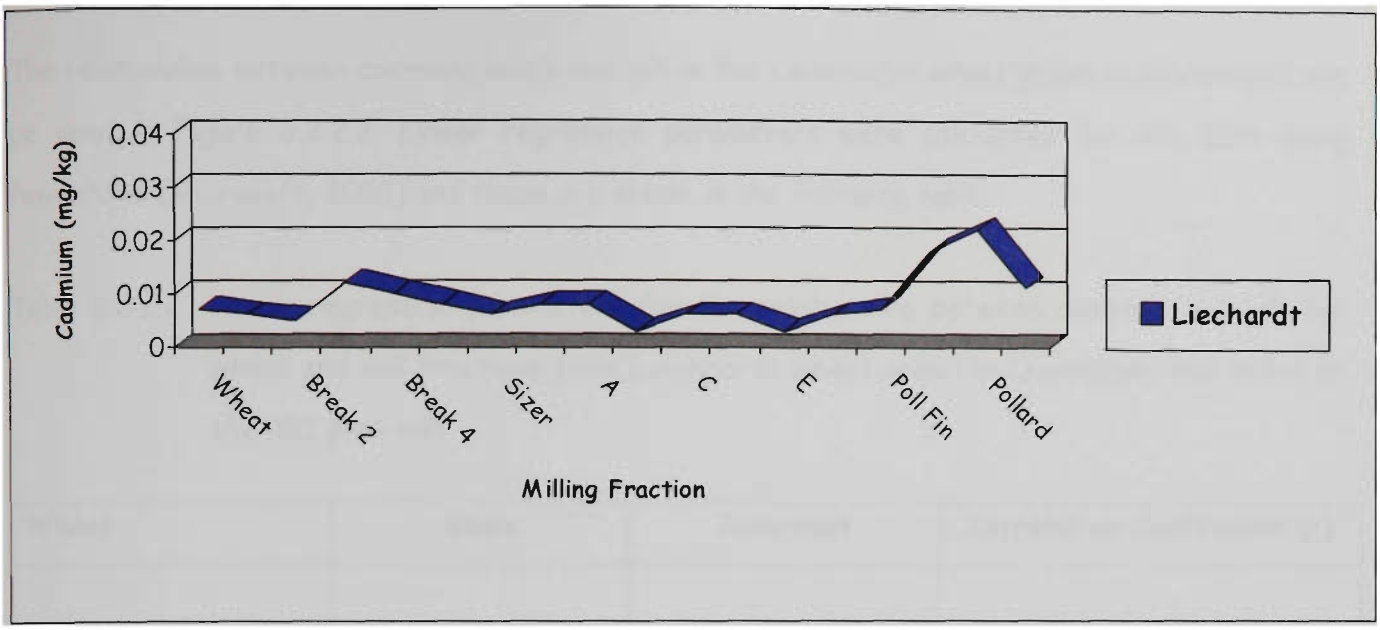


Figure 6.2.2.1 Relationship between cadmium level, wheat and corresponding mill fractions for Liechardt wheat grown in Queensland and milled on the BRI pilot mill.

In Figure 6.2.2.1, the relationship between cadmium levels in Leichhardt wheat grown in Queensland and the corresponding mill fractions produced from the BRI pilot mill can be seen. The figure shows that the mill fractions have a slight reduction from the wheat over the first break roll, an increase over the third break roll and then a gradual decrease over the remaining break and reduction rolls. It then shows a marked increase over the pollard finisher, bran and pollard fractions and a decrease in the germ fraction.

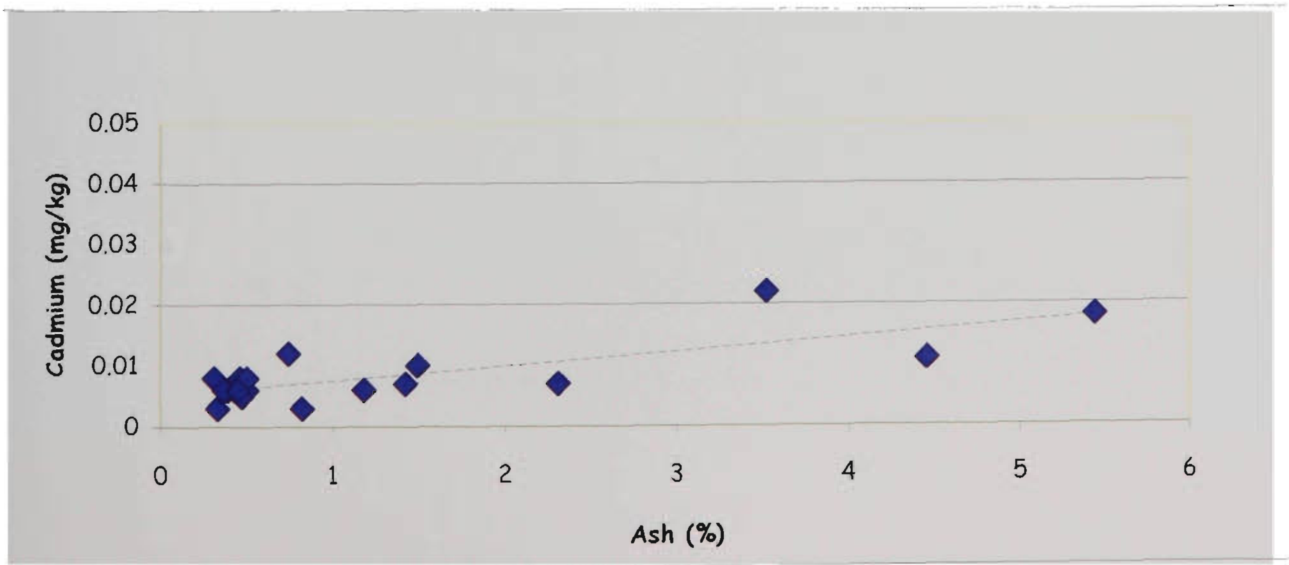


Figure 6.2.2.2 Relationship between cadmium and ash for Leichhardt wheat grown in Queensland and corresponding fractions milled on the BRI pilot mill

The relationship between cadmium levels and ash in the Leichhardt wheat grown in Queensland can be seen in Figure 6.2.2.2. Linear regression parameters were calculated for the data using PowerPoint (Microsoft, 2002) and these are shown in the following table.

Table 6.2.2.2 Linear regression parameters for the relationship between cadmium and ash for wheat and mill fractions from Leichhardt wheat grown in Queensland and milled on the BRI pilot mill

Wheat	**Slope**	**Intercept**	**Correlation Coefficient (r)**
Leichhardt	0.002	0.005	**0.746**

ANOVA analysis of the data in table 6.2.2.2 gave a P value of 0.001 at 16 DF for both wheats. As P is 0.001, it can be shown that a highly significant linear correlation exists at the 99.9% confidence level between cadmium and ash. This correlation shows that the cadmium concentration in the wheat and corresponding mill fractions can be directly related to the ash content. The slope and the intercept for the line can be related to the cadmium and ash in the wheat.

6.2.3 Wheat sourced from the AWB pilot milling program for South Australia

Table 6.2.3.1 Wheat, wheat fractions, cadmium and ash results for APW, Hard and Kirchauff wheat sourced from South Australia and milled on the BRI pilot mill

Sample	**APW**		**Hard**		**Kirchauff**	
	Cadmium (mg/kg)	**Ash (%)**	**Cadmium (mg/kg)**	**Ash (%)**	**Cadmium (mg/kg)**	**Ash (%)**
Wheat	0.011	1.38	0.011	1.42	0.022	1.35
1st Break	0.012	0.44	0.007	0.50	0.016	0.44
2nd Break	0.012	0.41	0.007	0.43	0.013	0.38
3rd Break	0.014	0.59	0.010	0.67	0.017	0.58
4th Break	0.007	0.91	0.011	1.10	0.018	0.91
Break Middlings	0.006	0.43	0.008	0.43	0.014	0.40
Sizer	0.006	0.40	0.007	0.41	0.016	0.36
B2	0.007	0.53	0.008	0.59	0.017	0.53
A	0.007	0.37	0.007	0.36	0.016	0.35
B	0.008	0.39	0.007	0.40	0.015	0.33
C	0.007	0.39	0.007	0.38	0.016	0.35
D	0.009	0.49	0.008	0.50	0.015	0.42
E	0.010	0.84	0.009	1.09	0.017	0.83
F	0.012	1.21	0.020	1.16	0.019	1.21
Pollard Finisher	0.013	1.49	0.015	1.80	0.020	1.48
Bran	**0.037**	**4.55**	**0.032**	**4.58**	**0.064**	**4.26**
Pollard	**0.035**	**3.03**	**0.030**	**3.37**	**0.029**	**3.08**
Germ	**0.037**	**3.65**	**0.040**	**3.96**	**0.058**	**3.61**
Offal/Flour Ratio	**3.820**	**5.755**	3.547	5.525	**2.372**	**1.917**

Table 6.2.3.1 highlights the relationship between wheat and mill fractions for cadmium level and ash in APW, Hard and Kirchauff wheats grown in South Australia. It can be seen that a greater proportion of the cadmium in the wheat is fractionated into the bran, pollard and germ fractions than into the various break and roll fractions and this is reflected in the higher ash results for these fractions (all shown in bold). The offal/flour ratios for both cadmium and ash are highest in the APW (3.820 and 5.755) and lowest in the Kirchauff wheats (2.372 and 1.917) (all in blue).

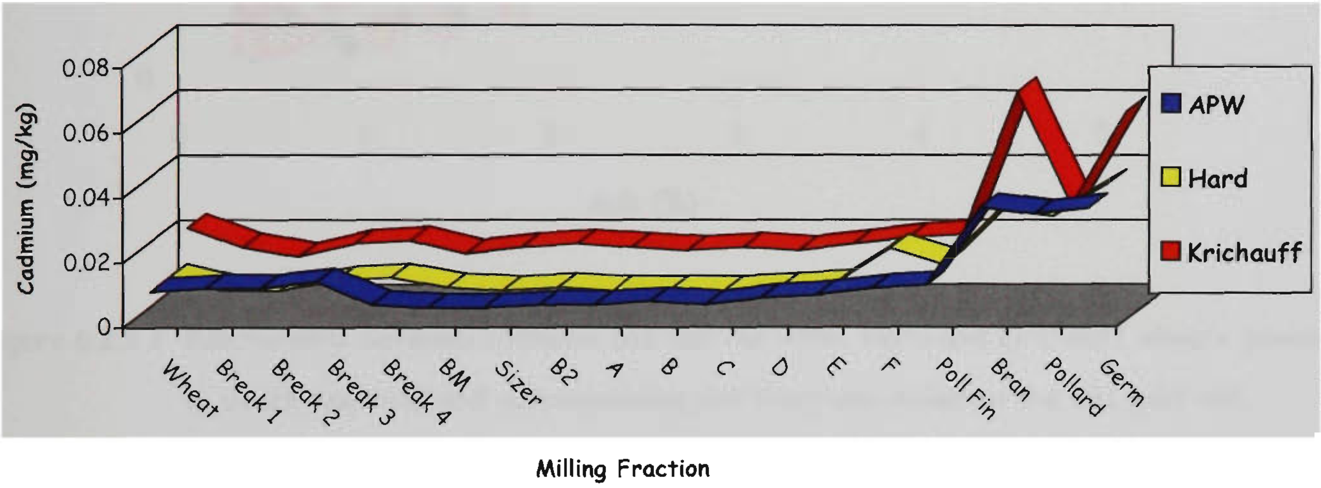


Figure 6.2.3.1 Relationship between cadmium, wheat and corresponding milling fractions for APW, Hard and Kirchauff wheats grown in South Australian and milled on the BRI pilot mill.

The relationship between cadmium levels in APW, Hard and Kirchauff wheats grown in South Australia and the corresponding mill fractions produced from the BRI pilot mill is shown in Figure 6.2.3.1. It can be seen that the mill fractions from the APW and Hard wheats are reasonably consistent until the pollard finisher where they show a slight increase and then a sharp increase over the bran, pollard and germ. The Kirchauff wheat shows a slight reduction from the wheat over the first break roll, an increase over the third break roll, however, it is then consistent over the remaining break and reduction rolls. It then shows a marked increase over the bran, a decrease over the pollard but an increase in the germ fraction.

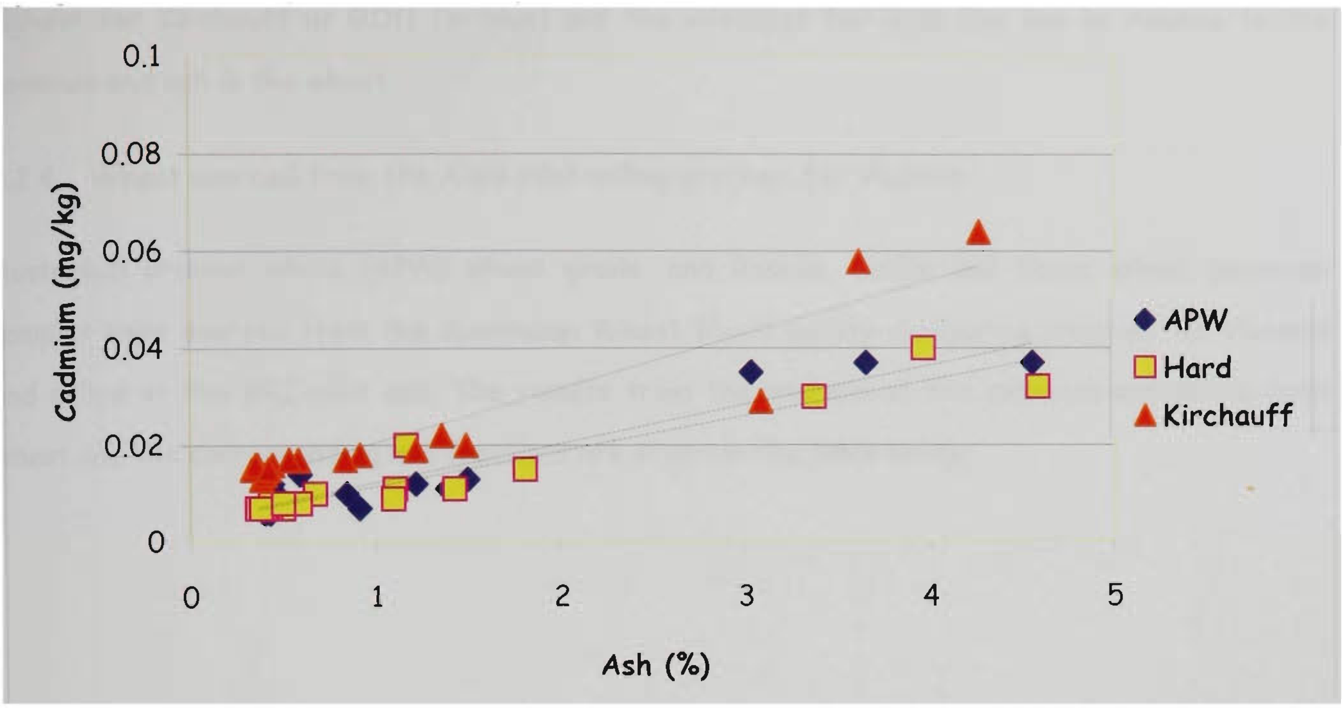


Figure 6.2.3.2 Relationship between cadmium and ash for APW, Hard and Kirchauff wheats grown in South Australia and corresponding mill fractions milled on the BRI pilot mill

The relationship between cadmium levels and ash in the APW, Hard and Kirchauff wheats grown in South Australia can be seen in Figure 6.2.3.2. Linear regression parameters were calculated for the three sets of data using PowerPoint (Microsot, 2002) and are shown in the following table.

Table 6.2.3.2 Linear regression parameters for the relationship between cadmium and ash for wheat and mill fractions from APW and Sunlin wheats grown in South Australia and milled on the BRI pilot mill

Wheat	**Slope**	**Intercept**	**Correlation Coefficient (r)**
APW	0.007	0.004	**0.955**
Hard	0.008	0.004	0.948
Kirchauff	**0.011**	0.009	0.938

ANOVA analysis of the data in table 6.2.3.2 gave a P value of < 0.001 at 16 DF for all three wheats. As P is < 0.001, it can be shown that a very highly significant linear correlation exists at the 99.9% confidence level between cadmium and ash. APW has the highest correlation with an r of 0.955 (in blue). These correlations show that the cadmium concentration in the wheat and corresponding mill fractions can be directly related to the ash content. The slope of the line was highest for Kirchauff at 0.011 (in blue) and the intercept for each line can be related to the cadmium and ash in the wheat.

6.2.4 Wheat sourced from the AWB pilot milling program for Victoria

Australian premier white (APW) wheat grade, and Rosella, Sunlin and Yanac wheat genotype samples were sourced from the Australian Wheat Board quality monitoring program for Victoria and milled at the BRI pilot mill. The results from the analysis of the cadmium and ash in each wheat and the corresponding mill fractions are shown in the table below.

Table 6.2.4.1 Wheat, wheat fractions, cadmium and ash results for APW, Rosella, Sunlin and Yanac wheats sourced from Victoria and milled on the BRI pilot mill

Sample	**APW**		**Rosella**		**Sunlin**		**Yanac**	
	Cadmium (mg/kg)	**Ash (%)**	**Cadmium (mg/kg)**	**Ash (%)**	**Cadmium (mg/kg)**	**Ash (%)**	**Cadmium (mg/kg)**	**Ash (%)**
Wheat	0.006	1.29	0.008	1.18	0.011	1.22	< 0.005	1.13
1st Break	< 0.005	0.45	0.007	0.38	0.008	0.45	< 0.005	0.41
2nd Break	< 0.005	0.40	0.005	0.37	0.011	0.41	< 0.005	0.36
3rd Break	0.005	0.75	0.007	0.55	0.015	0.69	< 0.005	0.61
4th Break	0.005	0.98	0.006	0.83	0.011	1.06	< 0.005	0.83
Break Middlings	0.005	0.44	0.005	0.37	0.010	0.42	0.006	0.39
Sizer	0.006	0.40	0.006	0.37	0.008	0.44	0.006	0.35
B2	0.007	0.60	0.006	0.52	0.010	0.57	0.005	0.46
A	0.006	0.34	0.005	0.33	0.007	0.36	< 0.005	0.33
B	0.008	0.43	0.006	0.35	0.009	0.38	0.008	0.36
C	< 0.005	0.36	0.007	0.36	0.010	0.38	< 0.005	0.38
D	0.005	0.46	0.008	0.52	0.009	0.49	0.005	0.47
E	0.006	1.03	0.008	0.66	0.011	1.05	0.005	0.77
F	0.007	1.33	0.009	0.71	0.013	1.34	0.005	0.67
Pollard Finisher	0.008	1.87	0.010	1.13	0.014	1.69	0.008	1.33
Bran	**0.017**	**4.34**	**0.017**	**3.74**	**0.024**	**4.35**	**0.013**	**3.78**
Pollard	**0.024**	**3.14**	**0.018**	**2.66**	**0.022**	**2.81**	**0.011**	**2.75**
Germ	**0.020**	**3.72**	**0.013**	**2.87**	**0.031**	**3.85**	**0.013**	**3.33**
Offal/Flour Ratio	1.188	**1.707**	**0.758**	1.866	**2.403**	**5.155**	0.841	1.916

Table 6.2.4.1 highlights the relationship between wheat and mill fractions for cadmium level and ash in APW, Rosela, Sunlin and Yanac wheats grown in Western Australia. It can again be seen that a greater proportion of the cadmium in the wheat is fractionated into the bran, pollard and germ fractions than into the various break and roll fractions and this is reflected in the higher ash results for these fractions (all shown in bold). The offal/flour ratios for both cadmium and ash are highest in Sunlin, lowest for cadmium in the Rosella wheat and lowest for ash in the APW (all shown in blue).

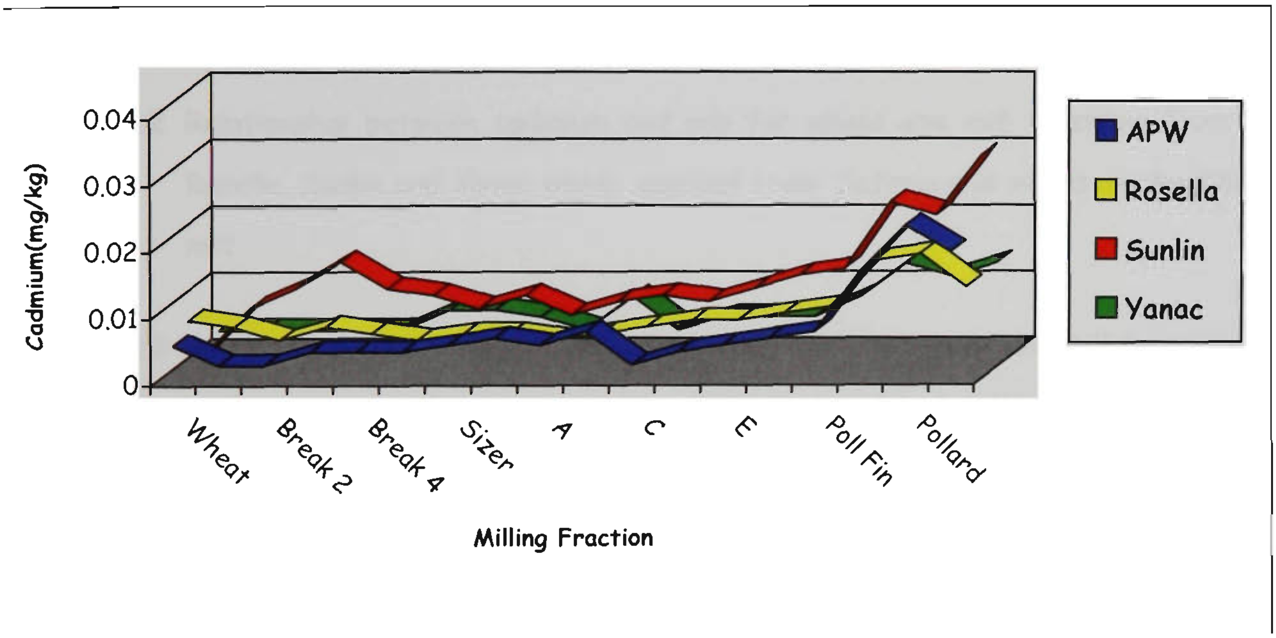


Figure 6.2.4.1 Relationship between cadmium levels, wheat and corresponding mill fractions for APW, Rosella, Sunlin and Yanac wheats grown in Victoria and milled on the BRI Pilot mill

Figure 6.2.4.1 shows the relationship between cadmium levels in APW, Rosella, Sunlin and Yanac wheats grown in Victoria and the corresponding mill fractions produced from the BRI pilot mill. It shows that the cadmium level in the mill fractions from the APW, Rosella and Yanac wheats are reasonably consistent until the pollard finisher where they show a slight increase and then a sharp increase over the bran and pollard with a decrease in the germ germ. The cadmium level in the mill fractions from the Sunlin wheat show a sharp increase from the wheat until the forth break roll, a decrease until the A reduction roll and then a slow increase over the remaining reduction rolls. The level then shows a marked increase over the bran, a decrease over the pollard and a final increase in the germ fraction.

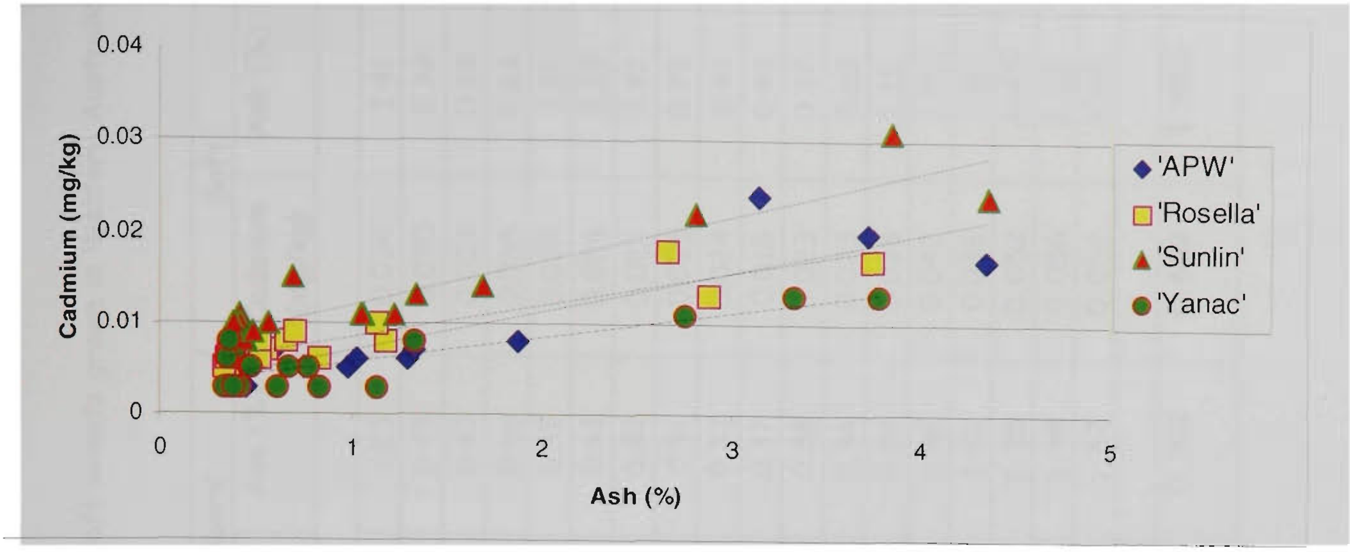


Figure 6.2.4.2 Relationship between cadmium and ash for wheat and mill fractions from APW, Rosella, Sunlin and Yanac wheat sourced from Victoria and milled on the BRI pilot mill

Figure 6.2.4.2 shows the relationship between cadmium and ash for wheat and mill fractions from APW, Rosella, Sunlin and Yanac wheat sourced from Victoria and milled on the BRI pilot mill. Linear regression parameters were calculated for the four sets of data using PowerPoint (Microsoft, 2002) and are shown in the following table.

Table 6.2.4.2 Linear regression parameters for the relationship between cadmium and ash for wheat and mill fractions from APW, Rosella, Sunlin and Yanac wheats grown in Victoria and milled on the BRI pilot mill

Wheat	**Slope**	**Intercept**	**Correlation Coefficient (r)**
APW	0.004	0.003	0.888
Rosella	0.004	0.005	0.928
Sunlin	0.005	0.007	**0.934**
Yanac	0.003	0.003	0.866

ANOVA analysis of the data in table 6.2.4.2 gave a P value of < 0.001 at 16 DF for all four wheats. As P is < 0.001, it can be shown that a very highly significant linear correlation exists at the 99.9% confidence level between cadmium and ash. Sunlin has the highest correlation with an r of 0.934 (in blue). These correlations show that the cadmium concentration in the wheat and corresponding mill fractions can be directly related to the ash content. The slopes are not significantly different and the intercept for each line can be related to the cadmium and ash in the wheat.

6.2.5 Wheat sourced from the AWB pilot milling program for Western Australia

Table 6.2.5.1 Wheat, wheat fractions, cadmium and ash results for Hard, Cadoux, Eradu, Halberd and Soft wheats grown in Western Australia and milled on the BRI pilot mill

Sample	Hard		Cadoux		Eradu		Halberd		Soft	
	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)	Cadmium (mg/kg)	Ash (%)
Wheat	0.053	1.30	0.029	1.11	0.024	1.46	0.022	1.25	0.036	1.41
1st Break	0.028	0.43	0.024	0.37	0.023	0.39	0.013	0.48	0.030	0.39
2nd Break	0.028	0.79	0.034	0.38	0.014	0.38	0.013	0.43	0.029	0.38
3rd Break	0.030	0.45	0.032	0.51	0.021	0.67	0.016	0.66	0.035	0.64
4th Break	0.033	1.31	0.031	0.69	0.018	1.16	0.014	0.98	0.024	1.12
Break Middlings	0.028	0.47	0.031	0.38	0.019	0.39	0.012	0.43	0.024	0.39
Sizer	0.033	0.40	0.036	0.36	0.016	0.47	0.013	0.42	0.023	0.47
B2	0.033	0.66	0.042	0.66	0.017	0.88	0.014	0.51	0.025	0.75
A	0.030	0.36	0.035	0.35	0.017	0.40	0.011	0.37	0.024	0.41
B	0.028	0.42	0.036	0.41	0.017	0.51	0.012	0.37	0.018	0.49
C	0.029	0.42	0.037	0.35	0.025	0.40	0.013	0.38	0.023	0.37
D	0.027	0.50	0.041	0.54	0.017	0.63	0.014	0.46	0.023	0.60
E	0.035	1.38	0.053	1.18	0.019	1.71	0.016	0.84	0.038	1.35
F	0.039	1.15	0.039	1.24	0.025	1.93	0.019	0.96	0.042	1.70
Pollard Finisher	0.042	2.15	0.036	1.22	0.022	1.80	0.020	1.60	0.036	1.66
Bran	**0.060**	**4.42**	**0.072**	**3.21**	**0.037**	**1.46**	**0.048**	**1.25**	**0.052**	**4.40**
Pollard	**0.053**	**3.37**	**0.079**	**3.02**	**0.048**	**1.39**	**0.046**	**1.48**	**0.056**	**3.22**
Germ	**0.071**	**3.91**	**0.077**	**3.06**	**0.043**	**1.38**	**0.053**	**1.43**	**0.052**	**3.46**
Offal/Flour Ratio	**0.623**	**1.612**	**0.675**	**1.613**	**0.711**	**0.541**	**1.103**	**0.702**	**0.609**	**1.550**

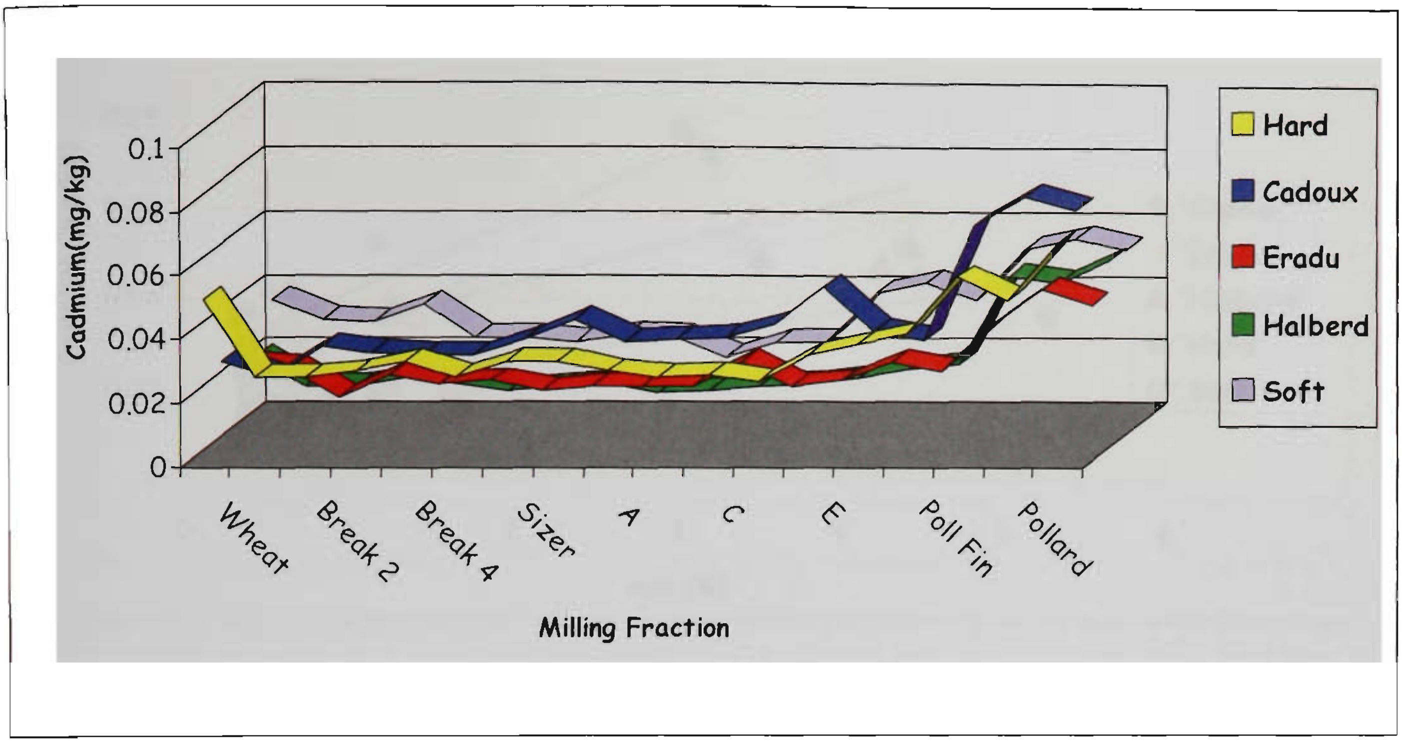


Figure 6.2.5.1 Relationship between cadmium levels in wheat and corresponding milling fractions for wheat grown in Western Australia and milled on the BRI pilot mill.

