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BY

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ABSTRACT

The bioavailability of cadmium from sewage sludge-amended soils

Cadmium is a potentially toxic trace element present, at low concentrations, in a variety of environmental media. The processing of waste-waters produces sewage sludge, in which cadmium may be present at high concentrations. Sewage sludge applied to soils used for the production of food crops represents a means for the elevation of human dietary exposure to cadmium. This study has examined aspects of the bioavailability of cadmium from sludge-amended soils.

A number of sludge-amended and control soils were sampled and placed in tubs in the field. Duplicates of each soil were limed to a mean pH(H_2O) of 6.9. Concentrations of cadmium in the edible tissues of cabbages and lettuces were lower for those plants grown on limed soils. Concentrations of cadmium in peeled potato tubers were not always reduced by the application of lime. Cadmium bioavailability was estimated by four soil test reagents, DTPA, EDTA-$(Na)_2$, $CaCl_2$ and NH_4NO_3. The DTPA test proved to be the best indicator of plant cadmium concentrations. Multivariate statistics were used to develop models, based upon soil variables, for the prediction of plant cadmium concentrations and CF-values. Models were tested against an independent data set.

Data quality for cadmium analyses was assessed by the routine use of certified reference materials. Methods of ETA-AAS analysis were developed and optimised for the control of interferences.

Preliminary cadmium speciation studies, using SEC-ICP-MS, are described. Cadmium-binding species in the cytosol extract of potato tubers do not appear to survive *in vitro* gastro-intestinal enzymolysis. Cadmium- binding species in aqueous extracts of both intrinsically and extrinsically labelled samples of cooked potato tuber have molecular weights of $< 2 \times 10^3$ daltons.

An iterative model was written to examine the influence of soil and plant variables on the cadmium burden of the plough layer through time.

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrometry
ADAS	Agricultural Development and Advisory Service
ADI	Acceptable daily intake
BCR	Community Bureau of Reference
CEC	Commission of the European Community
CF	Concentration factor
CRM	Certified reference material
CTF	Controlled temperature furnace
d	Daltons
DAC	Delayed atomisation cuvette
DTPA	Diethylenetriaminepentaacetic acid
DW	Dry weight
EC	European Community
EDTA-$(Na)_2$	Disodium ethylenediaminetetraacetic acid
ETA-AAS	Electrothermal atomisation atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
FAO	Food and Agriculture Organisation
FIA	Flow injection analysis
FPLC	Fast protein liquid chromatography
FW	Fresh weight
GC	Gas chromatography
GI	Gastro-intestinal
HPLC	High performance liquid chromatography
IAEA	International Atomic Energy Agency
ICP-ES	Inductively coupled plasma emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
LC	Liquid chromatography
LOI	Loss-on-ignition
MAFF	Ministry of Agriculture, Fisheries and Food
MCA	Multi-channel analyser
MT	Metallothionein
MTF	Mass transfer factor
NAA	Neutron activation analysis
NBS	National Bureau of Standards
NIES	National Institute for Environmental Studies
PCC	Population critical concentration
PTFE	polytetrafluroethylene
PTWI	Provisional tolerable weekly intake
SEC	Size exclusion chromatography
SEM	Scanning electron microscope
SN	Sample number
SRM	Standard reference material
STPF	Stabilised temperature platform furnace
TPC	Totally pyrolytic cuvette
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation
XRF	X-ray fluorescence

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CHAPTER 1: CADMIUM IN THE ENVIRONMENT: AN OVERVIEW

1.1: INTRODUCTION

An increase in the world production and use of cadmium has created an increased input of this element into the environment; these inputs may be either as inadvertant and therefore uncontrolled emissions or via waste disposal practices. One such practice is the disposal of cadmium to the sewerage system, leading to an elevation in the concentrations of cadmium in sewage sludges. The cost-efficient disposal of sewage sludges is a considerable problem to which the application to land provides an expedient solution. However, the application of sewage sludges to agricultural land has the potential to increase the cadmium exposure of sections of the population via an increase in the soil cadmium concentration.

The objective of this study was to examine the factors controlling the output of cadmium from soils amended with sewage sludges into the human food chain.

1.1.1: PHYSICAL AND CHEMICAL CHARACTERISTICS

Cadmium belongs to group II B of the Periodic Classification falling between zinc and mercury (Aylett 1979). The proximity of cadmium to zinc in the Periodic Table is reflected in the similar chemical behaviour of these two elements (Hamilton 1987). Table 1.1 lists some selected physical and chemical properties.

Atomic number	48
Atomic weight (Cd^{106},Cd^{108},Cd^{110},Cd^{111},Cd^{112},Cd^{113},Cd^{114},Cd^{116})	112.4
Boiling point	765°C
Melting point	321°C
Latent heat of fusion	13.2 cal g^{-1}
No. of oxidation states	2
Electronegativity coefficients	
Pauling	1.69
Allred-Rochow	1.46
Mulliken	1.4

Table 1.1: *Selected physical and chemical properties of cadmium (From Aylett 1979, Nriagu 1980b).*

Cadmium's presence in the d-block of elements means that it is considered to be a transition element and as such has more than one oxidation state.

Cadmium is not mined for in it's own right and is mainly produced as a by-product of the refinement of zinc. Cadmium has a number of uses, including:

- pigments for plastics
- cadmium coatings
- nickel-cadmium batteries
- PVC stabilisers
- alloys

(Stubbs 1980).

Approximately 90% of cadmium is used in the ways listed above; the remainder is used for a wide variety of processes, ranging from catalysts to cathode ray tubes (Department of the Environment 1984).

1.1.2: CONCENTRATIONS OF CADMIUM IN ENVIRONMENTAL MEDIA

Cadmium is a relatively rare element and, as such, is usually present in low concentrations. Cadmium is 67th in order of elemental abundance. The mean concentration of cadmium in the earth's crust is estimated to be 0.1 $\mu g g^{-1}$ (Bowen 1979), concentrations of cadmium in rocks vary from a mean of 0.005 $\mu g g^{-1}$ to schists and 219 $\mu g g^{-1}$ in black shales (Alloway 1990). Table 1.2 shows data for the cadmium concentration of a variety of environmental media.

Reservoir	Pool Mass (g)	Cadmium Concentration	Total Cadmium in Pool (g)	Residence Time§
Atmosphere	$5.1 \times 10^{18} m^3$	0.03 ngm^{-3}	1.5×10^8	7 days
Hydrosphere				
Oceans				
Dissolved	1.4×10^{24}	0.06 μgkg^{-1}	8.4×10^{13}	2.1×10^4
Suspended particulates	1.4×10^{18}	1.00 μgg^{-1}	1.4×10^{12}	-
Particulate organic matter	7×10^{16}	4.5 μgg^{-1}	3.2×10^{11}	1.3 years
Fresh waters				
Dissolved	0.32×10^{20}	0.05 μgkg^{-1}	1.6×10^9	-
Sediments	6.5×10^{17}	0.16 μgg^{-1}	1.0×10^{11}	3.6 years
Glaciers	1.65×10^{22}	0.005 μgkg^{-1}	8.2×10^{20}	-
Groundwater	4×10^{18}	0.1 μgkg^{-1}	4×10^8	-
Sediment pore waters	3.2×10^{23}	0.2 μgkg^{-1}	6.4×10^{13}	-
Swamps & marshes biomass	6×10^{15}	0.6 μgg^{-1}	3.6×10^9	-
Biosphere				
Marine plants	2×10^{14}	2.0 μgg^{-1}	4×10^8	18 days
Marine animals	3×10^{15}	0.6 μgg^{-1}	3.6×10^9	-
Land plants	2.4×10^{18}	0.3 μgg^{-1}	7.2×10^{11}	20 days
Land animals	2×10^{16}	0.3 μgg^{-1}	6×10^9	-
Freshwater biota	2×10^{15}	3.5 μgg^{-1}	7×10^9	3.5 years
Human biomass	$4x\ 10^9$ persons	50 mg/person	2 x 108	1 to 40 years
Terrestrial litter	2.2×10^{18}	0.6 μgg^{-1}	1.3×10^{12}	42 years
Lithosphere	5.7×10^{25}	0.5 μgg^{-1}	2.8×10^{19}	109 years
Sedimentary rocks	2.5×10^{24}	1.0 μgg^{-1}	2.5×10^{18}	-
Shale & clay	1.9×10^{24}	1.3 μgg^{-1}	2.47×10^{18}	-
Limestone	0.35×10^{24}	0.08 μgg^{-1}	2.1×10^{16}	-
Sandstone	0.3×10^{24}	0.07 μgg^{-1}	2.1×10^{16}	-
Soils (to 100cm)	3.3×10^{20}	0.2 μgg^{-1}	6.6×10^{13}	3000 years
Organic fraction	6.8×10^{18}	0.9 μgg^{-1}	6.1×10^{12}	> 200 years

§- the residence time (T) is given by the following formula, $T = \frac{A}{dA/dt}$ where A is the mass of cadmium in a given reservoir and t is time.

Table 1.2: *Cadmium burdens and residence times in the principal global reservoirs (From Nriagu 1980a).*

Concentrations of cadmium in unpolluted soils are quite closely related to the concentration of cadmium in the parent material from which they were derived. The background concentration of cadmium in soils is <1 μgg^{-1} (Alloway 1990), levels may be significantly elevated by some human activities or by the weathering of those parent materials that contain anomalously high cadmium concentrations, black shales for example. Table 1.3 shows the typical cadmium concentrations from a

number of national surveys. It should be stressed that the deposition of cadmium from the atmosphere is a significant process (Cawse 1980, Martin *et al* 1980), even at sites a considerable distance from point sources (Semb and Pacyna 1988); in this respect it is difficult to describe any soil as being uncontaminated.

Description	Range	Mean
Agricultural soils, Denmark	0.03 - 0.9	0.22
Agricultural soils, Sweden	0.03 - 2.3	0.22
Uncultivated soils, Canada	0.01 - 0.1	0.07
Agricultural soils, USA	0.005 - 2.4	0.27
Agricultural soils, Ohio USA	<0.1 -2.9	0.2
Agricultural soils, UK	0.01 - 2.4	1.0*
Poland	-	0.016
England & Wales	0.08 - 10	1.2
Ontario, Canada	0.30 - 0.65	-

* not the mean value, common value.

Table 1.3: *Typical concentrations of cadmium in soils (From Peterson and Alloway 1979 and Alloway 1990).*

The concentrations of cadmium in contaminated soils are significantly higher than those shown in Table 1.3 (see Chapters 2 and 6).

1.2: THE BEHAVIOUR OF CADMIUM IN ENVIRONMENTAL SYSTEMS

A biogeochemical perspective on the behaviour of cadmium enables the global cycling of cadmium to be examined (Nriagu 1980a). Such phenomenological models consist of a number of sources, sinks and pathways and enable comparisons to be drawn between their relative importance. Accurate quantification of cadmium fluxes are a long way short of being comprehensive and remain the subject of considerable

research effort. The biogeochemical cycling of cadmium in a number of natural systems has been described; for example, for a marsh ecosystem (Hazen and Kneip 1980) and for forested ecosystems (Martin and Coughtrey 1987, Sopper and Kerr 1980).

As part of the assessment of human exposure to a range of contaminants, Yost has applied a systems approach to the study of the cadmium cycle in the United States (Yost 1980, 1984). Figure 1.1 summarises the main flows of cadmium in Yost's model. The input of cadmium to the sewerage system is an important component of this model, with the sewage sludges generated being a major cadmium sink. This makes the carefully regulated disposal of sewage sludges a significant factor in the prevention of excessive dietary exposure to the human population

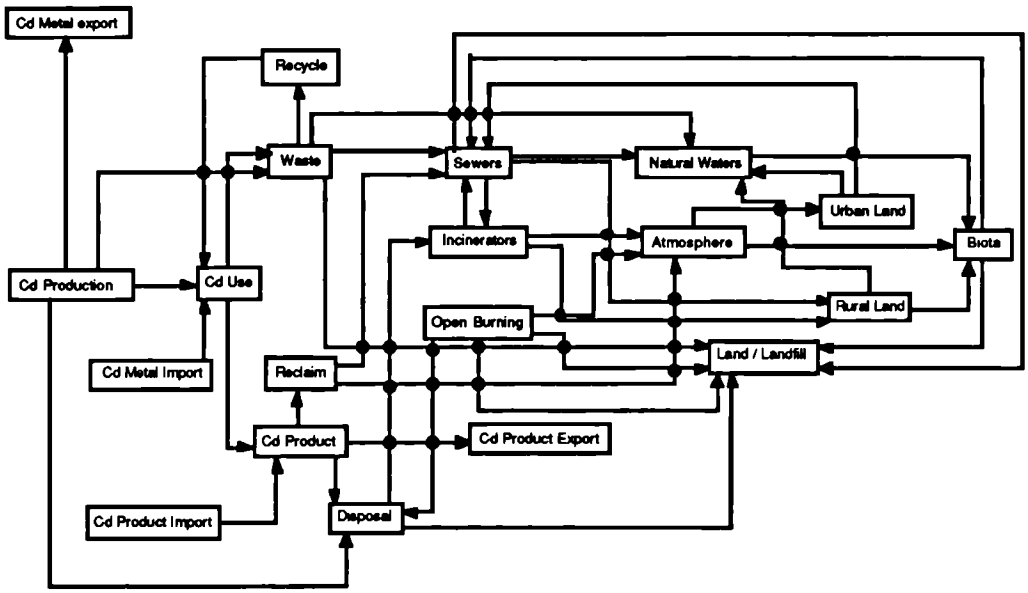


Figure 1.1: *Primary elements of the U.S. cadmium environmental flow system (Yost 1984).*

The model has been used to assess those pathways that could potentially affect human health. Yost was able to conclude that the diet was the main source of cadmium exposure and that the two primary sources of cadmium to agricultural soils were the atmosphere and the application of sewage sludges. The scale of these models may be varied, therefore one is able to construct a model to describe the behaviour of cadmium and other trace elements across the soil - plant continuum.

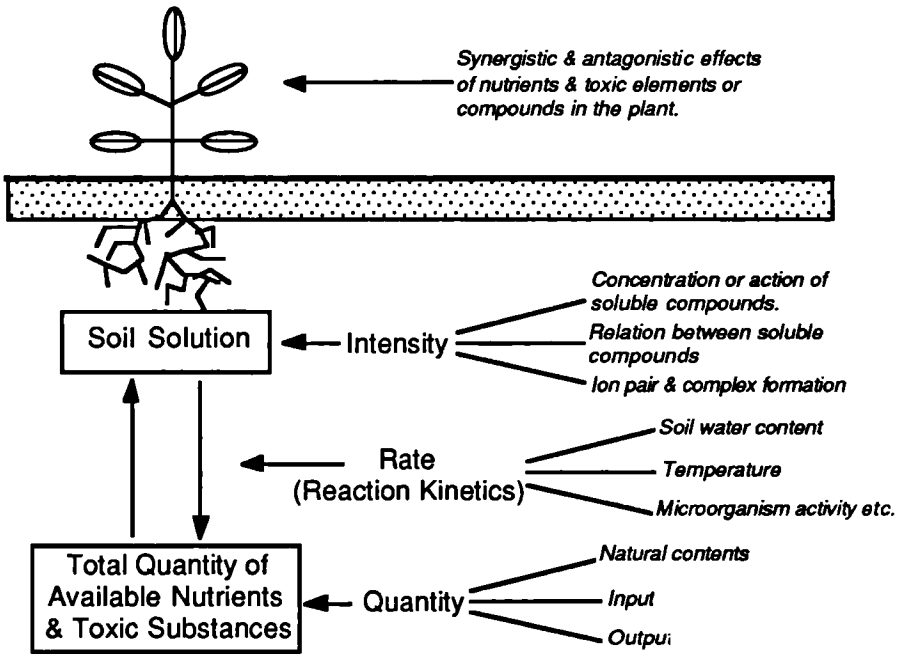


Figure 1.2: *A schematic representation of the processes affecting metal bioavailability to plants (Brummer 1986).*

Models such as these form the basis of a number of studies asssessing the risks associated with the application of sewage sludges to agricultural soils; this is examined in Chapter 2.

1.3: CADMIUM IN SEWAGE SLUDGES

1.3.1: THE PRODUCTION OF SEWAGE SLUDGE

Sewage sludge is produced as a by-product of wastewater treatment, the aim of which is to produce an effluent that, upon discharge, will not damage the environment (Vesilind and Pierce 1983). Treatment consists of three phases:

- Primary treatment - a physical process to remove suspended solids, producing the raw primary sludge.
- Secondary treatment - a biological process removing the oxygen demand.
- Tertiary treatment - consists of a number of effluent clean-up procedures, such as the removal of phosphorus.

Figure 1.3 summarises the wastewater treatment process.

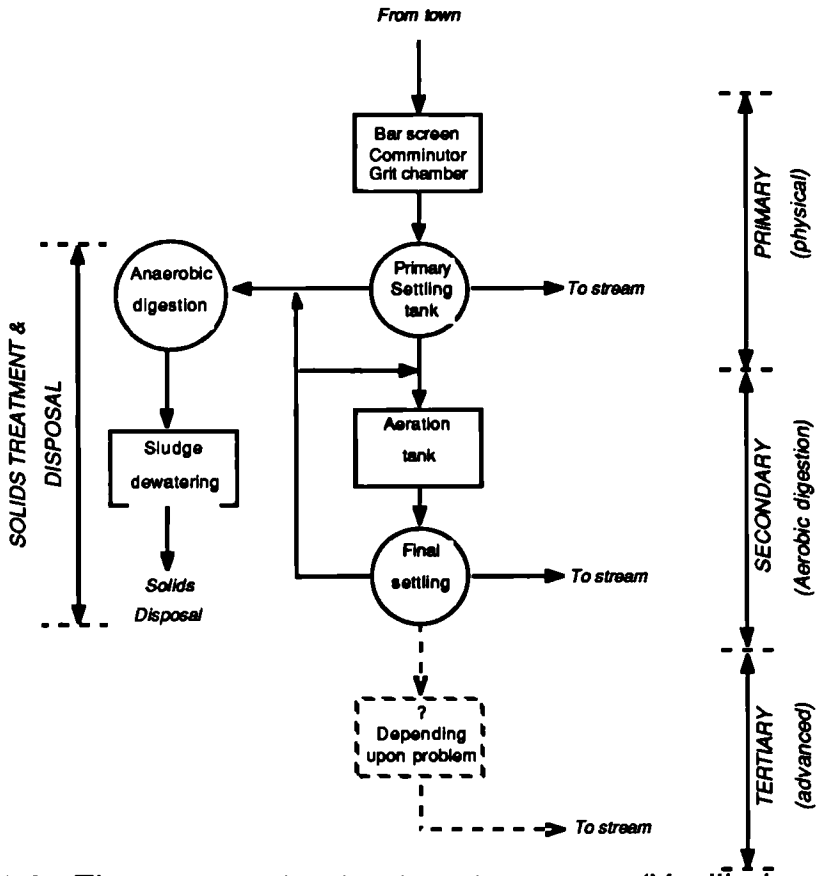


Figure 1.3: *The wastewater treatment process (Vesilind and Peirce 1983).*

1.3.2: CADMIUM CONCENTRATIONS IN SEWAGE SLUDGE

The concentration of cadmium in a given sample of sewage sludge is a reflection of the cadmium burden of the catchment served by the particular water treatment plant. The behaviour of heavy metals, including cadmium, during the activated sludge process have been examined by Stephenson and Lester (1987a,b). The concentrations of cadmium in sewage sludges vary from <1 to 3650 $\mu g g^{-1}$DW (Alloway 1990). Table 1.4 shows the range of cadmium concentrations found in a variety of sewage sludges reported in the literature.

Source of sludge	Range ($\mu g g^{-1}$DW)
16 US cities	6.8 to 444
150 treatment plants, US	3 to 3410
57 locations in Michigan, US	2 to 1100
6 towns, The Netherlands	0.3 to 168
42 treatment plants, UK	2 to 1500
200 sludges, UK	<1 to 180
45 teatment plants, Ireland	<1 to 90
Bordeaux, France	<2450
Helsinki, Finland	10 to 22
93 treatment plants, Sweden	2.3 to 171
7 cities in Ontario, Canada	0.3 to 236

Table 1.4: *Concentrations of cadmium in sewage sludges (Alloway 1990).*

The concentration of cadmium in some sewage sludges is very low, even on the basis of the national mean; for example, 80% of the 200 Irish samples of sludge analysed by O'Riordan *et al* (1986) contained less than $1 \mu g g^{-1}$.

The speciation or chemical form of cadmium in sludges and other wastes is an important factor in their behaviour in the environment (Forstner 1987). The incineration of sludges produces marked changes in the

extractability of cadmium, significantly increasing the proportion of cadmium in the 'residual' fraction (Forstner 1987). Analysis of sewage sludges by Fletcher and Beckett (1987) has shown cadmium to be bound to soluble organic matter. The affinity sequence of binding sites available to copper and the excluding ion was found to be Cu > Cd > Ni > Zn > Co ~ Mn > Ca > Mg.

1.3.3: APPLICATION OF SLUDGES TO LAND

The application of sewage sludges to soils provides a potentially valuable source of plant macronutrients (Chaney 1988, Chaney *et al* 1978, Chang *et al* 1979, Williams 1984). Table 1.5 gives data for the composition and dry matter analysis of a typical air-dried digested sludge.

Composition	
Moisture	50%
Dry matter	50%
pH	7.2
Dry matter analysis	
Organic matter content	60%
Nitrogen (as N)	3%
Phosphate (as P2O5)	2%
Potash (as K20)	0.5%
Calcium (as CaO)	5%
Magnesium (as MgO)	1.5%

Table 1.5: *Composition of an air-dried digested sludge (Arden 1977).*

If the concentrations of trace elements in the sludge are high, the trace element burden of the soil is likely to increase and become manifest in problems with phytotoxicity (Chaney *et al* 1978, Follet *et al* 1981, Kampe 1984, Sauerbeck 1987). The application of sewage sludge

usually results in an increase in the concentration of cadmium in the soil. The bioavailability of cadmium from sludge-amended soils is influenced by a wide range of factors, as discussed in Chapter 2. Cadmium speciation in the soil is likely to be an important factor in the determination of bioavailability to plants and has been quantified using a variety of techniques (Emmerich *et al* 1982, Lake *et al* 1984). The speciation of metals in the soil solution is likely to change with time (Katayama *et al* 1986) and may result in changes in the solubility of some of the metals (Campanella *et al* 1989).

CHAPTER 2: CADMIUM BIOAVAILABILITY & HUMAN DIETARY EXPOSURE AFTER THE APPLICATION OF SEWAGE SLUDGE TO AGRICULTURAL SOILS

2.1: INTRODUCTION

2.1.1: CADMIUM TOXICITY & HUMAN EXPOSURE

Cadmium has no known essential biological function and is toxic to both plants and animals above a certain threshold dose. The incidence of acute toxicity is rare and cadmium exposure is predominantly a chronic problem. The dose necessary to produce an acute response is estimated to be one thousand times greater than that needed to produce a chronic response (Kanzantzis 1979 in Naylor and Loehr 1981). The cardinal feature of its toxic action is the very long half-life retention time in the body, implying that the concentration of cadmium in target organs will increase with time and cadmium is therefore classed as a cumulative toxin. The kidney, or more specifically the renal cortex, is the principal component of the target organ in which cadmium has a mean residence time of 30 or more years (Elinder *et al* 1981). The ratio of the concentration of cadmium in the renal cortex, to that in the whole kidney is 1 : 1.25 (Kjellstrom *et al* 1984). A wide variety of estimates for the body retention time have been made ranging from 16 to 30 years (Joint FAO/WHO Expert Committee on Food Additives 1972).

In order to examine the dynamics of the behaviour of cadmium in animals, or more especially in mammals, a very wide range of experiments have been performed using *in vivo* techniques. A problem

with the application of much of the data collected from laboratory based *in vivo* experiments is that the cadmium exposures were much higher than those that one would expect to find in the majority of the population. A further problem is that the speciation of the metal used to administer the dose may well differ from that found in normal cadmium exposures. Much of the data from these experiments has been summarised and collated in a variety of forms, the most useful of which are models such as that produced by Kjellstrom and Nordberg (1979). The flow scheme of this model can be seen in Figure 1. The model recognises two main routes of exposure, gastrointestinal and pulmonary, adsorption through the skin is considered to be negligible. In this model the metabolic system is sub divided into a number of compartments, which act both as sinks and sources for cadmium. The rates of transfer of cadmium between these units are described by the transfer coefficients (C1 to C19); therefore, by multiplying the concentration in the source by the transfer coefficient the input to the next sink/source can be determined. The values of these coefficients are empirical. A series of iterative calculations will therefore enable predictions to be made of the concentrations of cadmium in the compartments, after a given period of time. The main weakness of this type of model is that it only deals with the mean values of coefficients, concentrations etc. and does not take into account the frequency distribution; this may lead to a number of problems in the application of the model unless care is taken in the interpretation of the data that it generates. Despite these problems this model, and others like it, have been used as a basis for many of the guidelines relating to cadmium exposure, issued by bodies such as the Environmental Protection Agency in the United States and the World Health Organisation.

As already stated, the kidney, or more specifically the renal cortex, is the main sink for cadmium in the body and so it is the concentration of cadmium in the kidney that should be minimised. The fate of cadmium in the body is determined by two basic factors (i) the concentration of cadmium binding ligands in an organ and (ii) the stability of the resulting complex (Kagi and Hopke 1984). A critical concentration of 200 $\mu g g^{-1}$ FW in the renal cortex has been shown to induce renal dysfunction which may be manifested in a variety of ways, the most common of which is proteinuria (Kjellstrom and Nordberg 1978). Metabolic models may be used to try to determine the daily exposure necessary to give this critical renal concentration of cadmium over a given period of time. A useful indicator of the maximum permissible cadmium concentration in the renal cortex, is the PCC-10 or population critical concentration; this is the concentration above which symptoms of renal dysfunction are expected to occur in 10% of the exposed population (Kjellstrom *et al* 1984). The rationale behind the concept of a critical concentration in the renal cortex has been questioned, as the toxicity of cadmium is influenced by a wide variety of factors, both intrinsic and extrinsic. Cadmium in the renal cortex can exist in a variety of locations and pools at the subcellular level and within this spatial differentiation there may exist different species. Cadmium bound to a cysteine-rich protein, metallothionein, has been shown to be less toxic than many other species in the renal cortex. The issue of cadmium dose is also subject to some complication as it would seem that certain species of the metal are more bioavailable than others (Fox 1983, 1988, McKenzie 1984). Extrinsic factors, such as the essential trace element composition of the diet, have, in laboratory animals, been shown to ameliorate the effects of cadmium toxicity; an extensive review of this aspect is given by Fox (1979). Cadmium toxicity is enhanced by

deficiencies of zinc, copper, selenium, iron and calcium. The fibre content of the diet also has an antagonistic effect upon its action; cadmium appears to be bound to a wide variety of dietary fibres (Fox 1988). The order of binding efficiencies is: cellulose < glucomannan < pectin < sodium alginate < sodium carboxymethyl cellulose < lignin (Kiyozumi *et al* 1982 in Fox 1988). Despite all of these complicating factors, a PCC-10 concentration of 180 to 220 $\mu g g^{-1}$ FW in the renal cortex is still used as the threshold concentration (Foulkes 1986).

It should be noted that the route via which the cadmium enters the body is very important, as different routes will lead to differential rates of entry into metabolic pathways. This illustrates the difference between dose and exposure. The exposure to cadmium is that amount that is ingested by the receptor organism, the dose is the amount of cadmium that crosses the GI tract. Bennett (1982) defines the exposure to a substance as a combination of the concentration of that substance and the time for which it is at the target point. A further classification may be useful with respect to the toxicity of a substance, ie metabolic dose, which may be defined as that quantity of a substance that is "available" to the receptor organisms metabolism. In this respect the speciation of cadmium may play a crucial role in the relative risks associated with a given exposure. A more detailed examination of the influence of speciation can be found in Section 2.2.

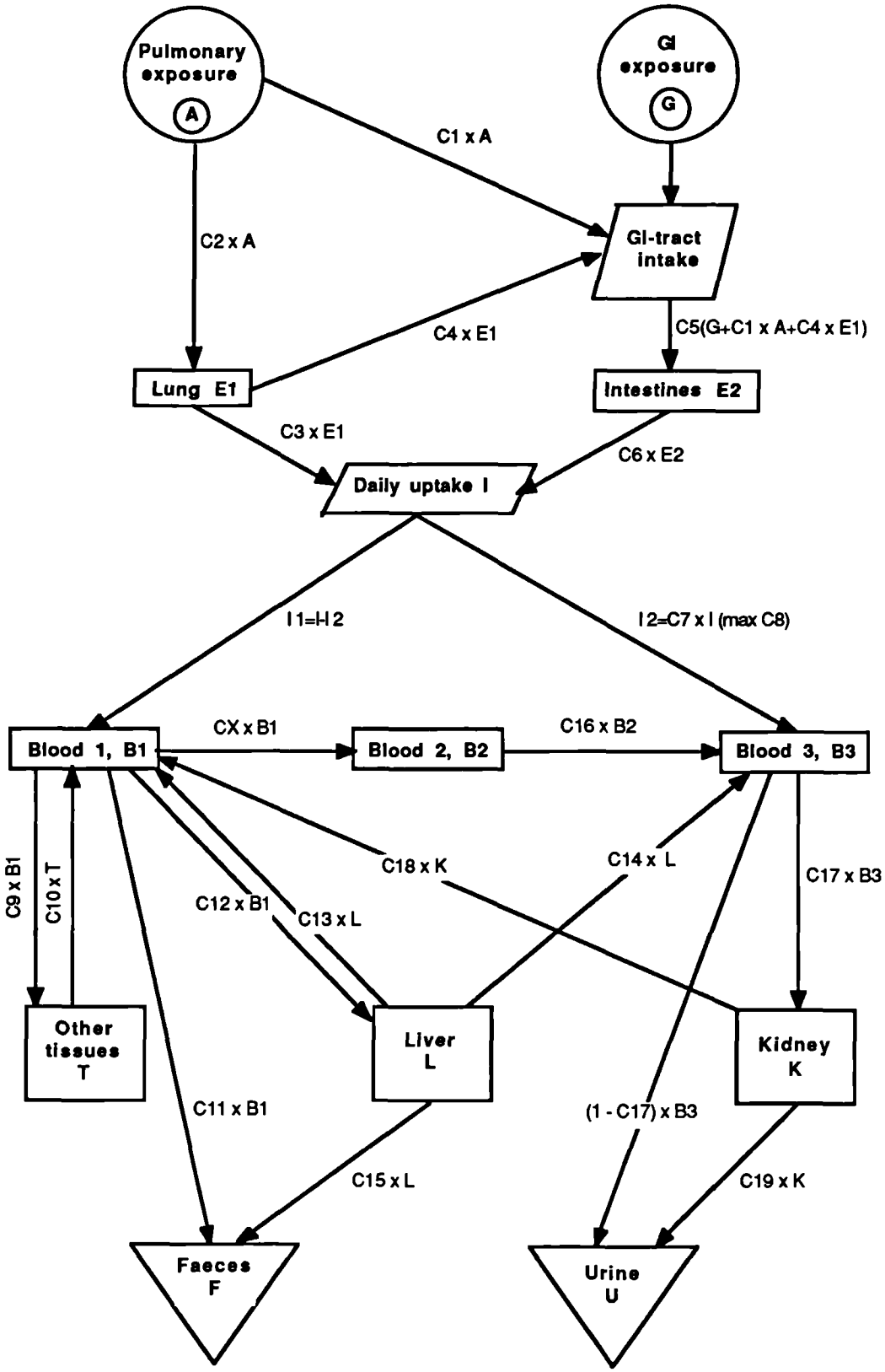


Figure 1: *A flow scheme for a kinetic model of cadmium metabolism in a human being. (From Kjellstrom and Nordberg 1978).*

As can be seen from Figure 2.1, the main sites of cadmium exposure are the gastrointestinal tract and the pulmonary system. The main sources of this exposure are the diet and the atmosphere, concentrations of cadmium in ambient air remain relatively low, ~3 ng kg^{-1} at rural sites in the UK (Cawse 1987), and so the diet remains the primary source of exposure to those members of the population who do not smoke (Louekari *et al* 1989). The main problem with exposure to the pulmonary system, is that it absorbs cadmium far more efficiently than the GI tract, 15 to 30% compared with 4 to 6% (Hutton 1982). Absorption of cadmium by the GI tract is highly variable, research on human subjects giving a mean of 4.6±4.0% (McLellan *et al* 1978). As will be discussed in Section 2.4.2.2, this has consequences for the cadmium exposure and dose of people who smoke cigarettes. Table 2.1 shows the main sources of emissions of cadmium to the atmosphere in the UK.

Source	Release ($t\ a^{-1}$)
Non-ferrous metal production	3.7
Iron and steel production	2.5
Fossil fuel combustion	2.2
Cement manufacture	1.0
Municipal waste disposal	4.5
Sewage disposal	0.2
TOTAL	14.1

Table 2.1: *Cadmium releases into the UK atmosphere. (From Hutton and Symon 1987)*

The diet exposes people in the European Community to between 18 and 48 μg Cd day^{-1}, of which only about 5% is from the consumption of water. Given that the diet is the most important route of cadmium exposure, an assessment of the sources of contamination to agroecosystems is desirable. Contamination of the soil by cadmium is by far the most important route as all crops will take up cadmium from the soil; however, the degree to which the cadmium of plant origin impinges upon the diet of both people and livestock, depends largely upon which component of the plant or animal is consumed, a more detailed examination of this feature can be found in Sections 2.2 and 2.3.

The accumulation of cadmium from the atmosphere in the edible tissues of crop plants is an additional exposure route which should not be ignored and is yet to be quantified in the whole range of food crops. At sites in which the soil concentration is considered to be at background levels the foliar uptake of cadmium is significant (Dollard and Davies 1989, Hansen and Tjell 1983, Harrison and Chirgawi 1989b, 1989c, Hovmand *et al* 1983). Once absorbed by the plant, a metal of atmospheric origin may be readily translocated and transpired throughout the whole plant (Harrison and Johnston 1987, Harrison and Chirgawi 1989a). Although it is important to recognise the role of cadmium in respired air, it is unlikely that this exposure route could pose a threat to the human food chain in the short-term.

With the exception of those occupationally exposed to cadmium, the most immediate short-term threat arises from soil-borne cadmium. Having established that the transfer of cadmium from the soil to the plant is the most important pathway, the issue of soil contamination is raised. Levels of cadmium in the soil have been increasing for a considerable period of

time even at sites considered to represent background conditions (Jones *et al* 1987a); this is probably a manifestation of elevated concentrations of cadmium in the atmosphere leading to both the wet and dry deposition of cadmium in the pedosphere (Nriagu 1979). Figure 2.2 shows the rise in global emissions of cadmium to the atmosphere from anthropogenic sources since 1900. Although this pathway is important, it does not produce the very high levels of contamination found in some soils unless they are located near to an intense point source.

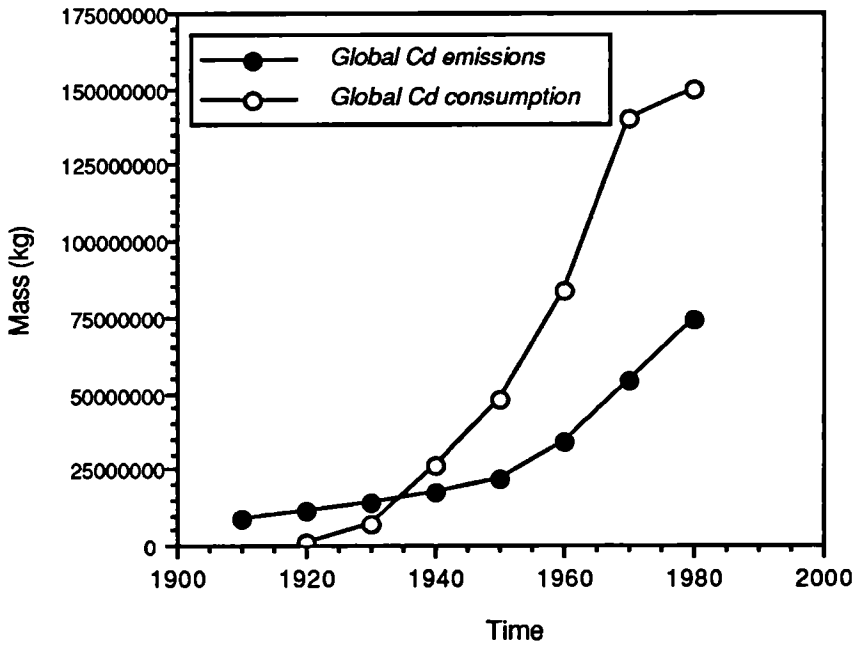


Figure 2.2: *Emissions of cadmium to the atmosphere (From Nriagu 1979).*

Two of the most important sources of contamination to soils are the application of phosphatic fertilizers and municipal sewage sludges. Many phosphatic fertilizers applied to land contain naturally high levels of cadmium, as can be seen in Table 2.2. Fertilizers made from magmatic phosphates will tend to contain only negligible concentrations of

cadmium; whereas those made from sedimentary phosphates will contain a wide range of concentrations, some of which may be very high (Hansen and Tjell 1983). Sewage sludges often contain elevated concentrations of cadmium (see Chapter 1) as a result of the disposal of the waste from industrial activities. A more detailed discussion of the inputs of cadmium from these sources to agricultural land can be found in Section 2.3 and Chapter 6.

Source country	Concentration (mg kg-1)
USA	6.5
Senegal	71.0
Morocco	18.0
Tunisia	18.0

Table 2.2: *Concentrations of cadmium in rock phosphate imported into the UK (From Hutton and Symon 1987)*

2.1.2: EXPOSURE LIMITS

A wide variety of exposure limits have been proposed by a number of organisations, most of which are based upon that issued by the FAO/WHO in 1972. An examination of the rationale behind this recommendation is desirable. The FAO/WHO makes its recommendations on the basis of a predetermined decision-making structure, the product of which is the allocation of an unconditional, conditional or temporary acceptable daily intake (ADI). The allocation of an ADI for cadmium was considered to be unsuitable for a wide variety of reasons, one of which was the cumulative nature of the dose. Therefore the term provisional tolerable weekly intake (PTWI) was used; implying

by the word "provisional" the tentative nature of the evaluation and by "tolerable" the inference that the exposure is not wholly acceptable.

A PTWI of 400 to 500 µg was finally made on the assumption that all of this exposure came from the diet, that 200 µgg-1 FW in the renal cortex was the critical concentration, that absorption by the GI tract was 5% efficient and that daily excretion was 0.005% of the total body burden. When applying this PTWI to models care should be taken as this is only an assessment of exposure and not of dose/metabolic dose. As shown above, metabolic dose is dependent upon many factors, including metal speciation and the exposure route. Another feature of the PTWI is that it is based upon the body of the average Caucasian and as such may be too high for populations with a smaller mean body size. For example the PTWI for the Japanese population is 325 µg (Kjellstrom and Nordberg 1978). This corresponds to a maximum exposure of 1µg cadmium kg^{-1} body weight per day.

2.2: CADMIUM IN THE DIET

2.2.1: QUANTIFYING DIETARY EXPOSURE TO CADMIUM

In order to assess the effect of dietary cadmium on the consumer some measure of the degree of exposure is necessary. A number of different methods exist. However, is a problem in that the exposure assessment methods do not always give the same answer for a given situation. The four main methods of exposure assessment are:

- the standard or total diet study;
- the duplicate meal;
- faecal analysis; and

• diary studies.

The method used in the majority of national surveys is the standard diet method, for example the Survey of Household Food Consumption and Expenditure in the UK and the Food and Drug Administrations Compliance Program in the United States. This technique usually involves an assessment of the mean consumption of foods from a variety of food classes, followed by their chemical analysis to arrive at some mean cadmium concentration (Global Environmental Monitoring System 1985). By multiplying together the mean consumption and the mean cadmium concentration of a certain class, a measure of the dietary exposure of cadmium from this class is obtained. The main problem with this method is that although it gives an indication of the typical dietary exposure to cadmium, it does not enable an assessment of the exposure of critical groups to be made.

Methods which can be used to assess the exposure to cadmium of a more specific group of people, are the duplicate diet study and the diary study, often used in combination; they are also useful if a particular component of the diet is under examination. The method requires the preparation of an extra meal/or component thereof for each meal prepared in a given household, the meal/component is then analysed. Although this technique may well provide a more targeted data set, it does require a considerable investment in the form of analytical effort. In an effort to examine the dietary intake of cadmium of a small population living in an area with a very high soil cadmium concentration, ie Shipham, the duplicate diet and dietary record studies of exposure were compared. The duplicate diet study was found to underestimate the exposure predicted by the dietary record studies (Barltrop 1986). This difference has often been observed and is thought to be either related to

a problem with the use and interpretation of detection limits during the analytical phase of the assessment (Dabeka *et al* 1987, Louekari *et al* 1987), or due to a slight change in the dietary habits of the participants (Morgan *et al* 1988, Sherlock and Walters 1983). Table 2.3 shows this discrepancy as found during studies of Shipham residents.

	Mean	Range
Diary estimate	0.25	(0.14 - 0.52)
Duplicate diet study	0.20	(0.04 - 1.08)
National average estimate based on total diet study	0.14	(0.09 - 0.18)

All units are mg Cd wk^{-1} $person^{-1}$.

Table 2.3: *Comparison of exposure assessment techniques used at Shipham. (From Sherlock and Walters 1983).*

The assessment of dietary cadmium exposure using faecal analysis is less frequently employed than either the duplicate meal or standard diet methods. The main problem with this method is the high degree of variability in the absorptive capacity of the GI tract (McLellan *et al* 1978). The most promising technique of assessment would be a simultaneous combination of the duplicate meal and faecal analysis methods, as this would give data for both exposure and dose. The practicalities of the widespread application of this method are prohibitive.

A more cost effective means of obtaining data for groups of people who may be said to be most at risk has been proposed by Coomes *et al* (1982), who found that a linear relationship exists between the arithmetic mean of consumption and specific measures of extreme consumption of a particular food. In this method, consumption greater than or equal to the

90th percentile of the consumption frequency distribution is used as a measure of extreme consumption. At the 95th percentile the mass of food consumed in any particular group is given by the equation:

$$Y = 2.0X + 111$$

where Y= consumption at the 95th percentile and X = mean consumption.

In this way data can be taken from total diet studies, such as the National Food Survey, and used to predict the enhancement in cadmium exposure produced by extreme consumption of a particular food. The main disadvantages of this approach stem from the fact that it cannot be used to predict extreme exposure from combinations of foods (Sherlock and Walters 1983).

2.2.2: COMPARISONS OF DIETARY EXPOSURE

All of the data quoted in this section are gathered from assessments which have employed standard diet techniques to quantify dietary exposure. The dietary exposure to cadmium varies quite considerably between countries, as can be seen from Table 2.4.

COUNTRY	Intake (mg Cd week^{-1})
West Germany	0.40
West Germany*	0.20
Poland*	0.13
Japan	0.27
New Zealand	0.11
Australia	0.15
Belgium	0.35
Denmark	0.21
Italy	0.38
USA	0.23
Britain	<0.15

NB: data for children signified by *.

Table 2.4: *National means for dietary exposure. (From Sherlock 1984)*

Dietary exposure to cadmium has three basic components:

- the total mass of food consumed;
- the composition of the diet, ie how much of the daily intake is derived from what food classes; and
- the concentration of cadmium in the food classes.

The qualitative and quantitative aspects of the composition of a diet will therefore lead to an exposure profile, and it is the differences in this exposure profile that may create differentials in dietary exposure. As a example of this phenomena it is useful to compare the dietary exposure of four countries, Finland, West Germany, Japan (Louekari and Salminen 1986) and Britain (MAFF 1983).

	Exposure (μg Cd day^{-1})			
	Finland	Germany	Japan	Britain
Cereals	6.6 (267)	11.0 (254)	17.6 (503)	5.0 (240)
Roots and tubers	2.4 (238)	10.4 (221)	2.1 (72)	<2.0 (159)
Sugars and honey	0.5 (109)	0.4 (122)	0.5 (73)	<1.0 (95)
Pulses	0.1	0.1	0.1	-
Nuts and oilseeds	0.0 (2)	0.1 (12)	2.3 (32)	-
Vegetables	1.4 (87)	15.4 (188)	10.3 (299)	2.0 (157)
Fruits	0.3 (219)	3.0 (287)	0.9 (178)	1.2 (84)
Meat and offals	2.0 (169)	4.3 (268)	1.0 (82)	2.3 (138)
Eggs	0.1 (29)	0.6 (47)	0.5 (45)	-
Fish and seafood	0.7 (78)	1.2 (27)	21.4 (239)	<0.2 (15)
Milk	1.1 (711)	1.2 (329)	0.7 (136)	<2 (360)
Oils and fats	0.7 (60)	1.2 (80)	0.4 (40)	<1.0 (90)
Spices	0.0 (1)	0.4 (2)	0.0 (2)	-
Stimulants	0.4 (41)	1.1 (28)	0.2 (9)	-
Alcoholic beverages	0.2 (180)	1.5 (489)	0.6 (160)	-
TOTAL	15.0 (2191)	50.0 (2354)	60.0 (1870)	19.0 (1456)

Figures in parentheses give the data for the mass of food consumed from from each food class. For Britain all data for vegetables other than potatoes given in the Vegetable row, the data for potatoes are in the Roots and tubers row.

Table 2.5: *Cadmium exposure profiles from Finland, West Germany, Japan and Britain (From Louekari and Salminen 1986, Ministry of Agriculture Fisheries and Food 1983).*

The most striking feature of these data is the very low overall exposures from the Finnish and British diets when compared to those from the West German and Japanese diets. In terms of the quantities of food consumed the West German and Finnish diets are very similar, yet the exposure from the Finnish diet is over three times lower. Consumption of cereal products is approximately the same but cereals consumed in West Germany must contain a higher mean cadmium concentration, which leads to an added exposure of 4.4 μgCd day^{-1} from this source. The lower than average cadmium concentration of foods from Finland seems to apply across a wide range of food classes and is consistent with some

micronutrient deficiencies in the Finnish diet (Koivistoinen 1980). Exposure from vegetables is very high in the West German profile due to a higher overall consumption and concentration. It is harder to make such direct comparisons between the data from Britain and the other three countries, this is due to a different classification of the food groups. It is possible, however, to draw a general comparison between the Finnish and the British data.

The average Japanese diet gives a cadmium exposure higher than both the Finnish and the West German diets, even though the mass of food consumed is lower. The three main components of the diet, in terms of cadmium exposure, are cereals, vegetables and fish and seafood. The latter producing an exposure of 21.4 μg; the enhanced exposure is due to the high consumption of foods from this component and also to the higher cadmium concentration in these foods. These differences in exposure profiles clearly have implications for standard setting both intra and internationally; a higher cadmium exposure could be indicative of a different and more susceptible diet composition and not indicative of higher mean soil concentrations, for example. Another factor which can affect the response to a certain exposure is the mean body size of the exposed population, this implies that the mass of the target organ will differ and so the mean concentration of cadmium will not be the same for a given exposure. Therefore, the PTWI for Japan is 325 μg Cd, 75 μg lower than that for people in the USA.

Another very important factor in the exposure profile, is the influence of smoking cigarettes upon the overall dose; this issue will be discussed in Section 2.4.

2.2.3: CONCENTRATIONS OF CADMIUM IN FOODS

The concentration of cadmium in foods will vary widely, as can be seen in Figure 2.3.

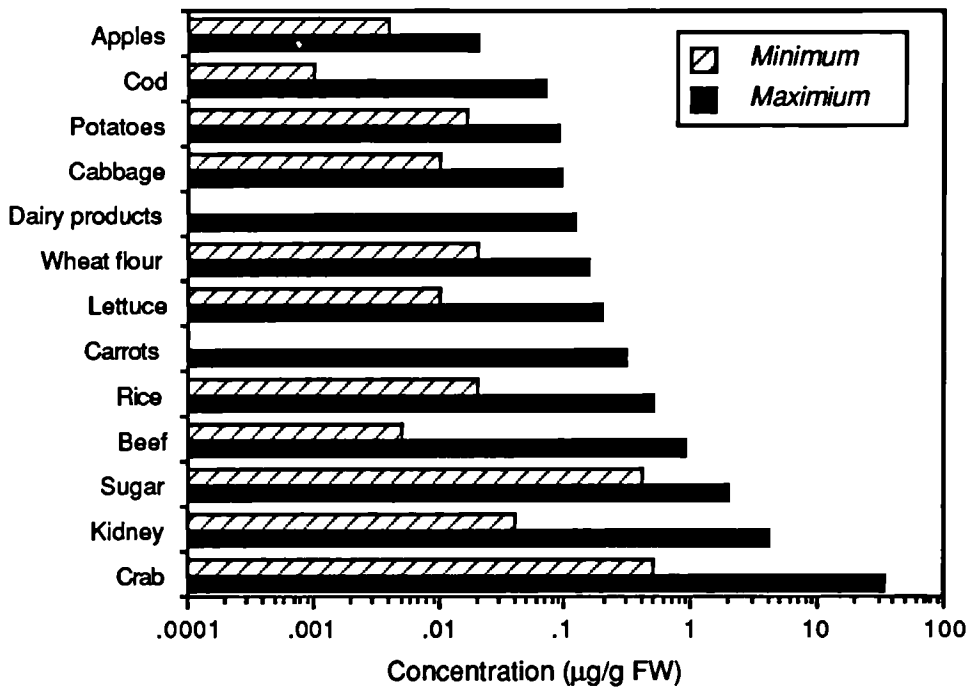


Figure 2.3: *Cadmium concentrations in selected food items. (Data from CEC 1978).*

The concentration of cadmium in a particular food item or type is important but must be considered as part of the overall exposure profile, as discussed in Section 2.2.2. Therefore, although crab and offals such as kidney and liver represent high concentration foods, their effect upon the weekly exposure is probably minimal, for the population as a whole. Cadmium exposure to groups who consume larger than average amounts of such foods may give rise to concern; such groups are often referred to as critical groups.

The foods which give rise to the most concern are those that form the largest component of the total intake in terms of grams of food per week. In the "typical" western European diet, the two most important groups are

cereal products and vegetables. 30 to 40% of the total dietary exposure of cadmium to people in the European Community is from the consumption of cereals (Hutton 1982). Figure 2.4 shows a typical exposure profile.

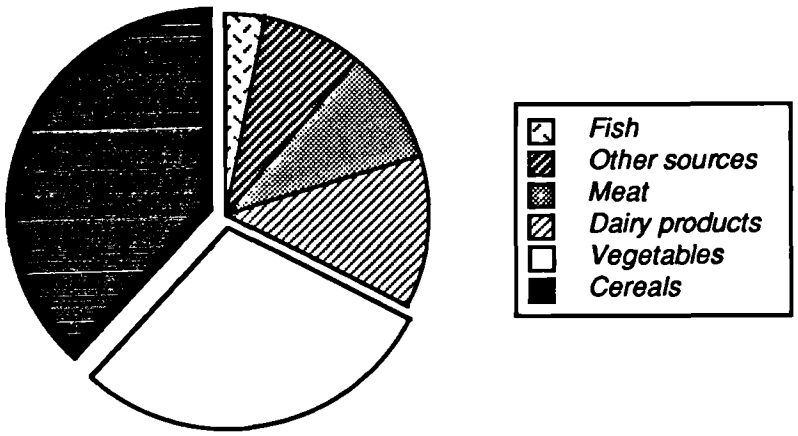


Figure 2.4: *Dietary exposure sources of cadmium in the Finnish diet (From Koivistoinen 1980).*

Most of the data presented in this section have only referred to food from sources considered to be *uncontaminated* by cadmium; the aim of this paper is to examine the potential threat to the diet from soils that have been amended with sewage sludges. It is necessary to be able to target those foods that will be affected by this land management /waste disposal practice and to assess the degree to which they will be impacted.

As discussed, the dietary cadmium exposure profile is influential in terms of the overall metabolic dose. Different components of this profile will respond in varying ways to an increase in the soil cadmium

concentration and so the exposure profile will also change. If a component of the diet contributes to the overall exposure to a large extent but does not markedly respond to a change in the soil concentration, then it may be said to have a buffered response. The term "buffered" is used in preference to "insensitive", as the exposure from these components is ameliorated by the position of the component in the food chain (Brams and Anthony 1983, Bache *et al* 1987) or by virtue of the part of the component consumed (eg fruits). However, if a component of the diet contributes significantly to the overall exposure and also responds to a change in the soil cadmium concentration, then it may be said to have a sensitive response. Using this theoretical relationship between response to a change in the soil concentration and dietary exposure, the components of the diet may be divided into groups. In this example the components of the diet correspond to those used by the Food and Drug Administration in the US and are assumed to be part of a typical diet in the USA or the UK (Drury and Hammons 1979).

SENSITIVE	**INTERMEDIATE**	**BUFFERED**
Grain & cereals	Dairy products	Meat & fish
Potatoes	Legumes	Garden fruits
Root vegetables		Fruits
Leafy vegetables		Oils & fats
		Beverages
		Sugars & adjuncts

Gradients of response to changes in the soil cadmium concentration exist within these groups and their classification will vary depending upon the exposure profile of the country concerned. Leafy vegetables, although not responsible for a large component of the dietary exposure, are placed in the sensitive group as they respond very markedly to increases

in the soil cadmium concentration and so have the potential to raise the total exposure. The inclusion of meats and fish in the buffered group is based upon the assumption that the consumption of offals and shellfish continue at their currently low levels. It must not be forgotten that once again the exposure profile is very important and that the above classification will only apply to the average consumer, people who consume certain foods at the levels found in the tails of frequency distributions may well have exposures well over the average (McKone and Ryan 1989, Nriagu 1988).

In light of the concern over dietary exposure a number of attempts have been made to restrict the dietary exposure to cadmium. Most of these aim to control the levels of cadmium in the soil and will be examined in Section 4; however the West German approach is to also propose limits as to the concentrations of cadmium in specific food groups (Kloke *et al* 1984). The proposals known as the Guidelines '79 are for three of the contaminants examined by the Joint FAO/WHO Expert Committee on Food Additives in 1972. Maximum concentrations for a variety of food groups can be seen below. The threshold concentrations of cadmium in these food groups are well below those that would produce overt phytotoxic effects in even the most cadmium-sensitive members of the groups.

Food group	Pb ($\mu g g^{-1} FW$)	Cd ($\mu g g^{-1} FW$)	Hg ($\mu g g^{-1} FW$)
Green vegetables	1.2	0.1	-
Sprout vegetables	1.2	0.1	-
Fruits	0.2	0.1	-
Root vegetables	0.5	0.05	-
Pomaceous fruits	0.5	0.05	-
Stone fruits	0.5	0.05	-
Berries	0.5	0.05	-
Cereals	0.5	0.1	0.03
Potatoes	0.2	0.1	0.02

Table 2.6: *Guidelines '79 values for concentrations of heavy metals in foods (From Bundesgesundheitsamt 1979).*

In light of the complicated and often unpredictable relationship between soil cadmium concentrations and those in plants, such threshold concentrations for foods may well be the way towards an effective exposure control strategy.

2.2.4: CADMIUM SPECIATION & BIOAVAILABILITY FROM FOODS

Bioavailability has been defined as "... a quantitative measure of the utilization of an element under specified conditions to affect the organism's normal structure and physiological processes" (Fox *et al* 1981). As briefly discussed in Section 2.1, the bioavailability of cadmium is influenced by a wide variety of intrinsic and extrinsic factors. Cadmium speciation is an intrinsic factor and as such may well play a key role in governing the degree of bioavailability and, therefore, the final metabolic dose from a given exposure (Chmielnicka and Cherian 1986). Figure 2.5 is a schematic representation of the possible role of speciation in the assessment of the metabolic dose arising from the consumption of foods grown upon soils with elevated cadmium concentrations. The same basic model could apply to background or uncontaminated situations if the

dichotomy between sensitive and intermediate/buffered food groups was omitted.

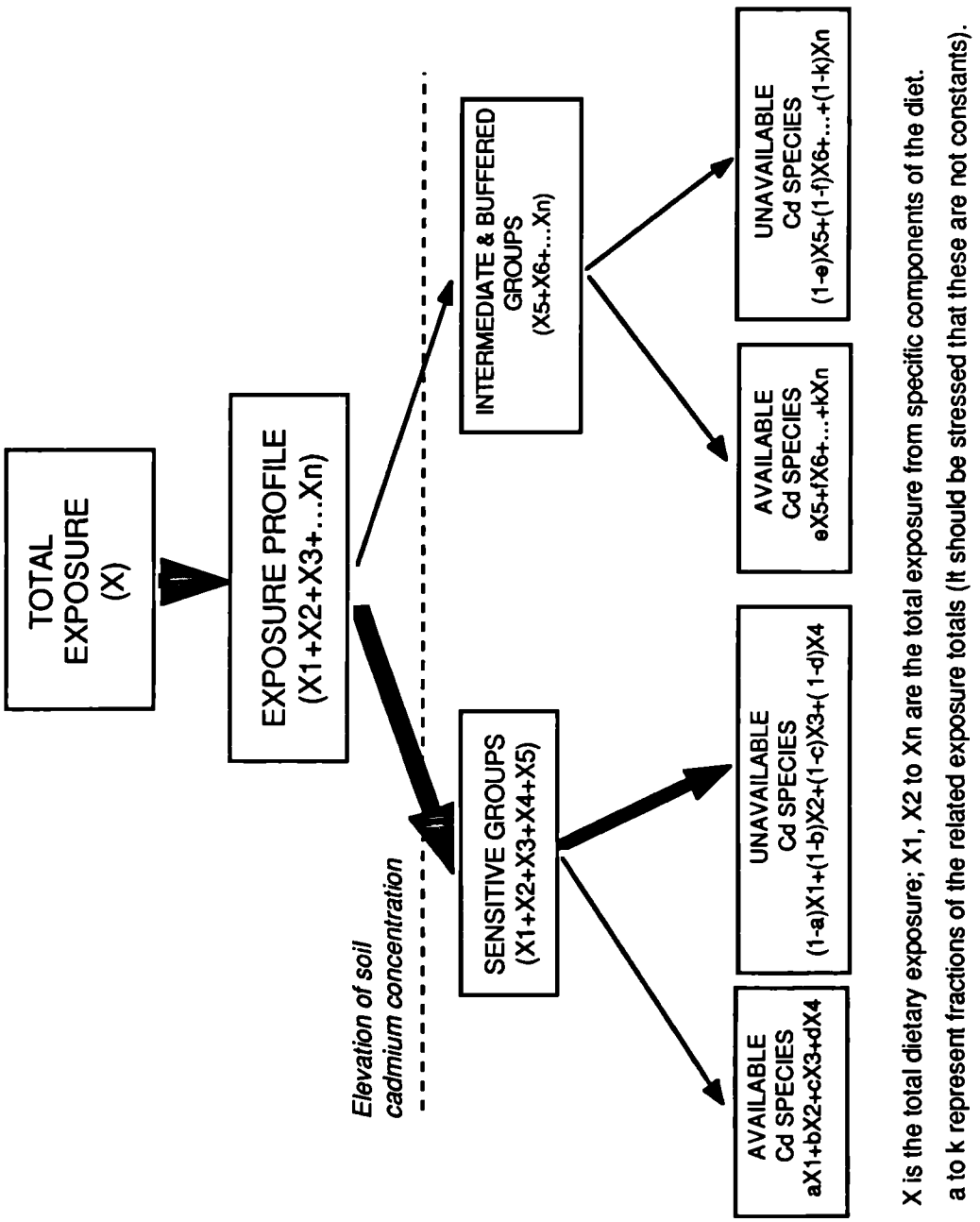


Figure 2.5: *The possible role of metal speciation in exposure/dose/metabolic dose assessment.*

The figure aims to show that an increase in the concentration of cadmium in agricultural soils will lead to increased exposure to cadmium via the diet. In particular, exposure to cadmium bound to phytochelatins will increase.

In foods the predominant cadmium species are those bound to low molecular weight proteins called metallothioneins or phytochelatins. In meat and meat products cadmium is probably bound to what are called Class I metallothioneins, in plant-based foods cadmium is probably bound to phytochelatins or phytometallothioneins (PC), which are classified as Class III metallothioneins. It is important to define exactly what is meant by the term metallothionein when it is used in this chapter, as there is some confusion over nomenclature. An early definition of metallothionein is given in a report from the "First International Meeting on Metallothionein and Other Low Molecular Weight Metal-binding Proteins" in which the following five distinguishing features were recognised:

- molecular weight 6 to 7 Kd;
- high metal content ;
- characteristic amino acid composition (high cysteine content, no aromatic amino acid or histidine);
- optical features characteristic of metal thiolates; and
- unique amino acid sequence.

(Nordberg and Kojima 1979)

Nomenclature has been expanded to include metal-binding proteins in plants, therefore a metallothionein is any polypeptide that resembles equine metallothionein in several of its features. Within this definition there exist three classes:

Class I: polypeptides with locations of cysteine closely related to those in equine metallothionein.

Class II: polypeptides with locations of cysteine only distantly related to those of equine renal metallothionein.

Class III: atypical nontranslationally synthesized metal thiolate polypeptides such as cadystin and phytometallotionein or phytochelatin.

(Fowler *et al* 1987).

In this paper emphasis will only be placed upon the Class III metallothioneins in foods, which shall be referred to as phytochelatins. The role of Class I metallothioneins in the metabolism of cadmium will be briefly discussed below.

Interest in the role of metallothioneins (MT) in cadmium toxicity in animals was stimulated in 1964, when Piscator proposed the hypothesis that the MT induced by cadmium exposure played a protective or detoxifying role in chronic exposure situations. This stimulated a great deal of research into the part played by MT in cadmium metabolism. The hypothesis revolves around the assumption that cadmium-thionein is metabolically inactive and therefore acts as a sink for cadmium. The hypothesis that MT enables cadmium to be detoxified appears to have been broadly accepted, despite the far from conclusive nature of research to date. Given that the retention of cadmium in the kidney may lead to renal failure, it is difficult to describe this process as being one of detoxification. The exact role of MT is still a matter of considerable conjecture, as its potential role in the detoxification of cadmium may not be its fundamental role in the metabolism. Given the antiquity of MT and its consistent composition and form, its fundamental role may be in the metabolism of

zinc and copper (Bremner 1987). The role of MT in the metabolism of cadmium is still the subject of a considerable research effort, as reviewed by Webb (1987). The main characteristic of MT is that it is induced by a variety of stimuli, of which cadmium may be one. Acute exposure to cadmium in the absence of a preceding period of low-level exposure will produce an acutely toxic response; the same acute level of exposure after a period of low level exposure will not produce this response. This phenomena stems from the lag phase between exposure to cadmium and the synthesis of MT and implies that low levels of cadmium exposure may well aid the resistance to brief but acute exposures (Webb 1979). MT is also inducible by zinc, although zinc induced MT has a shorter half-life than that induced by cadmium (Whanger and Oh 1979).

The bioavailability and fate of cadmium after ingestion may be determined, at least in part, by its speciation (Cherian *et al* 1978, Chmielnicka and Cherian 1986, Klein *et al* 1986). In this respect, cadmium bound to phytochelatins in foods represents a considerable source of exposure, as this is likely to be the dominant species in vegetables, grain, etc. Although cadmium may be bound to other proteins in foods, many of these will be denatured during the cooking process, unlike metallothioneins which are relatively heat stable (Crews *et al* 1989, Klein *et al* 1986, Maitani *et al* 1984). Comparisons of the fate of cadmium fed to mice as inorganic cadmium ($CdCl_2$) or as cadmium thionein, suggest that the final distribution of cadmium in the body may differ (Fox 1983). A greater proportion of the dose administered as cadmium thionein tended to accumulate more readily in the most sensitive organs, the kidneys; whereas that administered as $CdCl_2$ tended to accumulate more readily in the liver, the concentration of cadmium in the kidney was similar irrespective of the form administered

(Cherian 1979, 1983). A similar experiment to that conducted by Cherian but using wheat grain as the source of cadmium, has not repeated these observations (Wagner *et al* 1984).

A number of studies of phytochelatins and other cadmium-binding proteins in vascular plants have been made, including studies on wheat grain (Wagner *et al* 1984), cabbage (Wagner 1984), soybean (Casterline and Barnett 1982), *Agrostis gigantea* (Rauser 1984), rice (Kaneta *et al* 1983), tomato (Bartolf *et al* 1980) and lettuce (Henze and Umland 1987). The phytochelatins isolated from tomato, maize and cabbage are similar to animal or Class I MT in four respects:

- synthesis is stimulated by cadmium exposure;
- high cysteine content;
- do not have any aromatic amino acids; and
- ~10Kd characteristic produced by gel-filtration studies.

(Rauser 1987)

The main differences between the phytochelatins from the plant tissues and the MT, is that they differ in their elution characteristics at high ionic strength. Table 2.7 shows the differences in amino acid composition between the phytochelatins in three vascular plants and human MT.

Amino acid	Human MT	Cabbage PC	Tomato PC	Maize PC
Cys	32.8	28.9	25.6	40.3
Asx	6.6	4.2	5.4	2.8
Glx	3.3	39.0	53.3	35.1
Gly	8.2	11.1	12.8	10.4
Ser	13.1	1.9	1.9	1.5
Thr	3.3	1.5	0.3	1.0
Pro	3.3	1.7	<LOD	1.6
Ala	11.5	2.1	0.9	1.5
Val	1.6	0.9	<LOD	0.9
Met	1.6	0.7	-	-
Ile	1.6	3.4	<LOD	0.6
Leu	0	1.9	0.2	1.0
Tyr	0	0.9	<LOD	0.5
Phe	0	0	<LOD	0.5
Lys	13.1	1.0	0.5	1.0
His	0	0.8	0.2	0.5
Arg	0	0	<LOD	0.7

<LOD - less than limit of detection. All values are expressed in mole percent. Amino acid abbreviations are as follows: Cys - cysteine; Asx - aspartic acid; Glx - glutamic acid; Gly - glycine; Ser - serine; Thr - threonine; Pro - proline; Ala - alanine; Val - valine; Met - methionine; Ile - Isoleucine; Leu - leucine; Tyr - tyrosine; Phe - phenylalinine; Lys - lysine; His - histidine & Arg - arginine.

Table 2.7: *Amino acid composition of cadmium-binding proteins from different sources. (Data from Rauser 1987, Grill 1987).*

The main differences between the phytochelatins and human MT are the higher serine concentration of MT and the higher glutamic acid concentration in the phytochelatins. The differences between the phytochelatins are less pronounced, the significance of this subtle difference in terms of the cadmium metabolism of a person ingesting these proteins is unclear.

To conclude, it is widely recognised that the speciation of cadmium in foods may well be a factor governing the degree of bioavailability. The precise mechanism(s) in which cadmium speciation has an influence is

not clear; however, different species have been shown to produce different degrees of accumulation in target organs. A further complicating factor is the changes in speciation that occur in the gastro-intestinal tract upon ingestion (Mills 1986, van Dokkum 1989). The speciation of cadmium in plants differs from that in animals and this may alter the metabolic dose derived from the same exposure from these two food sources.

2.3: PLANT ACCUMULATION OF CADMIUM FROM CONTAMINATED SOILS

2.3.1: INPUTS OF CADMIUM TO AGRICULTURAL LAND

As previously discussed, the concentrations of metals in soils have been rising for about one hundred years as a result of the deposition of atmospheric metals and in this respect cadmium is not exceptional. The problem with contamination by cadmium, and indeed with that of some other metals such as lead, is that it tends to remain in the plough layer for a considerable period of time (McGrath 1987). This means that soils accumulate cadmium, making the potential problems associated with the transfer of cadmium into the foodchain long-term. The importance of the transfer of cadmium into the foodchain via soil was recognised by Allaway (1968). Inputs of cadmium to agricultural soils are derived from a variety of sources, as can be seen in Figure 2.6. If data can be found to quantify the values A to H, a mass balance for cadmium in agricultural soils can be calculated; the rate of change in the mass of cadmium in a given volume of soil is given by the following equation:

$$\Delta Cd = (A + B + C + D + G) - (H + F)$$

All units are g Cd ha^{-1} a^{-1}.

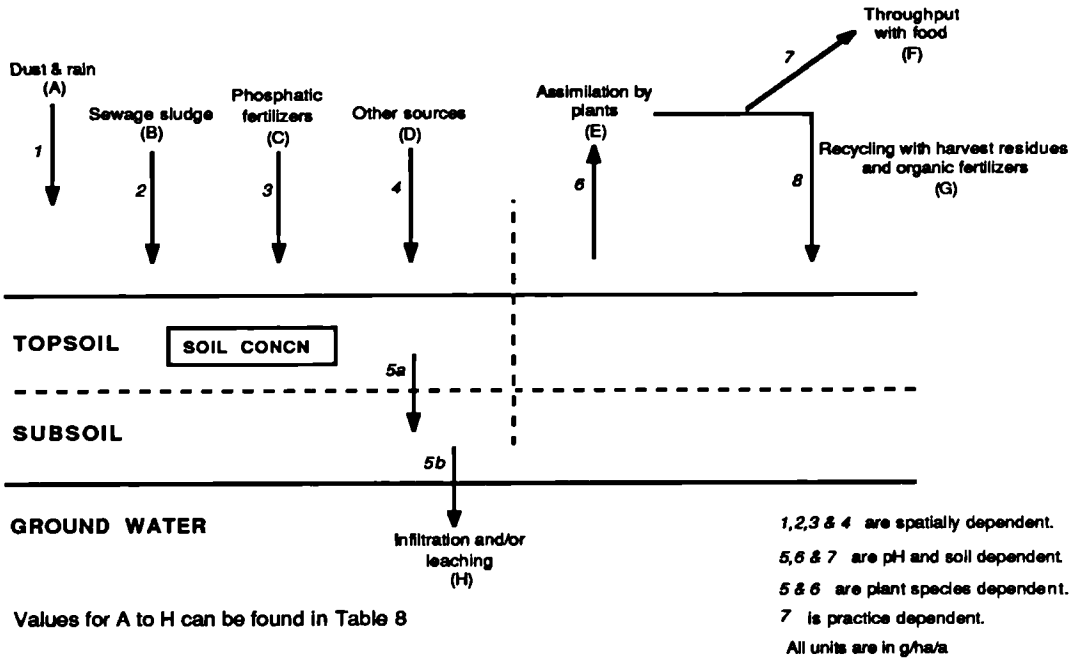


Figure 2.6: *A cadmium budget for agricultural soils (From Sauerbeck 1982 in Kloke et al 1984)*.

	United Kingdom[1]	West Germany[2]	Denmark[3]
A	3	3 to 27	2
B	0.94	10 to 25	0.12
C	4.30	3 to 7	3
D	No data	~1	No data
E	No data	0.5 to >10	1.3
F	No data	<0.6 to >5	No data
G	No data	0.3 to >8	0.4
H	No data	<1 to >2	2

1: Hutton & Symon 1986; 2: Sauerbeck 1982 in Kloke *et al* 1984 and 3: Hansen & Tjell 1983. All values are given in g ha^{-1} a^{-1}.

Table 2.8: *Mass flows of cadmium in agricultural soils.*

All of the data given in Table 2.8 assume that the quantities of cadmium, in sewage sludges for example, are distributed evenly throughout the

agricultural land area. As noted in Figure 2.6, deposition of cadmium from the atmosphere, sewage sludges, phosphatic fertilizers and other sources are spatially dependent, implying that the actual inputs to a given area of land may differ from the values given in Table 2.8. However, it is not unreasonable to assume that deposition from the atmosphere and from the use of phosphatic fertilizers will tend to display less spatial variation than that from sewage sludge. This means that although the total input of cadmium from sewage sludges may be low on a national basis (Davis 1984), it may well be very high on a local basis (Nriagu and Pacyna 1988).

Given that the above figure represents the cadmium budget for agricultural soils, it is possible to determine the ways by which the transfer of cadmium into the foodchain can be controlled. The inputs of cadmium to the system are the most likely means by which the overall soil burden can be controlled. Atmospheric deposition of cadmium is probably the least controllable input and cannot be completely eliminated as cadmium is discharged into the atmosphere from a variety of natural sources (Nriagu 1979). The use of phosphatic fertilizer is considered to be an important part of arable crop production, even though their use may lead to quite high concentrations of cadmium in the soil (Rothbaum *et al* 1986, Mortvedt 1987). To control this source of cadmium it would be most feasible to only use those sources of phosphate not containing high levels of cadmium, see Table 2.2. At the local level, controlling inputs of cadmium from sewage sludges may be important. Figure 2.7 shows that the disposal of sludges to agricultural land is an important part of the overall management of this form of waste, taking 28.5% of the total cadmium content of sludges. The future trends in the production of sewage sludges point to an increase in the quantity of

sludge generated but not to an increase in the concentration of cadmium in these sludges. The disposal routes are expected to change in emphasis, with the prediction that by 1990 75% of the sludge produced in the European Community will be disposed of to land (Hutton 1982). This would seem to imply that the total quantity of cadmium applied to the soil from sewage sludges will increase.

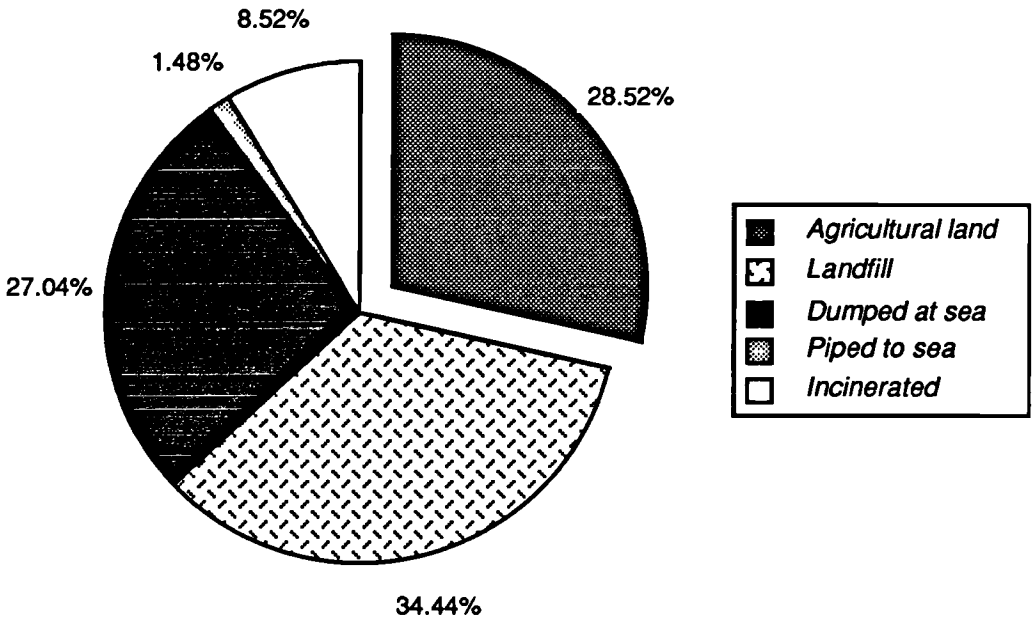


Figure 2.7: *Inputs of cadmium to the environment via sewage sludges (Data from Hutton and Symon 1986).*

Given that some industrial activities seem to almost inevitably generate cadmium wastes, it is difficult to see how the concentration of cadmium in the soil will not continue to increase. The control of cadmium discharges into sewage is probably the most feasible means under the current legislative emphasis upon pollution output from point sources (Zabel 1989). Chaney (1988) states that sludge quality should be regulated rather than cumulative metal application.

If the criteria of land management are based upon the anthropocentric approach of controlling the concentration of cadmium in foods and not upon soil protection, then routes *6* and *7* in Figure 2.6 offer the means by which the risk to the human foodchain may be minimsed. As stated in Figure 2.6, assimilation of cadmium by plants is controllable by a variety of means, including the manipulation of soil pH and the use of crop plants that do not accumulate significant concentrations of cadmium in their edible components. A more detailed examination of the importance and influence of these options will be given below.

2.3.2: UPTAKE & ACCUMULATION OF CADMIUM BY FOOD CROPS

As discussed in Section 2.1, the main source of cadmium to a plant growing on a soil with elevated levels of cadmium is the soil itself; although the deposition of atmospheric cadmium will play a more important role at sites with background levels of cadmium in the soil and at sites where the concentration of cadmium in the atmosphere is high (Dollard and Davies 1989).

It has been observed that plants accumulate cadmium at different rates and that the final concentration of cadmium in plant tissues will differ between two different species growing concurrently on the same soil, as can be seen in Figure 2.8. The accumulation of cadmium by plants growing on sewage sludge-amended soils also show this effect (Dowdy and Larson 1975, Keefer *et al* 1986, Kim *et al* 1988). In addition to the difference in the overall accumulation of cadmium, different components of a given plant will contain differing concentrations of cadmium, as can be seen in Table 2.9. These characteristics are important in the context of the dietary exposure profile, as they will determine, to an extent, the degree of exposure from the food when levels of cadmium in the soil are

elevated by the application of sewage sludge. It is perhaps fortunate that one of the plant components that accumulates the lowest concentrations of cadmium are seeds or grains, which can be seen from Figure 2.4 form an important part of the exposure profile.

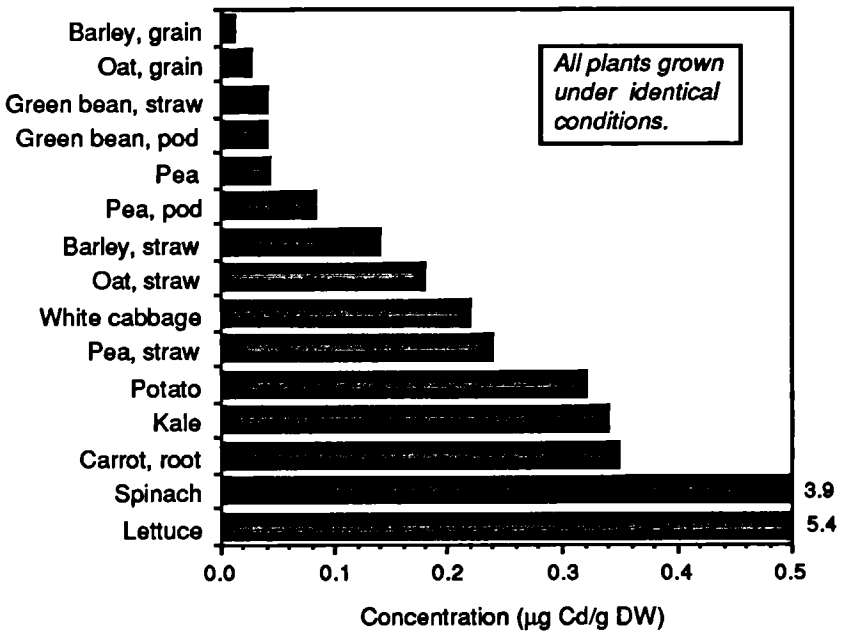


Figure 2.8: *Concentrations of cadmium in plants grown under identical soil conditions (Data from Hansen and Tjell 1983).*

Crop class	n	Minimum	Maximum	Mean
Fruits	190	0.0043	0.012	0.005
Vegetables				
Seed	394	0.0160	0.130	0.028
Root/bulb	878	0.0290	0.710	0.208
Fruit	322	0.0210	0.540	0.237
Leaf	297	0.0930	0.880	0.560
Field crops				
Grain	1302	0.0140	0.210	0.047

All units are in µg Cd g^{-1} DW.

Table 2.9: *Concentrations of cadmium in the edible components of food crops (Page et al 1987).*

A problem with the interpretation of grain or cereal concentrations is that it is unclear whether or not they are for whole grain samples or flours etc., as this will affect the significance of the concentration. Davis *et al* (1982) report that the concentration of cadmium in flour is only 57% of that found in the whole grain. Therefore, when assessing the dietary exposure to cadmium from grain products care should be taken to accurately describe the nature of the food being consumed, especially as whole grain products are becoming increasingly popular amongst consumers. Micco *et al* (1987) analysed a variety of grain products and found that the bran component did indeed contain a higher concentration of cadmium and of a number of other heavy metals than other components. Similar results are reported by Kloke *et al* (1984). Cadmium accumulation by rice grains also follows a similar trend, with unpolished rice having a higher cadmium concentration than polished rice (Asami 1984). The assumption that whole grain products will necessarily contain higher cadmium concentrations is however contradicted by some sources (CEC 1979). There also arises the question of the relative and absolute bioavailability of cadmium from this source when compared to that from flours, in this respect the dietary fibre content may play a key role. Analysis of archived samples has shown that there do not appear to be marked increases in the concentration of cadmium in cereal grains through time (Jones and Johnston 1989, Lorenz *et al* 1986)

The concentration of cadmium in edible tissues of plants is predominantly determined by the concentration of cadmium in the soil (Bingham and Page 1975, John 1973) and in this respect soils with elevated levels of cadmium, due to the application of sewage sludge, are no exception (Hyde et al 1979). In addition to the soil concentration of cadmium a wide variety of physico-chemical soil parameters have been

found to be related to uptake from soils; these include pH (Andersson and Nilsson 1974; Gerriste and van Driel 1984; Sanders *et al* 1986; Street *et al* 1978; Tyler and McBride 1982), cation exchange capacity (Korcak and Fanning 1985), organic matter content (Gerriste and van Driel 1984; Neal and Sposito 1986; Strickland *et al* 1979), soil temperature (Siriratpurija *et al* 1985), soil sorptive capacity (Jarvis and Jones 1980), concentrations of particular organic acids (Tyler and McBride 1982), antagonistic, synergistic or additive reactions between elements (Elliot *et al* 1986) and many more (Bingham et al 1983; Chammugathas and Bollag 1987[1], 1987[2]; Kuo 1986; McKenzie 1980). It is difficult to use any one of these parameters in isolation to predict the accumulation of cadmium from sludge-amended soils and retain a sufficient degree of accuracy over all plant and soil types. The two most influential soil parameters are pH and the soil concentration of cadmium, the former having a negative relationship and the latter having a positive relationship with plant accumulation (Jackson and Alloway 1990).

A factor unique to soils amended with sewage sludge is that of the effect of the residual period, ie the time between the application of sludge and the plant being harvested. Given the long residence time of cadmium in soils, any radical changes in the bioavailability of cadmium over the residual period would be very important to the overall assessment of dietary exposure. A number of field trials have been conducted from which the bioavailability of cadmium during this period of time may be examined.

With the application of sludge to a site, there is a sudden increase in plant accumulation of cadmium (Juste and Solda 1986); this may be attributed to a wide range of parameters, other than just the elevation of the soil cadmium concentration, many of which are site specific. An initial

lowering of the soil pH is the most probable. It is the nature of the changes in bioavailability after this initial rise that give rise to the most contradictory data. Observed changes fall into two groups, one in which the bioavailability remains constant and another in which it gradually declines.

Over the residual period, the offtake of cadmium by corn was seen to fall in a field trial reported by Bidwell and Dowdy (1987), as can be seen in Figure 2.9.

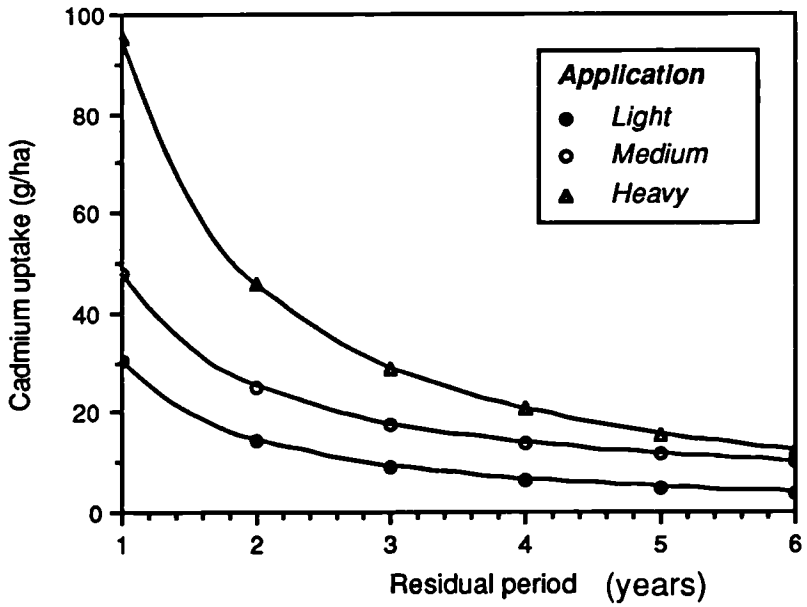


Figure 2.9: *Modelled values for the accumulation of cadmium by corn during the residual period (Data from Bidwell and Dowdy 1987).*

The rate of decrease is largely dependent upon the initial cadmium input. In a similar study by Hinesly *et al* (1979) the bioavailability of cadmium to *Zea mays* also fell, with the concentrations in grain falling from 0.44 to 0.07 $\mu g g^{-1}$DW over the four year residual period. Kelling *et al* (1977) observed, over a four year residual period, a fall in both the concentration of cadmium in plant tissues and also in the cadmium extractable from

soils using the DTPA soil test (an examination of such soil tests will follow).

Analysis of sludge-amended soils from the Woburn Market Garden Experiment has shown that over a 22 year residual period, the proportion of cadmium extractable from the soil by EDTA has remained constant (McGrath 1987). Data from field trials conducted by Dowdy *et al* (1978), which examined cadmium uptake by *Phaseolus vulgaris* showed no change over the four year residual period. A similar observation for a wider variety of crops has been made by Larsen (1984). An isotope-aided study by Lonsjo (1984) observed an initial rise in the accumulation of cadmium after the application of sludge, this fell to a near constant accumulation thereafter; as can be seen in Figure 2.10.

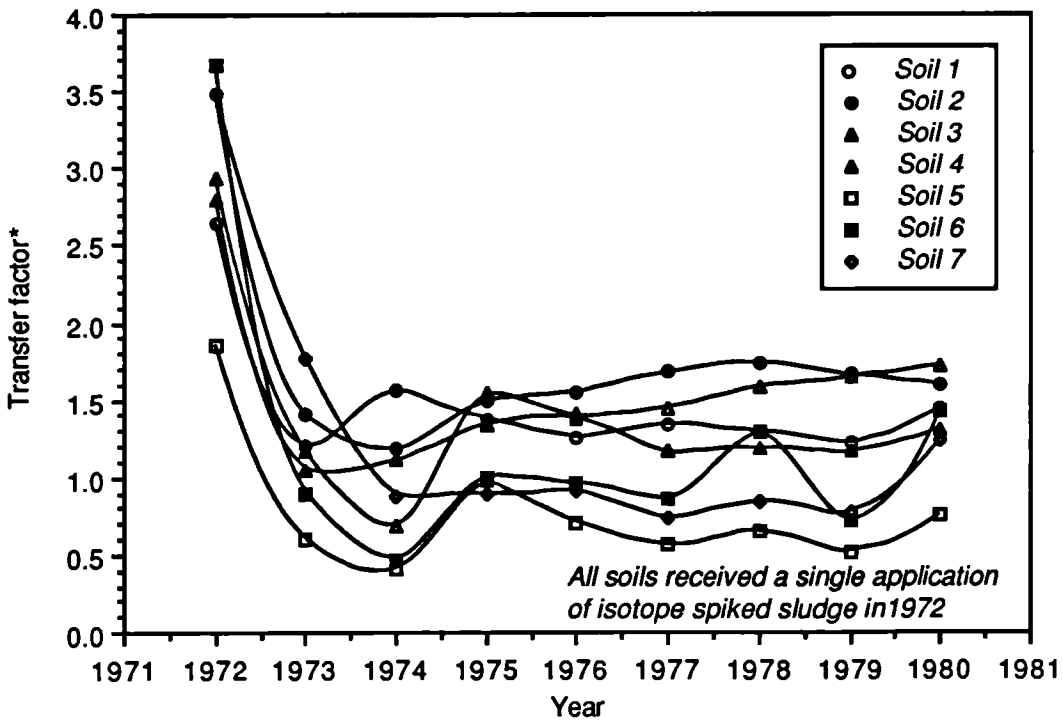


Figure 2.10: *Uptake of ^{109}Cd during the residual period (Data from Lonsjo 1984).*

*Transfer factor = (plant concentration DW)/(soil concentration DW)

As can be seen from the above figure, the transfer factor from the soils to the plants does not remain constant and instead fluctuates about an mean value. The data from this experiment is particularly valuable as it was collected from seven markedly different soils which received cadmium at levels such as those normally found in the use of sewage sludge in agriculture. The longest residual period examined in the papers referred to above is that reported by McGrath (22 years), a relatively short period of time when one considers the average residence time for cadmium in agricultural soils. In none of the papers studied was there an evidence of a sudden increase in the bioavailability of cadmium, as measured either directly by plant assay or by the use of soil extractions. This would seem to suggest that the notion of a "time bomb" effect, in which the bioavailability of cadmium is markedly increased after a residual period, is not a short term phenomenum. The increase in the bioavailability of cadmium in the longer term is a possibility given that bioavailability is influenced by a number of soil parameters any one of which could potentially change (Jackson and Alloway 1990).

2.3.3: PREDICTING THE ACCUMULATION OF CADMIUM BY PLANTS

If the potential dietary exposure of cadmium from soils amended with sewage sludge is to be predicted using models, a relationship or group of relationships have to be found that will enable the prediction of cadmium concentrations in crop plants to be made. Such models should be applicable over a range of soil cadmium concentrations and physico-chemical characteristics.

Modelling the uptake of heavy metals from the soil into the plant may be approached from two basic angles, (i) on the basis of current theories, a theoretical model may be developed and then tested against observed

data; or (ii) data is collected and then analysed in order to try to develop an empirical model. Most of the models presented below may be said to be equilibrium models, with the implicit assumption that temporal fluctuations in rates of reaction/transfer do not occur. Jackson and Smith (1987) give a generalised form for equilibrium models to describe the transfer of radionuclides or stable elements as :

$$C_a = f_a \sum_g J_g C_g$$

Where J_g = daily intake of food g; C_g = concentration of the element in food g; f_a = equilibrium diet-to-animal product transfer factor and C_a = concentration of the element in animal product. The relatively simple principles behind equilibrium models do not allow them the flexibility afforded by the more complex dynamic models. Dynamic models usually describe the rates of flux between the compartments of a system and take the form:

$$\frac{\partial}{\partial t} q_i(t) = \sum_{i=1}^{n} \mu_{ij} q_j(t) + I_i(t) \quad \text{for } i = 1 \text{ to } n$$

Where $q_i(t)$ = content of compartment i at time t; $I_i(t)$ = rate of intake into compartment i at time t; μ_{ij} with $i \neq j$ is the constant rate of transfer from compartment i to j and n = number of compartments.

Modelling the behaviour of metals in the soil-plant system is an important procedure if the potential hazard of growing food crops on contaminated soils is to be assessed. The first step is to quantify the accumulation of heavy metals in the plant and to identify the parameters which enable a prediction of this characteristic to be made. A wide variety of both soil and plant characteristics have been proposed, some of which can be found above. Models developed by Rappaport *et al* (1986) and Browne *et al*

(1984), based upon soil parameters, describe the plant uptake of zinc and cadmium respectively. The model proposed by Browne is shown below:

$$\log P = \alpha + \beta \log Cd_{DTPA}$$

where P = plant cadmium concentration, Cd_{DTPA} = DTPA extractable cadmium and α and β are linear regression coefficients. β was found to be principally a function of soil pH and cation exchange capacity and α was primarily a function of the plant species and may be related to the selectivity coefficient (Poelstra *et al* 1979). The zinc accumulation model of Rappaport *et al* (1986) is based upon DTPA extractable and organically bound zinc.

A cadmium and zinc accumulation model based upon a nutrient uptake model, soil characteristics and kinetic parameters has been developed by Mullins *et al* (1986) to describe plant uptake from sewage sludge-amended soils. Root growth constants, average root radius, water influx rate and soil solution metal concentrations were found to be the most influential parameters in the model. This model was based upon a more general model developed by Barber (1984) and later refined by Cushman. The model recognises three modes of ion-influx kinetics:

- passive ion movement independent of respiration energy;
- passive ion uptake along an electrochemical gradient, dependant on respiration energy; and
- active ion uptake against an electrochemical gradient requiring respiration energy.

In the comparison of cadmium and zinc accumulation by Mullins *et al* (1986), cadmium proved to be the most difficult to predict. A model

proposed by Hutton (1982) to describe the transfer of cadmium from soils to plants is shown below:

$$P_{SP} = \frac{(\beta S \Omega \phi)}{(\phi + \Omega \infty)}$$

where ß = water flow associated with plant production; S = plant selectivity coefficient (Poelstra *et al* 1979); Ω = soil adsorption coefficient (Jarvis and Jones 1980); ø = soil density; ∞ = soil moisture content and P_{SP} = soil - plant transfer coefficient.

A conceptual model to determine cadmium uptake from sewage sludge amended soils has been developed by Christensen and Tjell (1983). It divides the total plant cadmium concentration into three fractions, based upon their source, (i) cadmium from the topsoil, (ii) cadmium from the subsoil and (iii) cadmium from the atmosphere. Plant uptake from the topsoil is described by the following expression:

$$b = P.\, T_t\, .\, C_t = P.\, T_t.(S_t / K_{d,t})$$

Where b = root uptake from the topsoil, P = plant factor (constant for a specific plant), T = transpired amount of water, C = solute Cd concentration, S = soil Cd concentration, t = index for topsoil and K_d = Cd distribution coefficient. Plant uptake from the subsoil is described by:

$$c = P.\, T_s.\, (S_s / K_{d,s})$$

Where c = root uptake of Cd from the subsoil and s = index for subsoil. Foliar uptake of atmospheric cadmium can be considered to be a constant for a given plant species in a given area. The solute cadmium concentration, S, is said to be governed by two main processes, adsorption onto the solid phase and precipitation. The model is demonstrated to describe the behaviour of cadmium in sewage sludge

amended soil qualitatively however the tested data base was restricted in size.

The use of models to predict the accumulation of cadmium by plants grown on soils amended with sewage sludge may provide valuable data and insight; however diagnostic soil testing procedures enable approximations of cadmium bioavailability to be made. Such tests require only the measurement of one parameter to and allow comparisons to be made across a wide range of soils. The majority of soil tests for cadmium bioavailability require the use of an extracting solution; such solutions can be divided into three groups, dilute acids, chelating agents and neutral salts. Many of the soil tests using these extractants were designed as a diagnostic for nutrient deficiency problems, eg the DTPA soil test. Care must be taken when applying the tests to trace element sufficiency studies (Jackson and Alloway 1990, O'Connor 1988). The action of soil extractants is an area of some theoretical confusion as the precise nature of the bioavailability status of trace elements in relation to soil chemistry, or more specifically in relation to speciation in the soil solution, is unresolved.

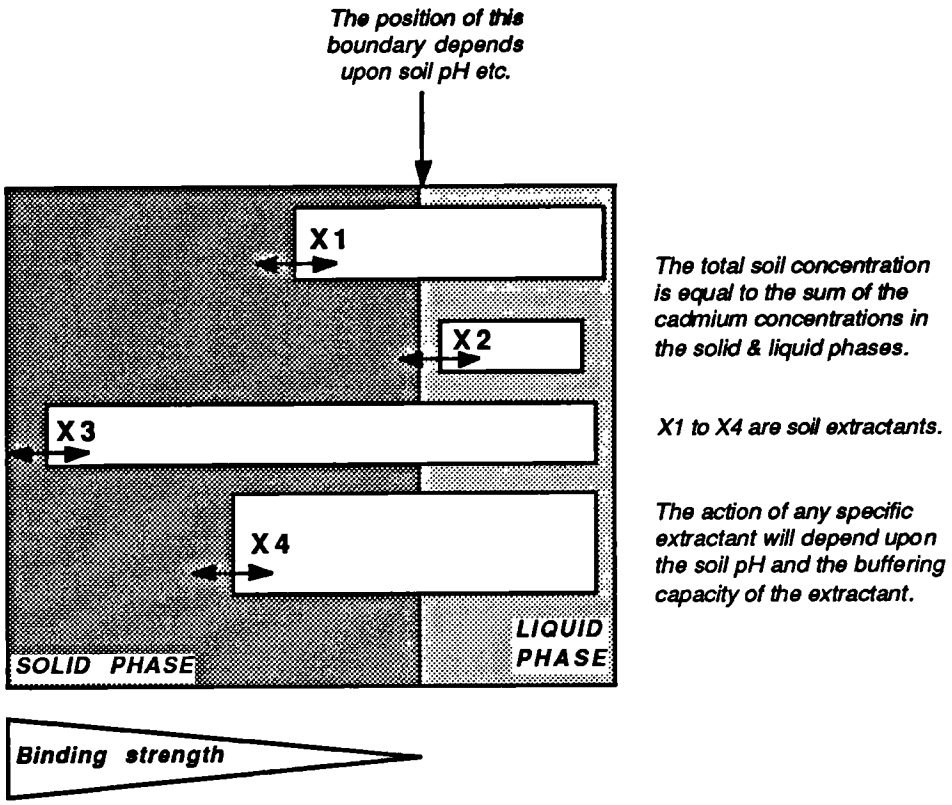


Figure 2.11: *The proposed action of four types of soil extractant.*

The figure above aims to show the relationship between the action of four types of soil extractant and the total pool of cadmium. X1 can be considered to be a neutral salt solution, X2 deionised water, X3 a dilute mineral acid and X4 a chelating agent. A great deal of research effort has been put into finding the ideal soil extractant for predicting the bioavailability of cadmium from sludge-amended soils. The results indicate that the use of the neutral salts are the best means available (Alloway *et al* 1984, Alloway and Morgan 1986, Hani and Gupta 1983, 1985, Jackson and Alloway 1990, Morgan and Alloway 1984, Sanders *et al* 1986). The work of Hani and Gupta (1983, 1985) recommends that 0.1 M $NaNO_3$ is used, this test has the disadvantage of extracting less cadmium than the more commonly used 0.05M $CaCl_2$ test, but has the advantage that it poses less vapour phase interference problems during analysis by electrothermal atomic absorption spectrometry. Despite the

analytical problems with the 0.05M $CaCl_2$ test it, has been quite widely used and found to produce satisfactory results (Morgan and Alloway 1984, Sanders *et al* 1986, Sauerbeck and Styperek 1984).

2.4: ASSESSING EXPOSURE TO CADMIUM ARISING FROM THE APPLICATION OF SLUDGE TO ARABLE SOILS

The aim of this section is to critically examine the methods of assessing the dietary exposure to cadmium arising from the application of sewage sludges to agricultural soils. It is stressed that adverse effects on other components of the environment are possible but their examination is beyond the scope of this paper.

2.4.1: CURRENT GUIDELINES & LEGISLATION

The application of sewage sludges to land is a viable means of both waste disposal and of providing macronutrients to crops; however the recognition of the potentially harmful effects to the environment, caused by such practices, require that legislation and guidelines are issued. Current guidelines are based upon one or more of the following assumptions:

- that heavy metal concentrations in the sludge may not exceed defined limits, when the sludge is to be applied to the land;
- that heavy metal inputs to agricultural land may not exceed defined limits;
- that heavy metals are less problematic when the load is applied in several increments, rather than in fewer increments; and/or
- that heavy metal concentrations in the soil may not exceed predefined limits or "Trigger concentrations".

(Webber *et al* 1983).

This paper will only consider the guidelines for cadmium, it is however recognised that such guidelines exist for a variety of potential contaminants beyond the scope of this paper. Table 2.10 summarises the guidelines related to maximum permissible cadmium loading to agricultural soils.

COUNTRY	ANNUAL(kg Cd ha^{-1})	TOTAL (kg Cd ha^{-1})
Canada	0.09	4.0
Denmark	0.01	0.2
Finland	0.02	0.1
West Germany	0.033	8.4
The Netherlands	0.02	0.2
Norway	0.02	0.2
Sweden	0.015	-
Switzerland	0.075	-
UK	0.17	5.0
USA	-	5 to 20
CEC draft directive*	0.1 (0.15)	-

Table 2.10: *Maximum permissible cadmium loading to agricultural soils (Porteous et al 1983).* * accepted 12/6/86

The majority of the limits presented above are independent of soil characteristics and as such may tend to under or over-estimate the safe limits. As will be examined in more detail in Section 2.4.2.3, the USA limits are based upon a diet scenario; the legislation does include the soil parameters pH and cation exchange capacity. pH is a very important factor in the assessment of cadmium bioavailability from the soil to the human food chain; a limit of 5 kg Cd ha^{-1} is given for soils with a pH less than 6.5 (Webber and Monks 1983).

The CEC directive for the disposal of sewage sludges also included recognition of the importance of soil parameters, other than the concentration of cadmium. Article 1 of the Directive states that:

> "The purpose of this Directive is to regulate the use of sewage sludge in such a way as to prevent harmful effect on soil, vegetation, animals and man, thereby encouraging the correct use of such sewage sludge."

(CEC 1986).

This paper only considers the Directive with respect to the risk of harm to people. A trigger concentrations of 3 μg Cd g^{-1} DW is set for soils with a pH of 6 to 7, this value combined with those for annual and cummulative cadmium loadings is considered to provide adequate protection to the human food chain.

2.4.2: SETTING STANDARDS

2.4.2.1: *Exposure commitment assessment*

The use of this technique of assessing the risks to people from pollutants, is based upon the commitment method as applied by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) to the study of radiation exposure (Bennett 1981). The basic method has been developed to cover other pollutants, of which cadmium is one. The assumptions of the method include that pollutant transfers follow first order kinetics and that risk is directly proportional to concentration.

The assessment procedure begins with the identification of sources and pathways of the pollutant, in this case cadmium. The flow digram below is taken from MARC Report number 26 (Hutton 1982) and only includes

background levels, to which the majority of the population are exposed. The increased exposure from the application of sewage sludge to arable soils is considered to be so negligable as to be not worthy of inclusion, as it only represents a small input of cadmium at the nation state level; the situation at the local level is considerably different, with sludges accounting for up to 90% of the total cadmium input to a given soil (Bennett 1981).

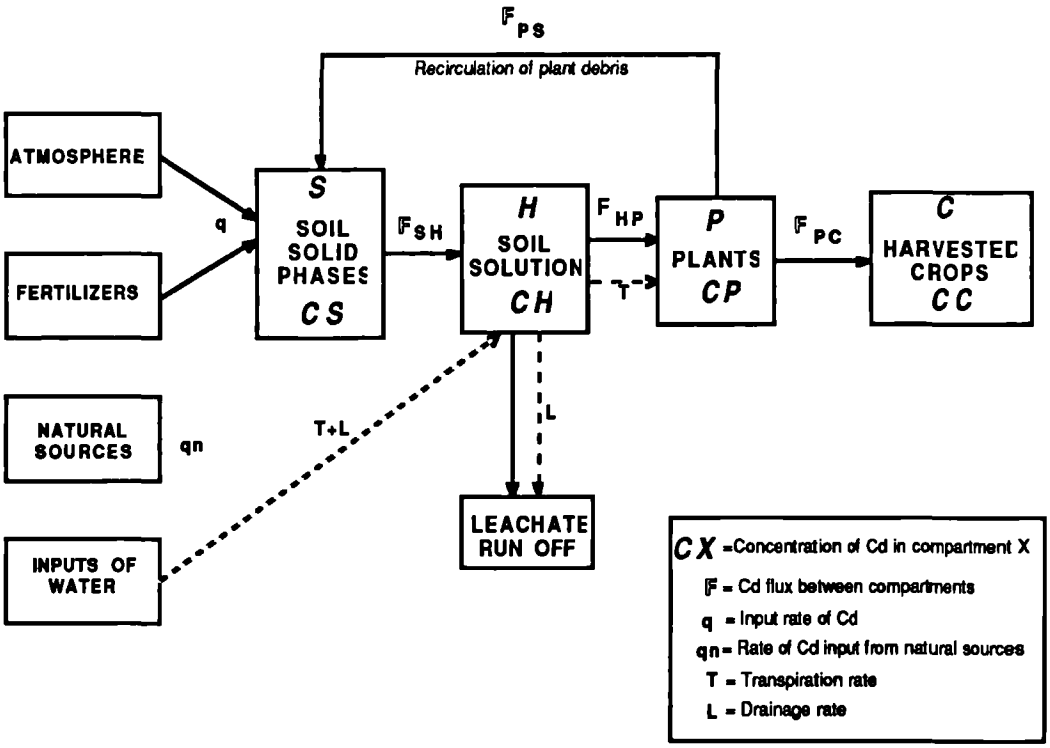


Figure 2.12: *Cadmium transfers in agroecosystems (Hutton 1982)*

Although the exposure commitment assessment technique is a useful means by which to assess the behaviour and possible risks associated with cadmium in the large-scale, it is not well suited to the examination of cadmium exposure from sewage sludge applications to arable soils. The nodal distribution of cadmium exposures from this source are suited to a less general or large-scale scenario. A smaller unit of study, perhaps analogous to a catchment or water authority district, may be more appropriate.

In addition to the problems of scale, there exist other problems which are principally associated with the calculation of transfer factors between compartments. Bennett (1981) identifies four factors which would complicate their calculation:

- non-linearity;
- synergistic, antagonistic or additive reactions between the contaminant and other variables;
- speciation; and
- compartment heterogeneity.

As examined above, cadmium can be shown to exhibit all of these characteristics to some degree; over a wide range of soils the accumulation of cadmium by plants is definitely non-linear, it has been shown to react antagonistically with zinc at sites of adsorption on soil solid-phases and speciation would appear to complicate the fate of cadmium in the body of an organism, for example. It would appear that cadmium speciation affects the risk associated with a given exposure to the human consumer; this, if proven, would contradict the basic assumption that risk is directly proportional to concentration. The relative risk of exposure to 20 μg cadmium via the pulmonary system is not necessarilly equivalent to an exposure of 100 μg cadmium via the gastrointestinal tract (assuming absorption efficiencies of 25 and 5% respectively).

To conclude, the value of exposure commitment assessment for the examination of the risks to human consumers arising from the application of sewage sludges to arable soils, is limited. The main problems arise from the suitability of the technique to large physical areas in which the contaminant is homogenously distributed; in this application the

contaminant does not behave in the stated manner, given the medium by which it enters the system.

2.4.2.2: *Trigger concentrations*

From the legislative point of view it is important to be able to define the concentration of cadmium in the soil at which there is a danger of adverse health effects to the human population. Such concentrations are said to be "trigger concentrations". The evaluation of a trigger concentration for cadmium in soils can be approached by the method outlined by Morgan and Simms (1988). The first stage is to identify and measure an accepted indicator of cadmium exposure and to determine the lowest level at which damage can be assessed using this indicator. The proportion of the population at risk may then be determined using what should be a reliable indicator; this will also enable the identification of groups of the population who are most at risk. The pathway of cadmium from the soil to people is then determined and the relative importance of sludge-borne cadmium assessed. The same basic rationale may be used for a wide variety of contaminants in a variety of different media, eg Simms 1986. When determining the trigger concentration of cadmium in the soil, parameters other than the total soil concentration should be considered; if they are not, then it would be prudent to set limits with a higher margin of safety.

In assessing the trigger concentration of cadmium in soils contaminated with sewage sludges, it is necessary to recognise the importance of the diet as the primary route of exposure. The concentration of cadmium in the renal cortex is the most widely accepted indicator of cadmium exposure, the PCC-50 is ~200 μg Cd g^{-1} FW*. The exposure of cadmium from the diet resulting in the stated concentration in the renal cortex, is

* (Kjellstrom and Nordberg 1978)

the principal factor in the determination of a trigger concentration for cadmium. It is clear that any increase in the concentration of cadmium in the soil will elevate the concentration of cadmium in the renal cortex of a proportion of the population. In setting the trigger concentration one has to assess the point at which the increasing number of people who are adversely affected becomes "significant". It is in the determination of this value that a subjective decision has to be made. If the mean concentration of cadmium in the soil giving a mean concentration of cadmium in the renal cortex of 200 μg Cd g^{-1} FW is used, then large sections of the population will be at risk.

The identification of higher risk groups is very important and in the case of cadmium these are people who consume larger than average quantities of accumulator foods such as shellfish and those who smoke cigarettes. In the UK it is the smoking of cigarettes that constitutes the greatest additional risk, as excessive consumption of accumulator foods is rare. Tobacco is a ready accumulator of cadmium with concentrations of between 1 and 2 μg Cd g^{-1} , this constitutes an intake (dose) of 0.1 to 0.2 μg cadmium per cigarette, assuming that the absorptive efficiency of the pulmonary system is high (~50%) (Bennett 1981). By smoking twenty cigarettes each day, a resident of the UK may double the dose of cadmium received, with 20 cigarettes a day being equivalent to an exposure of 20 to 30 μg cadmium from dietary sources (Hutton 1982).

An example of the application of this trigger concentration method is the model by Davis *et al* (1983), based upon data from Department of the Environment (UK) field trials at Royston. It was concluded that an upper trigger concentration of 12.90 μg Cd g^{-1} was acceptable for people consuming a standard diet; for people who consumed a diet in which the grain component was consumed as whole grain products, rather than as

refined white flour, the upper trigger concentration was 6.24 μg Cd g^{-1} (Davis 1984). The model included the assumptions shown below:

- 1.27% of UK agricultural land receives sludge each year and that the average application increases the soil concentration by 0.05 μg Cd g^{-1} ;
- it is inconceivable that a person could consume all of their food from sludged soils;
- the model uses mean consumption data, no account is taken of excessive consumption of any particular food;
- that the WHO/FAO PTWI is not a toxic threshold but does include a safety margin; and
- that there is no post-harvest contamination of the crop.

The recommended upper trigger concentrations produced by the model are over four times higher than that recommended by the CEC in their 1986 Directive; this is probably because the soils at the site of the field trial had a very high pH and a very high calcium carbonate content. These two factors will markedly reduce the bioavailability of cadmium and lead to high trigger concentrations.

It would seem that the use of soil trigger concentrations is a viable means of restricting the quantities of cadmium entering the human food chain; however, the effectiveness of the method depends upon the assumptions made by those who are applying it. When setting trigger concentrations for cadmium it would be prudent to include some consideration of those factors influencing its bioavailability, the soil pH for example. A number of other factors should be considered but not beyond the point at which the application of the limits become impractical.

2.4.2.3: *USEPA diet scenarios*

In order to restrict the contamination of the human food chain with cadmium as a result of the application of sewage sludges to agricultural land, the United States Environmental Protection Agency (USEPA) produced a model to relate the application of sludges to land and dietary exposure. An estimated PTWI of 525 µg was calculated independently of that of the WHO/FAO; in order to conform with the more widely used limit, the recommended maximum daily exposure was lowered to 70 µg cadmium per day. The median dietary exposure in the USA in 1974, was 40 µg cadmium per day; the model was therefore aimed at predicting the application rate that would increase the exposure by 30 µg.

The model uses data describing the relationships between application rates to soil and plant cadmium concentrations, to establish a dose response function for the soil-plant system. In order to assess the concentration of cadmium in the impacted food classes necessary to increase the exposure to that which is the maximum permissable, a multiplication factor can be applied to existing concentrations; the factor is calculated using the equation below:

$$MF = \frac{(X_1 - Y)}{(X - Y)}$$

Where MF = the multiplication factor; X = µg cadmium per day in diet at the present time; X_1 = projected µg cadmium per day in the diet and Y = exposure from food classes not impacted by the application of cadmium to the soil (Buffered groups) (Ryan *et al* 1982). The contribution of the particular crop to the total exposure is then taken from the Compliance Programme of the Food and Drug Administration, multiplied by the factor given in the equation and used to assess the application rate necessary to elevate the overall dietary exposure from this source. Not all crops respond in the same way to increasing cadmium concentrations in the

soil and so the integrated response by all components of the diet has to be calculated. Figure 13 shows the change in exposure, of two differing types of diet, when the concentration of cadmium in the soil is elevated by the application of sludge. The model is also able to take soil pH into account, an acid soil is one with a pH of between 5.5 and 5.7 and a neutral soil is one with a pH of between 6.1 and 6.4. The reason that the lines do not intercept with the origin, is associated with the contribution to the total exposure from buffered food groups and possibly with accumulation of cadmium from sources other than the soil, ie the atmosphere.

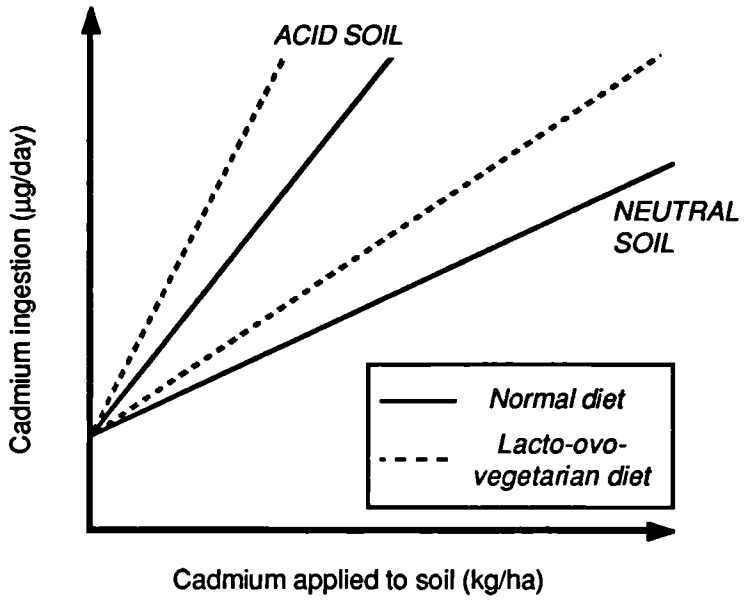


Figure 2.13: *Integrated response of dietary exposure to the application of cadmium to the soil (From Ryan et al 1982).*

Using the basic model a number of scenarios can be simulated in order to assess cadmium exposure, given a variety of different factors. Naylor and Loehr (1981) give three possible scenarios:

Scenario 1 - a person gets their entire diet from soils to which sewage sludge has been applied. The soil has a pH of 7 and all crops respond to the increase in the cadmium concentration as does lettuce;

Scenario 2 - the soil pH is greater than 7 and that the person consumes only grain products that were grown on sludge-amended soils; and

Scenario 3 - sludge is only applied to soils used for the production of animal feed crops.

In many ways the approach used here is very similar to that employed in the setting of trigger concentrations but it has been specifically adapted for the application of assessing dietary exposure from soils contaminated with cadmium from sewage sludges and is related to application rates, rather than to soil concentrations.

2.5: CONCLUSIONS

This chapter has aimed to appraise the mechanisms by which the application of sewage sludge to agricultural soils may increase human exposure to cadmium. In order to quantify the risk to the human food chain, two key rate determining processes should be examined:

- soil-plant transfer of cadmium
- the bioavailability of cadmium in foods to the human consumer.

The modelling of soil-plant bioavailability remains an area of some concern, with empirical models dominating the literature. The use of soil extractants such as EDTA, DTPA, $CaCl_2$ and NH_4NO_3 can give an indication of the relative availability of cadmium between soils; however the applicability of a given reagent to a large population of soils is questionable (Alloway *et al* 1990). Further elucidation of the processes determining the transfer of cadmium into plants may be provided by

developments in the area of cadmium speciation in soils (Landner 1987). However, the slow development of appropriate analytical methods has hindered progress in this area.

Cadmium speciation is similarly important in the determination of the bioavailability of cadmium from foods to the human consumer. In those components of the diet most affected by changes in the soil cadmium concentration, ie vegetables and cereals, there are few data for the speciation of cadmium. It is probable that cadmium is predominantly bound to low molecular weight proteins called phytochelatins. As yet, there are few data specifically describing the toxicology of cadmium bound to this group of proteins, this remains a key information gap.

An assessment of the relationship between soil cadmium concentration and human dietary exposure requires a thorough understanding of the key processes involved. As demonstrated in this review, there remain some areas of uncertainty in the quantification of these processes; therefore, an assessment of the risks posed to human health by the elevation of soil cadmium concentrations should be treated with a degree of caution.

CHAPTER 3: TRACE CADMIUM DETERMINATION BY ATOMIC ABSORPTION SPECTROMETRY

3.1: TRACE ELEMENT ANALYSIS OF ENVIRONMENTAL MEDIA

This chapter aims to review the literature on trace element analysis and then, in the light of the development of these techniques, to describe the development of two methods of trace cadmium determination by electrothermal atomisation atomic absorption spectrometry.

Quantitative analysis for trace elements in environmental media is an intrinsic part of many studies of environmental systems. In systems under the influence of human activity the levels of trace elements may become significantly elevated, to the point where the system as a whole loses its dynamic equilibrium. The protection of human health lies behind much of the work on the non-essential or toxic trace elements (Minderhoud 1983). Much emphasis has been placed upon trying to determine the threshold concentration of a specific element in a particular medium, beyond which human health can be said to be at risk.

A wide range of techniques is available for the quantitative determination of trace element concentrations in samples from the environment (Brown *et al* 1987, Cresser *et al* 1989, Ebdon *et al* 1987, Hoffmann and Lieser 1987, Sansoni 1987, Valkovic and Moschini 1989). The particular technique chosen is in part determined by the nature of the sample and the trace element(s) to be quantified. When attempting to quantify the concentration of trace elements in samples considered to represent background levels, the detection limits or sensitivities of the available

techniques are important factors to be considered. Table 3.1 lists the sensitivities and detection limits for the analysis of a range of potentially toxic trace elements by a range of analytical techniques.

Element	ASV[1] Detection Limits (ppb)	FAAS[2] Detection Limits (ppb)	ETA-AAS[3] Detection Limits (ppb)	ICP-ES[4] Detection Limits (ppb)	NAA[5] Sensitivity (ppb)
Arsenic	-	100	0.2	20	50
Cadmium	0.005	1	0.003	1	5
Mercury	1	2200	0.5	50	3
Lead	0.01	10	0.05	20	500
Zinc	0.4	0.6	0.2	1	100

1: Anodic stripping voltametry
2: Flame atomic absorption spectrometry
3: Electrothermal atomisation atomic absorption spectrometry
4: Inductively coupled plasma emission spectrometry (ultrasonic nebulizer)
5: Neutron activation analysis

Table 3.1: *Sensitivities and detection limits of analytical techniques (van Loon 1985).*

The above table shows that a wide range of sensitivities exists between both elements and techniques. The contrasting sensitivities of FAAS and ETA-AAS will be considered in Section 3.1.2. The issue of the detection limit is not of the utmost importance for those elements present at relatively high concentrations in the sample or for elements with low limits of detection, such as cadmium and zinc. Hoffman and Lieser (1987) cite the following factors as being important in the choice of technique:

- the element to be determined
- the concentration of the element in the sample
- the composition of the sample with respect to interferences and inter-elemental effects
- the amount of sample available for repetitive analyses

- the state of the sample or the state into which it can be transformed without changing the elemental composition
- the number of elements to be determined

The quality and quantity of the data required is a further consideration in the choice of analytical technique and it is here that there is a dichotomy between single and multi-element methods. A discussion of the multi-element approach to the analysis of environmental media has been presented by Sansoni (1987), who cites the main advantage of multi-element analysis as being that one "gets much more information about the multi-element composition of the sample with only reasonably increased costs". The major disadvantage is "the compromise to be made in optimal experimental conditions for each single element, which reduces precision, accuracy and detection limits of numerous elements.". The main advantage of single element techniques lies in the use of instrumental parameters optimal for the determination of the particular element of concern.

Sections 3.1.1 and 3.1.2 will examine specific instrumental methods of analysis, with the emphasis being placed upon cadmium determination. Section 3.1.2 will consider atomic absorption spectrometry and Section 3.1.1 will briefly examine other available techniques, the emphasis in Section 3.1.1 will be on the expanding area of multi-element analysis.

3.1.1: MULTI-ELEMENT ANALYSIS

The use of multi-element techniques for the determination of trace elements in environmental media is becoming an increasingly important component of environmental monitoring and research. Sansoni (1987) has examined the scope of this group of techniques, Table 3.2 lists the principal methods available.

Mass spectrometry (MS)	*Simultaneous*
Spark source (SSMS)	
Secondary ion source (SIMS)	
Plasma source (ICP-MS)	
Glow discharge source (GD-MS)	
Laser induced (LMS)	
Neutron activation analysis (NAA)	*Simultaneous*
Gamma spectrometry after neutron activation with	
reactor-	
epithermal-	
thermal-	
14MeV - neutrons	
charged particles	
X-ray fluorescence analysis (XFA)	
energy dispersive	*Simultaneous*
wave-length dispersive	*Sequential*
electron induced	
proton induced (PIXE)	
synchroton radiation induced (SIXE)	
total reflecting	
Atomic emission spectrometry (AES)	*Simultaneous*
arc excitation	
sparc	
ICP-plasma	
glow discharge	
Atomic fluorescence spectrometry (AFS)	*Sequential*
ICP excitation (ICP-AFS)	
Forward scattering spectrometry	*Simultaneous*
Atomic absorption with white light sources	

Table 3.2: *Techniques of multi-elemental analysis (Sansoni 1987).*

The most sensitive techniques across a range of elements are spark source mass spectrometry (Ure and Bacon 1987), neutron activation analysis and inductively coupled plasma mass spectrometry (ICP-MS).

ICP-MS for the determination of cadmium will be examined in more detail in Chapter 5.

3.1.2: ATOMIC ABSORPTION SPECTROMETRY (AAS)

The basic configuration of an atomic absorption spectrometer is shown in Figure 3.1.

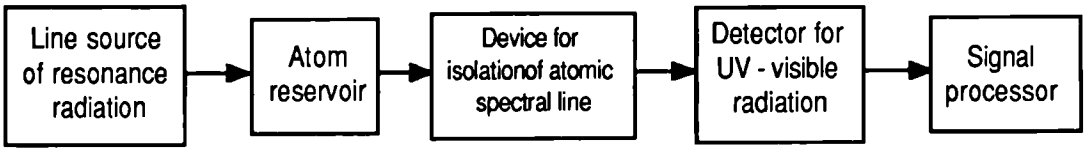


Figure 3.1: *The basic configuration of an atomic absorption spectrometer (van Loon 1985).*

Atoms absorb radiation at discrete wavelengths, the radiation being produced from a vapour of the particular analyte metal. The concentration of the analyte element introduced into the atomiser will therefore absorb a given proportion of the incident radiation from the radiation source. The absorbance is defined by the following equation:

$$A = \log \frac{I_0}{I}$$

where A = absorbance, I_0 = the intensity of the incident light beam and I = the intensity of the transmitted light. The role of the atomiser is to produce free atoms from the sample.

In practical terms the light source is usually a hollow-cathode or electrodeless discharge lamp. The atomiser or atom reservoir is either a flame or graphite furnace; more details of these two configurations follow in Sections 3.1.2.1 and 3.1.2.2 respectively. A monochromator is used to isolate the spectral line of the analyte and a photomultiplier is used for detection.

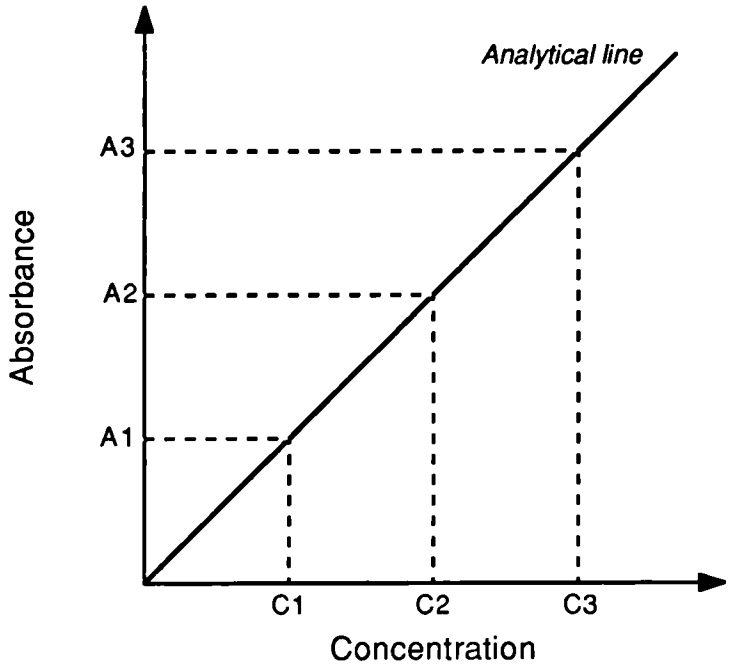


Figure 3.2: *Typical calibration curve.*

Calibration of a spectrometer is achieved by the analysis of samples with known concentrations of the analyte. Plotting the concentration of the analyte against the observed attenuation or absorbance of the incident radiation enables a calibration or working curve to be derived, see Figure 3.2. Therefore, for a sample of unknown analyte concentration, one is able to observe the absorbance and, using the calibration curve, calculate the concentration of the analyte.

3.1.2.1: *Flame atomisation atomic absorption spectrometry (FAAS)*

For flame AAS the atom reservoir/source, see Figure 3.1, is usually an air-acetylene flame; for those elements that form refractory compounds the use of a nitrous oxide-acetylene flame may be necessary (Ure 1990). The flame is required to produce ground state atoms only (Ebdon 1982). As flame temperatures show some spatial differentiation, the alignment of

the incident beam of radiation with the hottest region of the flame is one of the key procedures when setting up an instrument.

Sample delivery to the flame is via a capillary from which the sample is drawn by the venturi effect and nebulised. The efficiency of sample nebulisation is an important factor in determining the sensitivity of a given system. Secondary fragmentation of the sample may be attained by positioning an impact bead in the path of the nebulised sample. The sample subsequently enters the flame where it is atomised and the resulting attenuation of the incident radiation recorded by the system shown in Figure 3.1.

Interferences to FAAS fall into five basic categories (van Loon 1985):

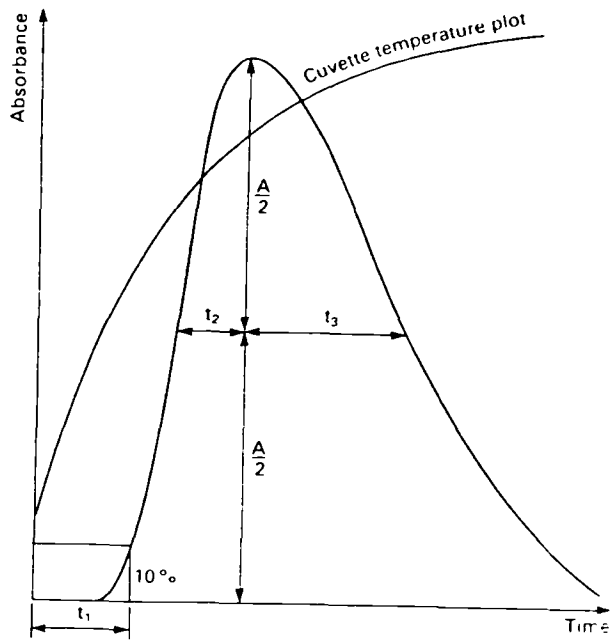
- *atomic spectral interferences* - caused by spectral line overlap and line interference. Given the narrow line widths used in FAAS these are likely to be rare.
- *physical interferences* - these may occur if there are differences in sample and standard viscosities.
- *ionisation interferences* - if the ionisation efficiency differs between the samples and standards it may be necessary to use an ionisation suppressor or buffer (Ebdon 1982).
- *chemical interferences* - possibly the major source of interference in FAAS. The main type of chemical interference is the formation of less volatile compounds of the analyte that fail to dissociate in the flame.
- *nonspecific (background) interferences* - caused by the apparent absorption and scatter of incident radiation by molecular species other than those of the analyte.

3.1.2.2: *Electrothermal atomisation atomic absorption spectrometry (ETA-AAS)*

Electrothermal atomisation can be defined as being the atomisation of a sample by thermal means after resistive heating (Ebdon 1982).

3.1.2.2.1: The theory of ETA-AAS

The main differences between FAAS and ETA-AAS lay in the means of atomising the sample (as defined above) and in the transient nature of the signal produced. The mechanism(s) by which an electrothermal atomiser produces free atoms at the ground state is the subject of much research effort (Welz *et al* 1988, Wu *et al* 1987). A number of processes have been suggested including the conversion of metal salts to the oxide, evaporation of the metal oxide or halide, thermal dissociation of the metal salt or oxide and the reduction of the metal oxide (Ebdon 1982). The time-absorbance curve shown in Figure 3.3 is typical of the signals from ETA-AAS; however, the basically Gaussian nature of the signal may be affected by a variety of factors, one of the most significant of which is the redeposition of the analyte onto the tube wall (Brumbaugh and Koirtyohann 1988, Welz *et al* 1988). The decay of the signal was formerly considered to be due to one process, ie the diffusion of atoms from the graphite tube and therefore from the path of the incident radiation. A further assumption, that atoms are freely and uniformly distributed within the tube, has been contrasted with observed spatial and temporal atom distributions (Holcombe *et al* 1982).



t_1 – appearance time measured at 10% of total AA signal
t_2 – risetime at half height
t_3 – decay time at half height
$t_2 + t_3$ – peak width at half height

Figure 3.3: *A typical ETA-AAS signal (Brown 1989).*

Analyses of such signals highlight the differences in the behaviour of metals when atomised. Peak shape characteristics are influenced by a number of characteristics:

- temperature rise time of the cuvette
- analyte volatility
- atomisation temperature
- cuvette material
- reactivity of the analyte with the graphite
- reactivity of the analyte with the inert gas.

The greater the volatility of an element the lower the appearance time (t_1), cadmium is a very volatile metal and has a typical t_1 value of 0.37 seconds; whereas vanadium is a refractory element and has a t_1 of 0.67 seconds (Brown 1989).

Calibration of the spectrometer for ETA-AAS can be achieved by the use of either peak areas or peak heights. L'vov has derived theoretical relationships between the mass of the analyte in the furnace and both peak heights and areas (L'vov 1970). Peak height is a function of the temperature (°K), the pressure of the fill gas, the mass of the analyte and the cross-sectional area of the cuvette. Peak area is a function of the temperature (°K), the cuvette length and cross-sectional area and the mass of the analyte. In deriving these theoretical relationships L'vov made three major assumptions (Sotera *et al* 1981):

- a diffusive transport mechanism operated through the cell and that this was uniform.
- atomisation was 100% efficient.
- no account was taken of interference effects.

The following section will examine interference effects and pay particular attention to the interferences acting upon cadmium determinations.

3.1.2.2.2: Interference effects

Interference effects are frequently cited as being a major problem with ETA-AAS (Ebdon 1982, van Loon 1985). This contradicts the forecasts made at the inception of the ETA-AAS technique which, at the time, was seen as being a way towards "absolute analysis". The interference problem first began to manifest itself when ETA-AAS shifted from the experimental stage into the production of commercial ETA systems. L'vov (1978) in his seminal paper "Electrothermal atomisation - the way toward absolute methods of atomic absorption analysis" initiated a fundamental redevelopment of ETA-AAS by identifying the predominant cause of the interference as being the non-isothermality of the furnace. This non-isothermality was predicted to operate in both the spatial and temporal

dimensions, L'vov's initial predictions have subsequently been observed to hold true. The reasons for non-isothermality lie in the design of the atomiser and will be discussed in the following section.

Ebdon (1982) identifies the four main types of interference in ETA-AAS as being physical interference, background absorption, memory effects and chemical interference. Physical interferences are associated with the position in which the sample was deposited in the tube; such problems, with the development of automated sample deposition (Stoeppler *et al* 1976), are probably rare. Background absorption by the sample matrix is particular problem in ETA-AAS but this may be solved by the routine use of background correction correction systems. Memory effects are a problem when determining analytes with very low volatilities, vanadium and boron for example. The use of a high temperature 'clean cycle' after signal measurement is usually sufficient to remove any residual analyte from the tube. Chemical interferences include the loss of the analyte as a volatile salt, interference by cations or anions, carbide formation and condensation. Condensation or vapour phase effects are the dominant source of interference in ETA-AAS of volatile elements. Figure 3.4 allows comparison of elements on the basis of their observed appearance temperatures and their calculated heat of vaporization.

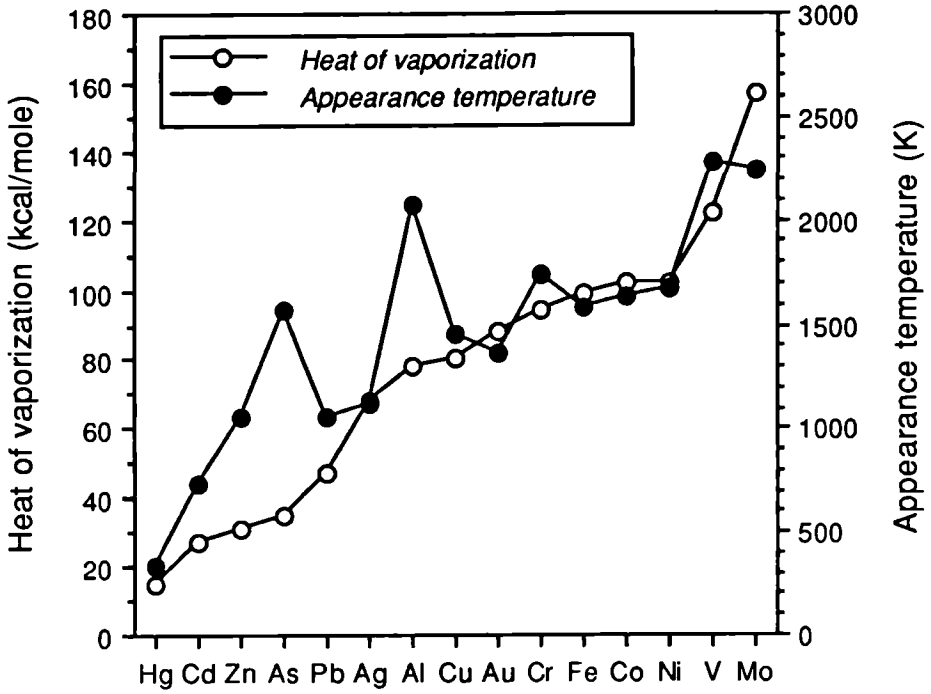


Figure 3.4: *Appearance temperatures and vaporization heats for a number of elements (Data from L'vov 1978).*

Cadmium has been found to be particularly prone to chemical interferences (Slavin *et al* 1983) and will be used as the model element for the following discussion. Hulanicki *et al* (1985) investigated the effect of the inorganic sample matrix on the determination of cadmium and found the interferences observed to be mainly due to the presence of anions. A common source of vapour phase interferences is the formation of gaseous monohalides of cadmium, especially chlorides (L'vov 1978). The incomplete dissociation of cadmium upon atomisation or condensation in the vapour phase leads to differing degrees of signal suppression. In order to overcome this interference, higher atomisation temperatures could be set; however the temporal and spatial non-isothermality within the atomiser may lead to analyte condensation in the

cooler regions. Methods for the control of interferences will be examined in Sections 3.1.2.2.3 and 3.1.2.2.4.

L'vov (1978) used halide dissociation energies as a means of contrasting the susceptibility of thirty eight elements to vapour phase interferences, Table 3.3 gives the dissociation energies of three elements of contrasting volatilities.

	Dissociation energy (kcal mole^{-1})			
Element	MF	MCl	MBr	MI
Cadmium	-	49±1	37±10	32±5
Copper	102±3	90±3	78	69±15
Vanadium	140±15	113±15	104±10	-

Table 3.3: *Halide dissociation energies (Data from L'vov 1978).*

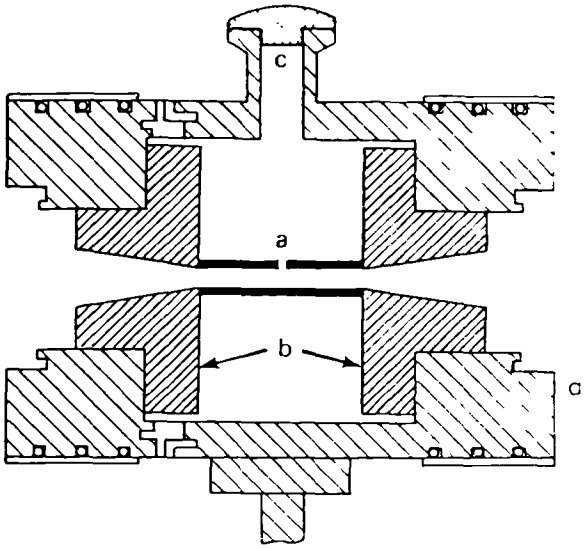
The lower dissociation energy of cadmium chloride implies that it may be susceptible to condensation in the cooler regions of the atomiser. The presence of halides in environmental samples is therefore a possible source of analytical error due to signal suppression. In concluding his 1978 paper L'vov cited three main obstacles to the analytical accuracy of ETA-AAS using the then commercially available atomisers:

- the use of the amplitude (peak height) method of recording absorbance pulses.
- time and space non-isothermalness of the absorbing zone of atomisers.
- the formation of gaseous monohalides of the elements to be determined.

3.1.2.2.3: Atomiser designs

Time and spatial isothermality is the key to the control of chemical interferences in ETA-AAS, especially when making cadmium determinations in 'real' samples. Atomiser design is fundamental to the thermal characteristics during the atomisation and measurement phase of the analysis. The potential, as yet unfulfilled, that ETA-AAS could become an absolute method of analysis (L'vov 1988, Slavin and Carnrick 1984), is largely determined by the lack of an isothermal furnace design on the open market.

Work in the late 1950's and early 1960's by L'vov used a probe cuvette design in which the sample was deposited onto an auxiliary electrode which was then inserted into a preheated cuvette where the sample is atomized. The design of this system appeared to give relatively interference-free determinations when compared with FAAS. The atomiser designs commercially available tend to be based upon the Massmann cuvette, the basic configuration of which is shown in Figure 3.5.



a. graphite tube
b. graphite cones
c. sample inlet port
d. water cooled steel jacket

Figure 3.5: *A Massmann atomiser (From Massmann 1968).*

The graphite tube (a) is heated by passing an electrical current through it along the line of the optical axis, this tends to produce some spatial variation in temperature. In particular, the ends of the tube may often be far cooler and lead to condensation of halides of the analyte (Slavin 1987). A further problem associated with the Massmann furnace, is the temporal temperature gradient that exists when heating the tube from the ashing or char stage to the atomisation temperature. Upon identifying this characteristic, L'vov (1978) proposed either shifting the analyte peak into the range of temperature equilibrium or pulse heating the furnace.

The use of a graphite platform to delay the atomisation of the sample is now widely employed (Baxter *et al* 1989, Blust *et al* 1985, Halls *et al* 1987, Kaiser *et al* 1981; Slavin *et al* 1983) The method relies upon sample heating by convection rather than conduction. Samples were

previously deposited, dried, ashed and than atomised from the wall of the graphite tube; when using a graphite platform the same processes occur but atomisation of the sample is delayed. The delay gives the tube wall and purge gas temperatures more time to equilibrate although there is a delay until isothermal conditions exist (Koirtyohann and Giddings 1984).

Probe atomisation is another technique by which the sample could be atomised in a more isothermal environment (Littlejohn *et al* 1983). In probe atomisation ETA-AAS, the sample is deposited on a graphite probe inside the furnace, it is subsequently dried and then ashed before the probe is withdrawn from the tube. The tube, with the probe outside it, is then heated to the atomisation temperature and the probe reinserted; the sample atomises and the signal is measured (Littlejohn 1989). Figure 3.6 shows the basic probe atomisation procedure. A variety of probe designs and materials have been developed (Ottaway *et al* 1986); these will be examined in more detail in Section 3.2.2.

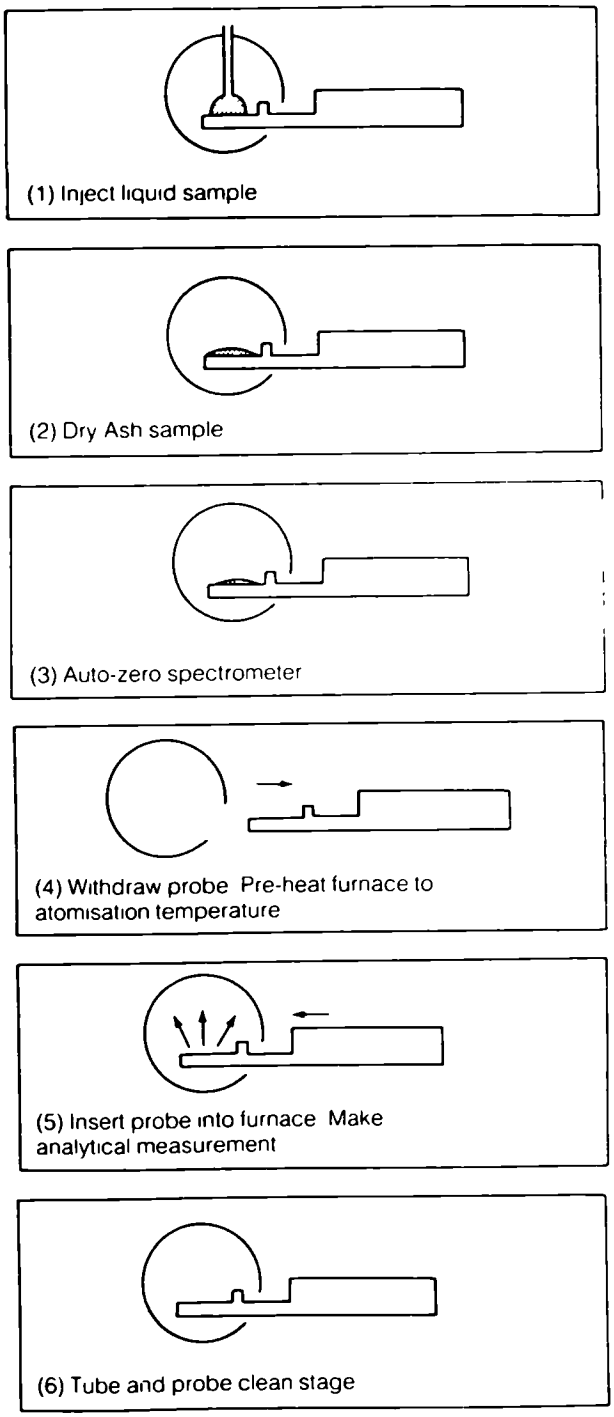


Figure 3.6: *The different procedures in probe atomisation (Brown 1989).*

Atomisation from probes or platforms still uses a furnace design based upon that by Massmann (1968), although some significant enhancements have been made in terms of materials (Littlejohn *et al* 1984), temperature control and automation. A relatively unique and, as

yet, not commercially available furnace design has been developed by Frech *et al* (1986). Based upon the aim of heating the tube in a direction perpendicular to the optical axis, a more isothermal environment is created within the tube and this has been shown to reduce vapour-phase interferences (Slavin 1987). Figure 3.7 shows the basic configuration of this furnace design, more commonly referred to as the integrated contact furnace.

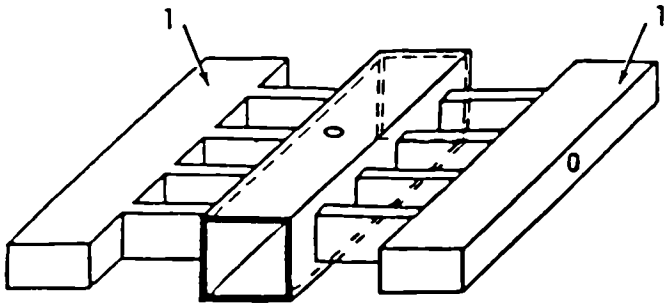


Figure 3.7: *Integrated contact furnace (Slavin 1987). The furnace is connected to the power supply via the wings (1). The furnace is machined from a single piece of graphite.*

Although still used in a furnace based upon the Massmann design, a variety of graphite geometries have been shown to reduce vapour-phase interferences. The delayed atomisation cuvette (DAC) is one such design; produced from a single piece of graphite, the walls at the ends of the cuvette are thinner than those at the centre (Murphy *et al* 1986). When the sample is atomised, the walls of the cuvette heat up from the ends of the tube inwards, as opposed to the reverse that is the case for more conventional cuvettes. An application of DAC's is presented in Section 3.2.1.

3.1.2.2.4: Modifiers for the control of interference effects

The previous two sections have sought to examine the nature of interference effects and their manifestation due to the majority of atomiser designs. Despite the advances made over the last decade, interferences due to the matrix of the sample may still represent a source of inaccuracy for ETA-AAS determinations (Zhe-Ming and Xiao-Quan 1987). Chemical modification of the sample is a potential means of controlling or, occasionally, eliminating interferences. The modifier may act in a variety of ways, including:

- stabilising the analyte during the ashing phase to allow a higher ashing temperature to be used.
- volatilising the interferent, leading to it being removed during the ashing phase.
- delaying the atomisation of the analyte during the atomisation phase, thereby increasing the degree of isothermality in the furnace (Brown 1989).

The particular modifier chosen should satisfy a number of criteria, as listed by Schlemmer and Welz (1986):

- the analyte element should be stabilised to as high a pyrolysis temperature as possible, hopefully at least 1000°C, to allow volatilisation of the bulk of the concomitants.
- the modifier should be applicable to as many elements as possible for simplicity.
- the modifier should be available in high purity, and not contain the analyte elements(s) in measurable concentrations.
- the modifier should not contain an element at high concentration which has to be determined in the furnace at a later time.
- the modifier should not markedly reduce the lifetime of the graphite tubes.

- the modifier should not produce excessive background attenuation around the wavelength of the analyte element.

The range of modifiers is considerable and has been extensively reviewed by Zhe-Ming and Xiao-Quan (1987).

For cadmium determinations, ammonium phosphate in either the monobasic ($NH_4H_2PO_4$) or the dibasic ($(NH_4)_2HPO_4$) form has been found to increase the appearance temperature (Kaiser *et al* 1981); this enables higher ashing temperatures to be used. Slavin *et al* (1983) found that the addition of 200µg $(NH_4)_2HPO_4$ to 40 pg cadmium enabled an ashing temperature of 650°C to be used, whereas the unmodified solution could only be ashed at 400°C. The maximum ashing temperature was further enhanced by the addition of 40µg NaCl, extending the ashing temperature to 900°C. Ammonium phosphate is more frequently used in conjunction with magnesium nitrate to achieve a similar elevation of the maximum permissible ashing temperature (Blust *et al* 1988). The use of ETA-AAS and slurry atomisation for the determination of cadmium in foods has been shown to benefit from the use of ammonium phosphate modification (Stephen *et al* 1987a). Lead analyses have also been enhanced by the use of ammonium phosphate as a modifier, both without (Halls *et al* 1987, Miller *et al* 1987) and with the inclusion of magnesium nitrate (Baxter *et al* 1989). Determinations of lead in foods by slurry atomisation ETA-AAS have been made using ammonium phosphate modification (Stephen *et al* 1987b). A combination of ammonium phosphate and magnesium nitrate was found to be an important factor in the determination of cadmium in natural waters when atomising the sample from a L'vov platform (Hunt and Winnard 1986). An application of ammonium phosphate and magnesium

nitrate sample modification for the determination of cadmium in foods is given in Section 3.2.1.

A combination of palladium and magnesium nitrates is becoming increasingly popular as a modifier for a range of elements (Schlemmer and Welz 1987). The mechanism by which cadmium is stabilised within the atomiser is still the subject of a number of theoretical and experimental studies (Hinds *et al* 1988, Rettberg and Beach 1989). Rettberg and Beach (1989) propose that the formation of intermetallics between the analyte and palladium may be the key stabilisation mechanism. Occlusion of the analyte within the mass of ashed palladium is the most likely means of stabilisation and in this respect droplet size and dispersion may by important factors (Rettberg and Beach 1989). Hinds *et al* (1988) have shown the that the masses of palladium and magnesium influence both the appearance temperature and sensitivity for lead determinations. Applications of the palladium modification technique include, the determination of zinc, copper, molybdenum, lead, caesium, chromium, cadmium and selenium in milk and milk powder (Wagley *et al* 1989); cobalt in plasma and urine (Sampson 1988), arsenic and selenium in soil digests (Dulude *et al* 1989) and lead in food slurries (Lynch and Littlejohn 1989).

Other means of sample modification include, the addition of lanthanum for the determination of lead in drinking water (Bertenshaw *et al* 1981) and the addition of EDTA for the determination of cadmium in sea water (Guevremont *et al* 1980).

An expanding area of sample modification is the use of an 'active gas' (Xiao-Quan and Zhe-Ming 1987). The use of oxygen during the ashing phase has been shown to enhance the maximum permissible ashing

temperature (Slavin *et al* 1983) and also to act as a means of removing the organic sample matrix and reducing the build-up of carbonaceous residues. This is particularly valuable when analysing sample slurries (Stephen *et al* 1987a, b). Hydrogen has been employed in the analysis of environmental samples as a means of removing chlorides (Zhe-Ming *et al* 1986).

3.1.2.2.5: Background correction systems

Background effects are created by the attenuation of incident radiation by species other than the analyte. Such effects may be corrected for using one of three basic techniques:

- the two-line method
- the continuum lamp method
- single source methods

(Brown 1989).

The two-line method has two major disadvantages, one being the sequential nature of the signal measurement (a major problem given the rapid and transient nature of ETA-AAS signals) and the other is associated with a disparity in the absorption coefficients of the two lines.

The continuum lamp or source method uses a deuterium arc lamp to obtain a simultaneous background correction, this is especially valuable when monitoring ETA-AAS signals in which the background changes very rapidly with respect to both time and wavelength (Brown 1989). Single source methods for background correction are available and these have been shown to be particularly valuable when analysing samples with high background attenuation (Ure 1990). The two principal single source methods are the Zeeman effect method and the Smith-Hieftje principle. The Zeeman effect divides the single line from the

radiation source into three or more components when the atomiser is placed in a magnetic field. The Smith-Hieftje background correction system uses the principle of "self-reversal" that occurs when the radiation source is pulsed at alternating high and low currents.

The scope for comparison and analysis of the various background correction systems is considerable but is not appropriate within the context of this work. Both the continuum source and the single source Smith-Hieftje methods have been used in method development and routine analysis, see Section 3.2 for details of their application.

3.2: THE DEVELOPMENT OF METHODS FOR THE DETERMINATION OF CADMIUM IN BIOLOGICAL SAMPLES

3.2.1: CADMIUM DETERMINATION USING DELAYED ATOMISATION CUVETTES (DAC) AND SMITH-HIEFTJE BACKGROUND CORRECTION

3.2.1.1: *Introduction*

The system used in this phase of the project had three distinctive features:

- delayed atomisation cuvettes
- Smith-Hieftje background correction
- aerosol sample deposition

This combination of measures was designed to enable the control of interferences.

3.2.1.2: *Materials and methods*

3.2.1.2.1: Instrumentation

Analyses were made on an Instrumentation Laboratory (IL) S-12 spectrometer in the single beam mode of operation, using Smith-Hieftje background correction. The instrument was equipped with an IL 655 Controlled Temperature Furnace (CTF) atomiser, IL 254 auto-sampler and a FASTAC for aerosol sample deposition. Pyrolytically coated delayed atomisation cuvettes (DAC) were used throughout the experiment (Allied Analytical Systems and Ringsdorff). Visimax II hollow cathode lamps were used as the radiation source. A Linear dual-channel integrating chart recorder was used to record peak shapes. BOC high purity argon was used as a purge gas.

3.2.1.2.2: Reagents

The acid used in sample digestion and in the make-up of the standards was AristaR grade nitric acid, standards being made by the sequential dilution of Spectrosol cadmium standard solution (BDH Chemicals, Poole, Dorset, UK). All water used in the analysis was initially glass distilled and then de-ionised using an Ionmiser 6C system.

Diammonium hydrogen phosphate modifier solution (2% m/V) was prepared from Pro analysi grade reagent (Merck) and purified by passing it through a column of H^+ substituted (10% AnalaR nitric acid) Chelex-100 cation-exchange resin (Bio-Rad Laboratories Ltd., Watford, UK) at a flow rate of 1ml $minute^{-1}$. The magnesium nitrate modifier solution (2% m/V) was prepared from AristaR grade reagent, no further purification was found to be necessary. The modifiers were added to the sample and standard solutions to give 0.2% (m/V) diammonium hydrogen phosphate and 0.02% (m/V) magnesium nitrate in the solutions to be analysed.

3.2.1.2.3: Instrumental parameters

Analyses of the samples were made against standards of the following concentrations, 0.50, 1.00, 1.50, 2.00 and 2.50 ng Cd ml^{-1}. Each standard solution contained 2% (V/V) AristaR nitric acid, 0.2% (m/V) Merck diammonium hydrogen phosphate and 0.02% (m/V) AristaR magnesium nitrate. Different combinations and concentrations of diammonium hydrogen phosphate and magnesium nitrate were used during the method development stages.

Wavelength=228.80 nm
Lamp current=3.20 mA
PMT voltage= 700 mV
Smith-Hieftje background correction.

Bandwidth=1.00 nm
Background current=0.45 mA

Sample deposition: Delay time= 7 seconds Deposit time= 20 seconds
Three repeats per sample.

Measurement:
Peak height - 2.00 seconds integration time.
Peak area - 5.00 second integration time.
Peak shape - Linear dual-channel integrating chart recorder.

High purity argon (BOC) used as the purge gas.

Table 3.4 : *S-12 and FASTAC parameters.*

Step no.	Step	Temperature (°C)	Ramp time (s)	Hold time (s)
1	Injection/dry	135	-	5
2	Ash§	650	15	5
3	Atomise¥	1550	5	5
4	Clean*	2500	Step	5

§ a variety of ashing temperatures were used in the course of method development.
¥ a variety of atomisation temperatures were used in order to optimise the sensitivity.
* Clean cycle not always necessary.

Table 3.5: *Time/temperature programme for CTF atomiser.*

3.2.1.3: *Optimisation of CTF atomiser parameters*

One of the key components of a successful ETA-AAS analysis is the optimisation of the time/temperature parameters of the atomiser. The determination of the ashing temperature is critical, especially so for volatile elements such as cadmium when the analyte could potentially be lost before the signal measurement phase of the analytical cycle.

Both pyrolytically coated and uncoated DAC tubes are available, the former were used for their greater sensitivity which is probably due to reduced porosity. The reduction in porosity will give an increased residence time in the tube and so lead to an enhancement of the peak area or integrated absorbance value, as shown in Figure 3.8. An additional advantage in the use of coated DAC tubes arises from the decreased potential for the analyte to react with the surface of the tube, this is especially important for those elements likely to form carbides.

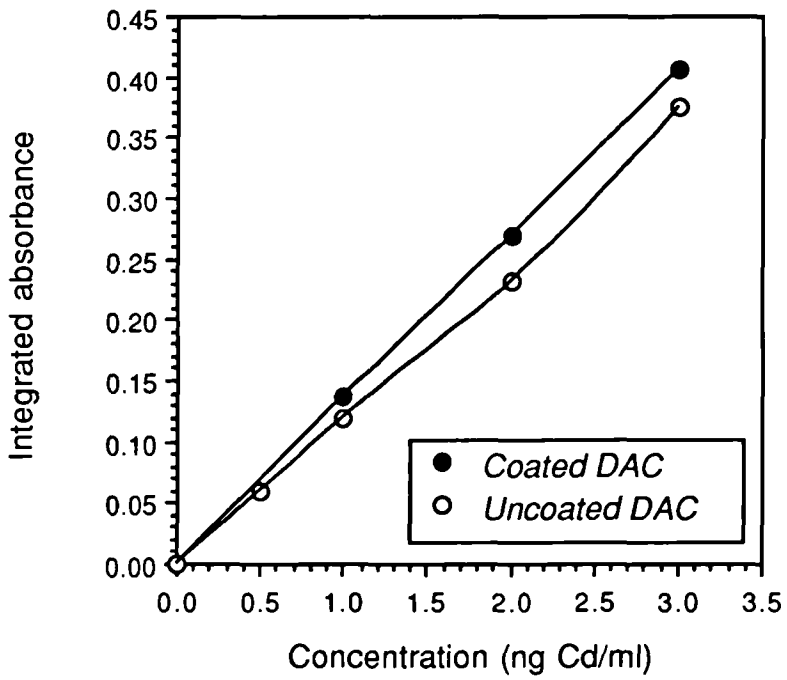


Figure 3.8: *Relative sensitivities for cadmium determinations using both pyrolytically coated and uncoated cuvettes.*

A number of experiments were conducted in order to optimise the ashing and atomisation temperatures, different concentrations and combinations of magnesium nitrate and diammonium hydrogen phosphate were also used in order to optimise the sample modification procedure. Three different concentrations of diammonium hydrogen phosphate and magnesium nitrate were used in a variety of combinations and at ashing temperatures ranging from 300°C to 1000°C. An atomisation temperature of 1600°C was used for all of these studies. Figures 3.9 to 3.12 show the results for a 1 ng Cd ml^{-1} standard in 2% (V/V) AristaR nitric acid.

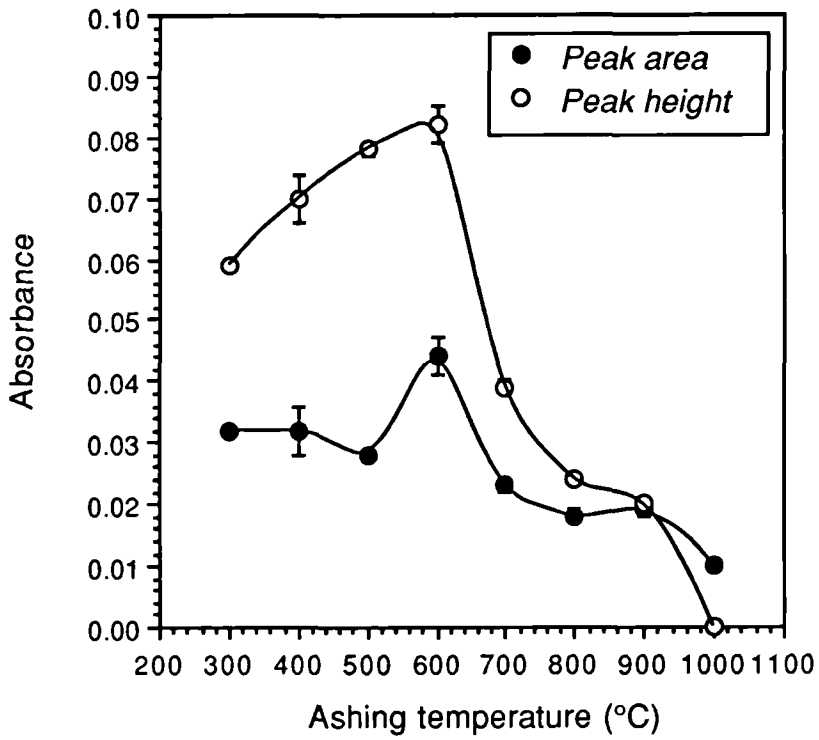


Figure 3.9: *Ashing curves for an unmodified 1 ng Cd ml^{-1} standard.*

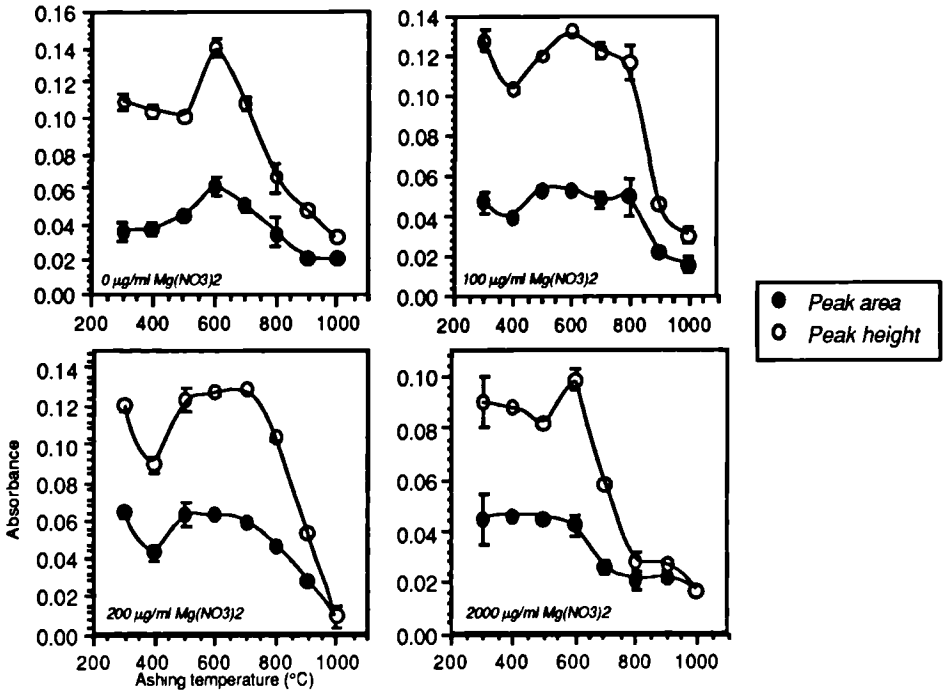


Figure 3.10: *Ashing curves for a 1 ng Cd ml^{-1} standard modified with 200 µg ml^{-1} diammonium hydrogen phosphate and varying concentrations of magnesium nitrate.*

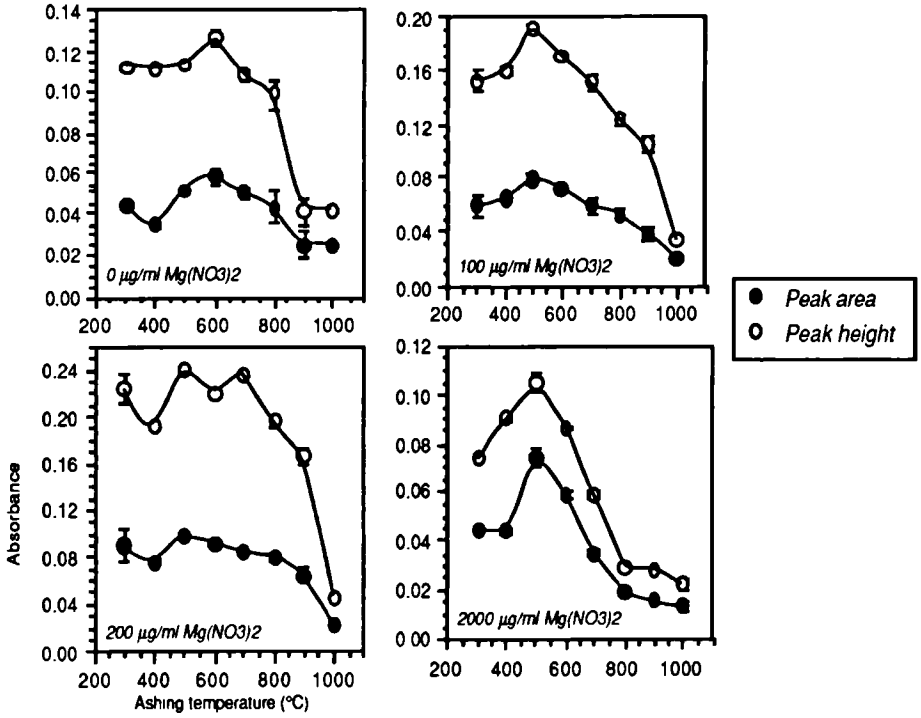


Figure 3.11: *Ashing curves for a 1 ng Cd ml^{-1} standard modified with 500 µg ml^{-1} diammonium hydrogen phosphate and varying concentrations of magnesium nitrate.*

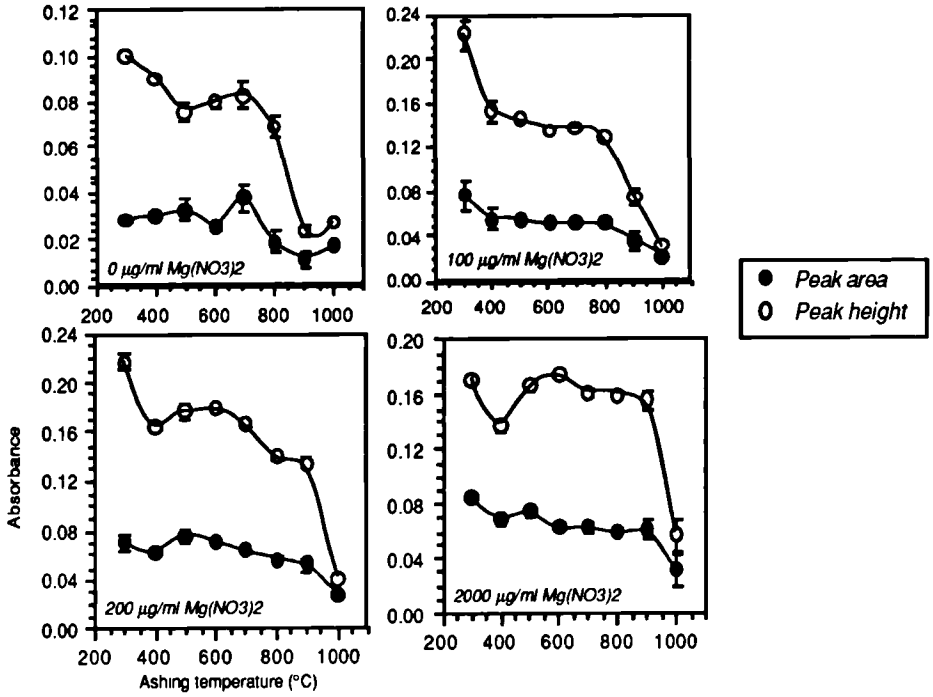


Figure 3.12: *Ashing curves for a 1 ng Cd ml^{-1} standard modified with 2000 µg ml^{-1} diammonium hydrogen phosphate and varying concentrations of magnesium nitrate.*

From Figure 3.9 it can be seen that the maximum ashing temperature is ~600°C, this coincides with the maximum sensitivity of 0.044 A.s. The addition of $(NH_4)_2HPO_4$ permitted the use of a higher ashing temperature at concentrations greater than 200 µgml^{-1}. Slavin *et al* (1983) were able to stabilise cadmium to a temperature of ~700°C using 200 µgml^{-1} $(NH_4)_2HPO_4$. A problem with the comparison of these data with those of others is that the temperatures recorded are often incompatible. Many of the data for ashing temperatures are not actually recorded by the instrument and only represent the temperature required rather than that achieved. In this respect there is a further problem when trying to compare data from wall-atomisation, such as that used in this work, with data from platform atomisation, Slavin *et al* (1983) for example. The addition of 500 or 2000 µgml^{-1} $(NH_4)_2HPO_4$ allowed an ashing temperature of ~700°C to be used before a sustained inflection

point is seen in the ashing curve plot. The addition of varying concentrations of $Mg(NO_3)_2.6H_2O$ allowed higher ashing temperatures to be attained. Figures 3.10 and 3.11 show that the addition of 100 and 200 $\mu g ml^{-1}$ $Mg(NO_3)_2.6H_2O$ to solutions containing either 500 or 200 $\mu g ml^{-1}$ $(NH_4)_2HPO_4$ gave an increase of ~100°C in the allowable ashing temperature, a higher concentration of $Mg(NO_3)_2.6H_2O$ did not produce this effect and suppressed the peak height sensitivity. This may well be a reflection of the suggested mechanism by which the addition of magnesium stabilises cadmium; it is often assumed that magnesium acts as does palladium and forms an inter-metallic bond with the analyte. This effectively reduces the volatility of cadmium that of magnesium and slows down the rate of atom formation on the time/temperature gradient between the ashing phase and atomisation temperature. A reduction in the rate of atom formation will therefore lead to decreased peak height sensitivity, a feature not observed when using peak area values. Figure 3.11 shows similar features to those in Figures 3.10 and 3.11, the optimum combination of modifiers would appear to be 2000 $\mu g ml^{-1}$ of both $Mg(NO_3)_2.6H_2O$ and $(NH_4)_2HPO_4$. This allows an ashing temperature of 900°C to be used without any interim loss of the analyte. This combination was employed and found to be acceptable for the analysis of certified reference materials, ie it gave analyte recoveries of 85 to 105% and data in the certified range.

Routine use of this modifier combination led to the manifestation of problems with the life-time of the DAC's, peak area sensitivity was seen to reduce quite markedly with time, a product of over-correction by the background correction system. In order to investigate this problem more thoroughly a study was made of the effects of two concentrations of $Mg(NO_3)_2.6H_2O$ on peak area sensitivity. A basic standard of 1 ng Cd ml-

^{1} and 2000 μgml^{-1} $(NH_4)_2HPO_4$ was used with either 200 or 2000 μgml^{-1} $Mg(NO_3)_2.6H_2O$. The solutions were repeatedly injected and analysed using the conditions presented in Tables 3.4 and 3.5, a new pyrolytically coated DAC was used for each experiment. These tubes were retained for subsequent analysis. The results of these studies are shown in Figure 3.13.

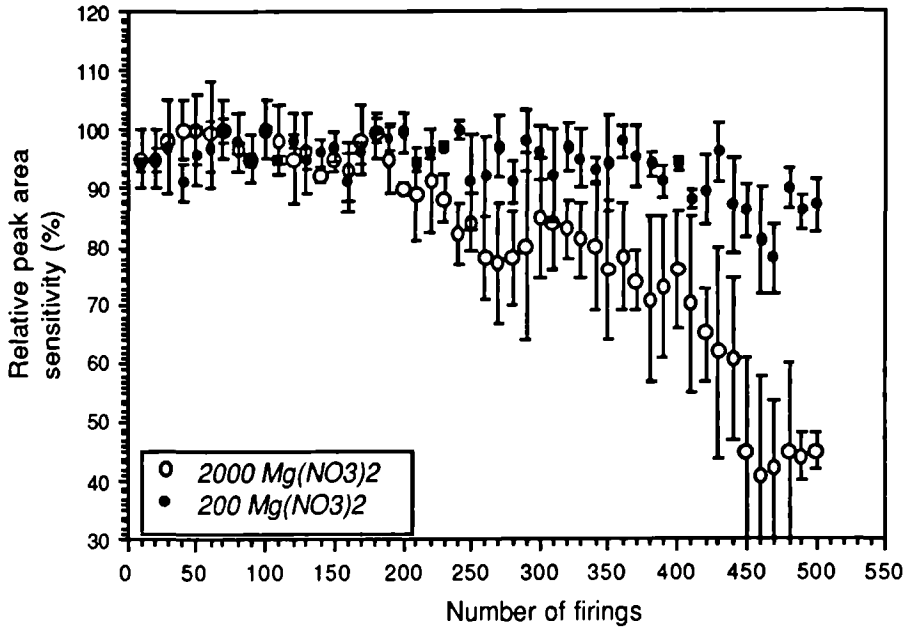


Figure 3.13: *DAC tube life-time studies.*

Use of the previously determined optimal concentration of magnesium nitrate appeared to impair the analyses more rapidly than did the lower concentration. Both sensitivity, expressed as being relative to the highest value over the whole experiment, and precision were adversely effected when the higher magnesium nitrate concentration was used.

An experiment was conducted to determine the optimum atomisation temperature, Figure 3.14 shows both the peak area and height atomisation curves for a standard containing 200 μgml^{-1} $Mg(NO_3)_2.6H_2O$ and 2000 μgml^{-1} $(NH_4)_2HPO_4$. From this study it was decided to use an

atomisation temperature of 1550°C in order that peak area absorbances were maximised.

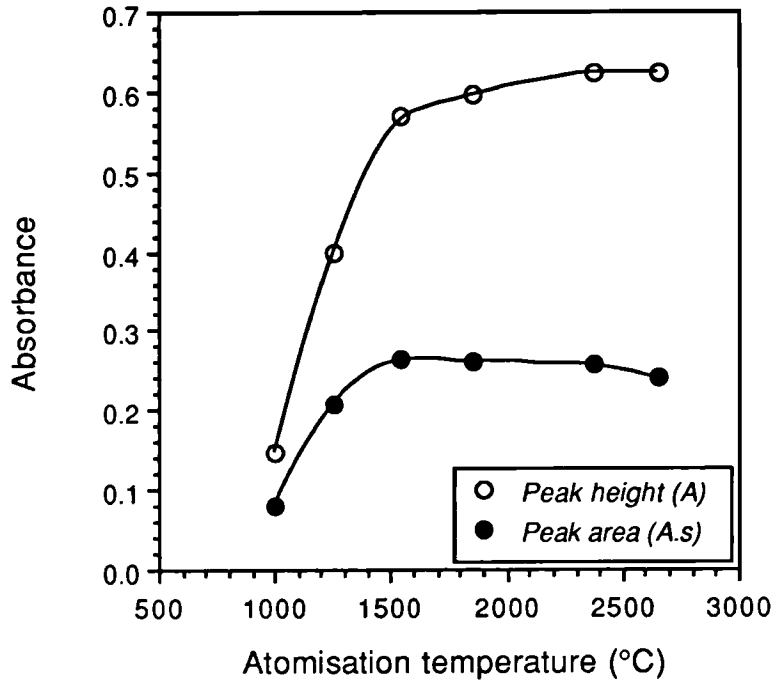


Figure 3.14: *Curves for the optimisation of the cadmium atomisation temperature.*

3.2.1.4: *SEM studies of DAC tubes*

The trend shown in Figure 3.13 for the 2000 µg ml^{-1} $Mg(NO_3)_2.6H_2O$ addition, ie an accelerating decline in peak area absorbance values, implied that the pyrocoating was being rapidly degraded. In addition to this problem, the appearance of over-correction by the Smith-Hieftje background correction system was observed and seen to worsen with the number of firings. Although this problem could be eliminated by the inclusion of a high temperature clean cycle, this was considered to be undesirable if tube life was to be maximised and cycle times kept to the minimum. In order to more closely examine the problems of declining sensitivity and over-correction, a DAC tube was bisected along its

longitudinal axis and analysed by scanning electron microscopy (SEM). Initial studies were made on tubes that had been exposed to a solution containing 2000 μg ml^{-1} of both $Mg(NO_3)_2.6H_2O$ and $(NH_4)_2HPO_4$; a subsequent study was made on DAC tubes exposed to higher concentrations of both modifiers, this enabled the effect to be manifested more rapidly. The results of the two studies were comparable.

A Hitachi S-450 SEM fitted with a Link Analytical AN 10000 microanalysis system was used to make both a visual inspection and an energy dispersive x-ray chemical analysis. As can be seen in Plate 3.1, the oxidising conditions at the site from which the sample is atomised have completely removed the pyrocoating, leaving behind a porous and potentially reactive surface from which the subsequently injected samples have to be atomised. An area 12mm from that in Plate 3.1 can be seen in Plate 3.2. At this point damage to the pyrocoating, such as that seen at the sample deposition area, is absent; however there has been an accumulation of material on the surface. This material was analysed using the microanalysis system and found to be magnesium nitrate. At 13mm from the area shown in Plate 3.1 the deposition of spheres of magnesium phosphate can be seen (Plate 3.3). This pattern may well be due to non-isothermal conditions in the tube after the atomisation of the sample, leading to the differential condensation of components of the sample modification compounds. As the tube cools down to the sample injection temperature, the areas adjacent to the thicker tube walls at the centre of the tube can be expected to loose heat more slowly and so lead to the spatial and temporal gradient which has caused the effects seen in Plates 3.1 to 3.3. The rapid losses in sensitivity are due mainly to the degradation of the pyrocoating at the sample deposition area, the loss of accurate background correction is probably due to the accumulation of

magnesium phosphate 13mm from the centre of the tube. The subsequent atomisation from these areas of secondary deposition will cause an increase in the background attenuation, eventually leading to the manifestation of over correction. As a relatively low atomisation temperature was considered to be a desirable feature and the use of a clean cycle was thought to impair tube life, it was decided to use a lower concentration of magnesium nitrate.

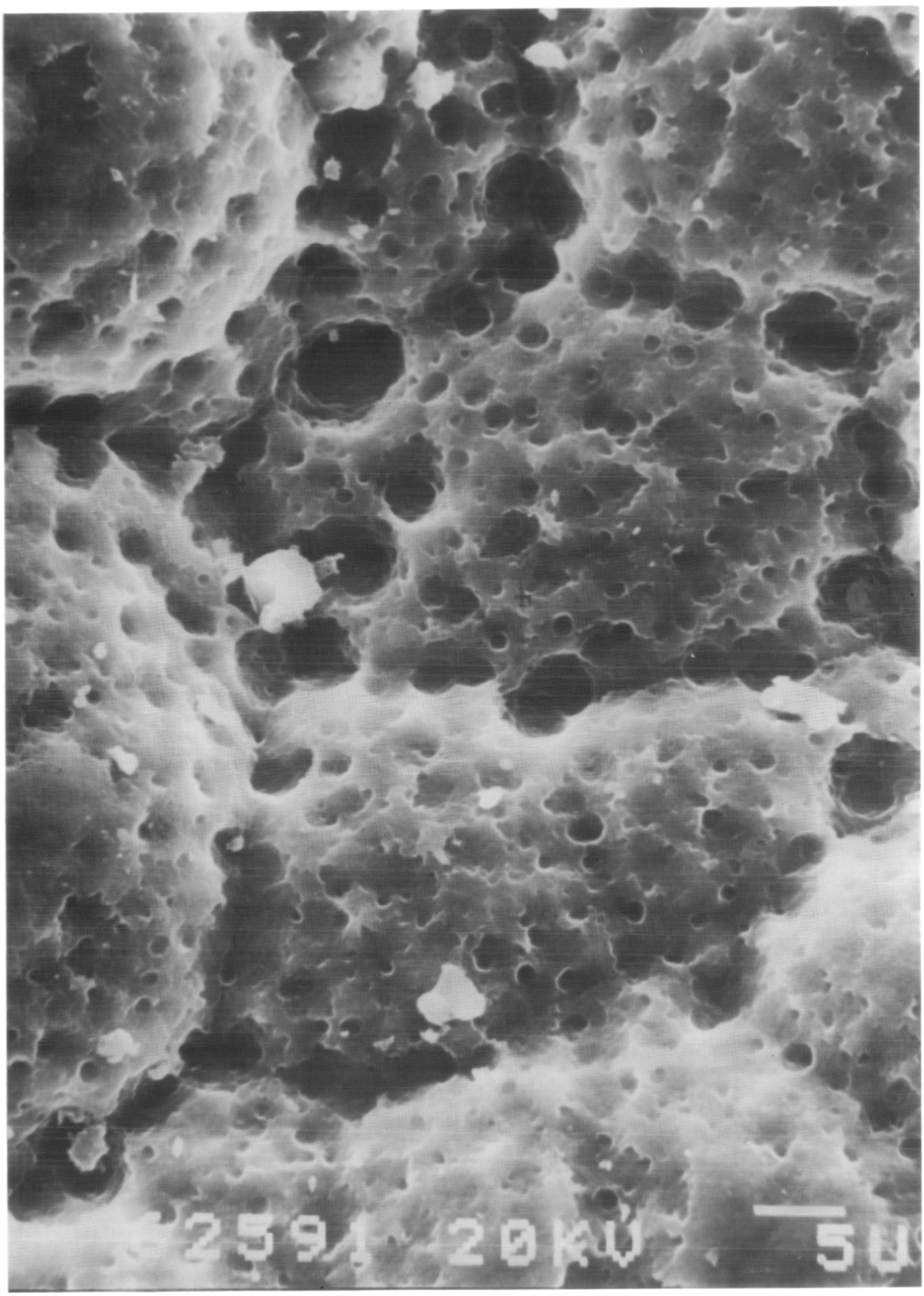


Plate 3.1: *Degradation of pyrocoating at the site of sample deposition*

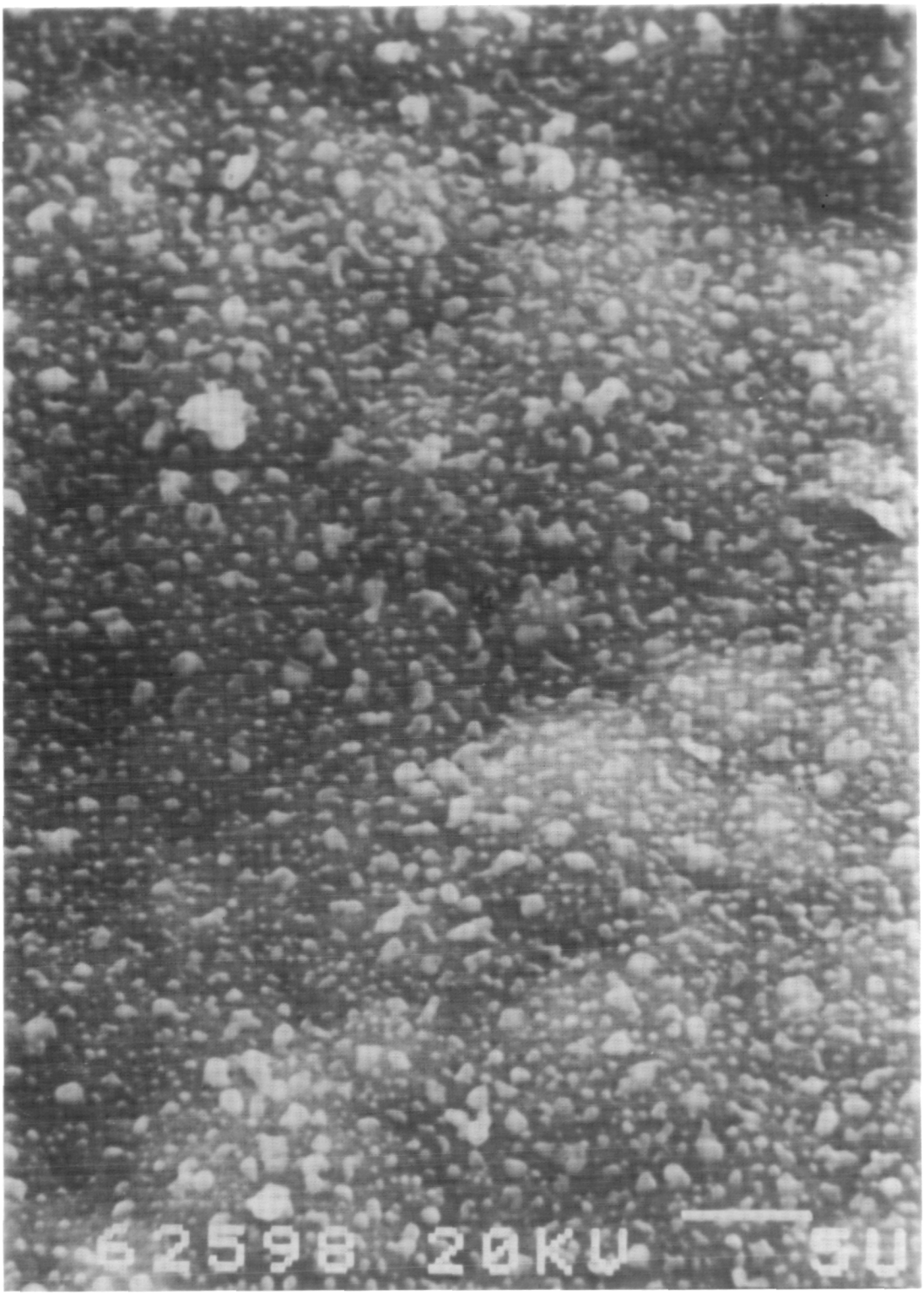


Plate 3.2: *Deposits of magnesium nitrate 12mm from the site of sample deposition*

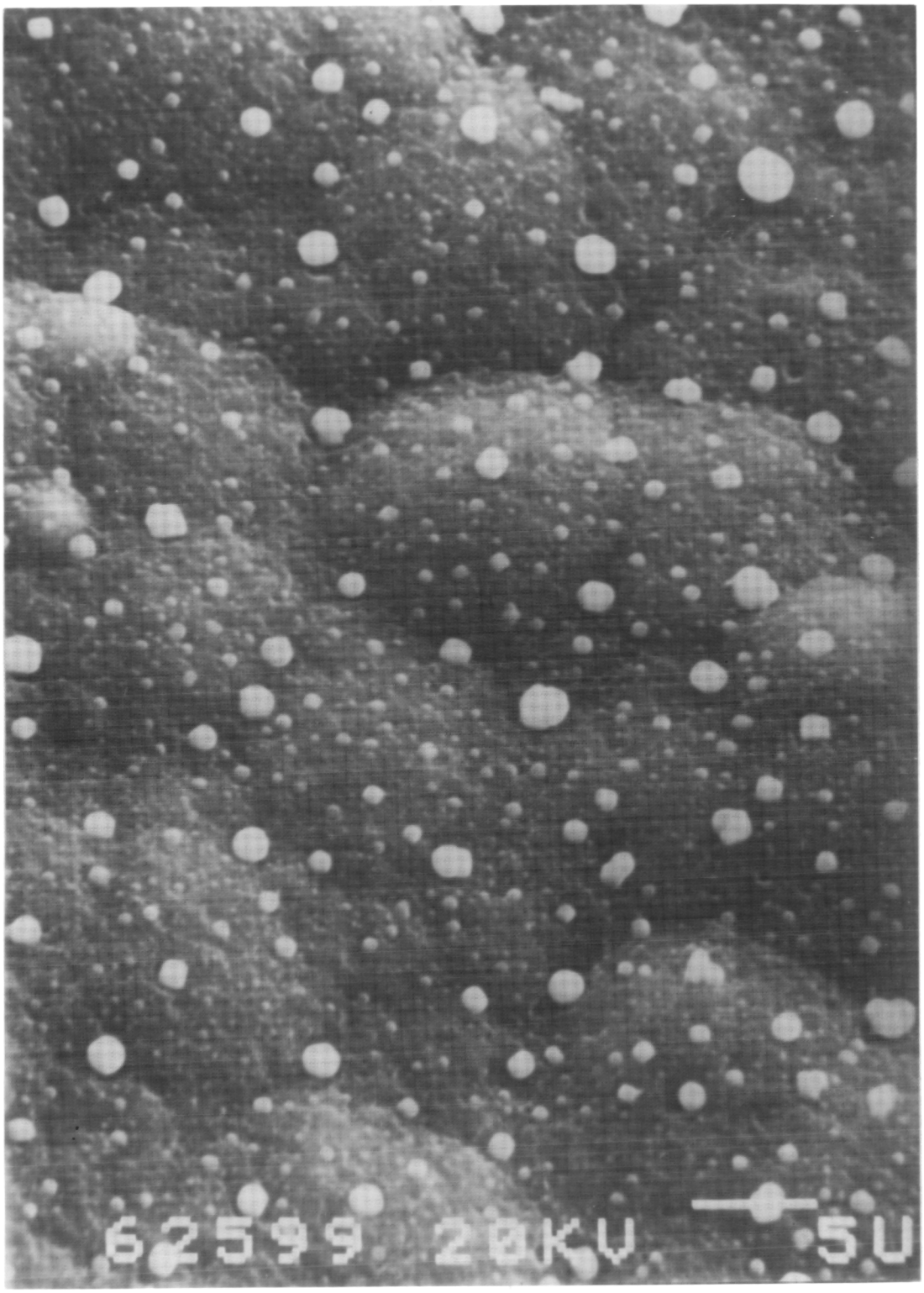


Plate 3.3: *Deposits of magnesium phosphate 13mm from the site of sample deposition*

3.2.1.5: *The determination of cadmium in certified reference materials*

In order to assess the validity of a method development strategy it is necessary to analyse a number of certified reference materials (CRMs). By comparing the determined values for the cadmium concentration with the certified values a measure of the degree of accuracy can be attained. For this experiment four certified materials were used:

- CRM-60 *Lagarosiphon major* (Community Bureau of Reference 1982).
- CRM-62 *Olea europaea* (Community Bureau of Reference 1982).
- SRM-1567 Wheat flour (National Bureau of Standards 1978).
- CRM-1 Pepperbush (National Institute for Environmental Studies 1980).

3.2.1.5.1: Digestion of CRMs

A wide range of procedures exist for the preparation of a sample for analysis, most of which involve the use of either wet or dry ashing (Bock 1979). Pressure-aided digestion procedures have enabled samples to be prepared more rapidly for analysis (Jackwerth and Gamiscek 1984), however a considerable investment is required if a high throughput of samples is to be attained. Much work has been done on sample dissolution aided by microwave heating in either a pressurised or unpressurised environment (Nakashima *et al* 1988). Much of the early work on this technique involved the use of equipment constructed in the laboratory and tended to concentrate upon the problem of removing the large volume of fumes generated during the heating cycle (Abu-Samra *et al* 1975; Barret *et al* 1978; Demura *et al* 1985; Tsukada *et al* 1985).

More recently, with the development of commercial systems, the attention has shifted towards the speed and efficiency of the system (Schramel *et al* 1987; Buresch *et al* 1987). The ideal solution to the problems of speed and safety would seem to be the use of a pressurised and completely closed system, from which there is no escape of the corrosive acid vapour (Nadkarni 1984). A comparison of a variety of decomposition techniques, including microwave digestions, has been made by Blust *et al* (1988).

Samples (0.050±0.001 g) were weighed into the PTFE liner of a Parr microwave acid digestion bomb (Scientific and Medical Products Ltd) using a Sartorious five-figure balance; the liner had previously been cleaned by the following process: ultrasonicated for 10 minutes in a 10% solution of Lipsol, rinsed with distilled water, soaked in 5% Lipsol, rinsed with distilled water, soaked in 5% AnalaR nitric acid and then rinsed in distilled de-ionised water. AristaR nitric acid (3.00 ml) was then added to each sample and the liner was then placed in the bomb. The bomb was then placed into a domestic microwave oven (Solavox T-2, 980 W) and heated at full power for 45 seconds; this was sufficient to give a clear solution. Although discharges of fumes from the bomb are only occasional and slight, the inside of the oven was coated with a PTFE spray to mitigate their action. All samples were prepared in quadruplicate, two of the four samples were spiked with the quantity of cadmium necessary to double the concentration. Reagent blanks were carried through the entire procedure.

A refrigerated cooling period of 25 minutes was then required before the bomb could be opened and the liner removed. Any acid which had condensed on the lid of the liner was then rinsed back into the liner with distilled de-ionised water. The liner, with the top removed, was then

placed on a Techne dri-block and heated for ~90 minutes at 120°C in order to reduce the volume of the sample to ~0.25 ml. The remaining solution was taken up in 5 ml of 4% (V/V) solution of AristaR nitric acid and then made up to 10 ml in a volumetric flask with distilled de-ionised water. Samples were usually stored in Sterilin bottles (30 ml) and not in the volumetric flask to avoid any adsorption of analyte onto the glass walls.

3.2.1.5.2: Analysis and results

The prepared samples were analysed by ETA-AAS using the instrumental parameters given in Section 3.2.1.2.3. Table 3.6 shows the observed values for each CRM.

Sample	Certified ($\mu g g^{-1}$DW)	Observed ($\mu g g^{-1}$DW)	Precision (%)	Accuracy (%)
CRM-60	2.20±0.10	2.23±0.27	12.23	+1.36
CRM-62	0.10±0.02	0.097±0.004	4.20	-3.00
SRM-1567	0.032±0.007	0.032±0.005	15.60	0
CRM-1	6.70±0.50	7.17±0.13	1.80	+7.01

Table 3.6: *Analysis of four CRMs.*

Each of the materials listed in Table 3.6 has been analysed in quadruplicate in three batches. Precision is assesed by the relative standard deviation between batches and is expressed as a percentage. Accuracy is calculated as the percentage difference between the certified and the observed value for a given material. The method was also used to analyse a large number of plant samples grown on soils contaminated with heavy metals due to the application of sewage sludges (These data

will be examined in more detail in Chapter 4). The mean recovery of a spike sufficient to double the concentration of cadmium in the sample, was 96.4±14.3% (n=107). In addition to the analysis of the certified reference materials, another check upon the precision of the analysis was made by the repeated analysis of an inhouse quality control material supplied by the Food Science Division of the Ministry of Agriculture Fisheries and Food. This material had been analysed by ETA-AAS and found to give a concentration of 4.43 μg Cd g^{-1} DW, the method described above gave a value of 4.48±0.31 μg Cd g^{-1} DW (n=44).

3.2.1.5.3: Conclusions

A combination of sample preparation using microwave-aided pressure decomposition and analysis using DAC tubes, Smith-Hieftje background correction and chemical modifiers has been found to be suitable for the determination of cadmium in biological CRMs. Values for accuracy (expressed as the percentage deviation of the observed value from the certified mean) tended to be better than those for precision (expressed as the relative standard deviation). This is most probably a function of sample heterogeneity, given that only 50mg of sample were take for each analysis. The mean recovery of a cadmium spike was 96.4%, implying that the method as developed was relatively free from interferences.

3.2.2: CADMIUM DETERMINATION USING PROBE ATOMISATION

3.2.2.1: *Introduction*

In 1978 L'vov published a paper examining the nature of and reasons for the susceptibility of electrothermal atomisers, mainly based upon the Massman graphite furnace system, to matrix interferences. He concluded that the "marked time and space non-isothermality of commercial

atomizers has been established" and it was these characteristics that led to interferences. In order to overcome these problems, three options were forwarded, pulse heating, platform atomisation or probe atomisation. Pulse heating and platform atomisation represent an improvement upon conventional wall atomisation but neither could be said to be truly isothermal. The non-isothermal features of commercial atomisers present particular problems when determining analytes with low appearance temperatures and low halide dissociation energies (L'vov 1978). Cadmium is one such element and has been shown to be susceptible to interferences from the inorganic components of the sample matrix (Hulanicki *et al* 1985). These features have been examined in more detail in Section 3.1.2.2.2.

Probe atomisation, in which the sample is removed from the atomiser cuvette during the period of non-isothermality, represents an enhancement of the stabilised temperature platform furnace (STPF) concept (Slavin *et al* 1983, Slavin 1988). Probe geometry, material and mode of operation are crucial to the optimisation of the method (Littlejohn *et al* 1984, Ottaway *et al* 1986, Carroll *et al* 1986). A number of studies using the introduction of known interferents have been conducted to demonstrate the value of probe atomisation (Ajayi *et al* 1988). A study by Ajayi *et al* has shown that probe atomisation cannot be said to be truly isothermal, although vapour temperatures are higher than those for either tube wall or platform atomisation.

3.2.2.2: *Materials and methods*

3.2.2.2.1: Instrumentation

All analyses were made on a Philips Analytical PU9290 atomic absorption spectrometer, fitted with a PU9390 graphite furnace, PU9385

furnace autoprobe and PU9380 furnace autosampler. Totally pyrolytic graphite (TPG) cuvettes of internal diameter 6.6 mm were used in all determinations. Each cuvette has a slot 2 mm high by 6 mm wide machined into its side to facilitate the entry of the probe directly below the injection port. Electrographite ridge probes were used for all analyses, this design has been shown to overcome a number of problems with sample migration during drying (Ottaway *et al* 1986). A Visimax II hollow cathode lamp (Thermo Electron, Warrington, UK) was used as the radiation source. BOC high purity argon was used as the purge gas.

At the start of the measurement cycle the probe is inserted into the cuvette and the sample deposited from the autosampler onto the probe, see Plate 3.4. The sample is then dried and ashed by the radiant energy of the tube wall before the spectrometer autozeroes itself. The furnace is then cooled and the probe withdrawn, see Plate 3.5. The cuvette is rapidly heated to a constant temperature before the probe is reinserted atomising the sample. All of the steps in the measurement cycle are controlled by the system software. For all quantifications peak area or integrated absorbance values were used.