Figure 6.2.5.1 shows the relationship between cadmium levels in the five wheats grown in Western Australia and the corresponding mill fractions produced from the BRI pilot mill. It shows that the cadmium level in the mill fractions from the Cadoux, Eradu, Halberd and Soft wheats are reasonably consistent until the pollard finisher where the Halberd and Soft wheats show a slight increase and the Cadoux and Eradu show a sharp increase. The four wheats then show a sharp increase over the bran and pollard with a decrease in the germ. The cadmium level in the mill fractions from the Hard wheat show a sharp decrease from the wheat then consistent until the D reduction roll, then a slow increase over the remaining rolls for the bran and pollard with a final decrease in the germ fraction.

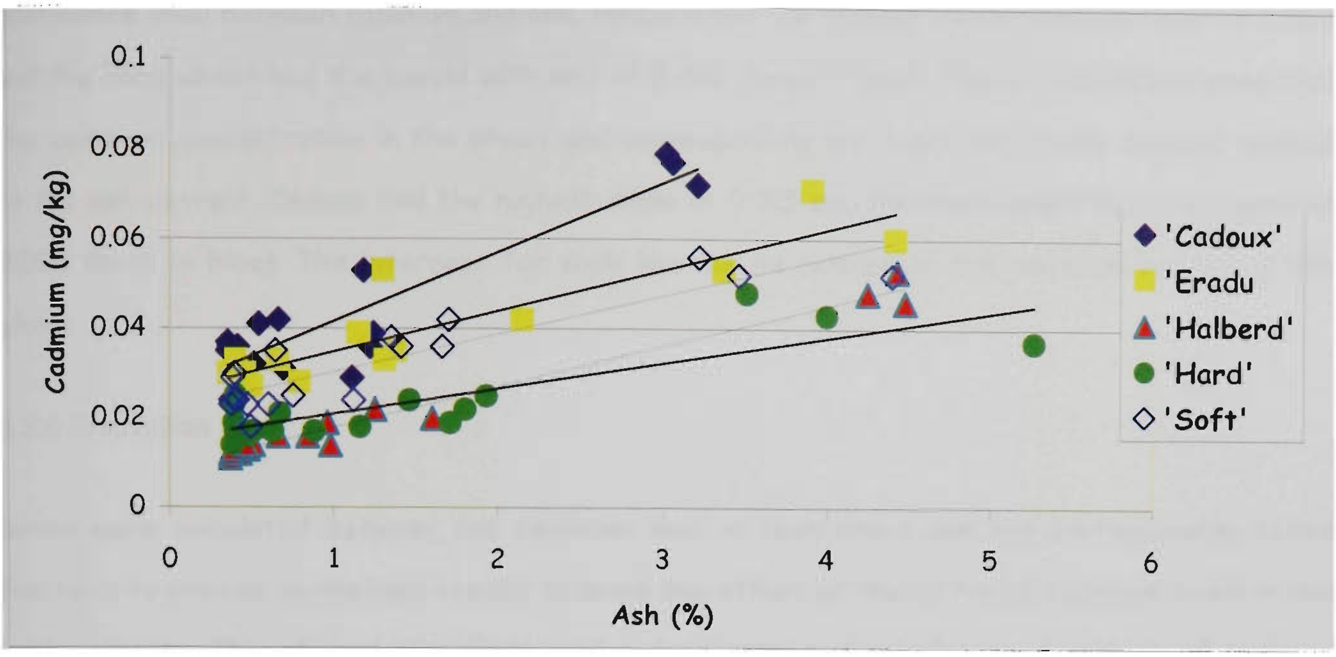


Figure 6.2.5.2 Relationship between cadmium and ash levels for wheat and corresponding mill fractions from the Cadoux, Eradu, Halberd, Hard and Soft wheats grown in Western Australia and milled on the BRI pilot mill

Figure 6.2.5.2 shows the relationship between cadmium and ash for wheat and mill fractions from Cadoux, Eradu, Halberd, Hard and Soft wheats sourced from Western Australia and milled on the BRI pilot mill. Linear regression parameters were calculated for the five sets of data using PowerPoint (Microsoft, 2002) and are shown in the following table.

Table 6.2.5.2 Linear regression parameters for the relationship between cadmium and ash for wheat and mill fractions from Cadoux, Eradu, Halberd, Hard and Soft wheats grown in Western Australia and milled on the BRI pilot mill

Wheat	**Slope**	**Intercept**	**Correlation Coefficient (r)**
Cadoux	0.015	0.026	0.910
Eradu	0.009	0.026	0.920
Halberd	0.009	0.009	0.989
Hard	0.006	0.015	0.846
Soft	0.009	0.022	0.902

ANOVA analysis of the data in table 6.2.5.2 gave a P value of < 0.001 at 16 DF for all five wheats. As P is < 0.001, it can be shown that a very highly significant linear correlation exists at the 99.9%

confidence level between cadmium and ash. Halberd has the highest correlation with an r of 0.989 and the hard wheat had the lowest with an r of 0.846 (both in blue). These correlations show that the cadmium concentration in the wheat and corresponding mill fractions can be directly related to the ash content. Cadoux had the highest slope at 0.015 and the Hard wheat had the lowest at 0.006 (both in blue). The intercept for each line can be related to the cadmium and ash in the wheat.

6.2.6 Discussion

Ratios were calculated between the cadmium level in each wheat and the corresponding milled fractions to provide normalised results to avoid the effect of the differing cadmium levels in the twelve wheats. This allowed the plotting of a dendogram and calculation of analysis of variance between the results for the states. The ratios are detailed in Table 6.2.6.1.

The highest ratios overall are for the bran, pollard and germ fractions (all shown in blue). This is expected because other work in this study shows that the cadmium is located in the aleurone layer and is fractionated into these offal fractions during milling. Yanac wheat grown in Victoria had the highest ratios for bran and germ (4.333 for both bran and germ) with Sunlin grown in New South Wales and APW grown in Victoria having the equal highest ratio for pollard (4.000). Hard wheat from Western Australia had the lowest ratios for bran, pollard and germ (1.132, 1.000 and 1.340 respectively).

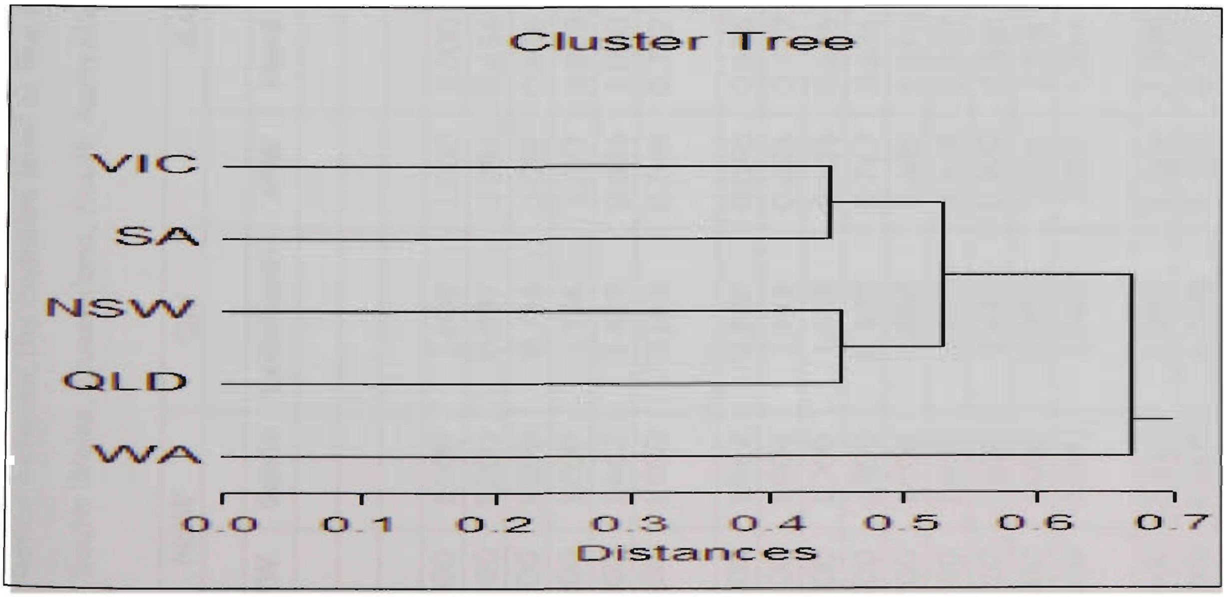


Figure 6.2.6.1 Hierarchical cluster dendogram (centroid linkage and Euclidean distance) of cadmium results for all fractions in the BRI pilot milling trial by state

Table 6.2.6.1 Ratios between the cadmium level in the wheat and the cadmium level in the corresponding milling fraction from wheats grown in New South Wales, Queensland, South Australia, Victoria and Western Australia and milled at the BRI Pilot mill

State	NSW		Qld	SA			Vic				WA				
Wheat	**APW**	**Sunlin**	**Leichhardt**	**APW**	**Hard**	**Kirchauff**	**APW**	**Rosella**	**Sunlin**	**Yanac**	**Hard**	**Cadoux**	**Eradu**	**Halberd**	**Soft**
Fraction															
Wheat	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
1st Break	0.600	1.000	0.857	1.091	0.636	0.727	0.500	0.875	0.727	1.000	0.528	0.828	0.958	0.591	0.833
2nd Break	1.000	1.000	0.714	1.091	0.636	0.591	0.500	0.625	1.000	1.000	0.528	1.172	0.583	0.591	0.806
3rd Break	1.200	1.000	1.714	1.273	0.909	0.773	0.833	0.875	1.364	1.000	0.566	1.103	0.875	0.727	0.972
4th Break	1.000	1.667	1.429	0.636	1.000	0.818	0.833	0.750	1.000	1.000	0.623	1.069	0.750	0.636	0.667
Break Middlings	1.200	1.000	1.143	0.546	0.727	0.636	0.833	0.625	0.909	2.000	0.528	1.069	0.792	0.546	0.667
Sizer	1.000	1.667	0.857	0.546	0.636	0.727	1.000	0.750	0.727	2.000	0.623	1.241	0.667	0.591	0.639
B2	1.200	1.000	1.143	0.636	0.727	0.773	1.167	0.750	0.909	1.667	0.623	1.448	0.708	0.636	0.694
A	1.200	1.000	1.143	0.636	0.636	0.727	1.000	0.625	0.636	1.000	0.566	1.207	0.708	0.500	0.667
B	1.200	1.000	0.429	0.727	0.636	0.682	1.333	0.750	0.818	2.667	0.528	1.241	0.708	0.546	0.500
C	1.000	1.000	0.857	0.636	0.636	0.727	0.500	0.875	0.909	1.000	0.547	1.276	1.042	0.591	0.639
D	1.200	1.000	0.857	0.818	0.727	0.682	0.833	1.000	0.818	1.667	0.509	1.414	0.708	0.636	0.639
E	1.200	1.000	0.429	0.909	0.818	0.773	1.000	1.000	1.000	1.667	0.660	1.828	0.792	0.727	1.056
F	1.000	1.000	0.857	1.091	1.818	0.864	1.167	1.125	1.182	1.667	0.736	1.345	1.042	0.864	1.167
Pollard Finisher	2.000	2.000	1.000	1.182	1.364	0.909	1.333	1.250	1.273	2.667	0.793	1.241	0.917	0.909	1.000
Bran	**2.400**	**2.667**	**2.571**	**3.364**	**2.909**	**2.909**	**2.833**	**2.125**	**2.182**	**4.333**	**1.132**	**2.483**	**1.542**	**2.182**	**1.444**
Pollard	**3.200**	**4.000**	**3.143**	**3.182**	**2.727**	**1.318**	**4.000**	**2.250**	**2.000**	**3.667**	**1.000**	**2.724**	**2.000**	**2.091**	**1.556**
Germ	**2.400**	**3.333**	**1.571**	**3.364**	**3.636**	**2.636**	**3.333**	**1.625**	**2.818**	**4.333**	**1.340**	**2.655**	**1.792**	**2.409**	**1.444**

ANOVA analysis of the data in Tables 6.2.1.1, 6.2.1.1, 6.2.3.1, 6.2.4.1 and 6.2.5.1 gives a P value of < 0.01 and indicates that a highly significant difference exists at the 99% confidence level between all the states. The dendogram in Figure 6.2.6.1 shows that Victoria and South Australia form one group, New South Wales and Queensland form another with Western Australia as the last group.

6.3 Wheat and corresponding mill fractions sourced from commercial flourmills in Victoria and Western Australia

Samples of ASW and Hard wheat and corresponding mill fractions were sourced from commercial flourmills in Victoria and Western Australia. The mills were run at normal commercial settings and samples were taken at the sampling points shown in Figures 3.4.3.1 and 3.4.3.2. The wheat and mill fractions were analysed for cadmium and ash content and the results are shown in the following table.

6.3.1 Victoria

Table 6.3.1.1 Cadmium and ash results for wheat and corresponding mill fractions for ASW and Hard wheat milled at a Victorian commercial flourmill.

Milling Fraction	**ASW**		**Hard**	
	Cadmium (mg/kg)	**Ash (%)**	**Cadmium (mg/kg)**	**Ash (%)**
Wheat	0.008	1.35	0.013	1.32
B1	< 0.005	0.30	< 0.005	0.28
B2	< 0.005	0.33	0.005	0.29
B3	< 0.005	0.51	0.005	0.46
A	< 0.005	0.27	0.005	0.23
B	< 0.005	0.28	0.005	0.23
C	< 0.005	0.29	0.007	0.25
D	< 0.005	0.35	0.007	0.38
E	< 0.005	0.38	0.010	0.38
F	0.005	0.59	0.012	0.55
G	0.008	1.33	0.010	1.27
X	0.010	1.56	0.015	1.63
Pollard	**0.016**	**4.37**	**0.032**	**4.42**
Germ	**0.021**	**3.88**	**0.040**	**3.91**
Offal/Flour Ratio	2.563	4.339	**3.039**	**4.964**

Table 6.3.1.1 highlights the relationship between wheat and mill fractions for cadmium level and ash in ASW and Hard wheats grown Victoria. It can be seen that a greater proportion of the cadmium in the wheat is fractionated into the pollard and germ fractions than into the various break and roll fractions and this is reflected in the higher ash results for these fractions (all shown in blue). The offal/flour ratios for both cadmium and ash are highest in the Hard wheat (in blue).

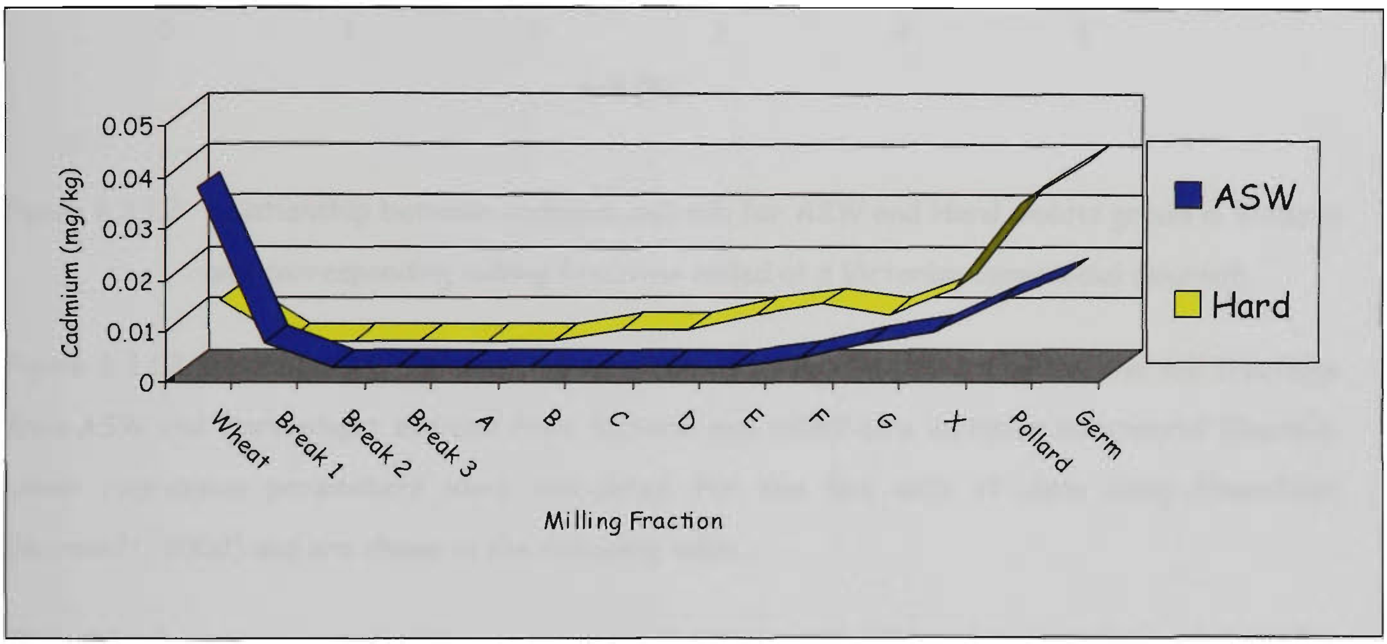


Figure 6.3.1.1 Relationship between cadmium levels in wheat and corresponding milling fractions for wheat milled at a Victorian commercial flourmill.

Figure 6.3.1.1 shows the relationship between cadmium levels in the two wheats grown in Victoria and the corresponding mill fractions produced from the Victoria commercial flourmill. It shows that the cadmium level in the mill fractions from the ASW wheat shows a sharp decrease from the wheat, it is then reasonably consistent until the pollard and germ where there is a smooth rise. The cadmium level in the mill fractions from the Hard wheat shows a slight decrease from the wheat, then it is reasonably consistent until the pollard and germ, where it shows a sharp rise.

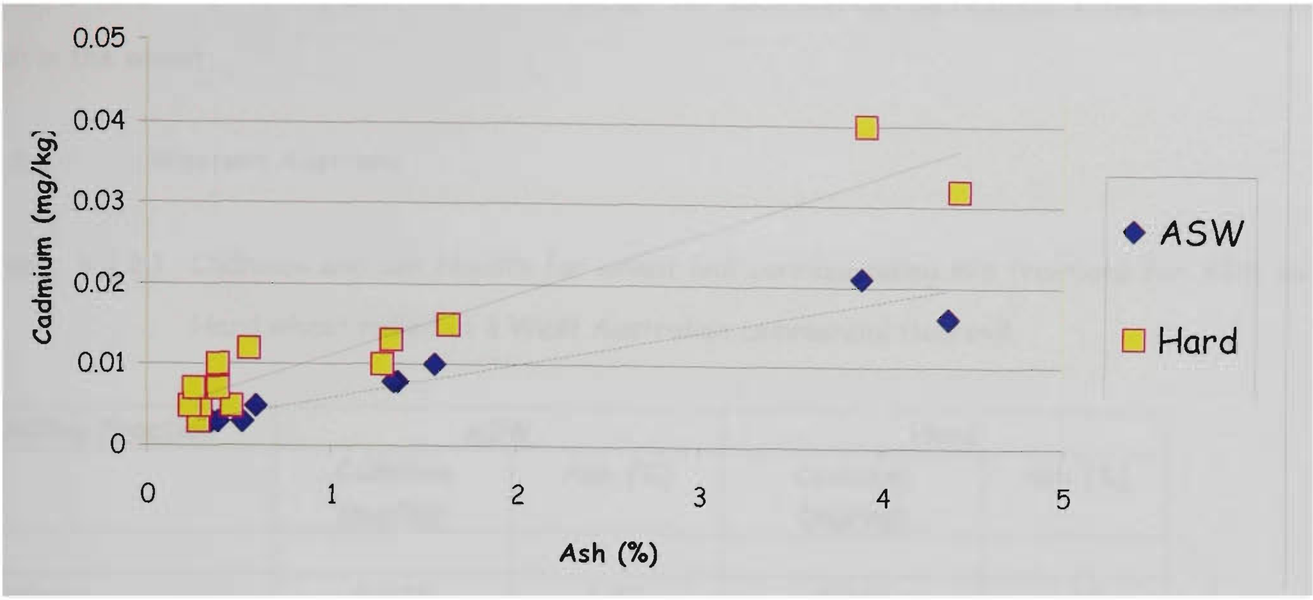


Figure 6.3.1.2 Relationship between cadmium and ash for ASW and Hard wheats grown in Victoria and corresponding milling fractions milled at a Victorian commercial flourmill

Figure 6.3.1.2 illustrates the relationship between cadmium and ash for wheat and mill fractions from ASW and Hard wheat sourced from Victoria and milled at a Victorian commercial flourmill. Linear regression parameters were calculated for the two sets of data using PowerPoint (Microsoft, 2002) and are shown in the following table.

Table 6.3.1.2 Linear regression parameters for the relationship between cadmium and ash for wheat and mill fractions from ASW and Hard wheats grown in Victoria and milled at a Victorian commercial flourmill

Wheat	**Slope**	**Intercept**	**Correlation Coefficient (r)**
ASW	0.004	0.002	**0.963**
Hard	**0.008**	0.004	0.9955

ANOVA analysis of the data in table 6.3.1.2 gave a P value of < 0.001 at 12 DF for both wheats. As P is < 0.001, it can be shown that a very highly significant linear correlation exists at the 99.9% confidence level between cadmium and ash. ASW has the highest correlation with an r of 0.963 (in blue). These correlations show that the cadmium concentration in the wheat and corresponding mill fractions can be directly related to the ash content. The slope of the Hard wheat at 0.008 (in blue) is twice that of the Soft and the intercept for each line can be related to the cadmium and ash in the wheat.

6.3.2 Western Australia

Table 6.3.2.1 Cadmium and ash results for wheat and corresponding mill fractions for ASW and Hard wheat milled at a West Australian commercial flourmill.

Milling Fraction	**ASW**		**Hard**	
	Cadmium (mg/kg)	**Ash (%)**	**Cadmium (mg/kg)**	**Ash (%)**
Wheat	0.038	1.32	0.043	1.43
B1	0.015	0.33	0.021	0.30
B2	0.016	0.32	0.020	0.82
B3	0.019	0.46	0.021	0.41
A	0.010	0.28	0.020	0.33
B	0.012	0.30	0.020	0.36
C	0.012	0.30	0.022	0.35
D	0.010	0.32	0.020	0.47
E	0.018	0.57	0.026	1.25
F	0.020	0.61	0.024	1.09
G	0.021	1.25	0.032	1.18
X	0.020	1.30	0.035	1.23
Pollard	**0.045**	**4.11**	**0.057**	**4.29**
Germ	**0.055**	**3.94**	**0.069**	**4.04**
Offal/Flour Ratio	**1.935**	**4.462**	1.712	3.791

Table 6.3.2.1 highlights the relationship between wheat and mill fractions for cadmium level and ash in ASW together with Hard wheats grown in Western Australia. It can be seen that a greater proportion of the cadmium in the wheat is fractionated into the pollard and germ fractions than into the various break and roll fractions, which is reflected in the higher ash results for these fractions (all shown in blue). The offal/flour ratios for both cadmium and ash are highest in the ASW wheat (1.935 and 4.462) (both in blue).

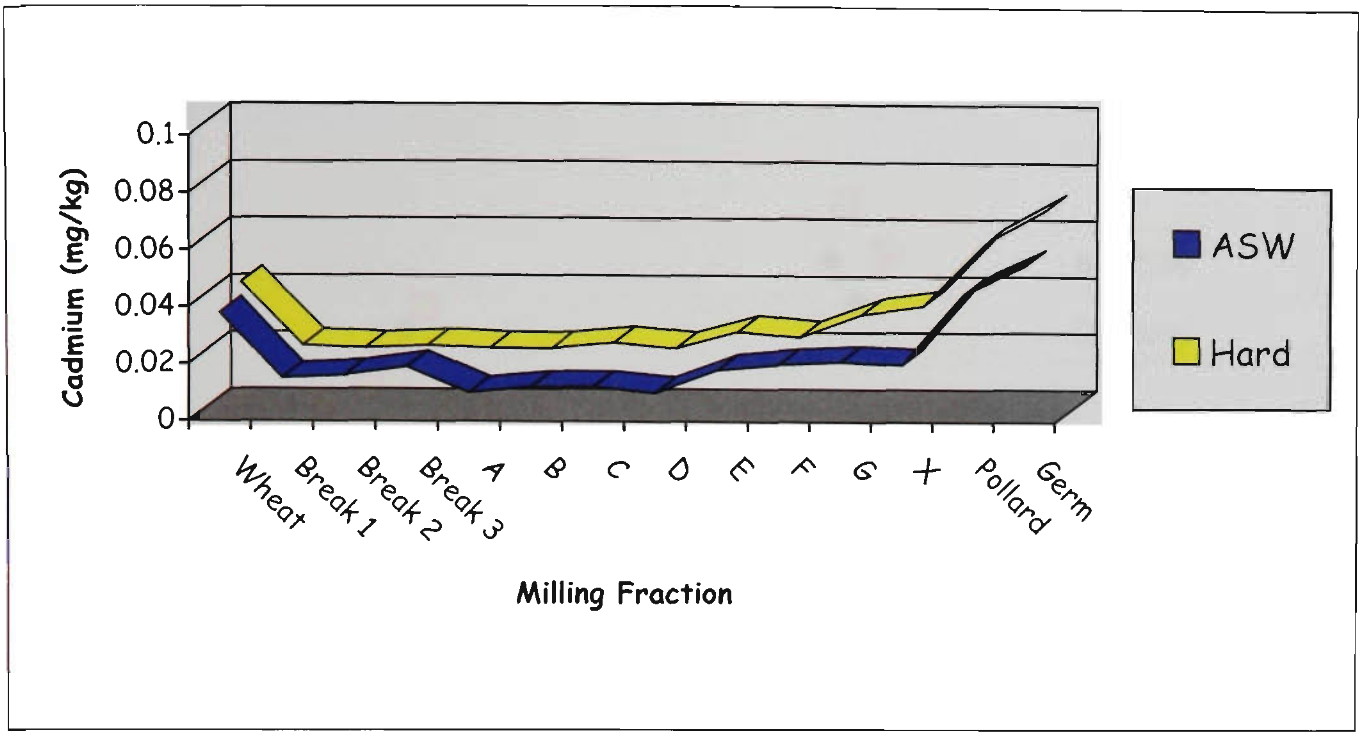


Figure 6.3.2.1 Relationship between cadmium, wheat and corresponding milling fractions for ASW and hard wheat grown in Western Australia and milled at a Western Australian commercial flourmill.

Figure 6.3.2.1 shows the relationship between cadmium levels in the two wheats grown in Western Australia and the corresponding mill fractions produced from the Western Australian commercial flourmill. It shows that the cadmium level in the mill fractions from both the ASW and Hard wheats show a decrease from the wheat and then are reasonably consistent until the D reduction roll they begin to show a smooth rise. The fractions beginning at the X reduction roller begin to show a sharp rise that is continued over the pollard and the germ.