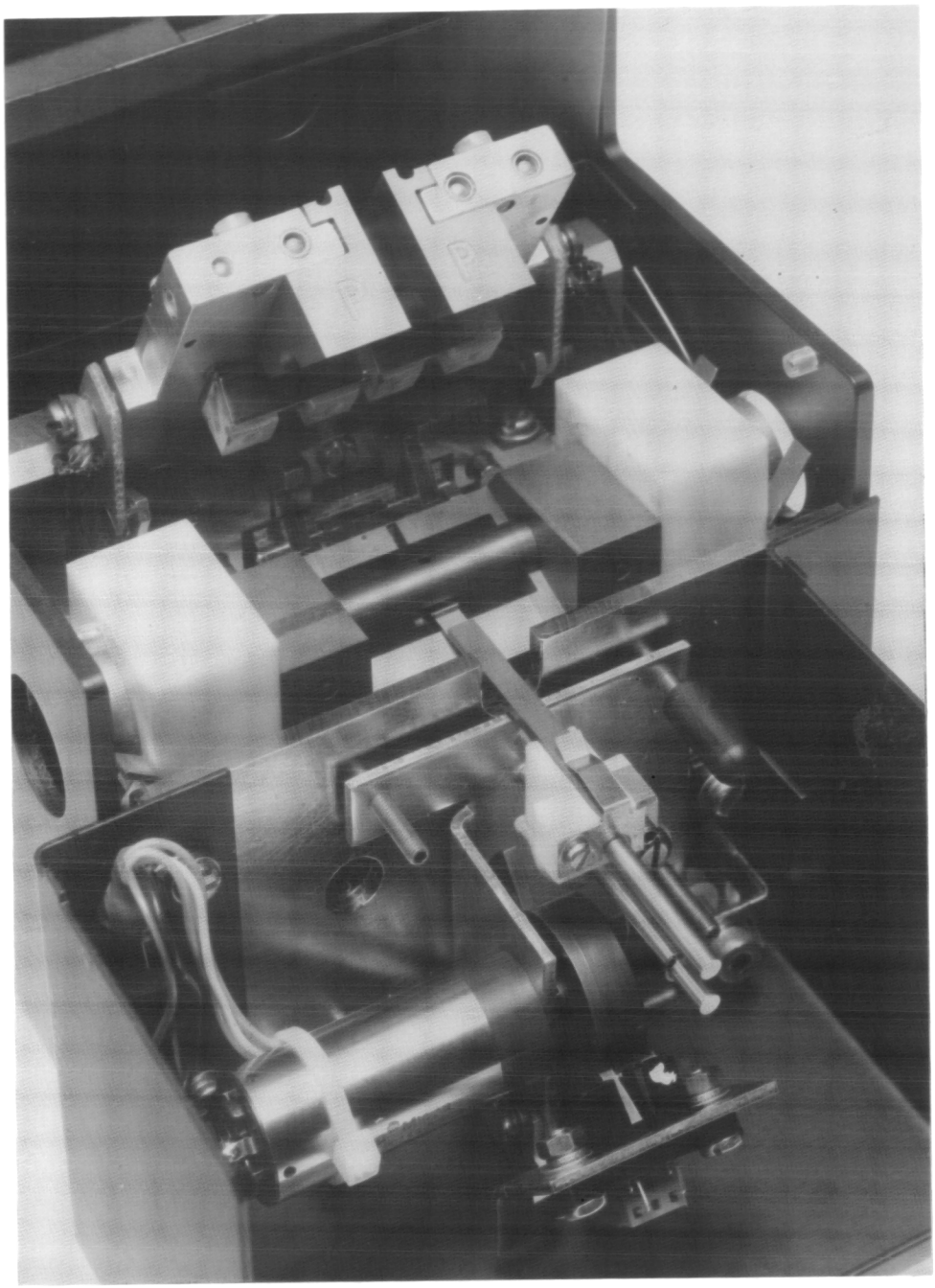


Plate 3.4: *Electrographite ridge probe inserted in the cuvette during sample deposition*

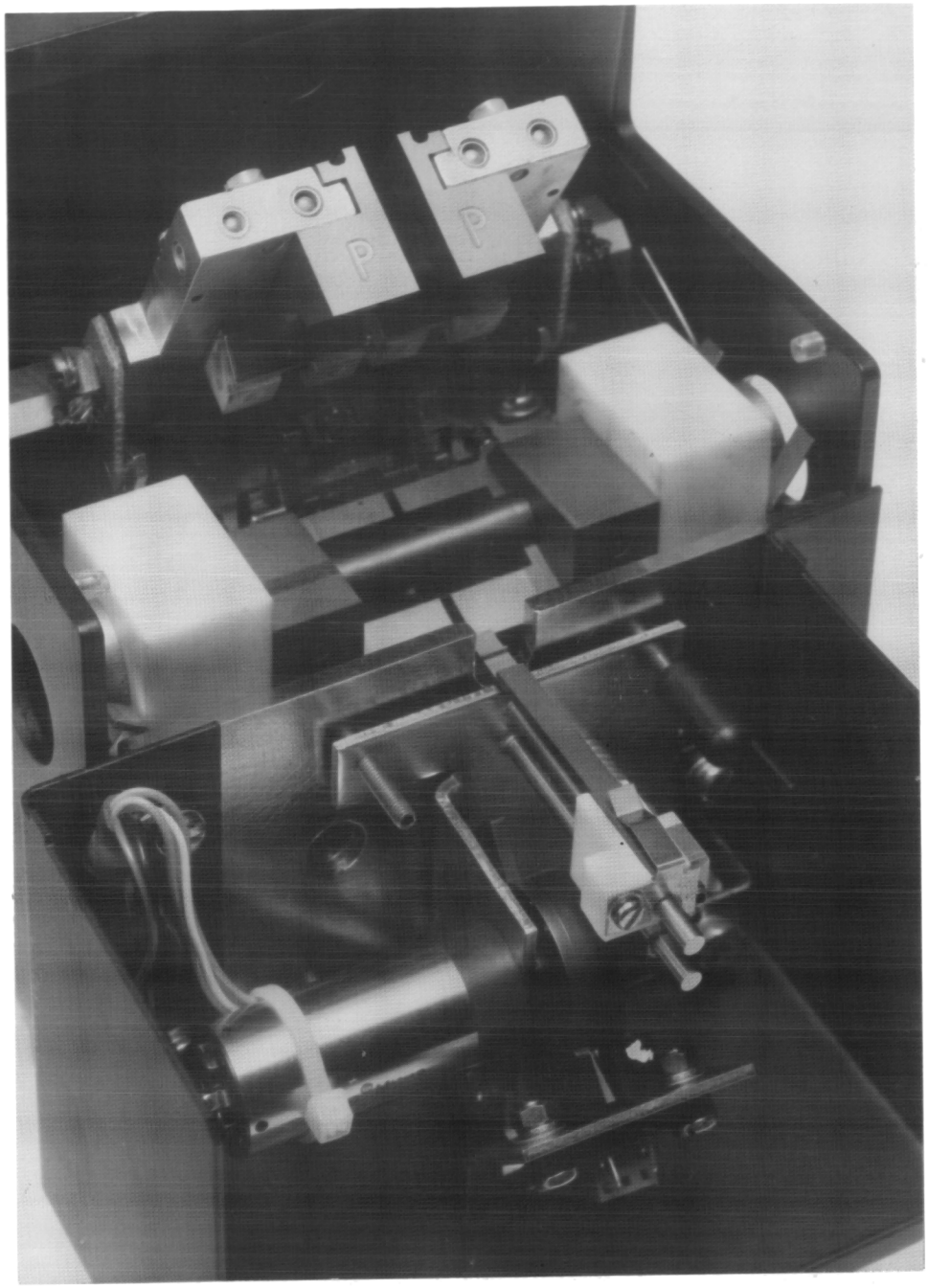


Plate 3.5: *Electrographite ridge probe withdrawn prior to sample atomisation*

3.2.2.2.2: Reagents

AristaR grade nitric and perchloric acids (BDH Chemicals, Poole, Dorset) were used in the preparation of all samples and standards. A modifier solution of AristaR magnesium nitrate (2000 mg $Mg(NO_3)_2.6H_20$ l^{-1}) and palladium nitrate (3000 mg Pd l^{-1}) (Johnson Matthey, Royston, Herts) was prepared and used to attain the higher ashing temperatures necessary for some determinations. Standards were made daily from 1000µg ml^{-1} Spectrosol cadmium solution (BDH). Glass distilled deionised water was used throughout the preparation of both samples and standards.

3.2.2.2.3: Instrumental parameters

All instrumental parameters were under software control and are shown in Tables 3.7 and 3.8.

Wavelength	228.8 nm
Bandpass	0.5 nm
Lamp current	5.4 or 4.0 mA
Background correction	On (D_2)
Measurement	Peak area
Integration time	2.0 seconds
Injection volume	15µl
No. of resamples	3

Table 3.7: *PU9290 spectrometer and PU9380 autosampler parameters*

Phase	Temperature (°C)	Ramp (°C s-1)	Hold time (s)	Gas flow (ml/min)	Commands
1	250	30	30	200	
2	400	30	30	300	NL RS
3	2000	-	2	0	RD TC
4	2700	-	2	300	TC

NL - non-linear ramp, RS - return to standby, RD - begin integration and TC - temperature control.

Table 3.8: *PU9390 graphite furnace parameters.*

The return to standby option was used in order to reconcentrate samples on the probe and so allow more accurate quantification of reagent blanks and those materials with very low cadmium concentrations (A-11 and AQA35). The autosampler was used to make up standards from a single master solution, to make any necessary dilutions and to add chemical modifiers. In all cases a constant volume of solution (15µl) was deposited onto the probe.

For CRM A-11 and intercomparison materials AQA-35, AQA-36 and IAEA-155 it was necessary to use a slightly different set of parameters to those described In Table 3.8. Cadmium determinations for the IAEA A-11 material had previously been identified as being particularly difficult (Byrne *et al* 1987). The high background attenuation of these samples caused over-correction by the background correction system and made it necessary to use a combination of palladium and magnesium nitrates as a modifier. Modifier solution (5µl) was added to each standard and sample using the autosampler, giving a mass of 15µg of Pd and 10µg $Mg(NO_3)_2.6H_20$ on the probe. For IAEA-155 and AQA-36 the modifiers were added to the samples and standards in the autosampler pipette tip and then deposited onto the probe. The low cadmium concentrations of

A-11 and AQA-35 made reconcentration of the sample on the probe necessary; one 5 µl volume of modifier solution was deposited onto the probe where it was dried, subsequently two 15 µl volumes of sample were deposited and dried before the sample was ashed and then atomised. No major differences in sensitivity were observed between the two methods of adding the modifier solution, as observed by Hinds *et al* (1988) for the determination of lead in soil slurries. The furnace parameters used were the same as those in Table 3.8 with the exception that an ashing temperature of 1000°C was used.

3.2.2.3: *Optimisation of probe atomisation parameters*

3.2.2.3.1: Calibration mode

In order that the instrument can be calibrated, either peak height or peak area readings must be taken from the transient peak. The use of nitric acid to acidify standards is recommended by Hulanicki *et al* (1985) as it helps to avoid some of the vapour phase interferences associated with the use of other acids such as hydrochloric. Given that the concentration of nitric acid in samples was variable, a study was conducted in order to determine the optimum ashing temperature and measurement mode for samples with varying concentrations of nitric acid. From Figures 3.14 and 3.15 it is clear that the use of peak area values is preferable to the use of peak heights as long as acid concentrations are kept at or below 5% (V/V).

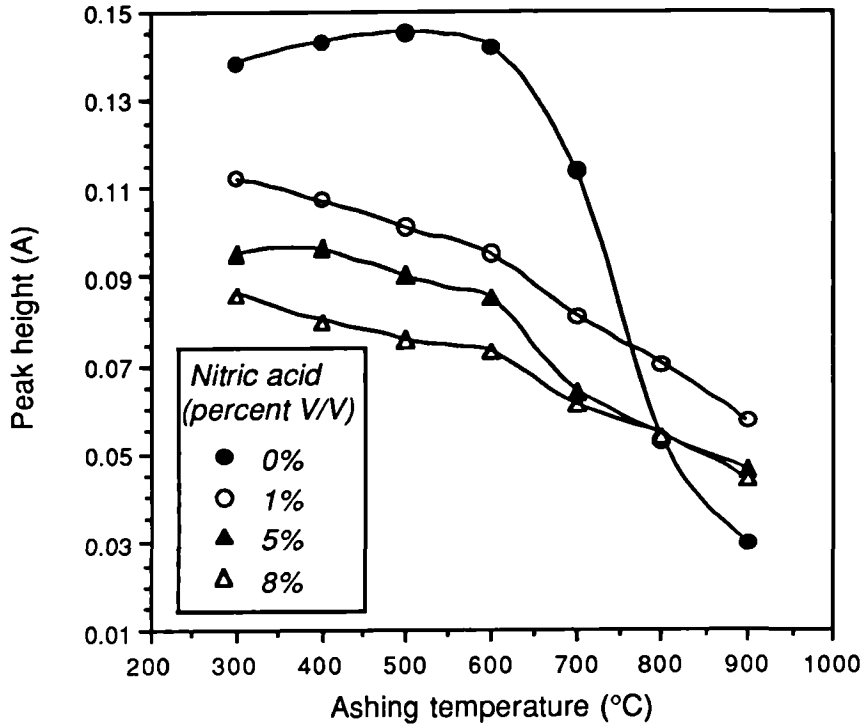


Figure 3.15: *The effect of ashing temperature and concentration of nitric acid upon peak height absorbance values, obtained when atomising 15µl of a 2 ng Cd ml^{-1} standard from an electrographite probe at 2000°C.*

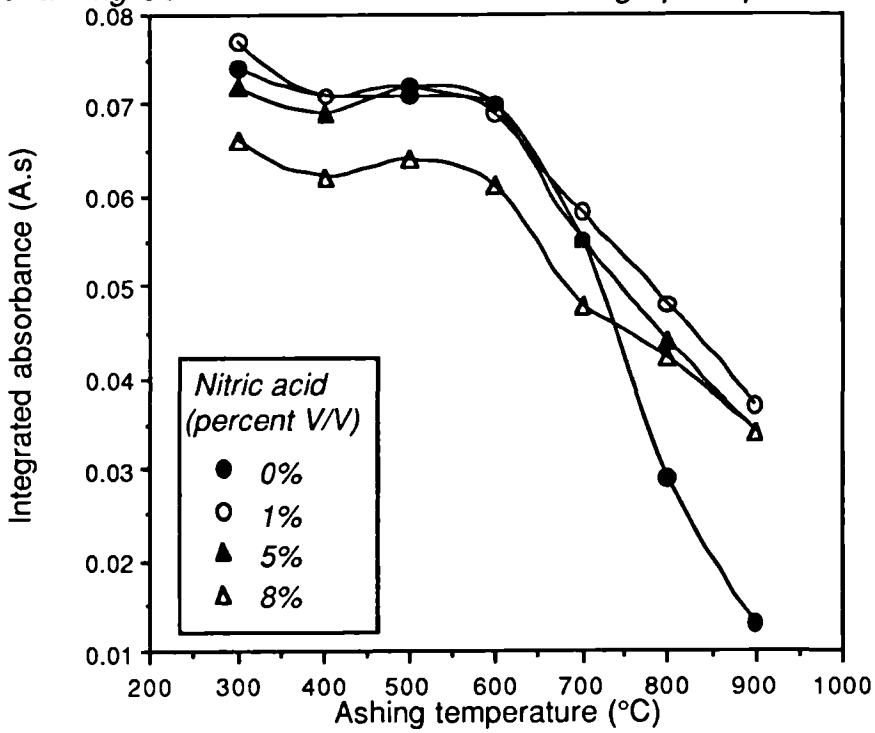


Figure 3.16: *The effect of ashing temperature and concentration of nitric acid upon peak area integrated absorbance values, obtained when atomising 15µl of a 2 ng Cd ml^{-1} standard from an electrographite probe at 2000°C.*

3.2.2.3.2: Optimisation of electrothermal atomiser parameters

In order to develop a suitable time-temperature programme a number of ashing studies were conducted. A range of biological sample digests were used to allow comparison between both each other and an aqueous standard. If it is to be possible to quantify the cadmium concentrations of a range of sample types using a single calibration curve, the ashing temperature to be used must be suitable for all samples. The results of an ashing temperature study are shown in Figure 3.17 below.

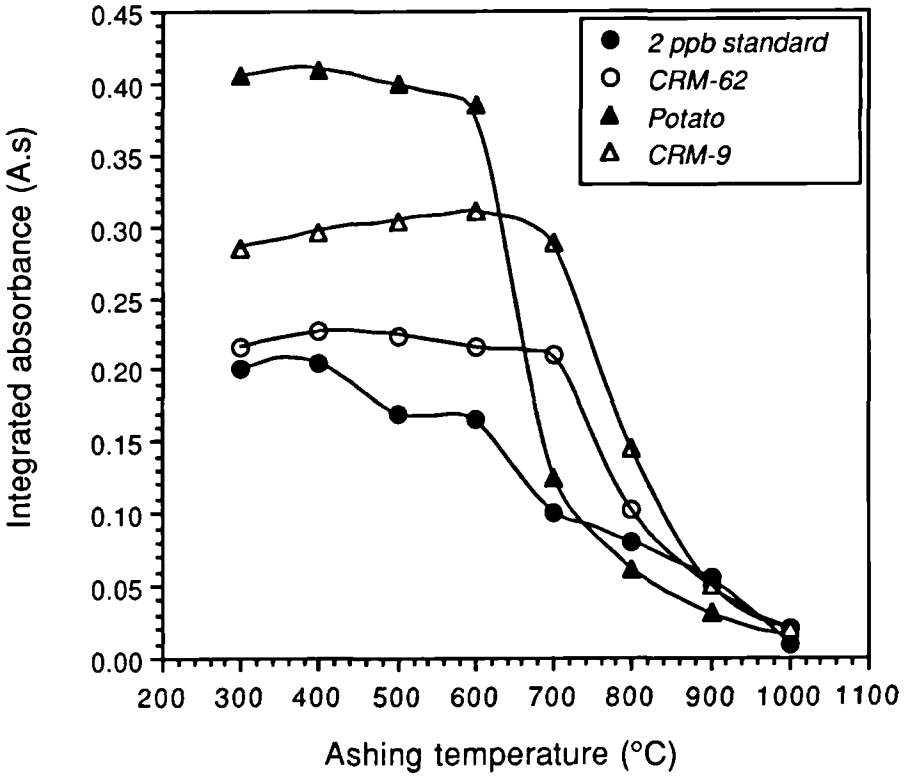


Figure 3.17: *Ashing curves for three typical biological samples and an aqueous standard (refer to Table 3.9 for descriptions of the biological samples).*

It is clear from the above figure that the optimum ashing temperature varies between samples, with the cadmium standard being more volatile

than the samples. The thermal stabilities of CRM-9 (Sargasso) and CRM-62 (Olive leaves) are very similar, both may be ashed at 700°C without the loss of the analyte. The inhouse potato sample has a lower optimum ashing temperature, ~600°C. If the samples are to be quantified against an aqueous calibration curve then an ashing temperature of 400°C should be used. Optimisation of the atomistion temperature was also performed with the aim of maximising both the integrated absorbance sensitivity and temporal resolution of the background and analyte signals. An atomisation temperature of 2000°C was used.

3.2.2.4: *Cadmium determinations for biological CRMs*

As with the method developed in Section 3.2.1, CRMs were analysed in order to assess the application of probe ETA-AAS for the analysis of biological samples for cadmium.

3.2.2.4.1: Digestion of CRMs

The CRMs chosen were considered to represent a broad spectrum of matrix types and analyte concentrations. In addition to these certified materials, a number of uncertified materials were also used in order to provide extra data on precision. Details of the materials used can be found in Table 3.9.

Code	Supplier	Description	Value (μg Cd g^{-1}DW)
Certified materials			
A-11	IAEA	Milk powder	0.0017±0.0002
CRM-10a	NIES	Rice flour - unpolished	0.023±0.003
SRM-1567a	NBS	Wheat flour	0.026±0.002
CRM-7	NIES	Tea leaves	0.030±0.003
CRM-62	BCR	Olive leaves	0.100±0.010
CRM-9	NIES	Sargasso	0.150±0.020
CRM-10b	NIES	Rice flour - unpolished	0.320±0.020
CRM-6	NIES	Mussel	0.820±0.030
CRM-60	BCR	Aquatic plant	2.200±0.100
Uncertified materials			
AQA-33	AEA	Cabbage (unspiked)	0.050±0.013
AQA-34	AEA	Cabbage (spiked)	0.43
AQA-35	AEA	Milk powder	<0.01
AQA-36	AEA	Milk powder (spiked)	0.40
Kale	MAFF	Kale	0.210±0.060
Potato	QMW	Potato (peeled)	No data
IAEA-155	IAEA	Whey powder	IC sample

NIES - National Institute for Environmental Studies,Tsukuba, Japan.

BCR - Community Bureau of Reference,Brussels, Belgium.

IAEA - International Atomic Energy Agency, Vienna, Austria.

NBS (now NIST) - National Institute of Standards and Technology, Washington, USA.

MAFF - Food Science Division, Ministry of Agriculture Fisheries and Food, Norwich, UK.

AEA - AEA Technology, Harwell, UK.

Table 3.9: *Certified and uncertified materials analysed.*

Samples (1.000±0.005g) were weighed into acid-washed 30ml borosilicate test-tubes, nitric acid (5.00ml) was added and the sample

heated for 1 hour at 110°C on a Techne Dri-block. Perchloric acid (1.00ml) was then added and the sample heated at 140°C until charring was observed, upon charring a further 1.00ml of nitric acid was added and the sample reheated. It should be noted that the use of perchloric acid for the digestion of fatty samples is hazardous. This process was repeated until no further charring was seen. The sample was then heated at 160°C and the volume reduced to ~2ml before being transferred to a 50ml volumetric flask and made up to volume with glass-distilled deionised water. Reagent blanks were carried through all of the above procedures. The moisture contents of the CRMs were determined according to the method stated in their certification papers and the results corrected accordingly.

In order to maintain the integrity of the data generated, a standard batch format was employed for all sample preparations and analyses. A batch contained eighteen samples, of which two were reagent blanks. All samples were analysed in quadruplicate, of which two of the samples were spiked with a quantity of cadmium sufficient to double their concentration. This enabled the recovery of cadmium from the whole procedure to be calculated and compensated for where necessary. An 'acceptable' recovery for these determinations was one that fell between 85 and 105%, samples whose recovery was outside of this range were reanalysed.

3.2.2.4.2: Analysis and results

All of the samples, except A-11, AQA-35, AQA-36 and IAEA-155, were analysed without the need for chemical modifiers or the use of standard additions. The relatively low atomisation temperature gave greater sensitivity for integrated absorbance values and allowed for temporal

resolution of the analyte and background signals, as can be seen in Figure 3.18.

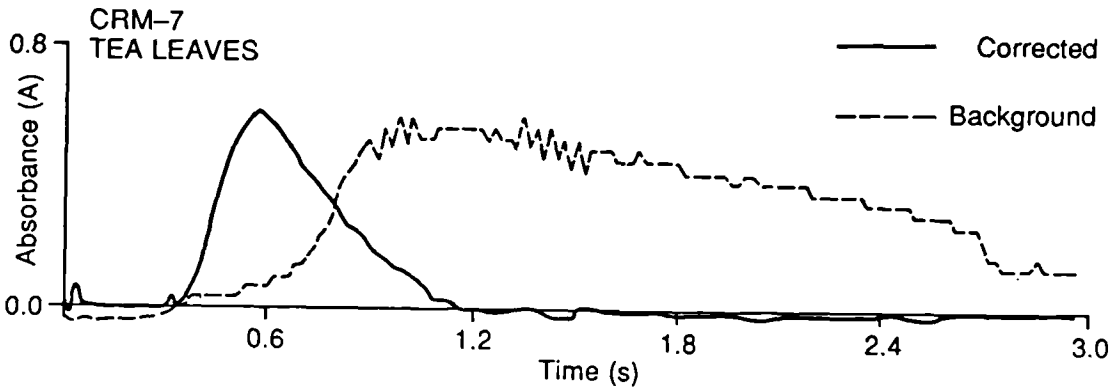


Figure 3.18: *Temporal resolution of analyte and background signals from the atomisation of 15µl of CRM-7 digest.*

A mean sensitivity of 1.62±0.33 pg for integrated absorbance values was obtained in the course of these analyses. Carroll *et al* (1986) report a peak height sensitivity of 1.4 pg using an end-entry tube probe design, an end-entry flat probe gave a sensitivity of 1.3 pg. A side-entry flat probe design, similar to that used in this work, gave a peak area sensitivity of 1.61 pg (Littlejohn *et al* 1984).

IAEA-155, AQA-35, AQA-36 and A-11 required the use of palladium and magnesium nitrate to allow an increased ashing temperature to be used and so reduce the background attenuation. The masses of palladium and magnesium used were the same as those used for the determination of a number of elements from a L'vov platform furnace (Schlemmer and Welz 1986). The ashing temperature was optimised in order to give the maximum peak area for the atomisation of a 20µl aliquot of A-11 or AQA-35 digest; this allowed sensitivity to be maximised and demonstrated the progressive reduction in over-correction. As can be seen from Figure 3.19, cadmium was stabilised up to 1100°C for both A-11 and an aqueous cadmium standard.

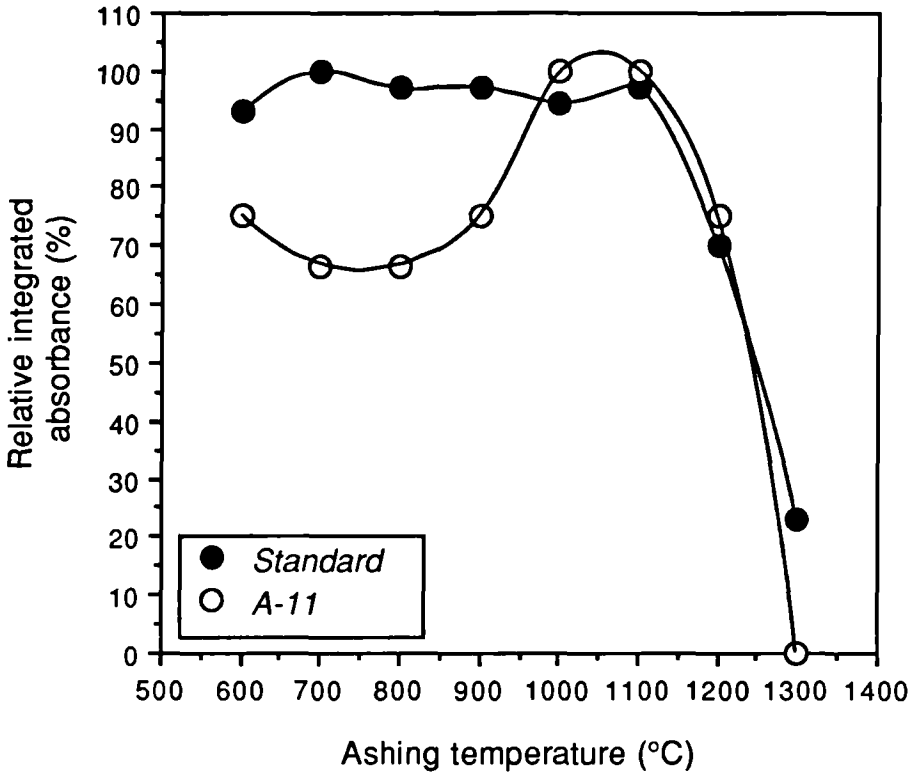


Figure 3.19: *Ashing curves for an aqueous standard and a digest of A-11 (milk powder) before and after chemical modification with 15µg palladium and 10µg magnesium nitrate.*

Figure 3.20 shows the effect of the addition of palladium and magnesium nitrates on the analyte and background signals when atomising IAEA-155 from an electrographite probe.

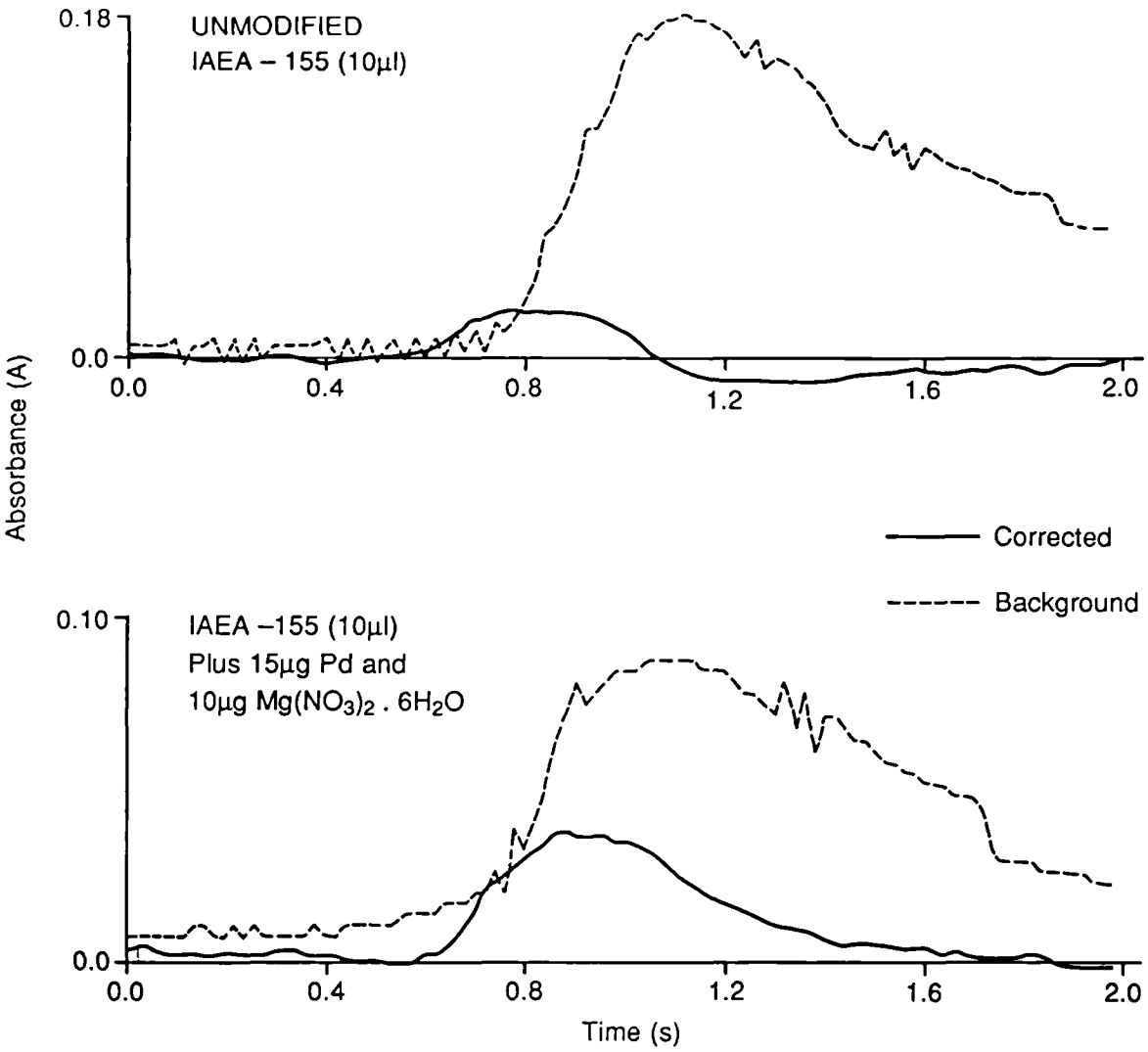


Figure 3.20: *The effect of palladium and magnesium nitrate on the analysis of whey powder.*

Table 3.10 gives a summary of the analyses of the CRMs and of the other data quality control materials. The value for precision is the interbatch value and is expressed as the relative standard deviation of samples analysed in different batches. Accuracy is expressed as the percentage deviation of the observed value from that given in the certificate of each material. The figure for recovery of the spike is the interbatch mean.

Code	n	Concentration	Recovery (%)	Precision (%)	Accuracy (%)
Certified materials					
A-11	8	<0.005	95±5	-	-
CRM-10a	12	0.025±0.009	92±14	3.4	+11
SRM-1567a	8	0.024±0.002	97±2	8.3	-6
CRM-7	12	0.029±0.003	101±1	8.8	-2
CRM-62	8	0.045±0.006	92±12	12.6	-9
CRM-9	8	0.150±0.009	90±6	1.0	0
CRM-10b	8	0.335±0.015	94±8	4.6	+5
CRM-6	8	0.832±0.139	87±7	16	+1
CRM-60	12	2.24±0.27	96±5	12	+2
Uncertified materials					
AQA-33	8	0.045±0.006	92±12	12.6	-9
AQA-34	8	0.470±0.037	105±1	7.9	+9
AQA-35	8	<0.008	100±2	10	-
AQA-36	8	0.466±0.021	98±7	4.4	+16
Kale	12	0.175±0.013	94±7	7.4	-
Potato	20	0.202±0.028	105±7	14.0	-
IAEA-155	12	0.029±0.005	92±2	19	-

NB: n = number of analyses. The figure for inter-batch precision is shown in the fifth column. Accuracy is defined as the percentage deviation of the observed value from the certified mean.

Table3.10: *Summary data for the analysed CRMs.*

3.2.2.4.3: Conclusions

The use of probe atomisation allows cadmium determinations to be made for biological certified reference materials, in the majority of cases it is possible to do so without the need to use chemical modifiers. Milk-based samples gave a high background attenuation which could only be removed by the inclusion of a higher temperature during the ashing

phase. In order to remove this interferring sample matrix and not loose the analyte, it was necessary to use a combination of magnesium and palladium nitrates as chemical modifiers.

3.3: DATA QUALITY

The quality of data generated using the techniques described in the preceding sections is a matter to which considerable attention should paid. Data quality has two components:

- accuracy, and
- precision.

Accuracy is defined by Miller and Miller (1988) as being the 'closeness to the truth' whereas precision is a measure of repeatability. This means that a precise analysis of a particular sample may not necessarily be an accurate analysis. Accurate but imprecise analyses are possible but it is unlikely that such data would be accepted as being of acceptable quality. If the data are said to be of good quality then they should be both precise and accurate.

Analytical error may arise in either a random or systematic manner. Random error will manifest itself in poor precision, systematic errors will tend to produce precise but inaccurate data. In ETA-AAS the most likely sources of random errors are in the sample preparation stages and when depositing the sample aliquot into the atomiser. Sturgeon and Berman (1987) have reviewed the problems associated with the storage and sampling of samples for trace element analysis. Systematic errors are more likely to arise during the samples treatment in the atomiser; for example, if the ashing temperature is too high a volatile element such as cadmium could quite easily be lost before the measurement phase of the time/temperature cycle. A variety of statistical methods are available for

the measure of both accuracy and precision and these have been considered by Miller and Miller (1988). Delimiting the boundary between what is or is not considered to be acceptable in terms of precision and accuracy is an area of some conflict. Precision, when expressed as the relative standard deviation, is likely to appear to be poor for samples with a low concentration of analyte, even though the standard deviation may only be one or two nanograms. At such low levels the effects of sample heterogeneity are likely to appear to be more manifest. Accuracy is probably best assessed with reference materials, the majority of which have been analysed by a range of techniques.

An important means by which data quality can be assessed for trace element analysis is the use of standard or certified reference materials (Veillon 1986). A certified reference material is defined as being "a reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is used by the certifying body." (Parr *et al* 1987).

Inter-laboratory comparisons for the determination of lead and cadmium in food samples have demonstrated discrepancies between laboratories (Sherlock *et al* 1985). A possible means by which the quality of these data for cadmium and lead could have been improved, is the routine use of certified reference materials. A growing range of materials exist for the control of cadmium data quality; both biological and environmental materials are available from a number of agencies (Muramatsu and Parr 1985). The five principal agencies that supply certified reference materials are the Community Bureau of Reference (BCR), the International Atomic Energy Agency (IAEA), the Office of Standard

Reference Materials (NBS), the National Institute for Environmental Studies (NIES) and the National Research Council Canada (NRCC).

Muramatsu and Parr (1985) list 41 certified biological reference materials as being available, with concentrations ranging from 189 to 0.0004 $\mu g g^{-1}$. A variety of sample matrices are available although it is still probable that the analyst will not be able to match the matrix of the samples with that of a suitable certified reference material.

3.4: CONCLUSIONS

This chapter has sought to critically assess the outcomes of two ETA-AAS method developments in the light of the development of the technique as a whole. ETA-AAS at its inception pointed the way towards an "absolute method of atomic absorption analysis" (L'vov 1978), the commercial application of this technique tended to generate a number of interference problems based around the non-isothermal environment within the graphite tube. In order to overcome this fundamental flaw a variety of adaptions have been made, including the use of chemical modifiers, pulse-heated furnaces and the L'vov platform. A number of these techniques were incorporated into the first method development, see Section 3.2.1. Section 3.2.2 describes the application of probe atomisation to the determination of cadmium in CRMs. By no means a panacea, probe atomisation does present a significant step towards the reduction of interferences caused by less isothermal furnace designs.

CHAPTER 4: CADMIUM BIOAVAILABILITY TO PLANTS GROWING ON SEWAGE SLUDGE-AMENDED SOILS

4.1: FIELD-BASED EXPERIMENTS

4.1.1: SAMPLE COLLECTION

The aim of these experiments was to examine the bioavailability of cadmium to plants from soils previously amended with sewage sludges. Zinc was also determined in view of the possible antagonistic reaction with cadmium (Kabata-Pendias and Pendias 1984). As briefly discussed in Chapter 1 and more extensively in Chapter 2, the application of sewage sludge to agricultural soils will tend to elevate their cadmium concentration and potentially increase the dietary cadmium intake of people. The key processes requiring investigation, if one is to assess the risks associated with the application of sewage sludges to soil, are those that determine bioavailability (a definition of what is meant by bioavailability is given at the start of Section 2.2.4).

Few theoretical models exist to explain the mechanism(s) by which cadmium enters a plant and as a result the majority of models have tended to be empirical. Cadmium bioavailability is affected by a number of physical and chemical soil variables. Therefore, when empirically examining the nature of cadmium bioavailability it is necessary to use a heterogeneous population of soils.

Samples with a history of sludge-amendment were collected from a number of locations in the UK, together with a number of control soils. A control soil for the purposes of this experiment is one to which no application of sewage sludge has been made. Reliable records of sludge

applications to soil only exist for the last fifteen years or so and this made site selection problematic (Alloway and Jackson 1987).

Twenty sites were selected and a bulk sample of topsoil, approximately 50kg, was taken from each one. Samples were collected by S Hopkinson and B J Alloway between November and December 1986, with additional samples collected in July 1988. Sampling procedures were designed to give a heterogeneous collection of soils and not to be representative of the particular sites. This meant that a sample tended to be collected from a small area of a each site. Table 4.1 gives details of the collected samples.

NUMBER	LOCATION	SOIL SERIES	YEAR SLUDGED
10/11	Checkley, Staffs	Wigton Moor series	1980
12/13	Canwick sewage works, Lincoln	Wickham series	1981
14/15	Canwick Manor Farm Lincoln	Elmton series	1974
16/17	Samlesbury, Preston	Flint series	1970
18/19	Stoke Bardolph 1, Notts	Wick/Wharfe series	1985-6
20/21	Stoke Bardolph 2, Notts	Wick/Wharfe series	1981
22/23	Ashton-Under-Lyme	Upland peat	1984
24/25	Ashton-Under-Lyme	Upland peat	Uncertain
26/27	Beverley	Fladbury series	1971
28/29	Colnbrook, Berks	Wickham series	1978-80
30/31	Pikesmead, Surrey	Wickham series	1985
32/33	Cassington A R_0, Oxon	Carswell series	Control*
34/35	Cassington B S_1R_4 Oxon	Sutton series	1979
36/37	Cassington A S_1R_4 Oxon	Carswell series	1979
38/39	Horley, Surrey	Wickham series	1976
40/41	Royston R_0, Herts	Swaffham Prior series	Control*
42/43	Royston S_1R_4	Swaffham Prior series	1979
44/45	Windsor	Wickham series	1982
46/47	Galley Hill, Essex	Windsor series	1975
48/49	Galley Hill , Essex	Windsor series	1975
50/51	Dytchleys, Essex	Wickham series	Control
52/53	Dytchleys, Essex	Wickham series	1987
54/55	Dytchleys, Essex	Wickham series	Spiked 1987

NB: Sample numbers 32/33 and 40/41 had sludge applied to one of the replicates, see below for details.

Table 4.1: *Bulk soil samples*

An additional group of soils was collected from the field by the author in July 1988. In addition to the soil samples, a number of grain and potato samples were collected from the same sites. Details of these samples can be found in Table 4.2.

NUMBER	LOCATION	SERIES	DESCRIPTION
57	Stoke Bardolph	Wick	Annual sludge application
58	Stoke Bardolph	Wick	High-metal site
59	Stoke Bardolph	Wick	Well drained area
60	Stoke Bardolph	Wick	Water-logged area
61	Northampton	Waterstock	Control site
62	Northampton	Waterstock	Possible control site
63	Northampton	Waterstock	Sludged site
64	Northampton	Waterstock	Heavily sludged site

Table 4.2: *Sites sampled for soils and wheat grain*

4.1.2: SAMPLE PREPARATION

All samples were passed through a coarse sieve and approximately halved, with each half being placed in a polyethylene tub (Stewart's "Shrub tub") of dimensions 35 X 35 X 30 cm. The tubs of soil were kept at the college field station (at Dytchleys, near Brentwood, Essex) in the open air for eight months before the first experiment was conducted. Two of the control soils (SN 32/33 and SN 40) were amended with sewage sludge at a rate equivalent to that applied to the experimental soils in 1979. The control soil from Cassington A (SN 32) had 3.68 kg sludge per tub applied to it, this is equivalent to the rate of the previous application to

the experimental soil (SN36/37). Sample 36/37 had been amended with sludge in 1979 at a rate of 300 t ha^{-1}. Sample number 40 from Royston was amended with 6.13 kg sludge, this is equivalent to the 500 t ha^{-1} applied to sample 42/43. Two of the soils from Dytchleys, numbers 52 and 53, were amended with sewage sludge at a rate equivalent to 300 t ha^{-1} . Sludge was applied to the surface of each soil and left for a week before being incorporated into the soil to a depth of ~20 cm. Soils 54 and 55 were spiked with inorganic cadmium salts

For those soils with a pH of less than 7, the odd numbered sample of each pair was limed to a neutral pH. In order to accurately calculate the quantity of lime required a number of laboratory experiments were performed. Five 10g samples of the air-dried <2mm fraction of each soil were weighed into acid-washed polyethylene bottles. 25ml of distilled deionised water were added to each sample. 0, 0.01, 0.02, 0.04 and 0.08g of AnalaR grade calcium carbonate were added to the five replicates from each sample. The bottle was then shaken vigorously for five seconds, before being placed on a Griffin orbital shaker. Samples were then agitated for sixteen hours at a rate of 200 rpm. The pH of each sample was then measured using a pH meter that had been previously calibrated against buffers of pHs 4, 7 and 9.2. Results are shown in Table 4.3.

	CALCIUM CARBONATE (g per 10g soil)				
NUMBER	0	0.01	0.02	0.04	0.08
11	5.79	6.35	6.91	7.80	8.95
13	4.59	5.32	6.00	6.65	8.02
17	6.16	6.46	6.80	7.20	7.90
19	5.92	6.66	7.13	7.83	8.98
21	5.56	6.47	7.35	8.04	9.20
23	5.92	6.20	6.46	6.85	7.54
25	4.99	5.12	5.28	5.69	6.28
29	6.11	6.63	7.15	8.12	9.82
31	4.22	4.58	4.93	5.43	6.12
33	6.28	7.18	7.76	8.72	10.09
35	5.06	5.89	6.41	7.14	8.38
37	6.28	6.75	7.10	7.94	9.48
39	5.19	5.92	6.80	7.00	7.95
45	5.45	5.75	6.22	6.87	7.70
47	6.48	6.88	7.48	8.16	9.32
49	5.79	6.19	6.54	7.12	8.22
51/53/55	6.58	6.97	7.60	8.05	9.04

Table 4.3: *Changes in soil pH after the addition of calcium carbonate*

The data were then examined by linear and second order polynomial regression analyses, to give an empirical relationship between pH and the mass of added calcium carbonate, Figure 4.1 shows some typical soil pH responses to the addition of calcium carbonate.

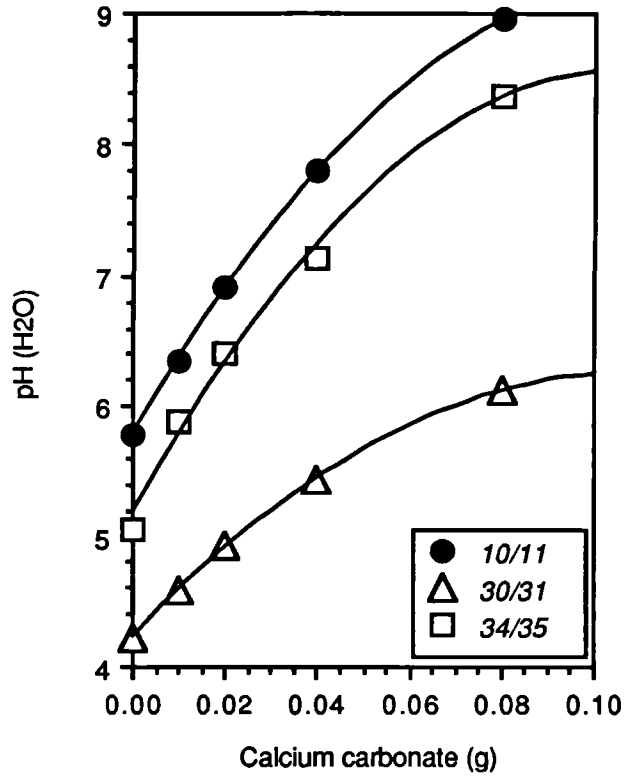


Figure 4.1: *The effect of calcium carbonate additions on soil pH*

The regression analyses that best fit the data are given in Table 4.4. These equations were used to determine the mass of calcium carbonate necessary to raise the pH of soils in the tubs to 7.

NUMBER	REGRESSION EQUATION	R^2 VALUE
11	$Y = 5.78 + 61.28X - 271.09X^2$	1.000
13	$Y = 4.67 + 64.04X - 281.14X^2$	0.992
17	$Y = 6.17 + 31.51X - 124.32X^2$	0.998
19	$Y = 6.01 + 58.61X - 270.47X^2$	0.995
21	$Y = 5.66 + 83.85X - 499.13X^2$	0.989
23	$Y = 5.93 + 26.84X - 84.99X^2$	0.999
25	$Y = 4.98 + 16.50X$	0.996
29	$Y = 6.11 + 54.14X - 96.28X^2$	1.000
31	$Y = 4.23 + 37.37X - 171.71X^2$	1.000
33	$Y = 6.36 + 75.18X - 359.43X^2$	0.997
35	$Y = 5.16 + 64.35X - 305.09X^2$	0.993
37	$Y = 6.32 + 39.75X$	0.999
39	$Y = 5.32 + 64.74X - 404.71X^2$	0.954
45	$Y = 5.41 + 43.42X - 183.87X^2$	0.998
47	$Y = 6.46 + 50.60X - 186.60X^2$	0.998
49	$Y = 5.88 + 29.83X$	0.995
51	$Y = 6.59 + 46.72X - 203.60X^2$	0.990

The dependent variable (Y) is the soil pH and the independent variable (X) is the mass of calcium carbonate added per 10 grams of soil.

Table 4.4: *Regression equations used to calculate the lime requirements for each of the experimental soils*

4.1.3: PLANT GROWTH

4.1.3.1: *Cabbages*

Cabbage seeds (*Brassica oleracea* cv. Greyhound) were germinated in John Innes No.2 and subsequently planted into the tubs during March 1988. Three plants were left to grow to maturity in each tub. The mature plants were harvested in late June 1988.

4.1.3.2: *Lettuces*

Lettuce seeds (*Latuca sativa* cv. Webbs Wonderful) were germinated in John Innes No.2 in seed trays prior to being planted out into the tubs during July 1988. Three plants were planted in each tub of soil. The mature plants were harvested in September.

4.1.3.3: *Potatoes*

Seed potatoes (*Solanum tuberosum* cv. Kingston) were planted into the tubs during April 1989 and harvested upon reaching maturity. Two seed potatoes were planted into each tub.

4.2: SOIL AND PLANT ANALYSES

4.2.1: SOIL CHARACTERISATION

4.2.1.1: *Sample preparation*

Soil samples were taken from the tubs prior to harvesting the plants. The samples were left to air dry for about one week at room temperature, before the < 2mm fine earth fraction was removed by passing the lightly ground sample through a nylon sieve. This fine earth fraction of the soil sample was used for all determinations of soil parameters. Samples were stored in the dark at room temperature. The moisture content of each of the soils under field conditions was determined gravimetrically.

4.2.1.2: *Soil pH*

5g of the fine earth fraction was weighed on a Sartorious three figure balance into an acid-washed plastic beaker. 15ml distilled deionised water was added to each sample and the sample shaken for five seconds. The sample was then placed on a Griffin orbital shaker and

agitated for 15 minutes at 200 rpm after which the sample was left to stand for 10 minutes. A pH meter was calibrated using standards of pHs 4, 7 and 9.2 and used to determine the pH of the sample. These data were recorded as pH (H_2O).

0.15ml of 1M $CaCl_2$ solution was then added to each sample which was then agitated for a further 30 minutes at 200 rpm on an orbital shaker. The sample was then left to stand for 10 minutes before the pH was determined using a calibrated pH meter. These data were recorded as pH ($CaCl_2$).

All of the above determinations were performed in duplicate.

4.2.1.3: *Soil organic matter content*

Soil organic matter content was determined gravimetrically using the method of Ball (1964). 2g of the fine earth fraction was placed into an pre-weighed acid-washed crucible using a Sartorious four figure balance. The sample was then placed into a drying oven set at 110°C for 16 hours and subsequently removed and left to cool in a dessicator. The sample was then reweighed and the percentage moisture content determined. The samples were placed in a muffle furnace at 375°C and heated for 16 hours. Samples were allowed to cool in a dessicator before weighing and the percentage loss-on-ignition calculated. All samples were analysed in duplicate.

4.2.1.4: *Ammonium nitrate soluble potassium concentration*

5g of the fine earth fraction were weighed into a plastic shaking bottle, to which was added 25ml 1M ammonium nitrate solution. The sample was agitated on an orbital shaker at 200 rpm for 1 hour, after which it was filtered through Whatman 42 filter paper into a boiling tube. 1ml of the

filtrate was pipetted into a 50ml volumetric flask and made up to volume with distilled deionised water. The sample was then analysed against aqueous standards by atomic emission spectrometry using a Pye Unicam SP1950. All samples were analysed in duplicate with reagent blanks.

4.2.1.5: *Sodium bicarbonate soluble phosphorus concentration*

The 'available' concentration of phosphorus was determined using a sodium bicarbonate extraction (Olsen and Sommers 1982). 2.5g of the fine earth fraction was weighed into a 250ml acid-washed extraction bottle. 50ml 0.5M sodium bicarbonate was added and the sample agitated on a Griffin orbital shaker for 30 minutes at 200 rpm. The sodium bicarbonate solution had been adjusted to pH 8.5 with 50% (V/V) sodium hydroxide. The sample was then filtered through Whatman 42 filter paper. 5ml of the filtrate was pipetted into a 50ml volumetric flask and acidified to pH5, 15ml distilled deionised water was added. 5ml of the mixed colour developing reagent was then added to the sample and left to stand at room temperature for 15 minutes. The mixed colour developing reagent was prepared as follows. 6g ammonium molybdate was dissolved in 125ml distilled deionised water and 0.146g antimony potassium tartrate was dissolved in 500ml 5N sulphuric acid. The two solutions were mixed in a 1 litre volumetric flask and made up to volume with distilled deionised water; this solution is now referred to as solution 1. To make the mixed colour developing reagent, 0.739g ascorbic was dissolved in 140ml solution 1.

Samples were analysed colorimetrically, using a Perkin Elmer Model 55 UV-visible spectrophotometer, against phosphorus standards made from potassium dihydrogen phosphate which had been reacted with the

mixed colour developing reagent. All samples were analysed in duplicate with reagent blanks.

4.2.1.6: *Manganese oxide concentration*

The manganese oxide concentration of the soils was determined using the method of Chao (1972). 1g of the fine earth fraction was weighed into an acid washed 100ml plastic shaking bottle to which was added 50ml of the extracting solution. The extracting solution was 0.1M hydroxylamine hydrochloride in 0.01M hydrochloric acid. The sample was agitated for three hours on a Griffin orbital shaker at 200 rpm, before being transferred to an acid-washed centrifuge tube. The sample was centrifugated at 2000rpm for 10 minutes, the resultant supernatant was filtered through Whatman 42 filter paper and subsequently analysed by atomic absorption spectrometry using an Instrumentation Laboratory S-12 aas. All samples were analysed in duplicate with reagent blanks.

4.2.1.7: *Dithionite extractable iron concentration*

The dithionite extractable iron concentration of the soils was determined using the method of Bascomb (1974). 1g of the fine earth fraction was weighed into a acid-washed shaking bottle. A buffer solution was made by mixing 1l M CH_3COONa with 390ml CH_3COOH and making up to 2.5l with distilled deionised water. 100ml of the buffer solution was added to the sample to which was added 4g sodium dithionite. The sample was agitated for 16 hours on a Griffin orbital shaker at 200 rpm before being transferred to an acid-washed centrifuge tube. The sample was centrifugated and the resultant supernatant filtered through Whatman 42 filter paper. Samples were then analysed by atomic absorption spectrometry. All samples were analysed in duplicate with reagent blanks.

4.2.1.8: *Total metal concentrations*

The total concentration of heavy metals in a soil is indicative of both the parent material and, in some cases, of the effects of human activity. In this respect, the application of sewage sludges will tend to increase the heavy metal concentration of soils. A variety of means exist for the determination of total concentrations of an analyte in solid samples, these include x-ray fluorescence spectrometry (XRF) and instrumental neutron activation analysis (INAA) (Ure 1990). Many instrumental methods of analysis require that the sample is in liquid form; therefore a means of sample dissolution is necessary.

It is at this point that one has to make the distinction between total and pseudo-total analyses. Where the sample is to be analysed in liquid form, a total analysis must include the complete dissolution of the sample matrix, for soil samples this includes the dissolution of silica and silicates. A method for the total analysis of soil samples is described in Section 4.2.1.8.1. Pseudo-total analyses are the more frequently used group of sample preparation procedures, given that sample preparation for total analyses are quite hazardous. Pseudo-total sample preparation usually involves the use of concentrated mineral acids such as HCl, HNO_3, $HClO_4$ or H_2SO_4. A method for the determination of pseudo-total analysis is described in Section 4.2.1.8.2.

Cao *et al* (1984) contrasted three sample preparation procedures for the determination of Cd, Cr, Cu, Ni, Pb and Zn in control soils and soils amended with sewage sludges. Three procedures were compared:

- 4M HNO_3 extraction
- $HClO_4$ digestion
- HF decomposition

The efficiencies of the three procedures were greater for sludge-treated soils than for control soils, perhaps reflecting the solid phase speciation of the metals concerned. For control soils, 4M HNO_3 extracted ~50% of the metal concentration of that after HF decomposition, this ratio increased to 70% for the sludge-treated soils. $HClO_4$ digestions were comparable to HF decomposition for Cd, Cr, Cu and Zn. Tjell and Hovmand (1978) compared HNO_3 extracted concentrations with the total concentrations of Li, Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb in a number of Danish arable soils. HNO_3 extraction yielded 80 to 100% of the total concentration.

4.2.1.8.1: HF and $HClO_4$ decomposition

The method of Walsh and Howie (1980) was used for the total analysis of soil samples. 0.50g of the fine earth fraction was weighed into an acid-washed PTFE beaker. 5ml 70% AristaR $HClO_4$ (V/V) and 15ml AtrisaR HF were pipetted onto each sample. The sample was then heated on a hot plate and allowed to evaporate to dryness, the sample was subsequently resuspended in 20ml 25% AristaR HCl (V/V) and allowed to stand for 1 hour at room temperature. The sample was then filtered through Whatman 42 filter paper into a 50ml volumetric flask and made up to volume with distilled deionised water. Samples were then analysed by both flame atomic absorption spectrometry (FAAS) and simultaneous inductively coupled plasma atomic emission spectrometry (ICPAES). Instrumental parameters for FAAS are given in Table 4.6. All samples were analysed in duplicate with reagent blanks.

4.2.1.8.2: HNO_3 sample extraction

2g of the fine earth fraction was weighed into an acid-washed 100ml conical flask and 20ml AristaR HNO_3 added. A reflux condenser was

placed on the neck of the flask which was then left to stand for sixteen hours at room temperature; this allowed the sample to be heated without the risk of losses due to boiling over the top of the flask. The sample was then heated on a hot plate at ~100°C for eight hours before the condenser was removed and the sample evaporated to near dryness. The residue was taken up into 10ml 6M HNO_3 and left for one hour at room temperature before being filtered through Whatman 42 filter paper into a 50ml volumetric flask, the sample was then made up to volume with distilled deionised water. All samples were prepared in duplicate with reagent blanks. Two certified reference materials were used to obtain data for the accuracy of the method. BCR No.143 "Overfertilized soil" and IAEA SL-1 lake sediment were chosen as they represent the extremes of heavy metal concentrations, as shown in Table 4.5. A major problem with the use of soil CRMs is that the sample preparation procedure may have a significant effect upon the value obtained.

ELEMENT	BCR No.143	IAEA SL-1
Cd	31.1±1.2	0.260±0.050
Cu	236.5±8.2	30.0±5.6
Pb	1333.0±39	37.7±7.4
Zn	1272.0±30	223.0±10

All units are in $\mu g g^{-1}$ DW.

Table 4.5: *Concentrations of selected elements in two certified reference materials*

Samples were analysed by FAAS using an S-12 double beam aas and Smith-Hieftje background correction. Instrumental parameters for these analyses are given in Table 4.6. Where the concentration was below the limit of detection for FAAS, probe atomisation ETA-AAS was employed.

	CADMIUM	COPPER	LEAD	ZINC
Wavelength (nm)	228.8	324.7	217.0	214
Bandwidth (nm)	1.0	1.0	1.0	1.0
Lamp current (mA)	2.5	5.0	4.0	3.5
Background current (mA)	1.5	2.5	3.0	2.5
PMT voltage (mV)	530	460	700	460

Table 4.6: *FAAS parameters for an IL S-12 aas*

4.2.1.9: *Extraction of the "bioavailable" cadmium and zinc fractions*

Partial extractions of metals from soils are used in order to try to predict the proportion of the total metal content that is available to plants. A number of approaches to these empirical measures of bioavailability have been suggested and are examined in Section 2.3.3.

4.2.1.9.1: EDTA-$(Na)_2$ extraction

The extraction of soils with 0.05M disodium ethylenediaminetetraacetic acid (EDTA-$(Na)_2$) buffered to pH7 has been found to provide a useful indication of the relative bioavailabilities of a number of heavy metals in soils. The method used here is based upon that used by the Ministry of Agriculture Fisheries and Food (Anon 1973).

5g of the fine earth fraction was weighed into an acid-washed 50ml polypropylene bottle, to which was added 30ml AnalaR grade 0.05M EDTA-$(Na)_2$. The sample was shaken for 1 hour at 150 rpm on a Griffin orbital shaker, before being transferred to an acid-washed centrifuge tube. After centrifugation at 2000 rpm for 10 minutes, the sample was filtered through Whatman 44 filter paper. Analysis for cadmium and zinc was by FAAS against a calibration with matrix matched standards.

Instrumental parameters for these analyses are shown in Table 4.6. All samples were analysed in duplicate with reagent blanks.

4.2.1.9.2: DTPA extraction

The DTPA soil test of Lindsay and Norvell (1978) was used to estimate the relative bioavailabilities of cadmium and zinc. The DTPA soil test was originally developed to identify near neutral or calcareous soils with Zn, Fe, Mn or Cu deficiencies (O'Connor 1988) and care must be taken when applying it to situations outside of this application. O'Connor identifies four classes of possible misuse:

- method alteration
- exceeding the pH buffering capacity of the extractant
- application to metals other then Zn, Mn, Cu and Fe
- the soil test reflects plant bioavailability only at soil concentrations in the deficiency region.

10g of the fine earth fraction was weighed into an acid-washed polypropylene bottle and 20ml of the extracting solution was added. This solution comprised of a mixture of 0.005M AnalaR grade diethylenetriaminepentaacetic acid (DTPA), 0.1M AnalaR grade triethanolamine (TEA) and 0.01M AnalaR grade calcium chloride; the pH of this solution was adjusted to 7.3. The sample was placed on an orbital shaker and agitated for 2 hours at 200 rpm, before being centrifugated for 10 minutes at 2000 rpm and the supernatant filtered through Whatman 42 filter paper . This solution was then analysed by FAAS for cadmium and zinc using the instrumental parameters in Table 4.6, the instrument had previously been calibrated against matrix matched standards. All samples were analysed in duplicate with reagent blanks.

4.2.1.9.3: $CaCl_2$ extraction

The extraction of soils with calcium chloride solutions has been shown to be indicative of the relative bioavailabilities of cadmium and zinc (Sauerbeck and Styperek 1984, Hani and Gupta 1985, Alloway and Morgan 1986). The method used was that of Alloway and Morgan (1986).

5g of the fine earth fraction was weighed into an acid-washed 100ml shaking bottle, 50ml 0.05M AnalaR grade calcium chloride was added and the sample agitated at 200 rpm on an orbital shaker for 16 hours. The sample was then transferred to an acid-washed centrifuge tube and centrifugated at 200 rpm for 10 minutes, the supernatant was filtered through Whatman 42 filter paper. Cadmium and zinc determinations were made by FAAS using the instrumental parameters in Table 4.6. All samples were analysed in duplicate with reagent blanks.

4.2.1.9.4: NH_4NO_3 extraction

Soil extraction with 1M NH_4NO_3 has been shown to be correlated with the cadmium concentration of plants growing on the same soil (Alloway 1986). The method used here was that used by Alloway and Morgan (1986).

5g of the fine earth fraction was weighed into an acid-washed 100ml shaking bottle, to which was added 50ml 1M NH_4NO_3. The sample was then agitated at 200 rpm on a Griffin orbital shaker for 1 hour. The sample was then transferred to an acid-washed centrifuge tube and centrifugated at 200 rpm for 10 minutes, the supernatant was filtered through Whatman 42 filter paper. Cadmium and zinc determinations were made by FAAS using the instrumental parameters in Table 4.6. All samples were analysed in duplicate with reagent blanks.

4.2.2: PLANT ANALYSIS

4.2.2.1: *Sample preparation*

Samples of lettuce and cabbage were taken from the field and thoroughly rinsed in distilled water before being stored in a freezer. Potato tuber samples were removed from the soil, rinsed in distilled water and subsequently frozen. The fresh weights of all samples were recorded.

Lettuce and cabbage samples were taken from the freezer and shredded with an acid-washed stainless steel knife. Potato tuber samples were peeled with an acid-washed stainless steel peeler and shredded with a grater. All samples were then dried at 65°C in a forced draught oven until no further change in weight was observed. For the samples of leaf tissue this usually required a drying time of 72 to 96 hours, potato tuber samples took between 96 and 144 hours to dry. The dried samples were then finely ground in a Fritsch Pulverisette centrifugal ball mill; in order to minimise the risk of contamination, acid-washed polyamide pots and agate balls were used for this process. The prepared samples were then stored in acid-washed 25g powder jars. It should be stressed that plastic surgical gloves were worn throughout the sample handling procedures, this, it was hoped, would reduce the risk of post-harvest contamination of the samples.

The grain samples were collected as ears of wheat and subsequently left to air dry for one week. The grains were then separated from the stems and the chaff removed by winnowing. The resultant "whole grain" was rinsed in distilled water, dried and then ground in a Fritsch Pulverisette centrifugal ball mill, as were the other plant samples.

4.2.2.2: *Sample digestion and analysis*

Two methods of sample digestion were evaluated and used in these analyses.

- microwave-aided pressure digestion in concentrated nitric acid
- wet digestion on a heated aluminium block.

Detailed descriptions of these two methods are given in Sections 3.2.1.5 and 3.2.2.4.1. In most cases, microwave digestions were only used when a limited sample mass was available or for a rapid screening of a batch of samples.

In order to maintain the integrity of the data generated a standard batch format was established and used throughout the analytical procedures. All batches of samples prepared contained system blanks and a reference or quality control material. All of the samples were analysed in at least triplicate with at least one replicate spiked with a known concentration of cadmium prior to sample dissolution. A typical batch is shown below:

BLANK	BLANK	BCR60	BCR60
BCR60.S	BCR60.S	10	10
10.S	10.S	11	11
11.S	11.S	12	12
12.S	12.S		

The suffix S is used to denote a spiked sample, the inclusion of which enables the recovery of the analyte to be determined and compensated for. Reference materials are included in order to identify systematic (accuracy) and random errors (precision) (Stoeppler 1983, Miller and Miller 1988).

All cadmium analyses were made by ETA-AAS. The lettuce and cabbage samples were analysed using the method described in Section 3.2.1, ie with the use of DAC tubes, Smith-Hieftje background correction and chemical modifiers. Samples of grain and potato tubers were analysed by probe atomisation ETA-AAS (see Section 3.2.2 for details). In addition to the certified reference materials, two inhouse quality control materials were used to assess the precision of the data acquired.

All zinc analyses were made by FAAS using the parameters defined in Table 4.6.

4.3: RESULTS

4.3.1: SOIL CHARACTERISATION

4.3.1.1: *Soil pH*

SN	SITE	pH (H_2O)	pH ($CaCl_2$)
10	Checkley	5.62±0.10	5.08±0.05
11	Checkley & lime	6.95±0.02	6.62±0.06
12	Canwick (sewage works)	4.61±0.23	4.05±0.02
13	Canwick (sewage works) & lime	6.75±0.05	6.16±0.28
14	Canwick (Manor Farm)	7.64±0.01	7.28±0.43
16	Samlesbury	6.03±0.12	5.52±0.28
17	Samlesbury & lime	6.91±0.14	6.30±0.00
18	Stoke Bardolph 1	5.77±0.08	5.03±0.18
19	Stoke Bardolph 1 & lime	6.65±0.00	6.21±0.06
20	Stoke Bardolph 2	6.01±0.34	5.40±0.03
21	Stoke Bardolph 2 & lime	6.75±0.39	6.03±0.16
22	Ashton-Under-Lyme 1	5.90±0.02	5.52±0.13
23	Ashton-Under-Lyme1 & lime	7.05±0.01	6.34±0.28
24	Ashton-Under-Lyme 2	5.15±0.02	4.23±0.11
25	Ashton-Under-Lyme 2 & lime	6.95±0.00	6.46±0.20
26	Beverley	7.57±0.54	6.96±0.08
28	Colnbrook	5.79±0.07	5.53±0.01
29	Colnbrook & lime	7.21±0.08	6.55±0.62
30	Pikesmead	4.32±0.09	3.88±0.19
31	Pikesmead & lime	6.55±0.04	6.01±0.09
32b	Cassington A R_0 & sludge	6.12±0.04	5.24±0.18
33	Cassington A R_0 & lime	7.50±0.31	6.74±0.05
34	Cassington B	5.77±0.21	4.63±0.11
35	Cassington B & lime	7.16±0.08	6.46±0.27
36	Cassington A	5.06±0.00	4.21±0.01
37	Cassington A & lime	7.22±0.46	6.51±0.06
38	Horley	5.14±0.06	4.62±0.14
39	Horley & lime	7.19±0.24	6.48±0.06
40b	Royston R_0 & sludge	7.76±0.02	7.10±0.32
41	Royston R_0	7.12±0.20	7.00±0.07
42	Royston	7.14±0.04	6.78±0.42
44	Windsor	5.53±0.26	5.28±0.12
45	Windsor & lime	6.65±0.34	6.01±0.06
46	Galley Hill 1	5.64±0.16	5.49±0.00
47	Galley Hill 1 & lime	6.45±0.12	5.71±0.01
48	Galley Hill 2	6.49±0.08	6.05±0.41
49	Galley Hill 2 & lime	6.94±0.12	6.42±0.03
50	Dytchleys	6.58±0.20	6.30±0.01
51	Dytchleys & lime	7.60±0.18	6.83±0.00
52	Dytchleys & sludge	6.62±0.10	6.26±0.04
53	Dytchleys & sludge & lime	7.09±0.10	6.54±0.26
54	Dytchleys & leaf mould & spike	6.58±0.12	5.45±0.18
55	Dytchleys & leaf mould & spike & lime	6.95±0.01	5.44±0.09
57	Stoke Bardolph S1	6.12±0.03	5.87±0.00
58	Stoke Bardolph S2	5.69±0.19	5.34±0.04
59	Stoke Bardolph S3	5.92±0.04	5.73±0.23
60	Stoke Bardolph S4	5.87±0.24	5.34±0.02
61	Northampton S1	6.50±0.04	6.12±0.42
62	Northampton S2	6.43±0.01	6.27±0.04
63	Northampton S3	6.05±0.00	5.87±0.02
64	Northampton S4	5.78±0.32	5.52±0.04

Table 4.7: *Soil pHs*

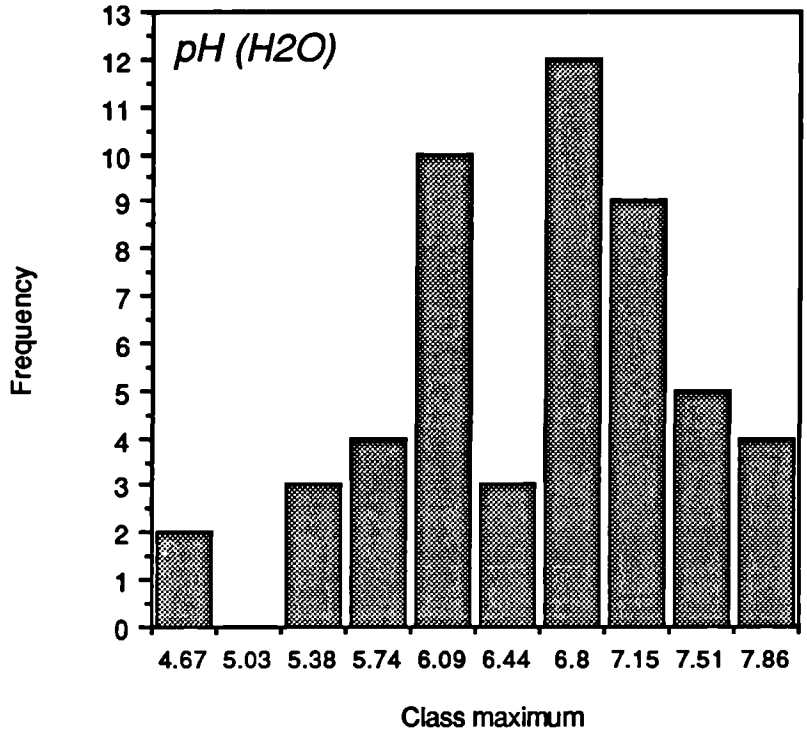


Figure 4.2: *Frequency distribution of soil pH values (H_2O)*

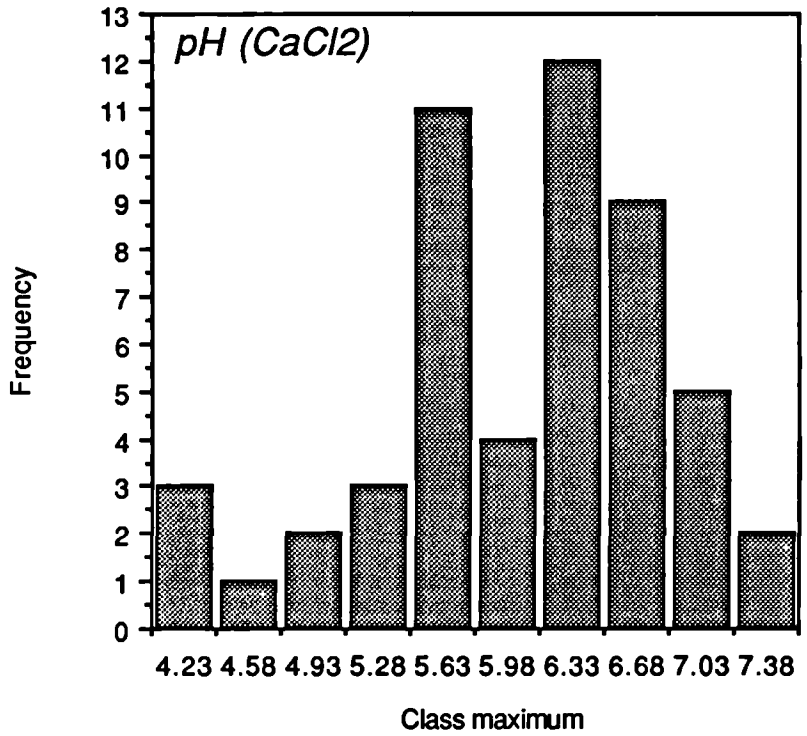


Figure 4.3: *Frequency distribution of soil pH values ($CaCl_2$)*

4.3.1.2: *Soil organic matter content*

SN	SITE	LOI (%)
10/11	Checkley	9.97±0.10
12/13	Canwick (sewage works)	15.85±1.2
14	Canwick (Manor Farm)	4.94±0.26
16/17	Samlesbury	2.69±0.50
18/19	Stoke Bardolph 1	8.49±0.05
20/21	Stoke Bardolph 2	9.87±0.01
22/23	Ashton-Under-Lyme 1	37.42±1.45
24/25	Ashton-Under-Lyme 2	39.31±2.41
26	Beverley	22.65±0.48
28/29	Colnbrook	4.11±0.02
30/31	Pikesmead	32.61±0.65
32b	Cassington A R_0 & sludge	8.95±0.26
33	Cassington A R_0 & lime	8.68±0.08
34/35	Cassington B	11.06±0.64
36/37	Cassington A	7.05±0.16
38/39	Horley	10.70±0.28
40b	Royston R_0 & sludge	11.36±0.51
41	Royston R_0	11.37±1.23
42	Royston	10.90±1.27
44/45	Windsor	31.56±2.47
46/47	Galley Hill 1	22.88±0.90
48/49	Galley Hill 2	15.42±1.09
50/51	Dytchleys	9.51±0.05
52/53	Dytchleys & sludge	10.02±0.39
54/55	Dytchleys & leaf mould & spike	9.13±0.11

Table 4.8: *Soil organic matter contents determined by loss-on-ignition*

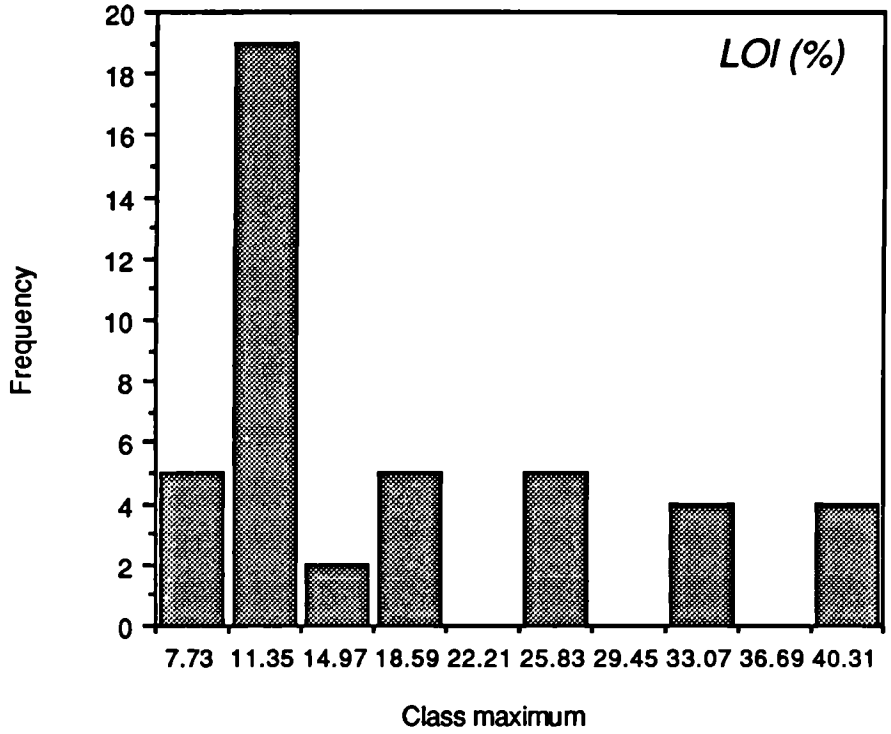


Figure 4.4: *Frequency distribution of loss-on-ignition values*

4.3.1.3: *Ammonium nitrate soluble potassium concentration*

SN	SITE	K ($\mu g g^{-1}$ DW)
10/11	Checkley	97.87
12/13	Canwick (sewage works)	65.97
14	Canwick (Manor Farm)	177.33
16/17	Samlesbury	324.61
18/19	Stoke Bardolph 1	184.11
20/21	Stoke Bardolph 2	191.86
22/23	Ashton-Under-Lyme 1	318.80
24/25	Ashton-Under-Lyme 2	197.67
26	Beverley	240.31
28/29	Colnbrook	155.04
30/31	Pikesmead	709.30
32b	Cassington A R_0 & sludge	224.67
33	Cassington A R_0 & lime	211.24
34/35	Cassington B	290.70
36/37	Cassington A	246.12
38/39	Horley	238.37
40b	Royston R_0 & sludge	543.53
41	Royston R_0	504.84
42	Royston	163.76
44/45	Windsor	277.13
46/47	Galley Hill 1	241.28
48/49	Galley Hill 2	566.86
50/51	Dytchleys	215.56
52/53	Dytchleys & sludge	286.69
54/55	Dytchleys & leaf mould & spike	218.79

Table 4.9: *'Available' soil potassium concentrations*

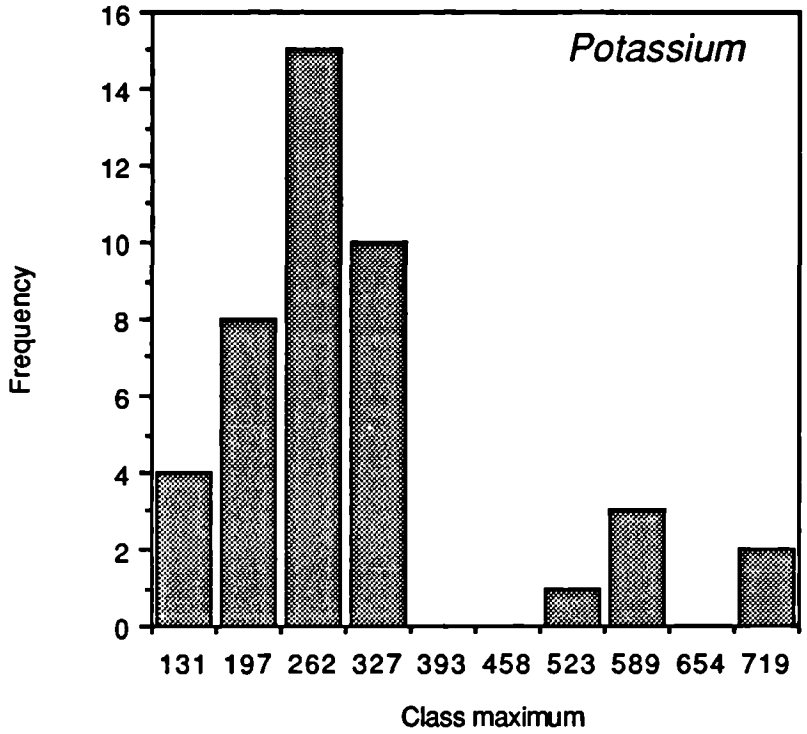


Figure 4.5: *Frequency distribution of extractable potassium values*

4.3.1.4: *Sodium bicarbonate soluble phosphorus concentration*

SN	SITE	P ($\mu g g^{-1}$ DW)
10/11	Checkley	77.04
12/13	Canwick (sewage works)	184.68
14	Canwick (Manor Farm)	99.72
16/17	Samlesbury	186.48
18/19	Stoke Bardolph 1	199.44
20/21	Stoke Bardolph 2	191.16
22/23	Ashton-Under-Lyme 1	276.48
24/25	Ashton-Under-Lyme 2	196.20
26	Beverley	192.96
28/29	Colnbrook	151.20
30/31	Pikesmead	176.76
32b	Cassington A R_0 & sludge	101.25
33	Cassington A R_0 & lime	211.24
34/35	Cassington B	78.84
36/37	Cassington A	213.84
38/39	Horley	135.00
40b	Royston R_0 & sludge	136.40
41	Royston R_0	123.84
42	Royston	284.40
44/45	Windsor	316.44
46/47	Galley Hill 1	208.80
48/49	Galley Hill 2	286.20
50/51	Dytchleys	124.00
52/53	Dytchleys & sludge	163.30
54/55	Dytchleys & leaf mould & spike	138.28

Table 4.10: *Soluble phosphorus concentrations*

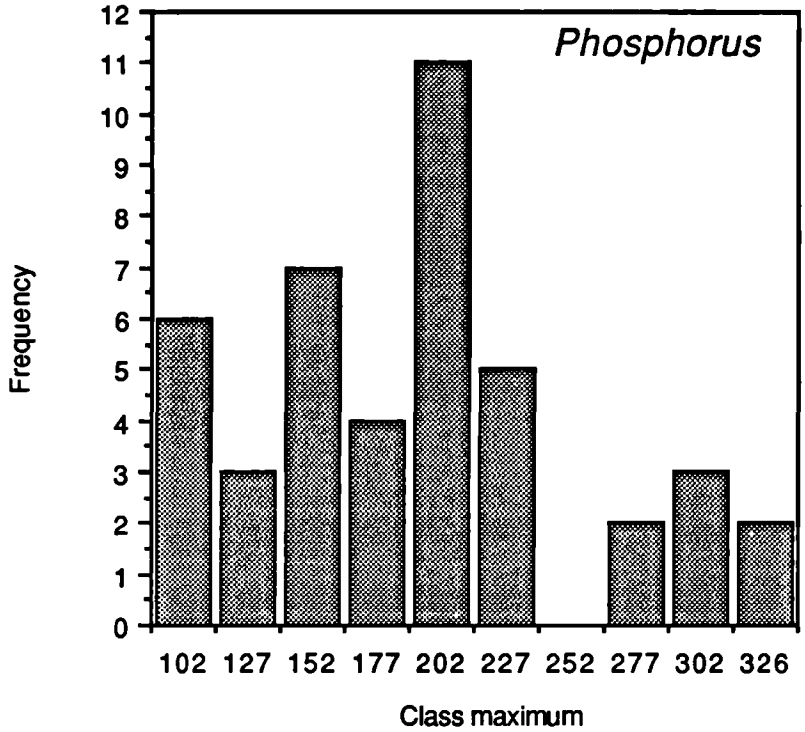


Figure 4.6: *Frequency distribution of soluble phosphorus concentrations*

4.3.1.5: *Easily reducible manganese oxide concentration*

SN	SITE	Mn ($\mu g g^{-1}$ DW)
10/11	Checkley	875±25
12/13	Canwick (sewage works)	8.5±0.5
14	Canwick (Manor Farm)	440±2
16/17	Samlesbury	440±2
18/19	Stoke Bardolph 1	1184.5±77
20/21	Stoke Bardolph 2	241.5±9
22/23	Ashton-Under-Lyme 1	355.5±5.5
24/25	Ashton-Under-Lyme 2	187±5
26	Beverley	218±9
28/29	Colnbrook	175.5±9.5
30/31	Pikesmead	198.5±9.5
32b	Cassington A R_0 & sludge	102±3
33	Cassington A R_0 & lime	90.5±12.5
34/35	Cassington B	98.5±12.5
36/37	Cassington A	601±26
38/39	Horley	655±90
40b	Royston R_0 & sludge	367±32
41	Royston R_0	331±2
42	Royston	294±6
44/45	Windsor	249±4
46/47	Galley Hill 1	219.5±7.5
48/49	Galley Hill 2	344±4
50/51	Dytchleys	77.5±2.4
52/53	Dytchleys & sludge	102±5
54/55	Dytchleys & leaf mould & spike	102.5±5.5
57	Stoke Bardolph S1	227±5
58	Stoke Bardolph S2	183±2
59	Stoke Bardolph S3	245±11
60	Stoke Bardolph S4	171.5±1.5
61	Northampton S1	125±6
62	Northampton S2	108±4
63	Northampton S3	148.5±1.5
64	Northampton S4	201±1

Table 4.11: *Concentrations of easily reducible manganese oxides*

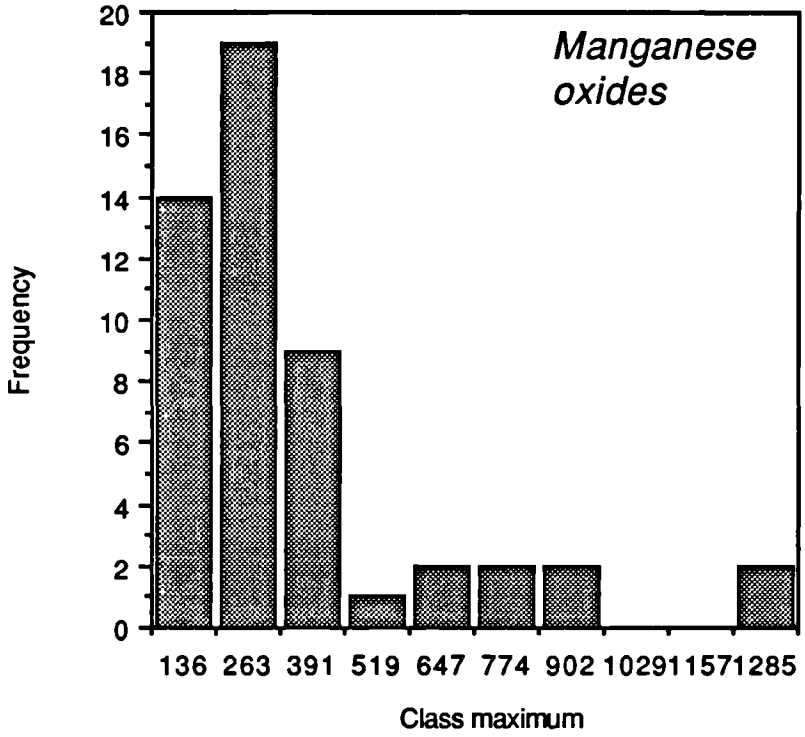


Figure 4.7: *Frequency distribution of manganese oxide concentrations*

4.3.1.6: *Dithionite extractable iron concentration*

SN	SITE	Fe (% DW)
10/11	Checkley	2.30±0.03
12/13	Canwick (sewage works)	0.69±0.03
14	Canwick (Manor Farm)	2.01±0.13
16/17	Samlesbury	2.89±0.02
18/19	Stoke Bardolph 1	1.62±0.17
20/21	Stoke Bardolph 2	1.62±0.05
22/23	Ashton-Under-Lyme 1	2.97±0.01
24/25	Ashton-Under-Lyme 2	2.61±0.01
26	Beverley	1.97±0.01
28/29	Colnbrook	1.26±0.16
30/31	Pikesmead	1.96±0.00
32b	Cassington A R_0 & sludge	2.87±0.04
33	Cassington A R_0 & lime	2.96±0.25
34/35	Cassington B	2.56±0.02
36/37	Cassington A	4.15±0.28
38/39	Horley	2.12±0.05
40b	Royston R_0 & sludge	0.67±0.11
41	Royston R_0	0.63±0.02
42	Royston	0.98±0.01
44/45	Windsor	1.28±0.01
46/47	Galley Hill 1	1.32±0.06
48/49	Galley Hill 2	1.70±0.05
50/51	Dytchleys	1.69±0.02
52/53	Dytchleys & sludge	1.65±0.06
54/55	Dytchleys & leaf mould & spike	1.40±0.04
57	Stoke Bardolph S1	1.45±0.01
58	Stoke Bardolph S2	1.45±0.01
59	Stoke Bardolph S3	1.05±0.02
60	Stoke Bardolph S4	1.20±0.07
61	Northampton S1	3.78±0.18
62	Northampton S2	3.50±0.03
63	Northampton S3	3.14±0.11
64	Northampton S4	2.51±0.08

Table 4.12: *Dithionite extractable iron concentrations*

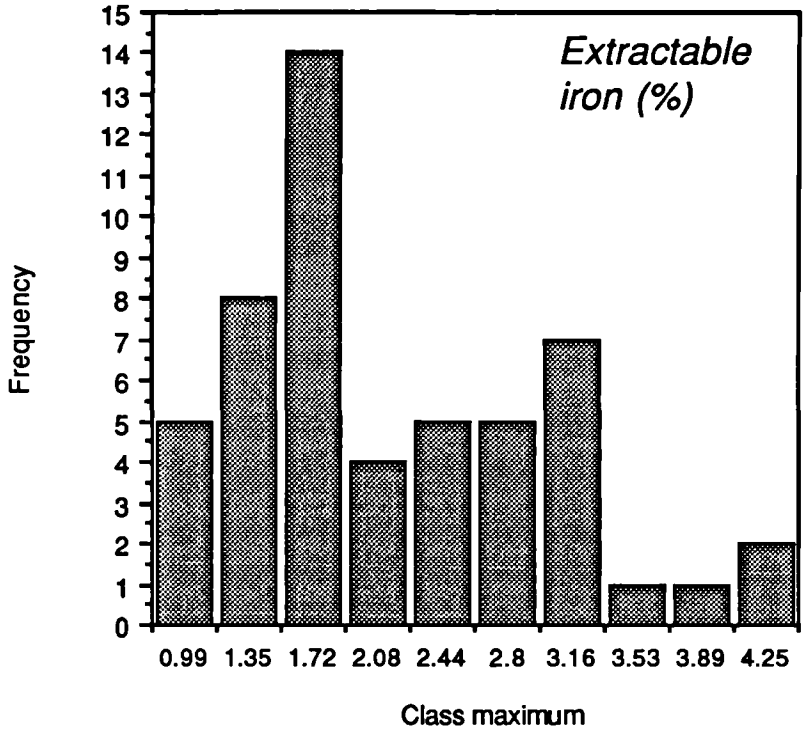


Figure 4.8: *Frequency distribution of dithionite extractable iron concentrations*

4.3.1.7: *Total metal concentrations*

4.3.1.7.1: Multi-element soil characterisation after $HF/HClO_4$ decomposition

Soil decomposition using HF and $HClO_4$ and was followed by ICPAES and FAAS analysis. Cadmium and lead determinations were only made by FAAS due to the relatively poor sensitivity of ICPAES for these elements.

SN	SITE	Ba	Ce	Co	Cu	Ni	Sc	Sr	V	Y	Zn
10/11	Checkley	646	54.2	72.8	140.8	81	10.3	83.3	114.5	19	692.8
12/13	Canwick (sewage works)	667.3	26	6	115.3	20	2.3	57.8	23.1	7.8	161.4
14	Canwick (Manor Farm)	208.2	40.6	9	37.6	27.6	6.9	142.2	59.9	14.6	86.7
16/17	Samlesbury	1949	40.2	19.5	486	105.2	6.9	114	61.9	16.8	784.1
18/19	Stoke Bardolph 1	569.4	52.2	14.3	164.4	154.5	6.9	71	54.3	16.8	488
20/21	Stoke Bardolph 2	587.8	55.4	15.8	166.7	152.6	8	73.1	58.5	17.9	474.9
22/23	Ashton-Under-Lyme 1	466.8	34.8	18	183.1	59.9	8	86.1	72.1	17.9	456.4
24/25	Ashton-Under-Lyme 2	398.8	34.8	17.3	120.9	56	8	60.2	72.1	15.6	219.4
26	Beverley	364.2	21.7	6	130.3	37.7	4.6	187.2	44.6	9.3	428
28/29	Colnbrook	454.8	45.7	8.3	73	36.5	6.9	66.6	49.5	18.7	174.5
30/31	Pikesmead	717.4	34.8	9.7	280.8	130.4	6.3	73.4	55.5	12.4	363.3
33	Cassington A R_0	300.8	67.4	11.8	33.9	49.3	14.6	76.4	107	34.2	119
34/35	Cassington B	444.5	62	11.8	108	72.1	13.6	90.1	100.1	30.1	305.1
36/37	Cassington A	373.2	60.9	15.2	86.4	67.5	9.4	69.5	103	31.1	241.6
38/39	Horley	558.2	58.7	13.9	149.1	55.6	9.4	76.4	82.2	20.7	436.6
41	Royston R_0	88.9	15.2	-	40.1	-	5.2	383.7	31.7	17.6	116.3
42	Royston	363.9	22.8	27.7	168.7	51.1	5.2	356.3	40.4	17.6	353.1
44/45	Windsor	363.9	22.8	27.7	168.7	51.1	5.2	254.5	50.3	12.4	1105.8
46/47	Galley Hill 1	618.4	38	19	798.1	543.4	3.1	232	36.5	11.4	2290.8
48/49	Galley Hill 2	563.4	37	16.1	516.2	238.6	6.3	166.4	60.1	15.6	1143.6

All concentrations are in $\mu g g^{-1}$ DW.

Table 4.13: *Trace element composition by ICPAES after $HF/HClO_4$ decomposition*

SN	SITE	Al_2O_3	Fe_2	MgO	CaO	Na_2	TIO	P_2O	MnO
10/11	Checkley	10.22	4.8	0.86	1.26	0.50	0.52	0.47	0.16
12/13	Canwick (sewage works)	3.27	1.49	0.2	0.3	0.21	0.15	0.5	0.01
14	Canwick (Manor Farm)	5.67	3.78	0.48	10.99	0.21	0.27	0.26	0.08
16/17	Samlesbury	6.9	5.6	0.66	1.31	0.34	0.32	2.11	0.2
18/19	Stoke Bardolph 1	7.36	3.29	0.7	0.96	0.41	0.44	0.84	0.06
20/21	Stoke Bardolph 2	6.98	4.12	0.82	1.22	0.4	0.42	0.81	0.06
22/23	Ashton-Under-Lyme 1	5.25	5.53	0.39	2.36	0.2	0.22	0.37	0.08
24/25	Ashton-Under-Lyme 2	5.93	5.65	0.37	1.14	0.2	0.22	0.37	0.05
26	Beverley	2.92	3.5	0.52	17.05	0.23	0.2	0.84	0.07
28/29	Colnbrook	5.78	3.62	0.38	0.73	0.47	0.45	0.45	0.05
30/31	Pikesmead	4.98	3.96	0.32	0.69	0.13	0.37	0.99	0.04
33	Cassington A R_0	12.87	6.29	0.76	1.11	0.38	0.66	0.23	0.08
34/35	Cassington B	12.03	5.65	0.76	1.19	0.35	0.64	0.61	0.04
36/37	Cassington A	5.92	10.31	0.38	0.87	0.31	0.33	0.72	0.16
38/39	Horley	9.45	5.23	0.49	0.67	0.2	0.63	0.63	0.11
41	Royston R_0	2.58	1.21	0.4	35.75	0.11	0.14	0.25	0.06
42	Royston	3.87	1.72	0.57	27.85	0.17	0.22	1.44	0.07
44/45	Windsor	4.45	2.84	0.63	4.82	0.39	0.35	2.91	0.06
46/47	Galley Hill 1	3.49	2.27	0.43	3.66	0.25	0.3	2.95	0.04
48/49	Galley Hill 2	5.72	3.4	0.63	3.37	0.39	0.34	1.72	0.07

All concentrations are in percentages

Table 4.14: *Major element composition by ICPAES after $HF/HClO_4$ decomposition*

SN	SITE	Cd	Zn	Cu	Pb
10/11	Checkley	46.0	828	176	863.3
12/13	Canwick (sewage works)	2.0	181.3	144.67	306.7
14	Canwick (Manor Farm)	0.58	85.3	20.0	66.7
16/17	Samlesbury	12.0	971.3	678.3	2116.7
18/19	Stoke Bardolph 1	11.33	644.7	206.0	203.3
20/21	Stoke Bardolph 2	9.0	349.9	211.7	250.0
22/23	Ashton-Under-Lyme 1	2.33	468.0	232.7	456.7
24/25	Ashton-Under-Lyme 2	0.65	344.7	135.0	900.0
26	Beverley	18.0	521.3	124.7	200.0
28/29	Colnbrook	6.0	704.7	84.7	203.3
30/31	Pikesmead	32.33	431.3	366.0	533.3
32b	Cassington A R_0 & sludge	2.67	357.8	156.4	98.4
33	Cassington A R_0 & lime	0.45	294.7	32.0	30.0
34/35	Cassington B	7.33	354.7	123.3	110.0
36/37	Cassington A	6.0	278.8	103.0	90.0
38/39	Horley	31.66	514.7	184.7	136.7
40b	Royston R_0 & sludge	3.89	255.6	109.8	92.1
41	Royston R_0	0.37	148.0	18.3	33.3
42	Royston	12.0	434.7	208.7	120.0
44/45	Windsor	8.0	1230	990.0	780.0
46/47	Galley Hill 1	139.0	2596.3	1025.0	196.7
48/49	Galley Hill 2	49.0	1263.0	685.0	320.0
50/51	Dytchleys	0.50	204.1	-	-
52/53	Dytchleys & sludge	3.27	393.3	-	-
54/55	Dytchleys & leaf mould & spike	113.8	210.3	-	-

All concentrations are in $\mu g g^{-1}$ DW.

Table 4.15: *Trace element composition by FAAS after $HF/HClO_4$ decomposition*

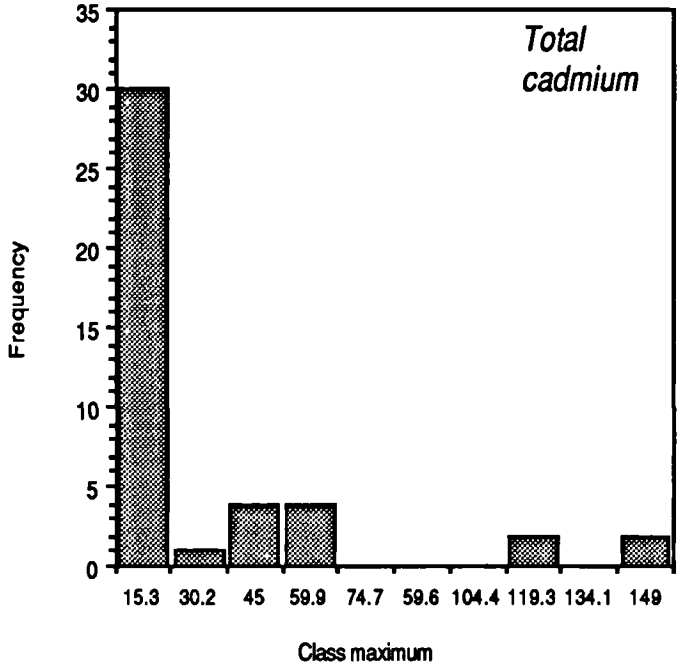


Figure 4.9: *Frequency distribution of total cadmium concentrations*

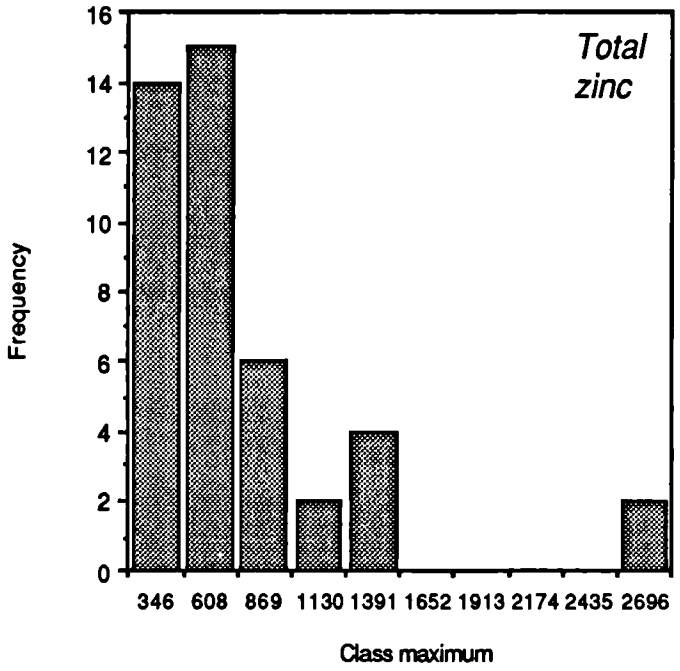


Figure 4.10: *Frequency distribution of total zinc concentrations*

4.3.1.7.2: Metal concentrations after HNO_3 extraction

All soil samples extracted with concentrated nitric acid were analysed for Cd, Cu, Pb and Zn by FAAS.

SN	SITE	Cd	Zn	Cu	Pb
10/11	Checkley	38.9±1.2	708.3±10	151.7±3.5	580.8±16
12/13	Canwick (sewage works)	1.5±0.1	162.5±8.2	153.3±6.7	291.5±9.5
14	Canwick (Manor Farm)	0.24±0.02	75.0±2.4	15.0±0.4	57.4±1.1
16/17	Samlesbury	11.1±0.3	704.2±4.1	615.0±24	1867±84
18/19	Stoke Bardolph 1	9.8±0.4	470±35	190.8±5.6	168.8±3.6
20/21	Stoke Bardolph 2	7.8±0.2	379.2±28	177.5±2.6	199.3±14
22/23	Ashton-Under-Lyme 1	2.12±0.1	354.2±15	203.3±6.4	445.8±17
24/25	Ashton-Under-Lyme 2	0.43±0.02	150±2.1	110±8	255.8±3.9
26	Beverley	15.5±0.7	370.8±15	121.7±1.8	162.1±2.8
28/29	Colnbrook	4.75±0.1	150±4.6	68.8±0.4	156±1.3
30/31	Pikesmead	29.2±0.45	308.3±4.1	340.8±3.9	484.2±2.8
32b	Cassington A R_0 & sludge	2.2±0.1	167±2.5	78.4±2.1	47.2±0.7
33	Cassington A R_0 & lime	0.36±0.02	129.2±2.5	18.7±0.6	25.4±1.1
34/35	Cassington B	6.3±0.2	266.6±2.8	115.8±1.4	95.6±0.6
36/37	Cassington A	5.3±0.3	241.7±1.7	97.0±0.7	88.1±0.6
38/39	Horley	25.8±0.3	300±26	152.5±3.9	137.3±0.6
40b	Royston R_0 & sludge	3.67±0.1	216.8±0.8	98.3±1.9	82.1±1.8
41	Royston R_0	0.24±0.02	118.8±5.4	12.8±1.3	34.4±0.8
42	Royston	8.58±0.54	304.2±3.1	166.7±1.5	128.4±0.6
44/45	Windsor	7.3±0.2	1125±17	874.2±2.6	706.7±4.8
46/47	Galley Hill 1	158.7±8.3	2350±38	1018.3±3.5	275.4±0.4
48/49	Galley Hill 2	43.2±1.2	1066.6±24.1	584.2±4.9	407.5±2.6
50/51	Dytchleys	0.41±0.03	178±9	-	-
52/53	Dytchleys & sludge	3.4±0.2	385±27	-	-
54/55	Dytchleys & leaf mould & spike	113±2.0	190.2±2.9	-	-
57	Stoke Bardolph S1	8.9±0.2	515.0±5	192.0±0	220.0±0
58	Stoke Bardolph S2	19.0±1	990.0±0	336.0±0	313.0±1
59	Stoke Bardolph S3	19.0±0	810.0±20	298.5±3.5	320.0±3
60	Stoke Bardolph S4	19.0±1	740±0	307.5±3.5	371.5±7.5
61	Northampton S1	0.27±0	97.0±1	17.6±0	24.2±0.2
62	Northampton S2	5.7±0.2	380±40	96.0±1	141.5±2.5
63	Northampton S3	23.0±0	840.0±40	226.0±1	429.0±5
64	Northampton S4	18.5±0.5	685.0±5	223.0±5	378.5±6.5

All concentrations are in $\mu g g^{-1}$ DW.

Table 4.16: *Trace element composition by FAAS following HNO_3 extraction*

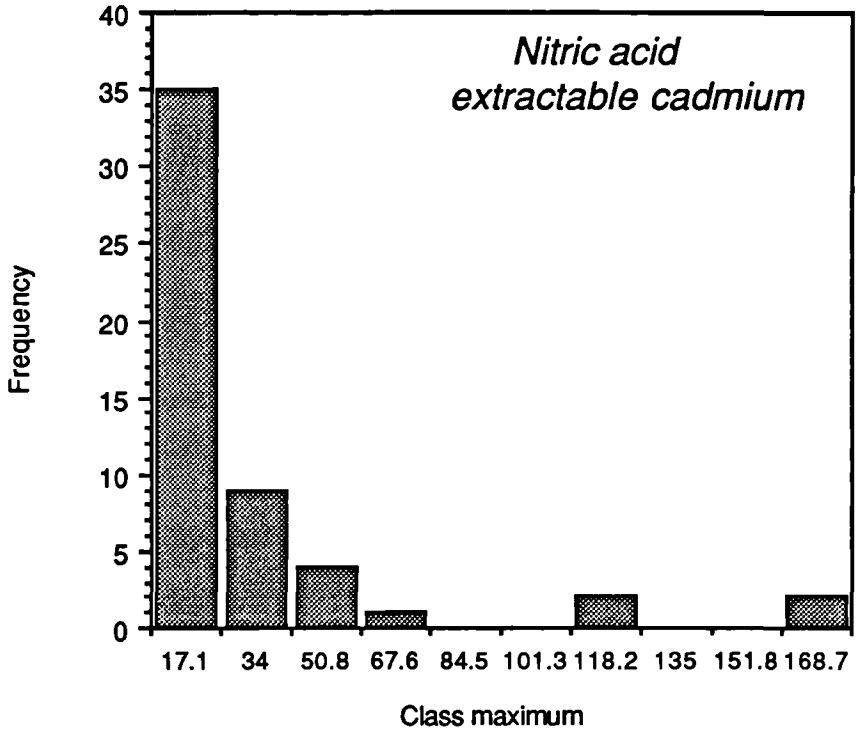


Figure 4.11: *Frequency distribution of nitric acid extractable cadmium concentrations*

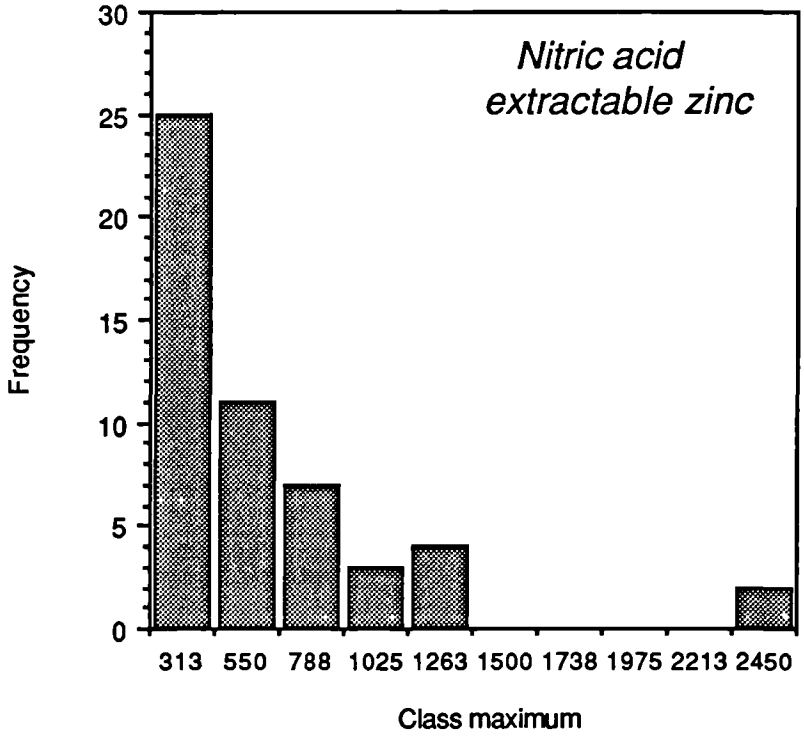


Figure 4.12: *Frequency distribution of nitric acid extractable zinc concentrations*

Data for a cadmium and zinc concentrations in a soil and a lake sediment reference material are shown in Table 4.17 below. All of these analyses were made by FAAS after nitric acid extraction.

	CADMIUM		ZINC	
SAMPLE	Certified	Observed	Certified	Observed
BCR No.143	31.1±1.2	31.1±2.5	1272±30	1272±36
IAEA SL-1	0.26±0.05	0.24±0.00	223±10	208±10

All concentrations are given in $\mu g g^{-1}$ DW.

Table 4.17: *Concentrations of cadmium and zinc in two certified reference materials*

4.3.1.8: *Concentrations of "bioavailable" cadmium and zinc*

4.3.1.8.1: Concentrations of EDTA-$(Na)_2$ extractable cadmium and zinc

		CADMIUM		ZINC	
SN	SITE	$\mu g g^{-1}$	%age	$\mu g g^{-1}$	%age
10	Checkley	31.2	80.1	307.50	43.41
11	Checkley & lime	37.2	95.51	53.40	7.54
12	Canwick (sewage works)	0.78	52.0	74.50	45.85
13	Canwick (sewage works) & lime	0.93	62.0	82.50	50.77
14	Canwick (Manor Farm)	0.18	36.0	15.50	20.67
16	Samlesbury	5.91	53.34	357.00	50.7
17	Samlesbury & lime	6.78	61.19	412.50	58.58
18	Stoke Bardolph 1	6.96	70.8	241.50	51.38
19	Stoke Bardolph 1 & lime	6.3	64.09	210.00	44.68
20	Stoke Bardolph 2	5.64	72.03	217.50	57.36
21	Stoke Bardolph 2 & lime	5.49	70.12	211.50	55.78
22	Ashton-Under-Lyme 1	1.29	64.50	246.00	69.46
23	Ashton-Under-Lyme1 & lime	0.72	36.0	142.50	40.24
24	Ashton-Under-Lyme 2	0.57	61.96	100.50	67.0
25	Ashton-Under-Lyme 2 & lime	0.48	52.17	40.50	27.0
26	Beverley	6.01	38.77	121.13	32.67
28	Colnbrook	3.42	72.0	88.50	59.0
29	Colnbrook & lime	3.15	66.32	63.00	42.0
30	Pikesmead	30.0	102.85	246.00	79.81
31	Pikesmead & lime	26.1	89.48	156.00	50.61
32b	Cassington A R_0 & sludge	2.35	105.86	-	-
33	Cassington A R_0 & lime	0.74	98.67	19.5	15.10
34	Cassington B	5.07	81.12	148.5	55.69
35	Cassington B & lime	4.92	78.72	135.0	50.63
36	Cassington A	3.72	69.79	114.0	47.18
37	Cassington A & lime	3.21	60.23	102.0	42.21
38	Horley	19.2	74.33	148.5	49.50
39	Horley & lime	22.8	88.27	117.8	39.27
40b	Royston R_0 & sludge	1.58	47.73	-	-
41	Royston R_0	0.20	39.0	12.38	10.43
42	Royston	3.92	45.69	73.5	24.17
44	Windsor	2.94	40.11	592.5	52.67
45	Windsor & lime	2.22	30.29	366.0	32.53
46	Galley Hill 1	85.8	54.07	1590.0	67.66
47	Galley Hill 1 & lime	88.2	55.59	1597.5	67.98
48	Galley Hill 2	28.2	65.32	726.0	68.06
49	Galley Hill 2 & lime	22.2	51.43	504.0	47.25
50	Dytchleys	0.12	26.61	16.4	0.09
51	Dytchleys & lime	0.33	91.92	21.0	0.12
52	Dytchleys & sludge	1.14	36.73	28.5	0.07
53	Dytchleys & sludge & lime	1.08	30.74	86.85	0.24
54	Dytchleys & leaf mould & spike	57.0	51.58	27.6	0.14
55	Dytchleys & leaf mould & spike & lime	108.0	93.59	18.3	0.10

Percentages are expressed as the percentage of the nitric acid extractable concentration.

Table 4.18: *EDTA-$(Na)_2$ extractable cadmium and zinc*

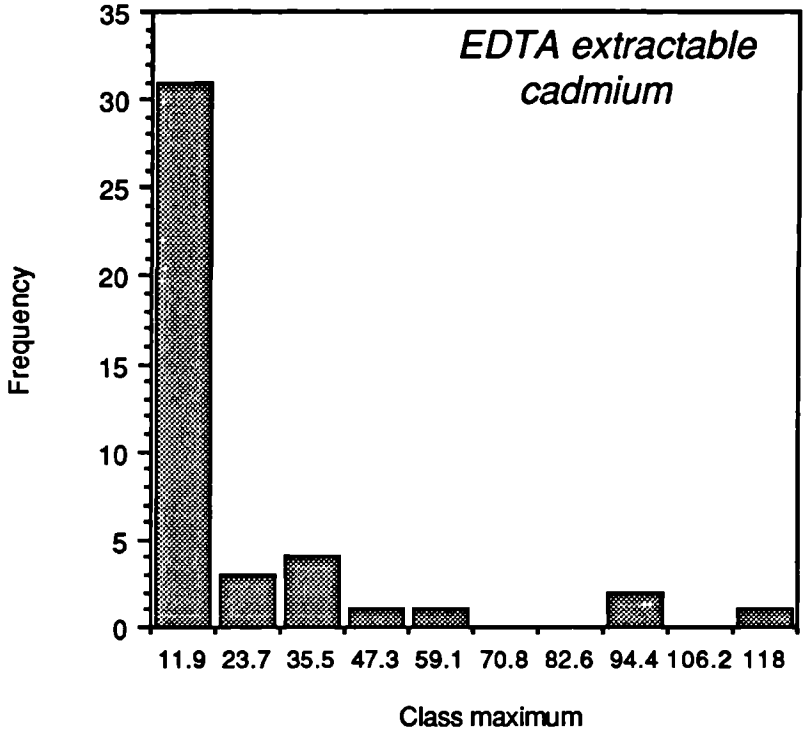


Figure 4.13: *Frequency distribution of EDTA-$(Na)_2$ extractable cadmium concentrations*

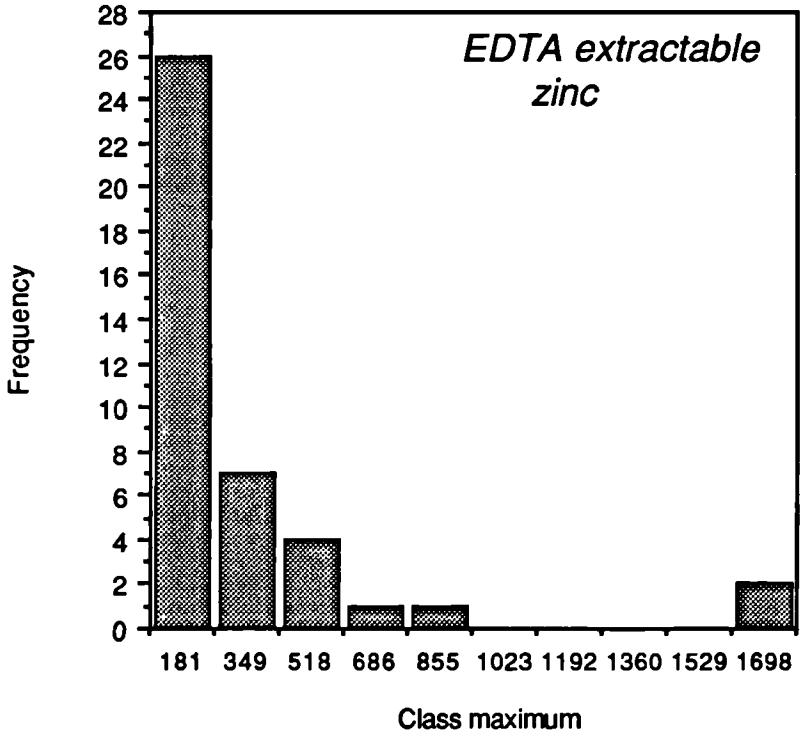


Figure 4.14: *Frequency distribution of EDTA-$(Na)_2$ extractable zinc concentrations*

4.3.1.8.2: Concentrations of DTPA extractable cadmium and zinc

		CADMIUM		ZINC	
SN	SITE	μgg^{-1}	%age	μgg^{-1}	%age
10	Checkley	31.79	81.62	226.40	31.96
11	Checkley & lime	22.59	58.00	116.90	16.50
12	Canwick (sewage works)	0.92	61.33	52.20	32.12
13	Canwick (sewage works) & lime	0.84	56.00	51.40	31.63
14	Canwick (Manor Farm)	0.14	28.00	2.20	2.93
16	Samlesbury	4.73	42.69	187.30	26.60
17	Samlesbury & lime	5.19	46.84	167.70	23.82
18	Stoke Bardolph 1	5.20	52.90	127.40	27.11
19	Stoke Bardolph 1 & lime	3.73	37.95	90.30	19.21
20	Stoke Bardolph 2	4.02	51.34	109.40	28.85
21	Stoke Bardolph 2 & lime	4.26	54.41	83.60	22.05
22	Ashton-Under-Lyme 1	1.24	62.00	204.80	57.83
23	Ashton-Under-Lyme1 & lime	0.64	32.00	72.40	20.44
24	Ashton-Under-Lyme 2	0.39	42.39	51.30	34.20
25	Ashton-Under-Lyme 2 & lime	0.47	51.09	22.80	15.20
26	Beverley	6.05	39.03	110.80	29.89
28	Colnbrook	3.18	66.95	56.70	37.80
29	Colnbrook & lime	2.81	59.16	30.10	20.07
30	Pikesmead	30.20	103.53	31.70	10.28
31	Pikesmead & lime	19.13	65.58	58.40	18.95
32b	Cassington A R_0 & sludge	0.93	41.89	-	-
33	Cassington A R_0 & lime	0.56	74.67	11.80	9.14
34	Cassington B	3.94	63.04	97.10	36.42
35	Cassington B & lime	3.78	60.48	38.10	14.29
36	Cassington A	2.25	42.21	51.20	21.19
37	Cassington A & lime	1.76	33.02	30.10	12.46
38	Horley	22.03	85.29	136.20	45.40
39	Horley & lime	14.75	57.10	51.50	17.17
40b	Royston R_0 & sludge	1.06	32.02	38.10	19.55
41	Royston R_0	0.30	60.00	8.80	7.41
42	Royston	3.61	42.08	56.50	18.58
44	Windsor	1.29	17.60	198.90	17.68
45	Windsor & lime	1.67	22.78	168.50	14.98

SN	SITE	CADMIUM		ZINC	
		μgg^{-1}	%age	μgg^{-1}	%age
46	Galley Hill 1	31.37	19.77	268.00	11.40
47	Galley Hill 1 & lime	17.00	10.71	226.40	9.63
48	Galley Hill 2	12.60	29.19	242.30	22.72
49	Galley Hill 2 & lime	6.52	15.10	208.80	19.58
50	Dytchleys	0.40	88.69	60.10	5.2
51	Dytchleys & lime	0.27	75.21	60.50	6.2
52	Dytchleys & sludge	0.68	21.91	200.70	5.8
53	Dytchleys & sludge & lime	0.69	19.14	180.90	10.5
54	Dytchleys & leaf mould & spike	54.00	48.87	90.10	12.9
55	Dytchleys & leaf mould & spike & lime	103.10	89.26	90.80	18.2
57	Stoke Bardolph S1	3.40	38.20	105.60	20.50
58	Stoke Bardolph S2	6.84	36.00	192.90	19.48
59	Stoke Bardolph S3	7.77	40.90	183.10	22.61
60	Stoke Bardolph S4	8.90	46.84	186.40	25.19
61	Northampton S1	0.14	51.47	9.96	10.27
62	Northampton S2	2.48	43.51	83.20	21.89
63	Northampton S3	9.47	41.17	175.80	20.93
64	Northampton S4	6.17	33.35	125.00	18.25

Percentages are expressed as the percentage of the nitric acid extractable concentration.

Table 4.19: *DTPA extractable cadmium and zinc*

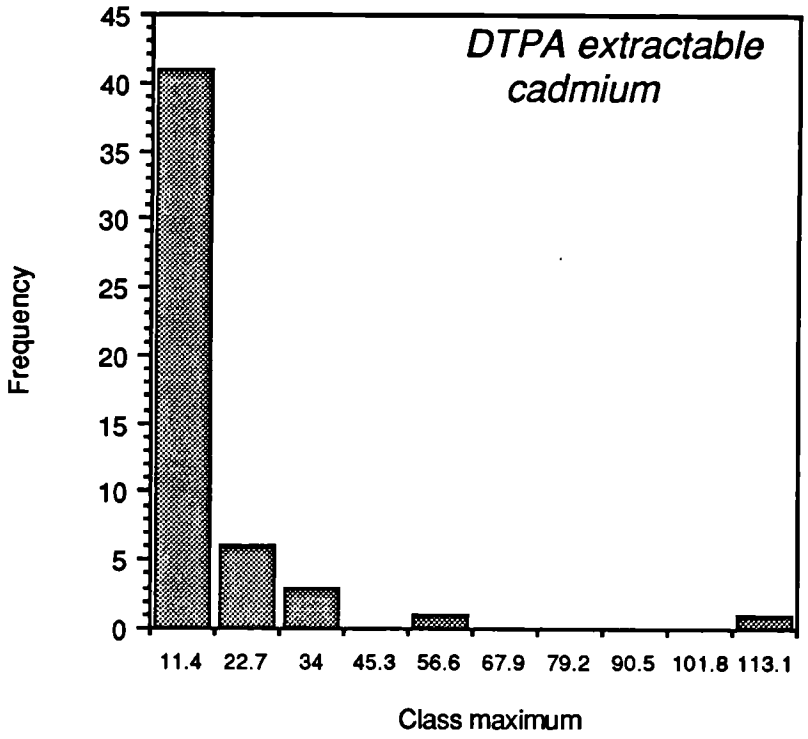


Figure 4.15: *Frequency distribution of DTPA extractable cadmium concentrations*

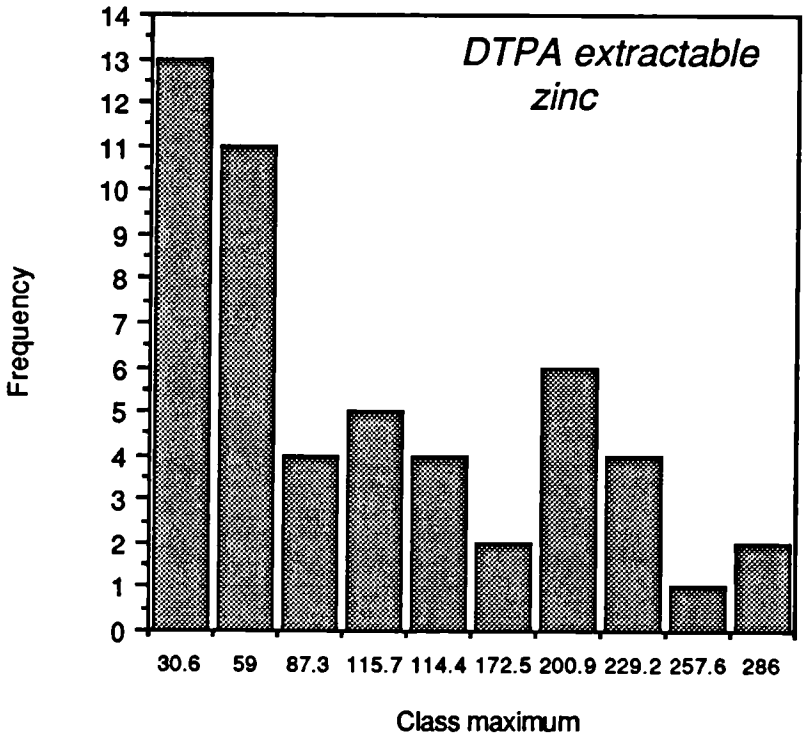


Figure 4.16: *Frequency distribution of DTPA extractable zinc concentrations*

4.3.1.8.3: Concentrations of $CaCl_2$ extractable cadmium and zinc

		CADMIUM		ZINC	
SN	SITE	$\mu g g^{-1}$	%age	$\mu g g^{-1}$	%age
10	Checkley	12.06	30.96	57.0	8.05
11	Checkley & lime	1.98	5.09	2.524	0.36
12	Canwick (sewage works)	0.66	44.0	48.0	29.54
13	Canwick (sewage works) & lime	0.06	3.97	1.85	1.14
14	Canwick (Manor Farm)	<LOD	<LOD	0.18	0.24
16	Samlesbury	0.78	7.04	16.50	2.34
17	Samlesbury & lime	0.24	2.16	2.87	0.41
18	Stoke Bardolph 1	1.5	15.26	16.50	3.51
19	Stoke Bardolph 1 & lime	0.66	6.70	3.29	0.70
20	Stoke Bardolph 2	0.90	11.49	10.50	2.77
21	Stoke Bardolph 2 & lime	0.48	6.10	2.87	0.76
22	Ashton-Under-Lyme 1	0.12	6.00	9.0	2.54
23	Ashton-Under-Lyme1 & lime	<LOD	<LOD	0.79	0.22
24	Ashton-Under-Lyme 2	0.24	26.09	30.0	20.0
25	Ashton-Under-Lyme 2 & lime	<LOD	<LOD	0.78	0.52
26	Beverley	0.06	0.39	0.60	0.16
28	Colnbrook	1.56	32.84	12.0	8.0
29	Colnbrook & lime	0.3	6.27	1.13	0.75
30	Pikesmead	17.10	58.62	111.0	36.01
31	Pikesmead & lime	1.55	5.32	3.34	1.09
32b	Cassington A R_0 & sludge	0.12	5.41	4.55	2.46
33	Cassington A R_0 & lime	<LOD	<LOD	0.60	0.47
34	Cassington B	1.80	28.80	24.0	9.0
35	Cassington B & lime	0.30	4.77	1.37	0.51
36	Cassington A	0.54	10.13	4.92	2.04
37	Cassington A & lime	0.12	2.25	0.84	0.35
38	Horley	10.50	40.65	49.50	16.50
39	Horley & lime	1.25	4.64	1.07	0.36
40b	Royston R_0 & sludge	0.27	8.01	3.45	1.77
41	Royston R_0	<LOD	<LOD	0.429	0.361
42	Royston	0.12	1.40	1.14	0.38
44	Windsor	0.24	3.27	13.50	1.20
45	Windsor & lime	0.12	1.65	12.71	1.13

SN	SITE	CADMIUM µgg^{-1}	CADMIUM %age	ZINC µgg^{-1}	ZINC %age
46	Galley Hill 1	6.0	3.78	34.50	1.47
47	Galley Hill 1 & lime	5.40	3.40	33.61	1.43
48	Galley Hill 2	1.26	2.92	6.30	0.59
49	Galley Hill 2 & lime	1.04	2.40	4.94	0.46
50	Dytchleys	0.06	13.30	4.08	0.02
51	Dytchleys & lime	<LOD	<LOD	0.478	<LOD
52	Dytchleys & sludge	0.24	7.89	5.65	0.01
53	Dytchleys & sludge & lime	0.06	1.66	0.66	<LOD
54	Dytchleys & leaf mould & spike	43.33	39.21	0.66	<LOD
55	Dytchleys & leaf mould & spike & lime	67.69	58.66	0.542	<LOD

Percentages are expressed as the percentage of the nitric acid extractable concentration. <LOD = less than limit of detection. For FAAS analyses LOD = 0.025 µg Cd g^{-1} DW.

Table 4.20: *$CaCl_2$ extractable cadmium and zinc*

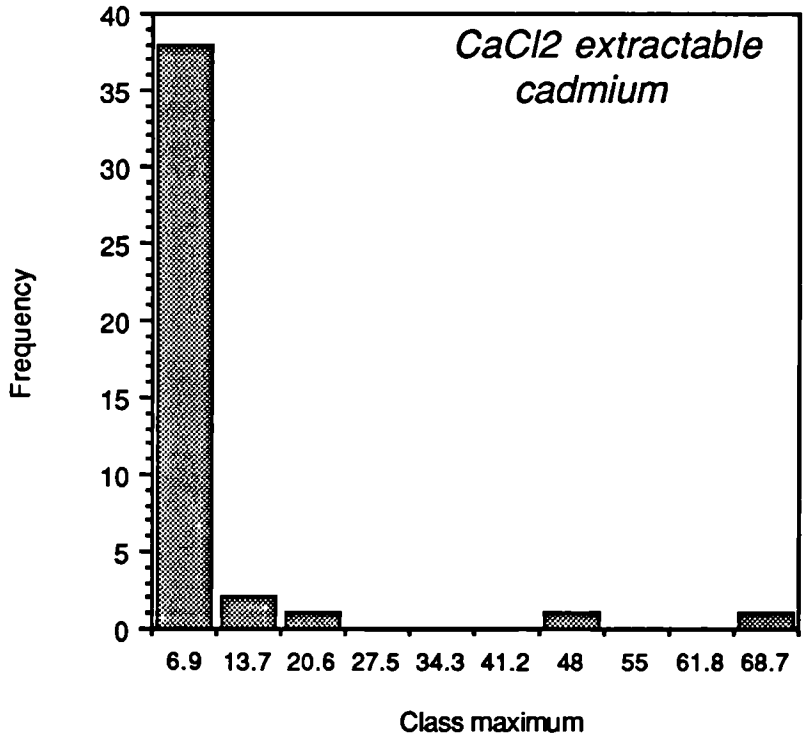


Figure 4.17: *Frequency distribution of $CaCl_2$ extractable cadmium concentrations*

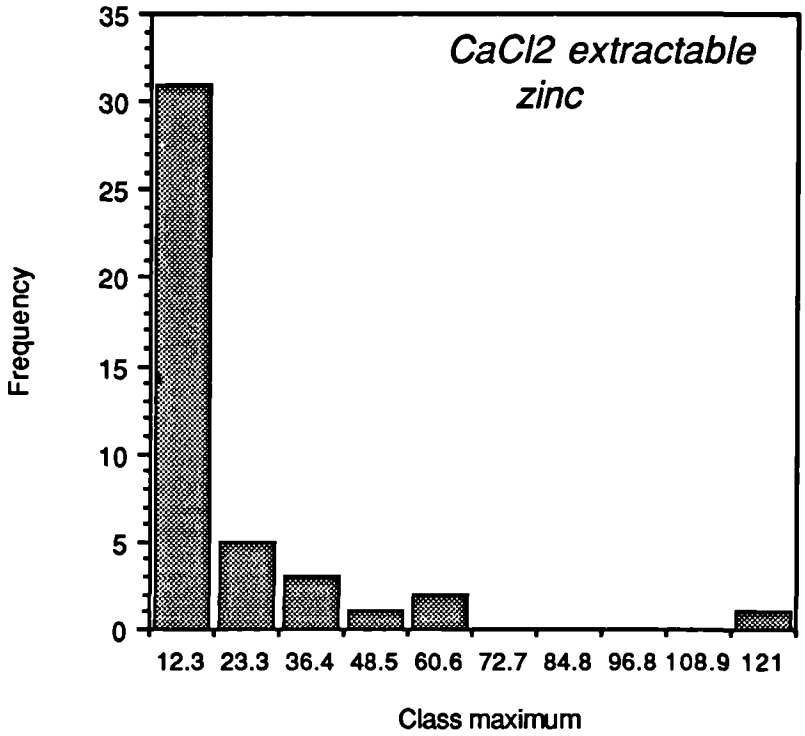


Figure 4.18: *Frequency distribution of CaCl2 extractable zinc concentrations*

4.3.1.8.4: Concentrations of NH_4NO_3 extractable cadmium and zinc

		CADMIUM		ZINC	
SN	SITE	μgg^{-1}	%age	μgg^{-1}	%age
10	Checkley	2.82	7.24	43.50	6.14
11	Checkley & lime	0.60	1.54	1.38	0.20
12	Canwick (sewage works)	0.30	20.0	39.0	24.0
13	Canwick (sewage works) & lime	<LOD	<LOD	1.26	0.78
14	Canwick (Manor Farm)	0.06	12.0	0.36	0.48
16	Samlesbury	0.24	2.17	13.50	1.92
17	Samlesbury & lime	0.06	0.54	1.93	0.27
18	Stoke Bardolph 1	0.42	4.27	9.00	1.92
19	Stoke Bardolph 1 & lime	0.06	0.60	0.651	0.14
20	Stoke Bardolph 2	0.24	3.07	3.0	0.79
21	Stoke Bardolph 2 & lime	<LOD	<LOD	0.72	0.19
22	Ashton-Under-Lyme 1	0.54	27.0	7.50	2.12
23	Ashton-Under-Lyme1 & lime	<LOD	<LOD	0.60	0.17

		CADMIUM		ZINC	
SN	SITE	$\mu g g^{-1}$	%age	$\mu g g^{-1}$	%age
24	Ashton-Under-Lyme 2	0.30	32.61	23.50	15.67
25	Ashton-Under-Lyme 2 & lime	<LOD	<LOD	0.54	0.36
26	Beverley	0.06	0.387	0.30	0.08
28	Colnbrook	0.30	6.32	3.90	2.60
29	Colnbrook & lime	0.06	1.27	0.24	0.16
30	Pikesmead	7.38	25.30	85.50	27.74
31	Pikesmead & lime	0.30	1.03	1.56	0.51
32b	Cassington A R_0 & sludge	0.09	4.28	1.34	0.73
33	Cassington A R_0 & lime	<LOD	<LOD	0.06	0.05
34	Cassington B	0.66	10.56	30.0	11.25
35	Cassington B & lime	0.06	0.96	0.30	0.11
36	Cassington A	0.06	1.13	2.28	0.94
37	Cassington A & lime	<LOD	<LOD	0.06	0.03
38	Horley	3.3	12.78	39.0	13.0
39	Horley & lime	0.299	1.159	0.539	0.18
40b	Royston R_0 & sludge	0.241	7.281	0.56	0.287
41	Royston R_0	<LOD	<LOD	<LOD	<LOD
42	Royston	<LOD	<LOD	<LOD	0.02
44	Windsor	0.06	0.819	4.98	0.443
45	Windsor & lime	<LOD	<LOD	3.483	0.31
46	Galley Hill 1	0.78	0.492	10.5	0.447
47	Galley Hill 1 & lime	1.139	0.718	15.288	0.651
48	Galley Hill 2	0.18	0.417	1.86	0.174
49	Galley Hill 2 & lime	0.12	0.278	1.199	0.112
50	Dytchleys	<LOD	<LOD	3.719	0.02
51	Dytchleys & lime	<LOD	<LOD	<LOD	<LOD
52	Dytchleys & sludge	0.06	1.933	2.806	0.006
53	Dytchleys & sludge & lime	<LOD	<LOD	0.182	0.005
54	Dytchleys & leaf mould & spike	30.351	27.467	8.272	0.042
55	Dytchleys & leaf mould & spike & lime	38.354	33.236	5.094	0.027

Percentages are expressed as the percentage of the nitric acid extractable concentration.
<LOD = less than limit of detection. For FAAS analyses LOD = 0.025 μg Cd g^{-1} DW.

Table 4.21: *NH_4NO_3 extractable cadmium and zinc*

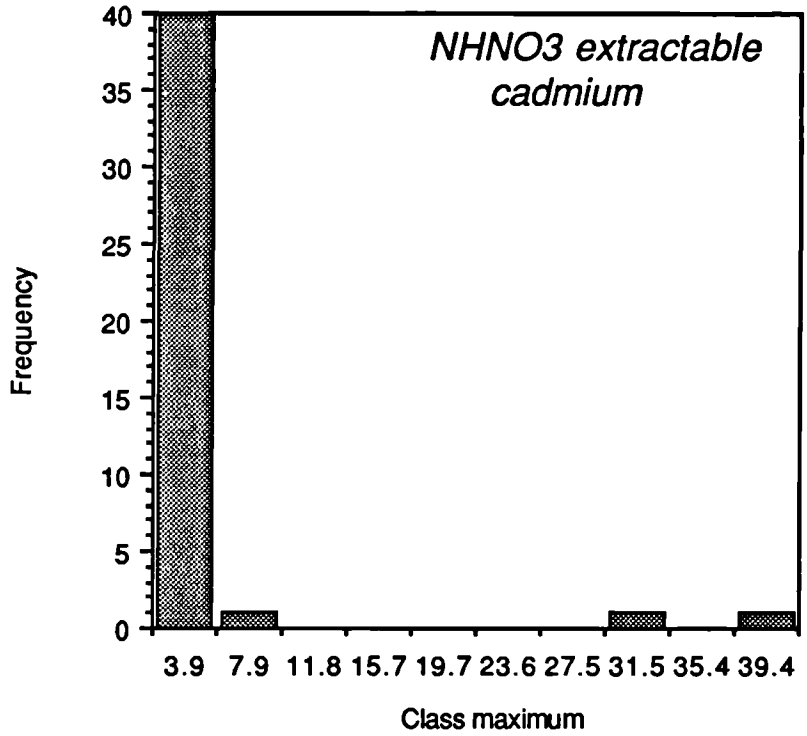


Figure 4.19: *Frequency distribution of NH_4NO_3 extractable cadmium concentrations*

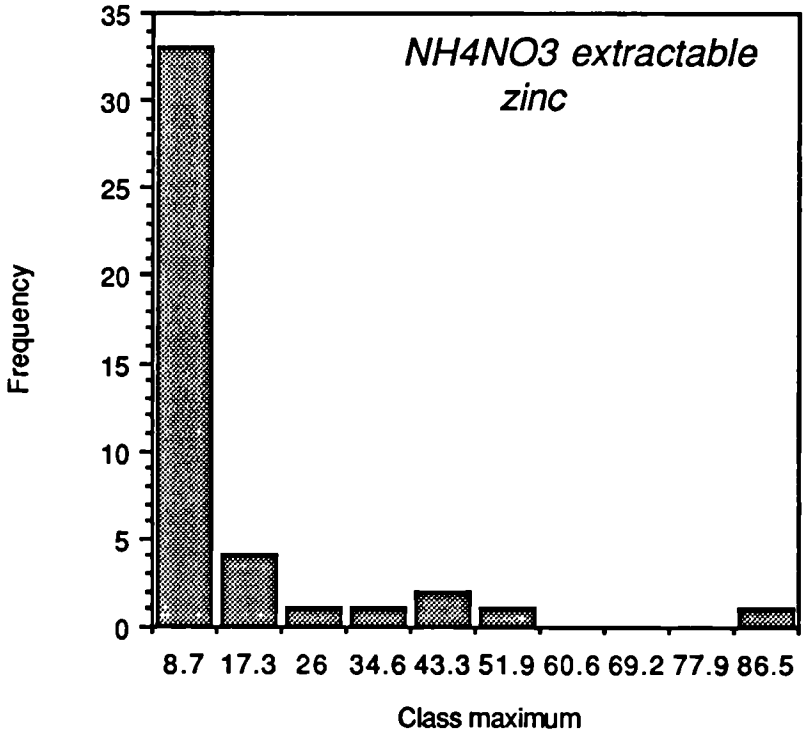


Figure 4.20: *Frequency distribution of NH_4NO_3 extractable zinc concentrations*

4.3.2: PLANT ANALYSIS

4.3.2.1: *Concentrations of cadmium in cabbages grown on sludge-amended soils*

SN	SITE	µg Cd g^{-1} DW	µg Cd g^{-1} FW
10	Checkley	4.89±0.31	0.48±0.03
11	Checkley & lime	1.55±0.14	0.15±0.01
12	Canwick (sewage works)	1.49±0.08	0.15±0.01
13	Canwick (sewage works) & lime	0.4±0.03	0.04±0
14	Canwick (Manor Farm)	0.62±0.06	0.06±0.01
15	Samlesbury	1.06±0.05	0.11±0
17	Samlesbury & lime	0.34±0.02	0.03±0
18	Stoke Bardolph 1	2.49±0.07	0.25±0.01
19	Stoke Bardolph 1 & lime	1.36±0.12	0.14±0.01
20	Stoke Bardolph 2	2.14±0.09	0.21±0.01
21	Stoke Bardolph 2 & lime	1.04±0.10	0.10±0.01
22	Ashton-Under-Lyme 1	0.18±0.01	0.02±0
23	Ashton-Under-Lyme1 & lime	0.03±0.01	0.003±0
24	Ashton-Under-Lyme 2	0.31±0.02	0.03±0
25	Ashton-Under-Lyme 2 & lime	0.15±0.01	0.02±0
26	Beverley	0.35±0.01	0.04±0
28	Colnbrook	1.01±0.04	0.10±0
29	Colnbrook & lime	0.54±0.05	0.05±0
30	Pikesmead	14.68±1.81	1.44±0.18
31	Pikesmead & lime	9.65±0.88	0.96±0.09
32b	Cassington A R_0 & sludge	1.05±0.06	0.10±0.01
33	Cassington A R_0 & lime	0.31±0.03	0.03±0
34	Cassington B	2.11±0.22	0.21±0.02
35	Cassington B & lime	0.66±0.04	0.07±0
36	Cassington A	0.55±0.03	0.06±0
37	Cassington A & lime	0.38±0.02	0.04±0
38	Horley	4.3±0.5	0.43±0.05
39	Horley & lime	0.71±0.06	0.07±0.01
40b	Royston R_0 & sludge	0.45±0.02	0.05±0
41	Royston R_0	0.29±0.02	0.03±0
42	Royston	0.90±0.06	0.09±0.01
44	Windsor	0.36±0.02	0.04±0
45	Windsor & lime	0.12±0.01	0.01±0
46	Galley Hill 1	1.70±0.11	0.17±0.01
47	Galley Hill 1 & lime	1.44±0.07	0.14±0.01
48	Galley Hill 2	1.30±0.11	0.13±0.01
49	Galley Hill 2 & lime	0.89±0.07	0.09±0.01
50	Dytchleys	0.46±0.02	0.05±0
51	Dytchleys & lime	0.24±0.10	0.02±0
52	Dytchleys & sludge	0.70±0.06	0.07±0.01
53	Dytchleys & sludge & lime	0.52±0.04	0.05±0
54	Dytchleys & leaf mould & spike	6.52±0.14	0.65±0.01
55	Dytchleys & leaf mould & spike & lime	5.63±0.30	0.56±0.03

FW - fresh weight. DW - dry weight.

Table 4.22: *Concentrations of cadmium in cabbages*

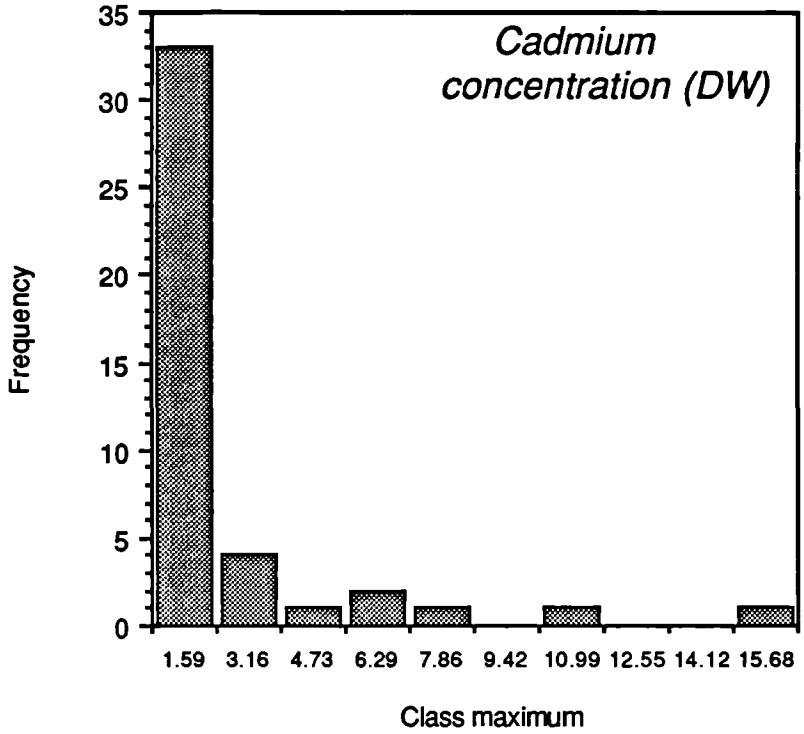


Figure 4.21: *Frequency distribution of the concentrations of cadmium in cabbages*

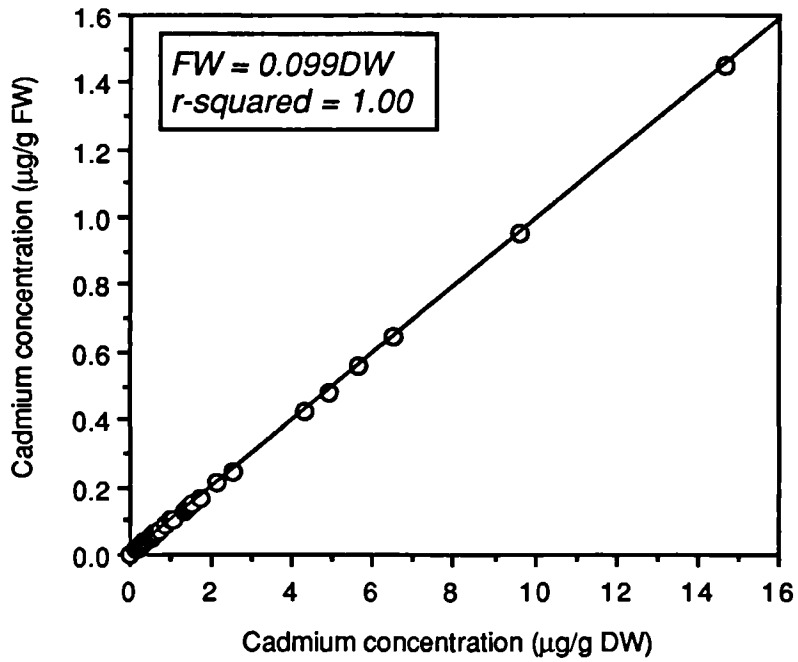


Figure 4.22: *The relationship between cadmium concentrations in dry weight and fresh weight for cabbages*

4.3.2.2: *Concentrations of cadmium and zinc in lettuces grown on sludge-amended soils*

SN	SITE	CADMIUM		ZINC	
		$\mu g g^{-1}$DW	$\mu g g^{-1}$FW	$\mu g g^{-1}$DW	$\mu g g^{-1}$FW
10	Checkley	33.44±1.69	0.78±0.04	233±5	5.42±0.11
11	Checkley & lime	22.90±1.69	0.68±0.05	113±1	3.35±0
12	Canwick (sewage works)	13.09±0.66	0.36±0.02	509±2	14.05±0.07
13	Canwick (sewage works) & lime	5.53±0.2	0.15±0.01	229±7	6.09±0.17
14	Canwick (Manor Farm)	0.82±0.11	0.02±0	42±5	1.21±0.14
16	Samlesbury	6.84±0.18	0.18±0.01	166±2	4.39±0.05
17	Samlesbury & lime	6.86±0.37	0.17±0.01	171±0	5.78±0
18	Stoke Bardolph 1	18.41±0.25	0.39±0.01	314±63	6.65±1.32
19	Stoke Bardolph 1 & lime	9.55±0.31	0.24±0.01	154±4	3.94±0.1
20	Stoke Bardolph 2	13.40±0.03	0.38±0	193±3	5.46±0.07
21	Stoke Bardolph 2 & lime	10.50±0.36	0.31±0.01	124±2	3.60±0.07
22	Ashton-Under-Lyme 1	1.520.05	0.04±0	69±1	2.02±0.01
23	Ashton-Under-Lyme1 & lime	0.62±0.04	0.02±0	59±5	1.75±0.15
24	Ashton-Under-Lyme 2	6.78±0.23	0.25±0.01	241±10	8.76±0.37
25	Ashton-Under-Lyme 2 & lime	2.21±0.08	0.07±0	90±10	2.96±0.33
26	Beverley	3.21±0.12	0.10±0	106±7	3.28±0.22
28	Colnbrook	23.74±0.42	0.57±0.01	214±46	3.94±0.08
29	Colnbrook & lime	9.38±ND	0.30±ND	123±3	5.13±1.09
30	Pikesmead	34.09±0.11	0.720	309±13	6.49±0.27
31	Pikesmead & lime	24.90±0.42	0.49±0.01	167±5	3.28±0.1
32b	Cassington A R_0 & sludge	5.24±0.29	0.16±0.01	86±2	2.60±0.07
33	Cassington A R_0 & lime	1.17±0.09	0.03±0	75±17	1.89±0.42
34	Cassington B	17.43±0.91	0.55±0.03	164±8	5.15±0.26
35	Cassington B & lime	15.94±0.39	0.38±0.01	132±9	3.16±0.23
36	Cassington A	8.04±0.35	0.24±0.01	85±3	2.52±0.08
37	Cassington A & lime	5.54±0.26	0.15±0.01	79±1	2.13±0.03
38	Horley	17.87±0.66	0.46±0.02	190±2	4.88±0.06
39	Horley & lime	12.69±0.74	0.34±0.02	104±2	2.81±0.06
40b	Royston R_0 & sludge	0.90±0.11	0.02±0	42±1	0.76±0.01
41	Royston R_0	0.35±0.02	0.01±0	32±1	0.81±0.01
42	Royston	2.90±0.17	0.08±0	42±2	1.11±0.06
44	Windsor	3.60±0.04	0.09±0	220±11	5.45±0.27
45	Windsor & lime	2.18±0.19	0.05±0	207±10	4.80±0.24
46	Galley Hill 1	14.14±0.72	0.29±0.01	239±0	4.86±0
47	Galley Hill 1 & lime	2.97±0.25	0.06±0.01	44±0	0.88±0.01
48	Galley Hill 2	19.49±0.10	0.40±0	169±48	3.46±0.97
49	Galley Hill 2 & lime	10.41±0.22	0.28±0.01	70±1	1.85±0.02
50	Dytchleys	4.58±0.43	0.14±0.01	125±4	2.23±1.45
51	Dytchleys & lime	3.26±0.17	0.10±0	110±8	2.10±0.19
52	Dytchleys & sludge	6.45±0.31	0.18±0.01	190±5	3.86±0.07
53	Dytchleys & sludge & lime	5.90±0.09	0.17±0	154±12	3.02±0.26
54	Dytchleys & leaf mould & spike	24.58±1.56	0.59±0.01	154±6	3.20±0.14
55	Dytchleys & leaf mould & spike & lime	23.56±0.74	0.57±0	124±4	2.87±0.07

FW - fresh weight. DW - dry weight.

Table 4.23: *Concentrations of cadmium and zinc in lettuces*

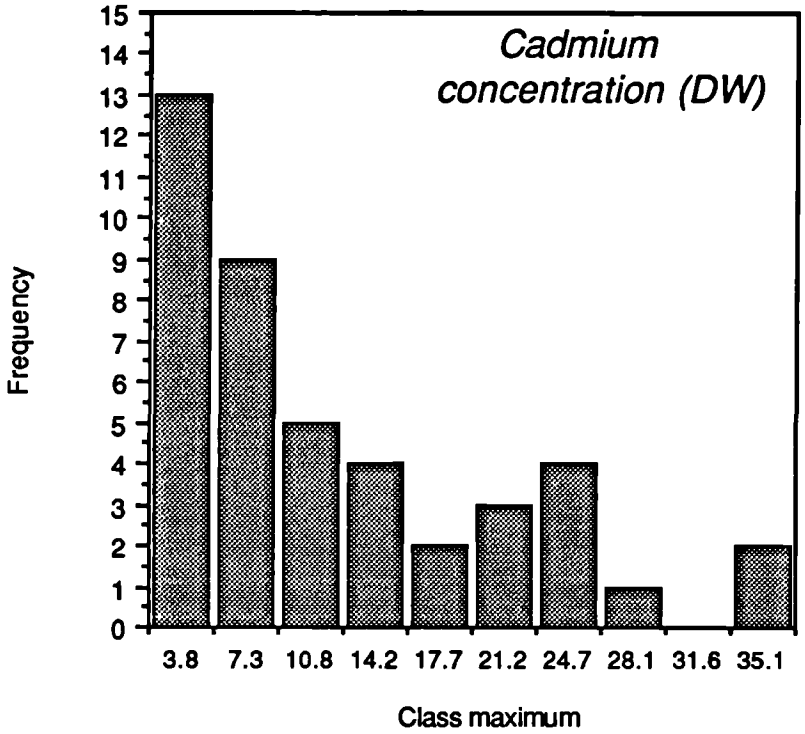


Figure 4.23: *Frequency distribution of the concentrations of cadmium in lettuces*

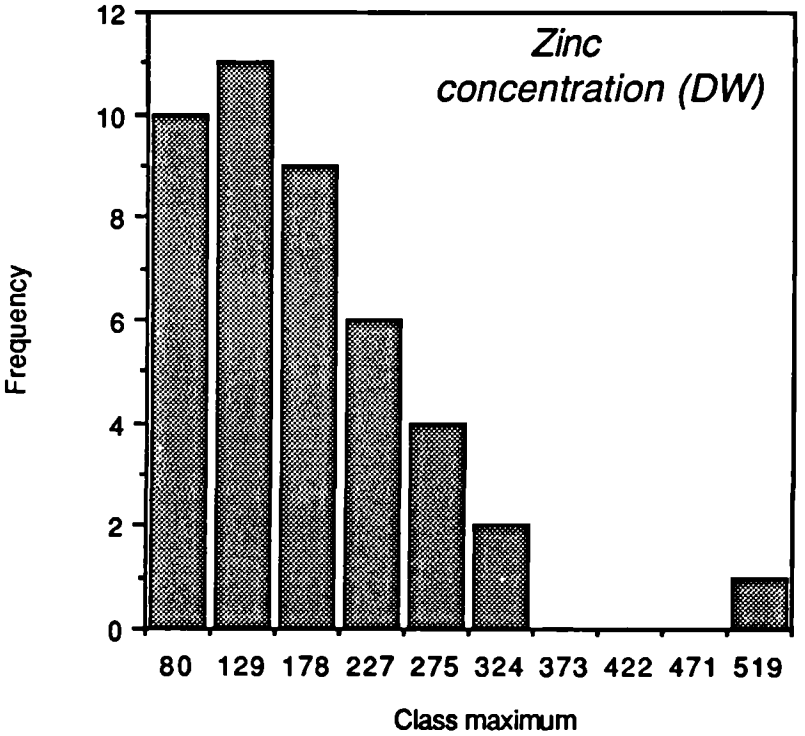


Figure 4.24: *Frequency distribution of the concentrations of zinc in lettuces*

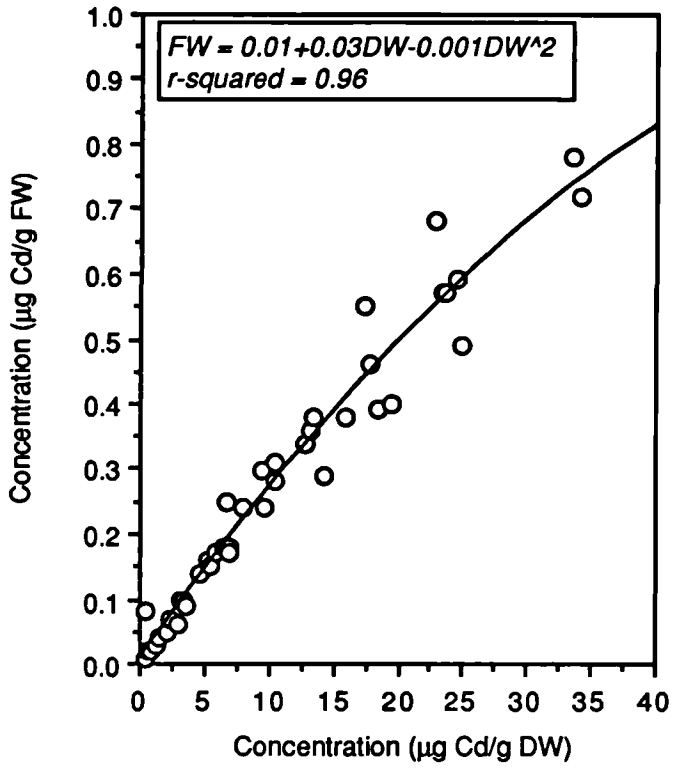


Figure 4.25:*The relationship between cadmium concentrations in dry weight and fresh weight for lettuces*

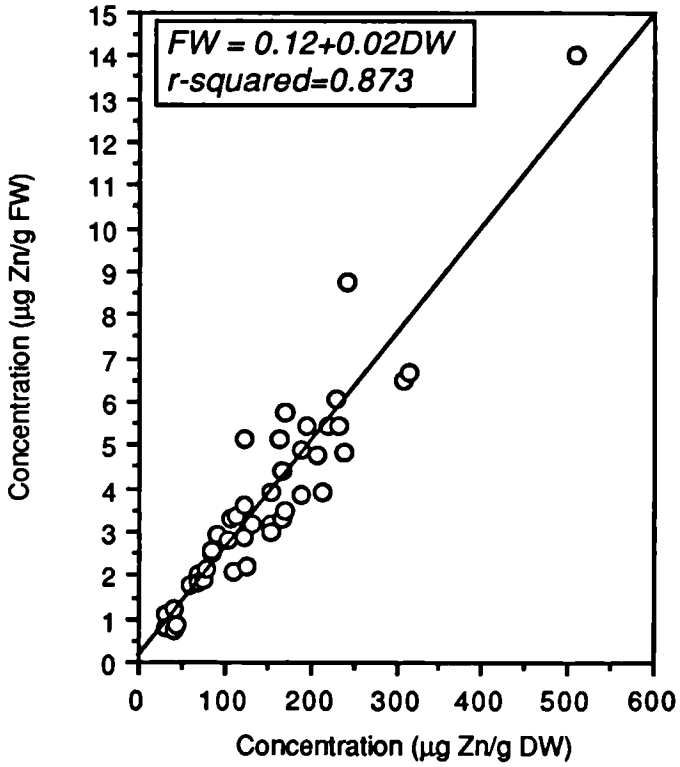


Figure 4.26:*The relationship between zinc concentrations in dry weight and fresh weight for lettuces*

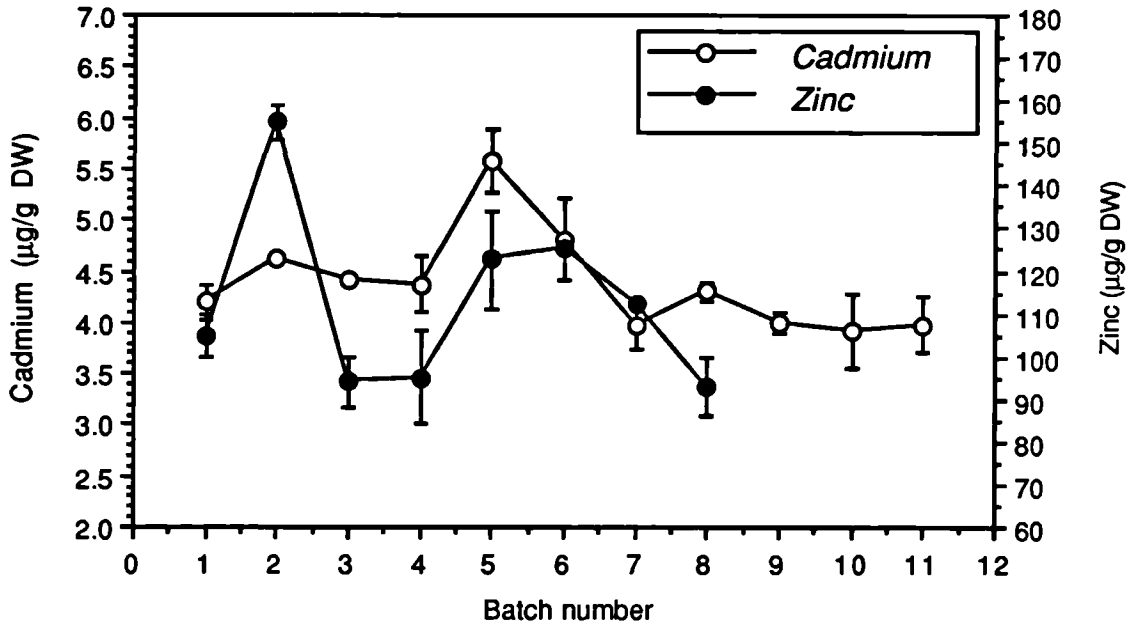


Figure 4.27: *Inter-batch comparisons of cadmium and zinc concentrations in a quality control material ("MAFF lettuce")*

4.3.2.3: *Concentrations of cadmium and zinc in potato tubers grown on sludge-amended soils*

SN	SITE	CADMIUM		ZINC	
		$\mu g g^{-1}$DW	$\mu g g^{-1}$FW	$\mu g g^{-1}$DW	$\mu g g^{-1}$FW
10	Checkley	0.854±0.015	0.156	27.09±1.11	4.95
11	Checkley & lime	1.218±0.001	0.233	14.68±0.24	2.81
12	Canwick (sewage works)	0.166±0.002	0.032	23.94±0.11	4.62
13	Canwick (sewage works) & lime	0.216±0.018	0.041	16.77±0.14	3.21
14	Canwick (Manor Farm)	0.031±0.003	0.006	4.11±0.24	0.82
16	Samlesbury	0.173±0.002	0.032	16.39±0.34	3.08
17	Samlesbury & lime	0.201±0.004	0.037	16.14±0.4	2.93
18	Stoke Bardolph 1	0.161±0.019	0.038	6.05±0	1.42
19	Stoke Bardolph 1 & lime	0.173±0.004	0.042	4.83±0.05	1.18
20	Stoke Bardolph 2	0.297±0.005	0.068	8.68±0.74	1.97
21	Stoke Bardolph 2 & lime	0.14±0.01	0.026	5.73±0.41	1.06
22	Ashton-Under-Lyme 1	0.135±0.005	0.028	8.21±0.06	1.73
23	Ashton-Under-Lyme1 & lime	0.045±0.004	0.010	9.29±0.07	2.11
24	Ashton-Under-Lyme 2	0.033±0.007	0.007	8.05±0.11	1.83
25	Ashton-Under-Lyme 2 & lime	0.036±0.006	0.008	5.74±0.04	1.28
26	Beverley	0.311±0.006	0.053	7.16±0.45	1.21
28	Colnbrook	0.231±0.01	0.053	6.43±0.41	1.48
29	Colnbrook & lime	0.174±0.02	0.044	5.66±0.54	1.42
30	Pikesmead	0.669±0.003	0.134	17.23±0.6	3.46
31	Pikesmead & lime	0.74±0.002	0.146	17.78±0.19	3.50
32b	Cassington A R_0 & sludge	0.174±0.002	0.038	5.47±0.47	1.19
33	Cassington A R_0 & lime	0.099±0.001	0.023	4.05±0.2	0.95
34	Cassington B	0.258±0.002	0.060	3.97±0.2	0.92
35	Cassington B & lime	0.202±0.01	0.051	3.85±0.4	0.96
36	Cassington A	0.12±0.007	0.030	4.87±0.08	1.23
37	Cassington A & lime	0.068±0	0.017	13.79±0.2	3.52
38	Horley	0.863±0.022	0.197	11.93±0.71	2.73
39	Horley & lime	1.115±0.01	0.229	6.81±0.23	1.40
40b	Royston R_0 & sludge	<LOD	<LOD	4.81±0.23	1.09
41	Royston R_0	<LOD	<LOD	2.66±0.15	0.97
42	Royston	0.163±0.005	0.031	9.78±0.01	1.84
44	Windsor	0.029±0.001	0.006	21.95±0.06	4.22
45	Windsor & lime	0.097±0.003	0.018	19.39±0.52	3.53
46	Galley Hill 1	0.234±0.004	0.052	6.82±0.14	1.53
47	Galley Hill 1 & lime	0.243±0.014	0.057	6.72±0.23	1.59
48	Galley Hill 2	0.206±0.007	0.038	5.29±0.22	0.98
49	Galley Hill 2 & lime	0.134±0.004	0.030	5.98±0.21	1.36
50	Dytchleys	0.029±0.002	0.009	4.56±0.09	1.09
51	Dytchleys & lime	0.027±0.001	0.008	4.23±0.24	1.04
52	Dytchleys & sludge	0.121±0.01	0.027	8.56±0.08	1.81
53	Dytchleys & sludge & lime	0.108±0.009	0.025	7.24±1.05	1.57
54	Dytchleys & leaf mould & spike	1.002±0.019	0.203	5.65±0.87	1.29
55	Dytchleys & leaf mould & spike & lime	0.995±0.008	0.202	5.12±0.05	1.19

FW - fresh weight. DW - dry weight.

Table 4.24: *Concentrations of cadmium and zinc in potato tubers*

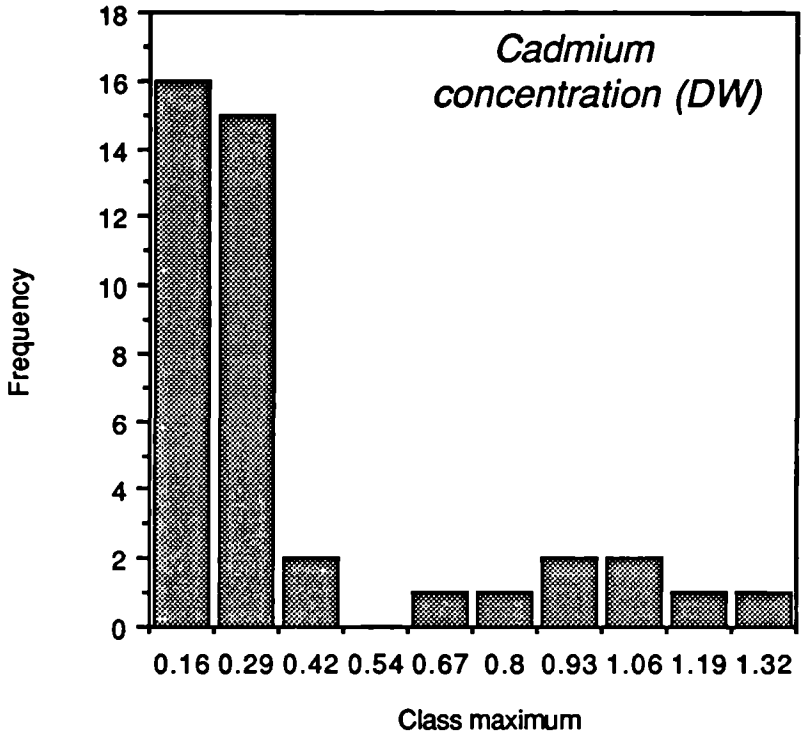


Figure 4.28: *Frequency distribution of the concentrations of cadmium in potato tubers*

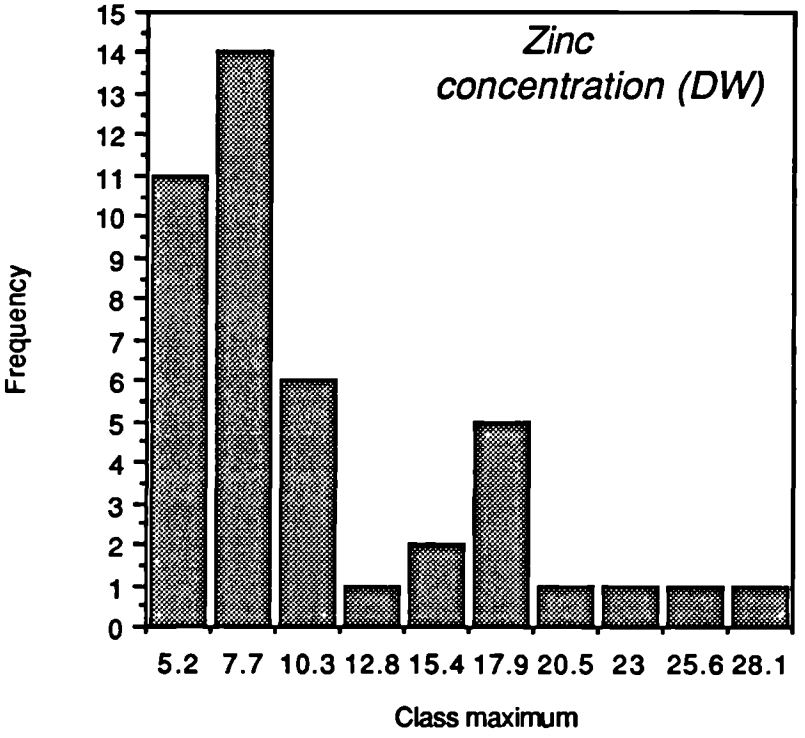


Figure 4.29: *Frequency distribution of the concentrations of zinc in potato tubers*

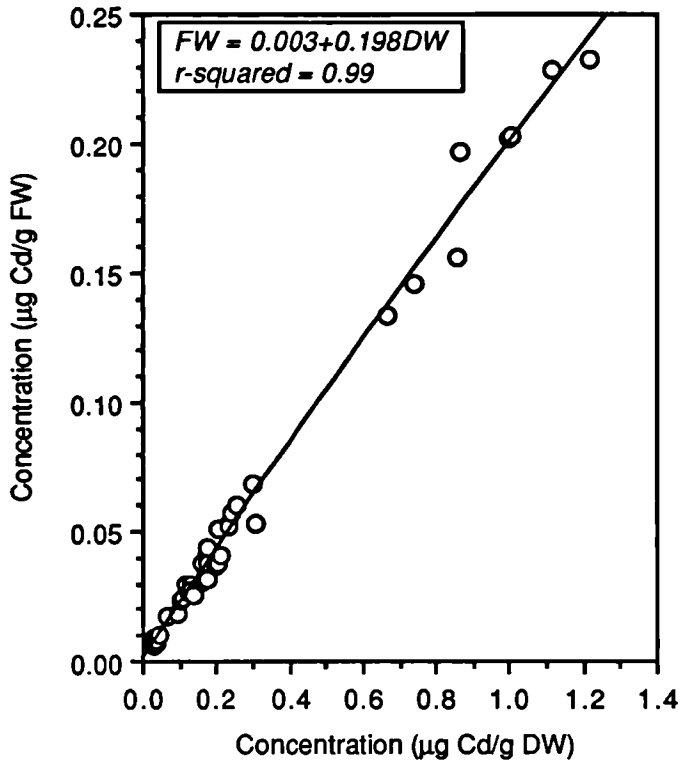


Figure 4.30: *The relationship between cadmium concentrations in dry weight and fresh weight for potato tubers*

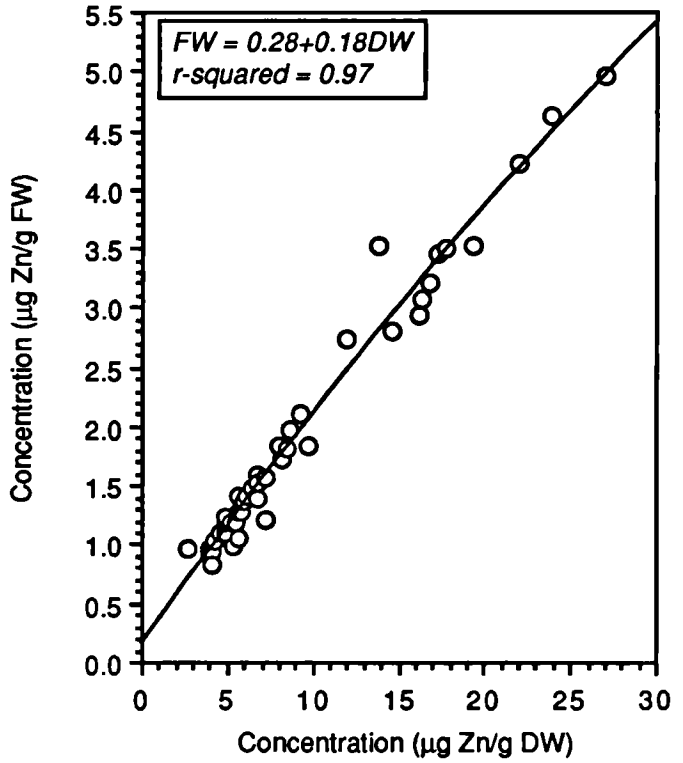


Figure 4.31: *The relationship between zinc concentrations in dry weight and fresh weight for potato tubers*

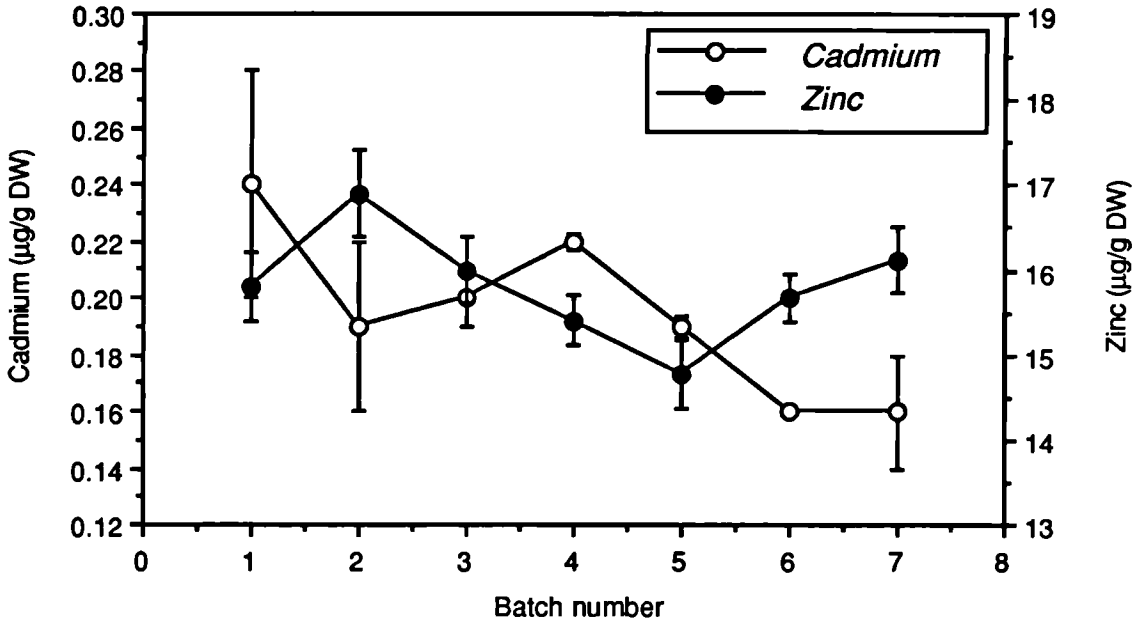


Figure 4.32: *Inter-batch comparisons of cadmium and zinc concentrations in an inhouse quality control material (potato tuber)*

4.3.2.4: *Concentrations of cadmium and zinc in whole grain samples grown on sludge-amended soils*

SN	SITE	CADMIUM (µgg-1 DW)	ZINC (µgg-1DW)
48	Galley Hill 2	0.253±0.003	58.64±1.28
57	Stoke Bardolph S1	1.070±0.049	96.44±2.27
58	Stoke Bardolph S2	0.721±0.018	62.91±1.50
59	Stoke Bardolph S3	0.690±0.015	55.30±2.10
60	Stoke Bardolph S4	1.450±0.097	72.84±2.41
61	Northampton S1	0.059±0	35.46±1.09
62	Northampton S2	0.314±0.024	53.40±1.90
63	Northampton S3	0.753±0.062	68.30±0.48

Table 4.25: *Concentrations of cadmium and zinc in whole grain*

4.3.2.5: *Concentrations of cadmium and zinc in biological data quality control materials*

CODE	CERTIFIED VALUE ($\mu g Cd g^{-1} DW$)	OBSERVED VALUE ($\mu g Cd g^{-1} DW$)
Certified materials		
A-11	0.0017±0.0002	<0.005
CRM-10a	0.023±0.003	0.025±0.009
SRM-1567a	0.026±0.002	0.024±0.002
CRM-7	0.030±0.003	0.029±0.003
SRM-1567	0.034±0.007	0.032±0.005
CRM-62	0.100±0.020	0.096±0.010
CRM-9	0.150±0.020	0.150±0.009
CRM-6	0.820±0.030	0.832±0.139
CRM-60	2.20±0.10	2.24±0.27
Uncertified materials		
AQA-33	0.045±0.006	0.050±0.013
AQA-34	0.43	0.47±0.04
AQA-35	<0.01*	<0.008*
AQA-36	0.40*	0.466±0.021*
Lettuce	4.48	4.60±0.50
Kale	0.210±0.060	0.175±0.013
Potato		0.202±0.028
IAEA-155		0.029±0.005

Specific details of all materials are given in Table 3.9.

Values superscripted with an asterix are quoted on an "as received" basis.

Table 4.26: *Cadmium concentrations in biological data quality control materials*

CODE	CERTIFIED VALUE ($\mu gZn g^{-1} DW$)	OBSERVED VALUE ($\mu gZn g^{-1} DW$)
Certified materials		
SRM-1567	10.60±1.00	10.76±0.42
SRM-1567a	11.60±0.40	11.01±0.34
CRM-9	15.60±1.20	15.6±0.90
CRM-62	16.00±0.70	16.30±0.70
CRM-10b	22.30±0.90	21.20±1.30
CRM-10a	25.20±0.80	26.60±1.20
CRM-7	33.00±3.00	29.60±1.10
A-11	38.90±2.30	36.69±0.72
CRM-60	313±8	324±5.9
Uncertified materials		
Potato		16.70±0.60
IAEA-155		32.00±2.90
Kale		33.76±2.93
Lettuce		119±21

Table 4.27: *Zinc concentrations in biological data quality control materials*

4.4: DATA ANALYSIS AND DISCUSSION

4.4.1: DESCRIPTIVE STATISITCS

In this section the data are summarised using a number of basic statistical tests. Average values are described using the medians and arithmetic means; ranges and variances are described using the maximum and minimum values of the data and the coefficient of variation. The nature of the frequency distribution is described using a value for the momental skewness and for the kurtosis. All data were analysed using Statview 512 (Abacus Concepts Inc., 1986).

Table 4.28 describes the basic soil parameters for the soils used in this study.

Variable	n	Mean	Median	Coefficient of variation (%)	Minimum	Maximum	Kurtosis	Skewness
pH (H_2O)	44	6.48	6.64	12.86	4.32	7.76	-0.16	-0.70
pH ($CaCl_2$)	44	5.88	6.09	14.625	3.88	7.28	-0.32	-0.65
Residual period (Years)	41	7.66	7	80.73	0	18	-1.15	0.30
LOI (%)	44	16.18	10.98	64.13	4.11	39.31	-0.22	1.05
Extractable Fe (%)	52	2.00	1.71	43.56	0.63	4.15	-0.11	0.64
Reducible Mn (μgg^{-1})	51	294.31	219.5	90.87	8.5	1184.5	3.27	1.89
Bulk density (gml^{-1})	53	0.95	0.96	18.24	0.55	1.26	-0.10	-0.66
Soluble K (μgg^{-1})	44	178.16	184.68	36.33	77.04	3166.44	-0.46	0.42
Soluble P (μgg^{-1})	44	287.7	238.37	55.85	65.97	709.3	0.46	1.15

Methods used to determine the solubility/"availability" of phosphorus and potassium are described in Sections 4.2.1.4 and 4.2.1.5.

Table 4.28: *Descriptive statistics for the basic soil variables.*

Soil pH (H_2O and $CaCl_2$) has a range of about 3.5 units between the maximum and minimum values found in this study. The frequency distribution tends towards being bimodal (refer to Figures 4.2 and 4.3), this is a reflection of the fact that the soils were in limed and unlimed pairs. Values for the organic matter content of the soils show considerable variation, the coefficient of variation for these soils is ~81%. Bulk density shows far less variation and has a coefficient of variation of ~18%. The median soluble potassium content is 185 $\mu g g^{-1}$, this relates to an index value of 7 on the ADAS scale; the median soluble phosphorus content was 238 $\mu g g^{-1}$, this has an index value of 8 on the ADAS scale (MAFF 1979). Table 4.29 shows the descriptive statistics for cadmium concentrations in soils, using a variety of extractants, and in a number of crops.

Variable	n	Mean	Median	Coefficient of variation (%)	Minimum	Maximum	Kurtosis	Skewness
HNO_3 extractable	53	21.74	7.83	165.4	0.27	158.67	6.94	2.73
Total	43	23.22	8.00	156.73	0.49	139	3.86	2.20
EDTA extractable	43	14.98	3.92	169.06	0.12	108	4.98	2.36
DTPA extractable	52	9.26	3.76	183.18	0.14	103.1	16.96	3.82
$CaCl_2$ extractable	43	4.20	0.30	292.2	<LOD	67.69	16.96	4.12
NH_4NO_3 extractable	43	2.08	0.10	354.24	<LOD	38.35	16.56	4.22
Cabbage (DW)	43	1.77	0.71	158.82	0.03	14.68	9.91	3.06
Cabbage (FW)	43	0.18	0.07	158.82	0.003	1.45	9.91	3.06
Lettuce (DW)	43	10.57	6.86	83.38	0.35	34.09	-0.02	0.92
Lettuce (FW)	43	0.27	0.24	76.61	0.01	0.78	-0.44	0.73
Potato (DW)	43	0.30	0.17	110.95	<LOD	1.22	1.05	1.56
Potato (FW)	43	0.06	0.04	106.71	<LOD	0.23	0.95	1.53

All concentrations are in μgg^{-1}.

Table 4.29: *Descriptive statistics for cadmium concentrations in plant and soil samples.*

The median soil cadmium concentration of the soils extractable with concentrated nitric acid, is 7.8 $\mu g g^{-1}$ with a 165% coefficient of variation. This median concentration is considerably lower than the mean concentration (21.7 $\mu g g^{-1}$) which is elevated by the soils from Galley Hill and those spiked with inorganic cadmium. The median nitric acid extractable cadmium concentration is approximately the same as the mean found by Chumbley and Unwin in their survey of UK sludge soils (1982). The frequency distribution is positively skewed and has a coefficient of momental skewness of 2.73. This median concentration is more than two times higher than the EC's recommended maximum cadmium concentration (CEC 1986) and higher than the normal or background concentrations given in Table 1.2 and 1.3. The median soil cadmium concentration after decomposition with HF and $HClO_4$ was higher than that after extraction with HNO_3. The median concentrations of cadmium extracted using all reagents decreased in the order shown below:

$$HF/HClO_4 > HNO_3 > EDTA\text{-}(Na)_2 > DTPA > CaCl_2 > NH_4NO_3$$

All of the frequency distributions for these soil data were positively skewed, positive values for the coefficients of kurtosis indicate that the frequency distributions for all these data are leptokurtic, ie extremely peaked with slim tails.

The median concentrations of cadmium in the three main crops, decrease in the order:

Lettuce > cabbage > potato

As expected from this observation, the fresh weight median cadmium concentrations are similarly ranked. All of the cadmium data for the plants

are positively skewed. Data for cadmium concentrations in both potatoes and cabbages are leptokurtic, however a mesokurtic distribution was observed for the dry weight concentrations of cadmium in lettuces. The median concentrations of cadmium in potatoes and cabbages are lower than those proposed as recommended maxima for these crops in the FRG (refer to Table 2.6). All data for plant concentrations are highly variable and have a coefficient of variation of over 50%. Tables 4.30 to 4.32 give data for the concentrations of cadmium in cabbages, lettuces and potatoes from a number of previous studies.

DESCRIPTION	MEAN	MEDIAN	RANGE	REFERENCE
• sludge soils	6.6^{DW}	-	$1.0 - 21.4^{DW}$	a
• inorganically polluted soils	18.1^{DW}	-	$0.5 - 75.7^{DW}$	a
• control soils	1.8^{DW}	-	$1.2 - 2.6^{DW}$	a
Experimental sludge soils	-	-	$0.10 - 0.16^{FW}$	b
ADAS survey of UK sludge soils	0.30^{DW}	-	-	c
Spanish survey	0.014^{FW}	-	$0.006 - 0.022^{FW}$	d
Netherlands survey	0.005^{FW}	0.004^{FW}	$0.001 - 0.017^{FW}$	e
Sludge soils, USA	-	-	$0.2 - 1.3^{DW}$	f
USA survey	-	-	$0.18 - 0.27^{DW}$	g
MAFF survey				
• control soils	$<0.1^{DW}$	-	$<0.1 - 0.3^{DW}$	h
• sludge soils	$<0.03^{FW}$	-	<0.01 - 0.08FW	h
• Shipham	0.61^{FW}	-	$0.02 - 8.24^{FW}$	h

All concentrations are in μgg^{-1}. A conversion relationship between fresh (FW) and dry weight concentrations is given in Figure 4.22.
a: Alloway and Morgan (1986); b: Keefer *et al* (1986); c: Chumbley and Unwin (1982); d: Zurera *et al* (1987); e: Wiersma *et al* (1986); f: Bingham (1979); g: Page *et al* (1987); h: MAFF (1983).

Table 4.30: *Typical concentrations of cadmium in cabbages*

The mean concentration of cadmium in cabbages grown on the soils used in this study was 1.77 $\mu g g^{-1}$ DW, this concentration is higher than the mean of the ADAS survey of UK sludge soils (Chumbley and Unwin 1982) and of the national surveys of The Netherlands (Wiersma *et al* 1986) and Spain (Zurera *et al* 1987). Data for a set of polluted soils (Alloway and Morgan 1986) had a mean concentration of cadmium in excess of that found in this study. The concentration of cadmium in cabbages grown on sludge soils in their experiment was over four times higher than that found in this study; this may be a reflection of the differences in growing conditions. Alloway and Morgan (1986) grew their plants in pots of a smaller volume than the tubs used in this study, this will tend to increase the density of the root ball and so lead to an enhanced efficiency in cadmium uptake. In addition to this difference, there may also be a discrepancy due to temperature, as Alloway and Morgan grew their plants in glasshouses. Siriratpuriya *et al* (1985) found that soil temperature had a marked effect upon the cadmium uptake of lettuce plants growing on sludge-amended soils and it does not seem improbable that a similar effect could be observed for cabbages. Data from the 1983 MAFF survey of cadmium in foods, enables a comparison of the data from this study with that from control sites, a market garden soil to which sludge had been applied and an area contaminated with non-ferrous mine waste (Shipham). The mean concentration of cadmium in cabbages in this study was greater than that found in plants from sludge-amended soils reported in the MAFF survey; however the mean cadmium concentration of cabbages from Shipham was considerably higher than that of this study.

DESCRIPTION	MEAN	MEDIAN	RANGE	REFERENCE
• sludge soils	-	-	2.9 - 58.3^{DW}	a
• inorganically polluted soils	-	-	1.9 - 100^{DW}	a
• control soils	-	-	1.1 - 6.6^{DW}	a
MAFF survey				
• sludge soils	1.79^{DW}	-	0.04 - 0.59^{FW}	b
• Shipham	0.68^{FW}	0.51^{FW}	0.03 - 2.9^{FW}	b
DoE field trial at Royston	2.25^{DW}	-	-	c
Survey of UK urban sites	-	-	0.3 - 2.3^{DW}	d
USA sludge soils	-	-	0.61 - 2.67^{DW}	e
Dredge materials, Netherlands	-	-	1.9 - 24.2^{DW}	f
Sludge soils, Norway	3.97^{DW}	-	-	g
Netherlands survey	0.05^{FW}	0.04^{FW}	0.01 - 0.19^{FW}	h
Spanish survey	0.01^{FW}	-	-	i
Sludge soils from southern USA	-	-	3.6 - 10.4^{DW}	j
ADAS survey of UK sludge soils	4.2^{DW}	-	-	k
Soils contaminated by mine waste	12.8^{DW}	-	1.2 - 28.8^{DW}	l
UK urban soils	-	-	0.3 - 2.3^{DW}	m
Polish soils atmospherically polluted	-	-	5.0 - 14.0^{DW}	n
Allotment gardens, Netherlands	0.05^{FW}	0.05^{FW}	0.01 - 0.18^{FW}	o

All concentrations are in $\mu g g^{-1}$. A conversion relationship between fresh (FW) and dry weight concentrations is given in Figure 4.25.
a: Alloway (1986); b: MAFF (1983); c: Davis and Stark (1980); d: Thornton (1986); e: Dowdy and Larson (1975); f: van Driel *et al* (1987); g: Vigerust and Selmer-Olsen (1985); h: Wiersma *et al* (1986); i: Zurera *et al* (1987); j: King (1986); k: Chumbley and Unwin (1982); l: Davies and White (1981); m: Thornton and Jones (1984); n: Kabata-Pendias (1984); o: van Lune (1987).

Table 4.31: *Typical concentrations of cadmium in lettuces*

Table 4.31 enables comparison of the lettuce cadmium concentrations in Table 4.29 with those found in the literature. The concentrations of cadmium found in these experiments are greater than those found in the national surveys (Thornton and Jones 1984, Thornton 1986, Wiersma *et al* 1986, van Lune 1987, Zurera *et al* 1987), as the majority of samples analysed in such a survey will not be exposed to such a degree of soil contamination this is to be anticipated. When compared with similar studies of sludge-amended soils (Vigerust and Selmer-Olsen 1985, Alloway 1986) the levels are less dissimilar. The national ADAS survey reported by Chumbley and Unwin (1982) produced a mean lettuce cadmium concentration of 4.20 μg Cd g^{-1} DW, approximately 50% of the mean value found in this study. This is probably due to the higher mean pH and lower cadmium concentrations of the soils in the survey (the mean soil cadmium concentration is less than 50% of that found in this study). It should be emphasised that studies of sludge-amended soils such as those of King (1986) and Dowdy and Larson (1974) will usually only sample soils with relatively low levels of contamination, many of the soils in this study are more heavily contaminated as they are from experimental sites, hence the higher plant concentrations.

Table 4.32 shows typical concentrations of cadmium in potato samples from the literature.

DESCRIPTION	MEAN	MEDIAN	RANGE	REFERENCE
Sludge soils from southern USA	-	-	0.08 - 0.51^{DW}	a
USA sludge soils	-	-	0.12 - 0.23^{DW}	b
Experimental soils				
• control soil	0.11^{DW}	-	-	c
• sludge soil	0.10^{DW}	-	-	c
ADAS survey of UK sludge soils	0.60^{DW}	-	-	d
Metal smelter site	-	-	0.03 - 0.20^{DW}	e
Control site in FRG				
• peeled	0.06^{DW}	-	-	f
• unpeeled	0.12^{DW}	-	-	f
Survey of EC	-	-	0.02 - 0.09^{DW}	g
Netherlands survey	0.03^{FW}	0.03^{FW}	0.01- 0.09^{FW}	h
Spanish survey	0.013^{FW}	-	0.01 - 0.02^{FW}	i
MAFF survey				
• control soils	<0.03^{FW}	-	<0.01 - 0.06^{FW}	j
• sludge soils	0.14^{FW}	-	0.06 - 0.21^{FW}	j
• Shipham	0.13^{FW}	-	0.03 - 0.30^{FW}	j

All concentrations are in $\mu g g^{-1}$. A conversion relationship between fresh (FW) and dry weight concentrations is given in Figure 4.30.
a: King (1986); b: Dowdy and Larson (1975); c: Page *et al* (1987); d: Chumbley and Unwin (1982); e: Davies and Crews (1983); f: Kampe (1983); g: CEC (1978); h: Wiersma *et al* (1986); i: Zurera *et al* (1987); j: MAFF (1983).

Table 4.32: *Typical concentrations of cadmium on potato tubers*

A major problem in the interpretation of data on cadmium concentrations in potato tubers, is that only rarely is the nature of the sample stated, ie peeled or unpeeled. Data from Davies and Crews (1983) and Kampe (1983) indicates that skin or peel of the potato contains an enhanced cadmium concentration. The samples in this study had been peeled before analysis. When compared with the peeled tuber cadmium

concentration from a control site in the FRG, the mean for this study was considerably elevated, some five times higher (Kampe 1983). The median concentration was also higher, by a factor of three. Data from the ADAS survey of sludge soils had a mean cadmium concentration twice that of the value of 0.30 $\mu g g^{-1}$ found in this study (Chumbley and Unwin 1982). Further comparison with the published data is of only limited relevance in light of the importance of differences in the sample preparation differences.

Table 4.33 gives descriptive statistics for the concentrations of zinc in soils and plants used in this study; these data will not be discussed in the same depth as those for cadmium.

Variable	n	Mean	Median	Coefficient of variation (%)	Minimum	Maximum	Kurtosis	Skewness
HNO_3 extractable	52	501.2	354.2	94.77	75	2350	6.52	2.39
Total	43	605.5	431.3	90.32	85.3	2596.3	5.86	2.36
EDTA extractable	41	239.8	121.1	146.1	12.4	1597.5	8.61	2.95
DTPA extractable	52	99.6	83.4	79.53	2.2	276	-0.87	0.58
$CaCl_2$ extractable	43	12.56	3.45	168.33	0.18	111	9.45	2.88
NH_4NO_3 extractable	43	8.59	1.86	191.61	<LOD	85.5	9.78	2.99
Lettuce (DW)	43	150	131.8	60.53	31.5	509.4	3.95	1.53
Lettuce (FW)	43	3.81	3.28	62.84	0.76	14.05	6.02	1.94
Potato (DW)	43	9.38	6.81	65.01	2.66	27.10	0.62	1.27
Potato (FW)	43	1.96	1.48	56.42	0.82	4.95	0.26	1.16

All concentrations are in $\mu g g^{-1}$.

Table 4.33: *Descriptive statistics for zinc concentrations in plant and soil samples.*

The median soil zinc concentration of the soils extractable with concentrated nitric acid, is 354 $\mu g g^{-1}$ with a 95% coefficient of variation. This median concentration is lower than the mean concentration (501 $\mu g g^{-1}$) which is elevated by the soils from Galley Hill and Stoke Bardolph. The frequency distribution is positively skewed and has a coefficient of momental skewness of 6.52. The median soil zinc concentration after decomposition with HF and $HClO_4$ was higher than that after extraction with HNO_3. The median concentrations of zinc extracted using all reagents decreased in the order shown below:

$$HF/HClO_4 > HNO_3 > EDTA\text{-}(Na)_2 > DTPA > CaCl_2 > NH_4NO_3$$

This order is the same as that observed for cadmium. All of the frequency distributions for these soil data were positively skewed, positive values for the coefficients of kurtosis indicate that the frequency distributions for all these data are leptokurtic, ie extremely peaked with slim tails. The frequency distribution for DTPA extractable zinc has a negative coefficient of kurtosis, indicating a mesokurtic/palykurtic distribution.

The concentration of zinc in lettuces is higher than that in potato tubers. Data for zinc concentrations in lettuces is leptokurtic, however a mesokurtic distribution was observed for the dry weight concentrations of cadmium in potatoes. All data for plant concentrations are highly variable and have a coefficient of variation of over 50%.