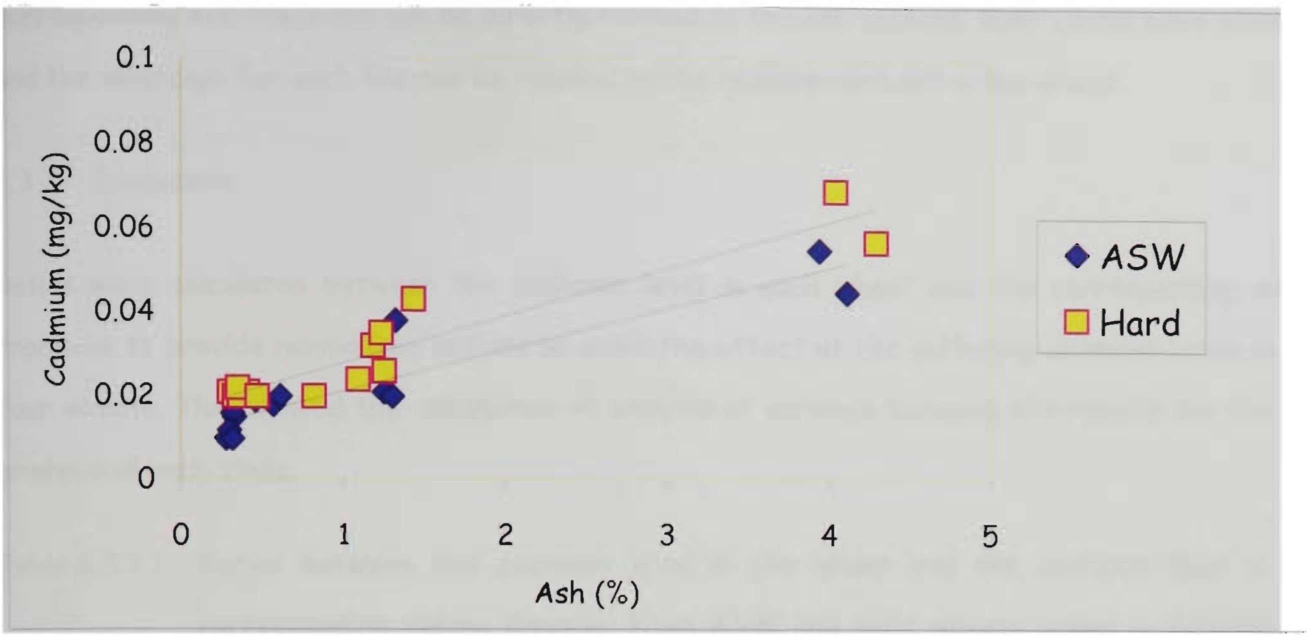


Figure 6.3.2.2 Relationship between cadmium and ash levels for ASW and Hard wheat grown in Western Australia and corresponding milling fractions milled at a Western Australian commercial flourmill

Figure 6.3.2.2 shows the relationship between cadmium and ash for wheat and mill fractions from ASW and Hard wheat sourced from Western Australia and milled at a Western Australian commercial flourmill. Linear regression parameters were calculated for the two sets of data using PowerPoint (Microsoft, 2002) and are shown in the following table.

Table 6.3.2.2 Linear regression parameters for the relationship between cadmium and ash for wheat and mill fractions from ASW and Hard wheats grown in Western Australia and milled at a Western Australian commercial flourmill

Wheat	**Slope**	**Intercept**	**Correlation Coefficient (r)**
ASW	0.010	0.011	0.927
Hard	0.010	0.017	**0.946**

ANOVA analysis of the data in table 6.3.2.2 gave a P value of < 0.001 at 12 DF for both wheats. As P is < 0.001, it can be shown that a very highly significant linear correlation exists at the 99.9% confidence level between cadmium and ash. The Hard wheat has the highest correlation with an r of 0.946 (in blue). These correlations show that the cadmium concentration in the wheat and corresponding mill fractions can be directly related to the ash content. Both slopes were identical and the intercept for each line can be related to the cadmium and ash in the wheat.

6.3.3 Discussion

Ratios were calculated between the cadmium level in each wheat and the corresponding milled fractions to provide normalised results to avoid the effect of the differing cadmium levels in the four wheats. This allowed the calculation of analysis of variance between the results for the two grades and each state.

Table 6.3.3.1 Ratios between the cadmium level in the wheat and the cadmium level in the corresponding milling fraction from ASW and hard wheats grown in Victoria and Western Australia and milled at commercial flourmills

Grade	**ASW**		**Hard**	
State	**Victoria**	**Western Australia**	**Victoria**	**Western Australia**
B1	0.375	0.395	0.231	0.488
B2	0.375	0.421	0.385	0.465
B3	0.375	0.500	0.385	0.488
A	0.375	0.263	0.381	0.465
B	0.375	0.316	0.385	0.465
C	0.375	0.316	0.539	0.512
D	0.375	0.263	0.539	0.465
E	0.375	0.474	0.769	0.605
F	0.625	0.526	0.923	0.558
G	1.000	0.553	0.769	0.744
X	1.250	0.526	1.154	0.814
Offal	**2.000**	**1.184**	**2.462**	**1.326**
Germ	**2.625**	**1.447**	**3.077**	**1.605**

The highest ratios in Table 6.3.3.1 are for Hard (2.462 for offal and 3.077 for germ) from Victoria and the lowest are for offal (1.184 for ASW and 1.326 for Hard) from Western Australia (all in blue). With a P value of 0.000, there is a significant difference between the offal and germ ratios for Victoria and Western Australia. In addition, there is also a significant difference between each of the two samples of ASW and Hard from each state.

It can be seen from all the tables and figures in this chapter that there is a highly significant relationship (P <0.01 or less in all cases) between cadmium and ash levels in the wheat and fractions from milling all grades and genotypes of wheat and from all states. This relationship is somewhat expected as cadmium and most other minerals that make up the ash are deposited in the aleurone layer during grain filling and are removed together with the other bran layers during milling.

Chapter 7 Cadmium and Dietary Fibre Results from the Analysis of Bread Products

7.1 Loaf bread

7.1.1 White loaf bread

7.1.1.1 White loaf bread sourced from Werribee (Victoria) food outlets

Twenty three samples of white loaf bread were purchased from a major supermarket and two small bakery outlets in Werribee, Victoria and analysed for cadmium and dietary fibre, the results are given in Table 7.1.1.1.

Table 7.1.1.1 Results for cadmium and dietary fibre levels for samples of white loaf bread sourced from Werribee (Victoria) food outlets

Supplier	Type	Dietary fibre (%)	Cadmium (mg/kg)
Bakers Delight		3.1	0.005
Bakers Delight	HyFibre	3.0	0.005
Bakery		5.9	0.005
Bakery		5.2	**0.007**
Burgen		3.6	< 0.005
Burgen	Soy & Linseed	6.8	0.010
Helga's		3.7	< 0.005
Helga's	Multigrain	5.7	0.005
Quality Bakers		3.0	< 0.005
Quality Bakers	Multigrain	5.0	0.006
Quality Bakers	Soy & Linseed	5.1	**0.007**
Quality Bakers	Wonder White	5.7	< 0.005
Sunicrust		**2.7**	< 0.005
Sunicrust	HyFibre	5.4	< 0.005
Sunicrust	Multigrain	4.3	**0.007**
Tip Top		3.0	< 0.005
Tip Top		5.0.	< 0.005
Tip Top	HyFibre	5.8	< 0.005
Tip Top	Multigrain	**7.4**	0.005
Tip Top	Multigrain	4.7	< 0.005
Uncle Tobys	Multigrain	4.6	< 0.005
Uncle Tobys	Soy & Linseed	7.1	**0.007**
Uncle Tobys	VitaGold	**7.4**	< 0.005

The data in Table 7.1.1.1 shows the relationship between cadmium and dietary fibre in white loaf bread purchased from Werribee (Victoria) food outlets. It can be seen that the dietary fibre ranges from a minimum of 2.7% for a Sunicrust bread (in blue) to a maximum of 7.4% for a Tip Top Multigrain bread and an Uncle Tobys VitaGold bread (both in blue). The results are normal for white loaf breads.

All cadmium values are reasonably low and these values would be expected from white bread made from flour milled from wheat grown in Victoria (see Table 6.3.1.1). The minimum value shown was less than 0.005 mg/kg, whereas the maximum was 0.007 mg/kg for bread from Sunicrust Multigrain bread and Uncle Tobys Soy and Linseed bread (both in blue).

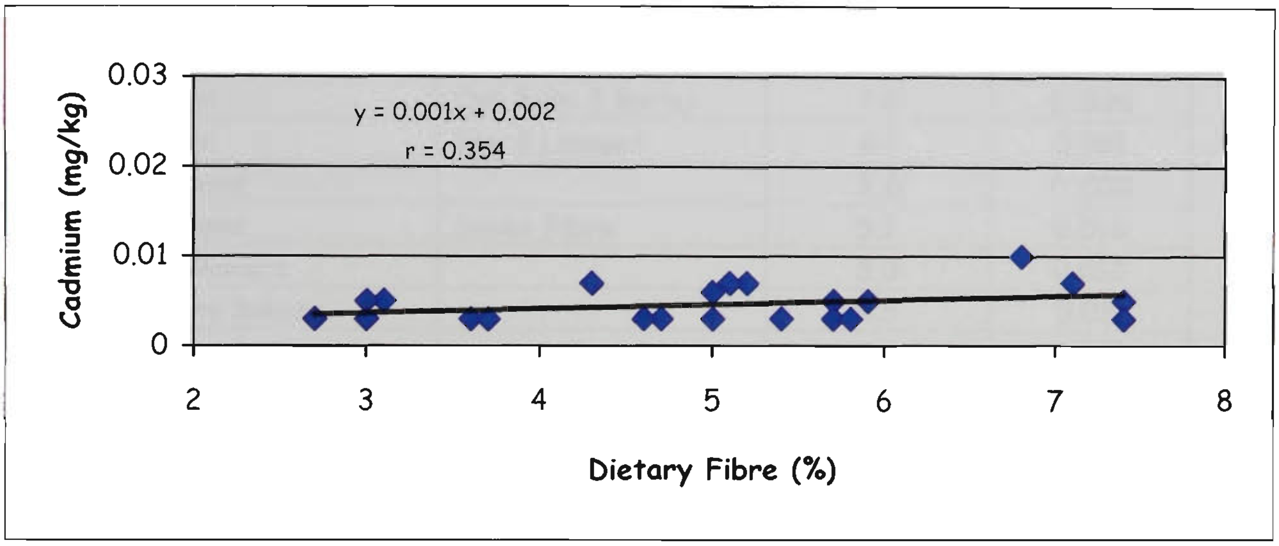


Figure 7.1.1.1 Relationship between cadmium and dietary fibre levels for samples of white loaf bread sourced from Werribee (Victoria) food outlets

ANOVA analysis of the data in Table 7.1.1.1 gave a P value of 0.049 at 21 DF. As P is < 0.05, it can be shown that a significant linear correlation exists at the 95% confidence level between cadmium and dietary fibre levels for samples of white loaf bread sourced from Werribee (Victoria) food outlets. With a R^2 value of 0.125, it can be also shown that only 12.5% of the cadmium concentration can be predicted from the dietary fibre level.

7.1.1.2 White loaf bread sourced from Fremantle (Western Australia) food outlets

Twenty-four samples of white loaf bread were purchased from a major supermarket and three small bakery outlets in Fremantle, Western Australia and analysed for cadmium and dietary fibre, the results are given in Table 7.1.1.2.

Table 7.1.1.2 Results for cadmium and dietary fibre levels for white loaf bread sourced from Fremantle (West Australia) food outlets

Supplier	**Type**	**Dietary fibre (%)**	**Cadmium (mg/kg)**
Bakers	Fibre Enriched	5.1	0.015
Bakery		4.7	0.010
Bakery		7.3	0.011
Bakery		5.3	0.012
Bakery	Soy & Linseed	6.4	0.015
Burgen	Granary Malt	5.0	0.010
Burgen	Oat Bran & Barley	7.0	**0.020**
Burgen	Soy & Linseed	6.1	0.019
Farmland		**3.0**	**0.009**
Farmland	Super Fibre	5.7	0.010
Mias Bakers		5.0	0.012
Quality Bakers		3.5	0.011
Quality Bakers		3.4	0.019
Quality bakers	Multigrain	3.6	0.011
Quality Bakers	Soy & Linseed	5.8	**0.009**
Quality Bakers	Soy & Linseed	5.1	0.010
Quality Bakers	Wonder White	5.7	**0.020**
Tip Top		**3.0**	0.010
Tip Top		5.8	0.010
Tip Top	Multigrain	4.7	0.014
Tip Top	Multigrain	6.2	0.019
Uncle Tobys	Multigrain	4.6	0.010
Uncle Tobys	Multigrain	6.4	0.012
Uncle Tobys	VitaGold	**7.4**	0.019

The data in Table 7.1.1.2 shows the relationship between cadmium and dietary fibre in white loaf bread purchased from Fremantle (Western Australia) food outlets. It can be seen that the dietary fibre ranges from a minimum of 3.0% for a Farmland and a Tip Top bread (both in blue) to a maximum of 7.4% for an Uncle Tobys VitaGold bread (in blue). The results are also normal for white loaf breads.

These cadmium values would be expected from white bread made from flour milled from wheat grown in Western Australia (see Table 6.3.2.1). The minimum value shown was 0.009 mg/kg for a Quality Bakers Soy and Linseed bread and the maximum was 0.020 mg/kg for a Quality Bakers Wonder White bread (both in blue).

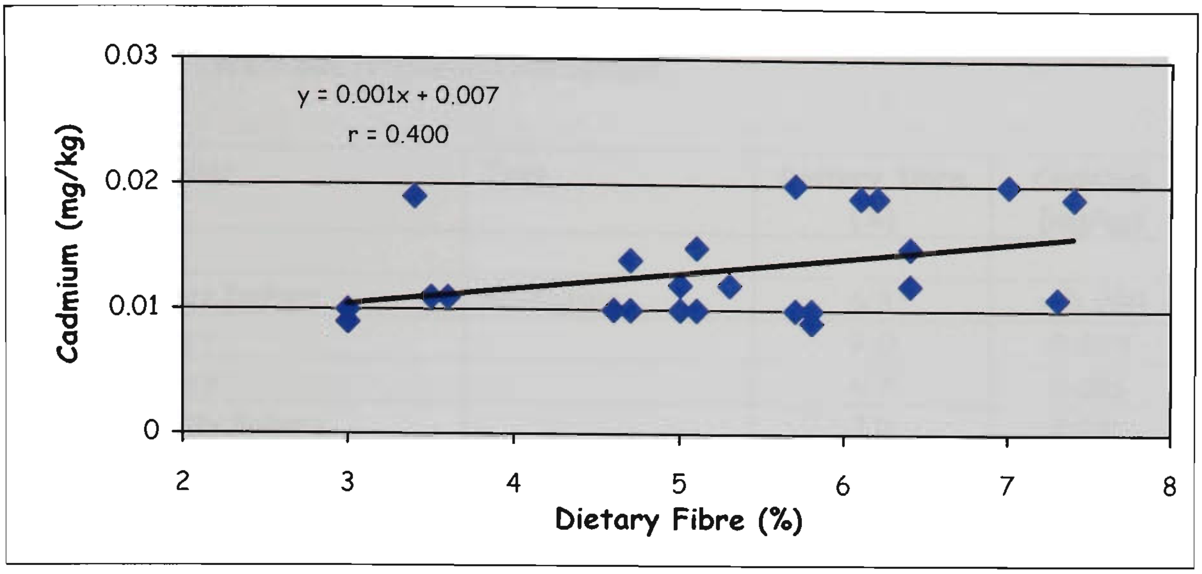


Figure 7.1.1.2 Relationship between cadmium and dietary fibre levels for white loaf bread sourced from Fremantle (Western Australia) food outlets

ANOVA analysis of the data in Table 7.1.1.2 gave a P value of 0.026 at 22 DF. As P is < 0.05, it can be shown that a significant linear correlation exists at the 95% confidence level between cadmium and dietary fibre levels for samples of white loaf bread sourced from Fremantle (Western Australia) food outlets. With a R^2 value of 0.160, it can also be shown that only 16.0% of the cadmium concentration can be predicted from the dietary fibre level.

7.1.2 Wholemeal loaf bread

7.1.2.1 Wholemeal loaf bread sourced from Werribee (Victoria) food outlets

Eight samples of wholemeal bread were purchased from a major supermarket and two small bakery outlets in Werribee, Victoria.

Table 7.1.2.1 Results for cadmium and dietary fibre levels for wholemeal loaf bread from Werribee (Victoria) food outlets

Supplier	**Type**	**Dietary fibre (%)**	**Cadmium (mg/kg)**
Bakers Delight	Multigrain	6.3	< 0.005
Bakery		9.0	0.015
Helga's		6.7	0.006
Quality Bakers		7.0	0.010
Quality Bakers		6.9	0.011
Sunicrust		6.0	< 0.005
Sunicrust		6.2	0.007
Tip Top	With Germ	6.8	0.006

The data in Table 7.1.2.1 shows the relationship between cadmium and dietary fibre in wholemeal loaf bread purchased from Werribee (Victoria) food outlets. It can be seen that the dietary fibre ranges from a minimum of 6.2% for a Sunicrust bread to a maximum of 9.0% for a bread from a small bakery outlet (both in blue).

The cadmium values show a wide range from a minimum of < 0.005 mg/kg for Bakers Delight and Sunicrust breads to a maximum of 0.015 mg/kg for bread from a small bakery outlet (both in blue).

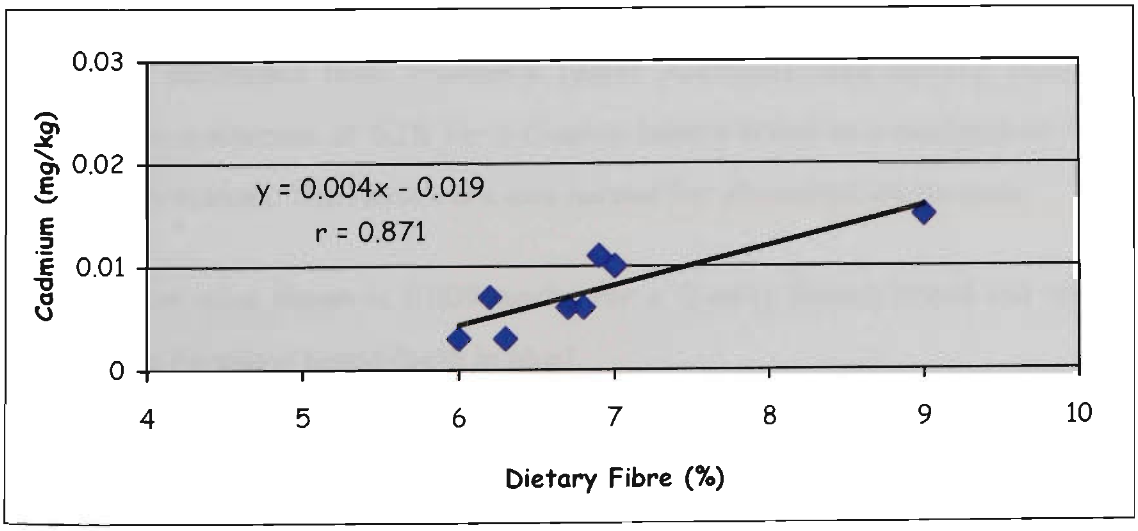


Figure 7.1.2.1 Relationship between cadmium and dietary fibre levels for wholemeal loaf bread sourced from Werribee (Victoria) food outlets

ANOVA analysis of the data in Table 7.1.2.1 gave a P value of 0.002 at 6 DF. As $P < 0.01$, it can be shown that a highly significant linear correlation exists at the 99% confidence level between cadmium and dietary fibre levels for samples of wholemeal loaf bread sourced from Werribee (Victoria) food outlets. With a R^2 value of 0.759, it can also be shown that 75.9% of the cadmium concentration can be predicted from the dietary fibre level.

7.1.2.2 Wholemeal loaf bread sourced from Fremantle (Western Australia) food outlets

Eight samples of wholemeal loaf bread were purchased from a major supermarket in Fremantle, Western Australia

Table 7.1.2.2 Results for cadmium and dietary fibre levels for wholemeal loaf bread sourced from Fremantle (Western Australia) food outlets

Supplier	**Type**	**Dietary fibre (%)**	**Cadmium (mg/kg)**
De Campo		7.1	0.016
Farmland		9.0	**0.021**
Mias Bakers		6.5	0.015
Quality Bakers		**5.1**	**0.009**
Quality Bakers		7.0	0.023
Quality Bakers	Wheat Germ	7.0	0.017
Tip Top		**9.3**	0.024
Tip Top	Wheat Germ	6.8	0.017

The data in Table 7.1.2.2 shows the relationship between cadmium and dietary fibre in wholemeal loaf bread purchased from Fremantle (West Australia) food outlets. Here, the dietary fibre ranges from a minimum of 5.1% for a Quality Bakers bread to a maximum of 9.3% for a DeCampo bread (both in blue). The results are also normal for wholemeal loaf breads.

The minimum value shown is 0.009 mg/kg for a Quality Bakers bread and the maximum is 0.021 mg/kg for a Farmland bread (both in blue).

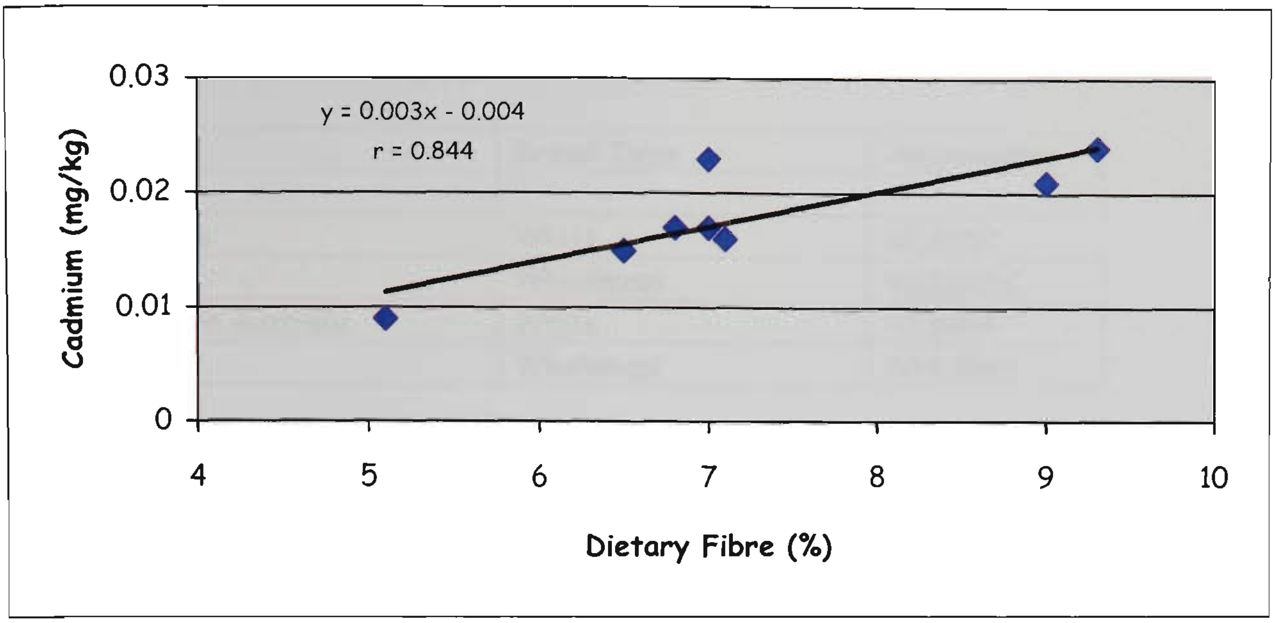


Figure 7.1.2.2 Relationship between cadmium and dietary fibre levels for wholemeal loaf bread sourced from Fremantle (Western Australia) food outlets

ANOVA analysis of the data in Table 7.1.2.2 gave a P value of 0.004 at 6 DF. As P is < 0.01, it can be shown that a highly significant linear correlation exists at the 99% confidence level between cadmium and dietary fibre levels for samples of wholemeal loaf bread sourced from Fremantle (Western Australia) food outlets. With a R^2 value of 0.713, it can also be shown that 71.3% of the cadmium concentration can be predicted from the dietary fibre level.

7.1.3 Discussion

Tables 7.1.1.1, 7.1.1.2, 7.1.2.1 and 7.1.2.2 show the range of cadmium levels in the white and wholemeal loaf breads from Victoria and Western Australia that would be expected from the cadmium levels found in the straight-run and wholemeal flours produced in commercial flourmills in each state (Tables 6.3.1.1 and 6.3.2.1).

The cadmium values from Tables 7.1.1.1, 7.1.1.2, 7.1.2.1 and 7.1.2.2 were plotted in a box and whisker plot to determine what correlation existed between the cadmium levels in the loaf breads. To simplify the plot, the breads were given the following abbreviations.

Table 7.1.3.1 Abbreviations given to loaf breads in the box and whisker plot.

State	Bread Type	Abbreviation
Victoria	White	WLBVIC
	Wholemeal	WHLBVIC
Western Australia	White	WLBWA
	Wholemeal	WHLBWA

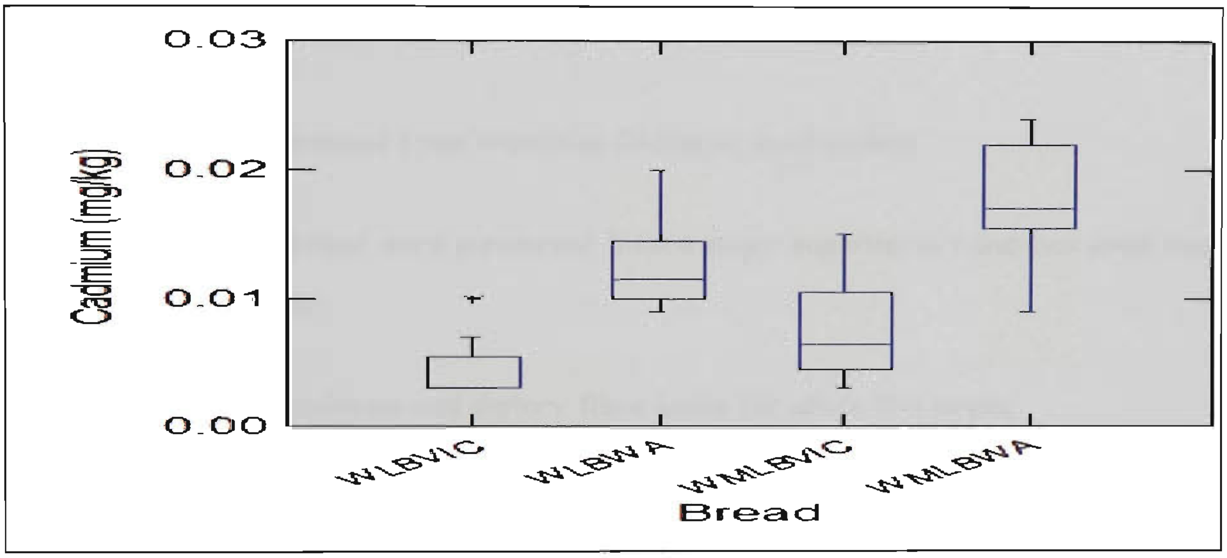


Figure 7.1.3.1 Box and whisker plot for white and wholemeal loaf breads from Victoria and Western Australia

Figure 7.1.3.1 is a plot of the range of cadmium levels for each of the loaf breads with the horizontal line in each box marking the median of the values and the length of the box showing the range of the central 50% of the values. The box edges or hinges show the first and third quartile of the results with the length of the whiskers or fences show the range of values that lie between 1.5 times the absolute value of the difference between the two hinges (Wilkinson, 1999). Values greater than the fence but less than twice the fence are plotted with asterisks. Values greater than twice the value of the fence are plotted with circles

It can be seen that bottom hinge of the loaf breads from Victoria overlap with each other but only the top hinge of the wholemeal loaf overlaps with the bottom hinge of the white bread from Western Australia. The wholemeal bread from Western Australia does not overlap with any of the other three breads. Apart from the slight overlap, it can be seen that the loaf breads from Victoria and Western Australia form two distinct groups. The value of < 0.01 for P for the ANOVA analysis of the cadmium levels in shows that a highly statistically significant difference at the

95% confidence level exists between the cadmium levels in the four different breads. In addition, it can be seen that the wholemeal breads have higher cadmium levels than white breads (p = 0 at the 0.05 level), this is expected from the higher levels of cadmium found in the bran and germ in Tables 6.3.1.1 and 6.3.2.1. Very little work from other authors on cadmium levels in bread could be found. Demirozu *et al.* (2003) reported cadmium levels of 0.012 mg/kg in bread samples from 20 bakeries in Turkey but did not give any other details about the bread.

7.2 Flat bread

7.2.1 White flat bread

7.2.1.1 White flat bread sourced from Werribee (Victoria) food outlets

Nine samples of white flat bread were purchased from a major supermarket and two small bakery outlets in Werribee, Victoria.

Table 7.2.1.1 Results for cadmium and dietary fibre levels for white flat bread sourced from Werribee (Victoria) food outlets

Supplier	**Type**	**Dietary fibre (%)**	**Cadmium (mg/kg)**
Bakery		2.6	0.006
Bakery		2.1	0.005
Middle East Bakers		3.9	< 0.005
Middle East Bakers		3.2	< 0.005
Middle East Bakers		3.7	< 0.005
Middle East Bakers		3.3	< 0.005
Middle East Bakers		3.7	< 0.005
Middle East Bakers		5.9	< 0.005
Middle East Bakers	Soy & Linseed	8.7	0.016

The data in Table 7.2.1.1 shows the relationship between cadmium and dietary fibre in white flat bread purchased from Werribee (Victoria) food outlets. It can be seen that the dietary fibre ranges from a minimum of 2.1% for a small bakery outlet bread to a maximum of 8.7% for a Middle East Bakers Soy & Linseed (both in blue). The minimum cadmium value shown is < 0.005 mg/kg for six of the samples and the maximum is 0.016 mg/kg for a Middle East Bakers Soy & Linseed flat bread (all in blue), although this high cadmium value may be due to the presence of linseed in the bread. Previous unpublished work by the author has shown that linseed from some areas of Australia may contain elevated levels of cadmium up to 0.06 mg/kg.