Table 4.34 summarises the data for cadmium concentrations in samples of grain reported in the literature and those found for sewage sludge-amended soils in this study.

DESCRIPTION	MEAN	RANGE	REFERENCE
Comparison of pot & field trials for wheat	0.10^{DW}	-	a
Netherlands survey	0.07^{FW}	0.02 - 0.35^{FW}	b
Retrospective analysis of archived samples of wheat grain	0.055^{DW}	0.02 - 0.11^{DW}	c
Field trial with sludge soils	<0.01^{DW}	-	d
Whole grain samples	0.03^{DW}	-	e
Control soils, wheat grain samples	-	<LOD - 0.024^{DW}	f
Sludge-amended soils, USA	-	0.02 - 0.05^{DW}	g
Spiked soils, wheat grain	-	0.10 - 5.60^{DW}	h
Wheat grain from field trials	-	0.02 - 0.05^{DW}	i
Wheat grain samples produced by organic farming methods in FRG	-	0.015 - 0.075^{DW}	j
MAFF survey, cereals food group	-	<0.02 - 0.05^{DW}	k
Wheat grain from Stoke Bardolph	0.69^{DW}	-	l
Wheat grain from Royston trial	-	0.05 - 0.70^{DW}	m
National survey, Sweden			
• winter wheat	0.06^{FW}	-	n
• spring wheat	0.04^{FW}	-	n
• barley from sludged soils	-	0.04 - 0.07^{DW}	n
Sludged soils in lysimeters, wheat grain	0.75^{DW}	-	o
Spiked soils			
• wheat	-	0.50 - 1.90^{DW}	p
• barley	-	0.60 - 3.50^{DW}	p
Wheat grain from pot trials	-	0.025 - 0.68^{DW}	q
EC background concentration in wheat	-	0.03^{DW}	r

DESCRIPTION	MEAN	RANGE	REFERENCE
Wheat grain from sludged soils	-	0.025 - 0.68^{DW}	s
CEC survey			
• flour	-	0.02 - 0.15^{DW}	t
• grain	-	0.01 - 0.04^{DW}	t
Wheat from USA survey	0.047^{DW}	0.014 - 0.21^{DW}	u
Wheat grain from field trials			
• control soils	0.004^{DW}	-	v
• sludged soils	0.016^{DW}	-	v
Corn grains	-	0.01 - 0.02^{DW}	w
THIS STUDY	**0.66^{DW}**	**0.06 - 1.45^{DW}**	

All concentrations are in $\mu g g^{-1}$.
a: Naylor *et al* (1987); b: Wiersma *et al* (1986); c: Lorenz *et al* (1986); d: Rappaport *et al* (1987); e: Micco *et al* (1987); f: King (1986); g: Bidwell and Dowdy (1987); h: Bingham and Page (1975); i: Dowdy and Larson (1975); j: Horner and Kurfurst (1987); k: MAFF (1983); l: Rundle and Holt (1983); m: Davis (1983); n: Berglund (1982); o: Webber and Monks (1982); p: Kloke (1982); q: Vigerust and Selmer-Olsen (1985); r: Hutton (1982); s: Vlamis *et al* (1985); t: CEC (1978); u: Page *et al* (1987); v: Campbell *et al* (1983); w: Hyde *et al* (1979).

Table 4.34: *Typical concentrations of cadmium in grain*

As was the case with the interpretation of data for cadmium concentrations in potato tubers, these data for wheat grain samples should be treated with a degree of caution. It would appear, although there are some contradictory data, that whole grain samples tend to give a higher cadmium concentration than when the bran component is removed during the preparation. This issue is discussed in more detail in Section 2.3.2. The mean concentration of cadmium in the whole grain samples analysed in this study, was higher than many of those in Table 4.34 and at least an order of magnitude higher than the concentrations of cadmium reported from national surveys of, presumably, non-contaminated sites. This mean concentration is very similar to that

reported by Rundle and Holt (1982) for the sludge-amended site at Stoke Bardolph and to that of Webber and Monks (1982) from lysimeter experiments with sludge-amended soils.

4.4.2: THE EFFECTS OF LIMING ON PLANT CADMIUM AND ZINC CONCENTRATIONS

In this section the effects of liming soils on the bioavailability of cadmium and zinc from sewage sludge-amended soils to three crop plants will be discussed. Figure 4.33 shows the mean cadmium concentrations for each of the plants grown and the pH (H_20), both before and after the application of quantities of lime determined by the experiments described in Section 4.1.2.

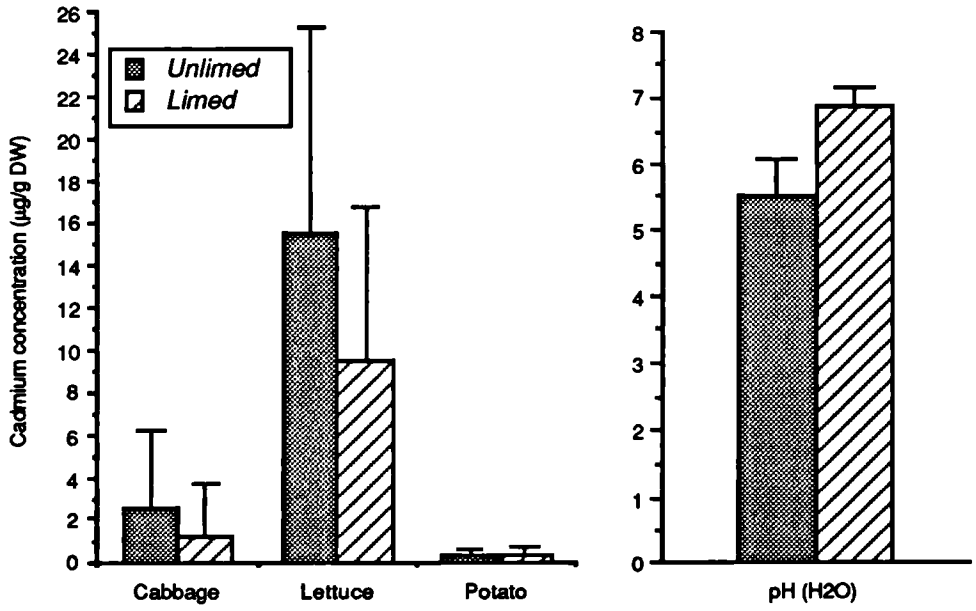


Figure 4.33: *A comparison of soil pH and plant cadmium concentrations before and after the application of lime*

The mean pH (H_2O) of the unlimed soils was 5.52±0.57, applying lime gave a mean pH of 6.89±0.25. These soil pHs were compared using a paired sample, two-tail t-test; there was a significant difference between

the means of these two groups, the p-value was 0.001. The mean cadmium concentration of the cabbages grown on the unlimed soils was 2.59±3.61 $\mu g g^{-1}$DW, cabbages grown on unlimed soils had a lower mean cadmium concentration, 1.28±2.37 $\mu g g^{-1}$DW. These two sets of data were compared using a t-test, as described above, a p-value of 0.004 was found, indicating a significant difference arises from the application of lime. The application of lime to sludge-amended soils lowered the mean concentration of cadmium in lettuces from 15.46±9.72 to 9.48±7.26 $\mu g g^{-1}$DW; these two data sets were compared using the t-test and found to be significantly different, the p-value was 0.001. Concentrations of cadmium in potato tuber samples did not appear to have been affected by the application of lime. The mean concentration of cadmium in plants from the limed soils was 0.32±0.38 $\mu g g^{-1}$DW, higher than that for plants grown on the unlimed soils. These two populations of data were also compared using the t-test and a p-value of 0.48 determined, this implied that there was not a significant difference in the cadmium concentrations of potato tubers grown on limed and unlimed soils.

Figure 4.34 enables comparison of concentrations of zinc in potato tubers and lettuces growing on limed and unlimed soils.

Data were normalised by logarithmic transformation and the mean cadmium and zinc concentrations in the crops compared. The concentrations of cadmium in lettuce and cabbage were shown to have been significantly reduced by the application of lime ($p<0.001$), whereas the cadmium concentration of potato tubers was not significantly reduced ($p=0.48$).

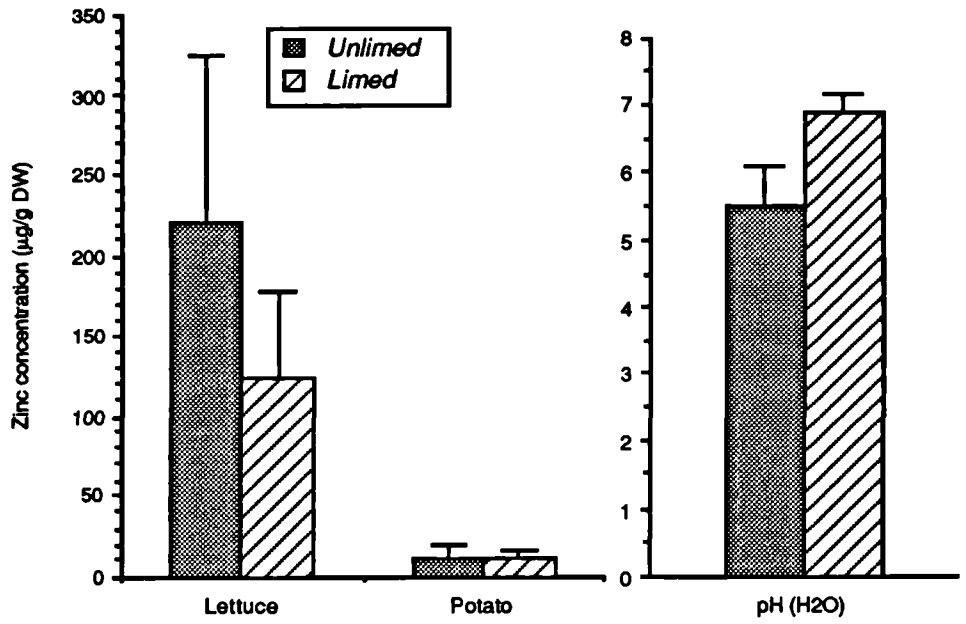


Figure 4.34: *A comparison of soil pH and plant zinc concentrations before and after the application of lime*

The mean concentration of zinc in the lettuces grown on the unlimed soils was 221±104 $\mu g g^{-1}$DW, the application of lime to these soils lowered this mean concentration to 124±54 $\mu g g^{-1}$DW. These two sets of data were compared using the t-test and found to be significantly different, the p-value was 0.004. Although liming soils lowered the mean zinc concentration in potato tubers from 11.79±7.61 to 10.21±5.51 $\mu g g^{-1}$DW, this difference was not shown to be significant by the t-test.

4.4.3: THE EFFECTS OF LIMING ON EXTRACTABLE CADMIUM AND ZINC CONCENTRATIONS

The concentrations of heavy metals extractable from a soil is influenced by the soils pH. The application of lime to sewage sludge-amended soils raises the pH and ie therefore likely to influence the concentrations of extractable cadmium and zinc. Figure 4.35 compares the mean concentrations of cadmium extracted from limed and unlimed soils using the methods described in Section 4.2.1.9.

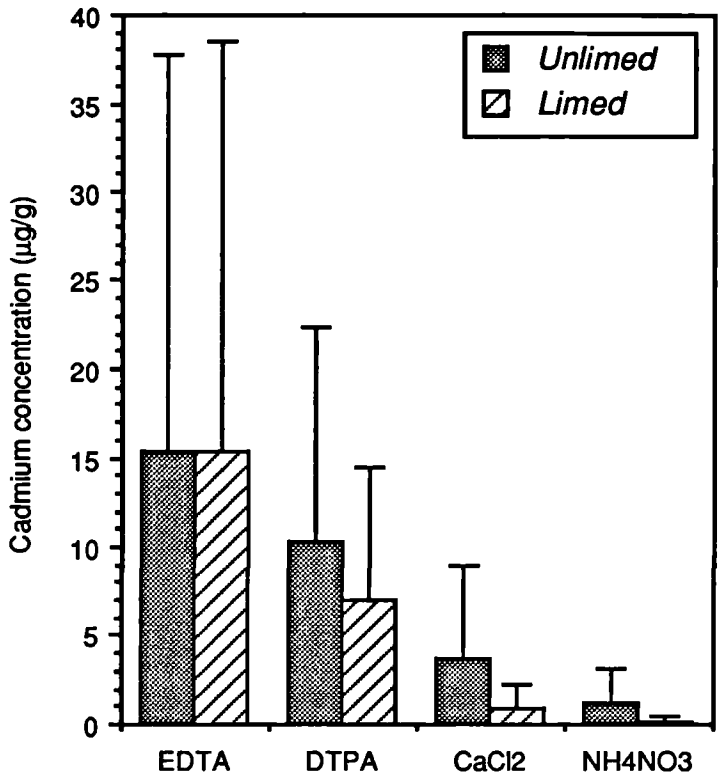


Figure 4.35: *A comparison of the mean concentrations of cadmium extracted from soils before and after the application of lime*

The mean concentration of cadmium extracted from limed soils by EDTA-$(Na)_2$ was 15.38±22.41 $\mu g g^{-1}$ exactly the same as that extracted from the unlimed soils. The comparison of means using a t-test for these two sets of data, gave a p-value of 1; therefore the null hypothesis, that there is no difference between the means, is accepted. The use of EDTA-$(Na)_2$ for soils with a high calcium carbonate content tends to give spurious data, it is often used as part of a sequential extraction procedure to dissolve trace elements bound to carbonates (Lund *et al* 1985). If the application of lime to a soil lowers the bioavailability of cadmium by increasing the proportion of cadmium present as cadmium carbonate, as was one of the mechanisms proposed by Christenssen and Tjell (1983), then the use of an extractant that dissolves this precipitate is not best suited to the

prediction of the "bioavailable" concentration of cadmium in a soil. DTPA extracted a lower mean cadmium concentration from the limed soils, 7.01±7.45 μgg^{-1} as compared with 10.34±12.09 μgg^{-1} from the unlimed soils. The use of a t-test to determine the significance of this difference gave a p-value of 0.02, the null hypothesis can therefore be rejected. Calcium chloride extracted a mean cadmium concentration of 3.68±5.29 μgg^{-1} from the unlimed soils and 0.90±1.39 μgg^{-1} from the limed soils. The significance of this difference was tested and gave a p-value of 0.004 and so the null hypothesis was rejected. The mean concentration of cadmium extracted from the unlimed soils by ammonium nitrate was 1.17±1.98 μgg^{-1}, approximately six times greater than the concentration extracted from the limed soils, 0.18±0.31 μgg^{-1}. The p-value for the significance of this difference was calculated to be 0.062.

Figure 4.36 compares the mean concentrations of zinc extracted from limed and unlimed soils using the methods described in Section 4.2.1.9.

Data were normalised by logarithmic transformation and the mean concentrations of cadmium and zinc extracted from limed and unlimed soils compared. In all but one case the difference between limed and unlimed soils was statistically significant, the only exception was for cadmium concentrations extracted by EDTA.

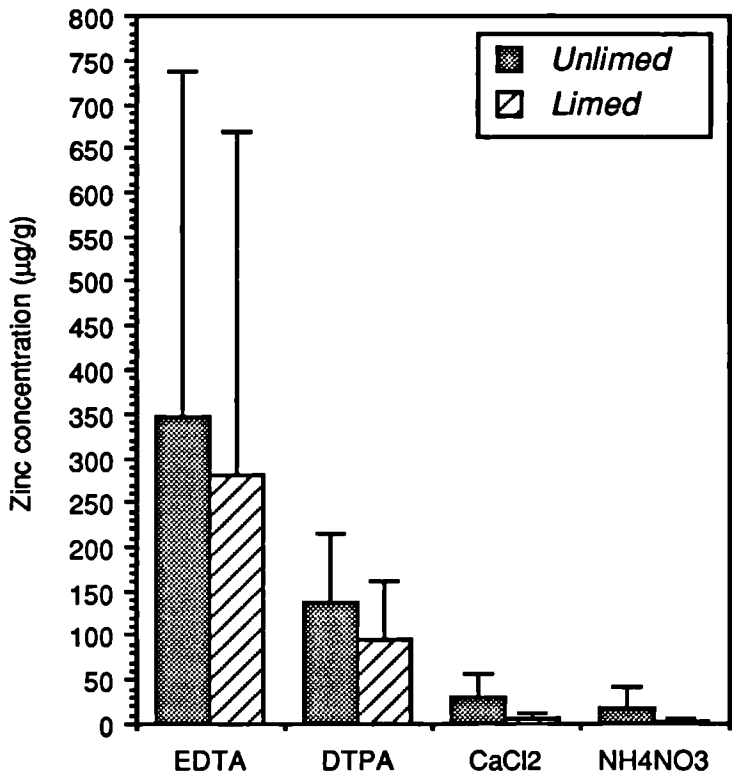


Figure 4.36: *A comparison of the mean concentrations of zinc extracted from soils before and after the application of lime*

EDTA-$(Na)_2$ extracted a greater mean zinc concentration from the limed soils than from the unlimed soils, 346.6±390.4 $\mu g g^{-1}$ as compared to 279.6±389.9 $\mu g g^{-1}$. A t-test to examine the significance of this difference gave a p-value of 0.016. This observation for zinc is contrary to that for cadmium and may imply that differences exist in the operationally defined speciation of these two elements in sewage sludge-amended soils. DTPA extracted a mean zinc concentration of 136.1±79.8 $\mu g g^{-1}$ from the unlimed soils and 94.5±67.6 $\mu g g^{-1}$ from the limed soils. This difference was highly significant and had a p-value of 0.001 and so the null hypothesis was rejected. Calcium chloride extracted a much lower mean concentration of zinc than either EDTA-$(Na)_2$ or DTPA. The mean zinc concentration extracted from the unlimed soils was 29.5±28.1 $\mu g g^{-1}$

compared with 4.9±8.5 μgg^{-1} from the limed soils. A p-value of 0.006 confirmed the significance of this difference. Ammonium nitrate extracted a lower mean concentration of zinc from the limed soils (1.98±3.78 μgg^{-1}), than from the unlimed soils (18.47±22.66 μgg^{-1}). Using a two tail t-test to compare these two means, indicated that the difference was significant and had a p-value of 0.02.

4.4.4: UNIVARIATE APPRAISAL OF FACTORS INFLUENCING THE BIOAVAILABILITY OF CADMIUM AND ZINC

In this section, the data are analysed by a number of comparative statistical measures in order to determine those factors that influence the bioavailability of cadmium and zinc. Bioavailability is best measured by the determination of the concentration of the particular metal in the plant of interest and in this respect, data for plant cadmium and zinc concentrations were correlated with and regressed against a number of potentially pertinent soil variables. The concentration factor value (CF-value) is another useful measure of bioavailability and will be analysed in the same way as the plant cadmium concentrations (use of the CF-value is discussed in section 6.1.2.4). The use of soil extractants may also provide an indication of the relative bioavailabilities of cadmium and zinc to plants, it is therefore necessary to determine those soil variables that influence the relative efficiency of specific soil extractants. Analyses of the relationships between the concentrations of cadmium and zinc extracted with soil extractants and the plant metal concentrations and CF-values, gives an indication of the validity of these soil testing methods.

Data were analysed using two software packages, Statview 512 (Abacus Concepts Inc., 1986) and SAS (SAS Institute Inc., 1988) running on a Macintosh SE FDHD and a MicroVax II respectively. All data were

normalised by logarithmic transformation prior to analysis using parametric statistics. Correlation coefficients were calculated using the product-moment equation; linear regression analyses were also made. Figures 4.37 to 4.60 are all log/log plots.

4.4.4.1: *Soil factors influencing the bioavailability of cadmium and zinc*

4.4.4.1.1: Cadmium

Table 4.35 gives the product-moment correlation coefficients, and their associated significance levels, for the relationships between the concentration of cadmium in cabbages and a number of soil parameters. The CF-value for cadmium in cabbages is also correlated with the same set of independent variables.

	Cabbage Cd ($\mu g g^{-1}$ DW)	CF-value
Residual period (years)	-0.121	-0.418*
pH (H_2O)	-0.433**	-0.190
pH ($CaCl_2$)	-0.463**	-0.177
LOI (%)	-0.212	-0.330*
Extractable Fe (%)	-0.076	-0.126
Manganese oxides ($\mu g g^{-1}$ DW)	-0.008	-0.418**
Bulk density ($g\ cm^{-3}$)	0.092	0.111
HNO_3 extractable Cd ($\mu g g^{-1}$ DW)	0.633***	-0.669***
Total Cd ($\mu g g^{-1}$ DW)	0.648***	-0.650***

$p < 0.001$ = *; $p < 0.01$ = ** & $p < 0.1$ = *.**

Table 4.35: *Correlations between soil variables and the concentration of cadmium in cabbages and CF-values*

The concentration of cadmium in cabbages is inversely correlated with soil pH and shows a positive correlation with the total and nitric acid

extractable concentrations of cadmium in the soil. The regression equations for the relationships between the concentration of cadmium in cabbage and the nitric acid extractable and total soil cadmium concentrations are shown in Figure 4.37 and in Table 4.36.

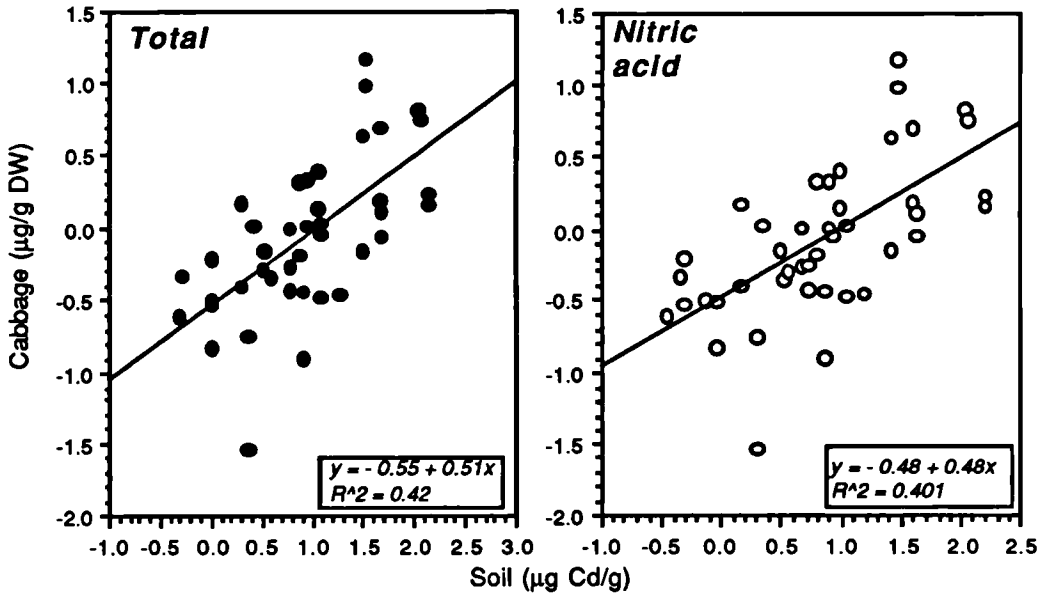


Figure 4.37: *The relationships between cabbage cadmium concentration and a) the total soil cadmium concentration and b) the nitric acid extractable cadmium concentration*

CF-values are significantly inversely correlated with the residual period (ie the time between sludge application and plant harvesting), the soil loss-on-ignition, the concentration of easily reducible manganese oxide concentration, the nitric acid extractable and total soil cadmium concentration. The equations for all significant linear regressions are listed in Table 4.36.

REGRESSION EQUATION	R^2 VALUE
y = cadmium concentration (DW)	
y = 3.00 - 3.81 pH (H_2O)	0.187**
y = 2.64 - 3.57 pH ($CaCl_2$)	0.214**
y = 0.48 Cd (HNO_3) - 0.48	0.401***
y = 0.52 Cd (Total) - 0.55	0.420***
y = CF-value for cadmium	
y = -0.29 - 0.83 Residual period	0.175*
y = -0.15 - 0.69 LOI	0.109*
y = 0.29 Mn oxides - 0.52	0.175*
y = -0.48 - 0.52 Cd (HNO_3)	0.448***
y = -0.43 - 0.54 Cd (Total)	0.423***

p<0.001 = *; p<0.01 = ** & p<0.1 = ***

Table 4.36: *Regression equations for those factors affecting the concentration of cadmium in cabbages*

The concentrations of cadmium in lettuce and associated CF-values were correlated using the product-moment correlation coefficient with a number of soil parameters; Table 4.37 summarises these correlations.

	Lettuce Cd ($\mu g g^{-1}$ DW)	CF-value
Residual period (years)	-0.089	-0.374*
pH (H_2O)	-0.472**	-0.362*
pH ($CaCl_2$)	-0.519***	-0.383*
LOI (%)	-0.151	-0.036
Extractable Fe (%)	0.179	0.176
Manganese oxides ($\mu g g^{-1}$ DW)	-0.039	-0.062
Bulk density (g cm^{-3})	0.125	0.005
HNO_3 extractable Cd ($\mu g g^{-1}$ DW)	0.577***	-0.215
Total Cd ($\mu g g^{-1}$ DW)	0.563***	-0.192

p < 0.001 = *; p < 0.01 = ** & p < 0.1 = ***.

Table 4.37: *Correlations between soil variables and the concentration of cadmium in lettuces and CF-values*

The concentration of cadmium in lettuces is significantly inversely related to soil pH and significantly related to the total concentration of cadmium in soil and the nitric acid extractable concentration. These correlations with the total concentration of cadmium in soil are not as strong for lettuces as those for cabbages. The cadmium CF-value for lettuces are only very weakly correlated with soil pH and the residual period. Figure 4.38 shows the regression equations for soil cadmium concentrations and the concentration of cadmium in lettuces.

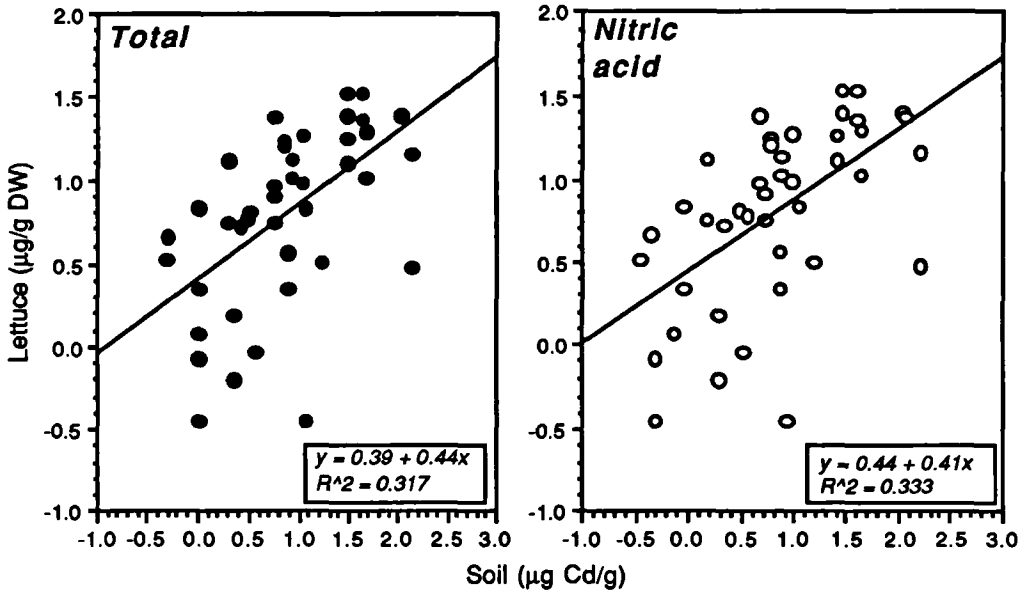


Figure 4.38: *The relationships between lettuce cadmium concentration and a) the total soil cadmium concentration and b) the nitric acid extractable cadmium concentration*

Linear regression equations for those variables significantly related to the concentration of cadmium in lettuces and to the associated CF-value, are shown in Table 4.38.

REGRESSION EQUATION	R^2 VALUE
y = cadmium concentration (DW)	
y = 4.13 - 4.13 pH (H_2O)	0.223**
y = 3.84 - 3.98 pH ($CaCl_2$)	0.270**
y = 0.44 + 0.43 Cd (HNO_3)	0.333***
y = 0.39 + 0.44 Cd (Total)	0.320***
y = CF-value for cadmium	
y = 0.56 - 0.76 Residual period	0.140*
y = 3.25 - 4.36 pH (H_2O)	0.131*
y = 2.81 - 4.04 pH ($CaCl_2$)	0.147*

p<0.001 = *; p<0.01 = ** & p<0.1 = ***

Table 4.38: *Regression equations for those factors affecting the concentration of cadmium in lettuces*

The concentrations of cadmium in potato tubers and associated CF-values were correlated using the product-moment correlation coefficient with a number of soil parameters; Table 4.39 summarises these correlations.

	Potato Cd ($\mu g g^{-1}$ DW)	CF-value
Residual period (years)	0.014	-0.324*
pH (H_2O)	0.117	0.042
pH ($CaCl_2$)	-0.168	0.022
LOI (%)	-0.218	-0.257
Extractable Fe (%)	-0.065	0.070
Manganese oxides ($\mu g g^{-1}$ DW)	0.150	-0.389*
Bulk density (g cm^{-3})	0.050	0.085
HNO_3 extractable Cd ($\mu g g^{-1}$ DW)	0.754***	-0.752***
Total Cd ($\mu g g^{-1}$ DW)	0.769***	-0.732***

p < 0.001 = *; p < 0.01 = ** & p < 0.1 = *.**

Table 4.39: *Correlations between soil variables and the concentration of cadmium in lettuces and CF-values*

The concentration of cadmium in potato tubers is strongly correlated with the nitric acid extractable and total concentration of cadmium in soil. The CF-value is weakly correlated with the concentration of easily reducible manganese oxide concentration and the residual period and strongly correlated with the nitric acid extractable and total concentration of cadmium in the soil. Figure 4.39 shows the relationship between the soil cadmium concentration and that of potato tubers.

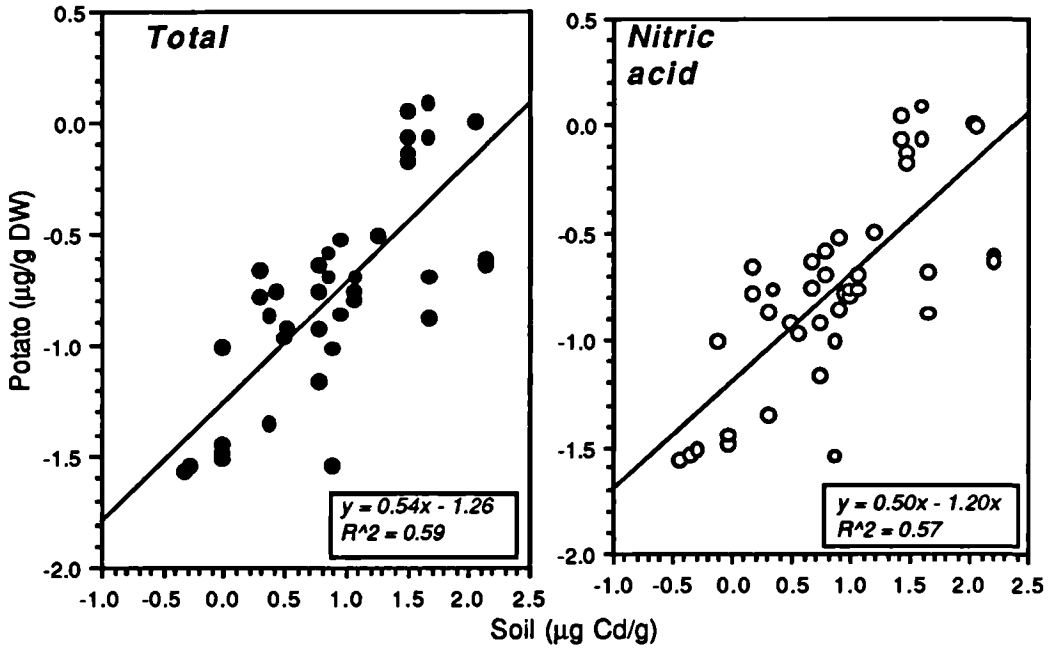


Figure 4.39 *The relationships between potato tuber cadmium concentration and a) the total soil cadmium concentration and b) the nitric acid extractable cadmium concentration*

A summary of the significant linear regression analyses using soil parameters as the independent variables and potato tuber cadmium concentration as the dependent variable is given in Table 4.40.

REGRESSION EQUATION	R^2 VALUE
y = cadmium concentration (DW)	
y = 0.50 Cd (HNO_3) - 1.20	0.569***
y = 0.54 Cd (Total) - 1.26	0.592***
y = CF-value for cadmium	
y = -1.40 - 0.66 Residual period	0.105*
y = -0.72 - 0.39 Mn oxides	0.151*
y = -1.19 - 0.50 Cd (HNO_3)	0.565***
y = -1.16 - 0.51 Cd (Total)	0.536***

p<0.001 = *; p<0.01 = ** & p<0.1 = ***

Table 4.40: *Regression equations for those factor affecting the concentration of cadmium in potato tubers*

Regression analysis confirms that the total and nitric acid extractable concentration of cadmium in soil is significantly related to the concentration of cadmium in potato tubers. The CF-value is inversely related to the soil cadmium concentration.

To conclude, all of the crops grown showed a significant positive correlation between the concentration of cadmium in the edible plant tissues and the total soil cadmium concentration and that extracted by nitric acid. Soil pH was shown to be inversely correlated with the plant tissue concentrations, although not always sufficiently strongly to be considered statistically significant; Figure 4.40 shows these relationships.

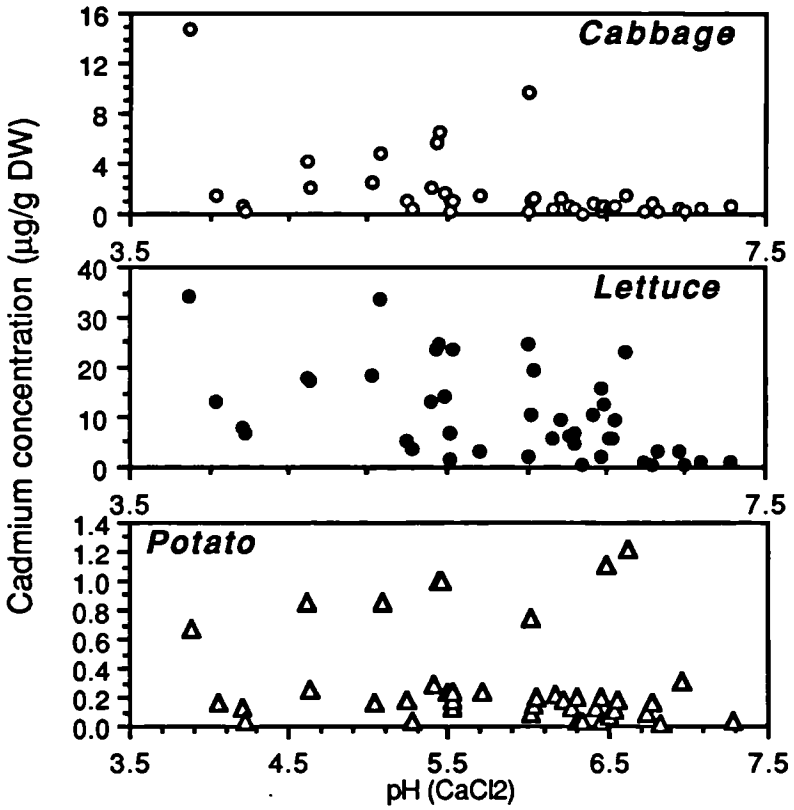


Figure 4.40: *Scattergrams of soil pH ($CaCl_2$) against a) cabbage cadmium concentration, b) lettuce cadmium concentration and c) potato tuber cadmium concentration*

The residual period was weakly inversely correlated with all of the CF-values for all of the crops; this implies that the after the application of sludge the ratio of plant to soil cadmium decreases with time. If this were to be proven, it would imply that the bioavailability of cadmium decreases with time. However, interpretation of CF-values is problematic due to the possibility of sources other than the soil making a contribution to the cadmium burden of the plant tissues. Cadmium from the atmosphere may enhance the cadmium concentration in the plant tissues and so lead to an increase in the CF-value; this is especially pertinent for plants growing on soils with low or only slightly elevated concentrations of cadmium. In order to rectify this discrepancy, Chamberlain (1983) proposed the use of

the CF_A value, taking into account cadmium from atmospheric sources. This issue is dealt with in more depth in Section 6.1.2.4.

4.4.4.1.2: Zinc

The soil factors influencing the bioavailability of zinc to lettuces and potato tubers were determined by correlation and regression analyses. Table 4.41 shows the product-moment correlation coefficients for the concentration of zinc in lettuces and a number of soil parameters.

	Lettuce Zn ($\mu g g^{-1}$ DW)	CF-value
Residual period (years)	-0.338*	-0.405*
pH (H_2O)	-0.626***	0.301*
pH ($CaCl_2$)	-0.608***	0.360*
LOI (%)	0.139	-0.253
Extractable Fe (%)	-0.024	-0.051
Manganese oxides ($\mu g g^{-1}$ DW)	-0.302*	-0.527***
Bulk density ($g\ cm^{-3}$)	0.014	0.060
HNO_3 extractable Zn ($\mu g g^{-1}$ DW)	0.188	-0.734***
Total Zn ($\mu g g^{-1}$ DW)	0.203	-0.633***

$p < 0.001$ = *; $p < 0.01$ = ** & $p < 0.1$ = *.**

Table 4.41: *Correlations between soil parameters and the concentration of zinc in lettuces and CF-values*

The concentration of zinc in lettuces is only strongly correlated with one soil parameter, the soil pH; the correlation coefficient is negative and has a p-value of 0.001. The CF-value of zinc for lettuces is strongly correlated with both the total and the nitric acid extractable soil zinc concentrations. As was the case with cadmium, these coefficients are negative. The easily reducible manganese oxide concentration is strongly inversely

correlated with the CF-value and shows a weak negative correlation with the concentration of zinc in lettuce. Table 4.42 gives the equations for the significant linear regression analyses of these data.

REGRESSION EQUATION	R^2 VALUE
y = zinc concentration (DW)	
y = 2.44 - 0.33 Residual period	0.114*
y = 4.40 pH (H_2O) - 2.85	0.392***
y = 3.95 pH ($CaCl_2$) - 2.42	0.370***
y = 2.53 - 0.19 Mn oxides	0.091*
y = CF-value for zinc	
y = 0.08 - 0.63 Residual period	0.164*
y = 1.17 - 1.98pH (H_2O)	0.091*
y = 1.16 - 2.07pH ($CaCl_2$)	0.130*
y = 0.66 - 0.47 Mn oxides	0.278***
y = 1.72 - 0.85 Zn (HNO_3)	0.538***
y = 1.68 - 0.79 Zn (Total)	0.401***

p<0.001 = *; p<0.01 = ** & p<0.1 = ***

Table 4.42: *Regression equations for those factors affecting the concentration of zinc in lettuce*

Table 4.43 shows the product moment correlation coefficients for the concentration of zinc in potato tubers and a number of soil parameters.

	Potato Zn ($\mu g g^{-1}$ DW)	CF-value
Residual period (years)	0.207	-0.288*
pH (H_2O)	-0.417**	-0.153
pH ($CaCl_2$)	-0.333*	-0.174
LOI (%)	0.430**	-0.088
Extractable Fe (%)	0.010	-0.032
Manganese oxides ($\mu g g^{-1}$ DW)	0.077	-0.311*
Bulk density (g cm^{-3})	-0.339*	-0.191
HNO_3 extractable Zn ($\mu g g^{-1}$ DW)	0.364*	-0.730***
Total Zn ($\mu g g^{-1}$ DW)	0.314*	-0.663***

$p < 0.001$ = *; $p < 0.01$ = ** & $p < 0.1$ = *.**

Table 4.43: *Correlations between soil variables and the concentration of zinc in potato tubers and CF-values*

The most significant correlations are between the zinc CF-value and the total and nitric acid extractable concentrations of zinc. Both of these two coefficients are negative and of a similar level of significance as those for lettuce. Table 4.44 shows the significant regression equations for potato zinc concentrations and their associated CF-values.

REGRESSION EQUATION	R^2 VALUE
y = zinc concentration (DW)	
y = 2.30 - 1.74 pH (H_2O)	0.174**
y = 1.83 - 1.22 pH ($CaCl_2$)	0.111*
y = 0.44 + 0.41 LOI	0.185**
y = 0.87 - 0.89 bulk density	0.115*
y = 0.22 + 0.27 Zn (HNO_3)	0.133*
y = 0.24 + 0.25 Zn (Total)	0.098*
y = CF-value for zinc	
y = -1.46 - 0.22 Residual period	0.083*
y = -1.07 - 0.24 Mn oxides	0.097*
y = 0.22 - 0.73 Zn (HNO_3)	0.534***
y = 0.28 - 0.72 Zn (Total)	0.44***

p<0.001 = *; p<0.01 = ** & p<0.1 = ***

Table 4.44: *Regression equations for those factors affecting the concentration of zinc in potato tubers*

4.4.4.2: *The effect of soil pH on the concentrations of cadmium and zinc extractable by four soil extractants*

The relationship between the percentage of the nitric acid extractable cadmium and zinc concentration and soil pH ($CaCl_2$) was determined by linear regression analysis, after first normalising the data by a logarithmic transformation. The data used for these analyses is given in Tables 4.18, 4.19, 4.20 and 4.21; where the concentration was recorded as being less than the limit of detection, no datum for that sample was entered into the regression analysis. Figure 4.41 shows the relationships between the percentage of cadmium extracted by EDTA-$(Na)_2$, DTPA, $CaCl_2$ and NH_4NO_3 and soil pH($CaCl_2$). The linear regression equations fitted to these pairs of data are given in Table 4.45

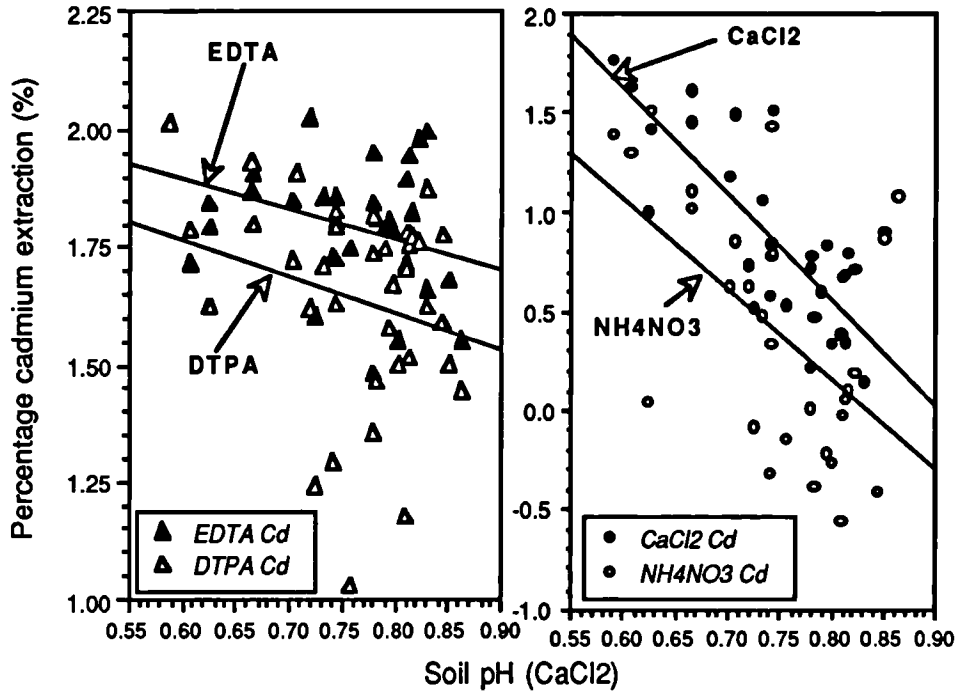


Figure 4.41: *The effect of soil pH($CaCl_2$) on percentage extractable cadmium concentrations*

The percentage of the nitric acid extractable cadmium concentration is inversely related to the soil pH ($CaCl_2$) for all soil extractants but with varying degrees of significance, as can be seen from Table 4.45. The percentage extractable by $CaCl_2$ is strongly related to the soil pH ($CaCl_2$) and less strongly, but still significantly, related to NH_4NO_3 extractable cadmium. The extraction efficiencies of DTPA and EDTA-$(Na)_2$ are far less well correlated with soil pH ($CaCl_2$), than either of the two neutral salt extractants; this characteristic is probably a function of the lower buffering capacity of these neutral salt extractants.

REGRESSION EQUATION	R^2 VALUE
y = percentage Cd extracted	
$CaCl_2$: y = 4.82 - 5.33 pH($CaCl_2$)	0.590***
NH_4NO_3: y = 3.83 - 4.59 pH($CaCl_2$)	0.300**
EDTA: y = 2.28 - 0.65 pH($CaCl_2$)	0.116*
DTPA: y = 2.22 - 0.76 pH($CaCl_2$)	0.064
y = percentage Zn extracted	
$CaCl_2$: y = 5.96 - 7.65 pH($CaCl_2$)	0.793***
NH_4NO_3: y = 7.03 - 9.59 pH($CaCl_2$)	0.731***
EDTA: y = 2.93 - 1.73 pH($CaCl_2$)	0.298***
DTPA: y = 2.37 - 1.42 pH($CaCl_2$)	0.178*

p<0.001 = *; p<0.01 = ** & p<0.1 = ***

Table 4.45: *Regression equations describing the affect of soil pH($CaCl_2$) on the percentage efficiency of four soil extractants*

Figure 4.42 shows the relationships between the percentage of zinc extracted by EDTA-$(Na)_2$, DTPA, $CaCl_2$ and NH_4NO_3 and soil pH($CaCl_2$).

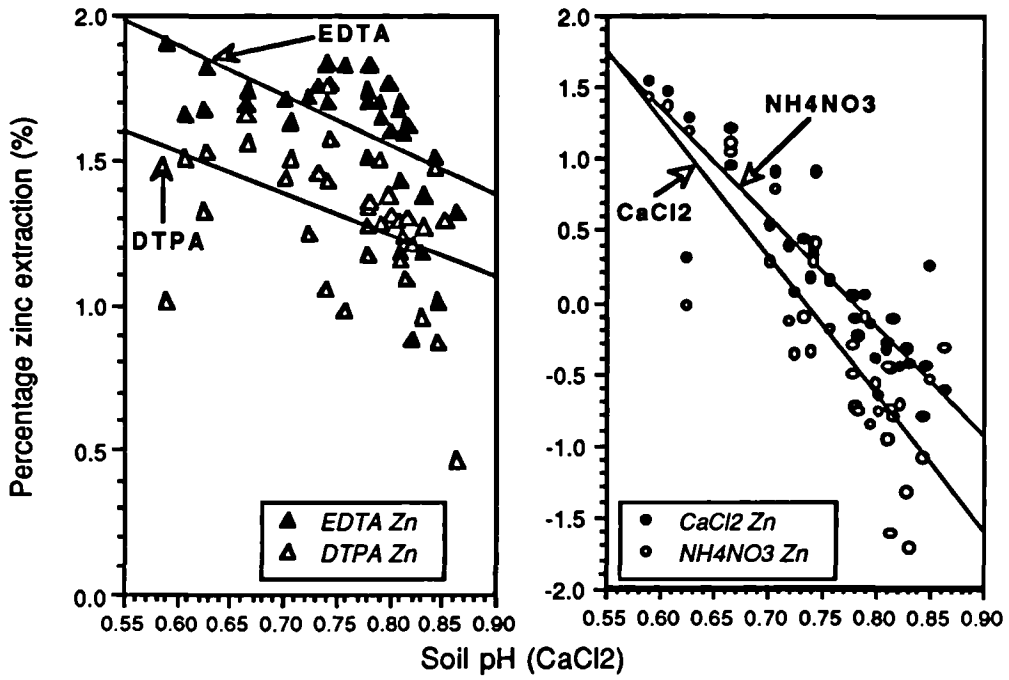


Figure 4.42: *The effect of soil pH($CaCl_2$) on percentage extractable zinc concentrations*

From Figure 4.42 and the r-squared values in Table 4.45, it is clear that for both of the neutral salt extractants, soil pH ($CaCl_2$) has a significant effect upon the percentage efficiency of the extraction. There is a clear inverse relationship, implying that as soil pH increases, the efficiency of the extraction decreases. This trend was also found to be statistically significant for EDTA-$(Na)_2$ but not for DTPA.

4.4.4.3: *The use of soil extractants to determine the relative bioavailability of cadmium and zinc to three crop plants*

In this section the relationships between the concentrations of, primarily cadmium, but also zinc in crop plants and those concentrations extracted from the soil by four procedures are examined. The soil tests used are described in Sections 4.2.1.9.

4.4.4.3.1: EDTA-$(Na)_2$

The concentrations of cadmium and zinc extracted from the soils using the procedure described in Section 4.2.1.9.1 were regressed against the concentrations of cadmium and zinc in crop plants grown on the same soils. All data were normalised prior to analysis. Figure 4.43 shows the relationships derived for cadmium.

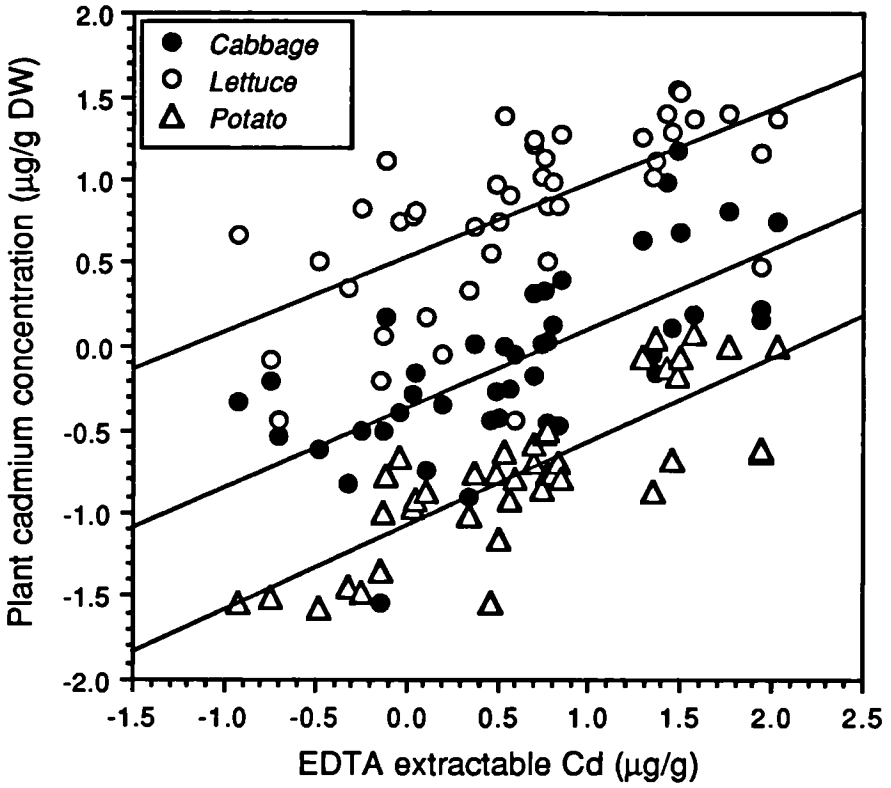


Figure 4.43: *The relationships between EDTA-$(Na)_2$ extractable cadmium and crop cadmium concentrations*

The EDTA-$(Na)_2$ extractable cadmium concentration is significantly related to the concentrations of cadmium in lettuce, cabbage and potato tubers. The high r-squared value and low p-values of these regression equations indicate that EDTA-$(Na)_2$ extraction of a soil provides an accurate reflection of the bioavailability of cadmium from sewage sludge-amended soils to these crops. Table 4.46 gives the regression equation and level of significance for each of the crops and for both cadmium and zinc.

REGRESSION EQUATION	R^2 VALUE
Cadmium	
Cabbage = 0.48 EDTA - 0.38	0.471***
Lettuce = 0.44 EDTA + 0.53	0.412***
Potato = 0.50 EDTA - 1.09	0.657***
Zinc	
Lettuce = 0.19 EDTA + 0.51	0.155*
Potato = 0.14 EDTA + 1.82	0.078*

p < 0.001 = *; p < 0.01 = ** & p < 0.1*.**

Table 4.46: *Regression equations relating EDTA-$(Na)_2$ extractable metal concentrations with plant metal concentrations*

Figure 4.44 shows the relationships between the concentrations of zinc extracted by EDTA-$(Na)_2$ and the zinc concentrations of lettuce and potato tuber.

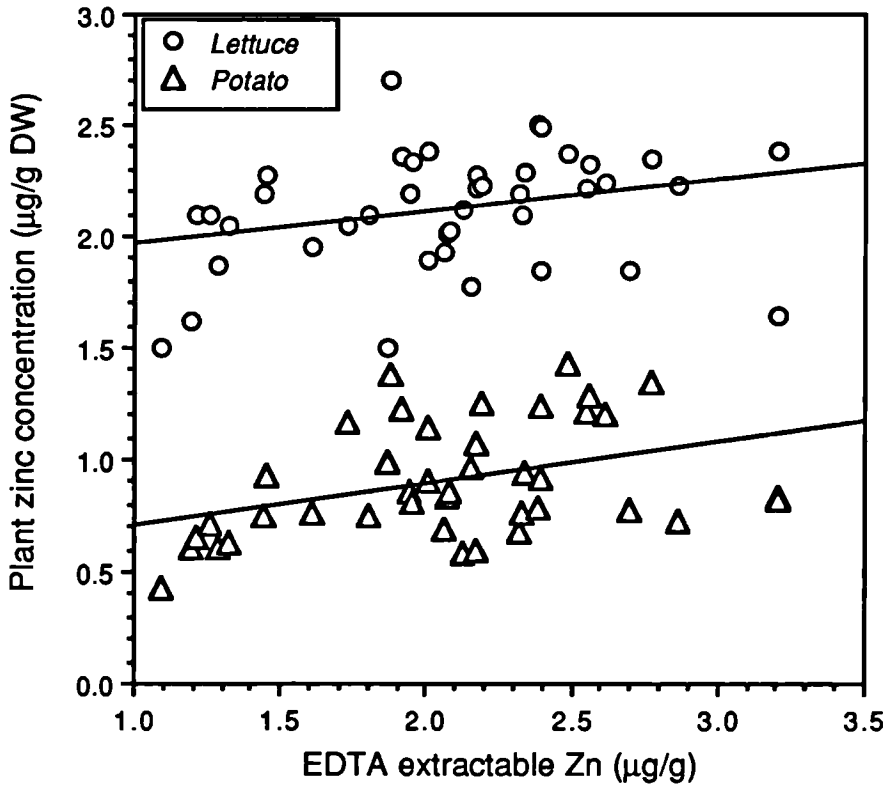


Figure 4.44:*The relationships between EDTA-$(Na)_2$ extractable zinc and crop zinc concentrations*

It is clear from Figure 4.44 and Table 4.46 that these relationships for zinc are not so clearly well defined as those for cadmium. The EDTA-$(Na)_2$ extraction would not appear to reflect the bioavailability of zinc to these crops from sewage sludge-amended soils.

4.4.4.3.2: DTPA

The concentrations of cadmium and zinc extracted from the soils using the procedure described in Section 4.2.1.9.2 were regressed against the concentrations of cadmium and zinc in crop plants grown on the same soils. All data were normalised prior to analysis. Figure 4.45 shows the relationships derived for cadmium.

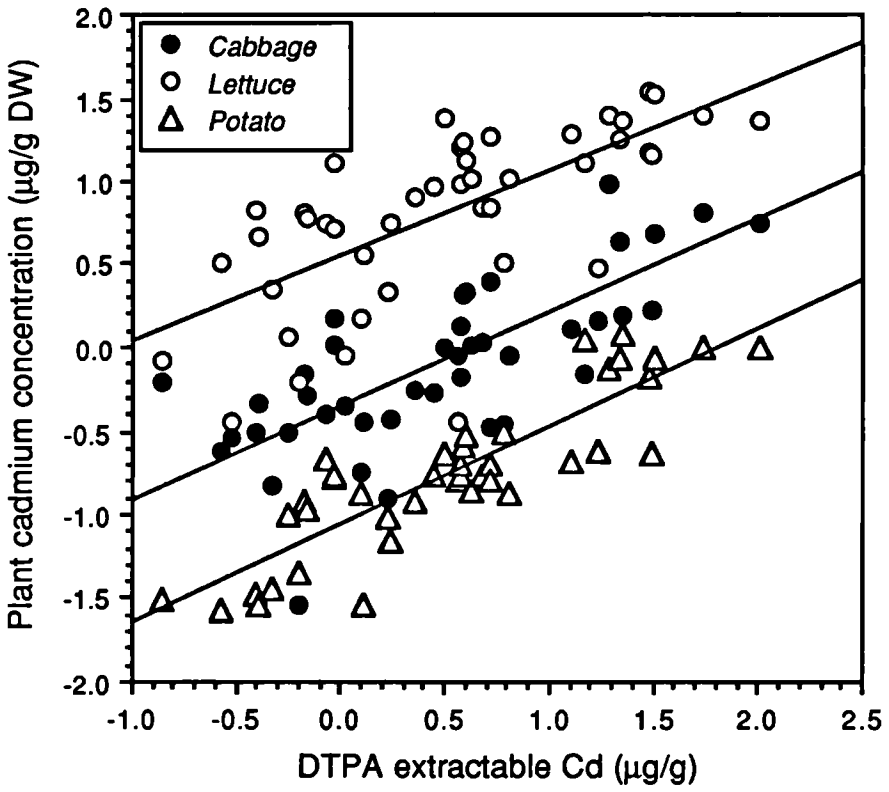


Figure 4.45: *The relationships between DTPA extractable cadmium and plant cadmium concentrations*

The regression equations for the relationships between DTPA extractable and plant concentrations of both cadmium and zinc are shown in Table 4.47. The levels of significance for the cadmium equations, indicate that the DTPA soil test is suitable for the determination of relative cadmium bioavailabilities to these crops from soils amended with sewage sludge.

REGRESSION EQUATION	R^2 VALUE
Cadmium	
Cabbage = 0.46 DTPA - 0.36	0.544***
Lettuce = 0.51 DTPA + 0.55	0.457***
Potato = 0.59 DTPA - 1.06	0.758***
Zinc	
Lettuce = 0.23 DTPA + 0.50	0.225**
Potato = 0.16 DTPA + 1.83	0.087*

p < 0.001 = *; p < 0.01 = ** & p < 0.1*.**

Table 4.47: *Regression equations relating DTPA extractable metal concentrations with plant metal concentrations*

The equations relating to zinc bioavailability are less significant than those for cadmium and are shown in Figure 4.46. The levels of significance and r-squared values are lower than those for cadmium and indicate that the DTPA soil extraction does not provide an accurate indication of zinc bioavailability to potato and lettuce from sewage sludge-amended soils.

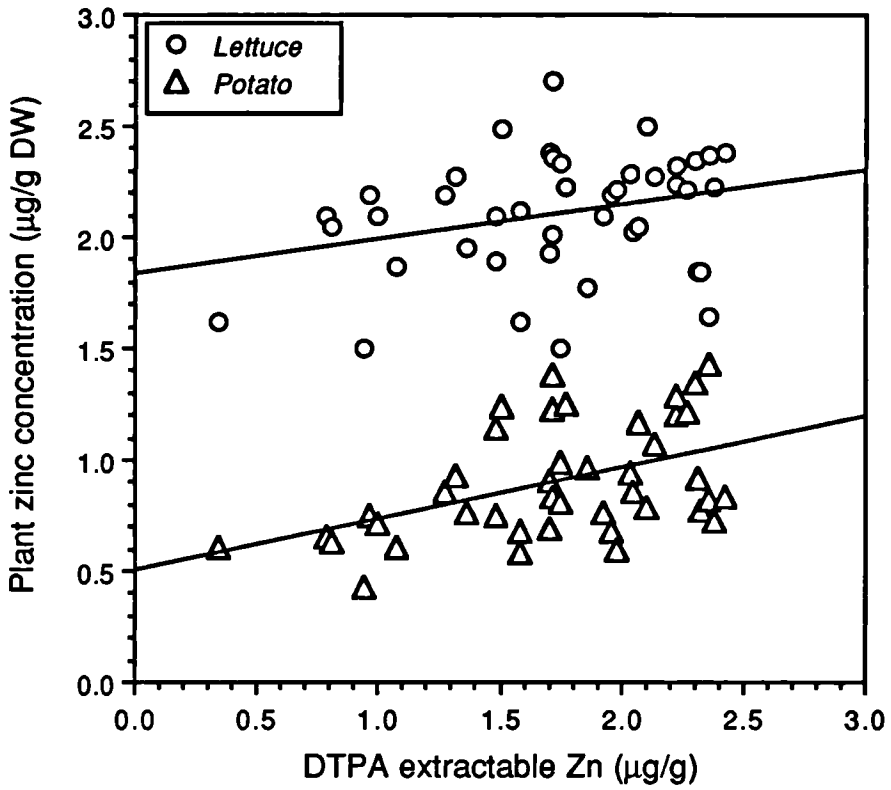


Figure 4.46:*The relationships between DTPA extractable zinc and plant zinc concentrations*

4.4.4.3.3: $CaCl_2$

The concentrations of cadmium and zinc extracted from the soils using the procedure described in Section 4.2.1.9.3 were regressed against the concentrations of cadmium and zinc in crop plants grown on the same soils. All data were normalised prior to analysis. Figure 4.47 shows the relationships derived for cadmium. Table 4.48 shows the regression equations for the lines fitted in Figures 4.47 and 4.48. The relationships between extractable and plant cadmium concentrations are all statistically significant. This implies that extraction of sewage sludge-amended soils with $CaCl_2$ is suitable for the estimation of cadmium bioavailability to potato tubers, lettuces and cabbages.

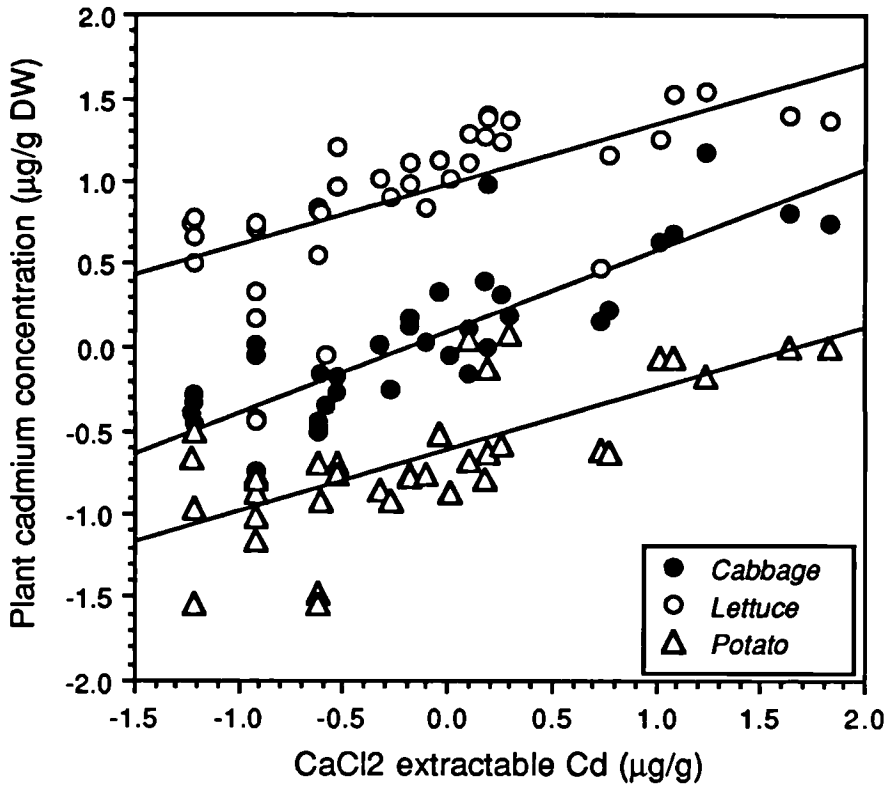


Figure 4.47: *The relationships between $CaCl_2$ extractable cadmium and plant cadmium concentrations*

REGRESSION EQUATION	R^2 VALUE
Cadmium	
Cabbage = 0.49 $CaCl_2$ + 0.09	0.694***
Lettuce = 0.36 $CaCl_2$ + 0.97	0.440***
Potato = 0.37 $CaCl_2$ - 0.62	0.496***
Zinc	
Lettuce = 0.17 $CaCl_2$ + 0.79	0.227**
Potato = 0.23 $CaCl_2$ + 1.96	0.345***

$p < 0.001$ = *; $p < 0.01$ = ** & $p < 0.1$*.**

Table 4.48: *Regression equations relating $CaCl_2$ extractable metal concentrations with plant metal concentrations*

The concentration of zinc in potato tubers was strongly correlated with the $CaCl_2$ extractable zinc concentration, lettuce concentrations were

also related to the $CaCl_2$ extractable concentration. Figure 4.48 shows these two relationships.

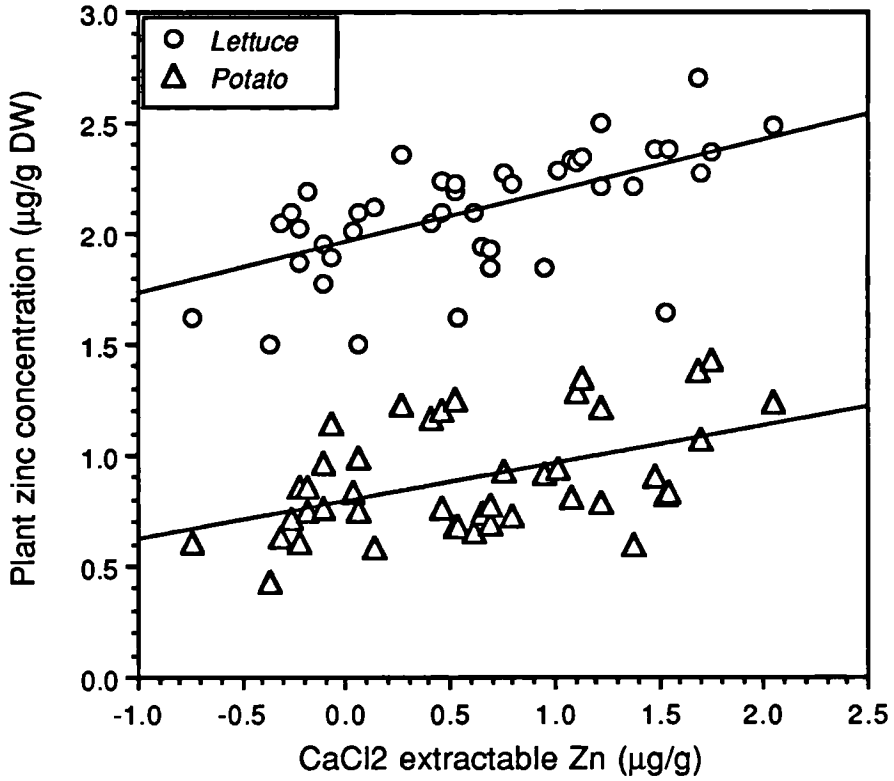


Figure 4.48: *The relationships between $CaCl_2$ extractable zinc and plant zinc concentrations*

4.4.4.3.4: NH_4NO_3

The concentrations of cadmium and zinc extracted from the soils using the procedure described in Section 4.2.1.9.4 were regressed against the concentrations of cadmium and zinc in crop plants grown on the same soils. All data were normalised prior to analysis. Figure 4.49 shows the relationships derived for cadmium. Table 4.49 shows the regression equations for the lines fitted in Figures 4.49 and 4.50. The relationships between extractable and plant cadmium concentrations are all statistically significant; however the linear regression for lettuce was less significant than those for potato tubers and cabbage. This implies that

extraction of sewage sludge-amended soils with NH_4NO_3 is suitable for the estimation of cadmium bioavailability to potato tubers and cabbages but is less accurate for lettuces.

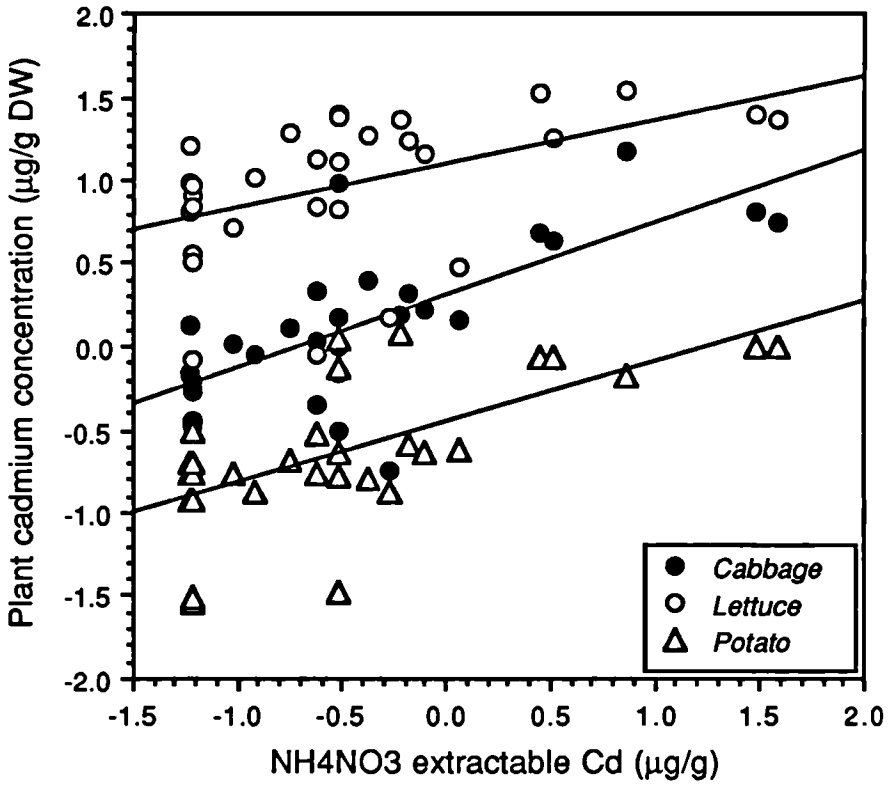


Figure 4.49: *The relationships between NH_4NO_3 extractable cadmium and plant cadmium concentrations*

REGRESSION EQUATION	R^2 VALUE
Cadmium	
Cabbage = 0.43 NH_4NO_3 + 0.29	0.518***
Lettuce = 0.26 NH_4NO_3 + 1.10	0.223**
Potato = 0.36 NH_4NO_3 - 0.46	0.419***
Zinc	
Lettuce = 0.20 $CaCl_2$ + 2.05	0.365***
Potato = 0.10 NH_4NO_3 + 0.88	0.120*

$p < 0.001$ = *; $p < 0.01$ = ** & $p < 0.1$*.**

Table 4.49: *Regression equations relating NH_4NO_3 extractable metal concentrations with plant metal concentrations*

The NH_4NO_3 extractable zinc concentration is significantly related to the concentration of zinc in potato tubers and less significantly so to the concentration of zinc in lettuce. These two relationships are shown in Figure 4.50 and show that soil extraction with NH_4NO_3 provides an indication of the bioavailability of zinc to lettuce.

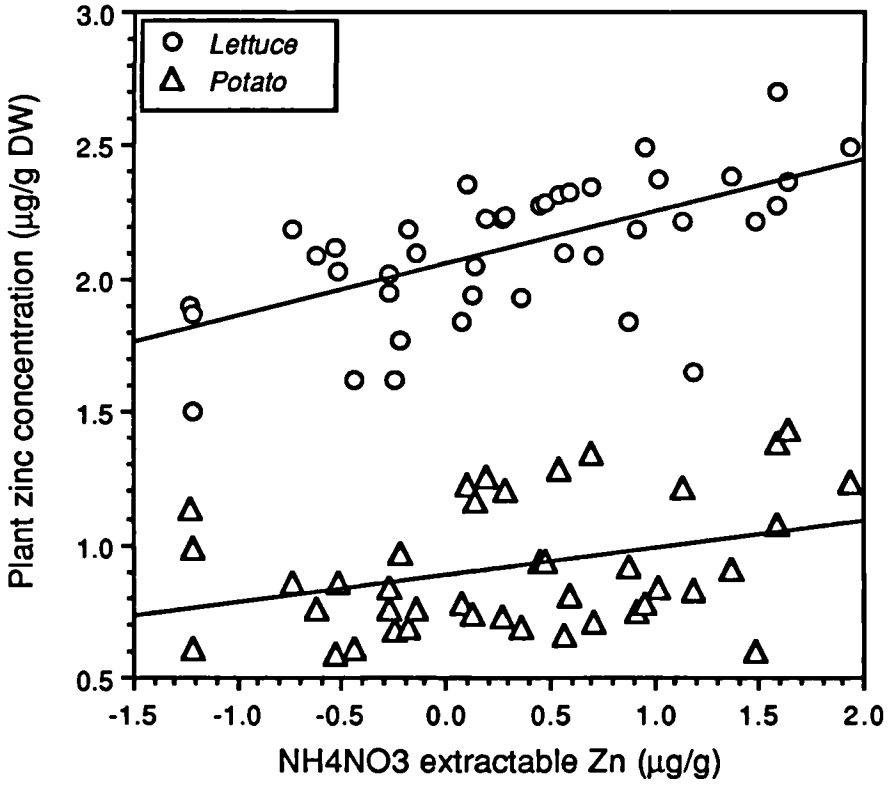


Figure 4.50: *The relationships between NH_4NO_3 extractable zinc and plant zinc concentrations*

4.4.4.3.5: Conclusions

By the use of soil extractants, the relative bioavailabilities of cadmium and zinc to these three crops has been determined. Four soil extractants were compared and contrasted in this study; two chelating agents, DTPA and EDTA-$(Na)_2$ and two neutral salts, $CaCl_2$ and NH_4NO_3. The r-squared value for the regression of the concentration of metal extracted

against the plant metal concentration, is a measure of the percentage of the variance in the dependent variable that is predictable from the independent variable. R-squared values for the cadmium concentrations extracted by the soil extractants, when regressed against the plant cadmium concentrations, are ranked in descending order (see below).

CABBAGE: $CaCl_2 > DTPA > NH_4NO_3 > EDTA\text{-}(Na)_2$

LETTUCE: $DTPA > CaCl_2 > EDTA\text{-}(Na)_2 > NH_4NO_3$

POTATO TUBER: $DTPA > EDTA\text{-}(Na)_2 > CaCl_2 > NH_4NO_3$

Therefore, it would appear on balance that the cadmium concentrations in the three crops are best predicted by the use of the DTPA soil test; only for cabbage is the cadmium concentration better predicted by the use of an alternative extractant. Browne *et al* (1984) examined the DTPA test as a means of predicting the accumulation of cadmium by lettuces grown in containers. The equation $\log P = \alpha + \beta \log Cd_{DTPA}$ was found to accurately predict the plant cadmium concentration (P). β was found to be principally a function of soil pH and the cation exchange capacity, α was considered to be a function of the particular species of plant. The mean value of β determined by Browne *et al* was 0.52 this is comparable to that found in this study, 0.51, and suggests that the equation can be usefully employed to predict the cadmium concentration of lettuces grown under a variety of soil conditions. α for this study was 0.55, considerably lower than that found by Browne *et al* who had a mean of 1.08. Browne *et al* concluded their paper by stating "Extrapolation from pot to field culture would present problems, however, in that relationships between species in the value of α derived from restricted root volumes in of glasshouse conditions are probably inapplicable." This is one possible explanation of the discrepancy in α but not in β between this study and

that by Browne *et al.* It is unlikely that the plant growth conditions of this study would differ from those in the field but it is likely that they are considerably different to those used by Browne *et al.* Alloway (1986), in a glasshouse experiment using a range of soils contaminated from a variety of sources, calculated r-squared values of 0.440 and 0.731 for lettuce and cabbage respectively, when correlated with DTPA-extractable cadmium concentrations. The data was not normalised before the coefficient was calculated and so comparisons with the values derived from this study are not valid. After normalising the data the concentrations of cadmium in cabbage and the DTPA extractable concentration , the values of α an β could calculated for the regression equation. β values were very similar 0.46 for this study and 0.47 for that by Alloway (1986); however α values were considerabluy lower for this study, -0.36 as compared to +0.61.

A study by Sauerbeck and Styperek (1984) examined the use of the $CaCl_2$ soil extraction for the prediction of the cadmium and zinc concentrations in lettuces and potato tubers. The data were analysed and r-squared values were calculated. An r-squared value of 0.51 was found for cadmium in lettuce as compared with 0.44 for this study. Comparison of these values, in terms of their statistical significance, is not possible because Sauerbeck and Styperek do not quote a p-value, nor do they give details of the nature of the statistical analyses, ie whether or not the data had been normalised before analysis. The r-squared value for potato tuber samples analysed in this study was 0.496 ($p<0.001$); this is considerably greater than that found by Sauerbeck and Styperek, 0.09. Alloway (1986) used the $CaCl_2$ soil extraction and obtained significantly better correlations with lettuce and cabbage concentrations than those found in this study, refer to Table 4.48.

Soil extraction with NH_4NO_3 was found by Alloway (1986), in glasshouse experiments using polluted soils, to be strongly correlated with the concentrations of cadmium in both cabbage and lettuce. The r-squared values for these two crops were 0.864 and 0.681 respectively, greater than those for this study, refer to Table 4.49.

EDTA-$(Na)_2$ extractable cadmium was significantly correlated (p-value < 0.001) with the cadmium concentrations in all of the crops grown in this study.

4.4.4.4: *Inter-specific comparison of cadmium and zinc concentrations*

It has been postulated that indicator species could be used to assess the bioavailability of cadmium from sewage sludge-amended soils (Chaney *et al* 1987, Ryan *et al* 1982). In this way dietary exposure to cadmium, arising from certain soil cadmium concentrations and other variables, could be estimated through the data from a single "index plant" (Kim *et al* 1988). A number of index plants or indicator species have been used, probably the most common of which are ryegrass (Sanders *et al* 1986) and lettuce (Chaney *et al* 1987). If an index plant is to provide data to be used as part of an assessment of dietary exposure to cadmium, then it's cadmium concentration should be significantly correlated with those of a range of food crops grown on the same soils. Figure 4.51 shows the relationships between the cadmium concentration in an index plant (lettuce) and those in potato tubers and cabbage.

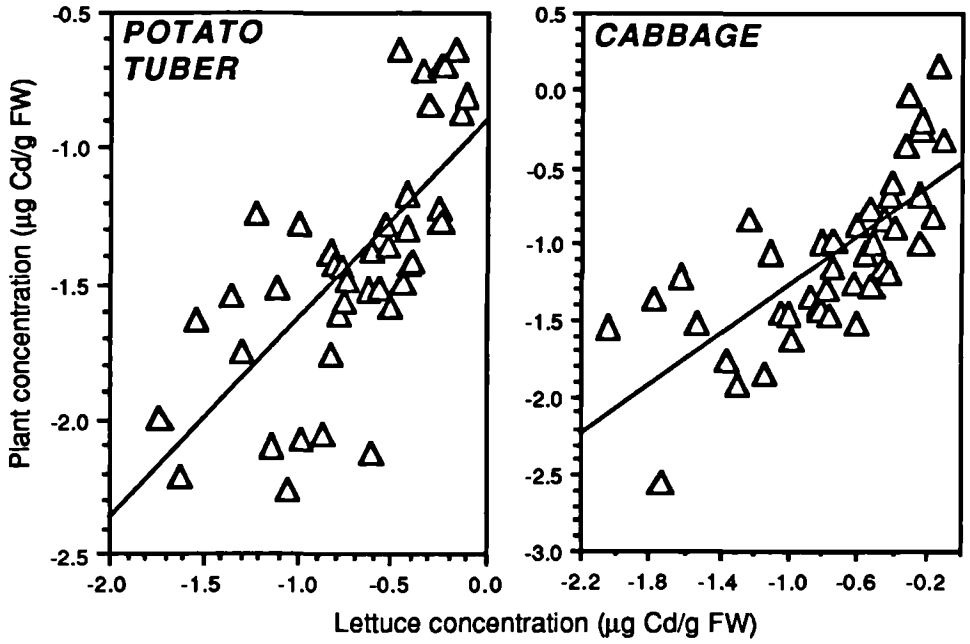


Figure 4.51: *The relationships between the cadmium concentration (FW) in a index plant and two food crops*

The relationship between potato tuber and lettuce cadmium concentrations, is described by the equation:

Potato Cd = 0.74(Lettuce Cd) - 0.90

and has a p-value of less than 0.001; the r-squared value is 0.49. The relationship between cabbage and lettuce cadmium concentrations is described by the equation:

Cabbage Cd = 0.80(Lettuce Cd) - 0.48

The r-squared value for this relationship is 0.53, the p-value is less than 0.001.

The relationship between the zinc concentrations of lettuces and potato tubers is shown in Figure 4.52.

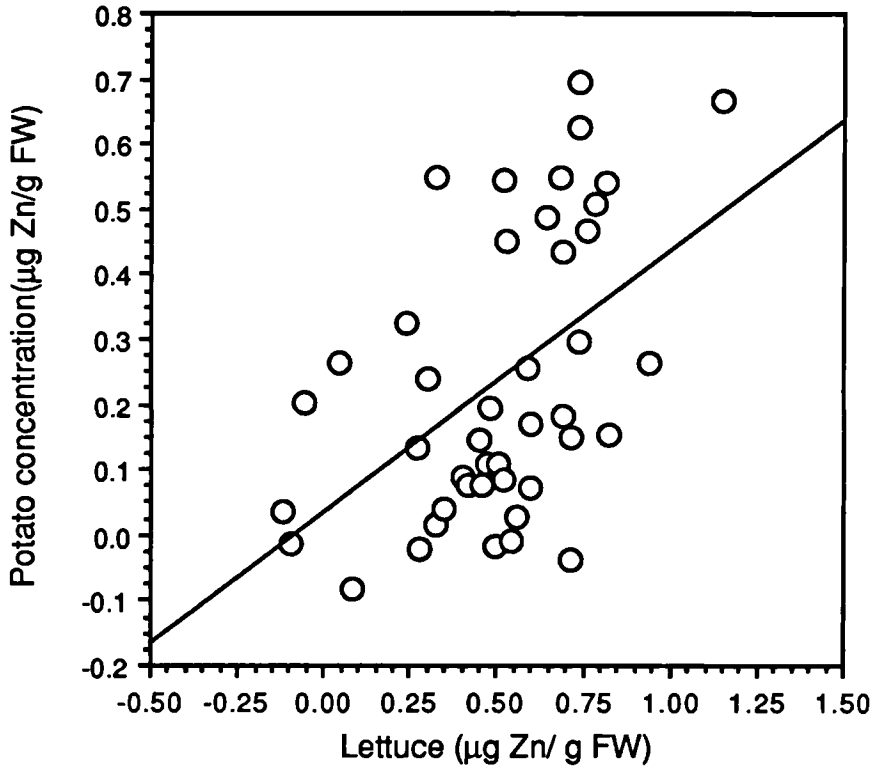


Figure 4.52: *The relationship between the zinc concentrations in potato tubers and those in lettuces*

The regression equation for this relationship is:

Potato Zn = 0.40(Lettuce Zn) + 0.03

The r-squared value for the relationship is 0.248 and the p-value is 0.0007.

These analyses imply that lettuce may be used as an index plant for the assessment of dietary exposure to cadmium from potato tubers and cabbages. This assumes that there is no post-harvest contamination of the sample during preparation prior to consumption. For an accurate assessment of cadmium exposure from the whole diet, analyses of a wider range of crops is necessary.

4.4.4.5: *Conclusions from univariate analyses*

Linear regression and correlation analysis of soil and plant data indicated that the total or nitric acid extractable concentration of cadmium in the soil are the key soil variables influencing the concentration of cadmium in cabbage, lettuce and potato tubers. The CF-values for cabbage and potato tubers were inversely related to the nitric acid extractable soil cadmium concentration. The lettuce CF-value was most significantly correlated with the soil pH ($CaCl_2$). Table 4.50 summarises those soil variables most significantly correlated with the cadmium concentrations of the three crops grown in this study.

CROP	CADMIUM CONCENTRATION	CADMIUM CF-VALUE
Cabbage	y = 0.52 Cd (Total) - 0.55 r-squared = 0.550***	y = 0.43 - 0.54 Cd (HNO_3) r-squared = 0.669***
Lettuce	y = 0.44 + 0.43 Cd (HNO_3) r-squared = 0.333***	y = 2.81 - 4.04 pH ($CaCl_2$) r-squared = 0.147*
Potato	y = 0.54 Cd (Total) - 1.26 r-squared = 0.592***	y = -1.19 - 0.50 Cd (HNO_3) r-squared = 0.565***

Table 4.50: *Soil parameters having the most significant effect on crop cadmium concentrations*

Zinc data were analysed in the same way as that for cadmium, Table 4.51 summarises the most significant correlations with crop zinc concentrations.

CROP	ZINC CONCENTRATION	ZINC CF-VALUE
Lettuce	y = 4.40 pH ($CaCl_2$) - 2.85 r-squared = 0.392***	y = 1.72 - 0.85 Zn (HNO_3) r-squared = 0.538***
Potato	y = 0.44 + 0.41 LOI r-squared = 0.185**	y = 0.22 - 0.73 Zn (HNO_3) r-squared = 0.534***

Table 4.51: *Soil parameters having the most significant effect on crop* zinc *concentrations*

The concentration of zinc in both lettuces and potato tubers is not best correlated with the zinc concentration of the soil. Soil pH ($CaCl_2$) and loss-on-ignition are best correlated with the concentrations of zinc in lettuces and potato tubers respectively. The CF-values for the crops are both significantly inversely correlated with the nitric acid extractable soil zinc concentration.

Four soil extractants, EDTA-(Na_2), DTPA, $CaCl_2$ and NH_4NO_3 were used in order to estimate the bioavailability of cadmium and zinc from sewage sludge-amended soils to three crops. The two neutral salt extractants showed an inverse correlation between the percentage metal extracted from the soil and soil pH ($CaCl_2$). DTPA was the single most accurate predictor for the plant cadmium concentration. The buffering capacity of DTPA, means that the negative correlations between soil pH ($CaCl_2$) and percentage extraction shown by the neutral salts, were not significant, refer to Figure 4.41. A more thorough discussion of the experiments using soil extractants is presented in Section 4.4.4.3.5.

It has been suggested that lettuce could be used as an index species for the assessment of heavy metal bioavailability. In this study of plants grown on soils which had been amended with sewage sludges, the

concentrations of cadmium in lettuces were significantly correlated with those in both cabbages and potato tubers.

4.4.5: MULTIVARIATE APPRAISAL OF THE BIOAVAILABILITY OF CADMIUM TO PLANTS GROWN ON SEWAGE SLUDGE-AMENDED SOILS

4.4.5.1: *Introduction*

The use of multivariate analysis enables a number of interacting variables to be considered simultaneously; the behaviour of contaminants in the environment may be effected by a number of factors. This is particularly true of the behaviour of cadmium in soils and the subsequent plant uptake (Alloway *et al* 1990). The technique chosen for these analyses was stepwise multiple linear regression analysis. This is a subsidiary of linear multiple regression analysis.

Linear multiple regression analysis attempts to predict the variation of a single dependent variable (Y), from a number of independent variables. The generalised form of the resulting equation is shown below:

$$Y = a + b_1X_1 + b_2X_2 + \ldots b_iX_i \pm e$$

where a = intercept value, b_1 to b_i = partial regression coefficients and e = error term. Independent variables in stepwise linear multiple regression analysis are selected by forward inclusion, so eliminating unnecessary variables. The forward inclusion procedure selects, as the next variable, that independent variable with the highest partial correlation with the dependent variable. With the inclusion of a new variable in the model, all of the previously entered variables are reevaluated and removed if the partial F-ratio becomes lower than 4.

All of the data to be analysed using this technique had been normalised by logarithmic transformation. The independent variables were used in two basic combinations as shown in Figure 4.53. Resulting in a maximum of five multiple regression equations for each dependent variable; two dependent variables were used for each crop, the cadmium concentration and CF-value.

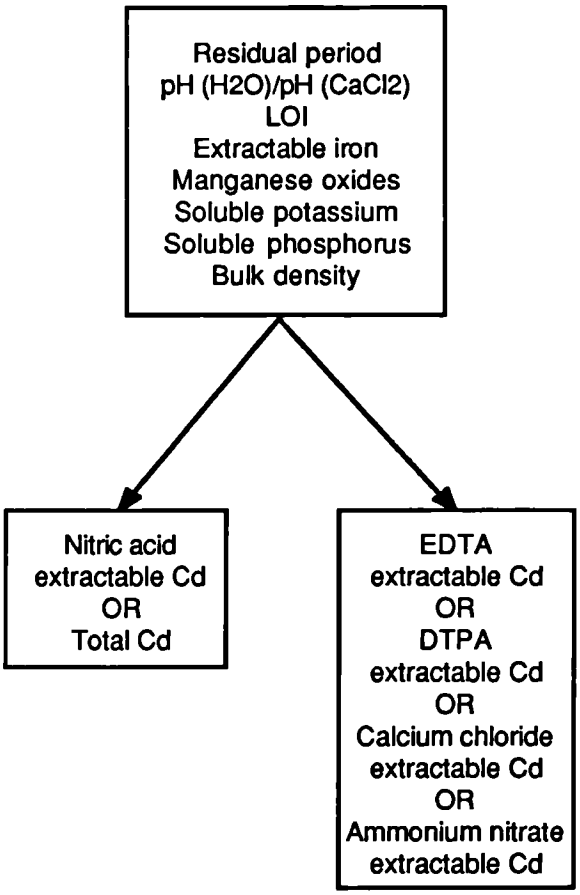


Figure 4.53: *Combinations of independent variables used in stepwise multiple linear regression analyses*

4.4.5.2: *Cabbage*

The multiple linear regression equations for cadmium concentrations in cabbages are shown in Table 4.52.

EQUATION	υ_1	υ_2	Critical value	F-ratio	R^2	p-value
y = log cadmium concentration						
a) y = 2.7 - 3.21 pH ($CaCl_2$) - 0.67 LOI + 0.47 Total Cd	3	39	2.84	23.86	0.647	0.001
b) y = 2.55 - 2.84 pH ($CaCl_2$) - 0.64 LOI + 0.42 EDTA Cd	3	39	2.84	24.76	0.656	0.001
c) y = 2.33 - 2.58 pH ($CaCl_2$) - 0.60 LOI + 0.49 DTPA Cd	3	39	2.84	30.05	0.698	0.001
d) y = 0.50 - 0.44 Residual period + 0.58 $CaCl_2$ Cd	2	26	3.37	39.08	0.754	0.001
e) y = 0.29 + 0.43 NH_4NO_3 Cd	1	29	4.17	31.13	0.518	0.001
y = log cadmium CF-value						
a) y = 2.77 - 3.17 pH ($CaCl_2$) - 0.70 LOI - 0.56 HNO_3 Cd	3	39	2.84	25.51	0.662	0.001
b) y = 3.14 - 3.25 pH ($CaCl_2$) - 0.85 LOI - 0.31 EDTA Cd - 0.56 Residual period	4	26	2.74	9.36	0.590	0.001
c) y = 4.05 - 3.03 pH ($CaCl_2$) - 1.51 LOI - 0.41 DTPA Cd - 0.36 Mn oxides - 2.67 Bulk density	5	37	2.45	13.06	0.638	0.001

All data had been normalised prior to analysis. The critical value is for p = 0.05.

υ = degrees of freedom

Table 4.52: *Multiple regression equations for the concentration of cadmium in cabbages grown on sewage sludge-amended soils*

Figure 4.54 shows the observed values plotted against those predicted by the multiple regression equations. The notations a to e used in the graphs correspond to those in Table 4.52.

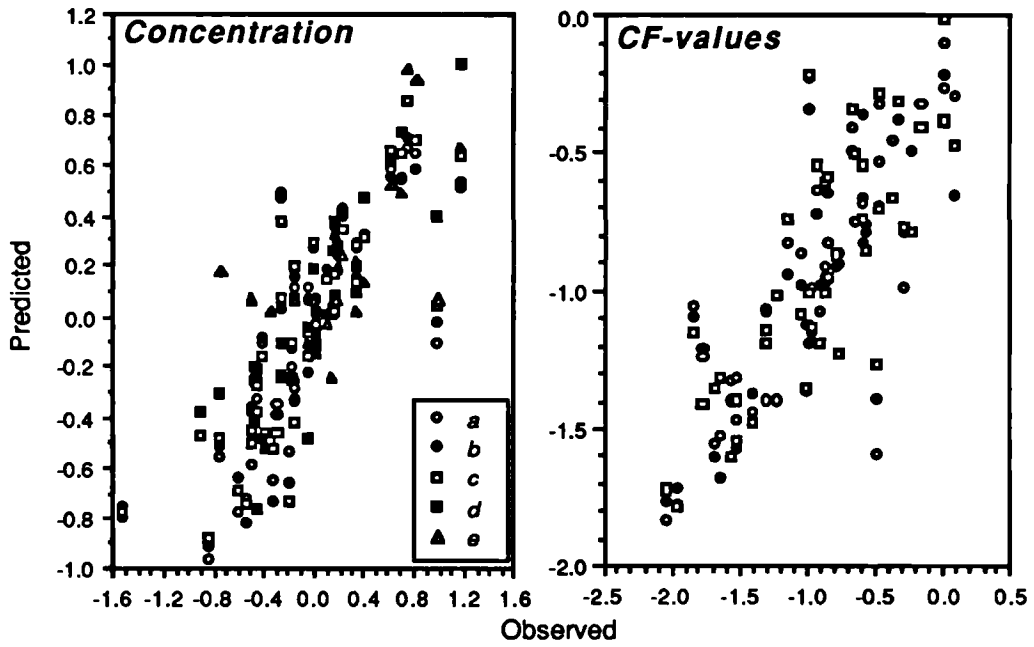


Figure 4.54: *Scattergrams of observed against predicted values for (i) the concentrations of cadmium in cabbages and (ii) the CF-values for cabbages*

Using the concentration of cadmium in cabbages as the dependent variable, the two most commonly included soil variables are the pH ($CaCl_2$) and organic matter content, as measured by the loss-on-ignition method. Both of the partial regression coefficients for these variables are negative, indicating an inverse relationship with the cabbage cadmium concentration. The effects of soil pH on plant cadmium concentrations are discussed in section 4.4.2. Almost half of the soils used in this study were limed in order to raise their pH, the effects on plant concentrations are illustrated by Figures 4.33 and 4.40. The inverse relationship with soil organic matter content may be explained in terms of the sorptive capacity of soil organic matter; increasing soil organic matter content leading to a

greater cation exchange capacity (Alloway *et al* 1988). Using the cadmium CF-value as the dependent variable, produced similar sets of independent variables to those analyses discussed above. Both soil pH ($CaCl_2$) and organic matter content were included and had negative partial correlation coefficients. Five variables were included in the equation in which the soil cadmium concentration was determined by extraction with DTPA, these included the bulk density of the soil and the easily-reducible manganese content.

In order to more rigourously test the validity of the multiple linear regression equations presented in Figure 4.52, data from a previous study was entered into the equations. The study chosen was that by Alloway (1986), in which a number of crops were grown in a glasshouse on soils contaminated from a variety of sources. Figure 4.55 is a plot of the predicted cabbage cadmium concentration against that observed by Alloway. The equation with the highest r-squared value was usually used for this comparison; however for cabbages this was not possible, as no data were available for the residual period (d). Equation c was used as it was the next most significant.

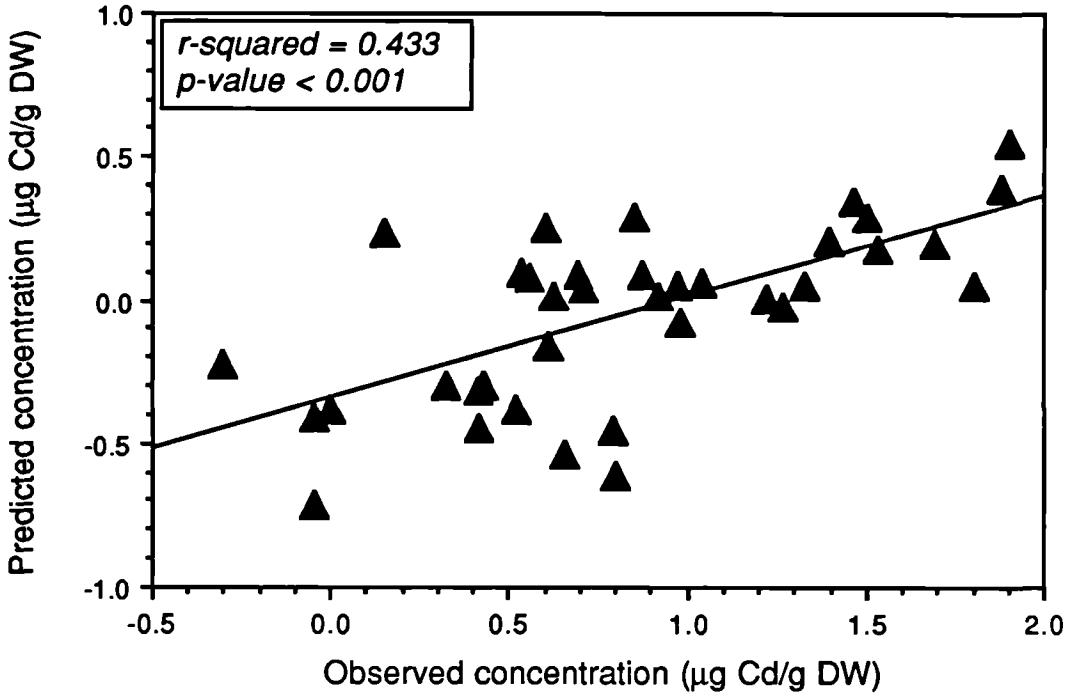


Figure 4.55: *The relationship between the predicted concentrations of cadmium in cabbage and the observed values from an independent data set*

The equation for this linear regression is given below:

Predicted = 0.36 Observed - 0.35

This implies that equation c underestimates the uptake of cadmium by cabbages grown in a glasshouse. This is to be expected in light of the differences in microclimatic factors, rooting density and nature of the soils used. Some of the soils used by Alloway were from areas contaminated by mine waste, Shipham for example, and other areas where the soils were contaminated as a result of atmospheric deposition or by the application of sewage sludge; this gives rise to high coefficients of variation for the data set (Alloway *et al* 1990).