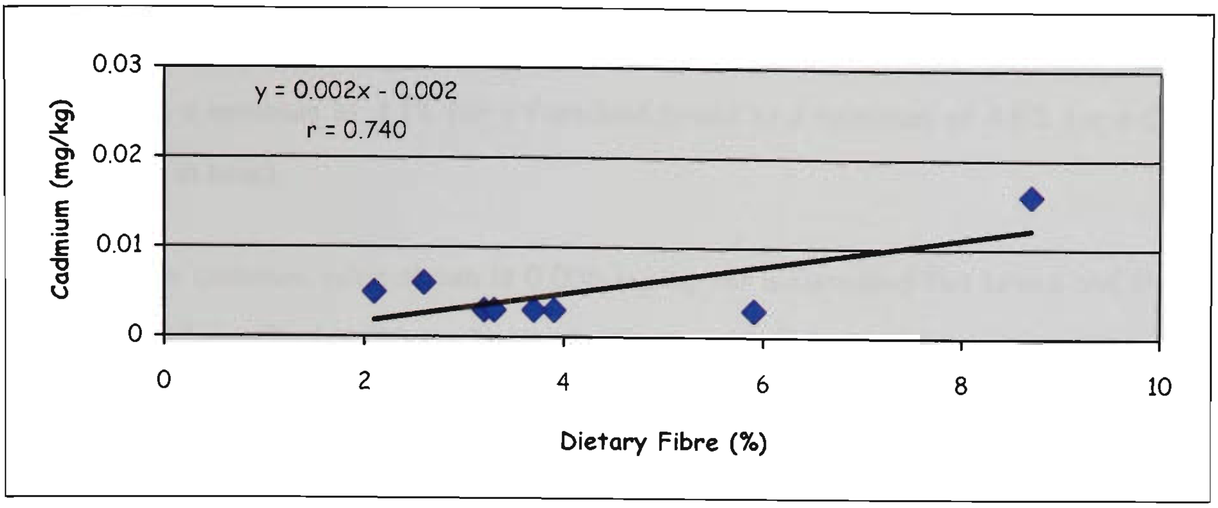


Figure 7.2.1.1 Relationship between cadmium and dietary fibre for white flat bread sourced from Werribee (Victoria) food outlets

ANOVA analysis of the data in Table 7.2.1.1 gave a P value of 0.011 at 7 DF. As P is < 0.05, it can be shown that a significant linear correlation exists at the 95% confidence level between cadmium and dietary fibre levels for samples of white flat bread sourced from Werribee (Victoria) food outlets. With a R^2 value of 0.547, it can be also shown that 54.7% of the cadmium concentration can be predicted from the dietary fibre level.

7.2.1.2 White flat bread sourced from Fremantle (Western Australia) food outlets

Eight samples of white flat bread were purchased from a major supermarket and a small bakery outlet in Fremantle, Western Australia.

Table 7.2.1.2 Results for cadmium and dietary fibre levels for white flat bread sourced from Fremantle (Western Australia) food outlets

Supplier	**Type**	**Dietary fibre (%)**	**Cadmium (mg/kg)**
Bakery		4.2	0.010
Bakery		4.6	0.012
Effes		3.7	0.013
Farmland		3.1	0.006
Lavash		4.0	0.015
Quality Bakers		4.8	0.021
Tip Top		3.5	0.009
Tip Top		3.3	0.015

Table 7.2.1.2 highlights the relationship between cadmium and dietary fibre in white flat bread purchased from Fremantle (Western Australia) food outlets. It can be seen that the dietary fibre ranges from a minimum of 3.1% for a Farmland bread to a maximum of 4.8% for a Quality Bakers bread (both in blue).

The minimum cadmium value shown is 0.006 mg/kg for a Farmland flat bread and the maximum is 0.021 mg/kg for a Quality Bakers flat bread (both in blue).

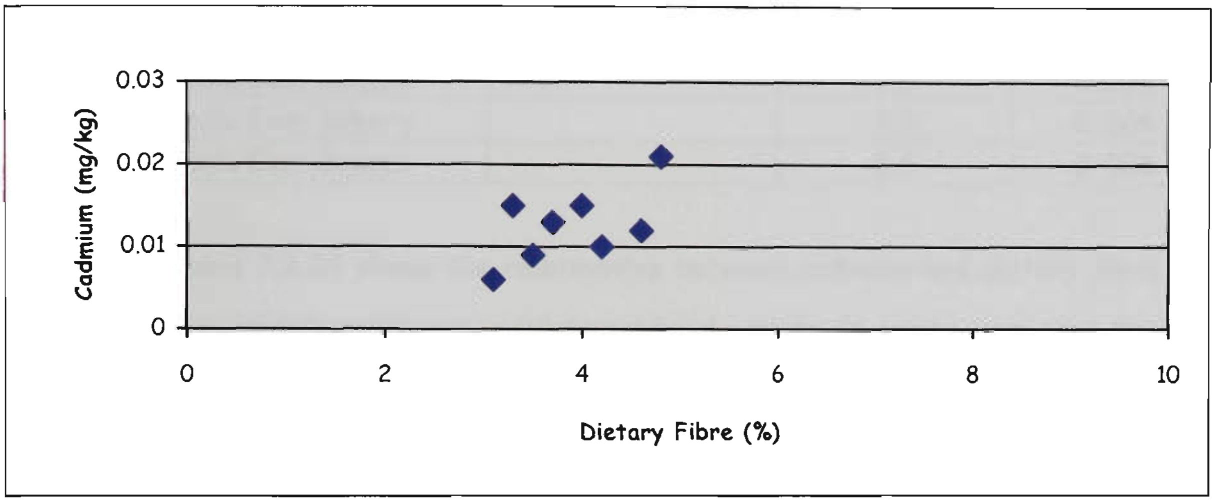


Figure 7.2.1.2 Relationship between cadmium and dietary fibre levels for white flat bread sourced from Fremantle (Western Australia) food outlets

ANOVA analysis of the data in Table 7.2.1.2 gave a P value of 0.056 at 6 DF. As P is > 0.05, it can be shown that there is no significant proof of a correlation between cadmium and dietary fibre levels for samples of white flat bread sourced from Fremantle (Western Australia) food outlets.

7.2.2 Wholemeal Flat

7.2.2.1 Wholemeal flat bread sourced from Werribee (Victoria) food outlets

Eight samples of wholemeal flat bread were purchased from a major supermarket and mall bakery outlet in Werribee, Victoria

Table 7.2.2.1 Results for cadmium and dietary fibre levels for wholemeal flat bread sourced from Werribee (Victoria) food outlets

Supplier	Type	Dietary fibre (%)	Cadmium (mg/kg)
Bakery		**6.3**	< 0.005
Bakery		6.7	< 0.005
Bakery		8.5	0.005
Middle East Bakers		**8.6**	< 0.005
Middle East Bakers		**8.6**	0.005
Middle East Bakers		7.5	0.005
Middle East Bakers		7.9	**0.006**
Middle East Bakers		8.5	**0.006**

The data in Table 7.2.2.1 shows the relationship between cadmium and dietary fibre in wholemeal flat bread purchased from Werribee (Victoria) food outlets. It can be seen that the dietary fibre ranges from a minimum of 6.3% for a small bakery outlet bread to a maximum of 8.6% for two Middle East Bakers breads (both in blue).

The minimum cadmium value shown is < 0.005 mg/kg for three of the samples while the maximum is 0.006 mg/kg for two Middle East Bakers breads (all in blue).

Figure 7.2.2.1 Relationship between cadmium and dietary fibre levels for wholemeal flat bread sourced from Werribee (Victoria) food outlets

ANOVA analysis of the data in Table 7.2.2.2 gave a P value of 0.056 at 6 DF. As P is > 0.05, it can be shown that there is no significant proof of a correlation between cadmium and dietary fibre levels for samples of wholemeal flat bread sourced from Werribee (Victoria) food outlets.

7.2.2.2 Wholemeal flat bread sourced from Fremantle (Western Australia) food outlets

Six samples of wholemeal flat bread were purchased from a major supermarket in Fremantle, Western Australia. The results of cadmium and dietary fibre analysis are shown in Table 7.2.2.2.

Table 7.2.2.2 Results for cadmium and dietary fibre levels for wholemeal flat bread sourced from Fremantle (Western Australia) food outlets

Manufacturer	**Type**	**Dietary fibre (%)**	**Cadmium (mg/kg)**
Farmland		7.1	0.015
Farmland		7.0	0.016
Oasis		7.3	0.015
Middle East Bakeries		**8.6**	**0.018**
Middle East Bakeries		**64**	**0.012**
Middle East Bakeries		8.3	0.021

The data in Table 7.2.2.2 shows the relationship between cadmium and dietary fibre in wholemeal flat bread purchased from Fremantle (Western Australia) food outlets. It can be seen that the dietary fibre ranges from a minimum of 6.4% for a Middle East Bakeries bread to a maximum of 8.6% also for a Middle East Bakeries bread (both in blue). The minimum cadmium value shown is 0.012 mg/kg for a Middle East Bakeries bread and the maximum is 0.018 mg/kg also for a Middle East Bakeries bread (both in blue).

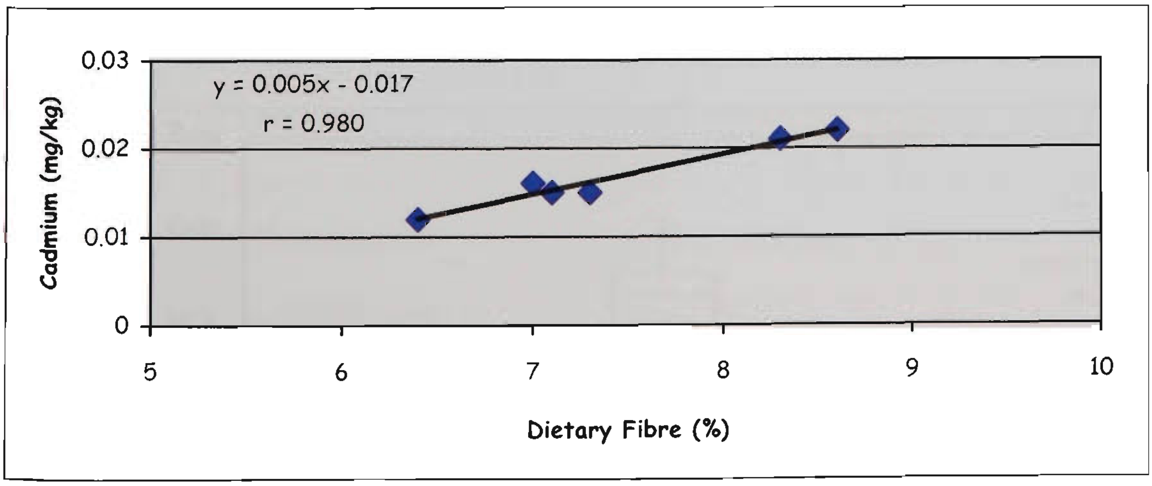


Figure 7.2.2.2 Relationship between cadmium and dietary fibre levels for wholemeal flat bread sourced from Fremantle (Western Australia) food outlets

ANOVA analysis of the data in Table 7.2.1.1 gave a P value of 0.001 at 4 DF. As P is = 0.001, it can be shown that a highly significant linear correlation exists at the 99.9% confidence level between cadmium and dietary fibre levels for samples of wholemeal flat bread sourced from Fremantle (Western Australia) food outlets. With a R^2 value of 0.960, it can be also shown that 96.0% of the cadmium concentration can be predicted from the dietary fibre level.

7.2.3 Discussion

Similar to the loaf breads in Section 7.1, Tables 7.2.1.1, 7.2.1.2, 7.2.2.1 and 7.2.2.2 show the range of cadmium levels in the white and wholemeal flat breads from Victoria and Western Australia that would be expected from the cadmium levels found in the commercial milled products from each state (Tables 6.3.1.1 and 6.3.2.1). The dietary fibre result was compared with the value given in the Nutritional Information Panel (NIP) for each bread, if this was included on the label. All results were within 10% of the given value and the mean result was within 5% of the given value.

The cadmium values from Tables 7.2.1.1, 7.2.1.2, 7.2.2.1 and 7.2.2.2 were plotted in a box and whisker plot to determine what correlation existed between the cadmium levels in the flat breads. To simplify the plot, the breads were given the following abbreviations.

Table 7.2.3.1 Abbreviations given to flat breads in the box and whisker plot.

State	**Bread Type**	**Abbreviation**
Victoria	White	WFBVIC
	Wholemeal	WHFBVIC
Western Australia	White	WFBWA
	Wholemeal	WFLBWA

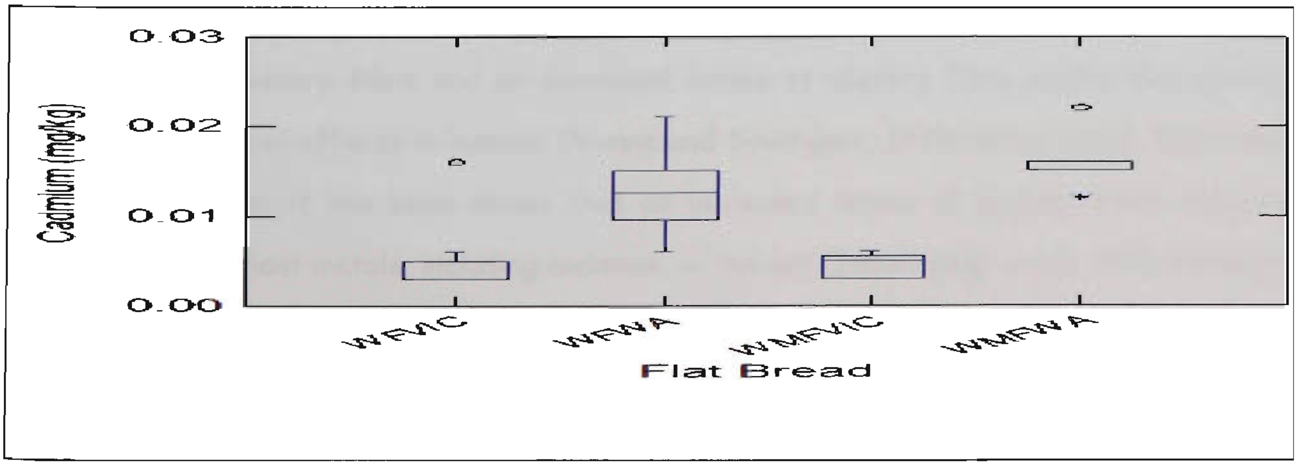


Figure 7.2.3.1 Box and whisker plot for white and wholemeal flat breads from Victoria and Western Australia

Both white flat bread from Victoria and white and wholemeal flat breads from Western Australia have cadmium levels that would be expected from the straight run and wholemeal flours produced from commercial flourmills in each state. This is not seen in the Victorian wholemeal flat breads and this is in contrast to the Victoria loaf breads. The reason for this is uncertain but may be due to the bread containing a substantial proportion of flour from other grains.

Wholemeal flat breads have significantly higher levels of cadmium than white flat breads from Western Australia (P = 0 at the 0.05 level) and this is expected from the higher levels of cadmium in the bran and germ (Tables 6.3.1.1 and 6.3.2.1). This relationship is not seen in flat breads from Victoria and as mentioned above, it may also be due to the wholemeal flat breads containing a substantial proportion of flour from other grains. White and wholemeal breads from Western Australia have significantly higher levels of cadmium than corresponding breads from Victoria (P = 0 at the 0.05 level in both cases). This is expected from the higher levels of cadmium in the wheat leading to higher levels in the flour and other milled fractions (Tables 6.3.1.1 and 6.3.2.1). MAFF (1994a, 1994b) gave levels of 0.03 mg/kg and 0.013 to 0.082 mg/kg in bread from two UK studies of metals in the total diet and Ahmed (2001) reported levels of 0.03 to 0.16 mg/kg in a study of cadmium in bread samples from Egypt.

A significant linear relationship between cadmium and dietary fibre levels in bread is shown in Figure 7.1.1.1, Figure 7.1.1.2, Figure 7.1.2.1, Figure 7.1.2.2, Figure 7.2.1.1 and Figure 7.2.2.2. This can be directly correlated to the positive relationship between cadmium and the wheat bran and germ levels in the milled products shown in Chapter 6. Figure 7.2.1.2 and Figure 7.2.2.1 do not show a significant relationship and this may be due to the very low levels cadmium and dietary fibre in the bread.

It has long been established by many workers that the outer bran layers of the wheat grain are a good source of dietary fibre and an increased intake of dietary fibre in the diet produces a number of beneficial effects in humans (Prynne and Southgate, 1979; Wrick *et al.*, 1983; Munroe, 2004). In addition, it has been shown that an increased intake of dietary fibre reduces the absorption of divalent metals, including cadmium, in the gut (Ismail-Beigi *et al.*, 1977; Fairweather, 1982; van Dokkum *et al.*, 1982; Moberg *et al.*, 1987, Ou *et al.*, 1999). Wing and Moberg (1993) reported that the reduction in the cadmium absorption from whole wheat and bran diets is not sufficient to compensate for the higher cadmium concentrations in these foods and hence efforts should be made to reduce cadmium in wheat.

Chapter 8 Conclusions

An analysis of the cadmium levels in Australian wheat from the wheat growing states, the levels in different milling streams and in wheaten products would be valuable information for the wheat growers and the Australian milling and bread making industries. Accurate analyses for the cadmium levels in the locally grown wheat would enable the Australian Wheat Board to have an extra quality parameter to quote to assist in increasing our competitive edge on the global market.

The general aims of this research were to establish if there were any difference in the cadmium levels of wheat grown in different Australian states and the reasons for this difference. Whether, the differences in cadmium levels was due to environmental factors that was in turn reflected in the different milling fractions and in the baked end products. The specific aims included establishing if the nitric acid digestion method for cadmium analysis of wheat and wheaten products would give reproducible results; determining if the soil type, soil acidity and fertilizer usage correlated with the elevated levels of cadmium in Western Australia and South Australia; showing that cadmium is deposited in the wheat aleurone layer that is fractionated during milling and, finally, determining if the levels of cadmium vary in white and wholemeal breads in the different states, specifically between Western Australia and Victoria.

One of the main sections of the thesis was the development of a suitable method for the analysis of trace amounts of cadmium and the validation of the method. This method was a single acid procedure that was developed and validated according to Varian (1988) and ICH (994 and 1996) for atomic absorption spectroscopy. In order to establish the procedures the methods were developed in order: development of instrument parameters, evaluation of the analytical standard, digestion conditions for both the blank and spiked samples as well as determining the minimum detection levels. Instrument parameters were established for the atomic absorption spectrophotometer and graphite furnace as recommended by the Varian staff and detailed in the Varian procedures manual. It was necessary to determine a suitable temperature for combustion of the samples that would not allow any volatilization and loss of cadmium that was being tested. Six temperatures were trialed and ten runs to determine the relationship between absorbance and cadmium standard concentrations were undertaken. Considerable amount of time was required to determine: optimization of digestion conditions; amount of acid required; digestion temperature; digestion time; minimum detection levels and uncertainty of the analysis and validation before the work could commence. Validation was rigorous, in that the method was also verified by trials in four independent laboratories: CSIRO, Division of Soils, South Australia, Victorian State Chemistry Laboratory, Werribee Victoria; the National Residue Survey Laboratory that is part of the Australian Government Department of Agriculture and by the Overseas Merchandise Inspection Company of Japan (OMIC). All QA data was plotted on control charts.

In any analyses, the selection of samples to give representative results is paramount. Wheat samples were sourced from the Australian Wheat Board (AWB) wheat monitoring program from the following areas: three in New South Wales, two in Queensland, three in South Australia, two in Victoria and three in Western Australia More samples were analyzed from Western Australia and South Australia as preliminary results indicated that these two areas had elevated cadmium levels when compared to the other states. The wheat from the silo sites from AWB, Grower Load Samples were, New South Wales, 47 sites, 150 samples; Queensland, 23 sites, 61 samples; South Australia, 19 sites, 638 samples; Victoria 41, sites, 426 samples and Western Australia, 41 sties and 682 samples. In the study, seventeen different genotypes were analyzed. The number of samples and sites analyzed from silo composites samples were: New South Wales, 114 sites and 171 samples; Queensland 17 and 21; South Australia, 124, 658; Victoria 118,184 and Western Australia, 193, 3,767 respectively. Wheat samples were also analyzed from other major wheat growing countries: Canada, Germany, Holland, Hungary and the United Knngdom. Sample numbers and cadmium results were plotted on a map of the Australian grain belt which gave a valuable visual picture of the work undertaken.

Samples were also taken from the AWB flourmill monitoring program during the following seasons: 1991/92; 1993/94; 1994/95 and 1995/96 seasons. The total number of samples that were analyzed from all states for the first season was 999; the second season 897, the third 414 and the final season 713 samples. Flourmill streams that were studied were milled from three different types of mill; a Buhler laboratory mill, at Agrifood Technology, Werribee; a pilot mill at the Bread Research Institute, North Ryde, New South Wales and commercial flour mills in Victoria and Western Australia. Samples from the millstreams were analysed for cadmium and ash. Dietary fibre and well as cadmium was analysed from bread products: purchased in Werribee Victoria: and Fremantle, Western Australia;: Twenty-three types of bread were analysed. From each area, nine wholemeal loaf breads from both Werribee and Fremantle were also analysed for cadmium and dietary fibre. Nine white flat bread samples were sourced from major supermarkets and two small bakeries in Werribee, as were the other bread samples mentioned above and six bakeries in Fremantle. Finally, eight wholemeal flat breads were purchased from food outlets in Werribee and six from outlets in Fremantle.

The results for the analysis of the cadmium levels in wheat from sites within Australia are: In mean levels of cadmium in mg/kg from New South Wales, Queensland, South Australia, and Western Australia are: 0.01; 0.01, 0.03, 0.02 and 0.05 respectively. The maximum levels of cadmium in the above five states, however, was: 0.04, 0.04, 0.12, 0.08, and 0.22 mg/kg respectively. The dendogram of the data for the mean cadmium results, sites for grower load wheat samples, by state, discussed above shows that are two distinct groups. Victoria, Queensland and New South Wales fall in one group and South Australia and Western Australia in the other. Similarly, for the maximum levels of cadmium in the same states the dendogram follows the same trend. The P value of <0.05 in the ANOVA analysis indicates that the levels of cadmium are significantly different for each state at the 95% confidence level.

Mean results for the site composite samples from each 500 tonnes of wheat received at the silos in 92/93 season for New South Wales, Queensland, South Australia, Victoria, and Western Australia are as follows: .0.01, <0.01, 0.02, 0.01 and 0.04 mg/kg respectively, while the results for the maximum values in the same order are: 0.04, 0.02, 0.02, 0.06 and 0.13 mg/kg respectively. The results for site composite samples sow a similar pattern to those for the grower load samples but the difference between states in reduced owing to the fact that there is a dilution of the cadmium levels from wheat blending at the silos. Again, the P values of < 0.05 in the ANOVA analysis of both the grower load and site composite samples follow the same trend as the mean cadmium levels indicating that the samples are significantly different at the 95% confidence level.

Cadmium and ash results were obtained for wheat sourced from Victoria and Western Australia and milled on three types of mill: Buhler, pilot and commercial, Two Australian standard white wheat samples (ASW 1 and 2), two hard wheat samples (Hard 1 and 2) and a single soft wheat sample (Soft 1) were milled. All wheat samples and the mill fractions were then analyzed for cadmium and ash. Victorian and Western Australian samples were milled on a Buhler mill at Agrifood Technology, Werribee.. Cadmium levels and ash were highest in the bran and shorts and lowest in the break and roll mill fractions. For Victoria, the cadmium level in mg/kg in the bran from the five wheat samples ASW 1 and 2, Hard 1 and 2 and Soft 1 were determined where the highest cadmium value was 0.053 mg/kg,, AWS2,, and the lowest ASW 1 of 0.022 mg/kg. For the shorts, the highest cadmium value was 0.56 mg/kg for ASW 2 and the lowest cadmium 0.0254 mg/kg for ASW 1. Similarly, for the ash content, in the bran, the highest value was.5.17 mg/kg for Hard 1 whereas the lowest was for Soft 1, with 3.07 gm/kg. For the shorts, the ash trend was the same with 5.20 mg/kg for Hard 1 and the lowest 3.34 gm/kg for Soft 1. For Western Australia, the highest cadmium bran value was 0.093 mg/kg for Soft 1 and the lowest was 0.034 mg/kg for Hard 1. For the Shorts, the highest cadmium was 0.098 for Soft 1 and the lowest 0.040 for Hard 1 both in mg/kg. The highest ash was 4.81 for ASW 1 and the lowest 3.42 for Hard 1, both in mg/kg.

For the pilot mill at the BRI, New South Wales, two wheat samples: Australian Prime Wheat (APW) and Sunllin, were analyzed for cadmium and ash. There were three fractions that contained the bulk of the cadmium: bran, pollard and germ. For APW, the highest cadmium bran level was 0.012 and the lowest 0.008 for Sunlin both in mg/kg respectively. Pollard followed the same pattern: highest cadmium value of 0.016 and lowest of 0.012 mg/kg for Sunlin. Finally, the germ values were 0.012 and 0.010 mg/kg for APW and Sunlin respectively. For APW the ash levels for bran, pollard and germ were 5.70, 3.77 and 4.18 mg/kg respectively while the ash percent for Sunlin was 5.54, 2.89 and 4.27 respectively. Cadmium values for Leichardt wheat from Queensland for the three mill fractions germ, pollard and bran were 0.018, 0.022 and 0.011 mg/kg and corresponding ash values were 5.44, 3.52 and 4.45 percent. Three varieties of South Australian Wheat: ASW, Hard and Kirchouff were also milled and the cadmium and ash content for bran, pollard and germ determined. The highest cadmium level in the bran was 0.064 for Kirchouff and the lowest for Hard, 0.032 mg/kg; pollard had the highest cadmium value of 0.035 for APW and the lowest for Hard Of 0.035 mg.kg. Again, Kirchouff had the highest cadmium level of 0.058 mg/kg in the germ and APW the lowest of 0.037 mg/kg. Ash content was highest in Hard Bran and lowest in Kirchouff with values of 4.58 and 4.26 percent respectively. Pollard ash values were 3.37 for Hard and 2.03 for APW percent respectively. Hard again had the highest ash value of 3.96 and Kirchouff the lowest of 3.61 percent.

Further wheat fractions from the BRI pilot were determined for four Victorian wheat cultivars: APW, Rosella, Sunlin and Yanac for the three fractions bran, pollard and germ. The highest value for cadmium in the bran was Sulin with 0.024 and the lowest for both APW and Rosella of 0.017

mg/kg. The highest pollard cadmium level was APW with 0.027 and the lowest 0.011 for Yanac both in mg/kg while the germ values were 0.031 for Sunlin and 0.013 the lowest values for both Rosella and Yanac,all in mg/kg. Five cultivars: Hard, Cadoux, Eradu, Halberd and Soft of Western Australian wheat were analyzed for all mill streams but again only three fractions where the cadmium and ash were highest will be mentioned here. The highest level of cadmium in the bran was for Cadoux and the lowest for Halberd with values of 0.072 and 0.048 mg/kg respectively. For pollard the same order was followed: 0.079 for Cadoux and 0.046 for Halberd being the highest and lowest values in mg/kg. Germ values were also in this order: 0.077 and 0.077 for Cadoux but 0.043 for Eradu being highest and lowest in mg/kg respectively. Considering the ash values in the bran, Soft had the highest value of 4.40 but Halberd was the lowest with 1.25 percent. Ash levels in the Germ were 3.91 for Hard and the lowest for 1.38 for Eradu, both in percent.

Samples of ASW and Hard wheat and all mill fractions from commercial flourmills in both Victoria and Western Australia were assessed for cadmium and ash content. Fourteen fractions were assayed from each mill: B1, B2, B3, A, B, C, D, E, F. G, X, pollard and germ. Only the pollard and germ will be mentioned as these two fractions contained the highest amount of cadmium and ash. Considering firstly the Victorian samples: the pollard fraction contained 0.016 mg/kg for ASW and 0.032 for Hard both in mg./kg while the germ values were 0.021 for ASW and 0.040 for Hard mg/kg of cadmium respectively. For pollard the ash content in ASW was 4.37 and 4.42 for Hard both in percent. Similarly, the germ ash content was 3.88 and 3.91 for the same two wheat samples in percent. The Western Australian cadmium values were higher for both ASW and Hard pollard and germ, but only germ ash values from Western Australia were higher than those from Victoria. Levels of cadmium in pollard and germ for ASW and Hard were 0.045 and 0.057 mg/kg respectively. Germ values were 0.055 and 0.069 cadmium mg/kg for ASW and Hard. The ash values were 4.11 and 4.29 for pollard and 3.94 and 4.04 for germ in percent respectively.

White loaves from Western Australia and Victoria were analyzed for cadmium and dietary fibre. Twenty-three samples of white loaf breads from local outlets in Werribee, Victoria and Fremantle, Western Australia were analyzed for cadmium and dietary fibre. The cadmium levels for the white breads from Victoria were very low, with most < 0.005 mg/kg while the highest values recorded were four loaves with cadmium content of 0.007 mg/kg. The lowest cadmium detected in the loaves from Western Australia was 0.010 mg/kg in a number of loaves and the highest 0.020 in two loaves all in mg/kg. The amount of dietary fibre was similar in the loaves from the two regions. Eight whole meal loaves were assessed for cadmium and fibre from the same two areas. For Victoria, the highest cadmium level was 0.015 mg/kg in one loaf and the lowest for two breads was less than 0.005 mg/kg. Again for Western Australia, the cadmium levels were higher than Werribee: the highest level detected was 0.24 mg/kg in one load and the lowest 0.015 mg/kg.

Flat white and flat wholemeal breads from both the same cities as above in Victoria and Western Australia were also assessed for cadmium and dietary fibre. Nine samples of white flat bread from Victoria and eight samples from Western Australia were assayed. Of the nine Victorian samples, only three had values of cadmium greater than 0.005. These values were: 0.016 the highest for one loaf and 0.005 and 0.005 was the lowest, all results in mg/kg. For Western Australia, the highest recorded cadmium level was 0.21 mg/kg in one loaf and the lowest value 0.09 mg/kg. Turning now to the wholemeal flat breads from the two regions, for Victoria eight loaves were analyzed, the highest level was 0.006mg/kg with three loaves being less than 0.005 mg/kg. Six loaves were used to determine the cadmium in the wholemeal flat bread from Western Australia and again the cadmium content was a little higher: 0.021 being the highest and 0.012 mg/kg the lowest.

In summary, this thesis represents an extremely large and thorough study of cadmium levels in the five Australian wheat- producing states as well as in overseas countries in Europe, United States of America and Canada that has not been undertaken before. It makes a significant contribution to the scientific literature The levels of cadmium in all Australian wheat cultivars were well below the permitted level, even though the cadmium content in Western Australia samples were slightly higher that the other states, being due to the soil type, pH and fertilizer usage. In addition, all Australian wheat had cadmium levels considerably lower that all other wheat growing countries. This is an important marketing edge for Australia that clearly shows the wheat produced in this country is of the highest quality of any on the global market. This information is very important for the Australian Wheat industry and the Australian Wheat Board.

Chapter 9.0 References

AACC, (1990), Total protein as nitrogen, Method number 46-12, Approved Methods of the AACC, 8th Edition, Volume 1 and 2, Association of Cereal Chemists, St Paul, USA

AACC, (1995a), Experimental milling: Introduction, equipment, sample preparation and tempering, Method number 26-10A, Approved Methods of the AACC, 9th Edition, Volume 1 and 2, Association of Cereal Chemists, St Paul, USA

AACC, (1995b), Experimental milling: Buhler method for hard wheat, Method number 26-21A, Approved Methods of the AACC, 9th Edition, Volume 1 and 2, Association of Cereal Chemists, St Paul, USA

AACC, (1995c), Experimental milling: Buhler method for soft wheat - short extraction flour, Method number 26-30A, Approved Methods of the AACC, 9th Edition, Volume 1 and 2, Association of Cereal Chemists, St Paul, USA

AACC, (1995d), Experimental milling: Buhler method for soft wheat - straight grade flour, Method number 26-31A, Approved Methods of the AACC, 9th Edition, Volume 1 and 2, Association of Cereal Chemists, St Paul, USA

ABARE, (2000), Australian Commodity Statistics 2000, ABARE, Canberra, Australia, 218

ABARE, (2002), Phosphorus added as fertiliser to farms in the Australian grain belt, ABARE, Canberra, Australia

ABARE, (2003a), Grain and Fodder Stocks, Australian Grains Industry 2003, ABARE, Canberra, Australia

ABARE, (2003b), Grains industry, performance and outlook, Australian Grains Industry 2003, ABARE, Canberra, Australia

ABARE, (2004), Australian processed food industry, summary statistics, Australian Food Statistics, ABARE, Canberra, Australia, 47-49

Abdel-Aal E.S.M., Hucl P. and Sosulski F.W., (1998), Food uses for ancient wheat, Cereal Foods World, **43(10)**, 763-766

Adams M.A., Bolger M., Carrington C.D., Coker C.E., Cramer G.M., DiNovi M.J. and Dolin S., (1993), Smoking, In: Guidance Document for Cadmium in Shellfish, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, United States of America, 16-18

Adams M.L., Zhao F.J., McGrath S.P., Nicholson F.A., Chalmers, A., Chambers B.J. and Sinclair A.H., (2001a), Cadmium and lead in British wheat and barley: survey results and factors affecting their concentration in grain, Project Report Number 265, Home-Grown Cereals Authority, UK, 6-9

Ahmad S., Waheed S., Mannan A., Fatima I. and Qureshi I.H., (1994), Evaluation of trace elements in wheat and wheat by-products, Journal of AOAC International, **77(1)**, 11-18

Ahmed M.T., Hady E-S.A., Yossof K. and Samahy S.K., (2001), Residues of polycyclic aromatic hydrocarbons and heavy metals in bread samples collected from Cairo and Ismailia, Egypt, Environmental Health Sciences, **1(1)**, 1-5

Ahn H.J, Kim J.H, Yook H.S. and Byun M.W., (2003), Irradiation effects on free radical scavenging and antioxidant activity of phytic acid, Journal of Food Science, **68(7)**, 2221-2224

Akubor P.I. and Badifu G.I.O., (2004), Chemical composition, functional properties and baking potential of African breadfruit kernel and wheat flour blends, International Journal of Food Science and Technology, **39(2)**, 223-230

Algerria A., Barbera R. and Farre R., (1988), Atomic absorption spectrophotometric determination of nickel in foods, Journal of Micronutrient Analysis, **4**, 229-239

Algerria A., Barbera R. and Farre R., (1990), Influence of environmental contamination on Cd, Co, Cr, Cu, Ni, Pb and Zn content of edible vegetables: safety and nutritional aspects, Journal of Micronutrient Analysis, **8**, 91-104

Ambalammaatil S., Lukow O.M., Hatcher D.W., Dexter J.E., Malcolmson L.J. and Watts B.M., (2002), Milling and quality evaluation of Canadian hard white spring wheats, Cereal Foods World, **47(7)**, 319-327

Analytical Methods Committee, Royal Society of Chemistry, (1987), Recommendations for the definition, estimation and use of the detection limit, Analyst, **112**, 199-204

Andersson A. and Pettersson O., (1981), Cadmium in Swedish winter wheat - regional differences and their origin, Swedish Journal of Agricultural Research, **11**, 49-55

ANZFA, (1998), Australian Food Standards Code, Standard A12, Australian and New Zealand Food Authority, Australian Government Publishing Service

ANZFA, (2003), The 19th Australian total diet survey: A total diet survey of pesticide residues and contaminants, Australian and New Zealand Food Authority, Australian Government Publishing Service

AOAC (2002a), Ash of flour - direct method, AOAC official method number 923.03, In: Official Methods of Analysis of AOAC International, Ed Horwitz W., Chapter 2, 2-14

AOAC (2002b), Insoluble dietary fibre in foods and food products, enzymatic-gravimetric method, phosphate buffer, AOAC official method number 991.42, In: Official Methods of Analysis of AOAC International, Ed Horwitz W., Chapter 32, 5-13

AOAC, (2002c), Protein (total) in flour, AOAC official method number 927.87, In: Official Methods of Analysis of AOAC International, Ed Horwitz W., Chapter 32, 14-26

APHA, (1989), Metals by electrothermal atomic absorption spectrometry, Standard Methods for the Examination of Wastewater, Ed: Franson M.A., American Public Health Association, USA, **3-33-3-39**

Arisawa K., Nakana A., Saito H., Liu X.-J., Yokoo M., Soda M., Koba T., Takahashi T. and Kinoshita K., (2001), Mortality and cancer incidence among a population previously exposed to environmental cadmium, International Archives of Occupational and Environmental Health, **74(4)**, 255-262

Ashton J.F. and Laura R.S., (1992), The cadmium problem, Search, **23(1)**, 31-33

ATSDR, (1989), Cadmium, Public health statement, Agency for Toxic Substances and Disease Registry, United States of America

Atwell, W.A. (2001), An overview of wheat development, cultivation and production, Cereal Foods World, **46(2)**, 59-65

Australian Agricultural Assessment, (2001), Soil acidification: an insidious soil degradation issue, www.audit.ea.gov.au/ANRA/agriculture/docs/national/Agriculture_Soil_Deg

AWB, (1986), The break system, In: Milling Australian Wheat, Australian Wheat Board, Australia, 43-55

AWB, (1993), Cadmium, Executive Committee Policy Paper, Australian Wheat Board, Australia

AWB, (1994), Grain commodities: Wheat industry, In: Australian Grains: A Complete Reference Book on the Grains Industry, Morescope Publishing, 336-341

AWB, (1998), Crop report: 1997 – 1998, Australian Wheat Board, Australia

AWB, (2000), Preferred white varieties, Australian Wheat Board, Australia

AWB, (2002), Wheat varieties in Australia: 1968 - 2001, Australian Wheat Board, Australia

AWB, (2005), Varietal master list, Wheat Receival Standards 2005-2006 Season, Australian Wheat Board, Australia, 28-34

Aylett B.J., (1975), The Chemistry of Zinc, Cadmium and Mercury, Pergamon Press, 254-260

Badt K.L., (1995), Raised or flat, In: Pass the Bread, Childrens Press, Chicago, USA, 12-15

Baechle H.T and Wolstein F., (1985), Cadmium Compounds in Mineral Fertilizers, Proceedings of the Fertiliser Society, **187**, 5-18

Baes C.F., (1973), The Properties of Cadmium, In: Cadmium - The Dissipated Element, Ed. Fulkerson W. and Goeller H.E., National Technical Information Service, USA, 29-36

Bailey A., (1975), Bread from the stones, In: The Blessings of Bread, Paddington Press, New York, USA, 1-28

Bass E.J., (1988), Wheat flour milling, In: Wheat: chemistry and technology, Ed: Pomeranz Y., Volume 11, American Association of Cereal Chemists. 1-67

Batten G.D., (1994), Mineral concentrations of wheat grains grown in Australia, North America and England, Australian Journal of Experimental Agriculture, **34**, 51-56

Batten G.D. and Marr K.M., (1995), The mineral content of cereals in relation to plant and animal nutrition, In: Proceedings of the 45th Australian Cereal Chemistry Conference, Ed: Williams Y.A. and Wrigley C.W., Adelaide, South Australia, 39-43

Batten G.D., Marr K., Williams R. and Farrell T, (2000), Mineral concentrations in Australian and overseas brown rice genotypes, Commun. Soil Sci. Plant Anal., **31(11-14)**, 2393-2400

Beach L.M., (1989), Varian SpectrAA Zeeman graphite furnace methods for environmental samples, Varian Instruments at Work, Number AA-90, Varian, USA

Beach L.M., (1990a), Evaluation of a new forked platform design for graphite furnace AAS, Varian Instruments at Work, Number AA-99, Varian, USA

Beach L.M., (1990b), The determination of trace elements in stainless steel by forked platform GFAAS, Varian Instruments at Work, Number AA-102, Varian, USA

Bellinger D., Bolger M., Goyer R., Barraj L. and Baines J., (2000), Calcium metabolism and bone, In: WHO Food Additives Series 46: Cadmium, International Programme on Chemical Safety, World Health Organisation, Geneva, Switzerland, 20-22

Benzo Z, Cecarelli C., Carrion N., Alvarez M., Rojas C. and Rosso M., (1992), Analytical characteristics in electrothermal atomisation studies of cadmium, copper, germanium, molybdenum, lead and vanadium from pyrolytic and tungsten coated L'vov platforms by atomic absorption spectrometry, Journal of Analytical Atomic Spectrometry, **7**, 1273-1280

Bernard A., Roels H., Buchet J.P, Cardenas A. and Lauwerys R., (1992), Cadmium and health: the Belgian experience, In: Cadmium in the Human Environment: Toxicity and Carcinogenicity, Ed; Nordberg G.F., Herber R.F.M. and Alessio L., International Agency for Research on Cancer, IARC Scientific Publications, Lyon, France, 15-28

Bett-Garber K.L., Champagne E.T., McClung A.M., Moldenhauer K.A., Linscombe S.D. and McKenzie K.S., (2001), Categorizing rice cultivars based on cluster analysis of amylose content, protein content and sensory attributes, Cereal Chem., 78(5), 551-558

Bienvenu P.M.M., Nofre C. and Cier A., (1963), Toxicite Generale Comparee des Ions Metalliques, Comptes Rendus Hebdomadaires des Seances, Academie des Sciences, **256**, 1043-1048

Bingham F.T, Page A.L. and Price C.A., (1980), Yield and cadmium concentration of rice grain in relation to addition rates of cadmium, copper, nickel and zinc with sewage sludge and liming, Soil Sci., **130**, 32-39

Bissell D., (1994), Control charts for process management and improvement, Chapter 7, In: Statistical Methods for SPC and TQM, Chapman and Hall, UK, 99-128

Boisset M. and Narbonne J.F., (1996), Exposure, In: Cadmium in Food, Council of Europe Publishing, 11-25

Boonen S., Verrept P, Moens L.J. and Dams R.F.J., (1993), Use of the simplified generalised standards additions method for calibration in solid sampling electrothermal vaporisation inductively coupled plasma atomic emission spectrometry, Journal of Analytical Atomic Spectrometry, **8**, 711-714

Bothams, (2004a), History of bread, The story behind a loaf of bread, www.bothams.com/bread/history1

Bothams, (2004b), History of bread, The story behind a loaf of bread, www.bothams.com/bread/history2

Bradbury D., MacMasters M. M. and Cull I. M., (1956), Structure of the mature wheat kernel. IV. Microscopic structure of the germ of hard red winter wheat, Cereal Chemistry, **33**, 373-378

Bread, (2000), About bread, www.bread.com/about_bread/history

Breadinfo, (2004), History of bread, www.breadinfo.com/history

BRI, (1992), Millers, In: A Bakers Dozen, Bread Research Institute, New South Wales, Australia, 123-131

BRI, (1997a), Bread and bread making: an introduction, In: Australian Breadmaking Handbook, Bread Research Institute of Australia, TAFE Educational Books, New South Wales, Australia, 2-6

BRI, (1997b), Commercial bread making processes, In: Australian Breadmaking Handbook, Bread Research Institute of Australia, TAFE Educational Books, New South Wales, Australia, 57-65

BRI, (1998), A comparison of bread making methods, Bakers technical Bulletin Number 98/02, Bread Research Institute, New South Wales, Australia

Brooks R.R., Pressley B.J. and Kaplan I.R., (1967), APDC-MIBK extraction systems for the determination of trace elements in saline waters by atomic absorption spectrometry, Talanta, **14**, 809-816

Brown S.L, Chaney R.L, Angle J.S. and Ryan J.A., (1996), Organic carbon and the phytoavailability of cadmium to garden vegetables in long term sewage sludge amended soils, Tektran, Agricultural Research Service, United States Department of Agriculture, United States of Amercia, www.nalusda.gov/ttic/tektran/data/000007/50/0000075075

BRS, (1998), Digital atlas of Australian soils, www.brs.gov.au/data/datasets/atlas/index

Brzoska M.M. and Moniuszko-Jakoniuk J., (1997), Inorganic compounds: The influence of cadmium content in diet on cumulation and toxicity of cadmium in the organism, Archives of Toxicology, **72(2)**, 63-70

Buchet J.P., Lauwerys R., Roels H., Bernard A. and Bruaux P., (1990), Renal effects of cadmium body burden in the general population, Lancet, **336**, 700-705

Butt M.S., Qamar M.I., Anjum F.M., Aziz A. and Randhawa M.A., (2004), Development of minerals-enriched brown flour by utilising wheat milling by-products, Nutrition and Food Science, **34(4)**, 161-165

Buzoianu M., (2000), Measurement uncertainty and legal limits in analytical measurements, OIML Bulletin, **XLI(1)**, 5-11

Cakmak I., Welch R., Hart J., Norvell W., Ozturk L. and Kochian L., (2000), Uptake and retranslocation of leaf applied cadmium (109 Cd) in diploid, tetraploid and hexaploid wheats, Journal of Experimental Botany, **51**, 221-228

Capar S.G., (1977), Atomic absorption spectrophotometric determination of lead, cadmium, zinc and copper in clams and oysters: Collaborative study, Journal of the AOAC, **60(6)**, 1400-1407

Cartwright B. and Spouncer L.R., (1987), A survey of mineral nutrient concentrations in South Australian barley grain. 1. Procedures for acid digestion of grain, Divisional Report Number 87, Division of Soils, CSIRO, Australia

Cattle A., McBratney A.B. and Minasny B., (2002), Kriging method evaluation for assessing the spatial distribution of urban soil lead contamination, Journal of Environmental Quality, **31**, 1576-1584

CCFAC, (2004a), Australian delegation report of the 36th Codex Committee on Food Additives and Contaminants, Codex Alimentarius Commission, Italy

CCFAC, (2004b), Draft and proposed draft maximum levels for cadmium comments at step 6 and at step 3, Codex Committee on Food Additives and Contaminants, Codex Alimentarius Commission, Italy

Chakravarty S., Deb M.K. and Mishra R.K., (1993), Hydroxyamidines as new extracting reagents for spectrophotometric determination of cadmium with 4-(2-pyridylazo)-naphthol in industrial effluents, coal and fly ash, Journal of the AOAC International, **76(3)**, 604-608

Chartres C. and Geeves G., (1992), Soil acidification in the higher rainfall wheat belt zone of south-eastern Australia, Australian Journal of Soil and Water Conservation, **5(4)**, 39-43

Chaudri A.M., Allain C.M.G., Badawy S.H., Adams M.L., McGrath S.P. and Chambers B.J., (2001), Cadmium content from wheat grain from a long term field experiment with sewage sludge, Journal of Environmental Quality, **30(5)**, 1575-1588

Chen Z-S., (1991), Cadmium and lead contamination of soils near plastic stabilising material producing plants in northern Taiwan, Water, Air and Soil Pollution, **57-58**, 745-752

Chen Z-S., (2000), Relationship between heavy metal concentrations in soils of Taiwan and uptake by crops, www.agnet.org/library/article/tb149

Chen J. and Gao J., (1993), The Chinese total diet study in 1990. Part 1 - Chemical contaminants, Journal of AOAC International, **76(6)**, 1193-1205

Childs E.A. and Gaffke J.N., (1974), Organic solvent extraction of lead and cadmium from aqueous solutions for atomic absorption spectrophotometric measurement, Journal of the AOAC, **57(2)**, 360-364

Chiy P., de la Fuente M., Barrado E., Vega M. and Phillips C., (1998), Determination of mineral balances in sheep offered feed with added cadmium and zinc, Fresenius Journal of Analytical Chemistry, **361(4)**, 343-348

Cho S., Deveries J.W. and Prosky L., (1997), An overview of dietary fibre analytical methodology, In: Dietary Fibre Analysis and Applications, AOAC International, Maryland, USA, 49-53

CITAC, (1995), International guide to quality in analytical chemistry - an aid to accreditation, CITAC Guide 1, CITAC Working Group, London, UK

Cocchi M., Franchini G., Manzini D., Manfredini M., Marchetti A. and Ulrici A., (2004), A chemometric approach to the comparison of different sample treatments for metals determination by atomic absorption spectroscopy in Aceto Balsamico Tradizionale di Modena, Journal of Agricultural and Food chemistry, **52(13)**, 4047-4055

Codex, (2004a), Provisional agenda, Codex Committee on Food Additives and Contaminants, Codex Alimentarius Commission, Italy

Codex, (2004b), Proposals for a definition and methods of analysis for dietary fibre in bread, CX/NFSDU 04/3 - addition number 1, Codex committee on nutrition and foods for special dietary uses, Codex Alimentarius Commission, Italy

Collins R.N., Merrington G., McLaughlin M.J. and Morel J.-l., (2003), Organic ligand and pH effects on isotopically exchangeable cadmium in polluted soils, Soil Science Society of America Journal, **67**, 112-121

Connolly J., (1988), The role of the NFA in cadmium regulation, In: Cadmium Accumulations in Australian Agriculture, Bureau of Rural Resources Proceedings No. 2, Ed. Simpson J. and Curnow W., Australian Government Publishing Service, Canberra, Australia, 76-79

Cook P.J and Freney J.R., (1988), Sources of cadmium in agriculture - sediments, groundwater, fertilizers and other additives, In: Cadmium Accumulations in Australian Agriculture, Bureau of

Rural Resources Proceedings No. 2, Ed. Simpson J. and Curnow W., Australian Government Publishing Service, Canberra, Australia, 6-19

Cook R.R., (1999), Assessment of uncertainties of measurement for calibration and testing laboratories, National Association of Testing Authorities (Australia), Australia, 10-11

Cornell H.J. and Hoveling A.W., (1998), The structure of the wheat kernel, In: Wheat Chemistry and Utilization, Technomic Publishing Company, USA, 2-5

Coventry D.R., (1992), Acidification problems with duplex soils used for crop-pasture rotations, Australian Journal of Experimental Agriculture, **32**, 901-914

Curnow W.J and McLaughlin M.J., (1988), Soil acidification in Australia: the implications for increased uptake of cadmium, Bureau of Rural Resources Proceedings Number 2, Canberra, Australia, 48-61

DAFF, (2003), Results for wheat, The National Residue Survey Annual Report 2002 - 2003, Bureau of Rural Resources, Department of Agriculture, Forestry and Fisheries, Australia, 127-128

DAFF, (2004), Overview of the baking industry, The Australian Baking Industry: A Profile, Food Policy and Communications Section, Department of Agriculture, Fisheries and Forestry, Australia, 5-10

David D.J., Pinkerton A. and Williams C.H., (1978), Impurities in Australian phosphate fertilizers, The Journal of the Australian Institute of Agricultural Science, **44**, 132-135

David D.J and Williams C.H., (1975), Heavy metal contents of soils and plants adjacent to the Hume Highway near Marulan, New South Wales, Australian Journal of Experimental Agriculture and Animal Husbandry, **15**, 414-421

dBase IV, (1995), dBASE IV Users Guide, Borland Corporation, California, USA

Demirozu B., Saldamli L., Gursel B., Ucak A., Cetinyokus F. and Yuzbasi N., (2003), Determination of some metals which are important for food quality control in bread, Journal of Cereal Science, **37(2)**. 171-177

De Vries M.P.C., Tiller K.G. and Beckwin R.S., (1975), Sources of error in the determination of cadmium and lead in plant material by atomic absorption, Communications in Soil Science and Plant Analysis, **6(6)**, 629-640

Dobson S., (1992), Cadmium - Environmental Aspects, Environmental Health Criteria 135, World Health Organization, Geneva, 16-23

Doidge P., (1982), Pyrolytic graphite platforms, Varian Instruments at Work, Number AA-25, Varian Australia, Victoria, Australia

Doidge P. (1998), Development of analytical programs, In: Analytical Methods for Graphite Tube Atomizers, Varian Australia, Victoria, Australia

Dolling P.J., (1990), The influence of soil acidity on barley production in Western Australia. MSc Thesis, University of Western Australia, 3-4

Dolling P.J., Porter W.M. and Robson A.D, (1990), Soil pH and aluminium and their spatial variation in Western Australian acidic soils, Australian Journal of Experimental Agriculture, **30**, 637-643

DPIE, (2001), Market Basket Survey, National Residue Survey, Department of Primary Industry and Energy, Australia

Dundas M.J. and Pawluk S., (1977), Heavy metals in cultivated soils and in cereal crops in Alberta, Canadian Journal of Soil Science, **57**, 329-339

Ebdon L., Goodall P., Hill S.J., Stockwell P.B. and Thompson K.C., (1993), Ultra-trace determination of cadmium by vapour generation atomic florescence spectrometry, Journal of Analytical Atomic Spectrometry, **8**, 723-729

Eckersley M., (1994), History of grain farming in Australia, In: Australian Grains: A Complete Reference Book on the Grains Industry, Morescope Publishing, Sydney, Australia, 206-212

Ellison S.L.R., Rosslien M. and Williams A., (2000), Analytical measurement and uncertainty, Eurachem/CITAC Guide - Quantifying uncertainty in analytical measurement, Eurachem/CITAC, Geneva, 70-78

Encarta, (2001), Cadmium, Microsoft Encarta Online Encyclopaedia, www.encarta.msn.com

EPA, (1994), Cadmium, EPA Health Effects Notebook for Hazardous Air Pollutants, EPA-452/D-95-00, PB95-503579, Environment Protection Agency, United States of America

EPA, (2002), Report on cadmium, mercury and lead, IRIS database, United States Environmental Protection Agency, <www.epa.gov/iris/subst>

Erdman J.A. and Moul R.C., (1982), Mineral composition of small grain cultivars from a uniform test plot in South Dakota, Journal of Agriculture and Food Chemistry, **30**, 169-173

Eriksson J.E., (1990), A field study on factors influencing cadmium levels in soils and in grain of oats and winter wheat, Water, Air and Soil Pollution, **53**, 69-81

Eriksson J.E. and Andersson A., (1990), Cadmium, nickel and zinc content of oat grain and the effect of precipitation and soil pH, Swedish Journal of Agricultural Research, **20(2)**, 81-89

ESRI, (1998), Arcview 3.1 Users Guide, Environmental Systems Research institute, United States of America

Eurachem, (1998), The fitness for purpose of analytical methods, a laboratory guide to method validation and related topics, Eurachem guide, Eurachem Working Group, UK

Eurachem, (2000), Quantifying Uncertainty in Analytical Measurement, Second Edition, Ed: Ellison S.L.R., Rosslein M. and Williams A., Eurachem/CITAC guide, Eurachem/CITAC Working Group, UK

Every D., Simmons L., Al-Hakkak J., Hawkins S. and Ross M., (2002), Amylase, falling number, polysaccharide, protein and ash relationships in wheat millstreams, Euphytica, **126(1)**, 135-141

Evers A.D., Kelfkens M. and McMaster G., (2001), Ash determination - a useful standard or a flash in the pan, Proceedings of the 48th Australian Cereal Chemistry Conference, www.satake.co.uk/laboratory/Ash%20Tony%20Evers

Fairweather-Tait S.J., (1982), The effect of different levels of wheat bran on iron absorption in rats from bread containing similar amounts of phytate, British Journal of Nutrition, **47(2)**, 243-249

Farre R. and Lagarda M.J., (1986a), Chromium content of foods, Journal of Micronutrient Analysis, **2**, 201-209

Farre R. and Lagarda M.J., (1986b), Chromium content of foods and diets in a Spanish population, Journal of Micronutrient Analysis, **2**, 297-304

Farrer K., (2005), Milling and flour based products, In: To Feed A Nation: a history of Australian food science and technology, CSIRO Publishing, Australia, 77-84

Feinberg M. and Ducauze C., (1980), High temperature dry ashing of foods for atomic absorption spectrotomic determination of lead, cadmium and copper, Analytical Chemistry, **52**, 207-209

Fleischer M., Sarofim A.F., Fassett D.W., Hammond P., Shacklette H.T., Nisbet I.C. and Epstein S., (1974), Environmental impact of cadmium: a review by the panel on hazardous trace substances, Environmental Health Perspectives, **7**, 253-264

Foulkes E.C., (1986), Absorption of cadmium, In: Cadmium, Handbook of Experimental Pharmacology, Volume 80, Ed: Foulkes E.C., Springer-Verlag, Berlin, 75-88

Foulkes E.C., (1988), On the mechanism of transfer of heavy metals across cell membranes, Toxicology, **52**, 263-269

Foulkes E.C. and McMullen D.M., (1986), Endogenous metallothionein as determinant of intestinal cadmium adsorption: a reevaluation, Toxicology, **38**, 285-292

Fox S., (1998), Breeders address cadmium levels in durum wheat, Western Grains Research Foundation Endowment Fund: Research Project 1993-07, www.westerngrains.com/endow/1993_07

Frary B.D., (1986), Factors affecting the analytical performance and lifetime of graphite tube atomisers, AA, Varian Instruments at Work, Number AA-63, Varian, USA

Friberg L., Elinder C.G. and Kjellstrom T., (1992a), Environmental Levels and human exposure, In: Cadmium, Environmental Health Criteria 134, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, 36-51

Friberg L., Elinder C.G. and Kjellstrom T., (1992b), Effects on laboratory mammals and *in vitro* test systems, In: Cadmium, Environmental Health Criteria 134, International Programme on Chemical Safety, World Health Organisation, Geneva, Switzerland, 97-129

Friberg L., Elinder C.G., Kjellstrom T. and Nordberg G. F., (1985), Cadmium and Health: A Toxicological and Epidemiological Appraisal, Volume 1, CRC Press, Florida, USA, 25-44

Friberg L., Piscator M., Nordberg G.F. and Kjellstrom T., (1976), Metabolism, In: Cadmium in the Environment, CRC Press, Florida, USA, 23-91

FSANZ, (2002), Australian Food Standards Code, Volume 2, Standard 1.4.1, Food Safety Australia and New Zealand, Australian Government Publishing Service

FSANZ, (2003), Application A497: Maximum residue limits, Food Standards Australia New Zealand

FSANZ, (2004a), Application A534, Maximum residue limits, Food Standards Australia New Zealand

FSANZ, (2004b), Contaminants and natural toxicants, Standard 1.4.1, Australian Food Standards Code, Food Standards Australia New Zealand

FSANZ, (2004c), Application 464: Definition of wholegrain, Food Standards Australia New Zealand

GAFTA, (2003), Grain and feed trade activity map, Members Yearbook, The Grain and Feed Trade Association, London, UK, 8-9

Gawalko E.J., Nowicki T.W., Babb J. and Tkachuk R., (1997), Comparison of closed vessel and focused open vessel microwave dissolution for determination of cadmium, copper, lead and selenium in wheat, wheat products, corn bran and rice flour by transverse-heated graphite furnace atomic absorption spectrometry, Journal of the AOAC International, **80(2)**, 379-387

Gibbons R.D., Coleman D.E. and Maddalone R.F., (1997), An alternative minimum level definition for analytical quantitation, Environ. Sci. Technol., **31**, 2071-2077

Gilmutdinov A.K., Nagulin K.Y. and Zakharov, (1994), Analytical measurement in electrothermal atomic absorption spectrometry - how correct is it, Journal of Analytical Atomic Absorption Spectrometry, **9**, 643-650

Gine M.F., Krug F.J., Sass V.A., Reis B.F., Nobrega J.A. and Berndt H., (1993), Determination of cadmium in biological materials by tungsten coil atomic absorption spectrometry, Journal of Analytical Atomic Absorption Spectrometry, **8**, 243-245

Goeller H.E., Hise E.C. and Flora H.B., (1973), Societal flow of zinc and cadmium, In: Cadmium - the Dissipated Element, Ed. Fulkerson W., National Technical Information Service, Virginia, USA, 61-64

Gong H., Rose A.W. and Suhr N.H., (1977), The geochemistry of cadmium in some sedimentary rocks, Geochimica Cosmochimica Acta, **41**, 1687-1692

Gonzalez M.M, Gallego M. and Valcarcel M., (2000), Continuous sorbent pre-concentration for the electrothermal determination of ultra trace amounts of cobalt in milled wheat fractions, Journal of Agricultural and Food Chemistry, **48(10)**, 4515-4521

Goodman Fielder, (2002), Implementing our strategy, Goodman Fielder 2002 Concise Annual Report, Goodman Fielder, New South Wales, Australia, 5-6

Gordenin D., Resnick M.A. and Kunkel T.A., (2003), Cadmium studies suggest new pathway to human cancer, Chemistry in Australia, **70(7)**, Royal Australian Chemical Institute, 31-32

Goyer R.A., (1991), Transplacental transfer of cadmium and fetal effects, Fundamental and Applied Toxicology, **16**, 22-30

Grant E. and Leavenworth R., (1988), Why the control chart works; some statistical concepts, Chapter 2, -In: Statistical Quality Control, Sixth Edition, McGraw Hill, USA, 31-70

Gray C.W., McLaren R.G. and Roberts A.H.C., (2001), Cadmium concentrations in some New Zealand wheat, New Zealand Journal of Crop and Horticultural Science, **29**, 125-136

Guglhupf, (2004), A brief history of bread, www.guglhupf.com/breaduca/history, 1

Hack A.G. and Cocker H.D., (1983), Residuos de metales pesados en trigo, Revista de Ciencias Agrarias, **4(3/4)**, 20-26

Hamblin A. and Kyneur G., (1993), Current and historical conditions of Australian soil fertility In: Trends in wheat yields and soil fertility in Australia, Australian Government Publishing Service, Canberra, 31-32

Hart J., Welch R., Norvell W. and Kochian L., (2000), correlating thiol and cadmium concentrations in tissues of near isogenic lines of durum wheat that differ in grain cadmium accumulation, www.ars.usda.gov/research/publications.htm?SEQ/NO_115=111225

Hart J., Welch R., Norvell W. and Kochian L., (2004), Compartmentation of cadmium in high and low cadmium accumulating, near isogenic lines, of durum wheat, American Society of Plant Biology Annual Meeting, 256-261

Heinrichs H., Schulz-Dobrick B. and Wedepohl K.H., (1980), Terrestrial chemistry of Cd, Bi, Tl, Pb, Zn and Rb, Geochimica Cosmochimica Acta, **44**, 1519-1522