The most significant equation for prediction of the CF-value was tested against the same independent data set; a comparison of the observed and predicted value is shown in Figure 4.56. As was the case with the prediction of the concentration of cadmium, the best equation could not be used as Alloway gave no data for the residual period; therefore equation a from Table 4.52 was used.

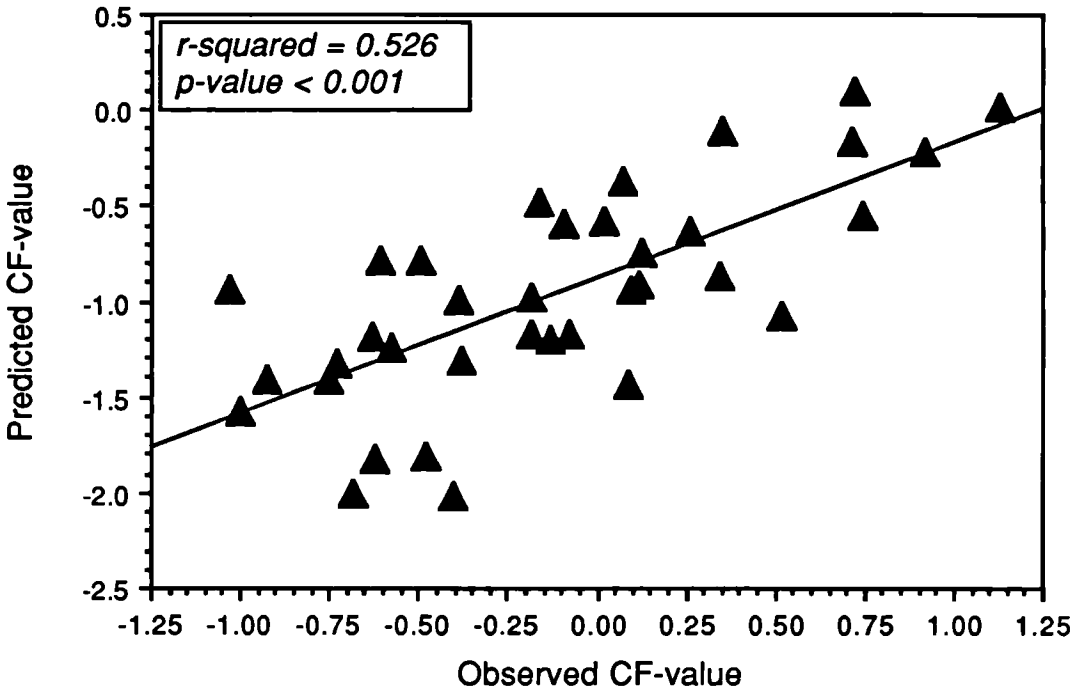


Figure 4.56: *The relationship between the predicted cadmium CF-values for cabbage and the observed CF-values from an independent data set*

The relationship between those CF-values observed by Alloway and those predicted using equation a in Table 4.52 is given below:

Predicted = 0.71 Observed - 0.88

As was the case for the concentration of cadmium in cabbage, the equation underestimates the CF-value. Multiple linear regression equations were previously derived for the CF-values of the data set used

to test those derived for this data set (Alloway *et al* 1990). The equation for the CF-value of cabbages is given below:

log CF-value = 6.12 - 4.92 log pH($CaCl_2$) - 1.16 log LOI - 0.49 log Mn oxides + 0.23 log NH_4NO_3 Cd

Soil pH and the loss-on-ignition are independent variables in all of equations for the CF-values in Table 4.52. Ammonium nitrate extractable cadmium is not a significant component of the equations derived from this study of sewage sludge-amended soils. Alloway *et al* divided the data into two subsets, inorganically contaminated soils and sewage sludge-amended soils. The equation for sewage sludge-amended soils contained the soil cation exchange capacity and nitric acid extractable cadmium concentration as the independent variables.

4.4.5.3: *Lettuce*

The multiple linear regression equations for cadmium concentrations in lettuces are shown in Table 4.53. As was the case when the cadmium concentration of cabbages was the dependent variable, both soil organic matter content and the soil pH ($CaCl_2$) are included in the first set of equations and both have negative partial regression coefficients.

EQUATION	υ_1	υ_2	Critical value	F-ratio	R^2	p-value
y = log cadmium concentration						
a) y = 3.9 - 3.62 pH ($CaCl_2$) - 0.51 LOI + 0.38 Total Cd	3	39	2.84	18.16	0.583	0.001
b) y = 3.69 - 3.3 pH ($CaCl_2$) - 0.53 LOI + 0.38 EDTA Cd	3	39	2.84	20.62	0.613	0.001
c) y = 3.52 - 3.11 pH ($CaCl_2$) - 0.50 LOI + 0.43 DTPA Cd	3	39	2.84	22.29	0.632	0.001
d) y = 0.97 + 0.36 $CaCl_2$ Cd	1	35	4.13	27.54	0.440	0.001
e) y = 1.10 + 0.26 NH_4NO_3 Cd	1	29	4.17	8.31	0.223	<0.01
y = log cadmium CF-value						
a) y = 3.60 - 4.72 pH ($CaCl_2$) - 0.32 HNO_3 Cd	2	40	3.23	6.25	0.238	<0.01
b) y = 3.29 - 4.22 pH ($CaCl_2$) - 0.41 EDTA Cd	2	37	3.29	7.86	0.298	<0.01

All data had been normalised prior to analysis. The critical value is for p = 0.05.

υ = degrees of freedom

Table 4.53: *Multiple regression equations for the concentration of cadmium in lettuces grown on sewage sludge-amended soils*

Figure 4.57 shows the observed values plotted against those predicted by the multiple regression equations. The notations a to e used in the graphs correspond to those in Table 4.53.

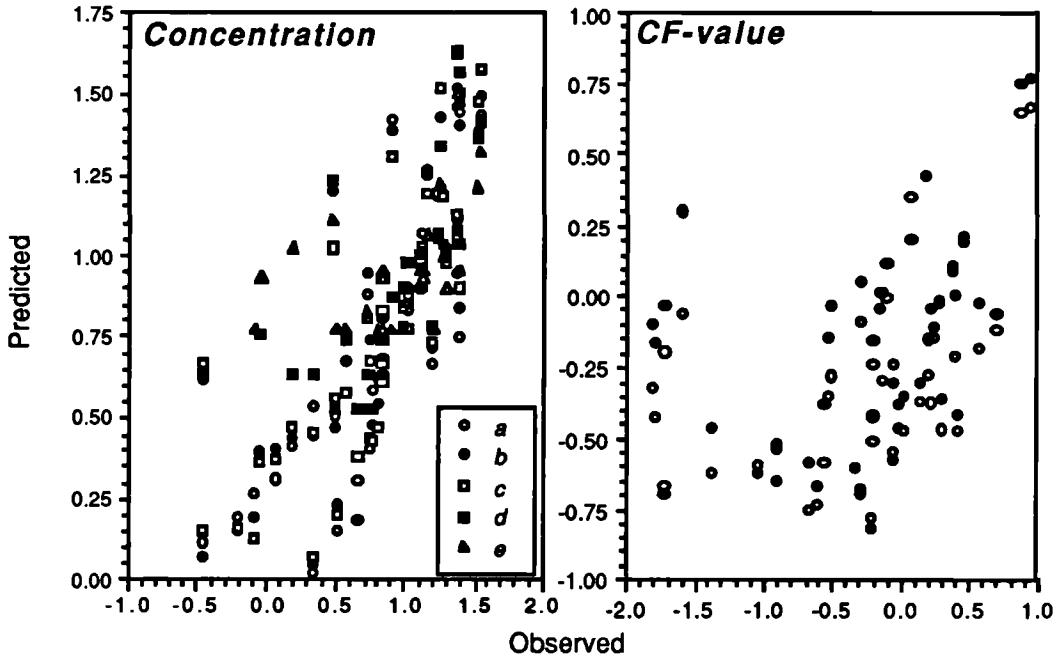


Figure 4.57: *Scattergrams of observed against predicted values for (i) the concentrations of cadmium in lettuces and (ii) the CF-values for lettuces*

From Figure 4.57 and the p-values in Table 4.53, it is clear that the equations fit the observed data less well for the CF-values than for the concentrations. The multiple linear regression equations in Table 4.53 were tested in the same way as those in Table 4.52 for lettuce, ie the predictions were tested against another set of data. A plot of the observed against the predicted values for the concentration of cadmium in lettuce is shown in Figure 4.58. Equation c from Table 4.53 was used to calculate the predicted concentrations.

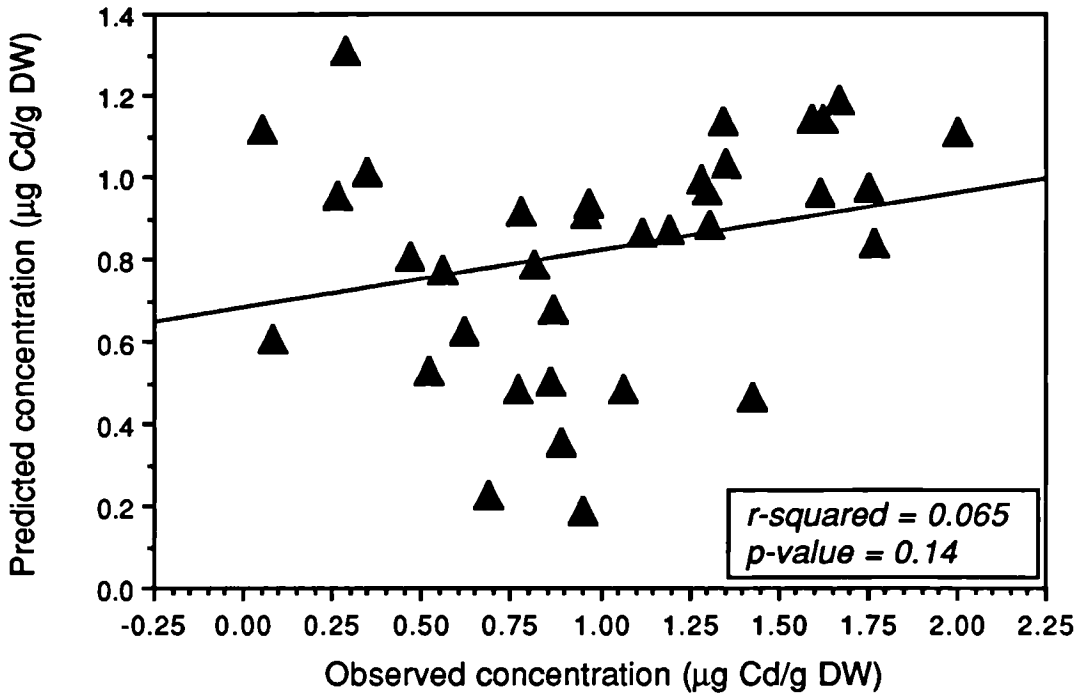


Figure 4.58: *The relationship between the predicted concentrations of cadmium in cabbage and the observed concentrations from an independent data set*

The relationship between those concentrations observed by Alloway (1986) and those predicted using equation c in Table 4.53 is given below:

Predicted = 0.14 Observed - 0.68

The relationship shown in Figure 4.58 is statistically insignificant due to it's low r-squared value and the high p-value; this implies that the equations shown in Table 4.53 are not suitable for the prediction of the cadmium concentrations in cabbages grown in the experiments by Alloway (1986). Figure 4.59 shows the relationship between the cabbage CF-values observed by Alloway *et al* (1990) and those predicted by equation b in Table 4.53.

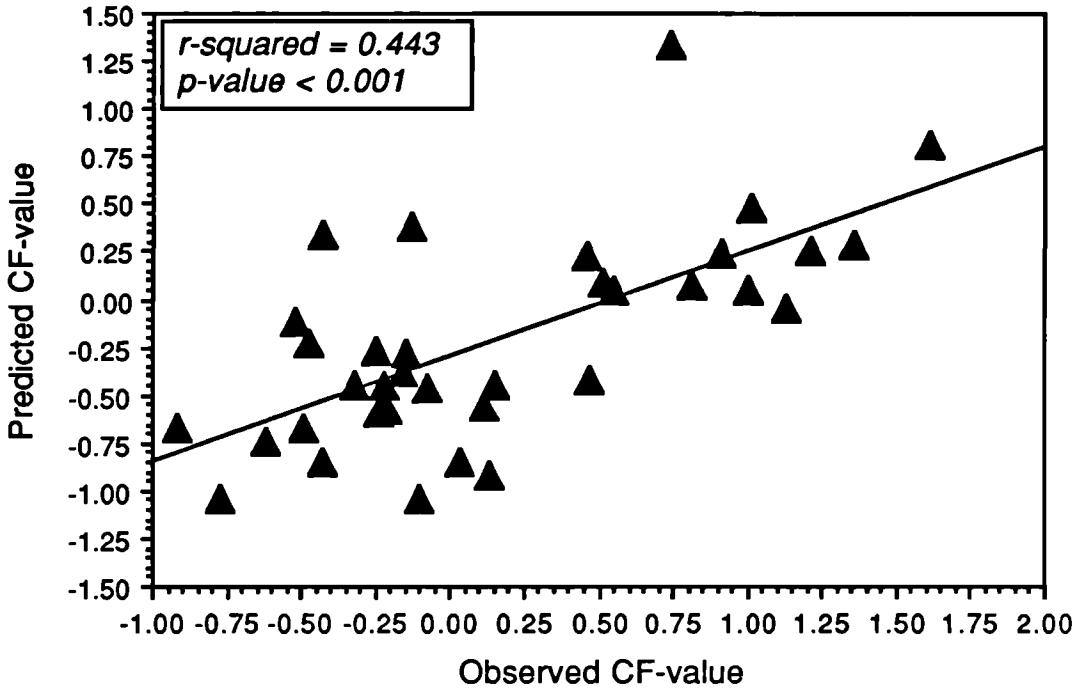


Figure 4.59: *The relationship between the predicted cadmium CF-values for lettuce and the observed CF-values from an independent data set*

The observed and predicted CF-values are related by the equation below:

Predicted = 0.55 Observed - 0.30

This relationship, unlike that shown in Figure 4.58, is statistically significant. Alloway *et al* (1990) derived multiple linear regression equations for the CF-values of cabbages grown on a range of polluted soils. All derived equations had two independent variables, the soil cation exchange capacity and the nitric acid extractable soil cadmium concentration. The equation is shown below

log CF-value = 2.72 - 2.74 log Cd HNO_3 - 0.53 log CEC

Cation exchange capacity was not measured for the soils in this study.

4.4.5.4: *Potato tubers*

The multiple regression equations for the concentration of cadmium in potato tubers are shown in Table 4.54. The regression equations with the cadmium concentration of potato tubers as the dependent variable, contain no more than two independent variables due to the strong correlation between the soil cadmium concentrations and those of the tubers. Soil pH ($CaCl_2$) is included in equations d and e but it has a positive partial regression coefficient, this is not the case for either lettuces or cabbages. The concentration of cadmium in potato tubers grown on limed soils was greater then that for those potatoes grown on unlimed soils (see Figure 4.33)

EQUATION	υ_1	υ_2	Critical value	F-ratio	R^2	p-value
y = log cadmium concentration						
a) y = 0.50 HNO_3Cd - 1.20	1	39	4.08	51.45	0.569	0.001
b) y = 0.50 EDTA Cd - 1.09	1	39	4.08	74.73	0.657	0.001
c) y = 0.59 DTPA Cd - 1.06	1	39	4.08	122.41	0.758	0.001
d) y = 0.43 $CaCl_2$ Cd + 2.0 pH ($CaCl_2$) - 2.12	2	33	3.30	22.73	0.579	0.001
e) y = 0.43 NH_4NO_3 Cd + 1.95 pH ($CaCl_2$) - 1.87	2	27	3.37	13.63	0.502	0.001
y = log cadmium CF-value						
a) y = - 1.20 - 0.50 HNO_3 Cd	1	39	4.08	50.68	0.565	0.001
b) y = - 1.36 - 0.41 EDTA Cd	1	39	4.08	30.74	0.441	0.001
c) y = -1.44 - 0.37 DTPA Cd	1	39	4.08	17.12	0.305	0.001
d) y = 1.33 - 0.34 $CaCl_2$ Cd - 3.07 pH ($CaCl_2$) - 0.67 LOI	3	32	2.92	6.54	0.380	0.010

All data had been normalised prior to analysis. The critical value is for p = 0.05.

υ = degrees of freedom

Table 4.54: *Multiple regression equations for the concentration of cadmium in potato tubers grown on sewage sludge-amended soils*

Figure 4.60 shows the observed values plotted against those predicted by the multiple regression equations. The notations a to e correspond to those in Table 4.54.

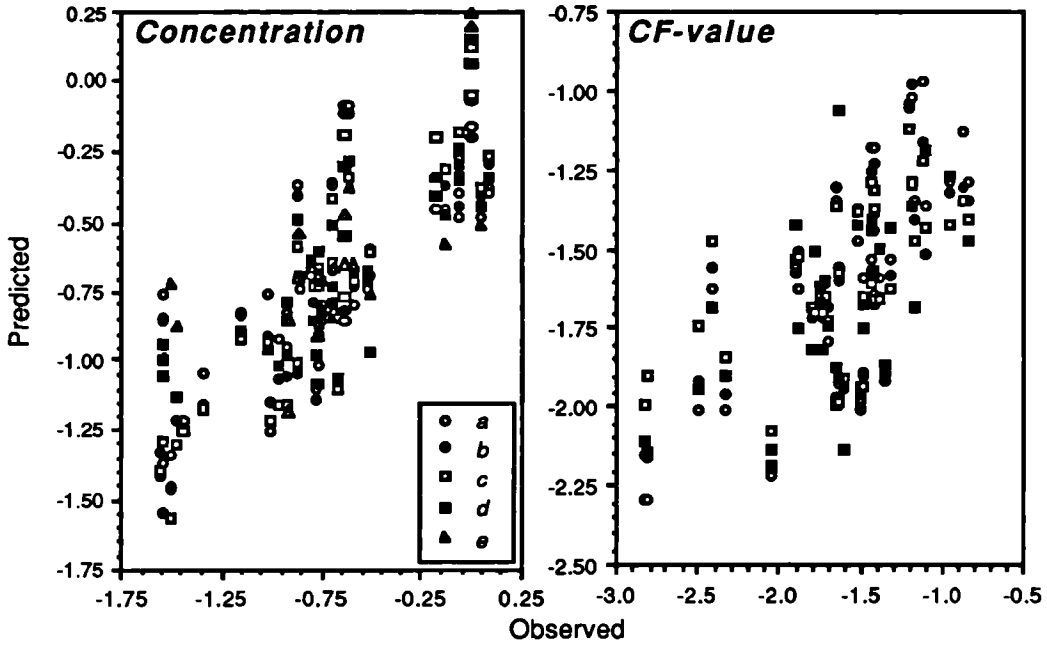


Figure 4.60: *Scattergrams of the observed against predicted values for (i) the concentrations of cadmium in potato tubers and (ii) the CF-values for potato tubers*

It was not possible to test these equations against an independent data set as no data were available for potatoes.

4.4.5.5: *Conclusions*

The use of multivariate analysis has enabled soil parameters to be combined in equations predicting the concentration of cadmium in three crops and their associated CF-values. Soil pH and organic matter content were shown to be significant in effecting the concentration of cadmium in both cabbages and lettuces. Both of these two independent variables had negative partial regression coefficients. Potato tuber cadmium concentrations were mainly effected solely by the nitric acid or

chelatable cadmium concentration. Where pH ($CaCl_2$) was included in the equation for potato tubers, it had a positive partial regression coefficient. Regression analyses using the cadmium CF-value tended to include fewer independent variables, those that were included tended to be the same as were included when the plant cadmium concentration was used as the dependent variable.

CHAPTER 5: PRELIMINARY CADMIUM SPECIATION STUDIES

5.1: INTRODUCTION

5.1.1: DEFINING THE SPECIATION OF TRACE ELEMENTS

The speciation of a trace element is now widely recognised as being an important factor governing it's behaviour in the environment (Bremmner 1986, Irgolic 1987, Mills 1986, Wolf 1986a). Definitions of the word "speciation" vary and are still the source of some confusion. The Dahlem Workshop on "The Importance of Chemical 'Speciation' in Environmental Processes" defined a species as being the "molecular representation of a specific form of an element" (Bernhard *et al* 1986). This definition has subsequently been adopted by a number of other such workshops (Reuther 1987). Ure and Griepink (*pers. com.* 1989) give three definitions of "speciation":

- *functionally defined* - for example 'plant-available' species.
- *operationally defined* - by the use of specific reagents or procedures, identify and quantify an element phase or form. An example of this definition is the use of hydrogen peroxide to isolate "organically bound" species.
- *specific chemical compounds or oxidation states* - for example tributyl tin oxide, methyl mercury etc.

Forstner (1987) uses "speciation" in one of two ways, when referring to either:

- *analyte species* - during analysis, those species to which the instrument is sensitive; or
- *matrix species* - determined by the matrices and leading to changes in reactivity, solubility and bioavailability.

When referring to soils and related media, "speciation" is often referred to as being of either the solid or liquid phase (Brummer 1986, Lake *et al* 1984, Lund *et al* 1985). Speciation of metals in the solid phases is closely linked to the sorption mechanisms, whereas liquid-phase speciation is largely determined by the chemical properties of the particular metal and the composition of the soil solution.

Unless otherwise stated, the definition of speciation used here will be that referring to specific chemical compounds.

5.1.2: THE ROLE OF SPECIATION

Speciation is an important factor in determining the behaviour of an element in the environment, cadmium speciation and it's relationship with bioavailability was examined in Chapter 2. More specifically, toxicological studies of cadmium are beginning to demonstrate that discrepancies in fate exist between cadmium species (refer to Section 2.2.4). Tills and Alloway (1983) have demonstrated that the liquid-phase speciation of cadmium in soils shows some variation. Although cadmium speciation is becoming quantifiable (see Section 5.1.3), the implications of different species existing within a given system remain to be evaluated. The role of laboratory based simulation studies is limited, given the discrepancies that exist between these and natural systems. Allard *et al* (1987), when examining the role of speciation in sorption phenomena, state that "...the difficulties in explaining or predicting the quantitative distribution and transport properties of trace metals under environmental conditions are substantial, despite the large volume of data from sorption studies under ideal laboratory conditions.".

5.1.3: THE DETERMINATION OF METAL SPECIES

A recognition of the importance of metal speciation has led to the need for the

development of suitable methods by which to quantify certain species. Irgolic (1987) states that "...'total element' thinking cannot lead to an understanding of bioinorganic processes on a molecular basis,".

Determination of specific chemical compounds usually requires a means for isolation of the compound from the sample matrix, followed by detection of the metal of interest. Separations are often achieved using chromatographic methods, gas chromatography for volatile species (Ebdon *et al* 1986) and liquid chromatography for non-volatile species (Ebdon *et al* 1987). Gas chromatography - atomic absorption spectrometry (GC-AAS) provides a highly sensitive and selective means for the determination of certain metal species (Fernandez 1977). It has been widely applied, for example to the determination of arsenic species (Ebdon *et al* 1988).

Liquid chromatography (LC) is a suitable means for the separation of certain cadmium species and will be considered further below.

5.1.3.1: *High performance liquid chromatography*

High performance liquid chromatography (HPLC) is a development of the established column chromatography technique. HPLC typically involves the use of a metal column packed with the *stationary phase* through which the liquid *mobile phase* is passed under pressure (Hamilton and Sewell 1982). Size exclusion (SEC) or gel permeation chromatography is a sub-division of the HPLC family of techniques. Separations in SEC are achieved by a single unambiguous mechanism (Engelhardt 1979); molecules are separated on the basis of their effective size and shape in solution (Lindsay 1987). Stationary phases in SEC are porous, pore size being closely controlled. Very small molecules should be able to enter any of the pores, whereas large molecules will not and consequently stay in the mobile phase, passing rapidly down and out of the column (Pryde and Gilbert 1979). Retention time on the

column is therefore related to molecular size, with the smallest molecules having the longest retention times. SEC has found applications to the study of metal species (Veening and Willeford 1983), some of which will be discussed below.

5.1.3.2: *Element specific detection systems for HPLC*

A range of detection systems exist for the monitoring of metals in the eluent from HPLC systems (Ebdon *et al* 1987, Wolf 1986b). Atomic spectroscopy is by far the most widely used method of detection. Irgolic and Brinckman (1986) state that "The major requirements for a successfully operating system include a flow rate through the column matching the demands of the detector and compatability of the mobile phase with the detector.".

The major problem in developing a suitable detector lies in the need for a high degree of sensitivity concurrent with no loss of resolution. Resolution is lost when fractions are collected from an HPLC column and subsequently analysed, what is needed is a system able to separate and analyse samples in "real-time". Coupling HPLC to flame AAS (FAAS) would seem to be an obvious solution to the interfacing problem (Hill *et al* 1986); however, there are problems associated with the flow rate demanded by the FAAS and that supplied by the HPLC. A number of possible solutions have been developed and applied to the analysis of "real" samples (Gustavsson and Nygren 1987, Nygren *et al* 1989). A problem with the use of FAAS as the detector is that it is often not sufficiently sensitive for the determination of some metals. A solution to this problem is the use of electrothermal atomisation atomic absorption spectrometry (ETA-AAS) as a detector (van Loon 1979).

HPLC-ETA-AAS interfacing systems have been developed and applied to a wide variety of samples. However, there is still a major problem with the loss of resolution associated with the time/temperature program of ETA-AAS, this

leading to what is effectively discrete sampling. One of the earliest systems was described by Brinckman *et al* (1977), this system used an all Teflon "well sampler" as the interface between the LC column and the ETA-AAS (referred to as GFAAS in this paper). Eluent from the LC column enters the well from the bottom and is sampled (10 to 50µl) using a conventional autosampler (Perkin Elmer AS-1). This system was used to resolve and detect trace organometallic compounds of arsenic, lead, mercury and tin. Parks *et al* (1979) applied this system to the determination of biocidal organotin moieties and organotin silicates, after SEC and reversed bonded phase separations respectively. An HPLC-ETA-AAS system with Zeeman effect background correction has been applied to the determination of cobalt speciation in samples of Vitamin B_{12} (Irgolic and Brinckman 1986; Koizumi *et al* 1978). An interface between an Instrumentation Laboratory 555 CTF atomizer and HPLC has been described by Haswell *et al* (1987); this system uses a solenoid-driven syringe to inject eluent from a sample loop into the atomiser and has been used to resolve three arsenic species from a single solution.

Despite the advances made in the development of HPLC-ETA-AAS systems, they still do not provide a continuous time-based supply of data for the concentration of metal in the eluent. A recent paper by Nygren *et al* (1988) describes an HPLC-ETA-AAS interface that allows such data to be collected. By using a design based upon the integrated contact furnace, previously described in Chapter 3, and a thermo interface; it is possible to obtain a true chromatogram for the element of interest. The system was successfully used to resolve and quantify butyltin compounds in wood preservatives.

Inductively coupled plasma spectrometry (ICP) has been applied to monitoring the concentration of specific elements in chromatography eluents (Ebdon *et al* 1987). ICP atomic emission spectrometry (ICP-AES) has been widely applied to the study of a range of sample types and metal species; for example

Nisamaneepong *et al* (1984) applied HPLC-ICP-AES the simultaneous determination of cadmium and arsenic species. The development of inductively coupled plasma mass spectrometry (ICP-MS) has enabled a more sensitive on-line detector to be used for HPLC (Dean *et al* 1989). HPLC-ICP-MS combines highly sensitive eluent analysis, at least equivalent to ETA-AAS, whilst remaining true to the chromatographic principle. Crews *et al* (1989) have applied SEC-ICP-MS to the determination of cadmium speciation in cooked and uncooked samples of pig kidney. Results showed that the majority of cadmium was associated with a metallothionein-like protein that survived both cooking and enzymolysis. Beauchemin *et al* (1988) have employed HPLC-ICP-MS for the determination of arsenic species in a sample of dogfish muscle reference material.

5.2: EXPERIMENTS TO DETERMINE THE SPECIATION OF CADMIUM IN FOODS

5.2.1: BACKGROUND

A study was conducted in order to evaluate the feasibility of determining the speciation of cadmium in a staple food, ie potato tubers. The methods employed were based upon earlier work examining the speciation of cadmium in pig kidney; this used a linked system comprising a Pharmacia fast protein liquid chromatography (FPLC) system and a VG ICP-MS (Crews *et al* 1989). This enabled the separation of a cytosol sample using a size exclusion column and the subsequent detection of cadmium by the ICP-MS and UV detection at 254 nm. The study showed that cadmium in kidney was bound primarily to low molecular weight proteins of 6.3Kd and 8.9Kd in the raw and cooked samples respectively. Two other peaks, both of higher molecular weight, were found in the raw sample but these were absent from the cooked sample.

The speciation of cadmium in foods may well be a primary determinant of the relative bioavailability and toxicity of cadmium to the human consumer (Fox 1988). As ~60% of the total human dietary exposure to cadmium is derived from vegetable and cereal crops and associated produce, it is desirable that a speciation profile is determined for these two food groups and that the relative toxicities of the different species be examined. Research, mainly upon non-food plants, has shown that cadmium is bound to low molecular weight cysteine and glutamic acid-rich proteins called phytochelatins (PCs) or phytometallothioneins (Grill 1987). The characteristics of a number of cadmium-binding proteins are listed in Table 5.1.

Sample	Molecular weight of Cd-binding species (daltons)	Reference
Cabbage leaves	1×10^4	Wagner (1984)
Lettuce leaves	3.2×10^3	Henze & Umland (1987)
Tomato roots	1×10^4	Rauser (1987)
Zea mays roots	8 & 3.5×10^3	Bernhard & Kagi (1987)
Wheat grain	1.1×10^4	Wagner *et al* (1984)
Soybean plants	$>50, 13.8$ & 2.3×10^3	Casterline Jr & Barnett (1982)
Agrostis gigantea roots	3.7×10^3	Rauser (1984)
Rice plants	3.31×10^4	Kaneta *et al* (1983)
Tomato roots	1×10^4	Bartolf *et al* (1980)
Bean plants • roots	5 & 10×10^3	
• leaves	0.7 & 5×10^3	Weigel & Jager (1980)

Table 5.1: *Cadmium-binding proteins isolated from plant tissues*

A number of these proteins have been isolated from plant materials, with molecular weights ranging from 0.7 to 33.1 Kd. A problem with the majority of these papers is that the experiments which they report have used very high plant cadmium concentrations, far higher than those found in foods consumed by people. As a number of PCs would appear to be induced by high cadmium

exposures, the profiles of cadmium species reported in the literature may not necessarily be the same as those found in the same plant species exposed to lower cadmium concentrations.

5.2.2: MATERIALS & METHODS

Three basic experiments were performed to (i) examine the speciation of cadmium in potato tuber and lettuce after *in vitro* enzymolysis; (ii) determine the speciation of cadmium in the cytosol of uncooked potato tuber and (iii) examine the speciation of cadmium in cooked potato tubers intrinsically and extrinsically labelled with cadmium.

5.2.2.1: *Plant growth for analysis*

5.2.2.1.1: Introduction

Potato tuber samples grown in sewage sludge-amended soils and sand cultures were used for the cadmium speciation experiments described in the following sections. A tuber with a high concentration of cadmium after growth on a sludge-amended soil (Pikesmead), was used for the determination of cadmium species in a cytosol extraction with 0.02M Tris-HCl, this procedure is described in Section 5.2.2.2.1. In addition to a control grown on a soil not previously amended with sludges (Canwick Manor Farm), this sample was also used for *in vitro* enzymolysis procedures described in Section 5.2.2.2.2. Details of the growth of potatoes and lettuces on sewage sludge amended soils are given in Section 4.1.3. A lettuce sample previously grown on a soil with a high cadmium concentration (Galley Hill site 1) was used in *in vitro* enzymolysis experiments.

All of the samples used for the comparison of cadmium speciation in tubers after intrinsic and extrinsic labelling were grown in sand cultures. The sand culture technique was used in order to determine the distribution of cadmium

through a potato plant and to enable the relationships between cadmium exposure and tissue concentrations to be examined.

5.2.2.1.2: Sand culture experiments

Growing plants in a sand culture enables their exposure to a particular element to be closely controlled. However, exposure cannot be completely controlled, as the atmosphere is a potentially important source of cadmium to a plant (Dalenberg and van Driel 1990, Dollard and Davies 1989).

Seed potatoes (*Solanum tuberosum* cv. Home Guard) were grown in polyethylene pots of 22cm diameter and 18cm depth. The pots were filled with coarse grained horticultural sand and placed on saucers in a greenhouse. The temperature was maintained at 15°C and a "day length" of 12 hours was ensured by the use of artificial lighting. Plants were watered with a modified Hoagland nutrient solution at a rate of 4 litres per week. Three weeks after the start of germination each of the plants was exposed to a quantity of cadmium in the medium of the nutrient solution. Exposures of 10, 20, 40, 80 µg cadmium and a control were used. In addition to this form of intrinsic labelling via the sand culture solution, the possibility of injecting cadmium into the stem was examined. For this experiment an exposure of 50µg cadmium was used. Considerable problems were encountered with this method of intrinsic labelling but the results for the most successful experiment are reported.

Upon reaching maturity, the whole plant was removed from the sand by washing with large volumes of water. Each plant was divided into three parts:

- stem and leaves
- roots
- tubers

Each component was weighed and then dried at 65°C in a forced draught oven, until no further change in weight was observed. The dry weight was

recorded and used to enable concentrations of cadmium to be expressed on both fresh and dry weight bases. Cadmium determinations were made in quadruplicate by probe electrothermal atomisation atomic absorption spectrometry after sample digestion in concentrated nitric acid; data quality was maintained by the use of certified reference materials (full details of analytical methods are given in Section 3.2.2). The total mass of cadmium in a component of a plant may be calculated by multiplying the concentration by the mass of the component. Data from the experiment are given in Table 5.2.

Exposure	Component	Fresh weight (g)	Dry weight (g)	Concentration ($\mu g g^{-1}$FW)	Concentration ($\mu g g^{-1}$DW)	Total (µg)
	T	166.62	13.01	0.013	0.049	2.232
0µg	S & L	86.58	9.30	0.056	0.632	4.889
	R	23.41	2.28	0.047	2.157	1.099
	T	172.37	12.68	0.017	0.060	3.006
10µg	S & L	94.40	9.78	0.079	0.836	7.423
	R	44.78	4.24	0.054	1.335	2.417
	T	152.22	10.45	0.017	0.076	2.522
20µg	S & L	107.65	9.87	0.090	0.956	9.726
	R	44.27	3.61	0.056	1.624	2.437
	T	158.69	10.17	0.028	0.114	4.514
40µg	S & L	116.84	11.70	0.169	1.508	19.796
	R	75.34	3.73	0.080	2.205	5.996
	T	120.37	9.16	0.052	0.263	6.231
50µg	S & L	60.90	8.70	0.278	3.350	16.938
	R	64.88	2.70	0.103	3.967	6.693
	T	204.99	13.51	0.081	0.248	16.512
80µg	S & L	60.90	10.84	0.278	3.350	16.938
	R	64.88	4.16	0.103	3.967	6.693

T - tuber, R - root tissue and S & L - stem and leaf tissue.

Table 5.2: *Cadmium concentrations in potato plants grown in sand cultures of varying cadmium concentrations*

The recovery of cadmium from the sand culture solution by each potato plant can be calculated using the following equation.

$$\text{Recovery} = \frac{\mu g \text{ Cd per plant} - \mu g \text{ Cd in control}}{\text{Exposure}} \times 100$$

The mass of cadmium in the seed potato had subsequently been deducted from the sum of the totals of the plant components shown in Table 5.2. The mean concentration of cadmium in the seed potatoes used was 0.021 $\mu g g^{-1}$ FW, this figure was multiplied with the mass of each seed potato planted to give a mean figure of 1.51 µg cadmium per plant. Recoveries from the sand culture solutions were 43%, 33%, 55%, and 69% for the 10, 20, 40, and 80 µg exposures respectively. Figures 5.1a, b and c show the relationships between cadmium exposure and the concentration of cadmium in roots.

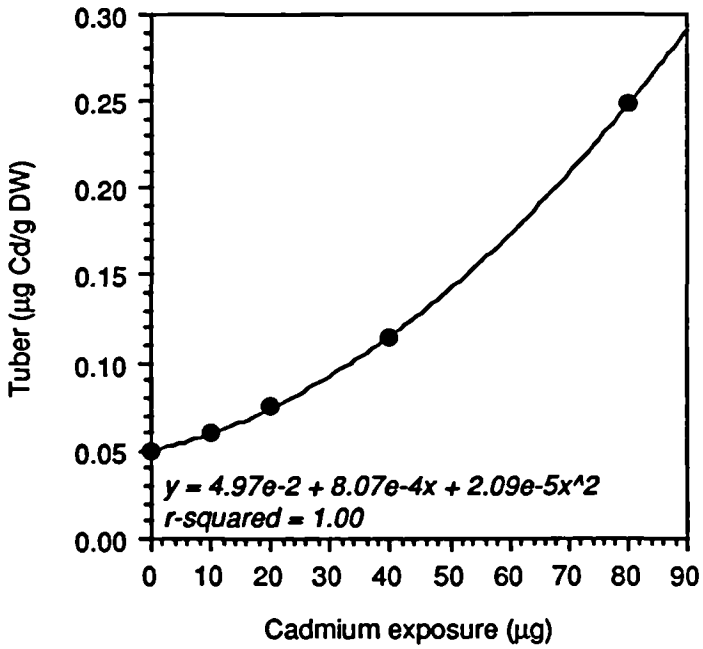


Figure 5.1a: *Concentrations of cadmium in potato tubers after varying exposures to cadmium via a sand culture solution*

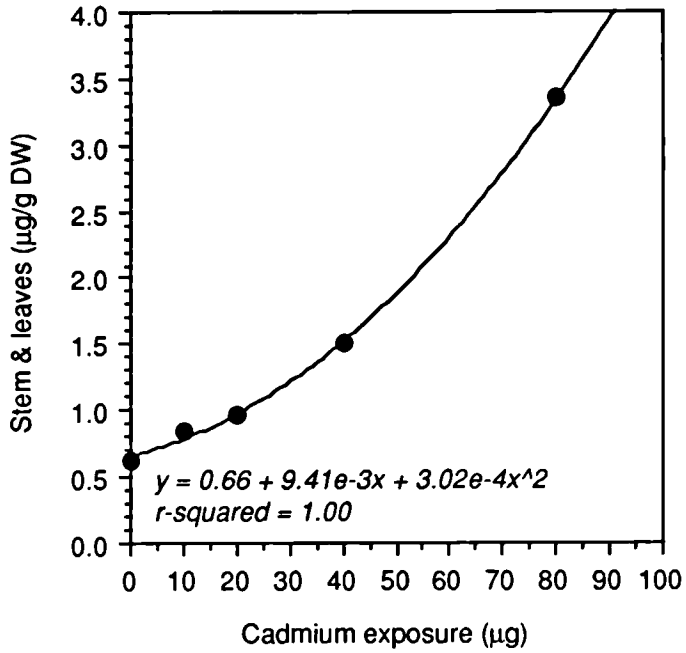


Figure 5.1b: *Concentrations of cadmium in the stem and leaf tissue of potatoes after varying exposures to cadmium via a sand culture solution*

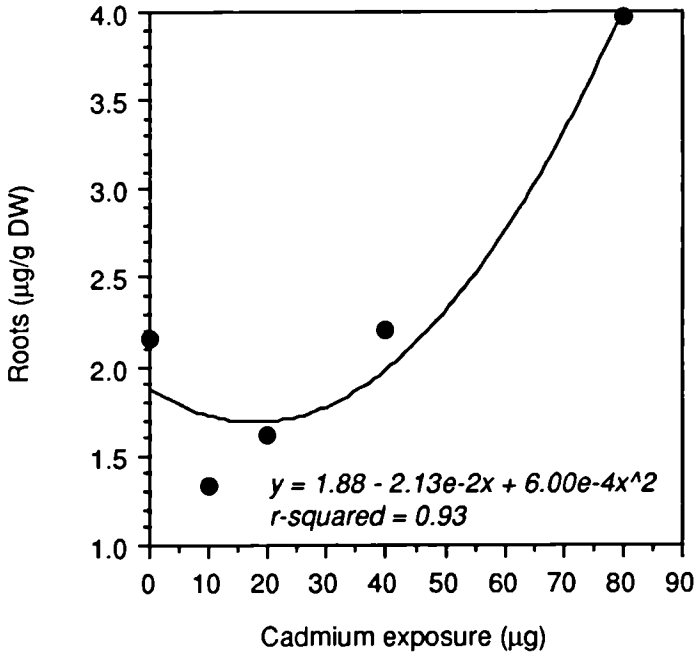


Figure 5.1c: *Concentrations of cadmium in potato roots after varying exposures to cadmium via a sand culture solution*

5.2.2.2: *Sample preparation*

All samples for *in vitro* enzymolysis and cytosol extraction had previously been thoroughly washed in distilled water and then rinsed in deionised water. The potato tuber samples were peeled and then finely chopped; the peel was retained so that it could be analysed at a later date. The lettuce sample was homogenised in a blender. All samples were initially stored in a refrigerator and subsequently frozen.

Samples for the comparison of intrinsically and extrinsically labelled potato tubers were cooked whole in a microwave oven for seven minutes at full power. The inner flesh was subsequently removed and homogenised in a blender, samples were stored in a refrigerator before further preparation.

5.2.2.2.1: Cytosol extraction

2.50 g of sample were weighed into a 10ml glass beaker, to which were added 7g of the extracting solution (0.25M sucrose, 0.02M Tris-HCL at pH 8.0). The sample was then homogenised for 3 minutes with a Janke and Kunkel electric homogeniser, the homogenised sample was then transferred to a centrifuge tube (stoppered Oakridge style; polysulfone - PSF). The sample was centrifugated in a Damon/IEC Centra-4X for 20 minutes at 10 000g. The supernatant (cytosol fraction) was passed through a 0.45μm filter and diluted 50:50 with 0.24M Tris-HCl (pH 7.5). The diluted solution was filtered through a 0.22μm filter, ready for injection onto the size exclusion column. A summary of the method is presented in Figure 5.2.

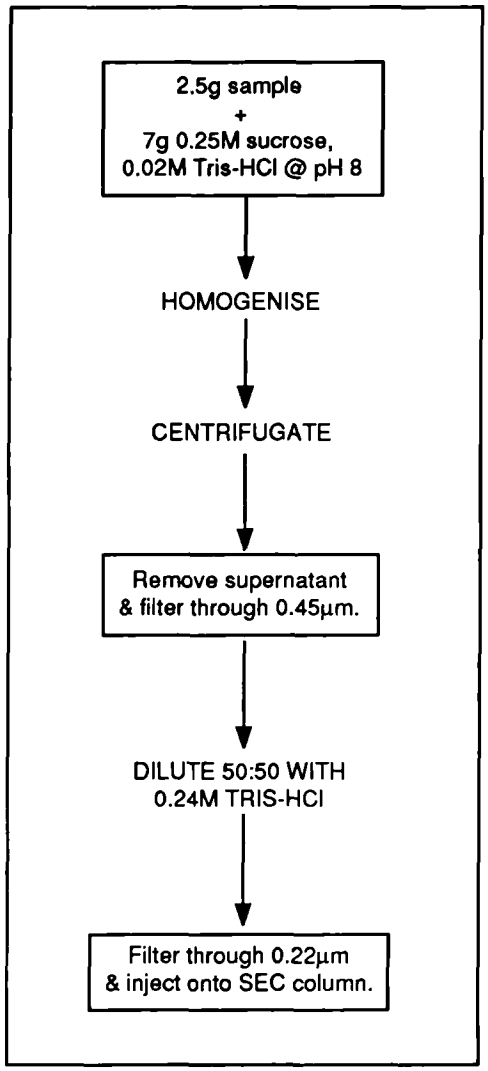


Figure 5.2: *Cytosol extraction procedure*

5.2.2.2.2: In vitro enzymolysis

In order to determine the solubility of cadmium from some of the samples, an *in vitro* enzymolysis procedure was used, based upon the method developed by Crews *et al* (1985a, 1985b). ~10 g of sample was weighed into a polypropylene bottle to which was added 20ml of "gastric" solution (1% pepsin in 0.15M NaCl at pH 1.8), the pH of this solution was adjusted using concentrated AristaR-grade HCl. For the lettuce samples the pH of the sample after the addition of this solution was 3.05 and that of the potato samples was 2.8; therefore the pH had to be lowered with an appropriate volume of HCl. The

samples were then incubated for 4 hours at 37°C in a shaking water bath, samples were periodically checked to assess their pH. The pH of the samples was raised by the addition of saturated $NaHCO_3$; 1 ml was required to raise the pH of the potato samples to 6.8 and 1.5 ml raised the pH of the lettuce samples to 7.25. The samples were left to stand at room temperature for 20 minutes before 20 ml of "intestinal juice" were added. This "juice" comprised two solutions:

- 3% pancreatin and 1% amylase in a 0.15M NaCl; and
- 1.5 g l^{-1} bile salts in 0.15M NaCl.

10 ml of each solution were added. The samples were incubated for 4 hours at 37°C in a shaking water bath. The pH during this procedure was ~6.50 for all of the sample types. Samples were centrifugated and filtered prior to their injection onto the SEC column. The procedure is summarised in Figure 5.3.

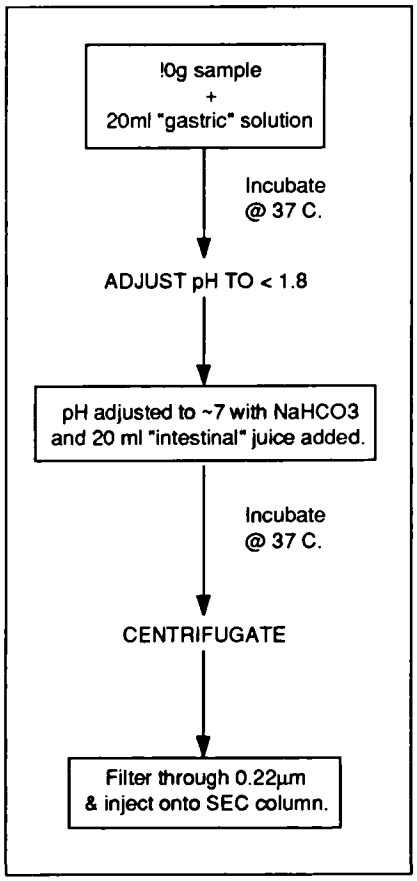


Figure 5.3: *In vitro enzymolysis procedure*

5.2.2.2.3: Aqueous extraction of intrinsically and extrinsically labelled samples of cooked potato tuber

An experiment was performed to determine and compare the speciation of cadmium in samples of intrinsically and extrinsically labelled samples of potato tuber. Intrinsically labelled potato tubers were grown in sand cultures, as described in Section 5.2.2.1. All samples were cooked whole in a microwave oven for 7 minutes at full power. The white inners were scraped out with an acid-washed plastic spatula and homogenised in a food blender to give a smooth paste. Two types of potato tuber were used, one intrinsically labelled by exposure to 80 μg cadmium and a control sample. Five sample types were prepared in duplicate:

(i) control

(ii) intrinsically labelled

(iii) intrinsically labelled + pH change during preparation

(iv) extrinsically labelled

(v) extrinsically labelled + pH change during preparation

The pH change was made in order to investigate the possibility of increasing the binding efficiency of the labelling process. Reagent blanks were carried through the entire sample preparations in duplicate.

3g of cooked sample were weighed into a beaker; if the sample was to be extrinsically labelled 3ml 75ng ml^{-1} cadmium standard were added to the sample. The pH of the spike solution had been buffered to that of the prepared sample solution (~6.2±0.1) with concentrated ammonium hydroxide. For those samples not requiring the extrinsic label, 3ml double distilled deionised water were used. The pH of samples (iii) and (v) was lowered to 2.5 with concentrated acetic acid and returned to ~6.2 with 50% ammonium hydroxide solution (V/V). Samples were then made up to 10ml with the required volume of distilled deionised water and homogenised for 3 minutes with a Janke and

Kunkel electric homogeniser. The homogenised samples were transferred to a stoppered Oakridge style centrifuge tube and centrifugated for 30 minutes at 20000 rpm in a Damon/IEC Centra-4X. The supernatant was filtered through a 0.22µm filter before being injected onto the SEC column. A summary of the sample preparation procedure is presented in Figure 5.4.

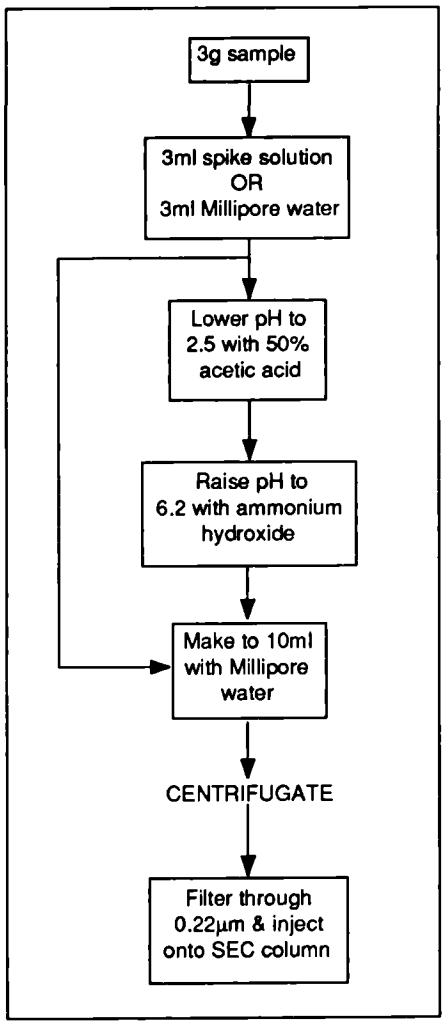


Figure 5.4: *Extrinsic labelling and aqueous extraction procedure*

5.2.3: ANALYSIS

The products of both the *in vitro* enzymolysis, cytosol extraction and aqueous extraction procedures were analysed by size exclusion chromatography - inductively coupled plasma - mass spectrometry (SEC-ICP-MS) in order to determine the speciation of cadmium and by flow injection ICP-MS (FIA-ICP-

MS) in order to determine the concentrations of cadmium in the samples. Figure 5.5 shows the basic configuration of the apparatus used for these analyses.

The chromatography component of the system comprised of a Pharmacia Fast Protein Liquid Chromatography system in which all surfaces in contact with the sample are made from glass or PTFE. A size exclusion analytical column was used (Superose-12) in series with a guard column (Superose-12 Prep-grade). For the comparison of extrinsically and intrinsically labelled samples of cooked potato tuber, a column of Chelex-100 cation exchange resin as inserted into the system between the pump and the valve, this was used to remove any cadmium from the buffer before it comes into contact with the sample. 1M ammonium acetate was used as the buffer for the majority of the analyses, although earlier work used 0.12M Tris-HCl; flow rate was maintained at 0.75 ml minute^{-1}. The chromatography system was coupled to an ICP-MS (VG Plasmaquad) via a FIA valve and a UV detector which was operated at 254nm.

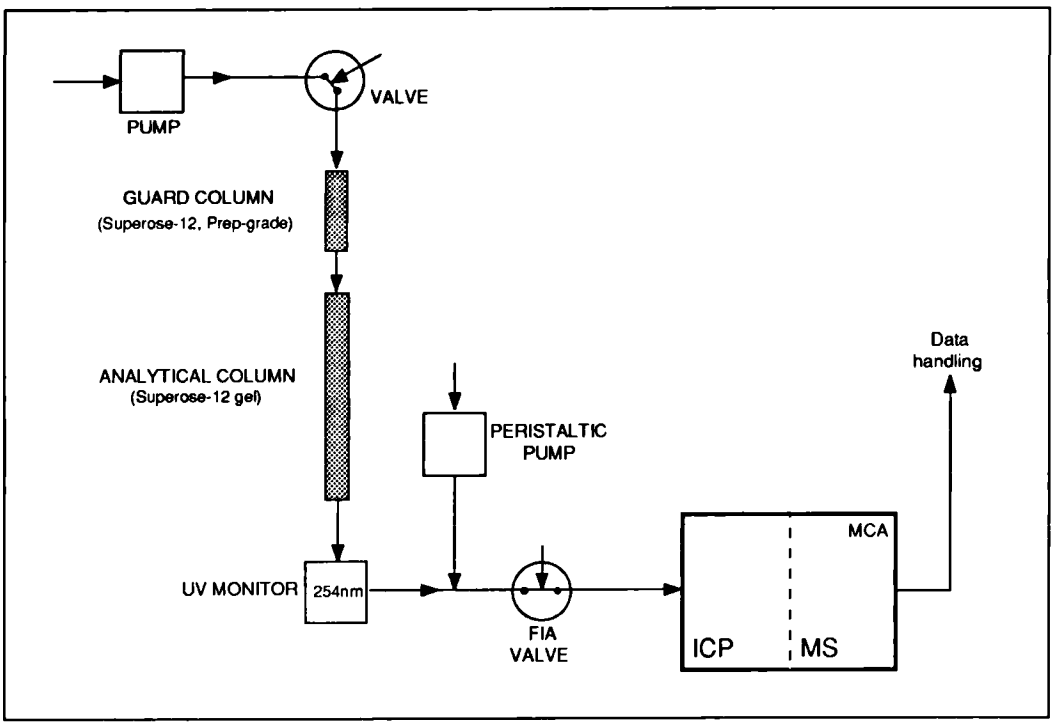


Figure 5.5: *Diagram of the FIA/SEC-ICP-MS apparatus*

5.2.3.1 *Flow injection analysis - inductively coupled plasma - mass spectrometry*

In order to determine the concentration of cadmium in a particular sample, the FIA system was used after calibration with 100µl of 10ngml^{-1} cadmium standards. The ICP-MS was operated in the single ion monitoring mode, counting ^{114}Cd; this enabled peak heights and peak areas to be quantified (Dean *et al* 1988). Net peak areas were determined via the multi-channel analyser (MCA) and used for all quantifications. Flow injection analyses were usually made during the period before the void volume of the column was eluted; sample analyses were usually bracketted between standards in order

to compensate for any instrumental drift.

5.2.3.2:*Size exclusion chromatography - inductively coupled plasma - mass spectrometry*

The prepared samples were analysed using the apparatus shown in Figure 5.5. 100µl of sample were injected onto the column via a sample loop, UV detection at 254nm was used in parallel with single ion monitoring for ^{114}Cd, this enabled correlations of cadmium with molecules of a certain molecular weights to be made. The SEC column was calibrated with BioRad standard containing proteins of known molecular weights; all parameters other than the wavelength of the UV monitor (now 280nm) were the same as those used for the samples. A 10ngml^{-1} cadmium standard was also put through the chromatography system, in order to determine the recovery of cadmium from the chromatography columns. Before use, all columns had undergone the extensive column clean-up procedure shown in Figure 5.6.

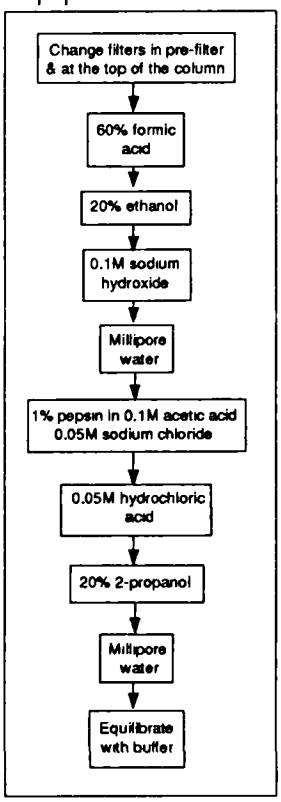


Figure 5.6: *Superose-12 column clean-up procedure (From Crews 1987)*

5.2.4: RESULTS

5.2.4.1: *Calibration of the SEC column*

The Superose-12 column was calibrated for both of the buffers, a BioRad standard containing a mixture of protein molecules of known molecular weights was used. Table 5.3 gives data for the standard.

	Molecular weight (d)	Tris-HCl		Ammonium acetate	
		V_e (ml)	K_{AV} (ml)	V_e (ml)	K_{AV} (ml)
A: Protein aggregates	No data	7.1	0.05	7.4	0.07
B: Thyroglobulin	6.7 x 105	8.9	0.16	9.6	0.20
C: Gammaglobulin	1.58 x 105	11.7	0.33	11.3	0.30
D: Ovalbumin	4.4 x 104	13.7	0.45	13.4	0.43
E: Myoglobulin	1.7 x 104	14.9	0.52	14.5	0.49
F: Vitamin B-12	1.35 x 103	19.7	0.80	19.2	0.77

See below for definitions of V_e and K_{AV}.

Table 5.3: *Superose-12 column calibration data for a BioRad standard in 0.12 Tris-HCl and 1M ammonium acetate buffer solutions.*

$$K_{AV} = \frac{V_e - V_o}{V_t - V_0}$$

where V_e = elution volume of the peak (ml); V_t = total void volume (ml); and V_o = void volume (ml). For the Superose-12 column used in this experiment the total void volume was 23±0.05ml and the void volume was 6.2ml. The elution characteristics are plotted against the log of the molecular weight to give the calibration shown in Figures 5.7a and 5.8a; it should be stressed that the elution time of a protein is determined by its size; however by assuming that all proteins are globular, size can be correlated with weight. It has been shown

on a number of occasions that metallothionein is not globular and that its molecular weight may well be lower than that produced by analysis using a size exclusion column. Phytochelatins have not as yet been shown to be anything other than globular.

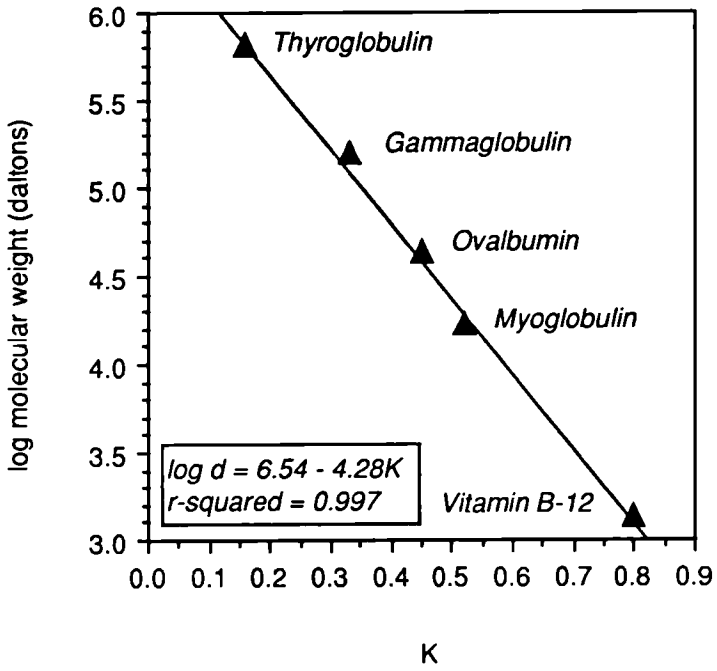


Figure 5.7a: *Calibration of a Superose-12 SEC column with 0.12M Tris-HCl buffer (flow rate = 0.75ml per minute)*

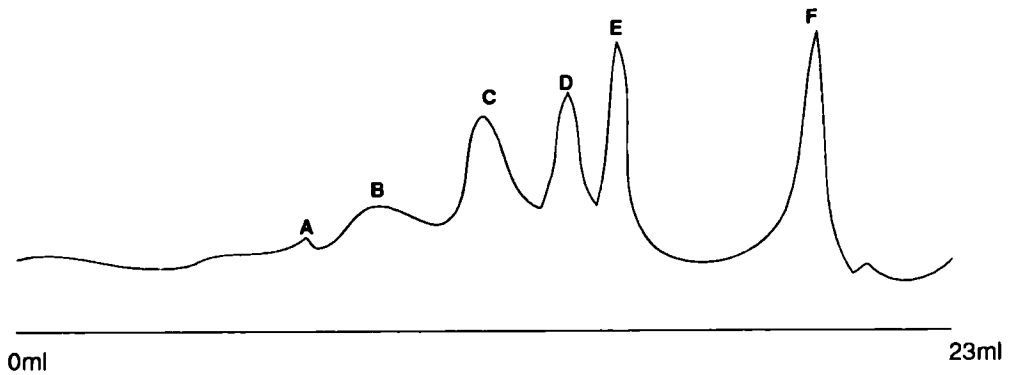


Figure 5.7b: *UV chromatogram of a BioRad standard (λ = 280nm); peaks A to F are identified in Table 5.3.*

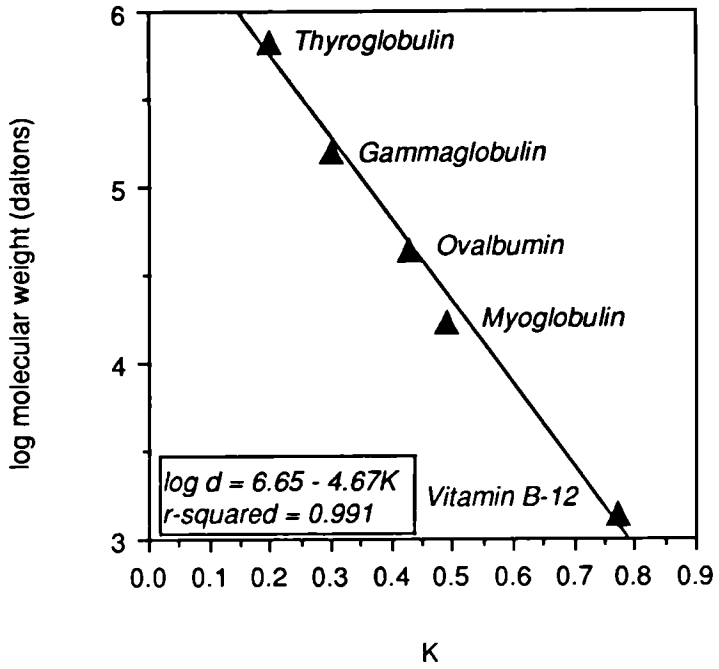


Figure 5.8a: *Calibration of a Superose-12 SEC column with 1M ammonium acetate buffer (flow rate = 0.75ml per minute)*

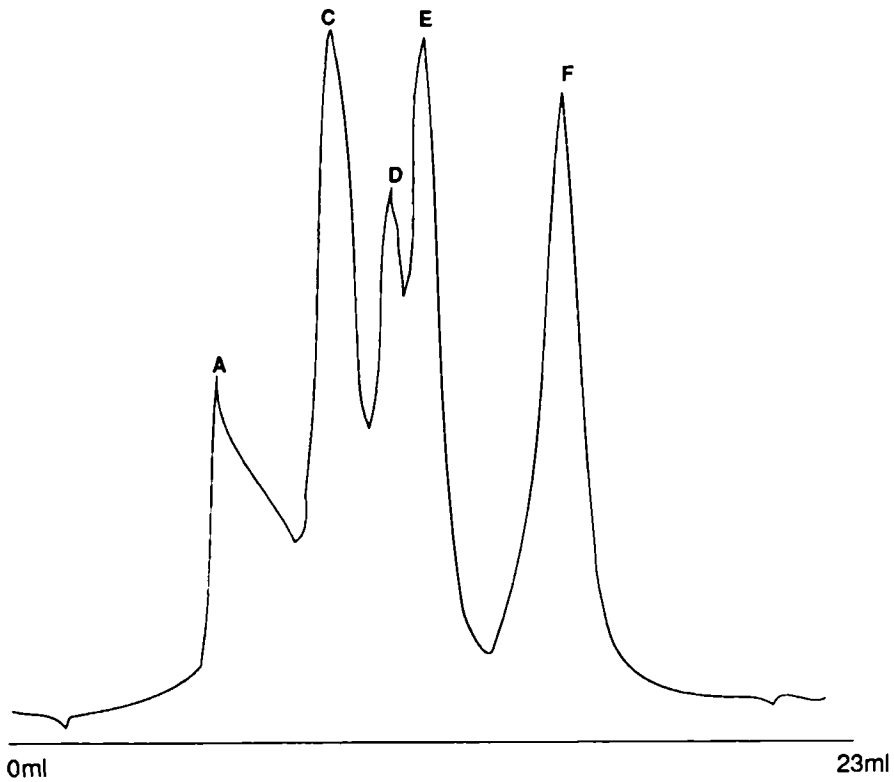


Figure 5.8b: *UV chromatogram of a BioRad standard (λ = 280nm)*

5.2.4.2: *Recovery of inorganic cadmium standard from SEC-ICP-MS system*

A 10ng ml^{-1} standard was injected onto the column to determine the recovery of cadmium from the system. In order to calculate the recovery, the number of counts from the SEC analyses were compared with those from the FIA analyses. Using a system configured without a scavenger column of Chelex-100, the SEC analyses gave a mean of 61655±11623 counts (RSD = 19%) and the FIA analyses a mean number of 68765±1608 counts (RSD = 2%). The mean recovery from a cadmium standard was calculated to be 90%. The system using a scavenger column gave a mean of 118024±8776 counts (RSD = 7%) from the SEC analyses and 135279±19368 counts (RSD = 14%) from the FIA analyses. The recovery from this configuration was 87%.

5.2.4.3: *Analysis of cytosol extractions*

Analyses of a potato sample (SN-30) with an elevated concentration of cadmium were performed in duplicate, with the individual analyses being made on different days. For this sample 0.12M Tris-HCl was used as a buffer, in the light of the results shown below the buffer was subsequently changed to 1M ammonium acetate. A mean recovery of 112% was obtained from the two SEC analyses. This would seem to imply an acceptable degree of accuracy but with a relative standard deviation of 32% the precision was poor. As shown in Figure 5.9, three peaks were observed in the cadmium chromatogram, two of which were eluted at comparable times (Peaks #2 and #3); the masses of cadmium associated with Peaks #3 and 1 were reproducible, that associated with Peak #2 was not. The elution times for Peak #1 were significantly different, but the masses of cadmium associated with the peak in separate analyses were the same. It is not inconceivable that the samples had aged between analyses. Three peaks are also shown for the UV profile of the sample, of these only the third occurs concurrently with a cadmium peak.

Table 5.4 gives the results of this analysis.

	Peak #1		Peak #2		Peak #3	
	V_e (ml)	Cd (ngg^{-1})	V_e (ml)	Cd (ngg^{-1})	V_e (ml)	Cd (ngg^{-1})
SEC #1	14.03	39.12	15.8	80.00	20.3	140.24
SEC #2	5.40	39.28	14.75	3.92	19.43	153.04
FIA	200.16±5.60 ngg^{-1}, n=3, RSD=3%					

All concentrations are in the fresh weight

Table 5.4: *FIA/SEC-ICP-MS of potato cytosol extractions*

Figures 5.9a and 5.9b show the UV chromatogram and cadmium speciation profile of the cytosol extraction. The black bars on the UV chromatogram indicate the elution time of each of the cadmium species, the height of each bar does not represent the concentration of cadmium and only indicates the mean elution time of the cadmium species.

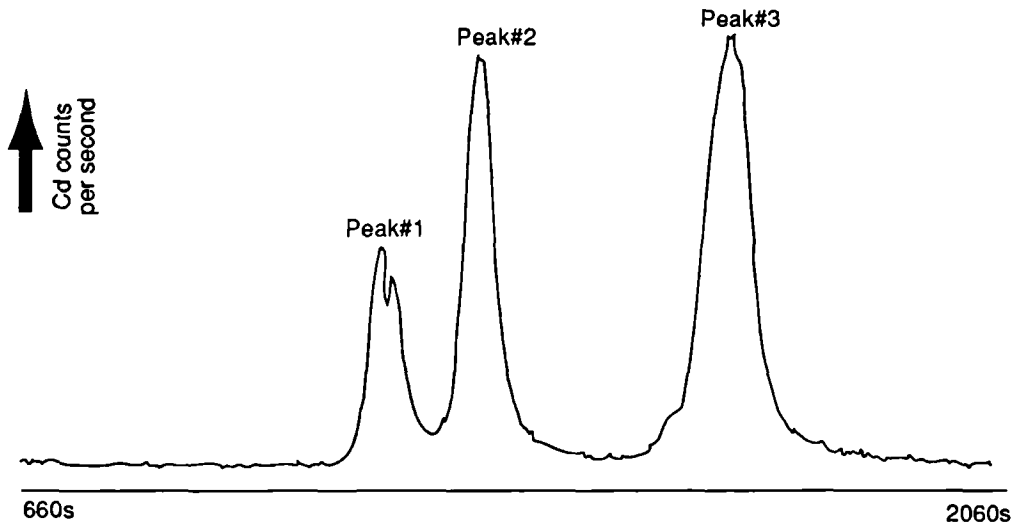


Figure 5.9a: *Cadmium speciation profile following SEC-ICP-MS of the cytosol extraction of a potato sample*

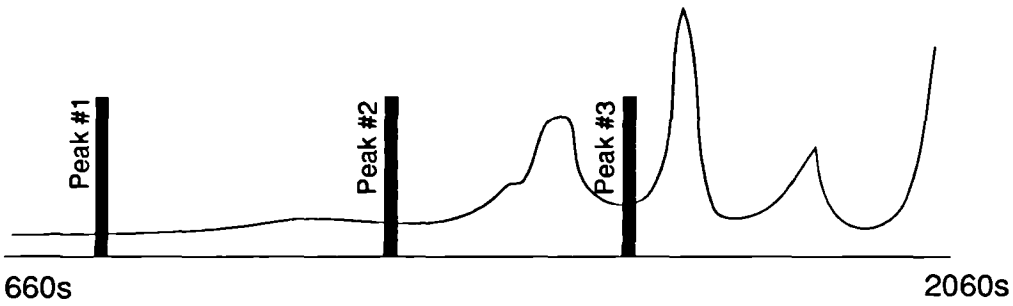


Figure 5.9b: *UV chromatogram of the cytosol extraction of a potato sample*

Using the data from Table 5.4, the molecular weights of the three cadmium species can be determined. Comparisons of these data with those obtained from similar studies can be made with reference to Table 5.1. Peak #2 has approximately the same molecular weight of cadmium binding proteins from cabbage leaves, tomato roots, wheat grain and bean plant roots. The molecular weight of Peak #3 is lower than all but one (bean leaves) of those previously found. The precision of the elution time of Peak #1 is low and hence the particular molecular weight determined should be treated with a degree of caution.

Peak number	K_{AV}	Molecular weight (d)
1	0.21±0.09	4.41×10^5
2	0.59±0.02	9.94×10^3
3	0.81±0.02	$<2.00 \times 10^3$

Table 5.5: *Molecular weights of three cadmium species in a cytosol extraction of potato tuber (two thousand daltons is the minimum quantitative determination)*

5.2.4.4: *Analysis of in vitro enzymolysis products*

Table 5.6 gives data for the analysis of the product of *in vitro* enzymolysis by FIA and after SEC. From these data the recovery of cadmium from the SEC procedures can be determined. The recovery for the analysis of the potato

sample with a low cadmium concentration could not be determined as the blank values were higher than those found in the sample. The analysis of this sample was therefore considered to be less than the limit of detection (LOD) of the method. For these analyses 1M ammonium acetate was used as the buffer.

SAMPLE	FIA ($ngg^{-1}FW$)	SEC ($ngg^{-1}FW$)	RECOVERY (%)
Blank	1.04±0.12 (n=5)	14.0±4.08 (n=2)	-
Potato, high	85.6±2.36 (n=4)	104.4±3.4 (n=2)	107
Potato, low	4.68±0.46 (n=6)	9.36± - (n=1)	-
Lettuce	182.4±1.48 (n=5)	214.0±7.6 (n=2)	110

Table 5.6: *FIA and SEC analyses of the product of in vitro enzymolysis*

Table 5.7 shows the data relating to the speciation of cadmium in the *in vitro* enzymolysis product. Those data for cadmium concentrations in the potato sample with the low cadmium concentration are less than the limit of detection. The molecular weight of the species with the highest molecular weight is similar for all samples, for all samples a mean of 6.7×10^3 was determined. This similarity is a reflection of the elution time of the first cadmium peak and can be compared with reference to Figures 5.10a, 5.11a and 5.12. Peak #2 elutes at a similar time for both the potato sample with a high cadmium concentration and the lettuce sample. The mean molecular weight of this species for the lettuce and potato sample is 1.5×10^3 daltons. Referring to Figure 5.11a, it can be seen that at the elution time of Peak #2 there is a trough below the baseline.

	PEAK #1			PEAK#2		
Sample	V_e(ml)	Daltons	Cd (ngg^{-1})	V_e(ml)	Daltons	Cd (ngg^{-1})
Blank	19.56	$<2.0 \times 10^3$	5.44	No data	No data	No data
Potato, high	16.5±0.2	6×10^3	66.1±3.5	18.9±0.3	$<2.0 \times 10^3$	17.0±0.9
Potato, low	16.4± -	6.7×10^3	11.6± -	21.5 ± -	$<2.0 \times 10^3$	8.0± -
Lettuce	16.2 ±0	7.5×10^3	149±4.7	18.5±0	$<2.0 \times 10^3$	23.2±2.0

All concentrations shown are in the freshweight

Table 5.7: *Molecular weights and cadmium concentrations in the product of in vitro enzymolysis*

Figures 5.10 and 5.12 show the UV chromatograms and cadmium speciation profiles for each of the samples analysed. The black bars on the UV chromatograms indicate the elution time of each of the cadmium species, the height of each bar is roughly equivalent to the concentration of cadmium.

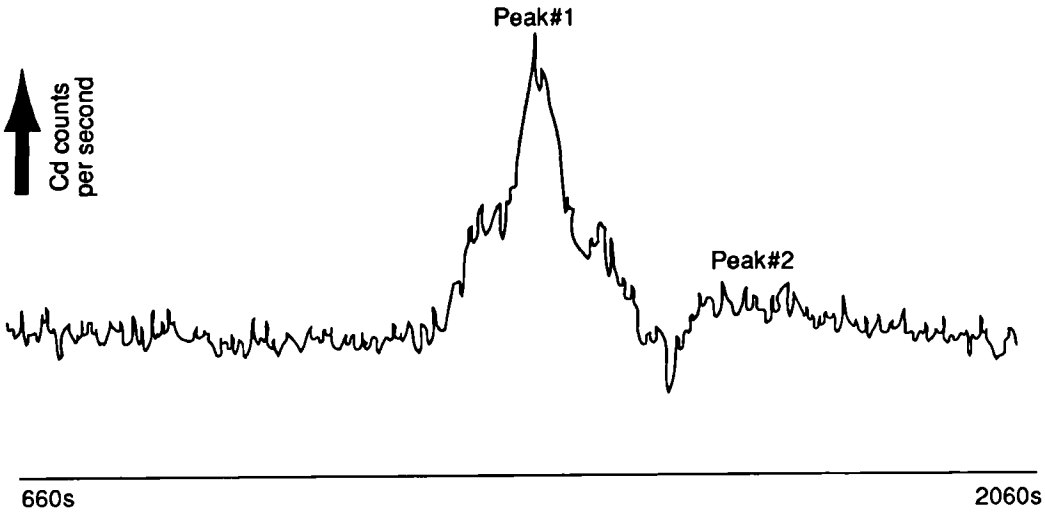


Figure 5.10a: *Cadmium speciation profile following SEC-ICP-MS analysis of a potato sample with a low cadmium concentration after in vitro enzymolysis*

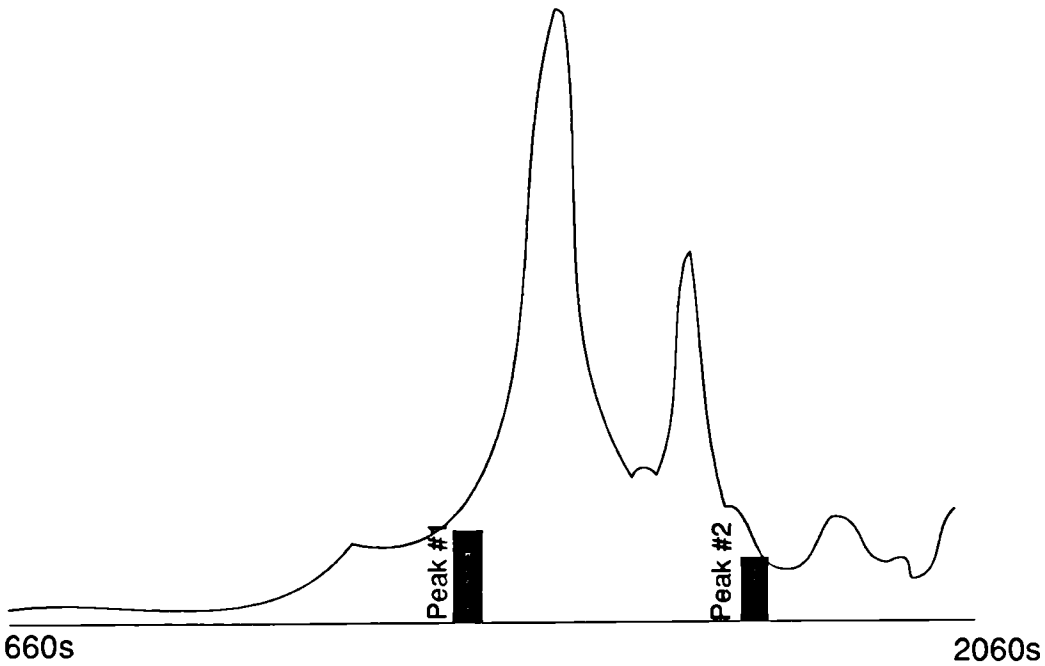


Figure 5.10b: *UV chromatogram of a potato sample with a low cadmium concentration after in vitro enzymolysis*

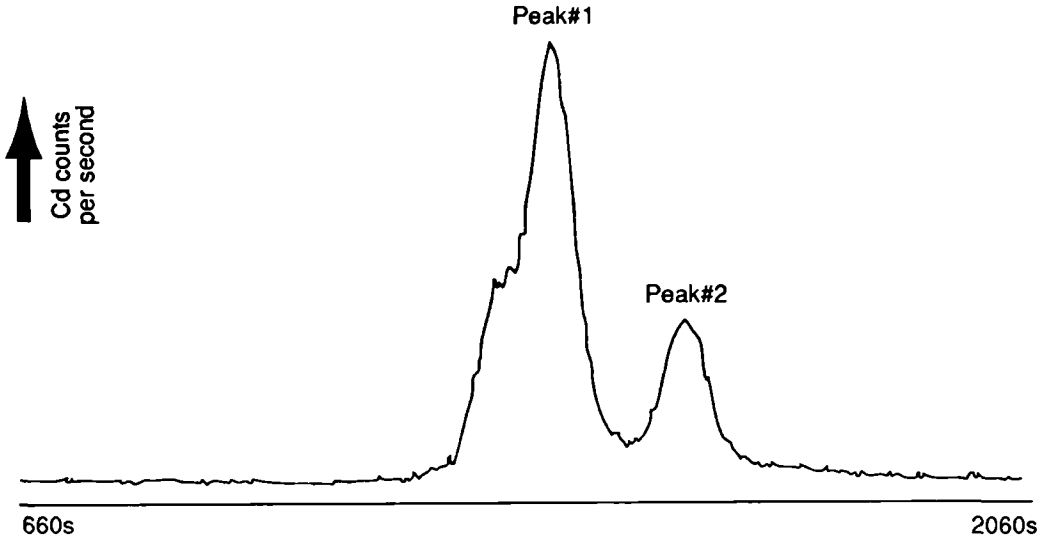


Figure 5.11a: *Cadmium speciation profile following SEC-ICP-MS analysis of a potato sample with a high cadmium concentration after in vitro enzymolysis*

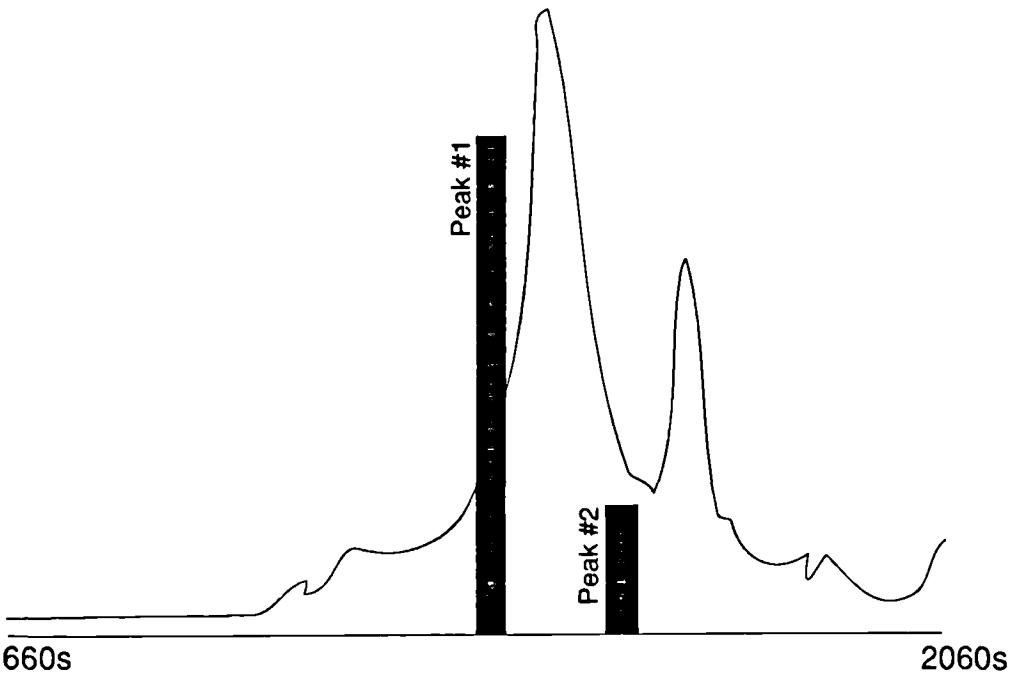


Figure 5.11b: *UV chromatogram of a potato sample with a high cadmium concentration after in vitro enzymolysis*

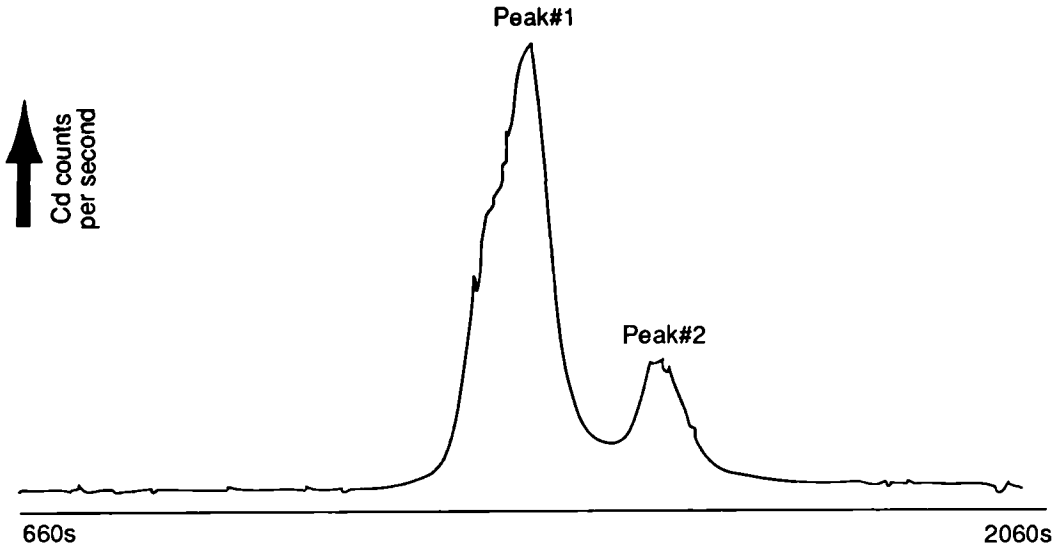


Figure 5.12: *Cadmium speciation profile following SEC-ICP-MS analysis of a lettuce sample with a high cadmium concentration after in vitro enzymolysis*

5.2.4.5: *Analysis of the aqueous extractions of intrinsically and extrinsically labelled samples of cooked potato tuber*

Intrinsically and extrinsically labelled samples of cooked potato tuber were analysed in order to contrast and compare the speciation of cadmium in the tissue after aqueous extraction. As stated in Section 5.2.2.2.3, five samples and a blank were prepared. These samples were analysed using using 1M ammonium acetate as the buffer at a flow rate of 0.75 ml per minute. The samples used in these experiments had previously been analysed for their total cadmium concentration using probe electrothermal atomisation atomic absorption spectrometry, refer to Section 3.2.2 for details of analytical method and control of data quality. The control sample had a cadmium concentration of 8±2 ngg^{-1} FW, the intrinsically labelled sample had a cadmium concentration of 35±6 ngg^{-1} FW.

Table 5.8 shows the cadmium concentrations of all samples after SEC and FIA, from these data the recoveries shown in the final column were calculated.

For all analyses, with the exception of those for one of the intrinsically labelled samples, the recoveries could be described as being quantitative. There was a tendency for the extrinsically labelled samples to give higher recoveries than for either the control or the intrinsically labelled samples.

SAMPLE	FIA (ngg^{-1} FW)	SEC (ngg^{-1} FW)	RECOVERY (%)
Control	5.1±0.2	4.8±0.1	93
Intrinsically labelled	15.4±3.0	14.9±0.3	97
Intrinsically labelled*	16.2±2.9	11.8±3.3	73
Extrinsically labelled	42.6±2.7	44.0±3.0	103
Extrinsically labelled*	51.3±3.4	55.7±2.7	109

Samples suffixed with an asterix are those for which the pH was adjusted during preparation.

Table 5.8: *FIA and SEC analysis of intrinsically and extrinsically labelled samples after aqueous extraction*

Table 5.9 gives data for the efficiency of the aqueous extraction procedure and allows comparison between those samples for which the pH was manipulated during preparation and those retained at their original pH. The data for efficiency was calculated from the FIAs of the aqueous extractions and not from the SEC analyses. The cadmium label is more readily extracted from the extrinsically labelled samples than from those intrinsically labelled, extraction efficiency is also enhanced by manipulation of the aqueous extraction medium.

SAMPLE	EXTRACTION EFFICIENCY (%)
Intrinsically labelled	44
Intrinsically labelled*	46
Extrinsically labelled	50
Extrinsically labelled*	62

Samples suffixed with an asterix are those for which the pH was adjusted during preparation.

Table 5.9: *Percentage extraction efficiency of intrinsic and extrinsic cadmium labels*

Table 5.10 shows the molecular weights of the extracted cadmium species. The cadmium species from intrinsically labelled and control samples have a lower molecular weight than those from extrinsically labelled samples. Changing the pH of the extraction medium during preparation would appear to reduce the molecular weight of the cadmium species. Species extracted from the intrinsically labelled and control samples have a molecular weight approximately equal to the apparent molecular weight of an inorganic cadmium standard.

SAMPLE	V_e (ml)	K_{AV}	MOLECULAR WEIGHT (d)
Control	20.08	0.83	$<2.0 \times 10^3$
Intrinsically labelled	19.91	0.82	$<2.0 \times 10^3$
Intrinsically labelled*	20.23	0.83	$<2.0 \times 10^3$
Extrinsically labelled	18.94	0.76	$<2.0 \times 10^3$
Extrinsically labelled*	19.21	0.77	$<2.0 \times 10^3$
10 $ngml^{-1}$ standard	20.10	0.83	$<2.0 \times 10^3$

Samples suffixed with an asterix are those for which the pH was adjusted during preparation.

Table 5.10: *Molecular weights of cadmium species extracted from intrinsically and extrinsically labelled samples of cooked potato tuber*

Figures 5.13 to 5.17 show the UV chromatograms and cadmium speciation profiles for all of the samples analysed. The black bars on the UV chromatograms indicate the elution time of each of the cadmium species, the height of each bar is roughly equivalent to the concentration of cadmium.

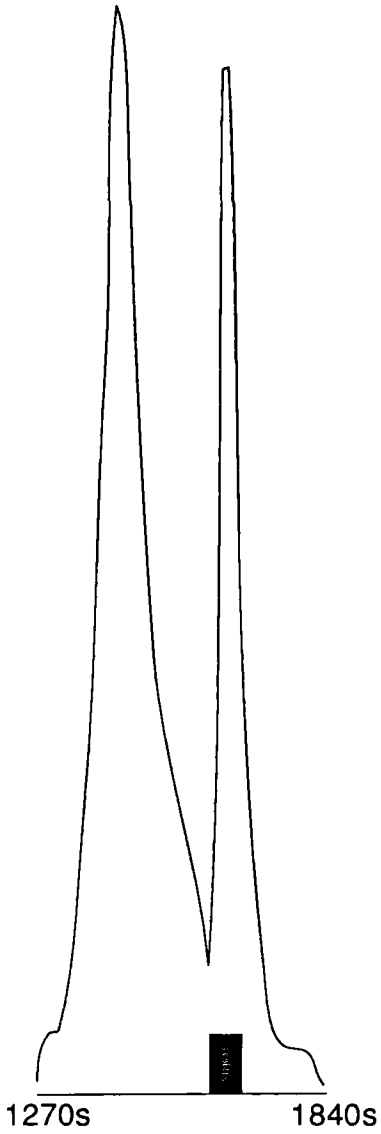


Figure 5.13a: *UV chromatogram of an aqueous extraction of the control sample of cooked potato tuber*

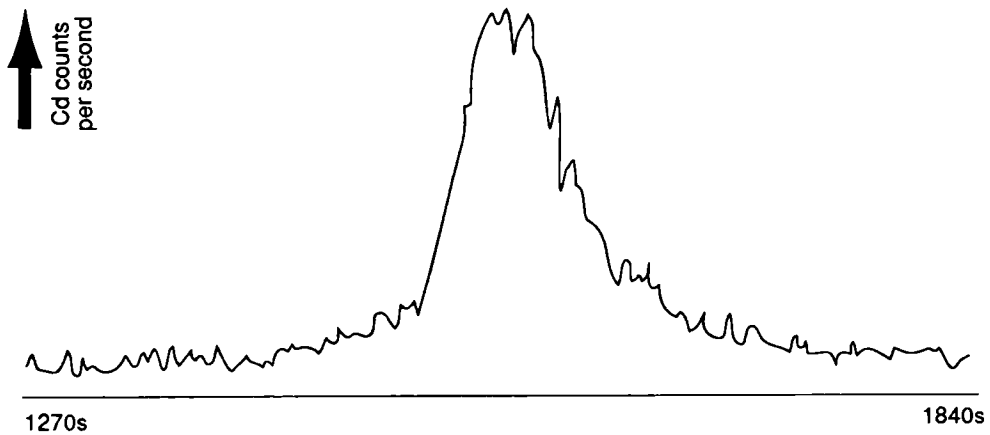


Figure 5.13b: *Cadmium speciation profile following SEC-ICP-MS analysis of an aqueous extraction of the control sample of cooked potato tuber*

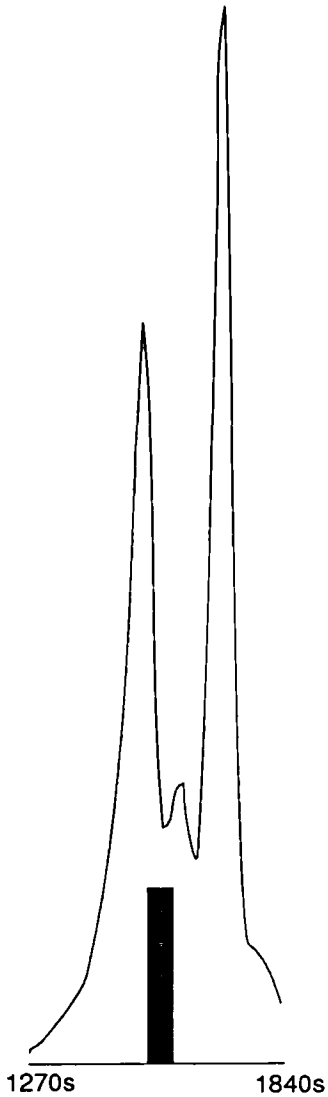


Figure 5.14a: *UV chromatogram of an aqueous extraction of an intrinsically labelled sample of cooked potato tuber*

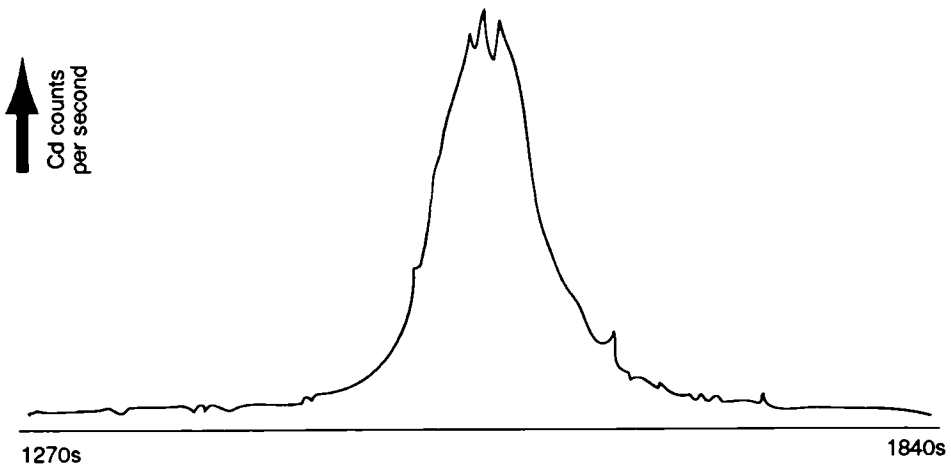


Figure 5.14b: *Cadmium speciation profile following SEC-ICP-MS analysis of an aqueous extraction of an intrinsically labelled sample of cooked potato tuber*

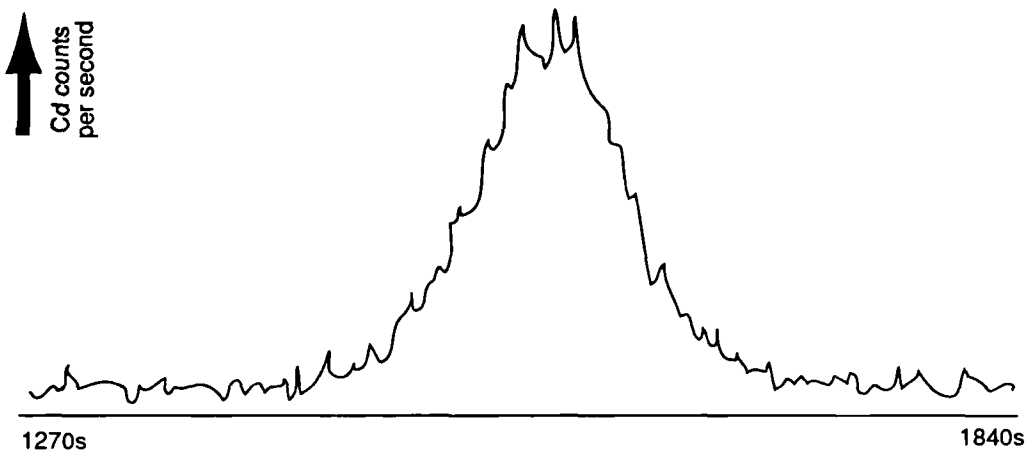


Figure 5.15: *Cadmium speciation profile following SEC-ICP-MS analysis of an aqueous extraction of an intrinsically labelled sample of cooked potato tuber (The pH of the aqueous extractant was manipulated during the preparation)*

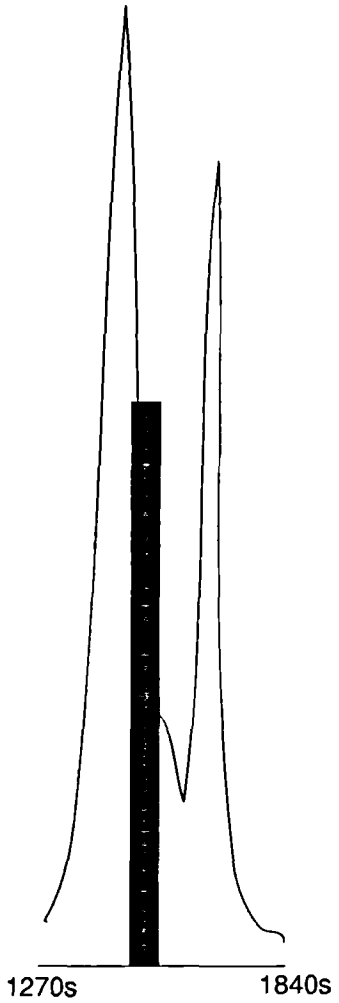


Figure 5.16a: *UV chromatogram of an aqueous extraction of an extrinsically labelled sample of cooked potato tuber*

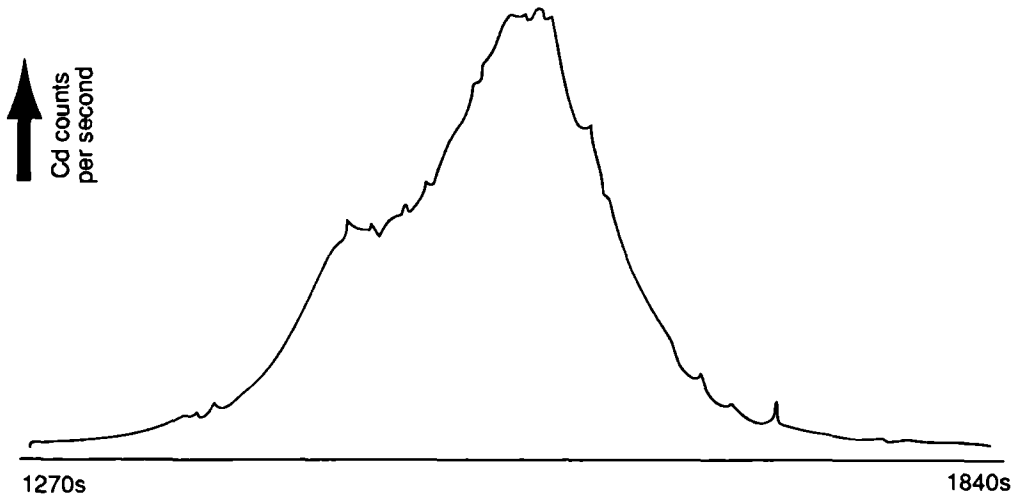


Figure 5.16b: *Cadmium speciation profile following SEC-ICP-MS analysis of an aqueous extraction of an extrinsically labelled sample of cooked potato tuber*

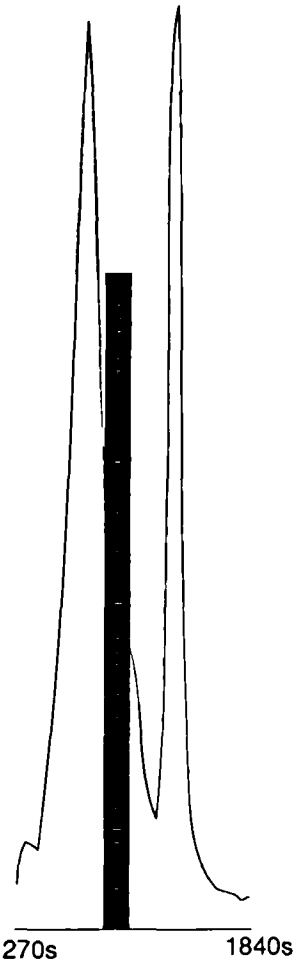


Figure 5.17a: *UV chromatogram of an aqueous extraction of an extrinsically labelled sample of cooked potato tuber (The pH of the aqueous extractant was manipulated during the preparation)*

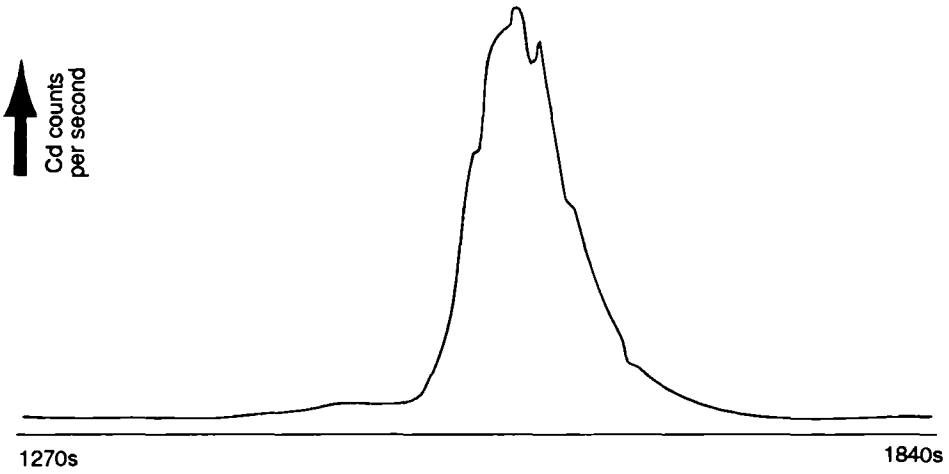


Figure 5.17b: *Cadmium speciation profile following SEC-ICP-MS analysis of an aqueous extraction of an extrinsically labelled sample of cooked potato tuber (The pH of the aqueous extractant was manipulated during the preparation)*

5.2.5: CONCLUSIONS

This group of experiments has applied SEC-ICP-MS to the determination of cadmium species in food samples, principally potato tuber. Particular attention has been paid to comparing and contrasting the cadmium speciation of intrinsically and extrinsically labelled samples of potato tuber. Analysis of the cytosol extract of an uncooked samples of cooked potato tuber indicated the presence of three cadmium species, one of which had a molecular weight corresponding to the cadmium-binding species in cabbage leaves (Wagner 1984), tomato roots (Bartolf *et al* 1980, Rauser 1987), wheat grain (Wagner *et al* 1984) and the roots of bean plants (Weigel and Jager 1980). Reference to Tables 5.1 and 5.5 enables broader comparisons and contrasts to be made. Such cross-referencing should be treated with a degree of caution in light of the differences between the method and samples used here and those used in the papers referred to in Table 5.1. Earlier work tended to use only samples containing high concentrations of cadmium in order to be able to use flame AAS for cadmium detection. A further departure from earlier methods, was the use of pre-packed HPLC columns and not the conventional columns packed with Sephadex gels. The use of ICP-MS clearly gives a higher resolution for the cadmium chromatogram and should allow for the more accurate estimation of cadmium species.