Henry R.J. and Kettlewell P.S., (1996), Grain components, In: Cereal Grain Quality, Chapman and Hall, London, UK, 300-317

History, (2004), Bread, www.history-magazine.com/bread

Hoenig M., (1985), The direct determination of cadmium in blood by electrothermal atomisation with the graphite platform, Varian instruments at Work, Number AA-49, Varian, USA

Hoenig M. and van Hoeyweghen P., (1987), Automated trace metal analysis of slurried samples by graphite furnace atomic absorption spectrometry, AA, Varian Instruments at Work, Number AA-77, Varian, USA

Holloway S, (2002), The history of bread, www.kitchenproject.com/history/bread

Hooper S., Barrett D. and Martin L., (2003), Grains industry : Performance and outlook, Australian Grains Outlook 2003, ABARE, Canberra, Australia, 1-4

Horiguchi H., Teranishi H., Niija K., Aoshima K., Katoh T., Sakuragawa N. and Kasuya M., (1994), Hypoproduction of erythropoietin contributes to anaemia in chronic cadmium intoxication: clinical study on Itai-Itai disease in Japan, Archives of Toxicology, **68(10)**, 632-636

Horiguchi H., Sato M., Konno N. and Fukushima M., (1996), Long-term cadmium exposure induces anemia in rats through hypoinduction of erythropoietin in the kidneys, Archives of Toxicology, **71(1/2)**, 11-20

Horiguchi H., Oguma E.,Sasaki S., Miyamota K., Ikeda Y., Machida M. and Kayama F., (2005), Environmental exposure to cadmium at a level insufficient to induce renal tubular dysfunction does not affect bone density among female Japanese farmers, Environmental Research, **97(1)**, 83-90

Horn M.K. and Adams J.A.S., (1966), Computer-derived geochemical balances and element abundances, Geochimica Cosmochimica Acta, **30**, 279-285

Horner E. and Kurfurst U., (1987), Cadmium in wheat from biological cultivation, Fresenius Zeitschrift fuer Analustische Chemie, **328**, 386-387

Horwitz W., (1998), Uncertainty - a chemists view, Journal of the AOAC International, **81(4)**, 785-794

Hoseney R.C., (1986), Structure of cereals, In: Principles of Cereal Science and Technology, American Association of Cereal Chemists, Minnesota, USA, 1 -15

Hough R.L., Young S.D. and Crout N.M.J., (2003), Modelling of Cd, Cu, Ni, Pb and Zn uptake, by winter wheat and forage maize, from a sewage disposal farm, Soil Use and Management, **19(1)**, 19-27

House W.A., Hart J.J., Norvell W.A. and Welch R.M., (2003), Cadmium absorption and retention by rats fed durum wheat grain, British Journal of Nutrition, **89(4)**, 499-505

Huber M.K.W., (1997), Improved calibration for wide measuring ranges and low contents, Journal for Quality, Comparability and Reliability in Chemical Measurement, **2**, 367-374

Hutton M., (1982), Cadmium in the European community: a prospective assessment of sources, human exposure and environmental impact, MARC report 26, Monitoring and Assessment Research Centre, Chelsea Collage, University of London, UK, 3-4

Hutton M., (1996), The release and pathways of environmental cadmium, In: Sources of Cadmium in the Environment, OECD Proceedings, Inter-Organisation Programme for the Sound Management of Chemicals, OECD, France, 19-48

Hutton M. and Symon C., (1986), The quantities of cadmium, lead, mercury and arsenic entering the U.K. environment from human activities, Science of the Total Environment, **57**, 129-151

IARC, (1993), Beryllium, cadmium, mercury and exposures in the glass manufacturing industry, IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, International Agency for Research on Cancer, WHO, France

IBIS, (2004), Flour mill product manufacturing in Australia, IBISWorld, www.ibisworld.com.au/industry/definition.asp?industry_id=102

ICC, (2004a), Sampling of grain, ICC Standard Number 120, General Principles of the Available ICC standard Methods, International Association for Cereal Science and Technology, Vienna, Austria

ICC, (2004b), Mechanical sampling of grains, ICC Standard Number 101/1, General Principles of the Available ICC Standard Methods, International Association for Cereal Science and Technology, Vienna, Austria

ICC, (2004c), Cereals – sampling of milled products, ICC Standard Number 130, General Principles of the Available ICC Standard Methods, International Association for Cereal Science and Technology, Vienna, Austria

ICC, (2004d), Cereals – mechanical sampling of milled products, ICC Standard Number 139, General Principles of the Available ICC Standard Methods, International Association for Cereal Science and Technology, Vienna, Austria

ICC, (2004e), Determination of ash in cereals and cereal products, ICC Standard Number 104/1, General Principles of the Available ICC Standard Methods, International Association for Cereal Science and Technology, Vienna, Austria

ICC, (2004f), Determination of total dietary fibre, ICC Standard Number 156, General Principles of the Available ICC Standard Methods, International Association for Cereal Science and Technology, Vienna, Austria

ICC, (2004g), Determination of lead and cadmium in cereals and cereal products, ICC Standard Number 154, General Principles of the Available ICC standard Methods, International Association for Cereal Science and Technology, Vienna, Austria

ICH, (1994), Text on validation of analytical procedures, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

ICH, (1996), Validation of analytical procedures: Methodology, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

Ihnat M., (1997), Metals and other elements, Committee on residues and related topics, Journal of the AOAC International, **80(1)**, 239-241

Ikeda M., Zhang Z.-W., Moon C.-S., Shimbo S., Watanabe T., Nakatsuka H., Matsuda-Inoguchi N and Higashikawa K., (2000), Possible effects of environmental cadmium exposure on kidney function in the Japanese general population, International Archives of Occupational and Environmental Health, **73(1)**, 15-25

Inglett G.E., (1974), Wheat in perspective, In: Wheat: Production and Utilisation, Ed: Inglett G.E., Avi Publishing Company, 1-7

Inglett G.E. and Anderson R.A., (1974), Flourmilling, In: Wheat: Production and Utilisation, Ed: Inglett G.E., Avi Publishing Company, 186-198

Isbell R.F., (1984), Soil classification in Australia, In: Proceedings of the National Soils Conference, Brisbane, Australia, 27-39

Isbell R.F., (1992), A brief history of soil classification in Australia since the 1920s, Australian Journal of Soil Research, **30**, 825-834

Isbell R. F., (1998), Key to soil orders, In: The Australian Soil Classification, CSIRO Publishing, Australia, 15-20

Ismail-Beigi F., Faraji B. and Reinhold J.G., (1977), Binding of zinc and iron to wheat bread, wheat bran and their components, American Journal of Clinical Nutrition, **30(10)**, 1721-1725

ISO, (1993), Guide to the expression of uncertainty in measurement, ISO, Geneva, Switzerland

Jansson G., (2002), Cadmium in arable crops: The influence of soil factors and liming, Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden, 26-30

Jarup L., Berlund M., Elinder C.G., Nordberg G. and Vahter M., (1998), Health effects of cadmium exposure - a review of the literature and a risk estimate, Scandinavian Journal of Work, Environment and Health, **24(1:1)**, 51-58

Jaio Y., Grant. And Bailey L.D., (2004), Effects of phosphorus and zinc fertiliser on cadmium uptake and distribution in flax and durum wheat, Journal of the Science of Food and Agriculture, **84(8)**, 777-785

Jorheim L., (1993), Determination of metals in foodstuffs by atomic absorption spectophotometry after dry ashing: NMKL interlaboratory study of lead, cadmium, zinc, copper, iron, chromium and nickel, Journal of AOAC International, **76(4)**, 798-813

JMHLW, (2003), Precision and quality assurance parameters for the establishment of new test methods, Development of Test Methods, Japanese Ministry of Health, Labor and Welfare, Tokyo, Japan

Jung K., Pergande M., Graubaum H.J., Fels L.M., Endl V. and Stole H., (1993), Urinary proteins and enzymes as early indicators of renal dysfunction in chronic exposure to cadmium, Clinical Chemistry, **39**, 757-765

Kaplan R., (1987), The one-way analysis of variance, In: Basic Statistics for the Behavioural Sciences, Chapter 8, Allyn and Bacon, USA, 169

Kashlan N.B., Srivastava V.P., Mohanna N.A. and Motawa Y.K., (1991), The proximate and elemental composition of wheat flour and major types of bread consumed in Kuwait, Food Chemistry, **36(2)**, 205-211

Kelly N. E., (2000), Minerals information report on cadmium, United States Geological Society, Cahners Business Information

Kent N, (1985), Wheat and rye milling, In: The Master Bakers Book of Bread Making, Ed: Brown J., The National Association of Master Bakers, United Kingdom, 122-127

Kido T., Nogawa K., Honda R. and Tsuritani I., (1990), The association between renal dysfunction and osteopenia in environmental cadmium-exposed subjects, Environmental Research, **51**, 71-77

King B. and Lawn R., (1999), International study of forensic ethanol standards, Analyst, **124**, 1123-1130

King W.G., Rodriguez J.M. and Wai C.M., (1974), Losses of trace concentrations of cadmium from aqueous solution during storage in glass containers, Analytical Chemistry, **46(6)**, 771-773

Kinrade J.D and van Loon J.C., (1973), Solvent extraction for use with flame atomic absorption spectrometry, Analytical Chemistry, **46(13)**, 1894-1898

Kjellstrom T., Lind B., Linnman L. and Nordberg G., (1974), A comparative study on methods for cadmium analysis of grain with application to pollution evaluation, Environmental. Research, **8**, 92-99

Knowles M., (1987) The determination of lead and cadmium in blood and manganese and aluminium in serum, Varian Instruments at Work AA-76, Varian, USA

Knutti R., Bucher P., Stengl M., Stolz M., Tremp J., Ulrich M. and Schlatter C., (1988), Cadmium in an unpolluted forest in Switzerland, In: Cadmium, Ed: Stoeppler M. and Piscator M., Springer-Verlag, Berlin, Germany, 171-191

Koityohann S.R. and Hopkins C.A., (1976), Losses of trace metals during the ashing of biological materials, Analyst, **101**, 870-875

Krishnamurti G.S.R. and Naidu R., (2003), Solid-solution equilibria of cadmium in soils, Geoderma, **113**, 17-22

Kukier U. and Chaney R., (2001), Growing rice grain with controlled cadmium concentrations, www.nps.ars.usda.gov/publications/publications.html?SEQ_NO_115=124041

Lagerwerff J.V. and Specht A.W., (1970), Contamination of roadside soil and vegetation with cadmium, nickel, lead and zinc, Environmental Science and Technology, **4**, 583-588

Lasztity R., (1999), General characterisation of the chemical composition of cereal grains. The structure of the cereal grain, In: Cereal Chemistry, Akademiai Kiado, Budapest, 13-24

Lauwerys R., Amery A., Bernard A., Bruaux P., Bruchet J.P., Claeys F., De Plaen P., Ducoffre G., Fagard R., Lijnen P., Nick L., Roels H., Rondia D., Saint-Remy A., Sartor F and Staessen, (1990), Health effects of the environmental exposure to cadmium. Objectives, design and organisation of the Cadmibel Study: a cross-sectional morbidity study carried out in Belgium from 1985-1989, Environmental Health Perspectives, **87**, 283-296

LECO (2003), Moisture and ash determination in flour, Organic Application Note TGA-601, LECO Corporation, USA

Lee S.C., Prosky L. And DeVries J.W., (1992), Determination of total, soluble and insoluble fibre in foods - enzymatic-gravimetric method, MES-TRIS buffer: Collaborative study, Journal of the AOAC International, **75**, 395-403

Lee S-Z., Allen H.E., Huang C.P., Sparks D.L., Sanders P.F. and Peijnenburg W.J.G.M., (1996), Predicting soil-water partition coefficients for cadmium, Environmental Science and Technology, **30**, 3418-3424

Leonard T., (1999), The milling process, Bread Bakers Guild of America, http://home.earthlink.net/~ggda/The_Milling_Process

Lind Y., Engman J., Jorhem L. and Glynn A.W., (1998), Accumulation of cadmium from wheat bran, sugar-beet fibre, carrots and cadmium chloride in the liver and kidneys of mice, British Journal of Nutrition, **80**, 205-211

Lindberg S., Landberg T. and Greger M., (2004), A new method to detect cadmium uptake in protoplasts, Planta, **219(3)**, 526-531

Lindqvist-Ostblom A. and Eklund M., (2001), cadmium in sewage sludge in a Swedish region: sources and reduction opportunities, Environmental Engineering and Policy, **2(3)**, 145-154

Liu C.L., Wang Y.P., Kiao Y.L., Wu C.R., Huang C.H., Sung C.C. and Lee C.H., (1998), The studies of heavy metals in soils and rice in central Taiwan, In: Proceedings of Soils and Fertiliser Reports of 1997, Department of Agriculture and Forestry, Taiwan Provincial Government, ROC, 328-337

Lorenz S.E., Hamon R.E., McGrath S.P., Holm P.E. and Christensen T.H, (1994), Application of fertilizer cations affect cadmium and zinc concentrations in soil solutions and uptake by plants, European Journal of Soil Science, **45**, 159-166

Lott J.N.A., Ockenden I., Raboy V. and Batten G.D., (2000), Phytic acid and phosphorus in crop seeds and fruits: a global estimate, Seed Science Research, **10**, 11-33

Lowry. R, (2005), Introduction to linear correlation and regression, www.faculty.vassar.edu/lowry/ch3pt1.html

L'vov B.V., (1978), Electrothermal atomisation - the way toward absolute methods of atomic absorption analysis, Spectrochimica Acta., **33B**, 153-162

MacLaren G.S. and Crawford D.M., (1996), Changes in the fertility status of Victorian agricultural soils, In: Temporal and Spatial Changes in Soil Acidity, Salinity and Fertility, National Landcare Program, Agriculture Victoria, 3-9

MacMasters M.M., Hinton J.J. and Bradbury D., (1971), Microscopic structure and composition of the wheat kernel, In: Wheat: chemistry and technology, Ed: Pomeranz Y., Volume 1, American Association of Cereal Chemists. 51-59

MAFF, (1994a), A survey of cadmium, arsenic, mercury and lead concentrations in selected foods, MAFF Report Number 33, Ministry of Agriculture, Foods and Fisheries, UK

MAFF, (1994b), 1994 total diet study: metals and other elements, MAFF Report Number 131, Ministry of Agriculture, Foods and Fisheries, UK

Mann S.S. and Richie G.S.P., (1995), Forms of cadmium in sandy soils after amendment with soils of higher fixing capacity, Environmental Pollution, **87**, 23-29

Marowsky G. and Wedephol K.H., (1971), General trends in the behaviour of Cd, Hg, Ti and Bi in some major rock forming processes, Geochimica Cosmochimica Acta, **35**, 1255-1259

Massadeh A.M., Tahat M., Jaradat Q.M., Al-Momani I.F., (2004), Lead and cadmium contamination in roadside soils in Irbid City, Jordan; a case study, Soil and Sediment Contamination, **13(4)**, 347-353

Matsumoto K. and Fuwa K, (1986a), The estimation of cadmium in biological samples, In: Cadmium, Ed: Foulkes E.C., Springer-Verlag, Berlin, Germany, 3-16

Mattern P.J., (1991), Cereal grains of the world, In: Handbook of Cereal Science and Technology, Ed. Lorenz K.J. and Kulp K., Marcel Dekker, New York, 1-2

Matusiewicz H., Kopras M. and Sturgeon R.E., (1997), Determination of cadmium in environmental samples by hydride generation with *in situ* concentration and atomic absorption detection, Analyst, **122**, 331-336

Matz S.A., (1991a), The origins of wheat, In: The chemistry and technology of cereals as food and feed, 2ND edition, Van Nostrand Reinhold, New York, USA, 1-2

Matz S.A., (1991b), Botany of the wheat plant, In: The chemistry and technology of cereals as food and feed, 2ND edition, Van Nostrand Reinhold, New York, USA, 6-15

McEwen J., (2004), Port Pirie and SA grains blending, www.crikey.com.au/whistleblower/2004/11/09-0002

McKenzie N., Jacquier D., Isbell R. and Brown K., (2004), Compendium of Australian soils, In: Australian Soils and Landscapes, CSIRO Publishing, Australia, 129-137

McMaster H., (2000), Grain production and yield in Australia, In: An Introduction to the Australian Grains Industry, Ed: O'Brien L. and Blakeney A.B., Royal Australian Chemical Institute (Cereal Chemistry Division), 1-4

McMaster G.J. and Gould J.T., (1995), Wheat usage in Australia and New Zealand, In: Wheat End Uses around the World, Ed: Faridi H. and Faubion J.M., Nabisco Incorporated, New Jersey, United States of America, 267-272

Meagher R.P, (2003), The bread making process, In: Bread and Bread Making – A practical guide to the technology of bread making in Australia, Ed Meagher R.P., Burns Phillip and Company, Australia, 32-39

Merry R.H. and Tiller K.G., (1978), The contamination of pasture by a lead smelter in a semi-arid environment, Australian Journal of Experimental Agriculture and Animal Husbandry, **18**, 89-96

Merry R.H, Tiller K.G., de Vries M.P.V. and Cartwright B., (1981), Contamination of wheat crops around a lead-zinc smelter, Environmental Pollution (Series B), **2**, 37-48

Minamisawa M., Minamisawa H., Yoshida S. and Takai N., (2004), Adsorption behaviour of heavy metals on biomaterials, Journal of Agricultural and Food Chemistry, **52(18)**, 5606-5613

Moberg A., Hallmanns G., Sjostrom R. and Wing K.R., (1987), The effect of wheat bran on the absorption of cadmium in rats, British Journal of Nutrition, **58(3)**, 383-391

Monro J.A., (2004), Adequate intake values for dietary fibre based on faecal bulking indexes of 66 foods, European Journal of Clinical Nutrition, **58(1)**, 32-39

Morales-Rubio A., Salvador A., De la Guardia M. and Ross R., (1993), Determination of cadmium and nickel in rice plants by flame atomic absorption spectrometry, Atomic Spectroscopy, **14(1)**, 8-12

Morris E.R. and Greene F.E., (1970), Distribution of lead, tin, cadmium chromium and selenium in wheat and wheat products, Federation Proceedings, Federation of American Societies for Experimental Biology, **29(2)**, 500-506

Mousia Z., Edherly S. Pandiella S.S. and Webb C., (2004), Effect of wheat pearling on flour quality, Food Research International, **37(5)**, 449-456

Mugford D.C. and Steele R.J., (1980), The mineral content of Australian wheat and baker's flour, Food Technology in Australia, **32(12)**, 630-636

Mustafa G., Singh B. and Kookana R., (2004), Cadmium adsorption and desorption behaviour on goethite at low equlibrium concentrations: effects of pH and index cations, Chemosphere, 57(10), 1325-1333

NABIM, (1979), Flourmill - description of process and main machines, In: The Practice of Flourmilling, Volume 1, National Association of British and Irish Millers, United Kingdom, 215-220

NABIM, (1988), Bran finishers, In: Mill Processes, Part 1, National Association of British and Irish Millers, United Kingdom, 37-39

Naidu R., Bolan N.S., Kookana R.S. and Tiller K.G., (1994), Ionic strength and pH effects on the sorption of cadmium and the surface charge of soils, European Journal of Soil Science, **45**, 419-427

Nan Z., Zhao C., Li J., Chen F. and Sun W., (2002), Relations between soil properties and selected heavy metal concentrations in spring wheat (Triticum aestivum L.) grown on contaminated soils, Water Air and Soil Pollution, **133(1/4)**, 205-202

NATA, (1995), Guidelines for quality control in the analytical laboratory, Technical Note 23, National Association of Testing Authorities (Australia), Australia

NATA, (2002), Measurement of uncertainty in chemical testing, Technical Circular 1, National Association of Testing Authorities (Australia), Australia

NATA, (2004) Guidelines for the validation and verification chemical methods, Technical Note 17, National Association of Testing Authorities (Australia), Australia

NARL, (2001), Measurement uncertainty: essential statistics, National Analytical Reference Laboratory, Australian Government Analytical Laboratory, Australia, 24-30

Navarro S. and Wood R., (2001), Introducing the Codex Alimentarius, In: Codex Deciphered, Leatherhead Publishing, UK, 1-18

NFA, (1995), Australian Food Standards Code, Standard A12, National Food Authority, Australian Government Publishing Service

Nishijo M., Satarug S., Honda R., Tsuritani I. and Aoshima K., (2004), The gender differences in health effects of environmental cadmium exposure and potential mechanisms, Molecular and Cellular Biochemistry, **255(1/2)**, 87-96

Northcote K.H, Beckmann G.G, Bettenay E., Churchward H.M., van Dijk D.C., Dimmock G.M., Hubble G.D., Isbell R.F., McArther W.M., Murtha G.G., Nicolls K.D., Paton T.R., Thompson C.H., Webb A.A. and Wright M.J., (1960 to 1968), Atlas of Australia Soils, Sheets 1 to 10, with explanatory notes, Melbourne University Press, Australia

Northcote K. H. and Skene J. K. M., (1972), Chemical criteria for salinity, sodicity and alkalinity, In: Australian Soils with Saline and Sodic Properties, Soil Publication Number 27, Commonwealth Scientific and Industrial Research Organization, Australia, 7-11

NRCC, (1979), Effects of cadmium in the Canadian environment, NRCC Publication 16743, National Research Council Canada, Ottawa, Canada, 16-21

NRS, (1966), Market Basket Survey, National Residue Survey, Bureau of Rural Resources, Australian Government Publishing Service

Oakenfull D., (2001), Bread through the ages, In: All about bread, Choice Books, Australia, 1-8

Ocker H.-D. and Seibel W., (1980), Ruckstandssituation bei getreide und brot. 2 Mitt: Schwermetallgehalte (blei, cadmium), Gerreide, Mehl und Brot, **34**, 118-122

O'Dea K. (1987), Crop physiology: some case histories, The Role of Cereals in the Human Diet, Proceedings, 37 Annual Conference, Royal Australian Chemical Institute, Cereal Chemistry Division, Australia, 1-5

Oliver D.P., Gore P.J., Moss H.J. and Tiller K.G., (1993), Cadmium in wheat grain and milling products from some Australian flourmills, Australian Journal of Agricultural Research, **44**, 1-11

Oosthuizen J. and Boyce M. (2002), An evaluation of heavy metals in sediment and fish in the Swan River and the health risks associated with fish consumption: A pilot study, Environmental Health, **2(3)**, 19-26

OSHA, (1992a), Substances technical guidelines for cadmium, Standard number 1910.1027 App B,. Occupational Safety and Health Administration, United States Department of Labor, United States of America

OSHA, (1992b), Substance safety data sheet - Cadmium - 1910.1077 AppA, OSHA Regulations (Standards - 29 CFR), Occupational Safety and Health Administration, United States Department of Labor, United States of America

Ou S., Gao K. and Li Y., (1999), An in vitro study of wheat bran binding capacity for Hg, Cd and Pb, Journal of Agriculture and Food Chemistry, **47(11)**, 4714-4717

Page A.L., El-Amamy M.M. and Chang A.C., (1986), Cadmium in the environment and its entry into terrestrial food chain crops, In: Cadmium, Ed. Foulkes E.C., Springer-Verlag, New York, 33-74

Pandya C.B., Patel T.S., Shah G.M. and Sathawara N.G., (1986), Quality assurance of analytical data, with special reference to the determination of lead and cadmium in biological samples, Journal of Analytical Atomic Spectrometry, **1**, 387-390

Pereira L.A., de Amorim I.G. and da Silva J.B.B., (2004), Development to determine methodologies to determine aluminium, cadmium, chromium and lead in drinking water by ET AAS using permanent modifiers, Talanta, **64(2)**, 395-400

Peterson D. and Fulcher R., (2002), Variation in Minnesota HRS wheats: bran content, Journal of Food Science, **67(1)**, 67-72

Pina K. and Amy N.K., (1989), Determination of molybdenum in human samples and defined diets using electrothermal atomic absorption spectrometry, Journal of Micronutrient Analysis, **5**, 127-138

Piso S. and de Boer J.L.M., (1994), *A priori* calculation of instrumental detection limits in graphite furnace atomic absorption spectrometry, Atomic Spectroscopy, **5**, 220-222

Plessi M. and Monzani A., (1990), Survey of total and bioavailable chromium in grain and cereal products by atomic absorption spectrophotometry, Journal of the AOAC International, **73(5)**, 798-800

Polacek I., Greco V., Kralova S., Biskupikova J., Lalcikova A. and Korcekova E., (1991), Content of copper, zinc, cadmium, lead, mercury and arsenic in food wheat harvested in 1989, Krmivarstvi, (1), 203-205

Pomeranz Y., (1988), Chemical composition of kernel structures, In: Wheat: chemistry and technology, Ed: Pomeranz Y., Volume 1, American Association of Cereal Chemists. 97-158

Posner E. and Hibbs, (1997a), The grinding process, In: Wheat Flour Milling, American Association of Cereal Chemists, St Paul, USA, 125-131

Posner E. and Hibbs, (1997b), The sieving process, In: Wheat Flour Milling, American Association of Cereal Chemists, St Paul, USA, 155-159

Posner E. and Hibbs, (1997c), Experimental and laboratory milling, In: Wheat Flour Milling, American Association of Cereal Chemists, St Paul, USA, 31-55

Posner E. and Hibbs, (2004a), Grinding machines, In: Wheat Flour Milling, Second Edition, American Association of Cereal Chemists, St Paul, USA, 125-132

Posner E. and Hibbs, (2004b), Operation of the roller mill, In: Wheat Flour Milling, Second Edition, American Association of Cereal Chemists, St Paul, USA, 137-154

Prosky L., Asp N.-G., Schweizer T.F, DeVries J.W. and Furda I., (1988), Determination of insoluble, soluble and total dietary fibre in foods and food products: Interlaboratory study, Journal of the AOAC International, **71**, 1017-1029

Prynne C.J. and Southgate D.A., (1979), The effects of a supplement of dietary fibre on faecal excretion by human subjects, British Journal of Nutrition, **41(3)**, 495-503

Pyler, E.J., (1973), Wheat flour, In: Baking Science and Technology, Siebel Publishing, 295-299

Qarooni J., (1990), Flat Breads, American Institute of Baking, Technical Bulletin, Volume XII (12),

Quail K., (1996), Introduction to flat breads: origins and definitions, In: Arabic Bread Production, American Association of Cereal Chemists, USA, 1-8

Quattrucci E., Conti M.E., Carcea M. and Francisci R., (1998), Monitoring of lead and cadmium in Italian Wheats, In: Cereal Science – its Contribution to Health and Wellbeing, 16th ICC Conference, Vienna, Austria, International Association for Cereal Science and Technology, 121-129

Rachou J., Hendershot. And Sauva S., (2004), Effects of pH on fluxes of cadmium in soils measured by using diffusive gradients in thin films, Communications in Soil Science and Plant Analysis, **35(17/18)**, 2655-2663

Rankama K. and Sahama T.G., (1950), Geochemistry, Chicago University Press, Chicago, USA

Rayment G.E., (1988), Sources of cadmium in agricultural products, In: Cadmium Accumulations in Australian Agriculture, Bureau of Rural Resources Proceedings No. 2, Ed. Simpson J. and Curnow W., Australian Government Publishing Service, Canberra, Australia, 35-66

Renella G., Adams P., Bianco M.R., Landi L., Violante P. and Nannipieri P., (2004), Availability and speciation of cadmium added to a calcareous soil under various managements, European Journal of Soil Science, **55(1)**, 123-131

Ries I., (2002), Flour power, The Bulletin/NewsWeek, http://bulletin.ninemsn.com.au/bulletin/EdDesk.nsfprinting/6BFAA6E626A8A0AEC

Rincon-Leon F., Abellan-Bellista P. and Zurera-Cosano G., (1990), Elemental composition of infant beikosts, Journal of Micronutrient Analysis, **8**, 43-53

Ritz B., (1998), Effect of cadmium body burden on immune response of school children, Archives of Environmental Health, www.findarticles.com/cf_0/m0907/n4_v53/21017752/print.j

Roane T.M. and Pepper I.L., (1999), Microbial responses to environmentally toxic cadmium, Microbial Ecology, **38**, 358-364

Robards K. and Worsfold P., (1991), Cadmium: Toxicology and analysis, Analyst, **116**, 549-563

Roberts A.H.C., Longhurst R.D. and Brown M.W., (1995), Cadmium survey of South Auckland market gardens and mid Canterbury wheat farms, AgResearch Report, New Zealand Department of Agriculture, New Zealand

Roels H., Lauwerys R.R., Buchet J.P. and Bernard A., (1981), Environmental expose to cadmium and renal function in aged women in three areas of Belgium, Environmental Research, **24**, 117-128

Roels H., Bernard A.M., Cardenas A., Buchet J.P. and Lauwerys R.R., (1993), Markers of renal changes induced by industrial pollutants. III Applications to workers exposed to cadmium, British Journal of Industrial Medicine, **50**, 37-45

Rossman T.G., Roy N.K. and Lin W, (1992), Is cadmium genotoxic, In: Cadmium in the Human Environment: Toxicity and Carcinogenicity, Ed. Norburg G.F., Herber R.F.M and Alessio L., International Agency for Research on Cancer, IARC Publications, Lyons, France, 367-375

Satzger R.D., (1988), Evaluation of inductively coupled plasma mass spectrometry for the determination of trace elements in foods, Analytical Chemistry, **60**, 2500-2504

Sauve S., Norvell W.A., McBride M.B. and Hendershot W., (1999), Speciation and complexation of cadmium in extracted soil solutions, Tektran, Agricultural Research Service, United States Department of Agriculture, United States of America, www.nalusda.gov/ttic/tektran/data/000010/78/0000107882

Satarug S., Ujjin P., Vanavanitkun Y., Nishijo M., Baker J. and Moore M., (2004), Effects of cigarette smoking and exposure to cadmium and lead on phenotypic variability of hepatic CYP2A6 and renal function biomarkers in men, Toxicology, **204(2/3)**, 161-169

Saxelby C. and Venn-Brown U., (1980), Nutritional aspects of milling, In: The Role of Australian Flour and Bread in Health and Nutrition, Bread Research Institute of Australia, New South Wales, Australia, 53-61

Schindler E., (1983), Schwermetallgehalte von getreide und getreideprodukten, sowie von muskelfleisch, leber und niere, Deutsche Lebensmittel-Rundschau, **79**, 338-343

Schulze K., (2003), Selecting a wheat variety, Agnote DPI 444, NSW Agriculture, New South Wales, Australia

Seibel W. and Ocker H.-D., (1979), Gerhalt an pflanzenschutzmittel - ruckstanden und schwermetallen in deutschen weizen - und roggenernten 1974 und 1975, Landwirschaftliche Forschung, **32**, 186-190