Analysis of samples of potato and lettuce after *in vitro* gastro-intestinal enzymolysis revealed the presence of two cadmium species (Table 5.7 and Figures 5.10 to 5.12). For the potato and lettuce samples containing relatively high concentrations of cadmium, both the UV chromatogram ($\lambda = 254$nm) and the cadmium chromatogram were very similar. Peak #1 for all samples was eluted at comparable times; however, the potato sample with a low cadmium concentration had a second cadmium species with a considerably lower molecular weight than either of those in the other two samples. Comparison

of the cadmium chromatogram shown in Figure 5.9a with that in Figure 5.11a, would tend to imply that *in vitro* gastro-intestinal enzymolysis alters the cadmium speciation profile, reducing the number of cadmium species from three to two. Peak#3 in the cytosol extraction has a similar molecular weight to Peak#2 in the gastro-intestinal digest of the potato sample containing a high concentration of cadmium. The same methods as those used for the potato samples in this study have also been used by Crews *et al* (1989) to investigate cadmium species in pig kidney both before and after cooking and after gastro and gastro-intestinal enzymolysis. These studies have shown that "...the majority of soluble cadmium in retail pig kidney is associated with a metallothionein-like protein that survives both cooking and simulated *in vitro* gastro-intestinal digestion."; these observations were not observed for the cadmium-binding species in potato tubers.

The speciation of cadmium in the aqueous extract of intrinsically labelled cooked potato tubers differs considerably from the cytosol extracts of the uncooked tubers. This is probably a reflection of both the extraction medium used and the effects of cooking. The aqueous extraction of both the intrinsically and extrinsically labelled samples only contained one species of cadmium. The molecular weight of the species extracted from the extrinsically labelled samples was similar to Peak#3 in the cytosol extraction of the uncooked sample, refer to Tables 5.5 and 5.10. Extrinsically labelled samples would appear to contain cadmium species of a higher molecular weight than the intrinsically labelled samples.Interpretation of these data is complicated by the fact that the eluted species were at the lower end of the calibratable range of the Superose 12 column.

CHAPTER 6: CADMIUM BUDGETS FOR AGRICULTURAL SOILS

6.1: INTRODUCTION

In Chapter 2, the potential threat to human health arising from the application of sewage sludges to agricultural soils was assessed. The principal area of concern is the elevation of the bioavailable cadmium content of soils. This increases the transfer of cadmium into the diet via the 'sensitive' groups of foods, ie those foods that currently contribute significantly to the cadmium exposure profile and are susceptible to changes in the soil cadmium concentration. The aims of this chapter are to examine one approach to the determination of the behaviour of cadmium in agricultural soils and, using this method, to examine temporal trends in soil cadmium concentrations. An estimation of the changes in the soil cadmium concentration will enable some approximation of future cadmium exposures to be made.

6.1.1: CADMIUM BUDGETS AND MASS BALANCES FOR AGRICULTURAL SOILS

Systematic approaches to the assessment of the behaviour of contaminants in the environment have been widely employed (Yost 1980 1984, Yost and Miles 1979, Bennett 1981, 1982, 1984, Hutton 1982, Jones and Bennett 1989, Nriagu 1979, Nriagu and Pacyna 1988, Rauhut 1980). In many of these models, the environment is sub-divided into a number of compartments and the transfers of a specific contaminant quantified between them. The scales of such models may vary from global inventories, such as those by Nriagu (1979, 1980), to national inventories (Hutton and Symon 1986, 1987, Kloke *et al* 1984). Further

refinement of this approach enables the transfers of a contaminant to and from a specific compartment of the environment to be studied in more detail; for example, Hovmand (1981) has produced an analysis of the cycling of lead, cadmium, copper, zinc and nickel in Danish agricultural soils.

6.1.1.1: *Global assessments of soil contamination by cadmium*

A number of global estimations of cadmium levels in soils and of soil contamination have been made by Nriagu (1979, 1980a). Comparison of the anthropogenic and natural emissions of trace elements to the atmosphere has demonstrated human activity to be the key agent in the global cycling of cadmium and a number of other elements (Nriagu 1989). Figure 6.1 gives a comparison of natural and anthropogenic emissions of cadmium to the atmosphere and a number of other trace elements considered in the FAO/WHO 1972 report on food contamination. The ratio of natural to anthropogenic emissions for cadmium was calculated to be 0.15; only for the emission of lead was the anthropogenic component more significant than that for cadmium.

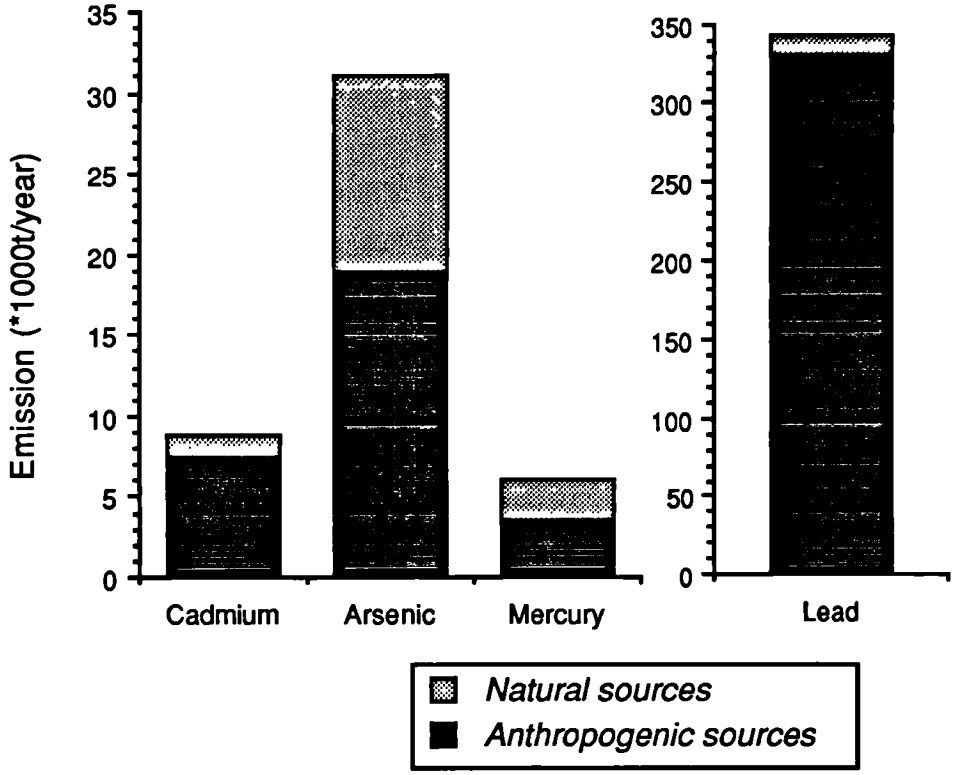


Figure 6.1: *Natural versus anthropogenic emissions of cadmium, lead, mercury and arsenic to the atmosphere (Data from Nriagu 1989).*

Given that the atmosphere is the second most important source of cadmium to soils, refer to Table 6.1, human industrial activities clearly play a key role in soil contamination at the global level (Nriagu and Pacyna 1988). What these data do not convey is any information about spatial distribution; implying that although the global emissions to soil may be low, local emissions may be very significant. This is particularly true of the municipal sewage sludge source category, where local inputs may be as high as 80 g ha^{-1} a^{-1} (Davis and Coker 1980) but the global mean is 0.11 g ha^{-1} a^{-1}.

Source category	Cadmium emission §
Agricultural & food wastes	1.50 (0-3.0)
Animal wastes, manure	0.70 (0.2-1.2)
Logging & other wood wastes	1.10 (0-2.2)
Urban refuse	4.20 (0.88-7.5)
Municipal sewage sludge	0.18 (0.02-0.34)
Coal fly ash & bottom ash	7.20 (1.5-13)
Wastage of commercial products	1.20 (0.78-1.6)
Atmospheric fallout	5.30 (2.2-8.4)
Fertilizer production	0.14 (0.03-0.25)
TOTAL INPUT, SOILS	22.00 (5.6-38)

§ Data are in 10^3 tonnes per annum. The value given without parentheses is the median, data in parentheses show the range.

Table 6.1: *Global estimates of cadmium inputs to soils (From Nriagu 1989)*

6.1.1.2: *Cadmium in soils in the European Community*

Hutton (1982) has produced a cadmium inventory for the member states of the European Community, listing inputs to the environment from a variety of sources. The behaviour of cadmium in the soil-plant system was assessed using the exposure commitment approach outlined in Chapter 2. The total cadmium input to soils away from a point source was calculated to be 8 g ha^{-1} a^{-1}. Inputs from the application of sewage sludges were "considered to be too small on a national or regional basis to warrant inclusion". Projections for dietary exposures to cadmium one hundred years hence were then made using four different scenarios, all of which indicated an increase in cadmium exposures. Two control strategies were compared using this model (Hutton 1983). Removal of cadmium from phosphate fertilizers would appear to be effective in keeping dietary exposures down, whereas the elimination of cadmium emissions to the atmosphere from the iron and steel industry is less effective.

6.1.1.3: *Cadmium budgets for agricultural soils at the national level*

The levels of cadmium in soils are increasing due to cadmium emissions from a variety of human activities; for example, the concentration of cadmium in Danish soils are increasing by between 1 and 3 $\mu g\ kg^{-1}\ a^{-1}$ (Tjell *et al* 1983). Analyses of archived soil samples from the Rothamsted Experimental Station in the UK, have indicated an increase of 27 to 55% in the soil cadmium burden since the 1850s (Jones *et al* 1987). Other studies of cadmium levels in soils have reported levels greater than those anticipated for uncontaminated areas (Asami 1984, Tjell and Hovmand 1978, Tjell and Christensen 1985). In an attempt to examine the means by which levels of cadmium have been increasing, the mass balance or soil budget approach has often been adopted; for example Sauerbeck (1982) has assessed the situation in the FRG. A summary of the data from the UK, FRG and Denmark is given in Table 2.6 and Figure 2.8.

A major problem with balance studies for specific field trials is that the cadmium budget does not balance and often gives a low recovery for the system as a whole (McGrath 1987, Williams *et al* 1987). Previous studies, observing this feature, have tried to explain it in terms of both the change in bulk density or by high losses due to leaching. A factor not previously examined is the physical movement of soil from the experimental plot in which the study was being conducted, the movement of cadmium in this way has previously been observed by McGrath (1987) and subsequently modelled by McGrath and Lane (1989). By accounting for the movement of soil from the field trial area, an increase in the recovery of cadmium and a number of other metals was observed. It is clear that work such as this will increase the estimated half-life of

cadmium in soils treated with sewage sludge and enable the more accurate application of field trial data to the agricultural situation.

6.1.2: PUBLISHED VALUES FOR OUTPUTS FROM AND INPUTS TO AGRICULTURAL LAND

In this section, published values for outputs from and inputs to agricultural soils are briefly reviewed. In some cases it is not possible to provide data for agricultural soils, therefore data for other systems will also be quoted. The aim of this section is to indicate the degree of variability between certain situations and to emphasise key data gaps. Three sources of cadmium inputs will be considered; atmospheric deposition, the application of phosphatic fertilizers and the application of sewage sludges. Outputs from agricultural soils via plant uptake and leaching are examined. There may well be a number of other processes contributing to changes in the soil cadmium burden, but these have not been quantified.

6.1.2.1: *Atmospheric deposition of cadmium*

The atmosphere is an important source of cadmium inputs to agricultural soils. A wide range of values, often reflecting the degree of local industrial activity, exist. Empirical evidence from monitoring in Greenland gives an annual deposition rate of 0.06 g ha^{-1} a^{-1}, compared with a value of 44.4 g ha^{-1} a^{-1} in New York city and 135.6 g ha^{-1} a^{-1} in an area adjacent to the Avonmouth smelter (Williams and Harrison 1984). Annual atmospheric deposition of cadmium in rural areas varies from 0.6 to 25 g ha^{-1} a^{-1} in the European Community (Lahmann *et al* 1986). Long-range transport of cadmium via the atmosphere from emission sources is an important process that has been successfully modelled (Pacyna *et al* 1985).

Estimates of cadmium inputs to agricultural soils from the atmosphere may be made using a variety of means. Analysis of an archived soil collection by Jones *et al* (1987a) has given some observed data that compare favourably with Nriagu's (1979) predicted values of deposition from the atmosphere. Taking the cumulative net input of cadmium between 1966 and 1980, a mean value of 19.9 g ha^{-1} a^{-1} can be calculated as being attributable to atmospheric sources. This value is considerably higher than that used in a cadmium budget for the UK by Hutton and Symon (1986), a nationwide mean of 3 g ha^{-1} a^{-1} was used for this study. It should be stressed that cadmium deposition rates are not uniform and it may be that the 'semi-rural' site (Rothamsted Experimental Station, Harpenden, UK) from which Jones *et al* collected their samples was not representative of a typical rural site in the UK. However, these data do correspond quite well with Nriagu's predicted values for global deposition, so it seems unlikely that the Rothamsted site is anomalous.

6.1.2.2: *Cadmium inputs via the application of phosphatic fertilizers*

The application of phosphatic fertilizers represents a significant source of cadmium input to agricultural soils. As discussed in Chapter 2, the concentrations of cadmium found in phosphatic fertilizers vary considerably and are dependent largely upon the origin of the rock phosphate from which they were produced (Alloway 1990, Kloke *et al* 1984).

In an experiment, in California, in which triple superphosphate was applied to a soil over a period of 36 years, the concentration of cadmium in the top 15 cm of the experimental plot was 1.2 μg g^{-1} as opposed to 0.07 μg g^{-1} in the control plot (Mulla *et al* 1980). An approximately five-fold increase in the cadmium concentration of the upper 2.5 cm of the soil

profile was observed in a similar experiment reported by Williams and David (1976).

Annual inputs of cadmium to an agricultural soil from phosphatic fertilizers vary as widely as do the concentrations in the fertilizers themselves. A mean annual input of 3.5 g ha^{-1} a^{-1} is reported by Kloke *et al* (1984) for soils in the FRG. Inputs to soils in the UK are estimated to be 22 tonnes of cadmium per annum, a mean of 4.3 g ha^{-1} a^{-1} if the total area of arable land in the UK is 5.12×10^6 ha (Hutton and Symon 1986). Cadmium inputs to an experimental plot at Rothamsted receiving applications of superphosphate over a 96 year period were estimated to be 5 g ha^{-1} a^{-1} (Rothbaum *et al* 1986). Hansen and Tjell (1983) have compared three different means by which phosphorus may be applied to agricultural soils at a level suitable for plant growth and the concomitant cadmium inputs, Table 6.2 summarises their data.

Cd source	Sludge	P-fertilizer	Manure
Present			
Atmosphere	+2.3	+2.3	+2.3
Fertilization	+7.0	+3.0	+0.7
Crop	-1.5	-1.5	-1.5
Wash-out [1]	-0.7	-0.7	-0.7
ACCUMULATION	**+7.1**	**+3.1**	**+0.8**
Future			
Atmosphere [2]	+1.0	+1.0	+1.0
Fertilization [3]	+3.0	+3.0	+0.7
Crop	-1.5	-1.5	-1.5
Washout [1]	-0.7	-0.7	-0.7
ACCUMULATION	**+1.8**	**+1.8**	**-0.5**

All units are in g ha^{-1} a^{-1}

1: highly dependent upon soil type.

2: future atmospheric deposition is expected to decrease with emission control technologies improving.

3: lower cadmium concentrations seem feasible based upon empirical evidence.

Table 6.2: *Cadmium budgets for agricultural soil with a phosphorus input of 20 kg per hectare (Hansen and Tjell 1983).*

6.1.2.3: *Cadmium inputs via the application of sewage sludges*

The application of sewage sludges to agricultural soils is a potentially advantageous means by which to supply nutrients to the soil (Chaney 1988, Mays and Giordano 1988, Page *et al* 1987). However, it has long been recognised that sludges may contain high levels of potentially toxic elements such as cadmium; an examination of the potential threat to human health from the application of sewage sludge to land has been presented in Chapter 2.

Sludges tend to be applied to land in the vicinity of their generation, due to high transport costs. This will tend to give a highly nodal national distribution pattern. In the United States, less than 1% of the agricultural land would be affected if 50% of the sludges generated annually were applied to the soil as a source of nitrogen (Page *et al* 1987). Most national estimates of the input of cadmium to agricultural soils, are probably in the region of two orders of magnitude lower than the inputs to those soils actually receiving sludges. Hutton and Symon (1986) estimate an annual input of 0.96 g ha^{-1} to agricultural soils in the UK. Hansen and Tjell (1983) give a much lower estimate for Denmark, 0.12 g ha^{-1} a^{-1}.

6.1.2.4: *Cadmium outputs via plant accumulation*

The accumulation of cadmium by plants is a complex and somewhat less than fully understood process; a number of models do exist (refer to Chapter 2) but the majority tend to be empirical, for example Alloway *et al* (1990). The output of cadmium from a soil has two components, the fresh weight production and the concentration of cadmium in the plant. For agricultural soils it is the concentration of cadmium in the harvested component of the plant that constitutes the output and contribution of cadmium to the food chain.

The accumulation of a trace element from the soil may be described by a number of means, one of which is the concentration factor (CF-value) (Chamberlain 1983, Dollard and Davies 1989) or accumulation ratio (Alloway *et al* 1990). The CF value is determined by dividing the concentration of cadmium in the plant by that in the soil. However, at background or only slightly elevated soil concentrations, atmospheric cadmium may make a significant contribution of cadmium to the plant

(Harrison and Chirgawi 1989[abc], Hovmand *et al* 1983). This means that the CF value will tend to overestimate the actual output from the soil via a given biomass. Table 6.3 shows the contribution of cadmium from the atmosphere to the total cadmium concentration of a number of food crops. In order to account for the contribution of cadmium from the atmosphere, a new coefficient, the CF_A value, was derived by Chamberlain (1983). This is determined by dividing the soil-derived cadmium concentration of the plant by the concentration of cadmium in the soil. Chamberlain's work was concerned mainly with lead, for which the coefficient was first derived, but can be applied equally well to the study of cadmium. For example, a recent study by Dollard and Davies (1989) has shown that lettuce plants grown in the vicinity of a non-ferrous metal smelter have a CF value of 13.8±4.5 and a CF_A value of 1.85±0.41. This, when applied in the context of a soil budget, would tend to imply that the output of cadmium from a soil via the harvested crop is approximately seven times greater than it would seem from than that which is actually the case. It should be stated that the use of the CF_A value for cadmium is usually less critical than it is for lead, due to the relatively greater bioavailability of cadmium in the soil.

Food Crop	Percentage of Cd burden from atmospheric sources	Reference
Barley (grain)	41-58	
Kale (leaf)	36-60	
Carrot (root)	37-52	Hovmand *et al*
Wheat (grain)	21	(1983)
Rye (grain)	17-28	
Cabbage (leaves)	36-60	
Radish (tuber)	25-47	
Turnip (root)	5-6	
Spinach (leaves)	23	Harris & Chirgawi
Carrot (root)	4-8	(1989[a])
Lettuce (leaves)	7-21	
Radish (tuber)	24-28	
Spinach (leaves)	8-10	Harrison & Chirgawi
Lettuce (leaves)	6-10	(1989[b])
Beans	99.5	
Lettuce	86-99	Dollard & Davies
Carrot	48-90	(1989)[§]
Cabbage	86-92	

§ It should be noted that this experiment was performed in the vicinity of a metal smelter.

Table 6.3: *The contribution of cadmium from the atmosphere to the total plant concentration.*

Values for the offtake of cadmium in the harvested crop tend to be low when compared with the total mass of cadmium in the plough layer. In a soil to which sewage sludge had been applied, McGrath (1987) estimated that the total cadmium offtake in the crops grown over a 20 year period was 180 grams. This represents only 0.28% of the total amount of metal added in sewage sludges. Data from Jones and Johnston (1989) give cadmium offtakes in wheat grain varying from 0.09 to 0.43 g ha^{-1} a^{-1}, although there was "little evidence of long-term

increases in crop cadmium...", the offtake did increase with time, see Figure 6.2.

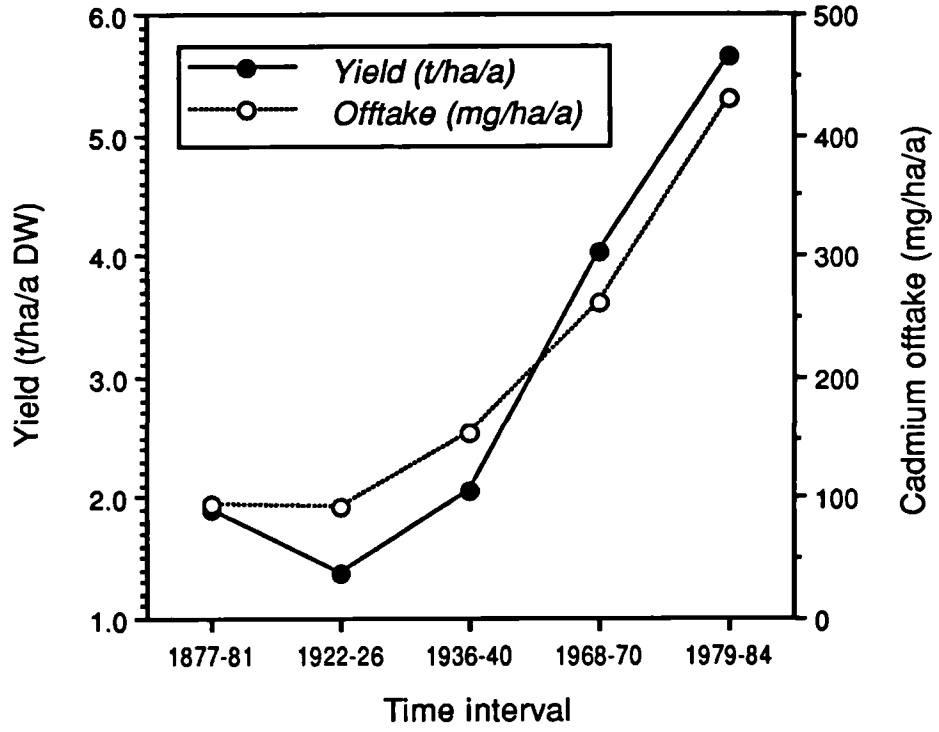


Figure 6.2:*Cadmium offtakes via wheat grain and yields from an experimental plot at Rothamsted (Data from Jones and Johnston 1989).*

6.1.2.5: *Cadmium outputs via leaching and associated processes*

Quantifying the losses of cadmium from a soil due to leaching and associated processes is an area of some uncertainty. The majority of studies examining this output have tended to imply that losses of cadmium due to leaching are not a major component of the overall losses from a soil. Accurate quantification of these processes in the field would be quite problematic and may lie at the root of some of the inconsistencies. The influence of soil properties, especially soil pH, is another source of variation. Data from the examination of soil profiles indicates that the majority of the soil cadmium burden will remain in the topsoil or plough layer (Davis *et al* 1988, McGrath 1987, Mulla *et al*

1980, Williams and David 1976). Analysis of a soil profile from a site receiving heavy applications of superphosphate fertilizers also shows this trend, see Figure 6.3.

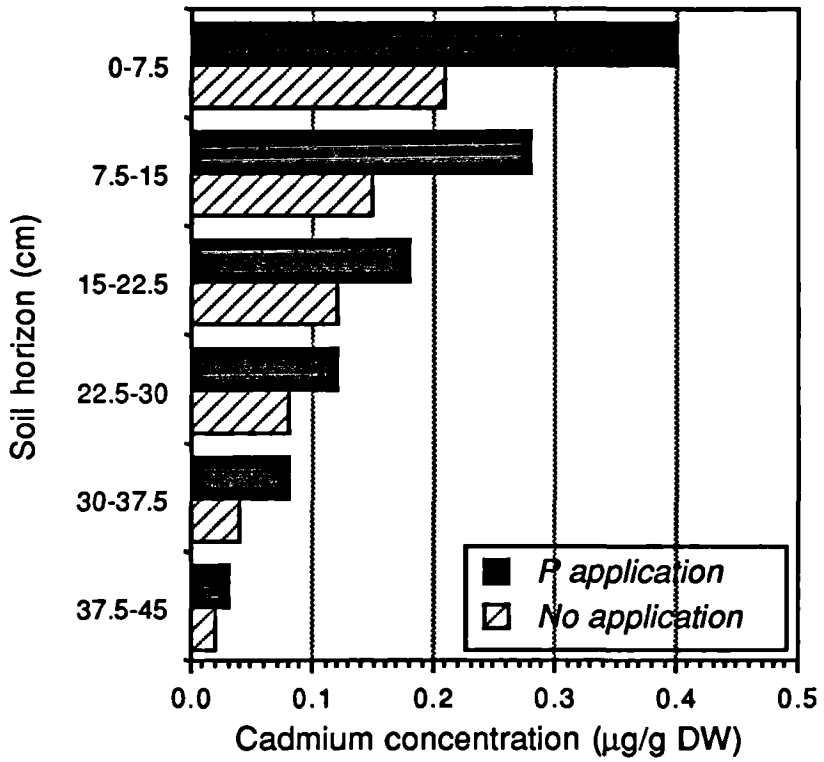


Figure 6.3: *The distribution of cadmium in a soil profile (Data from Rothbaum et al 1986).*

Estimations of annual losses by leaching and associated processes include 5 g ha^{-1} a^{-1} (Bowen 1975) and 1.5 g ha^{-1} a^{-1} (Hansen and Tjell 1983, Tjell *et al* 1983). A number of values are reported by Kabata-Pendias and Pendias (1984) ranging from 0.3 g ha^{-1} a^{-1} for agricultural land in Denmark, to 7 g ha^{-1} a^{-1} for a deciduous forest in Tennessee. McGrath (1987) has compared the predicted concentrations of cadmium in soil water necessary to account for the losses from an experimental plot with observed values; the observed values for 1984 fell over an order of magnitude short of the predicted concentration.

6.2: A CADMIUM BALANCE FOR SEWAGE SLUDGE-AMENDED SOILS

In order to describe the cadmium balances of a number of sewage sludge-amended soils a simple model has been developed. The model considers the inputs to and outputs from the plough layer of one hectare of agricultural land in one year; projections for future cadmium burdens can be made by performing the appropriate number of iterations. This enables the equilibrium cadmium burden of the soil to be estimated under a variety of conditions and also enables some estimation of the time taken for a soil to reach the cadmium concentration deemed to be potentially hazardous to human health, ie the trigger concentration.

6.2.1: DESCRIPTION OF THE MODEL

Figure 6.4 summarises the main fluxes of cadmium considered in the model. The physical movement of soil, and therefore of cadmium, from the plough layer has not been quantified for the field situation but is included in the figure as it has been referred to in the text. Atmospheric deposition, phosphatic fertilizers, infiltration and leaching and sewage sludges are considered to be constant through time; this is clearly a weakness but there are few projected data for annual fluxes via these processes. Some projections have been made, by Hansen and Tjell (1983) and Hutton (1982) for example, but rates of change were not included and these would be necessary if the data were to be incorporated into this model.

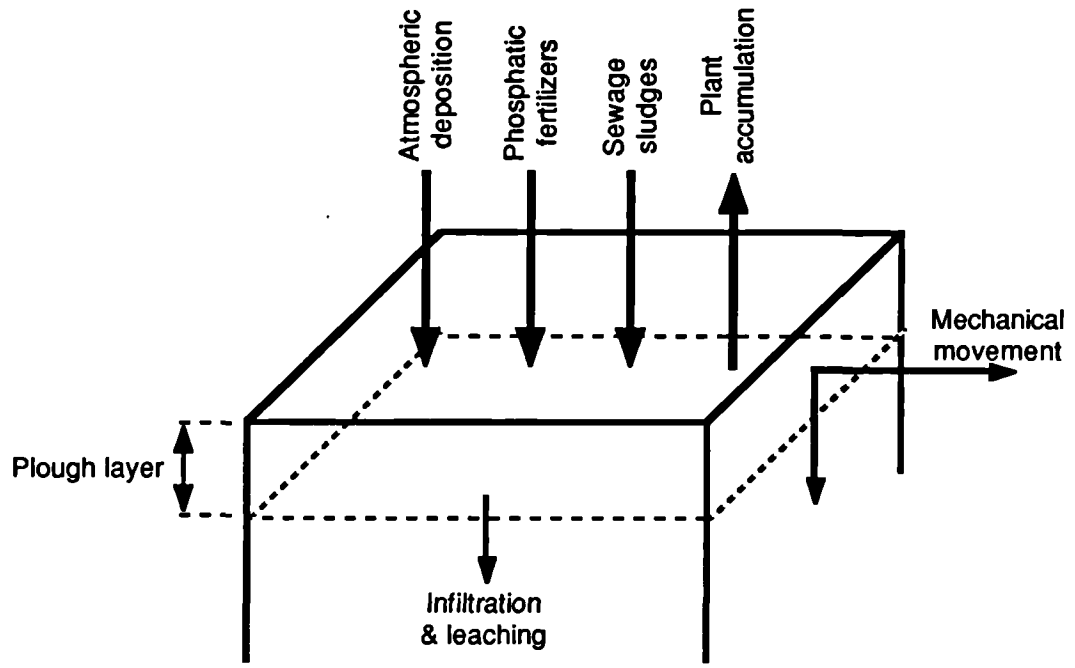


Figure 6.4: *Cadmium fluxes to and from the plough layer of an agricultural soil.*

The plough layer was considered to be the upper 23cm of the soil profile (Jones *et al* 1987a). Bulk densities on the air-dried <2mm fraction were determined for each of the soils studied, as were the percentage moisture contents immediately after sampling (See Chapter 4 for details). These data were then used to derive the mass of cadmium per hectare of soil, as shown in Figure 6.5. A number of assumptions have been made to enable the calculation of Cd1 and Cd2; these were:

- that the bulk density of a soil does not change when it is air-dried,
- that the soil does not swell in the field, and
- the density of the soil solution is 1 g cm^{-3}.

Due to the uncertain nature of the assumptions made to calculate the Cd2 value, the Cd1 value is more commonly used for the calculation of soil cadmium budgets. Values for Cd1 and Cd2 are given in Table 6.4, data for bulk density, moisture content and the cadmium concentration of any given soil are give presented in Chapter 4.

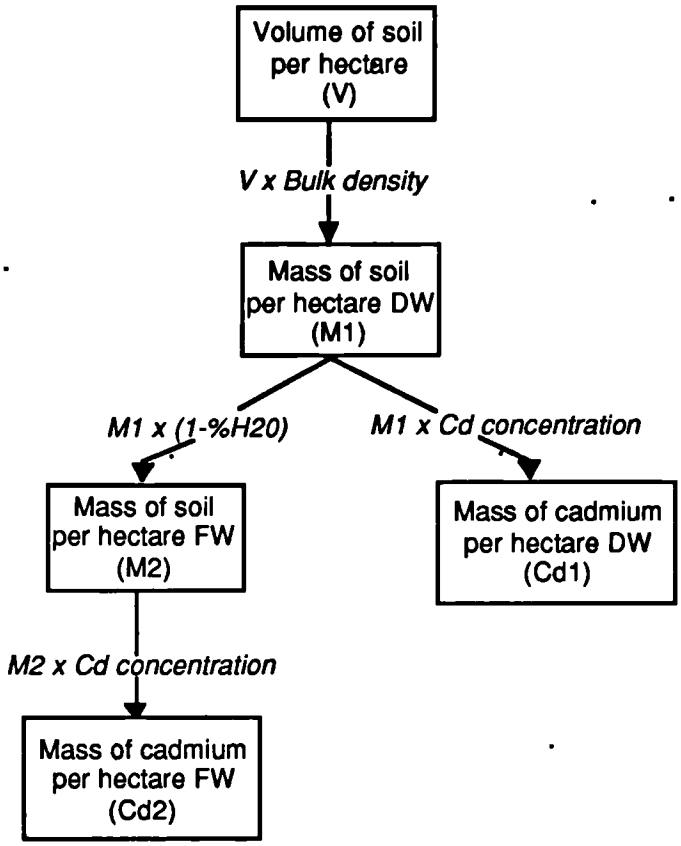


Figure 6.5: *Calculation of the mass of cadmium per hectare of soil (DW - dry weight and FW - fresh weight).*

SITE	Cd1 (kg ha-1DW)	Cd2 (kg ha-1FW)
Checkley	8.296	5.992
Canwick (Sewage works)	0.372	0.298
Canwick (Manor Farm)	0.145	0.116
Samlesbury	2.011	1.217
Soke Bardolph 1	2.444	1.829
Stoke Bardolph 2	1.931	1.438
Ashton-Under-Lyme 1	0.316	0.156
Ashton-Under-Lyme 2	0.124	0.056
Beverley	2.538	1.448
Colnbrook	1.300	1.057
Pikesmead	0.371	2.268
Cassington A R_0 Limed	0.202	0.139
Cassington B	1.702	1.130
Cassington A	1.171	0.951
Horley	6.719	4.750
Royston R_0	0.115	0.092
Royston	1.744	1.321
Windsor	1.527	0.850
Galley Hill 1	39.085	25.468
Galley Hill 2	9.254	6.236

Table 6.4: *The mass of cadmium per hectare of soil (Values are the same for both limed and unlimed soils, see Chapter 4)*

The cadmium offtake in the harvested proportion of the crops was calculated as the proportion of the total mass of cadmium in the plough layer of one hectare of land. The mass transfer factor (MTF) is similar to the CF value and is calculated using the equation below:

$$MTF = \frac{\text{Yield (g ha}^{-1}\text{) x Concentration in harvested crop (}\mu\text{g g}^{-1}\text{)}}{\text{Mass of cadmium in soil (g ha}^{-1}\text{)}}$$

The MTF is an indication of the total ouput of cadmium from the soil per unit area and can be calculated on either a dry weight or fresh weight basis. As with the CF-value, there is a tendency for the MTF to over-estimate the output of cadmium because a proportion of the plant

cadmium concentration is not derived from the soil. However, for the majority of soils used in this study this should not be a significant proportion, given the elevated concentrations of cadmium in the soils. Table 6.5 gives the cadmium offtake values for cabbages and lettuces.

Site	Lettuce (gha^{-1})	Cabbage (gha^{-1})
Checkley	21.67	14.30
Checkley limed	18.99	4.53
Canwick (Sewage Works)	10.06	4.37
Canwick (Sewage Works) limed	4.10	1.17
Canwick (Manor Farm)	0.67	1.81
Samlesbury	5.05	3.10
Samlesbury limed	4.74	0.99
Stoke Bardolph 1	10.88	7.28
Stoke Bardolph 1 limed	6.82	3.98
Stoke Bardolph 2	10.56	6.26
Stoke Bardolph 2 limed	8.64	3.04
Ashton-Under-Lyme 1	1.23	0.52
Ashton-Under-Lyme 1 limed	0.51	0.09
Ashton-Under-Lyme 2	6.87	0.92
Ashton-Under-Lyme 2 limed	2.04	0.44
Beverley	2.79	1.03
Colnbrook	15.89	2.97
Colnbrook limed	8.36	1.59
Pikesmead	20.07	42.95
Pikesmead limed	13.68	28.24
Cassington A R_0 + sludge	4.40	3.06
Cassington A R_0 limed	0.82	0.92
Cassington B	15.28	6.18
Cassington B limed	10.66	1.94
Cassington A	6.63	1.62
Cassington A limed	4.18	1.11
Horley	12.82	12.58
Horley limed	9.57	2.07
Royston R_0 + sludge	0.46	1.32
Royston R_0	0.25	0.86
Royston	2.14	2.62
Windsor	2.48	1.05
Windsor limed	1.41	0.36
Galley Hill 1	8.00	4.97
Galley Hill 1 limed	1.63	4.20
Galley Hill 2	11.09	3.79
Galley Hill 2 limed	7.72	2.59

NB: These data are based upon the total concentration of cadmium in the harvested portion of the crop and do not account for the fraction from atmospheric sources.

Table 6.5: *The offtake of cadmium from sewage sludge-amended soils by lettuces and cabbages.*

In order to be able to apply the data from the tub-based experiments reported in Chapter 4, it had to be assumed that the yield per hectare was the same for all of the soils. The mean fresh weight yield for the UK was used in the calculation of the offtake for each crop (Eurostat 1987). This assumes that the concentration of cadmium in the soil has not affected the yield. Table 6.6 shows the MTF values for both cabbage and lettuce calculated on the dry weight basis.

SITE	LETTUCE MTF	CABBAGE MTF
Checkley	0.0026	0.0017
Checkley limed	0.0023	0.0005
Carwick (Sewage Works)	0.0270	0.0117
Carwick (Sewage Works) limed	0.0110	0.0032
Carwick (Manor Farm)	0.0046	0.0125
Samlesbury	0.0025	0.0015
Samlesbury limed	0.0024	0.0005
Stoke Bardolph 1	0.0044	0.0030
Stoke Bardolph 1 limed	0.0028	0.0016
Stoke Bardolph 2	0.0055	0.0032
Stoke Bardolph 2 limed	0.0045	0.0016
Ashton-Under-Lyme 1	0.0039	0.0016
Ashton-Under-Lyme 1 limed	0.0016	0.0003
Ashton-Under-Lyme 2	0.0555	0.0074
Ashton-Under-Lyme 2 limed	0.0165	0.0035
Beverley	0.0011	0.0004
Colnbrook	0.0122	0.0023
Colnbrook limed	0.0064	0.0012
Pikesmead	0.0054	0.0116
Pikesmead limed	0.0037	0.0076
Cassington A R_0 + sludge	0.0238	0.0165
Cassington A R_0 limed	0.0041	0.0045
Cassington B	0.0090	0.0036
Cassington B limed	0.0063	0.0011
Cassington A	0.0057	0.0014
Cassington A limed	0.0036	0.0009
Horley	0.0019	0.0019
Horley limed	0.0014	0.0003
Royston R_0 + sludge	0.0058	0.0167
Royston R_0	0.0022	0.0075
Royston	0.0012	0.0015
Windsor	0.0016	0.0007
Windsor limed	0.0009	0.0002
Galley Hill 1	0.0002	0.0001
Galley Hill 1 limed	0.0000	0.0001
Galley Hill 2	0.0012	0.0004
Galley Hill 2 limed	0.0008	0.0003

Table 6.6: *MTF values for cabbages and lettuces grown on sewage sludge-amended soils*

The computer program was written in GFA Basic (Version 3) and is listed below. Data for inputs and outputs are entered and the model made to perform the required number of iterations, one iteration being equivalent to one year. The code is listed below:

```
md$="G"
strt:
PARAMETER INPUT
GOSUB par
q0$="*** CHECK DATA ***"
ALERT 2, q0$,1,"PROCEED: RE-ENTER", zq0%
IF z q0%=2 THEN
   CLS
   GOTO strt
ELSE
ENDIF
res:
CLS
PRINT "Select Data File Name and Path"
mf$=md$+":\*.DAT"
FILESELECT mf$,"", b$
CLS
lb%=LEN (b$)
IF lb%>0 THEN
   rb$=RIGHT$ (b$, 1 b%-3)
ELSE
   rb$=b$
ENDIF
IF rb$="" THEN
   q1$="NO FILE SELECTED"
   ALERT 1,q1$, 1," RESELECT:CANCEL", zq1%
   IF zq1%=2 THEN
      CLS
      PRINT "PROCESS CANCELLED"
      END
   ELSE
      GOTO res
   ENDIF
ELSE
ENDIF
md$=LEFT$(b$)
ct$=b$+".DAT"
lct%=LEN(ct$)
p%=INSTR(1,ct$,".")
cu$=RIGHT$(ct$,lct%-p%)
lcu%=LEN(cu$)
p2%=INSTR(1,cu$,".")
c$=LEFT$(ct$,lct%-p2%)
q2$="WRITE DATA TO:"+c$
ALERT 1,q2$,1,"YES:NO",zq2%
IF zq2%=2 THEN
   CLS
   q4$="PROCESS CANCELLED"
   ALERT 2,q4$,1,"RESTART:EXIT",zq4%
   IF zq4%=2 THEN
      CLS
```

```
      PRINT "PROGRAM EXITED"
      END
   ELSE
      GOTO strt
   ENDIF
   END
ELSE
ENDIF
DEFMOUSE 2
OPEN "O",#1,c$
GOSUB hdr
GOSUB wr
i=ia+ip+is-il
FOR x%=1 TO n%
   c=(c+i)*(1-l)
   GOSUB wr
NEXT x%
f$=STR$(LOF(#1))
CLOSE #1
CLS
DEFMOUSE 0
df$=STR$(DFREE(ASC(md$)-64))
q3$="FILE WRITTEN - "+c$+":"+f$+"BYTES LONG.:
      "+df$+"FREE BYTES IN DRIVE"+md$+":GENERATE SOME MORE DATA
?"
ALERT 1,q3$,1,"YES:NO",zq3%
IF zq3%=2 THEN
   CLS
   PRINT "PROGRAM TERMINATED"
   END
ELSE
   CLS
   GOTO strt
ENDIF
PROCEDURE par
   INPUT "Site (no punct's) ..........................",ms$
   ms$="Site ........................................."+ms$
   INPUT "Initial Cadmium Level (g/ha) ................",c
   mc$="Initial Cadmium Level (g/ha)..................."+STR$(c)
   INPUT "Atmospheric Deposition (g/ha) ...............",ia
   mia$="Atmospheric
Deposition(g/ha)..................."+STR$(ia)
   INPUT "Phosphate Fertiliser Application (g/ha) ......",ip
   mip$="Phosphate Fertiliser Application (g/ha)....."+STR$(ip)
   INPUT "Sludge Application (g/ha) ...................",is
   mis$="Sludge Application (g/ha)....................."+STR$(is)
   INPUT "Leaching and Runoff [output] (g/ha) ..........",il
   mil$="Leaching and Runoff [output] (g/ha)
.........."+STR$(il)
   INPUT "Mass Transfer Factor ........................",1
   ml$="Mass Transfer Factor
........................."+STR$(1)
   INPUT "Number of Years (integer !) .................",n%
   mn$="Number of Years
..............................."+STR$(n%)
RETURN
PROCEDURE hdr
   PRINT #1,ms$
   PRINT #1,mn$
   PRINT #1,mc$
   PRINT #1,mia$
   PRINT #1,mip$
   PRINT #1,mis$
```

```
    PRINT #1,mil$
    PRINT #1,ml$
    PRINT #1,"---------------------------------"
    PRINT #1,"Data (g/ha), starting at year 0:"
  RETURN
  PROCEDURE wr
    nc=100*c
    nr%=FIX(nc)
    IF nc-nr%>0.4999 THEN
      ADD nr%,1
    ELSE
    ENDIF
    nr=nr%/100
    PRINT #1,nr
RETURN
```

The model was used to project the temporal trends in the cadmium burdens of three soils, Pikesmead, Galley Hill 2 and Royston R_0. The limed replicates of both the Galley Hill and Pikesmead soils were included to demonstrate the affect of the application of lime in the long-term. The Royston soil was a control soil that had a pH greater than 7. The soils chosen reflect the extremes of soil pH and soil cadmium concentration. Input parameters are listed below:

- Number of iterations (years) - 2000
- Mass of cadmium in soil at t = 0 - *refer to Table 6.4*
- Lettuce MTF - *refer to Table 6.6*
- Cabbage MTF - *refer to Table 6.6*
- Leaching etc. - 1.5 g ha^{-1} a^{-1}
- Application of phosphate fertilizer - 4.30 g ha^{-1} a^{-1}
- Atmospheric deposition: high - 26.03 g ha^{-1} a^{-1}
  low - 0.58 g ha^{-1} a^{-1}

For each soil, two values for atmospheric deposition were used in order to reflect the extremes found in the European Community (Lahmann *et al* 1986).

6.2.2: RESULTS AND DISCUSSION

Figures 6.6 to 6.10 show the results of running the model with the values listed above.

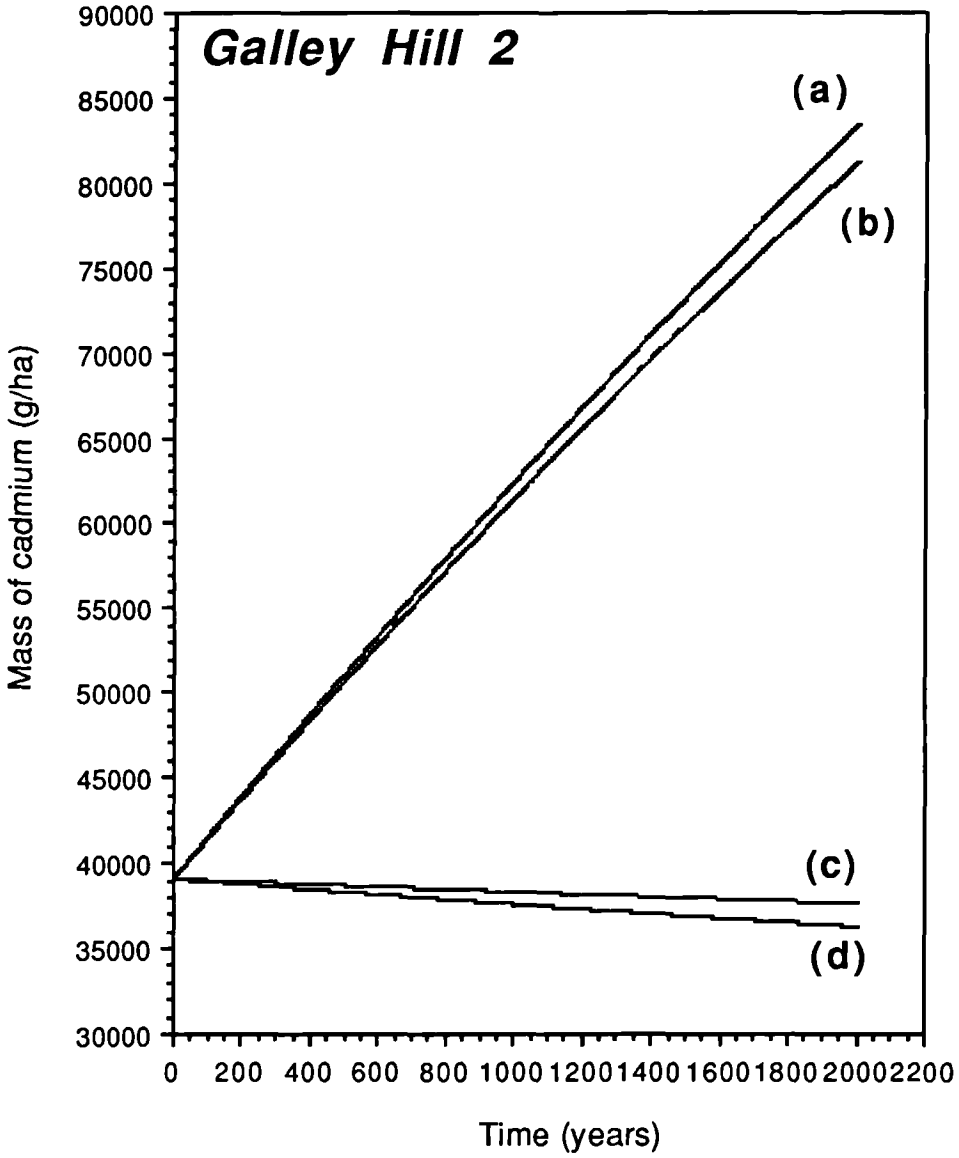


Figure 6.6: *Trends in the cadmium burden of a very heavily polluted soil under cabbages (a - limed soil, high atmospheric deposition; b - unlimed soil, high atmospheric deposition; c - limed soil, low atmospheric deposition and d - unlimed soil, low atmospheric deposition)*

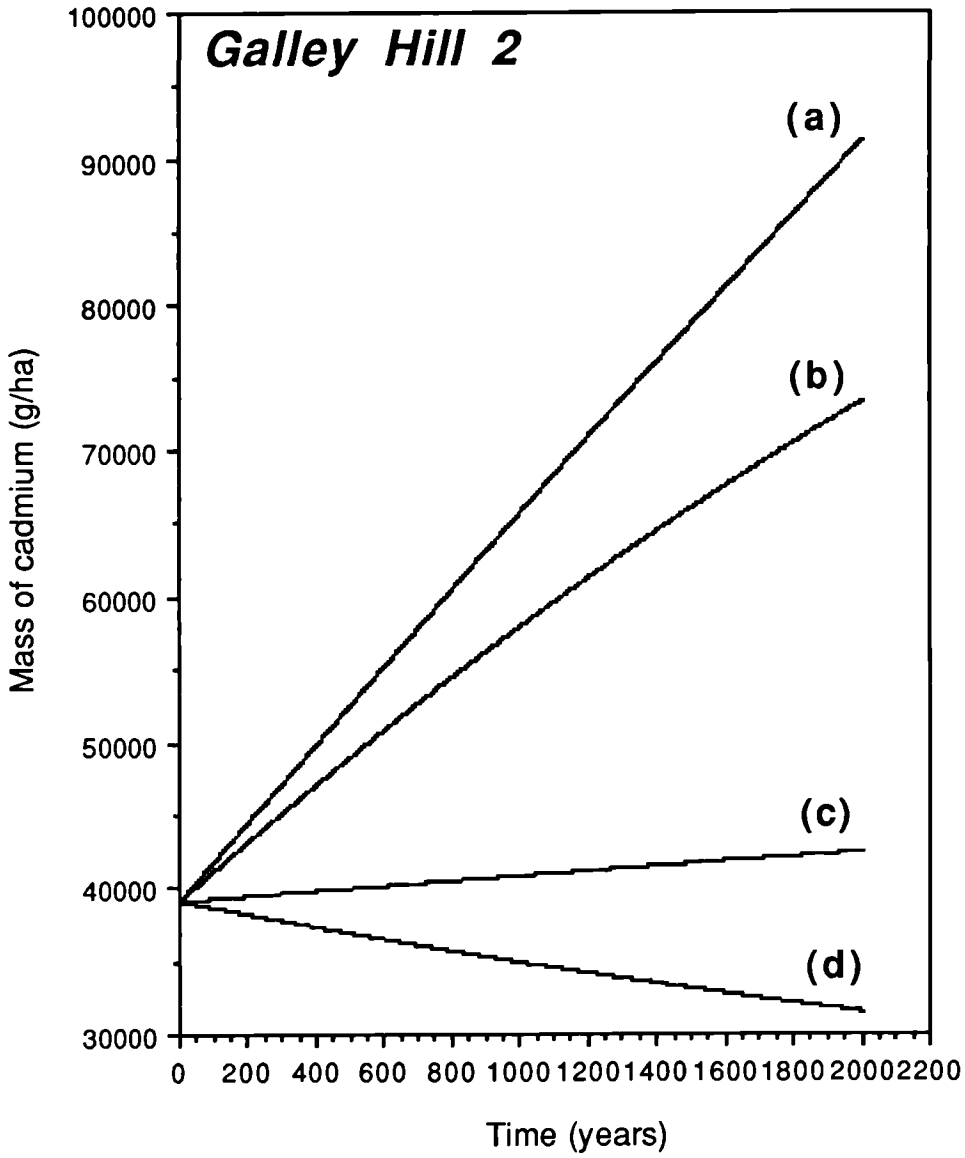


Figure 6.7: *Trends in the cadmium burden of a very heavily polluted soil under lettuces (a - limed soil, high atmospheric deposition; b - unlimed soil, high atmospheric deposition; c - limed soil, low atmospheric deposition and d - unlimed soil, low atmospheric deposition)*

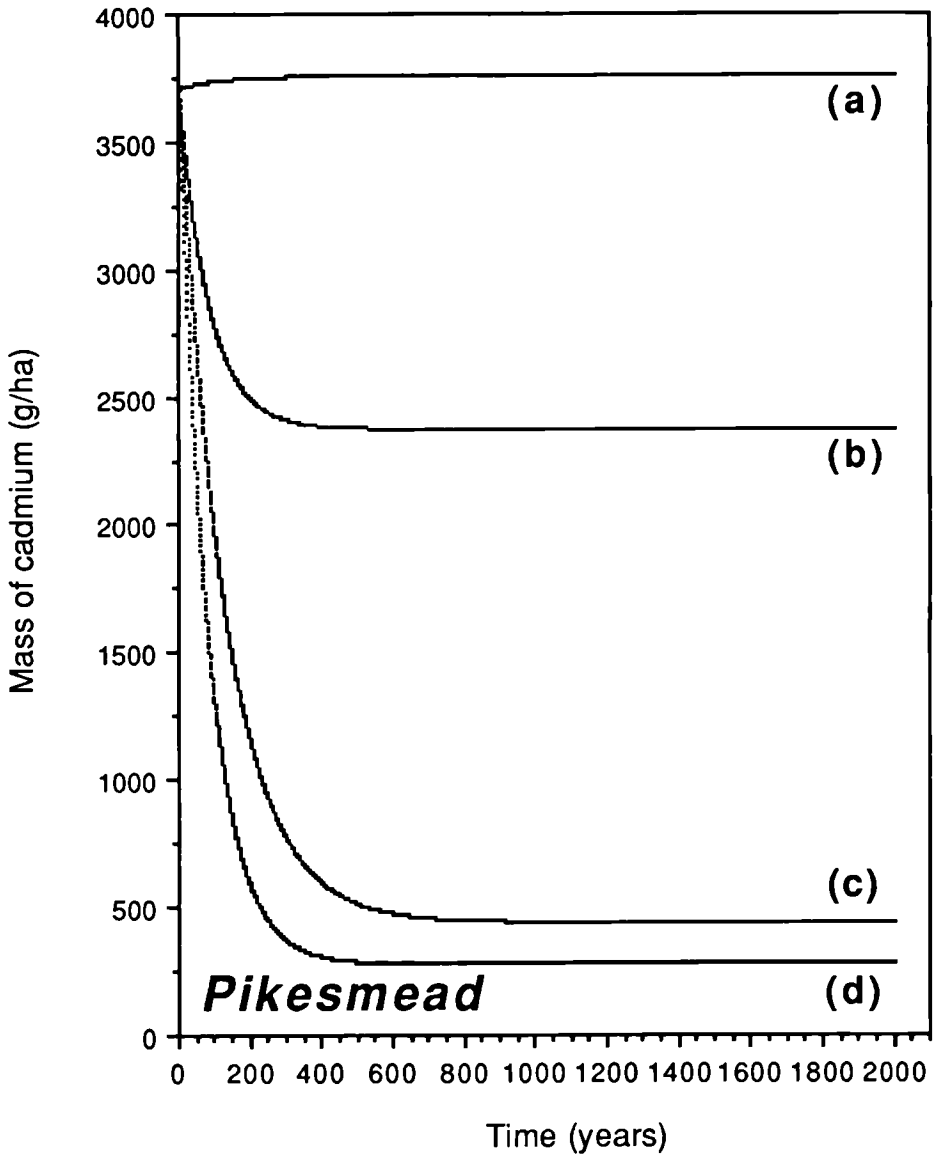


Figure 6.8: *Trends in the cadmium burden of a heavily polluted acid soil under cabbages (a - limed soil, high atmospheric deposition; b - unlimed soil, high atmospheric deposition; c - limed soil, low atmospheric deposition and d - unlimed soil, low atmospheric deposition)*

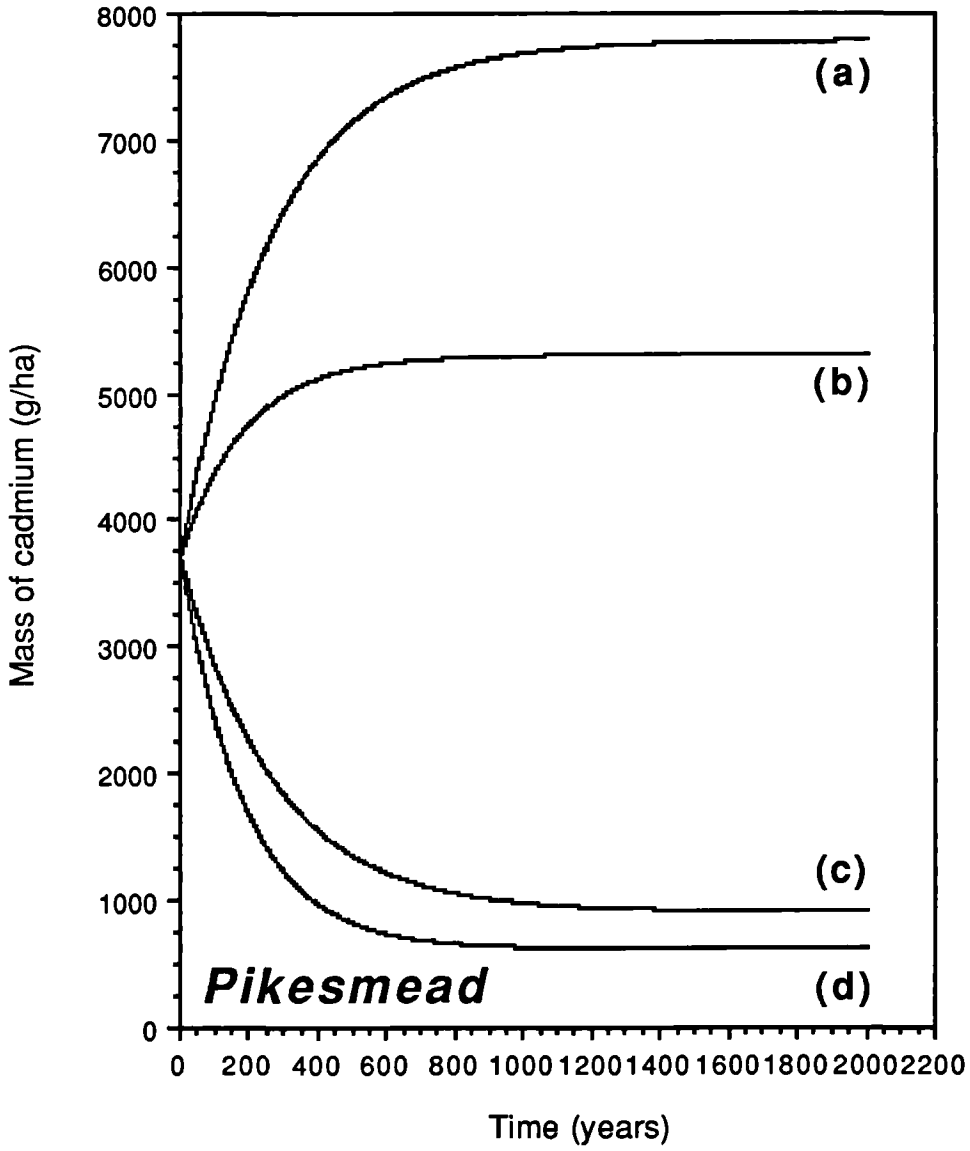


Figure 6.9: *Trends in the cadmium burden of a heavily polluted acid soil under lettuces (a - limed soil, high atmospheric deposition; b - unlimed soil, high atmospheric deposition; c - limed soil, low atmospheric deposition and d - unlimed soil, low atmospheric deposition)*

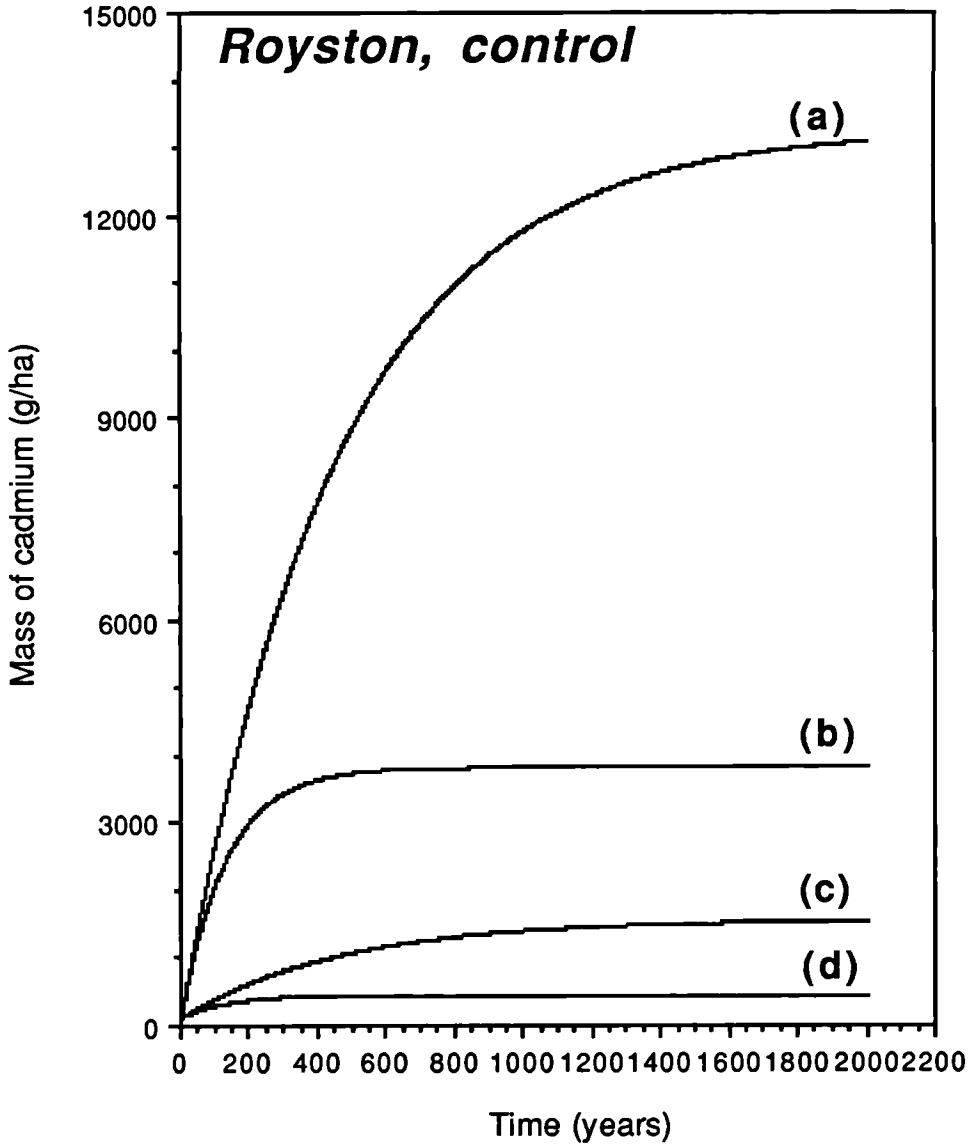


Figure 6.10: *Trends in the cadmium burden of a control soil (a - lettuce, high atmospheric deposition; b - cabbage, high atmospheric deposition; c - lettuce, low atmospheric deposition and d - cabbage, low atmospheric deposition)*

Comparison of the figures indicates that differences in the long-term trends in the cadmium burden of a soil may arise from a variety of sources. The crop grown clearly has an affect on the rates of change and equilibrium soil concentrations. Soil pH is an influential variable, through it's effect upon the values for crop offtake. Liming a soil would appear to reduce the output of cadmium into the foodchain and so slow the rate of change in the soil cadmium burden. Under conditions of high atmospheric deposition this leads to the accumulation of cadmium in the soil and to a high equilibrium soil cadmium concentration. These models are presented in order to apply the data from the literature and in so doing, to indicate key areas that need to be subsequently addressed.

Clearly, the assumptions made in the development of this approach are considerable. Many of the key processes determining the dynamics of cadmium in the soil-plant animal system are not accurately modelled. The processes contributing to losses of cadmium from the plough layer, and therefore effectively unavailable to plants, are not well quantified. It is improbable that the outputs via leaching are independent of soil variables such as pH, however the author could find no data relating soil variables to these outputs. The mechanical or physical movement of cadmium down a soil profile is another possible means by which cadmium could be lost from the plough layer. Data relating to the profile distribution of cadmium in sewage sludge-amended soils has tended to indicate little downward movement; however there are some exceptions to this and as yet there are very few long-term studies of this process (Alloway and Jackson 1990). Outputs in the harvested biomass are easier to quantify experimentally and a possible solution to this problem is presented here. Again there is not a sufficiently large data base available to allow for the accurate assessment of the cadmium offtakes

by a range of crops. A further problem is encountered in trying to assess what proportion of the plant cadmium concentration is derived from the soil and not from the atmosphere. Inputs to the soil via the application of fertilizers can be quantified over a variety of scales and should not present the considerable problems associated with the estimation of outputs via plant offtake and leaching, mechanical movement etc. The principal problems with the data for inputs arise when making studies addressing the large-scale dynamics of cadmium; this is when regional variations in the concentration of cadmium in the various media become difficult to quantify. The concentration of cadmium in sewage sludges is subject to both temporal as well as spatial variation, phosphate fertilizers also vary in their cadmium concentration depending upon their origin (see Chapter 2).

A more fundamental problem with the use of soil budgets is that they tend to over-estimate the mass of cadmium in a hectare of soil. This is because they only consider the fine earth fraction, which, especially for soils to which sewage sludges have been applied, will tend to contain a large proportion of the cadmium burden. This is one possible source of error in the calculation of cadmium budgets for experimental plots, although a number of other problems have been identified (McGrath and Lane 1989).

CHAPTER 7: CONCLUSIONS

- The aim of this study was to examine the transfer of cadmium into the human food chain, from soils previously amended with sewage sludges. In so doing, those factors influencing the bioavailability of cadmium were determined. Bioavailability is considered mainly in relation to the soil to plant transfer of cadmium.

- It has been postulated that the bioavailability of cadmium from food to the human consumer is in part determined by the speciation of cadmium. A preliminary investigation into the speciation of cadmium in food with elevated concentrations of cadmium was made.

- A supplementary aim of this study was to investigate and employ reliable methods for the determination of cadmium in biological and environmental samples. This involved the use of two methods of electrothermal atomisation atomic absorption spectrometry (ETA-AAS). Studies of the time/temperature cycle were made in order to maximise both the sensitivity and analyte recoveries. These studies sought to optimise of both ashing and atomisation temperatures. The methods, as developed, were applied to a range of certified reference materials (CRMs) in order to assess their accuracy. A wide range of sample types were analysed in order to develop a method capable of dealing with a number of different sample matrices, considered to be the source of most interferences to the analyte signal.

  Initial ETA-AAS studies used a system with specially designed graphite tubes, magnesium nitrate and diammonium hydrogen phosphate chemical modifiers and a Smith-Hieftje background correction system. The concentrations of cadmium in four CRMs were

determined and found to be within the certified ranges. The mean analyte recovery from the analysis of an in-house quality control material was 96%, indicating that the determinations were relatively free from interferences.

Subsequent studies in ETA-AAS were made using an adapted form of ETA-AAS, called probe atomisation. The work presented in this thesis constitutes one of the first attempts to apply this electrothermal atomisation system to the determination of cadmium in a range of certified reference materials. The technique has the advantage of not requiring the use of chemical modifiers for the analysis of the majority of samples against an aqueous calibration. Some milk-based samples did require the use of palladium and magnesium nitrates as chemical modifiers if acceptable recoveries were to be attained. The technique was used for cadmium determinations in sixteen quality control materials and found to give a mean recovery of 96%. Observed values for 8 CRMs fell within the certified ranges.

- In order to examine the bioavailability of cadmium to crop plants growing on sewage sludge-amended soils, and, therefore, to examine the transfer of cadmium into the human food chain, a field study was conducted. Three crops were grown: lettuces, cabbages and potatoes.

- A primary aim of this study was the creation of normal field conditions, whilst still using a sufficiently large group of soils to provide data over a broad spectrum of soil variables. This should enable the data to be extrapolated from these experiments and applied to real agricultural systems. Glasshouse studies, in small volumes of soil, have been

shown to generate plant cadmium concentrations that are unrepresentative of those from field experiments.

Comparisons of the data for concentrations of cadmium in cabbages and lettuces with those from a similar study conducted by Alloway (1986) in glasshouses, demonstrated that concentrations in this study were lower than those previously reported. The multivariate models developed in this study, were applied to the observed data from the glasshouse study. It was found that hey underestimated the cadmium concentration of those plants grown in glasshouses.

- Data for the concentrations of cadmium in lettuces were regressed against the DTPA-extractable soil cadmium concentration and compared with similar studies by Browne *et al* (1984). The basic form of the regression equation is shown below:

$$P = \alpha + \beta \log Cd_{DTPA}$$

In the study by Browne *et al* (1984), the mean value of α was 1.08 as opposed to 0.55 from this study; values for β were almost the same. The β value was considered by Browne *et al* (1984) to be dependent upon soil variables such as pH; whereas α is a function of the rooting density, pot size and soil temperature. Browne *et al* (1984) derived their values for α and β from glasshouse studies not representative of field conditions. The difference in α values and similarity in β values were also observed for cabbage cadmium concentrations when comparing the results of this study with that conducted by Alloway (1986) with plants growing in glasshouses.

- When the data for the cadmium concentrations in lettuces are regressed against the nitric acid extractable soil cadmium concentration, the gradient of the regression equation (β value) is

0.46. This compares with a value of 0.41 derived by Chumbley and Unwin (1982) from their field survey of sewage sludge-amended soils. These data imply that the concentration of cadmium in lettuces, per unit nitric acid extractable soil cadmium, are approximately 12% greater for this study.

- All of the soils collected for this study had a replicate, which was limed if the soil pH(H_2O) fell below 7.The mean pH (H_2O) of the limed soils was 6.9±0.3. For both cabbages and lettuces, the application of lime always reduced the plant cadmium concentration. Data for the concentration of cadmium in potato tubers did not show this consistent reduction in concentration, possibly due to changes in the dry matter production.

- All soils were extracted with four soil test reagents in order to assess the bioavailability of cadmium and zinc. Two chelating agents, DTPA and EDTA-$(Na)_2$, and two neutral salts, $CaCl_2$ and NH_4NO_3 were used. With the exception of EDTA-$(Na)_2$ these reagents always extracted less cadmium from the limed soils than from their unlimed replicates. Extractable zinc concentrations were lower from the limed soils when each of the four reagents was used.

- Data for the concentrations of cadmium and zinc in each of the three crops were investigated using univariate linear regression analysis. This was in order to determine those soils variables which significantly influence the concentration of the metal in the edible portion of the plant and the CF-value.

  The concentration of cadmium in both cabbages and potato tubers was most significantly related to the total concentration of cadmium in the soil; the concentration of cadmium in lettuces was related most

significantly to the nitric acid-extractable soil cadmium concentration. Cadmium CF-values for potato tubers and cabbages were most significantly related to the nitric acid extractable soil cadmium concentration. Lettuce cadmium CF-values are most significantly related to the soil pH ($CaCl_2$); however, this relationship is relatively weak and has a p-value of between 0.01 and 0.1.

- Data for the concentrations of cadmium in cabbages were regressed against the concentrations of cadmium extracted from the soils by the four soil test reagents. Each of the reagents extracted a cadmium concentration from the soils that was significantly related to the concentration of cadmium in the cabbages (p-value < 0.001). Soil extraction with $CaCl_2$ gave the best indication of the bioavailability of cadmium to cabbages grown on sewage sludge-amended soils.

  Concentrations of cadmium in lettuces were similarly analysed and all but one of the soil tests was found to give a significant indication of the bioavailability of cadmium to lettuces (ie p-value < 0.001). The ammonium nitrate soil test did not provide such a significant indication of the bioavailability of cadmium to lettuces. Soil extraction with DTPA gave the best prediction of the bioavailability of cadmium to lettuces grown on sewage sludge-amended soils.

  Cadmium extracted by each of the four soil test reagents was significantly related to the concentration of cadmium in peeled potato tuber samples. The DTPA soil test gave the best indication of the bioavailability of cadmium to potato tubers grown on sewage sludge-amended soils.

- It would appear from these experiments, that 0.005M DTPA is the single most useful soil extractant for the prediction of cadmium bioavailability to the crops grown in this study.

- Stepwise multiple linear regression analyses were used in order to develop models for the prediction of the concentration of cadmium in the three crops and their associated CF-values. This technique has enabled a number of influential independent variables to be considered simultaneously.

  The data for cadmium in cabbages were analysed in this way. If the concentration of cadmium in the cabbages was entered as the dependent variable, the residual period and the concentration of cadmium extracted from the soil by calcium chloride was found to be the most significant combination of independent variables. When using the CF-value as the dependent variable, soil pH ($CaCl_2$), organic matter content and the nitric acid extractable soil cadmium concentration was the most significant combination of independent soil variables.

  The model in which the concentration of cadmium in lettuces was the dependent variable, included soil pH ($CaCl_2$), organic matter content and the DTPA extractable soil cadmium concentration as the most significant combination of independent soil variables. Using the CF-value as the dependent variable, soil pH ($CaCl_2$), and the EDTA-$(Na)_2$ extractable soil cadmium concentration was the most significant combination of independent soil variables. However, the statistical significance of this model, when comparing the observed and the predicted values, was less than that for the other models.

When the concentration of cadmium in potato tuber was the dependent variable, multiple regression analysis produced a univariate equation, in which the DTPA extractable soil cadmium concentration was the single independent variable. Similarly, with the CF-value as the dependent value, another univariate equation was produced; however, in this equation the nitric acid extractable soil cadmium concentration was the independent soil variable.

- These field-based experiments were designed with the aim of providing data for the soil to plant transfer of cadmium, specifically for sewage sludge-amended soils. An appraisal of those soil variables influencing this process and of those soil tests best able to predict the plant cadmium concentrations has been made.

- The transfer of cadmium from foods to the metabolism of the human consumer is a less accessible area of study, however it may provide valuable data to be used in the assessment of the risks associated with dietary exposure.

The majority of these studies have examined the processes in the gastro-intestinal tract (Crews *et al* 1985a, 1985b, 1989; Olayinke *et al* 1989). The speciation of cadmium, as defined in Chapter 5, in the soluble component may then be determined. Although a considerable amount of work on metal speciation has been conducted, there remain a number of analytical problems. The development of HPLC-ICP-MS has provided a means by which real-time chromatographic separations can be analysed for cadmium on-line. Such a system was used in some preliminary studies of the speciation of cadmium in (i) the cytosol extract of uncoooked potato tuber, (ii) the products of *in vitro* enzymolysis of potato tuber and lettuce and (iii) aqueous

extracts of intrinsically and extrinsically labelled samples of cooked potato tubers.

- The speciation of cadmium in the cytosol extract of a sample of potato tuber showed the presence of three cadmium species, with molecular weights 4.4×10^5, 9.9×10^3 and $< 2 \times 10^3$ daltons. The majority of the cadmium in the extract was associated with the species of the lowest molecular weight.

  Uncooked samples of potato tuber were subjected to *in vitro* gastro-intestinal enzymolysis and the solubilised cadmium analysed by HPLC-ICP-MS. Two cadmium species were found with molecular weights of approximately 6.4×10^3 and $< 2 \times 10^3$ daltons. Similar speciation profiles were observed for samples of lettuce. None of the species in the cytosol extract survived the enzymolysis procedures; this contrasts with earlier work by Crews *et al* (1989) on samples of pig kidney.

  A comparison of the speciation of cadmium in the aqueous extracts of intrinsically and extrinsically labelled samples of cooked potato tuber was made using HPLC-ICP-MS. A single cadmium species of molecular weight $< 2 \times 10^3$ daltons was found in the samples. These data imply that it is feasible to extrinsically label samples of cooked potato tuber with Cd^{109} and this method will be used in *in vivo* studies.

- The use of soil budgets to examine the dynamics of cadmium in agricultural systems has been examined in order to identify gaps in the published data. A simple iterative model was developed and used to examine the influence of soil and plant variables upon the long-term cadmium burdens of the plough-layer. It was clear from these

studies that a number of problems exist with this soil budget approach; not least of which is that the precise nature of the processes involved are, as yet, only partially understood.

- Through an understanding of the short and long-term behaviour of cadmium in agricultural systems, and in particular of the soil to crop pathway, an assessment of some of the risks to human health associated with application of sewage sludges to agricultural land can be made. The concentration of cadmium in food has been shown to be the key component in the overall cadmium exposure of the human population (Bennett 1981) and it is this source of exposure that is likely to be elevated by the contamination of soil with cadmium.

- The long-term effects of contemporary and historical contamination of the soil with cadmium are a source for some concern. The residence time of cadmium in the soil is considerable and the bioavailability of cadmium influenced by a number of soil variables. Soil pH is of primary importance, with soil acidification likely to increase the bioavailability of cadmium to crops. It would appear to be necessary to carefully control the disposal of sewage sludges to soils intended for the production of food crops. Given the uncertain nature of changes in soil properties with time, it may be advisable to recommend that sludges with a high metal concentration be applied to land which will not be used for the agricultural production of food.

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THE BEHAVIOUR OF HEAVY METALS IN SEWAGE SLUDGE-AMENDED SOILS

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ABSTRACT

Soils amended with sewage sludges generally contain elevated concentrations of a wide range of heavy metals and are therefore of interest with regard to their potential impact on human health. This review considers the concentrations of heavy metals in sewage sludges and in the soils amended with them. The effects of sludge amendments on soil properties, the speciation of heavy metals and their bioavailability are reviewed. Variations in heavy metal accumulation between crop species are considered together with the effects of sludge-borne heavy metals on soil microorganism activity. Perhaps the most important question to be addressed, are the changes in the bioavailability of the heavy metals and their distribution in the soil profile during the residual period. The consequences of the application of sewage sludges to agricultural soils, with regard to their long-term bioavailability and movement in soil profiles, are incompletely understood.

1: INTRODUCTION

Sewage sludge is the insoluble residue from waste water treatment after either aerobic or anaerobic digestion processes. Sludge comprises resistant organic compounds (60% organic matter), nitrogen (3% N), phosphorus (2% P_2O_5) other macronutrients (O.5% K_20, 5% CaO, 1.5% MgO) (Arden, 1977) a wide range of micronutrient and non-essential trace metals, organic micropollutants, microorganisms and eggs of parasitic organisms. The substantial N and P concentrations in sludge render it a useful fertilizer material and its organic constituents give it beneficial soil conditioning properties. However, it is the relatively high concentrations of trace metals which limit both it's utility as a fertiliser and it's disposal to agricultural land.

Sewage sludge is produced wherever wastewater purification takes place and with world-wide production of sludge estimated to be around 20 x 10^9 t a^{-1} (Nriagu and Pacyna, 1988) its safe disposal is a matter of major concern. The disposal alternatives for this waste material are: application to soil, dumping at sea, landfilling or incineration. With increasing pressure to ban all sludge dumping at sea and the prohibitive costs of landfilling and incineration. There is a great incentive to dispose of sludge on land, unless its composition renders it too toxic for use, when it must be incinerated. However, incineration itself has major environmental implications due to the dispersion of aerosol particles of metals. Nriagu and Pacyna (1988) estimate that about 3 x 10^9 t of sludge are incinerated each year giving rise to the emission of 3 - 36 t Cd, 240 -300 t Pb and 150 - 400 t Zn into the atmosphere annually.

For many years, sewage sludge has been disposed of on sewage farms adjacent to wastewater treatment works and on local farms. With increasing population and more houses linked to sewerage systems, there is a need to either use a much larger area of land for sludge disposal, or to use defined areas of land primarily for the disposal of very large amounts of sludge rather than agriculture. At present, the general attitude in Europe seems to be to apply sludge to land in regulated amounts that will not preclude the use of land for agriculture. Although there are some fertilizer and physical conditioning benefits in the use of sludge by the farmer, it must still be remembered that the soil is being contaminated with a range of trace metals

and organic micropollutants. It is generally understood that the residence time for pollutant heavy metals in soils is in the region of hundreds and even thousands of years depending on the element and the type of soil (Bowen, 1979; Kabata-Pendias, 1987).

The main concern is to determine those concentrations of sludge-borne metal pollutants which pose the minimal threat to human health when applied to agricultural land. One of the problems in carrying out this risk assessment is that most of the field experiments with sewage sludge have only been running for a few decades at the most. Short-term changes in availability are important, but so too are the long-term changes which are more difficult to determine. Historic sites, such as sewage farms where sludges have been applied for a century or more are usually of little value for investigating the long-term behaviour of sludge-borne metals. This is because, in most cases, fresh applications of sludge have been made regularly to these sites.

Sewage sludge amended-soils differ to varying extents from their equivalent unsludged control soils in that they tend to have: i) higher concentrations of organic matter with variable rates of decomposition, ii) higher concentrations of macronutrients, iii) higher concentrations of micronutrients and non-essential trace elements, iv) their pH may have been increased or decreased, and v) the activity of soil microorganisms may be different (Hansen and Chaney, 1984). Sludges also have a positive soil conditioning effect on most soils. The improved aeration and drainage following sludge amendments can have indirect effects on the soil-plant relationships of heavy metals through affecting growth, nodulation in leguminous plants and other properties (Heckman *et al*, 1986; Heckman *et al*, 1987; Roberts *et al*, 1988). However, it is not known how long the effects of sludge on the organic matter status and physical properties will persist under different management and climatic regimes.

When considering inputs of metals to soils from sewage sludges, it is important to remember that metals are also entering soils in significant concentrations from other sources; including atmospheric deposition, phosphatic fertilizers and agrichemicals. Although sludges will usually be the major of contributor of metals in sludge-amended soils, metals from other

sources may complicate the soil-plant relationship. For example, Hovmand *et al* (1983) found that atmospheric deposition was responsible for 60% of the Cd found in crop plants on unsludged agricultural land in Denmark. Atmospheric deposition of metals is likely to be significant on sludge-amended soils since these are usually in relatively close proximity to sewage works which are located near to urban/industrial centres. Metals reaching foliage in deposited aerosol particles can be absorbed through the leaf, although the extent differs between plants, metals and weather conditions (Chamel,1986).

The main points requiring consideration in relation to the behaviour of heavy metals in sewage sludge-amended soils and their impact on food composition are:

- the concentrations of trace metals (essential and non-essential) present in sludges;
- the relative contribution of metals in sewage sludges to the total soil burden;
- the concentrations of heavy metals in sludge-amended soils;
- the bioavailability of these heavy metals to crops;
- effects of soil properties on the bioavailability of heavy metals and their modification by sludge amendment;
- changes in bioavailability during the residual period;
- differences between crop species and cultivars in the uptake and accumulation of heavy metals in edible organs;
- movement of heavy metals in the soil profile and the potential risk of groundwater pollution;
- the most suitable soil tests for predicting the amounts of metals likely to be taken up by specified crops.

The recent literature on sewage sludge-amended soils is reviewed here in the light of these points.

2: CONCENTRATIONS OF HEAVY METALS IN SEWAGE SLUDGES

As shown in Table 1, sludges can contain a wide range of concentrations of many elements. Sludge composition is determined in part by the effluents discharged into the sewers. In a residential area the metals are mainly

derived from human excretion, cosmetics, cleaning and kitchen wastes. Other sewerage systems with industrial discharges will give rise to sludges differing markedly in composition and may vary considerably with time depending on the industrial activities, weather and other factors.

Apart from variations in metal concentrations between sewage works, it has been shown that individual works have quite marked variations in sludge composition. Sommers *et al* (1976) reported coefficients of variation for metals in sludges from eight US cities to be: Cd 27 - 160% (mean 72%), Zn 26 - 58% (mean 41%), Cd/Zn ratios 18 - 190% (mean 86%), Cu 18 - 167% (mean 48%), Ni 12 - 144% (mean 48%) and Pb 9 - 56% (mean 32%). Some examples of industrial sources of metals in sludges are: Ag from photographic processing works, Cd, Ni, Cr etc from electroplating industries, Cr from leather tanneries, Pb from car battery manufacturers and Cd, Ni, Mn and Hg from dry cell battery manufacture.

In recent years there has been great pressure on manufacturers to reduce the metal concentrations in their effluents by introducing cleaner technology and recycling. Consequently, the average concentrations of most metals in sludges have decreased considerably in recent years. Nevertheless, the legacy of high metal sludges frequently applied to soils in unregulated amounts in the past remains with us.

3: THE RELATIVE CONTRIBUTION OF SEWAGE SLUDGES TO TOTAL HEAVY METAL LOADINGS IN TERRESTIAL ECOSYSTEMS

Nriagu and Pacyna (1988) estimated that the global discharge of sewage sludge to land was 20 x 10^{12} kg a^{-1} and the figure for urban refuse was 440 x 10^{12} kg a^{-1}. Table 2 gives the estimated inputs of selected metals and metalloids reaching soils through sludge and these values expressed percentage of the estimated total inputs of each element to soil from all sources.

It can be seen from Table 2 that sewage sludges are not a major contributor of metals in soils relative to all sources on a global scale. Only Hg, Ni and Zn in sludges exceed 2% of total inputs. Nevertheless, on a local scale, sludges

are the main source of metals in the soils to which they are applied (Nriagu and Pacyna, 1988). In some heavily sludged soils the concentrations of certain potentially harmful metals may be so high as to render the soil unsuitable for food crop production.

Concern over the transfer of cadmium into the human food chain is considerably greater than that for a number of other elements discussed in this paper (Bernhard and Lauwerys, 1984). Nriagu (1988) estimated that between 250 000 and 500 000 people have dietary exposures sufficiently high to lead to the onset of renal dysfunction. Cadmium is of particular concern due to it's:

- high toxicity
- long body retention time
- high mobility in the environment

Kloke *et al* (1984) have examined the mobility of a range of metals in the soil-plant system; their data for transfer coefficients are summarised in Table 3.

It is clear from Table 3 that cadmium is one of the most bioavailable heavy metals along this pathway. The human diet, and in particular the vegetable/grain component, represents the major source of cadmium exposure (Sherlock 1984). Therefore, the considerable mobility of cadmium in soil-plant system represents a potential hazard to human health when levels of cadmium in soils are raised by the application of sewage sludges.

These widely differing values indicate marked variations in the concern which different countries attach to soil pollution from heavy metals in sludges. However, more important are the total loadings of metals from sludges. The regulations or guidelines which govern the loadings of metals from sludges in soils differ between countries, although the EEC has issued a Directive for all 12 member countries. The Limit Values established by the EEC for metals in sludged soils used for agriculture are given as an example in Table 5.

Davis (1984) stated that Cd, Cu, Ni and Zn are more bioavailable in sludged soils than Pb, Hg and Cr. However, even for the more mobile elements less than 0.05% of the amounts of metals added annually in sludge are removed in the crop.

4: CONCENTRATIONS OF HEAVY METALS IN SLUDGE-AMENDED SOILS

Sludge applications to most agricultural soils are regulated by various guidelines and regulations and these are usually based on the heavy metal concentrations in the sludges. As a consequence of this regulation, most sludged soils will have metal concentrations within the limit values as shown in Table 5, However, some sites, especially old sewage farms adjacent to towns, have much higher concentrations of metals as a result of heavy applications over many years. Some of the high metal concentrations found in heavily sludged sites are shown in Table 6.

From Table 6 it can be seen that some very high concentrations of metals can occur in the soil of old, heavily sludged sites, such as sewage farms. Although these are only isolated cases, it is obvious that special attention needs to be paid to the management of these sites after their decommissioning as sludge disposal facilities. Some, such as that studied by Pike *et al* (1975) have been developed for housing and industry, after removal of the top 60 cm of soil. There is a good case for taking this type of land out of agricultural production, either for "set-aside" in the short term, or developing it for other purposes such as amenity land, industrial or urban development, in the longer-term.

5: THE EFFECTS OF SOIL PROPERTIES ON THE AVAILABILITY OF HEAVY METALS IN SLUDGE-AMENDED SOILS FOR UPTAKE BY CROPS

5.1: pH

Most authors have found that the bioavailability of metals by plants from sludged soils decreases as the pH is increased either by liming or applying calcareous (lime-treated) sludges. This effect could be due to both a pH effect and/or an increase in Ca^{2+} ions (John, 1970; Andersson and Nilsson, 1974; John and van Laerhoven, 1976; Chaney and Hornick, 1978; van Lune, 1985; Mahler et al, 1980 and 1987; Mulchii *et al*, 1987; Williams *et al*, 1987; Chaney, 1988; Milner and Barker, 1989). Jackson and Alloway (1990)

found that liming 18 different heavily sludged soils to pH 7 reduced the Cd content of cabbages grown on them by an average of 43% and the Cd content of lettuce by an average of 41%. Adams and Sanders (1984) observed an increase in concentrations of metals extracted with 0.01 M $CaCl_2$ as the soil pH was lowered. The threshold pH levels at which extractability increased in a range of soils were found to be: 5.8 - 6.5 for Zn, 6.2 - 6.5 for Ni, and 4.5 - 5.0 for Cu.

Unlike most other authors, Pepper *et al* (1983) did not find that liming reduced the uptake of Cd by silage maize on either a silt loam or a fine sandy loam. Jackson (1990) found in some cases that liming did not reduce the Cd and Zn concentrations in peeled potato tuber. Fiskell and Martin (1985) reported that liming increased the uptake of metals by *Lolium multiflorum* as a result of increased dry matter production. However, Mo differs from most other elements in almost always being more available at higher pHs (Pierzynski and Jacobs, 1986).

Although the findings are not all consistent, in most cases it is generally found that manipulation of soil pH is the most effective and rapid method of controlling the bioavailability of heavy metals in sludged soils (Davis, 1984; Jackson and Alloway, 1990)

5.2: Speciation and Adsorption Mechanisms

The speciation of metals in soils is likely to be more important than the total concentration of metals since it will determine whether they are available to plants or likely to leach down the profile. Ultimately, of course, the total content of metals is also important because that determines the size of the pool of metals which is present in solution and the different adsorption sites. Ure and Griepink (*pers. com.* 1989) give three definitions of "speciation":

- *functionally defined* - for example 'plant-available' species.
- *operationally defined* - by the use of specific reagents or procedures, identify and quantify an element phase or form. An example of this definition is the use of hydrogen peroxide to isolate "organically bound" species.

- *specific chemical compounds or oxidation states* - for example tributyl tin oxide, methyl mercury etc.

Mahler *et al* (1980) using the computer model GEOCHEM, predicted that the free metal ion was the predominant species of Cd in the soil solutions of sludged soils. Mullins *et al* (1986) using GEOCHEM also predicted that 85% of the Cd and 91% of the Zn were present as free metal ions in the solution of a soil sludged 5 years previously. Tills and Alloway (1983) using liquid chromatographic fractionations also found that Cd^{2+} predominated in the solutions of Cd polluted soils. Around 13% of the total Cd in the soil solution from a sludged soil was found to be organically complexed.

Chaney (1988) stresses the importance of the sludge constituents in the adsorption of Cd in sludged soils and hence the control of its bioavailability. He states that, in the long-term, plant uptake of Cd is curvilinear eventually reaching a plateau. The height of this plateau increases with increasing concentration of Cd in the sludge, but decreases with increasing concentrations of Fe in the sludge. Chaney's interpretation of these experimental findings is that sludge is a source of both Cd and also of Cd-specific adsorption capacity to the soil. Once the soil's adsorption sites for Cd have been saturated the adsorptive properties of the sludge itself control the bioavailability of Cd.

The model of Fujii *et al* (1983) reproduced in Figure 1 shows the interrelationships between the metals in the soil solution, the surfaces of soil particles, surfaces of sludge particles and the ion-absorbing surface of a plant root.

This model clearly shows the importance of the sludge particles as additional adsorptive material in the soil-plant system and the significance of the increased concentration of soluble organic ligands formed as a result of the decomposition of the sludge organic matter. The important question which needs answering is how long does this arrangement persist after the termination of sludge applications. The rate of organic matter decomposition and other chemical changes will obviously depend on climatic conditions, especially mean annual temperatures, soil type and management.

Mineralisation of organic matter will be more rapid under continual cultivation than under grassland or woodland.

5.2.1: The role of soil organic matter

Soil organic matter is a very important adsorptive medium for trace metals in all soils. Sludge differs from most other heavy metal containing pollutants in that it is an important source of adsorptive materials (organic matter, Fe and Mn). The adsorptive properties of Fe and Mn depend on their conversion to hydrous oxides but the organic matter of sludge has a high adsorptive capacity at the time it reaches the soil. King and Dunlop (1982) found that soil organic matter substituted for pH to a certain extent in controlling the bioavailability of metals from sludges. They therefore concluded that sludge could be applied to organic soils which had lower pH values than the specified pH of 6.5.