Sewell T., (2003), Wheat statistics, In: The Global Grain Market - From the Present Day to the Year 2008/09, Dry Cargo International, Protea Publishing, Essex, UK

Shcheglova I. and Golenkov V.F., (1984), The use of a.c. polarography to determine lead and cadmium in wheat grain, Voprosy Pitaniya, **4**, 60-66

Shellenberger J.A., (1969), Wheat, In: Cereal Science, Ed. Matz S.A., AVI Publishing Company, USA, 1-2

Shelly B., (1990), Low level measurement of cadmium in foods, Varian Instruments at work, Number AA-97, Varian, Australia

Shimbo S., Zhang Z.-W., Moon C.-S., Watanabe T., Nakatsuka H., Matsuda-Imoguchi N., Higashikawa K. and Ikeda M., (2000), Correlation between urine and blood concentration, and dietary intake of cadmium and lead among women in the general population of Japan, International Archives of Occupational and Environmental Health, **73(3)**, 163-170

Sillanpaa M. and Jansson H., (1992a), Results and discussion, In: Status of cadmium, lead, cobalt and selenium in plants and soils of thirty countries, FAO Soils Bulletin Number 65, Food and Agriculture Organisation of the United Nations, Rome, Italy, 13-17

Sillanpaa M. and Jansson H., (1992b), Methods of plant analysis, In: Status of cadmium, lead, cobalt and selenium in plants and soils of thirty countries, FAO Soils Bulletin Number 65, Food and Agriculture Organisation of the United Nations, Rome, Italy, 6-8

Simmonds D.H., (1989), Wheat and Wheat Quality in Australia, Commonwealth Scientific and Industrial Research Organisation, Australia

Simmons L., Al-Hakkak J., Hawkins S. and Ross M., (2002), Amylase, falling number, polysaccharide, protein and ash relationships in wheat millstreams, Euphytica, **126(1)**, 135-142

Singh B.R., Narwal R.P., Jeng A.S. and Almas A., (1995), Crop uptake and extractability of cadmium in soils naturally high in metals at different pH levels, Communications in Soil Science and Plant Analysis, **26(1314)**, 2123-2129

Skurikhin I., (1993), Methods of analysis for toxic elements in foods. Part IV: general method of ashing for the determination of toxic elements, Journal of the AOAC International, **76(2)**, 257-262

Slattery W.J and Ronnfeldt G.R., (1992), Seasonal variation of pH, aluminium and manganese in acid soils from north-eastern Victoria, Australian Journal of Experimental Agriculture, **32**, 1105-1112

Slattery W.J., Morrison G.R. and Coventry D.R., (1995), Liming effects on soil exchangeable and soil solution cations of four soil types in north-eastern Victoria, Australian Journal of Soil Research, **33**, 277-295

Smeyers-Verbeke J., Yang Q., Penninckx W. and Vandervoort F., (1990), Effectiveness of palladium chemical modification for the determination of cadmium by graphite furnace atomic absorption spectrometry, Journal of Analytical Atomic Spectrometry, **5**, 393-398

Smith C.J., Hopmans P. and Cook., (1996), Accumulations of Cr, Pb, Cu, Ni, Zn and Cd in soil following irrigation with treated urban effluent in Australia, Environmental Pollution, **94**, 317-323

Smolders E. and Peijnenburg W.J., (2002), Cadmium availability in soils and identification of high risk soils, www.icsu-scope.org/cdmeeting/smolders%20abstract

Spouncer L.R., Cartwright B. and Nable R.O., (1991), Nutrient concentration in barley grain from the 1983/84 season in South Australia and the 1989/90 season in South Australia and north-western Victoria, Divisional Report Number 120, Division of Soils, Commonwealth Scientific and Industrial Research Organisation, Australia

Spouncer L.R., Merry R.H. and Michalski C.H., (1996), Allocation of acidity profile classes to atlas of Australian soils mapping units, Technical Report 20/1996,Division of Soils, Commonwealth Scientific and Industrial Research Organisation, Australia

SPSS, (1999), Systat 9.0 Users Manual, SPSS, Chicago, United States of America

Stacey S., Merrington G. and McLaughlin M.J., (2001), The effect of aging biosolids on the availability of cadmium and zinc in soil, European Journal of Soil Science, **51**, 193-198

Staessen J., Amery A., Bernard A., Bruaux P., Buchet J.P., and Bulpitt C.J., (1991), Blood pressure, the prevalence of cardiovascular diseases and exposure to cadmium: A population study, American Journal of Epidemiology, **134**, 257-264

Standards Australia, (1985), Good laboratory practice Part 1: Chemical analysis, Australian Standard AS 2830.1 – 1985, Standards Australia

Standards Australia, (1989), Recommended practice for chemical analysis by atomic absorption spectrometry Part 2: Graphite furnace spectrometry, Australian Standard AS 2134.2 - 1989, Standards Australia

Standards Australia, (1990a), Test Methods – Guide to the format, style and content, Australian Standard AS 2929-1990, Standards Australia

Standards Australia, (1990b), Quality control - Guide to the use of control chart methods including Cusum techniques, Australian Standard AS 3940-1990, Standards Australia

Standards Australia, (1991), Guide 35 - Certification of reference materials - General and statistical principles, Australian Standard HB 19.35-1991, Standards Australia

Standards Australia, (1999), General requirements for the competence of testing and calibration laboratories, Australia Standard ISO/IEC 17025-1999, Standards Australia

Staudt E. and Ziegler E., (1973), Milling wheat, In: Flour Chemistry, Buhler Brothers, Switzerland, 32-43

Stefanov K., Seizova K., Yanishlieva N., Marinova E. and Popov S., (1995), Accumulation of lead, zinc and cadmium in plant seeds growing in metalliferous habitats in Bulgaria, Food Chemistry, **54**, 311-313

Stenson H., (1999), Quality control charts, Chapter 9, In: Systat Users Manual, SPSS, USA, 299-314

Su G.C.C., (1998), A comparison of statistical and empirical detection limits, Journal of AOAC International, **81(1)**, 105-109

Szlyk E. and Szydlowska-Czerniak A., (2004), Determination of cadmium, lead and copper in margarines and butter by galvanostatic stripping chronopotentiometry, Journal of Agricultural and Food Chemistry, **52(13)**, 4064-4071

Telisman S., Jurasovic J., Pizent A. and Cvitkovic P., (1997), Cadmium in the blood and seminal fluid of nonoccupationally exposed adult male subjects with regard to smoking habits, International Archives of Occupational and Environmental health, **70(4)**, 243-248

Thompson M., Ellison S. L. R., Fajgeli A., Willetts P. and Wood R., (1999), Harmonised guidelines for the use of recovery information in analytical measurement, Pure and Applied Chemistry, **71(2)**, 337-348

Tiller K.G., (1992), Urban soil contamination in Australia, Australian Journal of Soil Research, **30**, 937-943

Tiller K.G., Cartwright B., de Vries M.P.C., Merry R.H. and Spouncer L.R., (1975), Environmental Pollution of the Port Pirie Region 1. Accumulation of metals in wheat grain and vegetables grown on the coastal plain, CSIRO Division of Soils Report Number 6, Commonwealth Scientific and Industrial Research Organisation, Australia

Tsuritani I., Honda R., Ishizaki M., Yamada Y., Aoshima K. and Kasuya M., (1994), Serum bone-type alkaline phosphatase activity in women living in a cadmium-polluted area, Toxicology Letters, **71**, 209-212

United States Environment Protection Agency (US EPA), (2002), Report on cadmium, mercury and lead, IRIS database, <www.epa.gov/iris/subst>

Ure A.M and Berrow M.L., (1982), The Elemental Constituents of Soils in Environmental Chemistry, Volume 2, The Royal Society of Chemistry, London, 94-116

USEPA, (1987), Cadmium, Integrated Risk Information System, United States Environment Protection Agency, www.epa.gov/iris/subst/0141

van Dokkum W., Wesstra A. and Schippers F.A., (1982), Physiological effects of fibre-rich types of bread. 1. The effect of dietary fibre from bread on thew mineral balance of young men, British Journal of Nutrition, **47(3)**, 451-460

van Staden J.F. and Taljaard R.E., (2004), Determination of lead (II), copper (II), zinc (II), cobolt (II), cadmium (II), iron (III) and mercury (II) using sequential injection extractions, Talanta, **64(5)**, 1203-1212

Varian, (1988), Analytical data, In: Analytical methods for graphite tube atomisers, Ed: Rothery E. Chapter four, USA, 41-63

Varo P., Nuurtamo M., Saari E. and Koivistoinen P., (1980), Mineral element composition of Finnish foods III. Annual variation of the mineral element composition of cereal grains, Acta Agriculturae Scandinavica (Supplement), **22**, 27-35

Versieck J., (1989), Sample analysis - including current status of trace elemental analysis, recommended reference materials and considerations of quality control, Journal of Micronutrient Analysis, **6**, 261-304

Versieck J., Vanballenberghe L. and De Kesel A., (1988), Determination of cadmium in serum and packed blood cells by neutron activation analysis, In: Cadmium, Ed: Stoeppler M. and Piscator M., Springer-Verlag, Berlin, Germany, 205-212

Vesterberg O. and Engqvist A, (1988), Comparison of two methods for the determination of cadmium in blood, In: Cadmium, Ed: Stoeppler M. and Piscator M., Springer-Verlag, Berlin, Germany, 195-207

Vinas P., Campillo N., Garcia I. L. and Cordoba M.H., (1994), Slurry atomisation of vegetables for the electrothermal atomic adsorption spectrometric analysis of lead and cadmium, Food Chemistry, **50**, 317-321

Von Zglinicki T., Edwall C., Ostlund E., Lind B., Nordberg M., Ringertz N.R. and Wroblewski J., (1992), Very low cadmium concentrations stimulate DNA synthesis and cell growth, Journal of Cell Science, **103(4)**, 1073-1081

Voss G., Teeuwen J.J.M.H. and Vreman K., (1986), Cadmium and lead in compounded feeds, Netherlands Journal of Agricultural Science, **34**, 437-440

Voss G., Teeuwen J.J.M.H., Knottnerus O.C. and Wijers B.L., (1988), Cadmium and lead in Dutch and imported feeds, Netherlands Journal of Agricultural Science, **36**, 167-174

Voth L. M., (1983a), Determination of the priority pollutant metals - regulations and methodology, Varian Instruments at Work, Number AA-34, Varian, USA

Voth L. M., (1983b), Dealing with matrix interferences in the determination of the priority pollutant metals by furnace AA, Varian Instruments at Work, Number AA-35, Varian, USA

Voth L.M., (1985), Pyrolytic graphite platforms - guidelines for use with the GTA-95 graphite tube atomiser, AA, Varian Instruments at Work, Number AA-45, Varian, USA,

Waalkes M.P., Coogan T.P. and Barter R.A., (1992), Toxicological principles of metal carcinogenesis with special emphasis on cadmium, Critical Reviews in Toxicology, **22**, 175-281

Waalkes M.P. and Rehm S., (1994), Cadmium and prostrate cancer, Journal of Toxicology and Environmental Health, **43**, 251-256

Waketa H. and Schmitt R.A., (1970), Cadmium, In: Handbook of Geochemistry, Ed. Wedepohl K.H., Springer, New York, Chapter 48

Watanabe T., Nakatsuka H., Shimbo S., Iwama O., Imai Y., Moon C.-S., Zhang Z.-W., Iguchi H. and Ikeda M., (1996), Reduced cadmium burden in Japan in the past 10 years, International Archives of Occupational and Environmental Health, **68(5)**, 305-314

Watanabe T., Zhang Z.-W., Moon C.-S., Shimbo S., Nakatsuka H., Matsuda-Inoguchi N., Higashikawa K. and Ikeda M., (2000), Cadmium exposure of women in general populations in Japan during 1991-1997 compared with 1977-1981, International Archives of Occupational and Environmental Health, **73(1)**, 26-34

Weiss E, (2005), Evidence of ancient baking, Australian and New Zealand Baking Business, **2**, Australia, 6-7

Welz B., Schlemmer G. and Mudakavit J.R., (1988), Palladium nitrate - magnesium nitrate modifier for graphite furnace atomic absorption spectroscopy - Part 2: Determination of arsenic, cadmium, copper, manganese, lead, antimony, selenium and thallium in water, Journal of Analytical Atomic), Spectrometry, **3**, 695-701

Wendell N.A., Jiaping W., Hopkins D.G. and Welch R.M., (1999), Association of cadmium in durum wheat grain with soil chloride and chelate extractable soil cadmium, www.nal.usda.gov/ttic/tektran/data/000010/66/0000106647

Wheat CRC, (2001), Wheat facts, Quality Wheat Cooperative Research Centre, North Ryde, New South Wales

White J.G., Welch R.M. and Norvell W.A., (1977), Soil zinc map of the USA using geostatistics and gepgraphical information systems, Journal of the American Soil Science Society, **61**, 185-194

Whittemore A.S., Di Ciccio Y. and Provenzano G., (1991), Urinary cadmium and blood pressure : Results from the NHANES II study, Environmental Health Perspectives, **91**, 133-145

WHO, (2001a), Comments submitted on the draft guideline level and proposed draft maximum levels for cadmium, FAO/WHO, Codex Committee on Food Additives and Contaminants, 33rd Session, Agenda item 165d

WHO, (2001b), Draft guideline levels, Codex Alimentarius Commission, Codex Committee on Food Additives and Contaminants, www.who.int/fsf/Chemicalcontaminants/fa01_28e.

WHO, (2002), Comments submitted on the draft guideline level and proposed draft maximum levels for cadmium, Codex Alimentarius Commission, 33rd Session, Codex Committee on Food Additives and Contaminants, Agenda item 16d.

Wiersma D., van Goor B.J. and van der Veen N.G., (1986), Cadmium, lead, mercury and arsenic concentrations in crops and correspondins soils in the Netherlands, Journal of Agriculture and Food Chemistry, **34**, 1067-1074

Wilkinson L., (1999), Density Charts, Chapter 3, Graphics, Systat 9, SPSS Incorporated, USA, 89-121

Wilkinson L., Engelman L,. Corter J. and Coward M., (1999), Cluster Analysis, Chapter 4, Statistics 1, Systat 9, SPSS Incorporated, USA, 53-84

Williams C.H., (1974), Heavy metals and other elements in fertilizers - environmental considerations, In: Fertilizers and the Environment, Ed. Leece D.R., Australian Institute of Agricultural Science, Sydney, 123-131

Williams C.H., (1977), Trace metals and superphosphate: toxicity problems, The Journal of the Australian Institute of Agricultural Science, **43**, 99-109

Williams C.H. and David D.J., (1973), The effect of superphosphate on the cadmium content of soils and plants, Australian Journal of Soil Research, **11**, 43-56

Williams C.H. and David D.J., (1976), The accumulation in soil of cadmium residues from phosphate fertilizers and their effect on the cadmium content of plants, Soil Science, **121**, 86-93

Williams C.H., David D.J. and Iismaa O., (1972), The determination of cadmium in soils, plants and fertilisers by dithizone extraction and atomic absorption spectroscopy, Communications in Soil Science and Plant Analysis, **3(5)**, 399-407

Wing K.R and Moberg A., (1993), The effects of whole wheat, wheat bran and zinc in the diet on the absorption and accumulation of cadmium in rats, British Journal of Nutrition, 69(1), 199-209

Wolnik K.A., Fricke F.L., Capar S.G., Braude G.L., Meyer M.W., Stazger R.D. and Bonnin E., (1983), Elements in major agricultural crops in the United States. I. Cadmium and lead in lettuce, peanuts, potatoes, soybeans, sweet corn and wheat, Journal of Agricultural and Food Chemistry, **31(6)**, 1240-1244

Wrick K.L., Robertson J.B., van Soest P.J., Lewis B.A., Rivers J.M., Roe D.A. and Hackler L.R., (1983), The influence of dietary fibre source on human intestinal transit and stool output, Journal of Nutrition, **113(8)**, 1464-1479

Wu Q-T., Xu Z., Meng Q., Gerard E. and Morel J-L., (2004), Characterisation of cadmium desorption in soils and its relationship to plant uptake and cadmium leaching, Plant and Soil, **258(1)**, 217-224

Yamagata B. and Shigematsu I., (1970), Cadmium pollution in perspective, Bulletin of the Institute of Public Health, **19**, 1-10

Yizai M., Zhikun L., Jiazhen W. and Yongquan L., (1994), Determination of cadmium by electrothermal atomic absorption spectrometry using palladium and tartaric acid as a mixed chemical modifier and a tungsten foil platform with the possibility of standardless analysis, Journal of Analytical Atomic Spectrometry, **9**, 679-683

Youden W.J. and Steiner E. H., (1975), Measurement of precision and accuracy, Statistical Manual of the Association of Official Analytical Chemists, AOAC International, USA, 21-27

Zarcinas B.A., McLaughlin M.J., Mason S., Nolan A., Spouncer L., Ohmsen G., Hannay J and Wurst M., (2004), Executive summary, Grain Quality and Contamination around Port Pirie, CSIRO Land and Water Client Report, CSIRO Division of Land and Water, CSIRO, Australia, 7-8

Ziegler E. and Greer E.N., (1988), Principles of milling, In: Wheat: Chemistry and Technology, Ed: Pomeranz Y., Volume 1, American Association of Cereal Chemists. 115-199

Zorn M.E., Gibbons R.D. and Sonzogni W.C., (1999), Evaluation of approximate methods for calculating the limit of detection and limit of quantitation, Environmental Science and Technology, **33**, 2291-2295

Zou K.H., Tuncali K. and Silverman S.G., (2003), Correlation and simple linear regression, Radiology, **227**, 617-628

Appendix 1 Summary of sites, number of samples, mean and maximum cadmium results for grower load and composite site samples sourced from the AWB by state

Table A1.1 New South Wales

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Arajoel	-	-	-	1	0.02	
Armatree	3	0.03	0.04	2	0.03	0.04
Arthurville	-	-	-	1	0.01	
Baan Baa	3	< 0.01	0.01	2	0.01	0.01
Baradine	-	-	-	2	0.01	0.01
Barellan	6	0.01	0.02	3	0.01	0.01
Barmedman	-	-	-	2	0.01	0.01
Beckom	-	-	-	2	0.02	0.02
Belfrayden	-	-	-	1	0.02	
Bellata	12	0.01	0.02	3	0.01	0.01
Bendick Murrell	-	-	-	1	< 0.01	
Berrybank	-	-	-	1	0.01	
Biniguy	1	0.01		3	0.01	0.01
Bogan Gate	-	-	-	1	< 0.01	
Boggabilla	3	< 0.01	< 0.01	3	< 0.01	0.01
Boggabri	-	-	-	1	0.01	
Boorowa	14	0.02	0.03	2	0.02	
Borree Creek	3	0.02	0.03	2	0.02	0.02
Brocklesby	1	0.02	0.03	2	0.02	0.02
Burren Junction	1	0.03		2	0.02	0.02
Canowindra	-	-	-	1	0.02	
Caragabal	-	-	-	1	< 0.01	
Cobar	-	-	-	1	< 0.01	
Coleambally	-	-	-	2	0.02	0.02
Combara	-	-	-	1	0.01	
Condobolin	1	< 0.01		3	0.01	0.01
Coolamon	3	0.03	0.03	2	0.02	0.02
Coonamble	1	0.01		1	0.01	
Cootamundra	-	-	-	1	0.01	
Crooble	-	-	-	3	< 0.01	< 0.01
Croppa Creek	-	-	-	2	< 0.01	< 0.01
Cryon	-	-	-	1	0.02	
Culgoora	7	0.01	0.01	2	< 0.01	< 0.01
Cunningar	-	-	-	1	0.03	
Delungra	-	-	-	1	0.01	
Dunedoo	-	-	-	1	0.01	

Table A1.1 New South Wales (continued)

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Edgeroi	2	0.02	0.02	2	0.01	0.01
Elmore	-	-	-	1	0.01	
Emerald Hill	-	-	-	1	< 0.01	
Euabalong West	-	-	-	1	< 0.01	
Eumungerie	-	-	-	1	0.01	
Ferndale	-	-	-	1	0.01	
Garah	-	-	-	1	0.01	
Garoolgan	-	-	-	1	< 0.01	
Gilgandra	1	< 0.01		3	< 0.01	< 0.01
Girral	-	-	-	1	0.01	
Goolgowi	-	-	-	1	0.01	
Goonaumbla	-	-	-	1	< 0.01	
Gravesend	6	< 0.01	0.01	1	< 0.01	
Greenthorpe	-	-	-	1	0.02	
Grong Grong	-	-	-	1	0.01	
Gular	-	-	-	1	< 0.01	
Gurley	3	< 0.01	0.01	2	< 0.01	< 0.01
Harden	-	-	-	1	0.02	
Henty	-	-	-	2	0.04	0.04
Hillston	-	-	-	1	< 0.01	
Illabo	-	-	-	1	< 0.01	
Junee	-	-	-	1	0.03	
Kiacatoo	1	0.03		2	0.02	0.03
Kikoira	-	-	-	2	< 0.01	< 0.01
Koora Watha	-	-	-	1	0.01	
Kywong	-	-	-	1	< 0.01	
Lake Cargelligo	6	< 0.01	0.01	1	< 0.01	
Lockhart	1	0.01		2	0.01	0.01
Maimuru	-	-	-	1	0.03	
Marrar	-	-	-	1	0.02	
Matong	3	0.02	0.02	3	0.01	0.01
Merah North	-	-	-	1	< 0.01	
Merriwagga	5	< 0.01	< 0.01	1	< 0.01	
Merrywinebone	5	0.02	0.03	3	0.02	0.03
Mickibri	-	-	-	1	< 0.01	
Milbrulong	3	0.02	0.03	1	0.01	
Milvale	-	-	-	1	0.01	
Moree	5	0.01	0.02	4	< 0.01	0.01
Morundah	-	-	-	1	0.02	
Naradhan	-	-	-	1	< 0.01	
Narrandera	-	-	-	1	0.01	

Table A1.1 New South Wales (continued)

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Narromine	1	0.01		3	< 0.01	0.01
Nea	2	< 0.01	< 0.01	2	< 0.01	< 0.01
Nevertire	1	0.01		2	0.01	0.01
Noonbina	-	-	-	1	0.01	
North Star	1	< 0.01		1	< 0.01	
Nyngan	1	< 0.01		2	< 0.01	< 0.01
Old Junee	-	-	-	1	0.02	
Parkes	13	0.01	0.02	2	< 0.01	< 0.01
Pucawan	-	-	-	1	0.04	
Quirindi	-	-	-	1	< 0.01	
Rand	-	-	-	1	0.01	
Reefton	3	0.03	0.03	1	0.03	
Spring Ridge	1	< 0.01		3	< 0.01	0.01
Tabbita	-	-	-	1	0.02	
Temora	4	0.03	0.03	4	0.02	0.03
Tharbogang	-	-	-	1	0.02	
The Rock	1	0.03		1	0.02	
Tottenham	3	< 0.01	< 0.01	3	< 0.01	< 0.01
Trangie	9	0.01	0.01	2	< 0.01	< 0.01
Tullamore	-	-	-	1	< 0.01	
Tullibigeal	-	-	-	2	< 0.01	< 0.01
Ungarie	-	-	-	1	< 0.01	
Urana	-	-	-	1	< 0.01	
Urangeline	-	-	-	2	0.02	0.02
Walgett	2	0.01	0.01	3	< 0.01	0.01
Wallendbeen	2	0.03	0.04	2	0.03	0.03
Warren	1	0.01		2	0.01	0.01
Wee Waa	-	-	-	1	< 0.01	
Weethalle	-	-	-	1	< 0.01	
Werris Creek	-	-	-	1	< 0.01	
Willbriggie	7	0.02	0.03	2	0.02	0.02
Wirrinya	1	0.02		1	0.03	
Woodstock	-	-	-	1	< 0.01	
Wyanga	1	0.02		1	0.01	
Yanco	1	0.03		1	0.02	
Yarrabandai	-	-	-	1	< 0.01	
Yethera	1	< 0.01		1	< 0.01	
NSW Total	**150**	**0.01**	**0.04**	**175**	**0.01**	**0.03**

Table A1.2 Queensland

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Baigin	4	< 0.01	< 0.01	1	< 0.01	
Biloela	2	< 0.01	< 0.01	3	0.01	0.01
Bongeen	2	< 0.01	< 0.01	1	< 0.01	
Brookstead	5	< 0.01	< 0.01	-	-	-
Chinchilla	1	0.02		1	< 0.01	
Dysart	1	< 0.01		-	-	-
Glenmorgan	1	< 0.01		-	-	-
Goondiwindi	3	< 0.01	< 0.01	1	< 0.01	
Jandowae	2	< 0.01	< 0.01	-	-	-
Kingaroy	5	0.02	0.03	1	0.02	
Koorngoo	2	0.02	0.01	-	-	-
Malu	4	< 0.01	< 0.01	1	< 0.01	
Meandarra	4	< 0.01	< 0.01	-	-	-
Miles	1	0.01		1	< 0.01	
Millmerran	3	< 0.01	< 0.01	1	< 0.01	
Moura	4	0.03	0.04	1	0.02	
Muckadilla	3	< 0.01	< 0.01	1	< 0.01	
Pittsworth	1	< 0.01		1	< 0.01	
Roma West	3	0.01	0.01	1	< 0.01	
Thallon	1	0.01		-	-	-
Toobeah	3	< 0.01	0.01	1	< 0.01	
Wallumbilla	1	< 0.01		-	-	-
Wandoan	1	0.01		-	-	-
Yelarbon	5	< 0.01	< 0.01	3	< 0.01	< 0.01
Qld Total	**61**	**< 0.01**	**0.04**	**21**	**< 0.01**	**0.01**

Table A1.3 South Australia

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Alawoona	-	-	-	4	0.01	0.02
Andrews	-	-	-	5	0.04	
Ardrossan	39	0.04	0.11	23	0.03	0.09
Arno Bay	-	-	-	4	0.02	0.03
Balaklava	-	-	-	6	0.02	0.03
Bannerton	-	-	-	1	0.01	
Blyth	-	-	-	2	0.03	0.04
Bodellin	-	-	-	6	0.03	0.04
Booleroo	-	-	-	5	0.03	0.04
Bordertown	-	-	-	3	0.10	0.20
Bowman	-	-	-	2	< 0.01	< 0.01
Brinkworth	-	-	-	5	0.02	0.03
Bruce Rock	-	-	-	4	0.04	0.05
Buckleloo	-	-	-	11	0.02	0.02
Burra	-	-	-	2	0.02	0.03
Burracoppin	-	-	-	2	0.02	0.03
Bute	-	-	-	14	0.03	0.04
Caltowie	-	-	-	7	0.03	0.06
Carrabin	-	-	-	1	0.02	
Coomandook	20	0.04	0.06	6	0.02	0.04
Coonalpyn	-	-	-	8	0.02	0.05
Cowell	-	-	-	8	0.02	0.03
Cowra	-	-	-	1	0.04	
Crystal Brook	-	-	-	6	0.02	0.03
Cummins	47	0.06	0.10	17	0.06	0.09
Cungena	-	-	-	4	0.02	0.02
Darke Peak	-	-	-	5	0.03	0.04
Dulyalbin	-	-	-	1	0.03	
Edililie	-	-	-	6	0.07	0.12
Elliston	-	-	-	2	0.02	0.02
Eudunda	-	-	-	4	0.02	0.03
Farrell Flat	-	-	-	3	0.02	0.02
Frances	-	-	-	3	0.02	0.04
Geranium	-	-	-	6	0.02	0.03
Gladstone	20	0.01	0.06	7	0.02	0.03
Gulnare	-	-	-	4	0.02	0.03
Hallett	-	-	-	2	0.03	0.03
Hamley Bridge	-	-	-	4	0.01	0.02
Hines Hill	-	-	-	2	0.02	0.02
Holleton	-	-	-	2	0.02	0.02
Hoyleton	-	-	-	1	0.01	
Jamestown	-	-	-	2	0.03	0.03

Table A1.3 South Australia (continued)

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Kapinnie	-	-	-	6	0.07	0.10
Kapunda	-	-	-	2	0.01	0.01
Karoonda	-	-	-	5	0.01	0.02
Keith	-	-	-	4	0.02	0.05
Kielpa	-	-	-	6	0.06	0.07
Kimba	-	-	-	2	< 0.01	0.01
Kingscote	-	-	-	2	0.02	0.02
Koonadgin	-	-	-	2	0.02	0.02
Korbelka	-	-	-	2	0.02	0.02
Kulkami	-	-	-	2	0.03	0.03
Kununoppin	-	-	-	1	0.03	
Kyancutta	-	-	-	4	0.01	0.01
Lameroo	-	-	-	1	0.03	
Lock	-	-	-	11	0.04	0.06
Long Plains	-	-	-	2	< 0.01	< 0.01
Loxton	-	-	-	1	< 0.01	
Mallala	-	-	-	3	0.02	0.05
Mangalo	-	-	-	2	0.01	0.01
Marvel Loch	-	-	-	4	0.02	0.02
Melrose	-	-	-	4	0.02	0.04
Meribah	-	-	-	1	< 0.01	
Millicent	-	-	-	1	0.04	
Minnipa	-	-	-	9	0.03	0.05
Monarto South	-	-	-	2	0.02	0.02
Moorine Rock	-	-	-	1	0.01	
Mount Walker	-	-	-	1	0.02	
Muntadgin	-	-	-	2	0.03	0.04
Murdinga	-	-	-	9	0.04	0.06
Murray Bridge	20	0.04	0.06	-	-	-
Nantawarrra	-	-	-	2	< 0.01	0.01
Narembeen	-	-	-	1	0.02	
Nukarni	-	-	-	1	0.03	
Nungarin	-	-	-	3	0.03	0.04
Nunjikompita	-	-	-	5	0.01	0.01
Orroroo	-	-	-	2	0.02	0.02
Owen	-	-	-	6	0.03	0.07
Padthaway	-	-	-	1	0.02	
Parilla	-	-	-	2	0.01	0.01
Paskeville	-	-	-	12	0.02	0.03
Peake	-	-	-	3	0.02	0.03
Penong	-	-	-	1	< 0.01	

Table A1.3 South Australia (continued)

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Pinnaroo	-	-	-	4	0.01	0.01
Pintumba	-	-	-	1	0.01	
Poochera	-	-	-	6	0.01	0.01
Port Adelaide	52	0.02	0.06	12	0.02	0.05
Port Giles	45	0.03	0.07	21	0.04	0.05
Port Lincoln	110	0.03	0.12	47	0.03	0.09
Port Neill	-	-	-	9	0.05	0.07
Port Pirie	52	0.04	< 0.01	23	0.04	0.08
Quorn	1	< 0.01		1	0.03	
Redhill	-	-	-	4	0.03	0.04
Robertstown	-	-	-	5	0.01	0.01
Roseworthy	-	-	-	9	0.03	0.07
Rudall	-	-	-	7	0.03	0.07
Saddleworth	30	0.04	0.07	8	0.02	0.03
Sheoak Log	-	-	-	4	< 0.01	< 0.01
Snowtown	-	-	-	5	0.02	0.03
Stockwell	-	-	-	2	0.03	0.04
Strathalbyn	20	0.05	0.10	2	0.02	0.02
Streaky Bay	-	-	-	5	0.01	0.02
Tailem Bend	30	0.02	0.05	2	0.01	0.02
Taldra	-	-	-	2	< 0.01	< 0.01
Tarlee	-	-	-	9	0.02	0.03
Thevenard	29	0.01	0.04	16	0.01	0.02
Tintinara	15	0.06	0.08	5	0.01	0.02
Tooligie	-	-	-	6	0.03	0.06
Tumby Bay	18	0.04	0.07	11	0.03	0.06
Two Wells	-	-	-	4	0.02	0.05
Ungarra	-	-	-	2	0.05	0.05
Waddikee	-	-	-	3	0.03	0.03
Wallaroo	52	0.03	0.11	32	0.03	0.05
Wanbi	-	-	-	2	0.01	0.02
Warramboo	-	-	-	6	0.02	0.02
Wharminda	-	-	-	2	0.05	0.05
Wilmington	-	-	-	2	0.02	0.03
Wirrabara	38	0.05	0.10	3	0.03	0.05
Wirrulla	-	-	-	4	0.01	0.01
Witera	-	-	-	3	0.02	0.03
Wolsley	-	-	-	6	0.01	0.01
Wudinna	-	-	-	8	0.02	0.05
Wunkar	-	-	-	3	0.01	0.01
Yacka	-	-		2	0.03	0.03

Table A1.3 South Australia (continued)

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Yaninee	-	-	-	2	0.01	0.03
Yeelana	-	-	-	5	0.03	0.06
Yongala	-	-	-	4	0.02	0.03
SA Total	**638**	**0.03**	**0.12**	**1,296**	**0.03**	**0.20**

Table A1.4 Victoria

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Alawoona	2	0.01	0.01	-	-	
Albacuttya	-	-	-	2	0.02	0.03
Annuello	-	-	-	2	< 0.01	< 0.01
Arapiles	-	-	-	1	0.02	
Balranald	-	-	-	1	< 0.01	
Bannerton	1	0.01		-	-	-
Banyena	-	-	-	1	0.01	
Barham	-	-	-	1	0.01	
Barraport	1	0.01	-	1	< 0.01	
Batchica	-	-	-	1	0.01	
Berrigan	-	-	-	1	0.01	
Berriwillock	-	-	-	2	0.01	0.01
Beulah	-	-	-	2	0.01	0.01
Boigbeat	-	-	-	1	< 0.01	
Bolton	1	0.02	-	1	< 0.01	
Boort	2	< 0.01	0.01	2	< 0.01	< 0.01
Borung	-	-	-	1	< 0.01	
Bridgewater	-	-	-	2	< 0.01	0.01
Brim	-	-	-	2	0.01	0.01
Buckrabanyule	-	-	-	1	0.01	
Bunnaloo	-	-	-	1	0.01	
Burrum	-	-	-	1	< 0.01	
Cannie	-	-	-	2	< 0.01	< 0.01
Carina	-	-	-	1	0.01	
Carpolac	-	-	-	1	0.03	
Carwarp	11	< 0.01	< 0.01	1	< 0.01	
Charlton	11	0.01	0.03	6	< 0.01	0.01
Chilligollah	-	-	-	1	< 0.01	
Chinapook	-	-	-	3	< 0.01	< 0.01
Cobram	-	-	-	1	0.01	
Coromby	-	-	-	1	<0.01	
Cowangie	1	0.02		1	0.01	
Culgoa	1	0.03		-	-	-
Dahlen	-	-	-	2	0.02	0.03
Deniliquin	1	0.03		1	< 0.01	
Detpa	-	-	-	3	0.01	0.01
Daipur	-	-	-	1	0.03	
Diggora West	-	-	-	2	0.03	0.06
Dimboola	6	0.02	0.02	3	< 0.01	0.01

Table A1.4 Victoria (continued)

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Donald	10	0.02	0.06	4	< 0.01	0.01
Dooen	-	-	-	2	< 0.01	0.01
Dookie	-	-	-	1	0.01	
Dunnolly	7	0.03	0.05	2	0.02	0.02
Ellem	-	-	-	1	0.03	
Elmore	-	-	-	1	< 0.01	
Galaquil	-	-	-	2	< 0.01	< 0.01
Geelong	-	-	-	2	< 0.01	< 0.01
Gerang Gerang	-	-	-	1	0.01	
Glenorchy	-	-	-	2	0.01	0.01
Goorambat	-	-	-	1	0.03	
Goyura	-	-	-	1	0.01	
Gymbowen	-	-	-	3	0.03	0.04
Hopetoun	-	-	-	1	0.01	
Horsham	34	0.02	0.06	-	-	-
Hunter	-	-	-	1	< 0.01	
Jeparit	-	-	-	2	< 0.01	0.01
Jung	3	0.01	0.02	1	< 0.01	
Kaniva	1	< 0.01	-	1	0.02	
Karawinna	-	-	-	1	< 0.01	
Katamitite	-	-	-	1	0.03	
Kerang	-	-	-	2	< 0.01	< 0.01
Kiamal	1	0.02	-	-	-	-
Kiata	6	0.01	0.03	-	-	-
Lascelles	-	-	-	1	0.01	
Lillimur	-	-	-	2	< 0.01	0.01
Lalbert	1	< 0.01		-	-	
Manangatang	-	-	-	1	< 0.01	
Marmalake	17	< 0.01	0.05	-	-	-
Marnoo	-	-	-	1	0.01	
Massey	-	-	-	1	< 0.01	
Mathoura	-	-	-	1	< 0.01	
Meatian	2	0.02	0.02	1	0.01	
Meringur	3	< 0.01	< 0.01	1	< 0.01	
Merrinee	-	-	-	1	< 0.01	
Miram	-	-	-	1	0.02	
Mitiamo	-	-	-	1	< 0.01	
Mittyack	-	-	-	1	0.01	
Moolort	-	-	-	1	0.02	
Morton Plains	-	-	-	3	0.02	0.04
Moulamein	-	-	-	1	< 0.01	
Murchison East	-	-	-	2	< 0.01	< 0.01

Table A1.4 Victoria (continued)

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Murrayville	3	0.02	0.02	2	0.02	0.02
Murtoa	10	< 0.01	< 0.01	-	-	-
Nagambie	-	-	-	1	0.02	
Nandaly	-	-	-	1	0.01	
Natimuk East	-	-	-	1	0.02	
Nhill	-	-	-	4	< 0.01	0.01
Naradjuha	-	-	-	3	0.02	0.04
Nullawil	5	< 0.01	0.06	1	< 0.01	
Nyah West	2	< 0.01	0.01	1	0.01	
Ouyen	7	0.01	0.02	1	< 0.01	
Panitya	-	-	-	1	0.02	
Patchewollock	-	-	-	1	0.01	
Piangil	1	0.03		-	-	-
Pimpinio	-	-	-	1	0.01	
Pirlta	1	0.01		-	-	-
Portland	201	0.02	0.08	3	< 0.01	0.01
Prairie	-	-	-	1	< 0.01	
Pullut	-	-	-	3	0.01	0.02
Quambatook	13	< 0.01	0.02	2	< 0.01	0.01
Rainbow	-	-	-	3	0.01	0.02
Remlaw	-	-	-	1	< 0.01	
Rochester	-	-	-	1	0.07	
Rosebery	2	0.01	0.01	2	< 0.01	0.01
Salisbury	14	< 0.01	0.01	2	0.02	0.02
Serviceton	-	-	-	1	0.02	
Skipton	-	-	-	1	0.02	
Speed	5	0.02	0.02	1	0.02	
St Arnaud	-	-	-	1	0.01	
Stawell	-	-	-	2	0.03	
Swan Hill	-	-	-	2	0.03	0.05
Telford	-	-	-	1	< 0.01	
Tutye	1	0.01		2	0.01	0.01
Ultima	-	-	-	2	< 0.01	< 0.01
Underbool	-	-	-	1	0.01	
Vectis	-	-	-	1	< 0.01	
Wail	-	-	-	2	< 0.01	< 0.01
Warracknabeal	16	< 0.01	0.02	-	-	-
Watchem	-	-	-	1	0.01	
Wedderburn	-	-	-	1	< 0.01	
Werrimull	6	< 0.01	< 0.01	1	< 0.01	
Willaura	1	0.06		-	-	-

Table A1.4 Victoria (continued)

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Woomelang	-	-	-	1	< 0.01	
Wycheproof	10	0.01	0.05	1	< 0.01	
Wychitella	2	0.01	0.01	-	-	-
Yaapeet	3	0.01	0.01	1	0.01	
Yanac	-	-	-	1	0.03	
Yarrawonga	-	-	-	2	0.04	0.06
Yelta	-	-	-	2	< 0.01	< 0.01
Vic Total	**426**	**0.02**	**0.08**	**611**	**< 0.01**	**0.06**

Table A1.5 Western Australia

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Ainsworth	20	0.05	0.09	8	0.03	0.04
Albany	-	-	-	66	0.05	0.10
Aldersyde	20	0.06	0.11	34	0.05	0.08
Ardath	-	-	-	11	0.03	0.05
Arrino	-	-	-	6	0.05	0.09
Avon	-	-	-	26	0.04	0.07
Badgebup	-	-	-	13	0.04	0.07
Ballaying	9	0.02	0.04	26	0.04	0.07
Ballidu	19	0.08	0.15	32	0.04	0.07
Barooga	-	-	-	1	0.02	
Beacon	-	-	-	20	0.02	0.05
Beaumont	-	-	-	2	0.05	0.07
Bencubbin	-	-	-	26	0.03	0.06
Bendering	-	-	-	7	< 0.01	0.01
Beverley	-	-	-	11	0.04	0.05
Bindi	-	-	-	24	0.03	0.06
Binnu	-	-	-	37	0.05	0.08
Bodallin	-	-	-	11	0.02	0.02
Bolgart	-	-	-	9	0.04	0.06
Bonnie	-	-	-	19	0.03	0.07
Borden	5	0.06	0.08	11	0.04	0.07
Bowgada	-	-	-	1	0.04	
Brookton	8	0.02	0.06	27	0.05	0.08
Broomhill	-	-	-	11	0.05	0.06
Bruce Rock	9	0.03	0.07	24	0.03	0.07
Bullaring	10	0.03	0.07	53	0.04	0.08
Bullfinch	-	-	-	8	0.02	0.04
Bulyee	-	-	-	45	0.04	0.10
Buniche	-	-	-	6	0.03	0.04
Bunjil	-	-	-	10	0.05	0.07
Buntine	11	0.09	0.16	2	0.05	0.05
Burracoppin	-	-	-	13	0.02	0.07
Cadoux	-	-	-	44	0.04	0.07
Calingiri	-	-	-	41	0.04	0.07
Canna	-	-	-	6	0.03	0.04
Carnamah	-	-	-	26	0.05	0.08
Carrabin	-	-	-	5	0.03	0.07
Cascades	-	-	-	3	0.03	0.03
Cleary	-	-	-	16	0.03	0.06
Coomberdale	-	-	-	19	0.04	0.07
Coondle	-	-	-	3	0.03	0.04

Table A1.5 Western Australia (continued)

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Coorow	14	0.05	0.09	10	0.05	0.08
Corrigin	-	-	-	67	0.03	0.06
Cowcowing	-	-	-	10	0.03	0.05
Cranbrook	-	-	-	20	0.04	0.07
Cunderdin	21	0.07	0.11	58	0.05	0.10
Dale	-	-	-	3	0.02	0.02
Doodleakine	17	0.02	0.04	17	0.02	0.04
Dowerin	-	-	-	41	0.05	0.09
Dudinin	-	-	-	40	0.03	0.06
Dulacca	-	-	-	6	0.04	0.04
Dulyalbin	-	-	-	9	0.01	0.02
Dumbleyung	-	-	-	42	0.04	0.07
Dunn Rock	-	-	-	3	0.02	0.02
East Hyden	-	-	-	6	0.04	0.06
Ejanding	10	0.04	0.12	18	0.04	0.09
Elmore	-	-	-	12	0.06	0.08
Esperance	-	-	-	42	0.04	0.06
Fremantle	-	-	-	109	0.04	0.07
Gabbin	-	-	-	10	0.03	0.05
Gairdner River	17	0.06	0.14	7	0.06	0.10
Geraldton	-	-	-	255	0.04	0.08
Gnowangerup	10	0.04	0.09	36	0.04	0.08
Goodlands	-	-	-	39	0.03	0.06
Goomalling	-	-	-	58	0.05	0.13
Goonumbla	-	-	-	1	0.05	
Grass Patch	-	-	-	5	0.03	0.05
Grass Valley	-	-	-	8	0.05	0.06
Greenhills	21	0.05	0.09	21	0.03	0.08
Gutha	-	-	-	7	0.04	0.05
Henty	-	-	-	1	0.02	
Hines Hill	-	-	-	5	0.02	0.03
Holland Rock	-	-	-	5	0.02	0.03
Holleton	-	-	-	3	0.02	0.03
Holt Rock	-	-	-	5	0.03	0.04
Hyden	-	-	-	20	0.03	0.04
Jacup	-	-	-	3	0.04	0.06
Jennacubbine	-	-	-	7	0.05	0.06
Jerramungup	-	-	-	7	0.02	0.03
Jitarning	-	-	-	31	0.03	0.07
Jubuk	17	0.06	0.09	56	0.04	0.07
Kalannie	-	-	-	50	0.04	0.07

Table A1.5 Western Australia (continued)

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Karlgarin	-	-	-	14	0.02	0.05
Katanning	-	-	-	11	0.04	0.05
Kellerberrin	-	-	-	22	0.04	0.07
Kirwan	-	-	-	10	0.03	0.06
Koj Kojin	-	-	-	4	0.04	0.06
Kondinin	-	-	-	21	0.02	0.05
Kondut	20	0.03	0.05	27	0.05	0.09
Konnongorring	-	-	-	39	0.05	0.12
Koonadgin	-	-	-	4	0.02	0.02
Koorda	-	-	-	32	0.03	0.06
Korbelka	6	0.03	0.05	21	0.03	0.07
Kuender	-	-	-	4	0.02	0.02
Kukerin	-	-	-	13	0.03	0.05
Kulin	-	-	-	33	0.03	0.05
Kulja	-	-	-	15	0.03	0.06
Kununoppin	-	-	-	6	0.03	0.04
Kuringup	1	0.03	-	3	0.04	0.04
Lake Cairlocp	-	-	-	3	0.03	0.04
Lake Grace	30	0.02	0.04	13	0.02	0.04
Lake King	-	-	-	16	0.01	0.03
Lake Varley	-	-	-	6	0.03	0.05
Langi Logan	-	-	-	2	0.04	0.05
Latham	-	-	-	3	0.04	0.04
Lort River	-	-	-	1	0.02	
Manmanning	-	-	-	8	0.04	0.05
Marchagee	20	0.08	0.22	23	0.04	0.09
Marvel Loch	-	-	-	12	0.02	0.03
Mawson	-	-	-	10	0.04	0.06
Maya	-	-	-	3	0.05	0.06
McLevie	18	0.06	0.10	18	0.04	0.06
Meckering	-	-	-	21	0.05	0.08
Merredin	-	-	-	8	0.03	0.05
Miling	18	0.07	0.13	25	0.05	0.07
Mindarrabin	-	-	-	22	0.03	0.07
Mingenew	-	-	-	12	0.04	0.06
Minnivale	10	0.04	0.10	15	0.04	0.05
Mogumber	-	-	-	13	0.05	0.12
Mollerin	-	-	-	5	0.03	0.06
Moora	-	-	-	15	0.04	0.07
Morrine Rock	-	-	-	8	0.02	0.04
Morawa	-	-	-	25	0.03	0.05

Table A1.5 Western Australia (continued)

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Moulyinning	-	-	-	22	0.05	0.07
Mount Kokeby	20	0.05	0.09	6	0.04	0.07
Mount Madden	21	0.03	0.04	12	0.02	0.04
Mount Sheridan	-	-	-	1	0.01	
Mount Walker	-	-	-	12	0.02	0.02
Mukinbudin	-	-	-	28	0.03	0.05
Mullewa	-	-	-	36	0.03	0.07
Muntadgin	-	-	-	9	0.02	0.03
Narembeen	-	-	-	23	0.02	0.04
Nembudding	-	-	-	12	0.04	0.06
Neminga	-	-	-	1	0.04	
Netherby	-	-	-	1	0.02	
Newdegate	-	-	-	16	0.02	0.03
Nomans Lake	-	-	-	37	0.03	0.06
Northampton	-	-	-	41	0.04	0.07
Nukarni	-	-	-	7	0.03	0.04
Nungarin	19	0.03	0.05	20	0.03	0.07
Nyabing	10	0.02	0.05	7	0.04	0.07
Ongerup	-	-	-	4	0.02	0.02
Perenjori	51	0.05	0.14	33	0.03	0.06
Piawanning	-	-	-	32	0.04	0.06
Pindar	11	0.06	0.08	5	0.03	0.04
Pingaring	-	-	-	19	0.02	0.05
Pingelly	-	-	-	9	0.03	0.06
Pingrup	-	-	-	14	0.02	0.03
Pintharuka	-	-	-	3	0.04	0.05
Pithara	20	0.03	0.05	79	0.05	0.09
Quairading	-	-	-	26	0.04	0.08
Ravensthorpe	-	-	-	1	0.03	
Salmon Gums	-	-	-	15	0.02	0.04
Shackleton	-	-	-	25	0.04	0.07
South Cross	-	-	-	17	0.02	0.06
South Yilgarn	-	-	-	3	0.01	0.02
South East Hyden	-	-	-	8	0.03	0.05
South Kumminin	-	-	-	22	0.02	0.05
Sullivan	-	-	-	1	0.06	
Tambellup	1	< 0.01		10	0.04	0.07
Tammin	19	0.05	0.08	22	0.05	0.07
Tampu	-	-	-	10	0.03	0.05
Tarin Rock	-	-	-	7	0.02	0.05
Three Spring	20	0.04	0.09	8	0.03	0.05

Table A1.5 Western Australia (continued)

Site	Grower Loads			Site Composite		
	Number	Cadmium (mg/kg)		Number	Cadmium (mg/kg)	
		Mean	Maximum		Mean	Maximum
Tincurrin	-	-	-	36	0.03	0.05
Trayning	-	-	-	32	0.04	0.10
Ultima	-	-	-	7	0.05	0.07
Wagin	10	0.03	0.04	8	0.05	0.07
Warralackin	-	-	-	10	0.02	0.04
Warup	18	0.05	0.07	2	0.07	0.07
Watercarrin	-	-	-	13	0.06	0.09
Watheroo	-	-	-	35	0.06	0.08
Welbungin	-	-	-	12	0.03	0.04
West River	-	-	-	1	0.03	
Wialki	-	-	-	23	0.03	0.07
Wickepin	-	-	-	40	0.04	0.06
Wilgoyne	-	-	-	21	0.02	0.04
Wogarl	-	-	-	24	0.03	0.07
Wongan Hills	30	0.05	0.18	50	0.05	0.10
Wongoondy	-	-	-	8	0.04	0.06
Woodanilling	-	-	-	6	0.04	0.07
Woolocutty	-	-	-	5	0.02	0.03
Wubin	-	-	-	11	0.05	0.07
Wyalkatchem	-	-	-	25	0.04	0.08
Yarding	20	0.04	0.07	14	0.03	0.05
Yealering	30	0.04	0.10	55	0.04	0.07
Yericoin	-	-	-	8	0.04	0.05
York	-	-	-	14	0.04	0.06
Yorkrakine	-	-	-	36	0.04	0.06
Yornaning	-	-	-	1	0.05	
Yoting	20	0.06	0.09	41	0.05	0.10
Yuluma	-	-	-	9	0.05	0.06
Yuna	-	-	-	45	0.04	0.08
WA Total	**682**	**0.05**	**0.22**	**4,459**	**0.04**	**0.22**

Appendix 2 Summary of mean and maximum cadmium results by season and month for commercial flourmills

Table A2.1 New South Wales

Season	Month	Number	Cadmium (mg/kg)	
			Mean	Maximum
91/92	October	-	-	-
	November	23	< 0.01	0.01
	December	12	< 0.01	< 0.01
	January	1	< 0.01	
	February	13	0.01	0.02
	March	34	0.01	0.02
	April	36	0.01	0.02
	May	43	0.01	0.02
	June	22	0.02	0.02
	July	-	-	-
	August	9	0.01	0.01
	September	28	< 0.01	0.01
	91/92 Total	**221**	**0.01**	**0.02**
93/94	October	8	0.02	0.02
	November	-	-	-
	December	4	0.01	0.01
	January	40	0.02	0.03
	February	33	< 0.01	< 0.01
	March	15	0.01	0.02
	April	23	0.02	0.03
	May	32	0.02	0.03
	June	15	< 0.01	0.02
	July	-	-	-
	August	9	0.02	0.03
	September	-	-	-
	93/94 Total	**179**	**0.02**	**0.03**
94/95	October	7	< 0.01	0.01
	November	1	< 0.01	
	December	1	0.01	
	January	-	-	-
	February	13	< 0.01	0.01
	March	5	0.02	0.03
	April	5	0.01	0.01
	May	9	< 0.01	0.01
	June	-	-	-
	July	-	-	-
	August	-	-	-
	September	-	-	-
	94/95 Total	**41**	**0.01**	**0.03**

Table A2.1 New South Wales (continued)

Season	Month	Number	Cadmium (mg/kg)	
			Mean	Maximum
95/96	October	16	0.01	0.01
	November	3	< 0.01	0.01
	December	23	0.01	0.02
	January	-	-	-
	February	12	0.01	0.01
	March	35	0.01	0.03
	April	-	-	-
	May	-	-	-
	June	3	< 0.01	< 0.01
	July	9	0.01	0.02
	August	5	< 0.01	0.02
	September	1	0.01	
	95/96 Total	**107**	**0.01**	**0.02**
NSW Total		**548**	**0.01**	**0.03**

Table A2.2 Queensland

Season	Month	Number	Cadmium (mg/kg)	
			Mean	Maximum
91/92	October	2	< 0.01	0.01
	November	4	0.01	0.01
	December	-	-	-
	January	-	-	-
	February	-	-	-
	March	11	0.01	0.03
	April	12	0.01	0.03
	May	19	< 0.01	0.01
	June	19	< 0.01	0.02
	July	27	0.01	0.02
	August	16	0.01	0.02
	September	2	0.01	0.02
	91/92 Total	**110**	**0.01**	**0.03**
93/94	October	-	-	-
	November	21	0.01	0.02
	December	-	-	-
	January	4	< 0.01	< 0.01
	February	26	< 0.01	0.01
	March	25	< 0.01	< 0.01
	April	13	< 0.01	< 0.01
	May	31	< 0.01	0.01
	June	21	< 0.01	0.01
	July	4	< 0.01	0.01
	August	1	0.01	
	September	17	< 0.01	0.03
	93/94 Total	**163**	**0.01**	**0.03**
94/95	October	-	-	-
	November	-	-	-
	December	-	-	-
	January	2	< 0.01	0.01
	February	4	0.01	0.02
	March	8	0.01	0.02
	April	2	0.02	0.02
	May	5	< 0.01	0.01
	June	7	0.01	0.03
	July	9	< 0.01	0.02
	August	-	-	-
	September	-	-	-
	94/95 Total	**33**	**0.01**	**0.03**

Table A2.2 Queensland (continued)

Season	**Month**	**Number**	**Cadmium (mg/kg)**	
			Mean	**Maximum**
95/96	October	12	0.01	0.01
	November	-	-	-
	December	-	-	-
	January	13	0.01	0.02
	February	5	< 0.01	< 0.01
	March	7	< 0.01	0.01
	April	24	0.01	0.01
	May	11	0.01	0.01
	June	9	0.01	0.02
	July	-	-	-
	August	12	< 0.01	0.01
	September	10	< 0.01	0.01
	95/96 Total	**103**	**0.01**	**0.02**
Total		**409**	**0.01**	**0.03**

Table A2.3 **South Australia**

Season	Month	Number	Cadmium (mg/kg)	
			Mean	Maximum
91/92	October	33	0.02	0.05
	November	1	0.01	
	December	7	0.02	0.02
	January	15	< 0.01	0.01
	February	25	0.03	0.09
	March	8	0.02	0.04
	April	23	0.02	0.03
	May	15	0.01	0.03
	June	25	0.01	0.03
	July	25	0.02	0.04
	August	26	0.02	0.03
	September	23	0.01	0.04
	91/92 Total	**226**	**0.02**	**0.09**
93/94	October	3	0.02	0.05
	November	7	0.02	0.04
	December	-	-	-
	January	1	0.05	0.08
	February	47	0.02	0.05
	March	22	0.03	0.10
	April	20	< 0.01	0.04
	May	19	0.02	0.04
	June	39	0.02	0.04
	July	42	0.03	0.05
	August	23	0.02	0.08
	September	-	-	-
	93/94 Total	**223**	**0.02**	**0.10**
94/95	October	-	-	-
	November	1	0.01	
	December	-	-	-
	January	1	0.07	
	February	19	0.02	0.05
	March	16	0.02	0.04
	April	5	0.03	0.05
	May	12	0.02	0.03
	June	23	0.02	0.04
	July	-	-	-
	August	-	-	-
	September			
	94/95 Total	**77**	**0.02**	**0.07**

Table A2.3 South Australia (continued)

Season	Month	Number	Cadmium (mg/kg)	
			Mean	Maximum
95/96	October	7	0.03	0.04
	November	4	0.02	0.03
	December	28	0.02	0.05
	January	-	-	-
	February	37	0.02	0.03
	March	5	0.01	0.03
	April	19	0.02	0.04
	May	-	-	-
	June	20	0.02	0.05
	July	14	0.02	0.03
	August	-	-	-
	September	-	-	-
	95/96 Total	**134**	**0.02**	**0.05**
SA Total		**660**	**0.02**	**0.10**

Table A2.4 Victoria

Season	**Month**	**Number**	**Cadmium (mg/kg)**	
			Mean	**Maximum**
91/92	October	-	-	-
	November	17	< 0.01	0.01
	December	5	< 0.01	< 0.01
	January	7	< 0.01	< 0.01
	February	3	< 0.01	< 0.01
	March	4	< 0.01	< 0.01
	April	13	< 0.01	0.01
	May	8	< 0.01	< 0.01
	June	-	-	-
	July	13	< 0.01	0.01
	August	14	< 0.01	0.01
	September	-	-	-
	91/92 Total	**84**	**0.01**	**0.01**
93/94	October	-	-	-
	November	3	< 0.01	0.01
	December	2	< 0.01	0.02
	January	-	-	-
	February	2	0.01	0.01
	March	4	< 0.01	< 0.01
	April	1	< 0.01	0.01
	May	9	0.01	0.02
	June	23	< 0.01	0.02
	July	9	< 0.01	0.01
	August	8	< 0.01	0.01
	September	6	< 0.01	0.01
	93/94 Total	**64**	**< 0.01**	**0.02**
94/95	October	2	< 0.01	0.01
	November	3	< 0.01	0.02
	December	1	< 0.01	
	January	9	0.01	0.03
	February	4	< 0.01	< 0.01
	March	13	0.01	0.04
	April	7	0.01	0.02
	May	2	0.02	0.03
	June	-	-	-
	July	9	0.01	0.02
	August	4	0.02	0.03
	September	3	< 0.01	0.01
	94/95 Total	**57**	**0.01**	**0.04**

Table A2.4 Victoria (continued)

Season	Month	Number	Cadmium (mg/kg)	
			Mean	Maximum
95/96	October	3	0.01	0.01
	November	1	0.01	
	December	-	-	-
	January	-	-	-
	February	13	0.01	0.03
	March	1	< 0.01	
	April	1	0.02	
	May	16	0.01	0.03
	June	9	< 0.01	< 0.01
	July	25	0.01	0.02
	August	-	-	-
	September	12	0.01	0.01
	95/96 Total	**81**	**0.01**	**0.03**
Vic Total		**286**	**0.01**	**0.04**

Table A2.5 Western Australia

Season	**Month**	**Number**	**Cadmium (mg/kg)**	
			Mean	**Maximum**
91/92	October	48	0.03	0.05
	November	40	0.02	0.04
	December	-	-	-
	January	23	0.02	0.05
	February	29	0.03	0.05
	March	56	0.03	0.04
	April	36	0.02	0.04
	May	25	0.02	0.07
	June	50	0.03	0.07
	July	27	0.03	0.08
	August	-	-	-
	September	24	0.03	0.06
	91/92 Total	**358**	**0.03**	**0.08**
93/94	October	33	0.04	0.05
	November	21	0.04	0.06
	December	20	0.03	0.06
	January	19	0.03	0.06
	February	23	0.02	0.05
	March	47	0.04	0.06
	April	35	0.02	0.05
	May	-	-	-
	June	29	0.03	0.04
	July	31	0.02	0.06
	August	15	0.03	0.06
	September	20	0.03	0.06
	93/94 Total	**293**	**0.03**	**0.06**
94/95	October	29	0.03	0.06
	November	29	0.02	0.07
	December	24	0.01	0.04
	January	20	0.02	0.05
	February	24	0.02	0.05
	March	46	0.02	0.05
	April	5	0.02	0.02
	May	14	0.03	0.05
	June	7	0.03	0.05
	July	-	-	-
	August	8	0.03	0.05
	September	12	0.02	0.05
	94/95 Total	**218**	**0.03**	**0.06**

Table A2.5 Western Australia (continued)

Season	Month	Number	Cadmium (mg/kg)	
			Mean	Maximum
95/96	October	31	0.03	0.04
	November	28	0.03	0.05
	December	-	-	-
	January	-	-	-
	February	38	0.02	0.03
	March	14	0.03	0.04
	April	21	0.02	0.03
	May	52	0.03	0.08
	June	34	0.03	0.05
	July	47	0.02	0.5
	August	-	-	-
	September	23	0.02	0.03
	95/96 Total	**288**	**0.03**	**0.08**
WA Total		**1,157**	**0.03**	**0.08**