In addition to the solid state organic matter acting as a sink for metals in sludge-soil mixtures, soluble low molecular weight organic molecules produced during the microbial decomposition of sludge in the soil form soluble complexes with the heavy metals. These complexes are more mobile, less readily adsorbed and, possibly, more readily taken up by plants than free metal ions (Neal and Sposito, 1986; Yamada *et al*, 1988). Fletcher and Beckett (1987) showed that soluble organic matter from sludge has two groups of exchange sites, one group binding Ca, Mg, Zn, Ni, Co, Mn, Cd, Pb and Fe and the other binding only Cu, Pb and H.

O'Connor *et al* (1984) found that organic ligands significantly reduced free metal activity in solution. Sludge leachates have been found to reduce the adsorption of metals by soils (Minnelgrin and Biggar, 1986; Zabowski and Zasoki, 1987). The presence of surfactants, such as NTA (nitrilotriacetic acid) in sludges can increase the solubility and mobility of metals in sludged soils, particularly Cd, Cu, Mn, Ni, Pb and Zn (Elliot and Denneny, 1982; Garnett *et al* 1985 and 1986; Senesi and Sposito 1987).

Campanella *et al* (1989) showed that UV irradiation of sludge led to increased mobility of trace metals as a result of the accelerated decomposition of organic matter and increased concentrations of soluble

organic ligands. However, amendment of soils with UV irradiated sludge reduced metal leaching, presumably as a result of the increased release of adsorbable free metal ions. Organic matter decomposition in reclaimed strip mining soils amended with sludge was found to be lowest in the first year, increasing gradually with time over the five years of the study (Seaker and Sopper, 1988). Dudley *et al* (1987) found that in a 30 week investigation of the soluble ligands released from a decomposing anaerobically digested sludge, the soluble organic compounds tended to decrease in size with time. Nickel was organically complexed during the first four weeks but was later present as both organic and inorganic species. Copper was organically complexed during the whole 30 weeks of the investigation. Zinc was present as inorganic species during the whole period.

Garnett *et al* (1987) found that in the solution phases of both sludges and sludge/soil mixtures, Cu and Ni were present in solution as complexes with relatively high molecular weight organic ligands. Manganese was present as unbound organic species and Zn occurred in both organic and inorganic forms. Some of the soluble ligands produced after the addition of sludge to soil will probably have been derived from the breakdown of the indigenous soil humus. Terry *et al* (1979) found that the rapid decomposition which occurred in the first few weeks following the application of sludge to soil caused a marked increase in the breakdown of the soil organic matter. Anaerobically digested sludges appeared to be very resistant to decomposition in soil. Terry *et al* (1979) found that 55-80% of the organic C in sludge added to soils was resistant to decomposition and concluded that it's turnover time was likely to be in the order of hundreds of years.

5.2.2: The role of hydrous oxides

Hydrous oxides of Fe and Mn are important adsorbents of metals in soils. Alloway *et al* (1984) showed that a frequently waterlogged soil with lower hydrous Fe and Mn oxide contents than a freely drained soil had a lower adsorptive capacity and relatively higher available cadmium concentrations. Kuo *et al* (1985) found that the hydrous Fe oxide content of soils (along with pH and total metal content) was an important parameter in the prediction of metal uptake by Swiss chard. Brown *et al* (1989) found that a decreased redox potential caused by short-term flooding led to a marked increase in Ni

and Cd availability in naturally enriched mineral soils but no such increase occurred in sludged soils, presumably because of the organic complexation of the metals. Hue *et al* (1988) compared the uptake of metals by lettuce in a greenhouse experiment with three mineralogically distinct soils: a volcanic ash-derived Andept, an alkaline vertisol containing montmorillonitic clays and a limed manganiferous Oxisol, which had been amended with sludge. Zinc and Cd in the crop increased with sludge application rate, Cu and Fe were not significantly affected by sludge rate and Ni only increased with sludge rate in the Oxisol. The Andept showed the greatest adsorptive capacity for heavy metals and P, the Oxisol showed the least adsorption and gave rise to phytotoxic concentrations of Mn.

5.2.3: The role of soil carbonates

Several workers have found that Cd mobility and bioavailability are restricted in calcareous soils (Davis, 1984; Cline and O'Connor, 1984; Homann and Zasoski, 1987; Alloway *et al*, 1988). This is likely to be due to a combination of chemisorption (Papadopoulos and Rowell, 1988), precipitation of $CdCO_3$ (Christensen and Tjell, 1983) and competition with Ca^{2+} ions for absorption sites on plant roots. These processes help to explain the decreased mobility and plant availability normally found when acid soils are limed. Mahler and Ryan 1988) found that Cd was predominantly present in the carbonate, organic or residual phases of a sludged semi-arid soil from Southern California. Liming the sludged soil had little effect on this speciation but did increase the Cd concentrations in saturation extracts, although this was not reflected in increased uptake by plants. Using a sequential extraction procedure, LeGret *et al* (1988) found that Cd was mostly present in the exchangeable fraction, Ni in the oxidizable phase, Pb in the acid soluble and Cr in the residual phase of a sludged sandy soil.

6: MOVEMENT OF METALS WITHIN THE PROFILES OF SLUDGED SOILS

Many investigations of the distribution of metals with depth in the profiles of sludged soils have shown that, in the short-term, relatively little downward movement of metals occurs below the depth of cultivation or of sludge

injection (Hemkes and Kemp, 1983; Al-Solaimi, 1987; McGrath, 1987; Gebhardt *et al*, 1988). Williams *et al* (1987), working in California, found that even after applications of sludges amounting to a maximum of 1800 dry t ha^{-1} on a loam soil Cd, Zn, Ni, Co and Fe tended to remain in the zone of incorporation over a nine year period. One of the two sludges used in the experiment sludges caused progressive acidification which increased the availability of the metals but did not lead to their increased movement in the profile. In grassland, Davis *et al* (1988) found that Cd, Cr, Cu, Mo, Ni, Pb and Zn all moved from the surface to within the top 10 cm of the profile but an average of 87% of metals (range 60 -100%) remained in the upper 5 cm. From this they suggested that the most appropriate depth for taking soil samples from sludge-amended grassland was 0 - 5 cm or 0 - 7.5 cm.

Under forestry, Fiskell et al (1984) found that most metals occurred in the top 7 cm of the profile of an acid sandy soil although some downward movement was observed between 6 and 30 months. Harris and Urie (1986) found that a large proportion of the metals in a sludge-amended soil under aspen forest were immobilised in the humus layer and that the underlying soil did not play a large part in the cycling or storage of metals for at least 5 years after the sludge application.

However, some authors report a greater degree of movement of metals down the profiles of sludged soils. Darmody *et al* (1983) found that with 150 and 300 dry t ha^{-1} applications of composted sewage sludge on a silt loam soil, Zn and Cu concentrations increased down to 75 cm depth with most of the movement occurring in the first year with the higher application rate. Copper was particularly mobile with the 300 t ha^{-1} application, probably due to "cheluviation" with the soluble ligands. In a loam soil under grass, Genevini *et al* (1986) found that Zn showed the greatest downward movement with Ni and Cd moving to the least extent. Although most metals were found within the 0 - 15 cm horizon some had moved below this three years after sludging. Legret *et al* (1988) found that in a coarse-textured soil which had received several applications of sewage sludge, Cd had moved down from the surface to depths of 60 - 80 cm, Ni to 40 - 60 cm, Pb to 20 - 40 cm but Cr remained in the surface horizon. Juste and Solda (1977) working on the same soil had previously reported that three years after the application of 100 t ha^{-1} of a high metal sludge on the acid sandy soil, Cd,

Cu and Ni concentrations increased significantly in both the 0 - 20 cm and 20 - 40 cm horizons. In the 0 - 20 cm horizon, the following increases in concentrations were observed: trace to 39 mg kg^{-1} Cd, 3.4 to 13.1 mg kg^{-1} Cu and 0.8 to 86.5 mg kg^{-1} Ni. In the 20-40 cm layer Cd increased: trace to 5.7 mg kg^{-1} Cd, 3.1 to 4.2 mg kg^{-1} Cu and 0.5 to 13 mg kg^{-1} Ni in the 20-40 cm layer. Alloway (1979) found that in a profile of a chalky boulder clay on a former sewage farm site several metals had moved down to the 50 - 100cm layer of the profile. All metal concentrations were highest in the top 50 cm; Zn showed the greatest extent of migration (to at least 200 cm) Pb, Cu and Cd were mainly concentrated in the top 100 cm but slightly elevated concentrations of Cu and Cd occurred in the 100 - 150 cm layer. Pike *et al* (1975) reporting data for a more representative survey of profiles on the sewage farm site found that most Pb was distributed within the top 60cm, Cd concentrations were anomalously high through the 180 cm depth investigated, most As was in the upper 30 cm and Cr in the top 60 cm. The findings for this site (subsequently developed for industry and housing after removal of the top 60 cm of soil) are important because they relate to a more realistic time period (around a 100 years) than most recent studies.

Some of the differences in the profile distribution of sludge-borne metals mentioned above could be explained by variations in climate, especially the balance of precipitation and evaporation. For example Williams *et al* (1987) were working on relatively arid soils near Berkeley in California, Juste and Solda (1977) and Legret *et al* (1988) studied a soil from south west France, while Davis (1988), Alloway (1979) and Pike *et al* (1975) worked in the more moist and cooler climate of England.

7: UPTAKE OF HEAVY METALS BY CROP PLANTS

Crops differ in their ability to take up, accumulate and tolerate heavy metals. Marked differences occur between species and even varieties within a species. Kim *et al* (1988) reported that for 12 different food crops grown in the greenhouse on 6 sludged soils, leaf concentrations of Cd varied between 7.3 and 18.85 fold and Zn contents by 5.8 to 20.3 fold. Davis and Carlton-Smith (1980) showed that for a wide range of crops grown on sludged soils, the species which accumulated the most Cd were: tobacco > lettuce > spinach > celery > cabbage; for Pb, they were: kale > ryegrass >

celery; for Cu: sugar beet > some varieties of barley; for Ni: sugar beet > ryegrass > mangold > turnip; and for Zn: sugar beet > mangold > turnip. In general, cadmium tends to accumulate in the leaves and so is more of a risk in leafy vegetables grown on contaminated soils than in seed or root crops (Alloway *et al*, 1990). Alloway *et al* (1990) showed that the uptake of Cd by crops decreased in the order: lettuces, cabbages, radish and carrots.

Gebhardt *et al* (1988) found Cu and Zn accumulated in the grain of cereal crops. However, they found Cd, Pb, Zn, and Cu contents were generally highest in the roots but the shoots and leaves of rye, barley and oats also had increased metal concentrations. Maize cultivars differ considerably in their uptake of Cd and Zn and therefore it would be possible to select the least accumulating cultivars for growing on sludged soils. (Hinesly *et al*, 1982; Logan and Miller, 1985). Williams *et al* (1985) found few differences between four wheat varieties but found that one barley cultivar ("Igri") accumulated higher levels of Cu and Zn from sludged soils which appeared to reduce yields. Mahler *et al* (1980) found that Swiss chard and tomato plants accumulated two to three times more Cd on sludged acid soils than maize. Mondy *et al* (1984) found that potato tubers accumulated significant concentrations of B, Cd, Cu, Ni and Zn on sludged soils but that no significant trend was found for concentrations of Co, Cr, Fe, Mn and Pb.

van Lune (1985) found that the Cd in industrial sludges was less available to spinach, potato, carrot and spring wheat than an equivalent concentration of Cd as $CdNO_3$. Siriratpiriya et al (1985) found that Cd and Ni from sludge was accumulated by lettuce plants to a lesser extent than from spiked soils but Zn, Cu and Mn were more available from sludge, probably due to organic complexation. Street et al (1977) found that plants accumulated more Cd from mineral soils amended with $CdSO_4$ than from sludged soils spiked with $CdSO_4$ indicating the greater adsorptive capacity of the sludge constituents.

Less Cd was accumulated in the shoots of grape vines than in sunflower, maize, wheat or ryegrass, but with very heavy loadings of metals from refuse-sewage sludge compost toxic symptoms and imped growth were observed in vines. Toxicity was more severe on acid soils than on those with higher pHs (Mohr, 1985). Stadelemann *et al* (1987) found that nine years of

heavy applications of sludge followed by two years of fertilisers led to increased uptake of Cd, Zn and Mn concentrations in celeriac but Cu and Ni levels were not elevated. In soya bean plants, Reddy *et al* (1989) found Cu concentrations to be higher in the seed than the leaves or stem, but Zn contents were lower in the seed than other tissues. In maize, Cu and Zn contents increased more in the leaves than the seeds. In spinach, Yamada *et al* (1984) found that the Zn content increased steadily with increased applications of sludge, Cu increased for 4 applications but decreased after the fifth crop but the uptake of Cd, Ni and Cu by millet, rye, turnip and radish decreased with successive applications, probably as a result of increasing pH. Chu and Wong (1987) found greater accumulations of metals in roots than leaves of Chinese cabbage (*B. chinensis*) and tomato growing in sludged and composted soils but carrots contained more in their leaves than the edible root. de Haan (l975) reported very large increases in the metal concentrations in ryegrass grown on soil amended with a high metal sludge containing (in mg kg^{-1}): 5533 Zn, 637 Pb, 1084 Cu, 135 Cd, 934 Ni. In comparison with controls the concentrations of heavy metals in grass had increased by a factors of 145 Ni, 37.7 Zn, 14.5 Cd, 10 Cu and 3.5 Pb. In one case the Mn content of the grass was actually reduced to 15% of the control with the sludge.

Apart from the regularly investigated heavy metals, such as Cd, Cu, Cr, Mn, Ni, Pb and Zn, there are a lot of other metals and metalloids present in sludge that may be having an effect on soils, crops and, possibly, the food chain. Hansen and Chaney (1984) consider Cr (III), Zn, Ti, Si, Al, and Sn in sludges either so insoluble or of low toxicity that they constitute little risk to human or animal health. However, direct ingestion of metal-rich sludged soil through close grazing could result in potentially harmful concentrations of metals including: Cu, F, Zn, Pb, Fe, As, Co and Hg being ingested by livestock. Soon and Bates (1985) found that Mo uptake by maize was highest in the eighth year where sludging had ceased 1 - 3 years previously. Hansen and Chaney (1984) state that plants can tolerate high concentrations of Mo and uptake can be excessive under alkaline pH conditions. The main health problem associated with this is the incidence of Mo-induced copper deficiencies in ruminants. Boron and Co concentrations were not affected by sludging (Soon and Bates, 1985).

Chumbley and Unwin (1982) reported highly significant correlations between the total Cd content of sludged soils on a heavily sludged site and the Cd concentrations in lettuces and cabbages grown in the field. Lund *et al* (1981) also found significant correlations between Cd in sludged soils and the Cd contents in the leaves of several crop species. In a comparison of soils contaminated with heavy metals from both sewage sludges and other sources, such as metalliferous mining, smelters and scrap yards, Alloway *et al* (1990) found that Cd tended to be less bioavailable from the sludged soils than from those contaminated from inorganic sources. Korcak and Fanning (1985) found that Cd uptake by maize from $CdSO_4$-spiked soils was 5-18 times greater than from equivalent amounts of metal in sludged soils. This greater bioavailability of metals from spiked soils compared with sludged soils has also been reported by a number of authors (Mahler *et al*, 1978; Alloway, 1986).

According to Chaney (1983) Pb from sludge does not give rise to an increase in plant Pb unless the concentration is very high. Sludge even causes the reduction in Pb concentrations in crops due to either increased adsorption capacity or antagonism with P. Mercury, is strongly bound in sludged soils and uptake is very limited (Logan and Chaney, 1983).

With regard to the toxicity of metals to plants, Davis and Carlton-Smith (1984) found that the relative toxicities to *L. perenne* of Zn:Cu:Ni were 1.0:2.6:1.0 on a loading rate basis at pH7. At subcritical concentrations in the soil the element present at the highest concentrations relative to its critical concentration determined the effect on yield but if more than one element exceeded its critical concentration the phytotoxic effects were additive. Interactions with macronutrients are also likely to be significant.

8: EFFECTS OF SEWAGE SLUDGE AMENDMENTS ON SOIL MICROORGANISM ACTIVITY

Soil microorganisms play a vital role in the cycling of carbon, nitrogen and other nutrients through the decomposition of litter, the formation of humus, nitrogen fixation and many other roles. It is recognized that high concentrations of Cu and Zn in soils around some smelters and brass foundries are adversely affect various species of microorganisms in the soil,

resulting in reduced decomposition and other effects (Ruhling and Tyler 1973, Hutchinson, 1979).

McGrath *et al* (1988) observed a 50% reduction in nitrogen fixation in a soil which had last received sludge 20 years previously. The total metal concentrations in this soil were (mg kg^{-1}): 334 Zn, 99 Cu, 27 Ni and 10 Cd. In the EDTA extract the concentrations of these elements were (mg kg^{-1} dry soil): 165 Zn, 60 Cu, 7.3 Ni and 5.3 Cd. Brookes *et al* (1986) reported that acetylene reduction by soil microorganisms was reduced by 50% at at EDTA extractable concentrations of (in mg kg^{-1}) 50 Zn, 20 Cu, 7.3 Ni and 3 mg kg^{-1} total Cd. Reddy *et al* (1987) found that dehydrogenase and phosphatase enzyme activity was inhibited in all their experimental sludged soils and was related to their heavy metal concentrations. Increasing rates of sludge reduced urease activity in some soils but increased it in others.

Barkay *et al* (1983) studying the effects of Cd salts on the activity of microorganisms isolated from sludged soils found that gram-negative bacteria, especially Pseudomonas and Flavobacterium species, were more resistant to high levels of Cd than grampositive bacteria. The Pseudomonads were able to develop resistance to Cd in sludged soils but the Flavobacteria were naturally Cd resistant. Coppolla *et al* (1988) found that Cd from sewage sludge inhibited the activity of ammonifying bacteria in volcanic soils and the activity of free-living nitrogen fixers in a terra-rossa soil. Conversely, James and Bartlett (1984) demonstrated the inhibitory effect of Cr (VI) in metal salts on nitrification but found that the Cr in sewage did not have any inhibitory effect.

Heckman *et al* (1986) found that nodulation in Soya bean was enhanced by the addition of sludge but this was most pronounced at pH 7 and may have been largely due to the physical soil conditioning effects of the sludge. On reclaimed strip-mining soil with very low levels of fertility, sludge amendments gave rise to increased microorganism activity and the metals did not appear to be inhibiting it relative to local controls (Seaker and Sopper, 1988).

8: CHANGES IN THE AVAILABILITY OF HEAVY METALS TO CROPS DURING THE RESIDUAL PERIOD

Any changes which are observed in the bioavailability of metals through time could be due to combinations of several factors including changes in: soil pH, the proportions of soluble and insoluble organic matter, the speciation of the metals, the reactivity of soil minerals, such as hydrous oxides of Fe and Mn and factors which affect the rate of growth of the plant, such as nutrients and soil physical properties.

Almost all authors reporting investigations into the bioavailability of metals in sludged soils found that the metals remained available for uptake, giving anomalously high concentrations of metals in plant organs for many years after application (Chaney *et al* 1982; Burridge and Berrow 1984; Heckman *et al* 1987) Some found that the availability remained roughly the same (Chang *et al* 1982; McGrath 1987) while others reported decreases after the last sludge application (Hinesly 1979; Bidwell and Dowdy 1987; Morel *et al* 1988). Bidwell and Dowdy (1987) found a dramatic decrease in Cd uptake on a kg ha^{-1} basis after the first year following sludge application. Some, such as Adams and Sanders (1984) found the extractable metal concentrations to decrease over time and so predicted a decrease in bioavailability. Korcak and Fanning (1975) found the DTPA extractable concentrations of Zn, Cd and Cu decreased with time but extractable Ni increased. Burridge and Berrow (1984) found Cu concentrations in ryegrass and clover decreased with time but Ni and Zn remained highly available. In a review of papers concerned with changes in uptake of metals with time after sludging, McGrath (1987) points out that 9 out of 11 papers showed availability to remain more or less constant over several years. In contrast to the findings reported above, Soon and Bates (1985) reported that Mo uptake by maize and bromegrass was at its highest in the eighth year after the onset of an experiment and three years after the end of sludging, Schmitt and Sticher (1983) calculated that the Cd adsorption capacity of a parabraunerde soil would be saturated after 45 years of application implying much greater bioavailability after this time. de Haan (1975) commented that heavy metals in sludged soils became more bioavailable with time, mainly through acidification of the substrate by nitrification of nitrogen and the rapid leaching of lime from the sludges.

Chaney (1988) commenting on the discussion about the reversion (reduction over time) (sic) in the availability of soil metals from sewage sludges, states that "there has been little demonstration or explanation of this result". Chang *et al* (1987) had concluded that two types of reversion occurred. One type was the reduction in the formation of soluble ligands due to microbial action and the other was the slow reaction of metals with minerals in solid solution reactions. However, these mechanisms will vary with environmental conditions. The formation of some metal carbonate precipitates that occurs in arid soils will not take place in the leached podzolic soils of humid temperate zones, such as are found in north west Europe. The climatic changes predicted over the next 30 - 50 years and increasing acid precipitation from air pollution would result in changes in metal bioavailability in soils. It must be remembered that soils are dynamic entities in equilibrium with the environmental conditions acting upon them. With the long half-lives of metals in soils (Kabata-Pendias, 1987) it cannot be assumed that conditions will remain constant for all the time that the soil will remain significantly contaminated.

10: SOIL TESTS FOR MONITORING THE BIOAVAILABILITY OF HEAVY METALS IN SLUDGED SOILS

As shown in Table 7, a range of extractants have been found to be suitable for routine monitoring of soils for their available metal concentrations. These include the standard DTPA method, which is also used for assessing the micronutrient supply capacity of normal (unsludged) agricultural soils (Juwarkar and Shende, 1986; Sanders *et al* 1986; Barbarick and Workman, 1987; Mulchii *et al*, 1987; Rappaport *et al*, 1988; Adamu *et al*, 1989; Browne *et al*, 1984). EDTA is used as the official soil test in the UK and was found to correlate with the uptake of metals by ryegrass (Sanders *et al*, 1986) and similar results were obtained by Fujii and Corey (1986).

Experiments indicate that the use of the neutral salts are the best means available for predicting the bioavailability of cadmium (Hani and Gupta, 1983, 1985; Morgan and Alloway, 1984; Alloway *et al* , 1985; Alloway and Morgan, 1986; Sanders *et al* , 1986: Jackson and Alloway, 1990). The work of Hani and Gupta (1983, 1985) recommends that 0.1 M $NaNO_3$ is used, this test has the disadvantage of extracting less cadmium than the more

commonly used 0.05M $CaCl_2$ test. The $CaCl_2$ test has been quite widely used and found to produce satisfactory results, although the molarities of the extracting solutions varied (Sauerbeck and Styperek, 1984; Morgan and Alloway, 1985; Sanders *et al*, 1986). Ammonium nitrate (1 M) was found by Morgan and Alloway (1985) to be significantly correlated with the concentrations of cadmium in several vegetable crops grown on a range of polluted soils.

11: CONCLUSIONS

- The application of sewage sludges to soils is of relatively minor significance when considering the other sources of heavy metal contaminants on a global basis. However, for those soils amended with sludge, the input of heavy metals from this source is of major importance, resulting in elevated concentrations of a wide range of elements.

- The availability of heavy metals in sludged soils to crops varies between heavy metals, crops and soil types. Cadmium, zinc and thallium show the greatest tendancy to move along the soil to plant pathway.

- Heavy metal availability to crops is influenced by a wide range of soil variables of which, pH is probably the most important. In general the bioavailabilities of heavy metals, except Mo, in acid soils are reduced by liming to pH 6.5 to 7.

- Sludges differ from most sources of metal contamination in that they have a matrix with a strong adsorptive capacity. The application of sewage sludge to a soil simultaneously increases the concentrations of heavy metals and alters the adorptive capacity of that soil.

- In many cases, the majority of the heavy metals applied to soils in sludges tend to remain in the topsoil with a marked reduction in concentration with depth. However, this is influenced by soil type, vegetation and climate. Some soils with a relatively long residual period after sludge application have shown a marked movement down the profile.

The behaviour of heavy metals during the residual period has been examined by a number of researchers who have concluded either, that the bioavailability of heavy metals declines or remains constant during the residual period. As in the case of metal movement in soil profiles, short-term studies represent a very small fraction of the residence time of the heavy metals in soils. The legacy of the current contamination of soils by sewage sludges is not completely understood for the longer-term. Changes in climatic conditions and land management are likely to have significant effects.

Of the many soil tests evaluated for the prediction of the bioavailability of heavy metals in sludged soils, DTPA and dilute $CaCl_2$ appear to be the most useful, at least for elements such as Cd, Zn and Cu.

ELEMENT	RANGE (mg kg^{-1} DW)
Ag	< 930
As	3 - 30
Au	0.25 - 7
B	16 -680
Ba	9 - 1004
Cd	<1 - 3410
Co	1 - 260
Cr	8 - 40,600
Cs	0.45 - 2.9
Cu	50 - 8000
Hg	0.1 - 55
La	6.4 - 380
Mn	60 - 3900
Mo	1 - 40
Ni	6 - 5300
Pb	29 - 3600
Sb	3 - 44
Se	1 - 10
Sn	40 - 700
U	0.8 - 3.3
V	20 - 400
W	0.9 - 99.6
Zn	91 - 4900
Zr	4.8 - 319

Table 1: Typical concentrations of heavy metals in sewage sludges (Bradford et al, 1975; Furr et al 1976, Bowen 1984; Kabata-Pendias and Pendias 1984)

ELEMENT	INPUT (10^6 kg a^{-1})		PERCENTAGE
	Sewage sludge	Total	OF TOTAL (%)
As	0.01 - 0.24	52 - 112	0.02 - 0.21
Cd	0.02 - 0.34	5.6 - 38	0.36 - 0.89
Cr	1.4 - 11	484 - 1309	0.03 - 0.84
Cu	4.9 - 21	541 - 1367	0.09 - 1.54
Hg	0.01 - 0.8	1.6 - 15	0.63 - 5.33
Mn	4.4 - 11	702 - 2633	0.42 - 0.63
Ni	5 - 22	106 - 544	4.04 - 4.72
Pb	2.8 - 9.7	479 - 1113	0.58 - 0.87
Sb	0.04 - 0.2	4.7 - 47	0.43 - 0.85
Se	0.01 - 0.14	6 - 76	0.17 - 0.18
V	0.22 - 1.5	43 - 222	0.51 - 0.68
Zn	18 - 57	689 - 2054	2.61 - 2.78

Table 2: Estimated global inputs of metals to soils from sewage sludges (adapted from Nriagu and Pacyna 1988)

ELEMENT	TRANSFER COEFFICIENT*
Cd	1 - 10
Co	0.01 - 0.1
Cr	0.01 - 0.1
Cu	0.1 - 1
Hg	0.01 - 0.1
Ni	0.1 - 1
Pb	0.01 - 0.1
Tl	1 - 10
Zn	1 - 10
As	0.01 - 0.1
Be	0.01 - 0.1
Se	0.1 - 10
Sn	0.01 - 0.1

* orders of magnitude only, precise value depends upon plant species and soil variables.

Table 3: Transfer coefficients for heavy metals in the soil-plant system (Kloke et al 1984)

ELEMENT	RANGE[a]	EC LIMIT VALUES[b]
As	10 (B,N) - 75 (Can)	-
Cd	8 (Dk) - 30 (F, Sz)	20 - 40
Co	20 (B, N) - 150 (Can)	-
Cr	200 (N) - 1200 (G)	1000 - 1750[c]
Cu	500 (B) - 3000 (S)	1000 -1750
Hg	5 (Can) - 25 (F, G)	16 - 25
Mn	500 (B, N) - 3000 (F)	-
Mo	20 (Can)	-
Ni	30 (Dk) - 500 (F, S)	300 - 400
Pb	300 (B, N) - 1200 (F, G)	750 - 1200
Se	14 (Can) - 100 (Fr)	-
Zn	1000 (Sz) - 10000 (S)	2500 - 4000

Notes: a - associated country abbreviated in parentheses. b - EC document code 86/278 EEC

B - Belgium, Can - Canada, Dk - Denmark, F - Finland, Fr - France, G - West Germany, N - Norway, S - Sweden, Sz - Switzerland. c - provisional, to be confirmed.

All concentrations are in mg kg^{-1} DW.

Table 4: Maximum acceptable concentrations of metals in sewage sludges applied to agricultural land (from Webber et al 1984, EC 1986)

ELEMENT	LIMIT VALUES (mg kg^{-1} DW)	
	RECOMMENDED	MANDATORY
Cd	1	3
Cu	50	140
Cr	100	200*
Hg	1	1.5
Ni	30	75
Pb	50	300
Zn	150	300

Notes: Higher concentrations are permitted on land dedicated to sludge where commercial crops are only grown exclusively for animal consumption.
Where land is consistently above pH 7, these limits may be exceeded to a maximum of 50% so long as there are no other hazards to health or the environment (eg groundwater).
* Cr concentrations not yet finalised

Table 5: Limit metal concentrations for sludged soils in the European Community (from EEC 1986, 1989)

METALS						
Cd	Cu	Cr	Ni	Pb	Zn	REFERENCE
26	-	-	–	496	-	Chumbley and Unwin (1982)
61	-	2020	-	2470	-	Pike *et al* (1975)
64	770	6000	333	938	1748	Alloway *et al* (1988)
159	1025	-	543	2117	2596	Jackson (1990)

All concentrations are mg kg^{-1} DW

Table 6: Some typical high concentrations of heavy metals found in heavily sludged soils

REAGENT	ELEMENT(S)	CROP	REFERENCE
0.005M DTPA	Zn, Cd, Cu, Mn	Tobacco	Adamu *et al* 1989
0.005M DTPA	Ni	Lettuce	Browne*et al* 1984
0.005M DTPA	Cu	Soya beans	Adams & Kissel 1989
0.005M DTPA	Cu, Cd	-	Morel *et al* 1988
0.005M DTPA	Cd	Wheat	Jurwarka & Shende 1989
0.005M DTPA	Zn, Cu, Mn, Ni, Cd	Tobacco	Mulchi *et al* 1987
0.005M DTPA	Cd	Potato	Jackson 1990
Ammonium bicarbonate DTPA	Cd, Cu, Ni, Pb, Zn	Swiss chard	Barbarick & Workman 1987
Ammonium bicarbonate DTPA	Mo	-	Pierzynski & Jacobs 1986
DTPA, EDTA, $CaCl_2$	Zn, Cu, Ni	Ryegrass	Sanders *et al* 1987
EDTA	Cd, Zn	-	Fujii & Corey 1986
0.43M acetic acid	Ni	Ryegrass, clover	Burridge & Berrow 1984
0.01M $CaCl_2$	Zn, Cu, Ni	-	Sanders *et al* 1987
0.01M $CaCl_2$	Cd, Zn	-	Sauerbeck & Styperek 1984
0.01M $CaCl_2$	Cd	Lettuce, radish carrot, cabbage	Alloway *et al* 1985
0.1M $NaNO_3$	Cd	-	Hani & Gupta 1985
1M NH_4NO_3	Cd	Lettuce, radish, carrot, cabbage	Morgan & Alloway 1985

Table 7: Soil tests significantly correlated with plant heavy metal concentrations

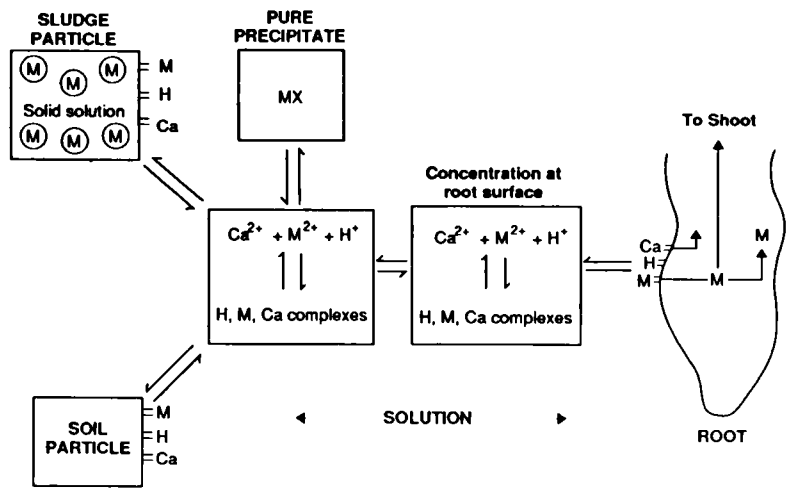

Figure 1 Metal reactions in a sludge-soil-water-plant system. M represents a metal ion or atom, H a hydrogen atom or ion, and x a substance that combines with M to form a precipitate (e.g., hydroxide, carbonate)

27 pages of text

5 tables

3 figures

'The bioavailability of cadmium to lettuces and cabbages ...'

In press *Plant and Soil*

The bioavailability of cadmium to lettuces and cabbages from soils previously amended with sewage sludges.

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KEY WORDS

Bioavailability, cabbage, cadmium, lettuce, sewage sludge amended soils.

ABSTRACT

The application of sewage sludges to soils may lead to the elevation in the concentration of cadmium. The bioavailability of cadmium is determined by the interaction of a number of soil physico-chemical and plant variables, of which pH is the most important. Duplicate samples of sludge amended soils were taken and placed in tubs in the field, one of each pair of the acid soils was limed to pH7±0.5. Lettuces and cabbages were grown to maturity and analysed for cadmium. Liming the soils always reduced the uptake of cadmium by the plants. Three soil extractants, 1M NH_4NO_3, 0.05M EDTA-$(Na)_2$ and 0.05M $CaCl_2$ were used in order to predict cadmium bioavailability. The 0.05M $CaCl_2$ soil extraction proved to be the most effective for both lettuces and cabbages. Multiple linear regression equations

were derived, to describe the uptake and accumulation of cadmium by both crops. The relative influence of soil variables differed between the two plant species. The data from this experiment are comparable with those for crop samples taken from the field, unlike a number of pot experiments conducted in glasshouses.

INTRODUCTION

The transfer of heavy metals into the human food chain as a result of the application of sewage sludges to agricultural land poses a potential threat to human health, of these metals cadmium is of particular concern (Chaney, 1988; Kloke *et al*, 1984; Naylor and Loehr, 1981; Ryan *et al*, 1982). The accurate prediction of soil to plant transfers of cadmium is fundamental to the assessment of the human health risk. Lettuce and cabbage are frequently studied in this respect, as they are representative of plants which most readily accumulate cadmium and a variety of other heavy metals (John, 1972; Alloway *et al*, 1990; Ryan *et al*, 1982; Davis and Carlton-Smith, 1980; Alloway, 1986).

As can be seen from Table 1, the concentrations of cadmium in lettuces can vary by almost two orders of magnitude, primarily depending upon the levels of soil contamination. The prediction of the concentration of cadmium in a plant growing in contaminated soil remains a problem. Using the models of soil nutrient bioavailability developed by Barber (1984), Mullins *et al* (1986) attempted to predict the accumulation of cadmium and zinc by Zea

mays seedlings growing on soils amended with sewage sludge. The accuracy of their model was considerably greater for zinc (R-squared 83%) than it was for cadmium (R-squared 66%). A number of purely empirical attempts have been made but these tend to be limited by the restricted nature of the sample which may not reflect the heterogeneity found in the field. The uptake of cadmium by a number of plant species has been shown to be influenced by a wide range of soil variables, including:

- temperature (Siriratpuriya *et al*, 1985)
- chloride salinity (Bingham *et al*, 1983)
- pH (Street *et al*, 1978; McClean, 1976; Tyler and McBride, 1982; Andersson and Nilsson, 1974; John, 1972)
- organic matter (McClean, 1976)
- calcium concentration (Tyler and McBride, 1982)

Given the considerable natural diversity of soils, a multivariate approach to the study of cadmium bioavailability is necessary.

A number of soil extractants have been employed in order to try to determine that fraction of the total concentration of cadmium considered to be 'bioavailable'. These soil extractants fall into three groups:

- neutral salts
- chelating agents
- weak acids

A previous study (Morgan and Alloway, 1984) has shown neutral salts to be the most accurate predictors of cadmium

bioavailability, confirming the work by a number of other workers (Sauerbeck and Styperek, 1984; Haeni and Gupta, 1983). The applicability of chelating agents for the determination of bioavailability of a number of trace elements has been the subject of much scrutiny, especially when they are used outside of the application for which they were first developed (O'Connor, 1988). Despite these methodological inconsistencies, the use of soil extractants for the prediction of cadmium bioavailability can provide useful information if the data generated are handled with care.

A key soil variable influencing the bioavailability of cadmium, is soil pH. Soil pH has been shown to influence the sorption of cadmium and therefore the concentration of cadmium in the soil solution (Alloway *et al*, 1984). The availability of cadmium is inversely related to the pH of the soil. Therefore, a feasible method of managing the availability of cadmium would be to manipulate the pH of the soil by the addition of calcium carbonate. The precipitation of cadmium carbonate from the soil solution has been proposed as a major factor in the control of bioavailability at high cadmium concentrations and neutral soil pHs (Christenssen and Tjell, 1984; Papadopoulos and Rowell, 1988). This is especially important when sewage sludges are applied to soil, as their application tends to lower the pH for the first year, producing a marked increase in the availability of cadmium during the first year of the residual period. It is the nature of the changes in

bioavailability after this initial increase that give rise to the most contradictory data. Observed changes tend to fall into two groups, one in which the bioavailability remains constant and another in which it gradually declines.

Over the residual period, the availability of cadmium to corn was seen to fall in a field trial reported by Bidwell and Dowdy (1987), the rate of decrease is largely dependent upon the initial cadmium input. In a similar study by Hinesly *et al* (1979) the bioavailability of cadmium to Zea mays also fell, with the concentrations in grain falling from 0.44 to 0.07 $\mu g g^{-1}$DW over the four year residual period. Kelling *et al* (1977) observed, over a four year residual period, a fall in both the concentration of cadmium in plant tissues and also in the cadmium extractable from soils using the DTPA soil test .

This paper reports outdoor experiments with lettuces and cabbages grown in large tubs of sewage sludge-amended soils that had been collected from a range of sites. The objectives were to determine the major soil variables controlling the accumulation of cadmium by two crops and to assess the application of three commonly used soil partial extractants to the prediction of the bioavailable cadmium fraction in sludge-amended soils.

METHODS

Soil sampling and field experiments

Twenty bulk soil samples of topsoil (~35kg) were taken from around the UK, mainly from sites for which there was some information of the previous land management practices; of particular importance was the year that the sludge was applied to the soil. As broad a spectrum of samples as possible were taken in order to reflect both extreme levels of soil contamination and control situations; the diversity of soil physico-chemical variables can be seen in Table 2. A number of the soils were from field trial sites and old sewage farms at which the levels of cadmium were considerably higher than those more commonly found in the field.

The samples were approximately halved, with each half being placed in a polyethylene container of dimensions 35 X 35 X 30 cm. The containers of soil were kept at a field station in the open air for eight months before the first experiment was conducted. Following a laboratory experiment, a quantity of lime was added to one of each of the duplicates in order to raise the pH of the acid soils to 7.0±0.5, subsequent analyses have shown that the concentration of cadmium in the soil was not elevated by the addition of lime. Lettuce (cv. Webbs Wonderful) and cabbage (cv. Greyhound) seedlings were germinated in John Innes No.2 in seed trays prior to being planted out , three plants were planted in each tub of soil.

Sample preparation

Soils: Soil samples were taken from the plant containers prior to harvesting the plants. The samples were left to air dry for about one week, before the < 2mm fine earth fraction was removed by passing the sample through a nylon sieve. This fine earth fraction of the soil sample was used for all determinations of soil variables.

Plants: The lettuces and cabbages were harvested, taking only the edible components. Fresh weights were recorded. The outer leaves of each plant were removed before the sample was washed with distilled water to remove any soil. The washed samples were then frozen until ready for use. Prior to the analysis samples were taken from the freezer, shredded and left to dry at 65°C in a forced draught oven, until no further change in weight was observed. The dried sample was weighed and then finely ground in a Fritsch Pulverisette centrifugal ball mill; in order to minimise contamination, acid-washed polyamide pots and agate balls were used for this process. At no point during this process did the sample come into contact with any metal surfaces.

Sample dissolution and analysis for the determination of total cadmium concentrations

Soils: The determination of the total metal concentration of a soil sample must involve the total dissolution of the sample, as this would involve the use of hydrofluoric and perchloric acids, less rigourous sample leaching or pseudo-total dissolution procedures were used.

2.00±0.10g of sample were weighed into an acid washed 200ml conical flask, 25ml AristaR grade nitric acid (BDH Chemicals, Poole, Dorset, UK) were added and then left overnight in a fume cupboard. This procedure was found to minimise the loss of sample by mitigating the otherwise vigorous action upon heating. A reflux condenser was placed in the neck of the flask, which was then carefully heated in a sand bath. The sample was heated for 3 days at ~120°C before the reflux condenser was removed and the total volume of acid in the flask reduced by heating at ~160°C for two hours. The sample was then washed from the flask with distilled deionised water and filtered through Whatman 42 filter paper into a 50 ml volumetric flask. The solutions were analysed by double beam flame atomic absorption spectrometry, using an Instrumentation Laboratory (IL) S-12 spectrophotometer. All samples were analysed in duplicate with blanks and the reference material, BCR No 143 'Trace elements in a sewage sludge amended soil'.

Plants: 1.00±0.01g of sample were weighed into a long neck test-tube that had been cleaned by the following process: soaked in 5% solution of Decon, rinsed in distilled water, soaked in a 10% solution of AnalaR grade hydrochloric acid and then rinsed three times in distilled deionised water. 5.00ml of AristaR grade concentrated nitric acid were then added to the sample which was then left at room temperature for 12 hours. The sample was initially heated at 125°C for 30 minutes. A further 5ml of nitric acid

was then added and the sample heated at 150°C until charring was observed. Upon charring a further 1ml of nitric acid is added and the temperature raised by 10°C. This process was repeated until no further charring was observed and the sample was colourless. The sample was transferred to a 50 ml volumetric flask and made up to the volume with distilled deionised water. All samples were analysed by electrothermal atomisation atomic absorption spectrometry (ETA-AAS).

Analyses were made on an IL S-12 spectrophotometer in the single beam mode of operation, using Smith-Hieftje background correction. The instrument was equipped with an IL 655 Controlled Temperature Furnace (CTF) atomiser, IL 254 auto-sampler and a FASTAC for aerosol sample deposition. Pyrolytically coated delayed atomisation cuvettes (DAC) were used throughout the experiment (Allied Analytical Systems and Ringsdorff). Visimax II hollow cathode lamps were used as the radiation source. A Linear dual-channel integrating chart recorder was used to record peak shapes. BOC high purity argon was used as a purge gas. The ETA-AAS method was optimised using a range of certified reference materials. Two reference materials were chosen to reflect the cadmium accumulation rates exhibited by lettuces and cabbages. BCR No. 60 and NIES1 were routinely used to assess both the accuracy and the precision of the method. In addition to these two materials the in-house lettuce material was used to assess the precision of the method. For the in-house lettuce sample an

interbatch precision of 6.8% was attained, the intrabatch precision was 5.1%. The concentrations determined for both of these materials fell within the certified range. A further check on the quality of the data generated is given by the mean recoveries from the spiked sample replicates. The mean was 96.4±14.3% where n=107, the RSD was 14.8%.

Determination of soil variables effecting and indicative of cadmium bioavailability

pH: Soil pH was determined in both distilled water and in 0.01 M calcium chloride, 15g of soil was shaken for 15 minutes with 15ml of solution. The pH was determined with a pH meter.

Soil organic matter content: This parameter was determined gravimetrically by the loss on ignition method (Bell, 1964). A known mass of sample was initially dried at 110°C for 16 hours and then ignited at 375°C for 16 hours in a muffle furnace. The change in weight after each of these two stages was recorded and the organic matter expressed as the percentage loss in weight.

'Bioavailable' cadmium. The bioavailability of cadmium was determined using three soil extractants, 1M ammonium nitrate, 0.05M calcium chloride and 0.05M disodium ethylenediaminetetraacetic acid (EDTA-$(Na)_2$). In each test a 5g soil sample was shaken with 30ml of the extracting solution for 1, 16 or 1 hour respectively before being transferred to a centrifuge tube and centrifugated at 400 g

for 10 minutes, the resulting supernatant was then filtered through Whatman 44 paper. All samples were analysed in duplicate with blanks, against matrix matched standards by flame atomic absorption spectrometry. The reagents used in the procedures were all AnalaR grade.

Hydrous iron oxide and manganese oxide concentration. The determination of these two variables gave some indication of the sorptive capacity of the soils and therefore of the composition of the soil solution. A dithionite extraction was used in order quantify the concentration of hydrous iron oxides in the soils (Bascomb, 1974). The concentration of manganese oxides in the soil was determined after extraction into acidified hydroxylamine hydrochloride at pH3 (Chao, 1972). Iron and manganese concentrations in the extractants were determined by flame atomic absorption spectrometry.

RESULTS AND DISCUSSION

Table 3 summarises the concentrations of cadmium in plants and extracted from the soils by the three reagents used in this experiment. The mean concentration of cadmium in cabbages grown on the soils used in this experiment was 1.77 $\mu g g^{-1}$ DW, this concentration is higher than the mean of the ADAS survey of UK sludge soils (Chumbley and Unwin 1982) and of the national surveys of The Netherlands (Wiersma *et al* 1986) and Spain (Zurera *et al* 1987). Data for a set of polluted soils (Alloway 1986) had mean concentrations of cadmium in excess of those

found in this study. Alloway (1986) grew plants in pots of a smaller volume than the tubs used in this study, this will tend to increase the density of the root ball and so lead to an enhanced efficiency in cadmium uptake. In addition to this difference, there may also be a discrepancy due to temperature, Alloway grew the plants in glasshouses. The 1983 MAFF survey of cadmium in foods in the UK, enables comparison of the data from this study with those from control sites, a market garden soil to which sludge had been applied and an area contaminated with Pb-Zn mine waste (Shipham). The mean concentration of cadmium in cabbages in this study was greater than that found in plants from sludge-amended soils reported in the MAFF survey; however the mean cadmium concentration of cabbages from Shipham was considerably higher than that of this study. Table 1 enables comparison of the lettuce cadmium concentrations in Table 3 with those found in the literature. The concentrations of cadmium found in these experiments are greater than those found in the national surveys (Thornton 1986, Wiersma *et al* 1986, van Lune 1987, Zurera *et al* 1987), as the majority of samples analysed in such a survey will not be exposed to such a degree of soil contamination this is to be anticipated. The UK survey of sludged soils reported by Chumbley and Unwin (1982), produced a mean lettuce cadmium concentration of 4.20 µg Cd g^{-1} DW, approximately 50% of the mean value found in this study. This is probably due to the higher mean pH and lower cadmium concentrations of the soils in the survey (the mean soil cadmium concentration is less than 50% of that

found in this study). It should be emphasised that studies of sludge-amended soils, such as those of King (1986) and Dowdy and Larson (1974), will often include soils with relatively low levels of contamination; many of the soils in this study are more heavily contaminated as they are from experimental sites, hence the higher mean concentrations of the plants in this study.

Influence of soil variables on cadmium uptake.

As can be seen in Table 4, there is a significant negative correlation between the concentration of cadmium in both lettuces and cabbages and the soil pH. In all cases, the application of lime to the soils has raised the pH and so reduced the concentration of cadmium in the plant tissues. This effect has been observed in a number of similar experiments (eg Andersson and Nilsson, 1974). The correlation coefficient has a greater statistical significance for lettuces than for cabbages. If the data for the limed soils are analysed independently of those for the unlimed soils, then the effect of the addition of lime can be observed. The mean concentration of cadmium in lettuces falls from 13.8±9.8 to 4.4±5.3 µg g^{-1}DW with the addition of lime; for cabbages the concentration falls from 5.8+0.7 to 0.44+0.23 µg g^{-1}DW.

The correlation between the total concentration of cadmium in the soil and that in lettuce tissues is statistically significant. The slope of the linear regression equation for these two variables is 0.46, this is very similar to that found

by Chumbley and Unwin (1982), 0.41. This implies that the proportion of the total soil cadmium taken up by lettuces is very similar for these two independent studies. As the data collected by Chumbley and Unwin (1982) were from field situations, the similar uptake efficiency of the two experiments would suggest that field conditions were reproduced in the tub based experiment reported here.

The effect of the residual period was not sufficiently influential to be of statistical significance in these univariate analyses. Neither the organic matter content nor the concentrations of hydrous iron oxides or manganese oxides were significant variables.

Correlations between soil extractants and cadmium uptake.

Three soil extractants were used in order to try to predict the concentrations of cadmium in plant tissues. All of the correlations between 'extractable' and plant cadmium concentrations shown in Table 4 were statistically significant ($p<0.1$). For both of the plants grown, soil extraction with 0.05M $CaCl_2$ proved to be that best correlated with the concentration of cadmium in the plant tissues. This has confirmed the glasshouse based experiments of Alloway (1986) and Morgan and Alloway (1984). For the lettuces EDTA extraction was better correlated with the tissue concentration of cadmium than NH_4NO_3, the reverse was true for cabbages.

Figure 1 shows the relationships between the concentration of cadmium in lettuces and that extracted by 0.05M $CaCl_2$.

The outliers were detected using Dixon's Q-test and are characterised by a 20% lower soil pH and a 27% higher 'total' soil cadmium concentrations, when compared with the main sample. It would appear that the 0.05M $CaCl_2$ extraction procedure is appropriate for soils of pH greater than 5.5, which is likely to apply to the majority of agricultural soils with sludge applications within current guidelines. The regression equation and R-squared value for the main body of the data are given in Figure 1.

The use of the more conventional EDTA soil extraction procedure is far less successful than 0.05M $CaCl_2$. In Figure 2 it can be seen that in some cases the concentration of cadmium extracted is greater from the soil after it has been limed, contradicting the observation of the reduction of plant tissue concentrations with the raising of the pH. This may well be due to the fact that EDTA has been used to dissolve cadmium carbonates as a step in sequential extraction procedures (Lund *et al*, 1985). If the application of lime to a soil increases the proportion of the total soil cadmium content present as the cadmium carbonate species then the use of the EDTA soil test is probably not well suited to soil of high calcium carbonate equivalence. The contrast between the percentage extractions by 0.05M EDTA and 0.05M $CaCl_2$ with pH is shown in Figure 3. The better prediction of lettuce and cabbage tissue concentrations by the 0.05M $CaCl_2$ soil extraction, is probably a function of the low buffering capacity of this extractant.

Multivariate descriptions of cadmium uptake and accumulation.

In this section, cadmium uptake is defined as the concentration of cadmium in the plant tissue, cadmium accumulation is the ratio of the cadmium concentration of the plant to that of the soil (Alloway *et al*, 1990). As stated in the Introduction, a number of key soil variables influence the concentration of cadmium in plant tissues; in order to try to describe the most influential combinations of variables, stepwise multiple linear regression analyses were made. The data had previously been normalised using a $\log_{10}$ transformation. A summary of those variables entered into the equations to describe the uptake and accumulation of cadmium by each of the plants is given in Table 5.

The variables entered into the multiple regression equation for the uptake and accumulation of cadmium by lettuce and cabbage are markedly different, implying that the factors controlling uptake and accumulation differ between species. In a previous glasshouse study similar observations were made for both cabbages and lettuces (Alloway *et al*, 1990). For both uptake and accumulation, the time that has elapsed since the application of sewage sludge is a variable in the equations for cabbages. A factor common to three of the four equations, is the soil pH, emphasising the importance of this variable. Soil pH ($CaCl_2$) always has a negative partial regression coefficient, indicating an inverse relationship with both cadmium

uptake and accumulation. In all but one case, the total concentration of cadmium in the soil was not included in the equations. The cadmium status of the soil was usually more significant when expressed as either the 0.05M $CaCl_2$ or the 0.05M EDTA extractable cadmium concentration.

The long-term changes in the bioavailability of cadmium from soils amended with sewage sludges were not clearly discernible from this experiment. The inclusion of a negative function for the residual time period since the last application of sewage sludge in the multiple regression equations describing the accumulation of cadmium by cabbage, implies a relative decrease in bioavailability over time. However, the data for this residual period is not very accurate and only covers up to twenty years. The data clearly show that the bioavailability of cadmium in sludged soils is influenced by a number of soil variables other than the total concentration of cadmium in the soil. Changes in some of these variables are probable in the long-term. Organic matter, which is shown to be negatively related to cadmium uptake and accumulation by lettuces and has a negative partial regression coefficient, is likely to decrease in the years following sludge application, suggesting that cadmium bioavailability could increase over time.

A wide range of variables influence the bioavailability of cadmium from soils amended with sewage sludges to plants. The concentration of cadmium in the plant is also a function of the particular plant species being grown. In this experiment lettuces were seen to more readily accumulate

cadmium than cabbages. Soil pH is the primary soil parameter and by the manipulation of the soil pH, a change in the bioavailability of cadmium can be observed. The use of soil partial extractants gives some information as to the degree of cadmium bioavailability, however the results of such procedures should be treated with a degree of caution. Extreme levels of contamination and of acidity may lead to poor correlations with the observed bioavailability.

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632 LIST OF FIGURES

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634 extractable cadmium and the concentration of cadmium in
635 lettuce.

636 Figure 2: The concentration of cadmium extracted by 0.05M
637 EDTA-$(Na)_2$ from limed and unlimed pairs of soils.

638 Figure 3: Percentage efficiency of 0.05M EDTA-$(Na)_2$ and
639 0.05M $CaCl_2$ soil extractions as a function of soil pH.

DESCRIPTION	MEAN	MEDIAN	RANGE	REFERENCE
Glasshouse experiments				
• sludge soils	-	-	2.9 - 58.3 DW	
• inorganically contaminated soils	-	-	1.9 - 100 DW	Alloway (1986)
• control soils	-	-	1.1 - 6.6 DW	
MAFF survey				
• sludge soils	0.18 FW	-	0.04 - 0.59 FW	MAFF (1983)
• Shipham, UK	0.68 FW	0.51 FW	0.03 - 2.90 FW	
Field trial on soil of pH > 7	2.25 DW	-	-	Davis and Stark (1980)
Survey of UK urban sites	-	-	0.30 - 2.30 DW	Thornton (1986)
Sludge soils, USA	-	-	0.61 - 2.67 DW	Dowdy and Larson (1975)
Dredged materials, The Netherlands	-	-	1.89 - 24.2 DW	van Driel *et al* (1987)
National survey, The Netherlands	0.05 FW	0.04 FW	0.01 - 0.19 FW	Wiersma *et al* (1986)
National survey, Spain	0.01 FW			Zurera *et al* (1987)
Sludge soils, USA	-	-	3.56 - 10.4 DW	King (1986)
Survey of sludge soils, UK	4.20 DW			Chumbley and Unwin (1982)
Soils contaminated by mine waste	12.8 DW		1.24 - 28.8 DW	Davies and White (1981)
Soils contaminated by atmospheric deposition, Poland	-		5.0 - 14.0 DW	Kabata-Pendias (1984)
Allotment gardens, The Netherlands	0.05 FW	0.05 FW	0.01 - 0.18 FW	van Lune (1987)

NB: All concentrations are in µg Cd g^{-1} for both fresh weight (FW) or dry weight (DW).

Table 1: Typical concentrations of cadmium in lettuces

SOIL VARIABLE	MEAN	MEDIAN	COEFFICIENT OF VARIATION (%)	RANGE
'Total' cadmium ($\mu g g^{-1}$)	18.9	7.6	184	0.5 - 159
pH (H_2O)	6.5	6.7	13	4.3 - 7.8
pH ($CaCl_2$)	5.9	6.2	15	3.9 - 7.3
Organic matter (%)	16.9	11.2	64	4.1 - 39.3
Hydrous iron oxides (%)	2.0	1.7	44	0.6 - 4.2
Manganese oxides ($\mu g g^{-1}$)	294	220	91	8.5 - 1185

Table 2: Descriptive statistics for soil variables

CADMIUM VARIABLE	MEAN	MEDIAN	COEFFICIENT OF VARIATION (%)	RANGE
Lettuce ($\mu g g^{-1}$ DW)	10.57	6.86	83.4	0.35 - 34.09
Cabbage ($\mu g g^{-1}$ DW)	1.77	0.71	158.8	0.03 - 14.68
0.05 M EDTA-$(Na)_2$ ($\mu g g^{-1}$)	14.98	3.92	169.1	0.12 - 108
1M NH_4NO_3 ($\mu g g^{-1}$)	2.08	0.10	354.2	<0.01 - 38.35
0.05 M $CaCl_2$ ($\mu g g^{-1}$)	4.20	0.30	292.2	<0.01 - 67.69

Table 3: Descriptive statistics for the concentrations of cadmium in plant and soil samples

	Lettuce ($\mu g Cd g^{-1}$)	Cabbage ($\mu g Cd g^{-1}$)
Residual period (years)	0.095	0.001
'Total' cadmium ($\mu g\ g^{-1}$)	0.580***	0.542***
$CaCl_2$ extractable Cd ($\mu g\ g^{-1}$)	0.676***	0.832***
NH_4NO_3 extractable Cd ($\mu g\ g^{-1}$)	0.467*	0.721***
EDTA extractable Cd ($\mu g\ g^{-1}$)	0.649***	0.687***
pH (H2O)	-0.510**	-0.433**
pH ($CaCl_2$)	-0.516***	-0.463**
Loss-on-ignition ( )	-0.135	-0.210
Hydrous iron oxides ( )	0.199	-0.076
Manganese oxides ($\mu g\ g^{-1}$)	-0.018	0.001
Cabbage Cd ($\mu g\ g^{-1}$)	0.700***	---

All data had been previously normalised by a $\log_{10}$ transformation

p<0 001, p<0 01 and p< 0 1 indicated by ***, ** and * respectively.

Table 4: Pearsons product moment correlation coefficients for cadmium concentrations in lettuces and cabbages.

Dependent variable	Independent variables (s)	p-value
Lettuce uptake ($\mu g\ Cd\ g^{-1}$)	(-)Loss-on-ignition (%), (+)0.05M EDTA extractable Cd ($\mu g\ Cd\ g^{-1}$), (-)pH ($CaCl_2$)	0.001
Lettuce accumulation	(-)Loss-on-ignition (%), (-)pH ($CaCl_2$), (-)'total' Cd ($\mu g\ Cd\ g^{-1}$)	0.001
Cabbage uptake ($\mu g\ Cd\ g^{-1}$)	(-)Time after application of sludge (years), (+)0.05M $CaCl_2$ extractable Cd ($\mu g\ Cd\ g^{-1}$)	0.001
Cabbage accumulation	(-)Time after application of sewage sludge (years), (+)0.05M $CaCl_2$ extractable Cd ($\mu g\ Cd\ g^{-1}$), (-)pH ($CaCl_2$), (-)manganese oxides ($\mu g\ g^{-1}$)	0 001

Positive and negative values of the partial regression coefficients are indicated by (+) and (-) respectively

Table 5: Summary of multiple regression analyses.

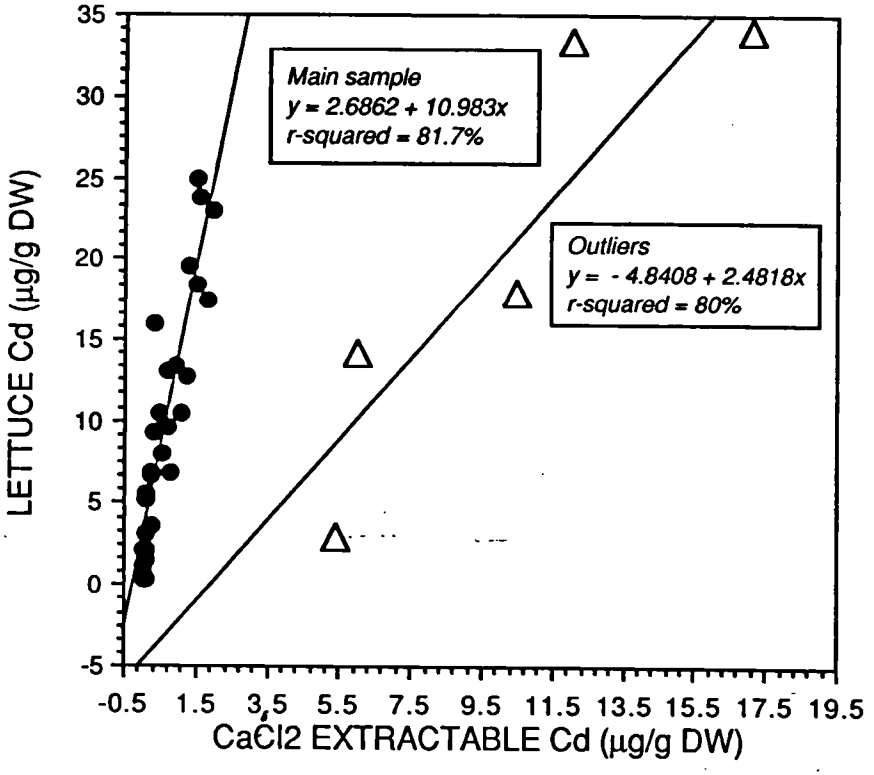
Main sample
y = 2.6862 + 10.983x
r-squared = 81.7%
Outliers
y = - 4.8408 + 2.4818x
r-squared = 80%
LETTUCE Cd (µg/g DW)
CaCl2 EXTRACTABLE Cd (µg/g DW)
35
30
25
20
15
10
5
0
-5
-0.5
1.5
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
19.5

Fig. 1

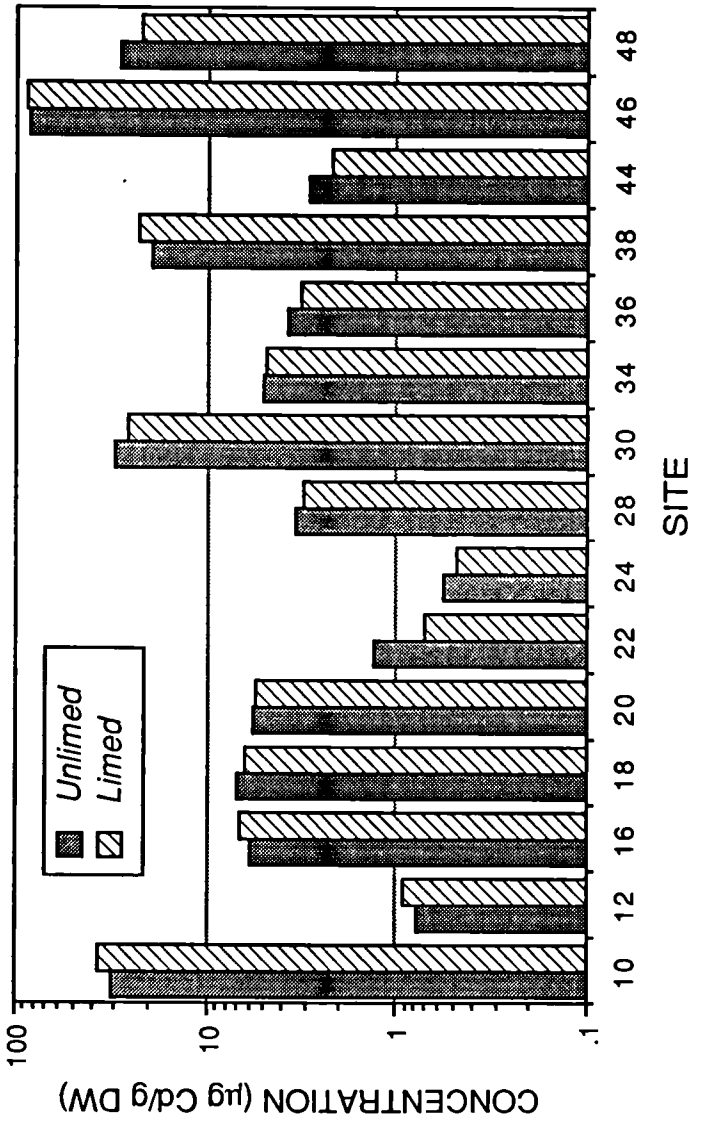
Unlimed
Limed
CONCENTRATION (µg Cd/g DW)
100
10
1
.1
SITE
10
12
16
18
20
22
24
28
30
34
36
38
44
46
48

Fig. 2

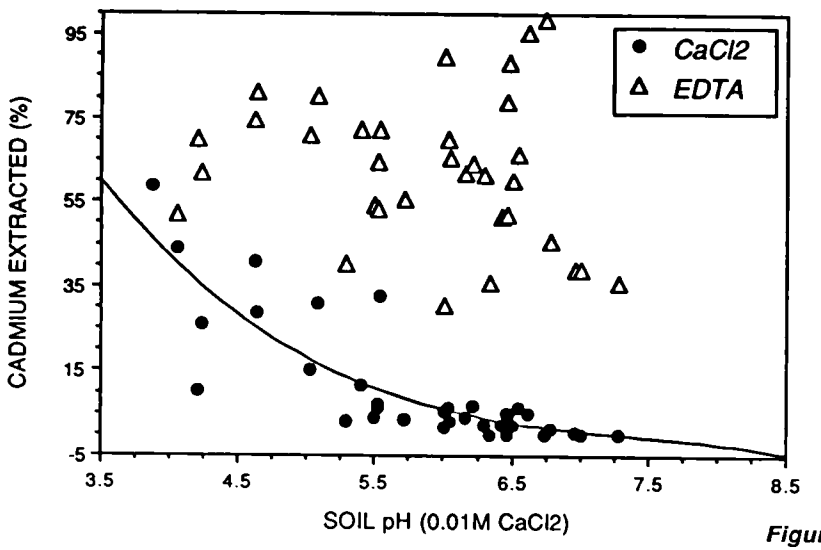
CaCl2
EDTA
CADMIUM EXTRACTED (%)
95
75
55
35
15
-5
3.5
4.5
5.5
6.5
7.5
8.5
SOIL pH (0.01M CaCl2)

Figure 3

In press *International Journal of Environmental Analytical Chemistry*

DETERMINATION OF CADMIUM IN PLANT TISSUES BY ELECTROTHERMAL ATOMISATION ATOMIC ABSORPTION SPECTROMETRY WITH MATRIX/ANALYTE MODIFICATION AND SMITH-HIEFTJE BACKGROUND CORRECTION.

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Paper submitted to the International Journal of Environmental Analytical Chemistry, March 1990.

Key Words

Cadmium, delayed atomisation cuvettes, microwave dissolution, matrix/analyte modifiers.

Abstract

Pressure decomposition in a microwave oven provides a rapid means of sample preparation for plant tissue analyses. The use of delayed atomisation cuvettes, Smith-Hieftje background correction and matrix/analyte modification enables accurate determinations of cadmium concentrations in plant materials to be made. However, care should be taken to restrict the concentrations of modifier used, as too high a concentration may lead to problems with both tube life and over-correction by the Smith-Hieftje background correction system.

Introduction

The aim of this study was to develop an accurate method for the determination of cadmium in plant samples from a range of contaminated soils. Matrix/analyte modification was evaluated in such a way as to optimise both tube life and analytical accuracy.

The routine use of electrothermal atomisation atomic absorption spectrometry (ETA-AAS) for the analysis of low levels of metals in a variety of environmental media is now an accepted part of monitoring and research procedures. However, with the increased sensitivity that ETA-AAS offers come a variety of problems, not the least of which are the interferences in the atomiser during signal measurement 1, 2. This paper will concentrate upon the analysis of plant materials for cadmium and will consider a specific problem inherent in the use of some matrix/analyte modifiers

The primary problem with cadmium is its low appearance temperature in the furnace (~580^{o}C), this means that the removal of matrix constituents during the ashing or char cycle is difficult without the associated loss of the analyte. Many papers have been published which deal specifically with the matrix effect problem and a wide variety of solutions are presented 3, 4, 5.

Furnace interferences are often divided into two groups: (i) *vapour-phase interferences* predominantly created by non-isothermal conditions in the furnace tube and (ii) *solid-phase interferences* a somewhat nebulous term which usually refers to the physical aspects of the volatilisation of the analyte itself. It should be noted that isothermality within the furnace tube has two components, (i) temporal and (ii) spatial 6. Much attention has been paid to the former, but less to the latter. A very wide variety of techniques have been developed to deal with vapour-phase interferences. These mainly involve the creation of an isothermal environment in the tube prior to the samples atomisation. Developments include the L'vov or platform furnace 7, delayed atomisation cuvettes 8, the graphite probe 9, the integrated contact furnace 10 and attempting to increase the thermal stability of the analyte.

With the exception of the standard additions procedure, the most commonly used technique is the use of matrix/analyte modifiers which are added to the sample in order to alter its thermal stability. In this paper an analyte modifier is considered to be any substance which alters the thermal stability of the analyte, this may either be an increase or decrease in stability; a matrix modifier is any substance which alters the thermal stability of the matrix. In most cases an analyte modifier will tend to be chosen in order to increase the stability of the analyte, however

there are some exceptions to this 11. The most widely used analyte modifier for the determination of cadmium is diammonium hydrogen or ammonium dihydrogen phosphate, which allows ashing temperatures up to ~700^{o}C to be employed without the loss of the analyte 12,4,13. The addition of magnesium nitrate to the sample as a matrix modifier helps to raise the maximum ashing temperature to ~1000^{o}C, an alternative to magnesium nitrate is the addition of sodium chloride 4 which enables a similar maximum to be attained. The addition of either of these two matrix modifiers to the analyte modifier is thought to create oxidising conditions in the tube and so remove the organic component of the sample matrix during the ashing phase. There also exists the possibility that the delay in the atomisation of the sample may be due to occlusion of the analyte in the matrix modifier. The major advantage of the use of these two analyte modifiers is that they allow a delay in the atomisation of the analyte and so increase the chances of isothermal furnace conditions existing at this crucial stage of the analytical cycle. Although not as widely used as diammonium hydrogen phosphate, the use of palladium nitrate in combination with magnesium nitrate is becoming widely used for a variety of elements in a number of sample matrices 14, 5.

Experimental

Instrumentation: Analyses were made on an Instrumentation Laboratory (IL) S-12 spectrometer in the single beam mode of operation, using Smith-Hieftje background correction. The instrument was equipped with an IL 655 Controlled Temperature Furnace (CTF) atomiser, IL 254 auto-sampler and a FASTAC for aerosol sample deposition. Pyrolytically coated delayed atomisation cuvettes (DAC) were used throughout the experiment (Allied Analytical Systems and Ringsdorff). Visimax II hollow cathode lamps were used as the radiation source. A Linear dual-channel integrating chart recorder was used to record peak shapes. BOC high purity argon was used as a purge gas.

Reagents: The acid used in sample digestion and in the make-up of the standards was AristaR grade nitric acid, standards being made by the sequential dilution of Spectrosol

cadmium standard solution (BDH Chemicals, Poole, Dorset, UK). All water used in the analysis was initially glass distilled and then de-ionised using an Ionmiser 6C system.

Diammonium hydrogen phosphate modifier solution (2% m/V) was prepared from Pro analysi grade reagent (Merck) and purified by passing it through a column of H^+ substituted (10% AnalaR nitric acid) Chelex-100 cation-exchange resin (Bio-Rad Laboratories Ltd., Watford, UK) at a flow rate of1ml $minute^{-1}$. The magnesium nitrate modifier solution (2% m/V) was prepared from AristaR grade reagent, no further purification was found to be necessary. The modifiers were added to sample and standard solutions to give 0.2% (m/V) diammonium hydrogen phosphate and 0.02% (m/V) magnesium nitrate in the solutions to be analysed.

Sample preparation: All plant samples were taken from the field and thoroughly washed in distilled water to remove any soil. The sample was then shredded and left to dry at 65°C in a forced draught oven, until no further change in weight was observed. The dried material was finely ground in a Fritsch Pulverisette centrifugal ball mill; in order to minimise contamination, acid-washed polyamide pots and agate balls were used for this process. Samples were strored in acid-washed glass jars prior to analysis; the moisture content of the samples after storage was determined by the method described below.

Determination of moisture content of prepared samples: The moisture content of a sample was determined after drying the sample at 85°C for 2 hours and recording the change in weight. The sample aliquots used for these determinations were not digested for ETA-AAS analysis.

Sample digestion: Samples (0.050±0.001 g) were weighed into the PTFE liner of a Parr microwave acid digestion bomb (Scientific and Medical Products Ltd) using a Sartorious five-figure balance; the liner had previously been cleaned by the following process: ultrasonicated for 10 minutes in a 10% solution of Lipsol, rinsed with distilled water, soaked in 5% Lipsol, rinsed with distilled water, soaked in 5% AnalaR nitric acid and then rinsed in distilled de-ionised water. AristaR nitric acid (3.00 ml) was then added to each sample and the liner was then placed in the bomb. The bomb was then placed into a domestic microwave oven

(Solavox T-2, 980 W) and heated at full power for 45 seconds; this was sufficient to give a clear solution. Although discharges of fumes from the bomb are only occasional and slight, the inside of the oven was coated with a PTFE spray to mitigate their action. All samples were prepared in quadruplicate, two of the four samples were spiked with the quantity of cadmium necessary to double the concentration. Reagent blanks were carried through the entire procedure.

A refrigerated cooling period of 25 minutes was then required before the bomb could be opened and the liner removed. Any acid which had condensed on the lid of the liner was then rinsed back into the liner with distilled de-ionised water. The liner, with the top removed, was then placed on a Techne dri-block and heated for ~90 minutes at 120°C in order to reduce the volume of the sample to ~0.25 ml. The remaining solution was taken up in 5 ml of 4% (V/V) solution of AristaR nitric acid and then made up to 10 ml in a volumetric flask with distilled de-ionised water. Samples were usually stored in Sterilin bottles (30 ml) and not in the volumetric flask to avoid any adsorption of analyte onto the glass walls.

Instrumental parameters: Analyses of the samples were made against standards of the following concentrations, 0.50, 1.00, 1.50, 2.00 and 2.50 ng Cd ml^{-1}. Each standard solution contained 2% (V/V) AristaR nitric acid, 0.2% (m/V) Merck diammonium hydrogen phosphate and 0.02% (m/V) AristaR magnesium nitrate.

Wavelength=228.80 nm Bandwidth=1.00 nm
Lamp current=3.20 mA Background current=0.45 mA
PMT voltage= 700 mV
Smith-Hieftje background correction.

Sample deposition: Delay time= 7 seconds Deposit time= 20 seconds
Three repeats per sample.

Measurement: Peak height - 2.00 seconds integration time.
Peak area - 5.00 second integration time.
Peak shape - Linear dual-channel integrating chart recorder.

High purity argon (BOC) used as the purge gas.

Table 1: Spectrometer and sample deposition parameters.

Step no.	Step	Temperature (°C)	Ramp time (s)	Hold time (s)
1	Injection/dry	135	-	5
2	Ash	650	15	5
3	Atomise	1550	5	5
4	Clean*	2500	Step	5

NB: * Clean cycle not always necessary.

Table 2: Time/temperature programme for CTF atomiser.

Results and discussion

The complex nature of the ashing curves shown in Figures 1, 2 and 3, for the modified standards are not easily explained but it may be associated with reactions between the graphite tube wall and the modified analyte. Similar effects have been observed on molybdenum ashing curves when the sample was atomised from a pyrolytic platform or from the wall of a pyrolytically coated tube 15. Another interaction between the tube material and the sample has been reported for lead analysis 16. The matrix /analyte modifiers have clearly had an effect upon the analyte because ashing of the acidified standard does not show these marked changes with ashing temperature. However, Figures 1, 2 and 3 do show the significant benefit of the use of chemical modifiers in increasing the appearance temperature, in most cases to ~800°C.

Atomisation studies showed the typical influence of atomisation temperature upon peak height and area; as expected the peak height increases with temperature and then remains constant once a maximum has been reached. Peak area responds similarly but begins to decline at temperatures in excess of 1500°C, this is due to the accelerated decay of the signal created by rapid diffusion of the atomised sample from the atomiser 16, 17.

The influence of matrix modifiers upon the atomisation of a sample can be seen in Figure 4, which shows the peak shapes produced by the atomisation of a) acidified and b) modified standards and samples. The acidified sample was ashed at 300°C for 30 seconds and then atomised at 1100°C. For both acidifed and modified solutions, the instrument was calibrated with five aqueous standards and the sample (a digest of NBS1573 tomato leaves) was analysed against the resultant calibration. As can be seen from Figure 4a the atomisation characteristics of sample and standard differ considerably in the absence of the modifiers, with the sample producing a two phase atomisation. This is thought to represent the presence of two cadmium species of differing thermal stabilities within the sample. The results of the analysis of NBS 1573 against a calibration of acidified standards can be seen in Table 3. Although the quoted value for the sample used is not fully certified it does represent the mean of several independent analyses. The atomisation of matrix/analyte modified samples and standards (Figure 3b) show marked differences from those of the acidified solutions. The forms of the peaks are very similar and more closely resemble those used in some theoretical models 16. The analysis of matrix/analyte modified samples against aqueous standards can be seen to be a viable strategy; this is borne out by the data presented in Tables 3 and 4.

Concentration of cadmium in NBS 1573 (µg g-1 dwt)		
	Peak area (A s)	Peak height (A)
ACIDIFIED	2.80±0.25 (10)	3.40±0.50 (10)
MODIFIED	3.05+0.24 (10)	3.08±0.41 (10)

Number of determinations shown in parentheses.

Table 3: Analysis of a reference material, NBS 1573 tomato leaves; reference value = 3.00 µg Cd g^{-1} dwt.

The final method using matrix/analyte modifiers was employed to analyse a variety of reference materials which had certified values for cadmium; summations of the results are shown in Table 4.

Sample	Certified (µgg-1 DW)	Observed (µgg-1 DW)	Precision (%)	Accuracy (%)
BCR60 (*Lagarosiphon major*)	2.20±0.10	2.23±0.27	12.23	+1.36
BCR62 (*Olea europaea*)	0.10±0.02	0.097±0.004	4.20	-3.00
NBS1567 (Wheat flour)	0.032±0.007	0.032±0.005	15.60	0
NIES1 (Pepperbush)	6.70±0.50	7.17±0.13	1.80	+7.01

Table 4: Analysis of four reference materials for cadmium.

Each of the materials listed in Table 4 has been analysed in quadruplicate in three batches. Precision is assesed by the relative standard deviation between batches and is expressed as a percentage. Accuracy is calculated as the percentage difference between the certified and the observed value for a given material. The method was also used to analyse a large number of plant samples grown on soils contaminated with heavy metals due to the application of sewage sludges. The mean recovery of a spike sufficient to double the concentration of cadmium in the sample, was 96.4±14.3% (n=107). In addition to the analysis of the certified

reference materials, another check upon the precision of the analysis was made by the repeated analysis of an inhouse quality control material supplied by the Food Science Division of the Ministry of Agriculture Fisheries and Food. This material had been analysed by ETA-AAS and found to give a concentration of 4.43 µg Cd g^{-1} DW, the method described above gave a value of 4.48±0.31 µg Cd g^{-1} DW (n=44).

At an earlier stage of the development of the method described in the experimental section, a wide range of time/temperature parameters and concentrations of modifiers were used. Figures 1, 2 and 3 show the effects of varying the concentrations of both analyte and matrix modifiers on ashing curves when peak areas were recorded. Initially concentrations of 1%(m/V) diammonium hydrogen phosphate and 0.2%(m/V) magnesium nitrate were used and found to give good short-term accuracy (≤200 firings) and precision for a reference material (BCR 62). However, an accelerating decline in peak area absorbance values was observed, implying that the pyrocoating was being rapidly degraded, see Figure 5. In addition to this problem, the appearance of over-correction by the Smith-Hieftje background correction system was observed and seen to worsen with the number of firings. Although this problem could be eliminated by the inclusion of a high temperature clean cycle, this was considered to be undesirable if tube life was to be maximised and cycle times kept to the minimum. An order of magnitude reduction in the concentration of magnesium nitrate gave a more satisfactory performance, enabling both sensitivity and precision to be sustained over 400 firings. In order to more closely examine the problems of declining sensitivity and over-correction, a DAC tube was bisected along its longitudinal axis and analysed by scanning electron microscopy (SEM). A Hitachi S-450 SEM fitted with a Link Analytical AN 10000 microanalysis system was used to make both a visual inspection and an energy dispersive x-ray chemical analysis. As can be seen in Plate 1, the oxidising conditions at the site from which the sample is atomised have completely removed the pyrocoating, leaving behind a porous and potentially reactive surface from which the subsequent samples have to be atomised. An area 12mm from that in Plate 1 can be seen in Plate 2. At this point damage to the pyrocoating, such as that seen at the sample deposition area, is absent; however there has

been an accumulation of material on the surface. This material was analysed using the microanalysis system and found to be magnesium nitrate. At 13mm from the area shown in Plate 1 the deposition of spheres of magnesium phosphate can be seen (Plate 3). This pattern may well be due to non isothermal conditions in the tube after the atomisation of the sample, leading to the differential condensation of components of the sample modification compounds. As the tube cools down to the sample injection temperature, the areas adjacent to the thicker tube walls at the centre of the tube can be expected to loose heat more slowly and so lead to the spatial and temporal gradient which has caused the effects seen in Plates 1 to 3. The rapid losses in sensitivity are due mainly to the degradation of the pyrocoating at the sample deposition area, the loss of accurate background correction is probably due to the accumulation of magnesium phosphate 13mm from the centre of the tube. The subsequent atomisation from these areas of secondary deposition will cause an increase in the background signal, eventually leading to the manifestation of over correction. As a relatively low atomisation temperature was considered to be a desirable feature and the use of a clean cycle was thought to impair tube life, it was decided to use lower concentrations of both analyte and matrix modifier. The details of the developed method are in the experimental section.

The use of pressure decomposition with microwave heating has been shown to be a viable means for the preparation of plant materials for cadmium analysis by ETA-AAS. The use of delayed atomisation cuvettes, matrix/analyte modifiers and Smith-Hieftje background correction enabled accurate results to be obtained for four certified reference materials.

Acknowledgements

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LIST OF FIGURES

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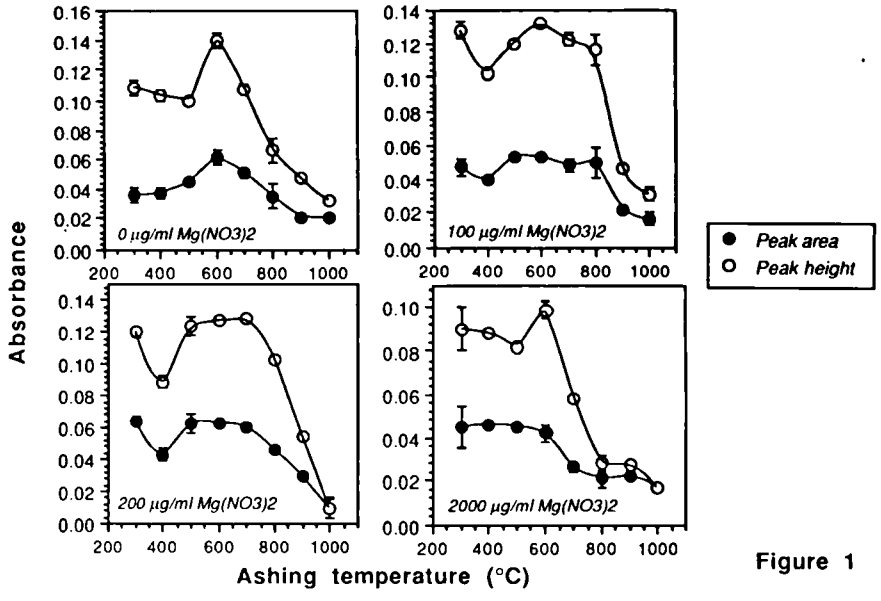
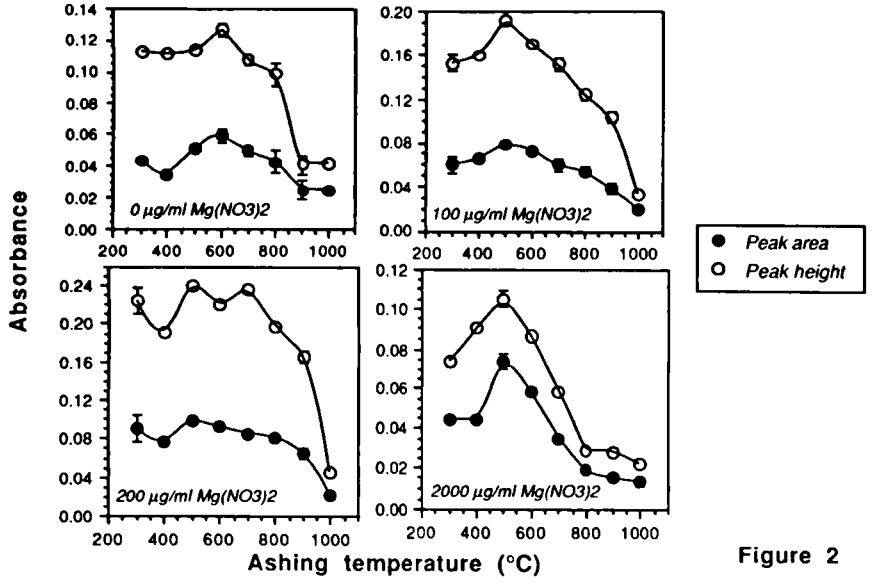


Figure 1



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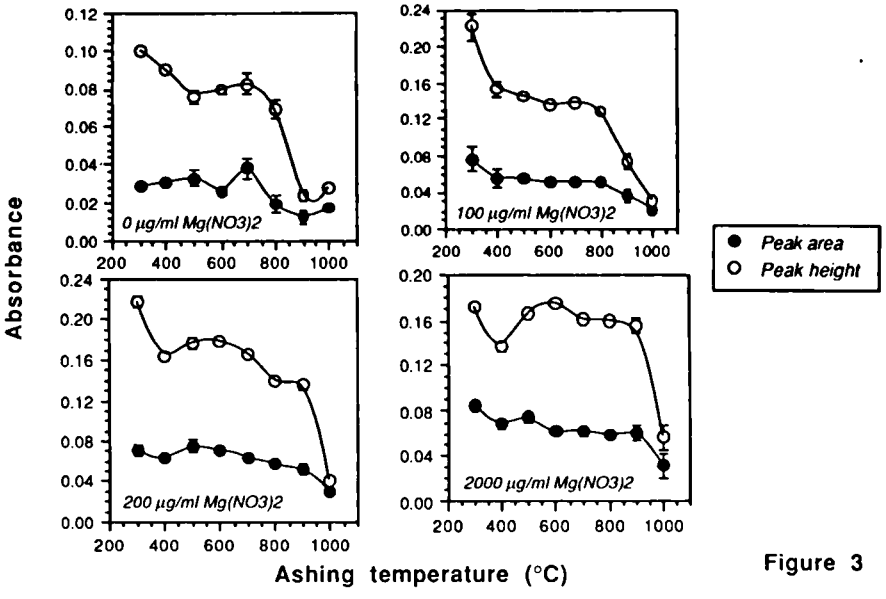

Figure 3

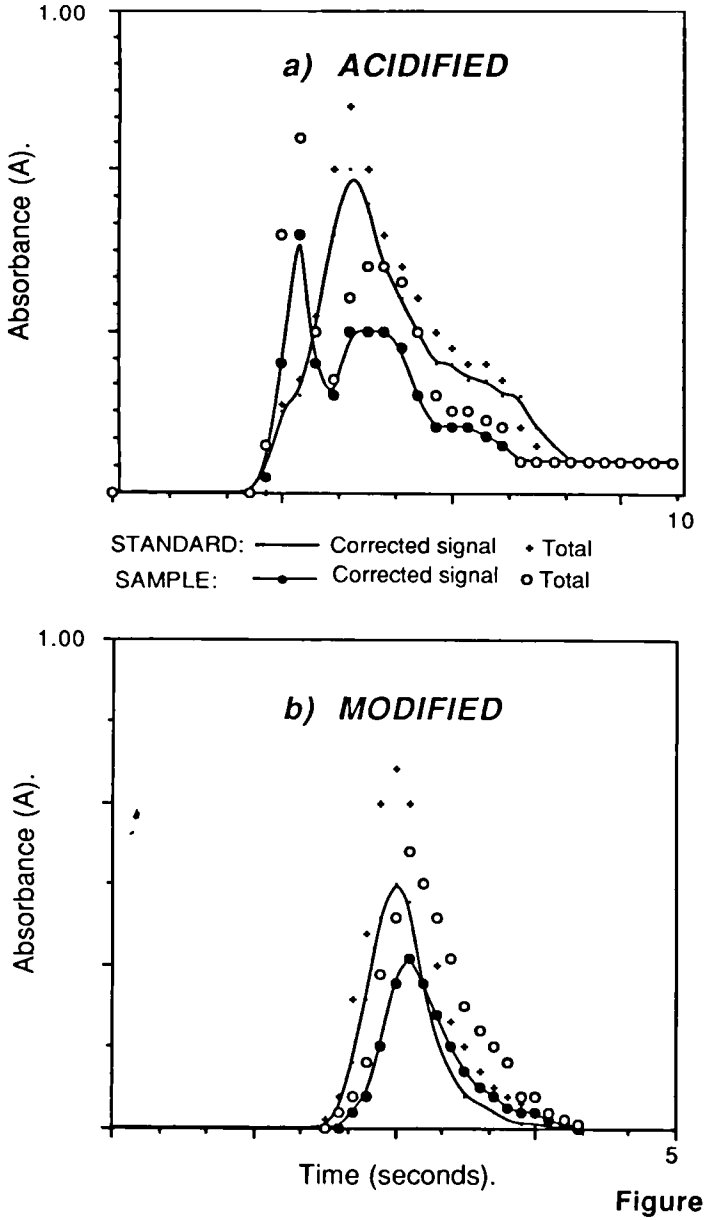

Figure 4

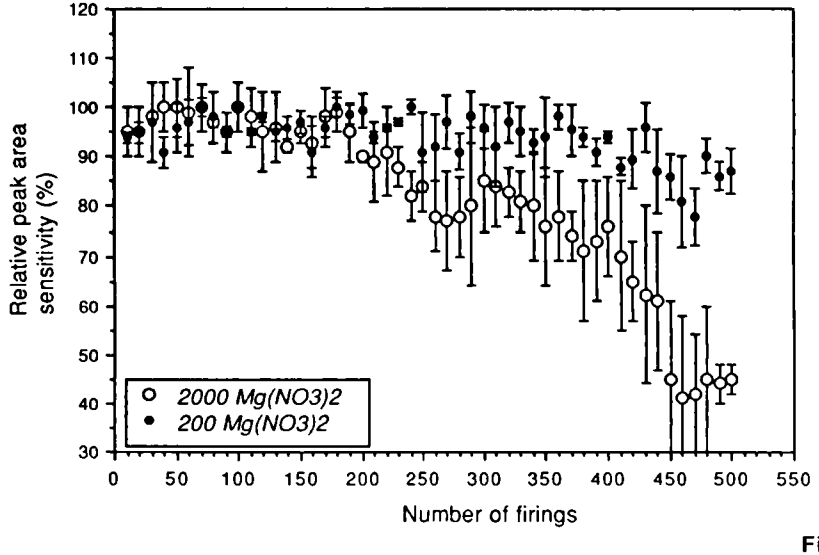

Figure 5

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THE ACCUMULATION OF CADMIUM BY VEGETABLES GROWN ON SOILS CONTAMINATED FROM A VARIETY OF SOURCES

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ABSTRACT

The accumulation of cadmium by four crops (cabbage, carrot, lettuce and radish) grown on soils contaminated from a variety of sources was investigated in greenhouse pot experiments. Stepwise multiple regression analyses of the data revealed that, out of the 23 soil variables determined, only eight were significantly related to cadmium accumulation in the edible plant tissues. The most frequently occurring soil parameter was total cadmium, which was inversely related to plant cadmium accumulation ($Cd_{Plant\ tissue}/Cd_{Soil}$). This implies that, for the heterogeneous group of soils used, as the concentration of cadmium in the soil increases the proportion available to the plant decreases. This may be due to the presence of metallic ore particles and/or the high sorptive capacity of the most contaminated soils. When the data were divided into two groups: sewage sludge amended soils and inorganically contaminated soils, the *R*-squared values were usually enhanced and some differences occurred in the variables included in the multiple regression equations; this may be indicative of the differences in speciation. Cadmium accumulation by plants grown on sewage sludge amended soils was lower than that for the inorganically contaminated soils. The heterogeneity of the inorganically contaminated group of soils resulted in lower *R*-squared values for the multiple regression equations; this group of soils exhibited a wide range of soil variables, such as pH, and had been contaminated by a variety of means, such as atmospheric deposition from metal smelters and the dumping of mine wastes.

INTRODUCTION

Elevated concentrations of cadmium in the human diet constitute a potential hazard to health in the long term. Cadmium accumulates in the kidneys and harmful effects are likely to occur when the concentration in the renal cortex exceeds 200 $\mu g\ g^{-1}$ fresh wt (Fassett, 1980). It is therefore essential that the cadmium content of foods is kept as low as possible. Cadmium is highly labile in the soil–plant system; its relative availability to plants is much greater than that of other potentially harmful heavy metal contaminants, such as lead (Alloway and Morgan, 1986; Wiersma et al., 1986).

Vegetable and salad crops are widely grown in domestic gardens and allotments as well as under commercial horticultural/agricultural conditions. The metal content of garden-type vegetables is important because some species, such as lettuces, accumulate relatively high concentrations of cadmium and

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other metals, although intraspecific variations may be considerable (Peterson and Alloway, 1979; Davis and Carlton-Smith, 1980). In general, leafy vegetables tend to accumulate higher concentrations of cadmium than root, grain or fruit crops.

Although vegetables comprise a much smaller proportion of the diet than cereals and potatoes (Sherlock and Walters, 1983), vegetables grown in domestic gardens and allotments can make a significant contribution to the cadmium intake of individuals and families. Preservation by deep freezing enables some families to be almost self-sufficient in vegetables, thus making the composition of the garden soil an even more important factor in their cadmium intake profile. Gardens and allotments may be exposed to a higher degree of environmental contamination than most agricultural land. The sources of low-level cadmium contamination of agricultural and horticultural soils are usually phosphatic fertilizers, atmospheric deposition from industrial sources and sewage sludge (Hutton and Symon, 1987). Domestic gardens and allotments may, in addition, be contaminated by atmospheric deposition from roads and nearby industrial sources, ash from burnt rubbish and pollution of the site prior to its use as a garden. For example, in the Somerset village of Shipham (U.K.), some houses had been built on old spoil heaps from lead/zinc mining and had up to 360 μg Cd g^{-1} in the garden soils (Morgan and Sims, 1988).

This paper reports the results of a study of the uptake of cadmium by four vegetable crops (cabbages, carrots, lettuces and radishes) grown in a greenhouse on soils contaminated with cadmium from a variety of sources. The edible portions of the crops were harvested, washed and analyzed. The results of the analyses were used in empirical models together with 18 soil physico-chemical parameters to predict the accumulation of cadmium.

All the models presented in this paper may be said to be equilibrium models, with the implicit assumption that temporal fluctuations in rates of reaction and transfer do not occur (Jackson and Smith, 1987). Modelling the behaviour of metals in the soil–plant system is an important procedure if the potential hazard of growing food crops on contaminated soils is to be assessed. The first step is to quantify the accumulation of heavy metals in the plant and to identify the parameters which enable a prediction of this characteristic to be made. A model developed by Browne et al. (1984), based upon soil parameters, describes, for a number of species, the uptake of cadmium:

$$\log P = \partial + \beta \log Cd_{DTPA}$$

where P is the plant cadmium concentration, Cd_{DTPA} is the DTPA extractable cadmium, and ∂ and β are linear regression coefficients. β was found to be principally a function of soil pH and cation exchange capacity, and ∂ was primarily a function of the plant species and may be related to the selectivity coefficient (Poelstra et al., 1979).

A cadmium and zinc accumulation model based upon a nutrient uptake model, soil characteristics and kinetic parameters has been developed by Mullins et al. (1986) to describe plant uptake from sewage sludge amended soils.

Root growth constants, average root radius, water influx rate and soil solution metal concentrations were found to be the most influential parameters in the model. A model proposed by Hutton (1980) to describe the transfer of cadmium from soils to plants is:

$$P_{SP} = \frac{(\beta S \Omega \phi)}{(\phi + \Omega \infty)}$$

where β is the water flow associated with plant production; S the plant selectivity coefficient (Poelstra et al., 1979); Ω the soil adsorption coefficient (Jarvis and Jones, 1980); ϕ the soil density; ∞ the soil moisture content; and P_{SP} the soil plant transfer coefficient.

A conceptual model to determine cadmium uptake from sewage sludge amended soils has been developed by Christensen and Tjell (1984). It divides the total plant cadmium concentration into three fractions, based upon their source: (i) cadmium from the topsoil, (ii) cadmium from the subsoil, and (iii) cadmium from the atmosphere. Plant uptake from the topsoil is described by the following expression:

$$b = P \cdot T_t \cdot C_t = P \cdot T_t \cdot (S_t, K_{d,t})$$

Where b is the root uptake from the topsoil, P the plant factor (constant for a specific plant), T the transpired amount of water, C the solute cadmium concentration, S the soil cadmium concentration, t the index for topsoil, and K_d the cadmium distribution coefficient. Plant uptake from the subsoil is described by:

$$c = P \cdot T_s \cdot \frac{(S_s)}{(K_{d,s})}$$

Where c is the root uptake of cadmium from the subsoil, and s the index for subsoil. Foliar uptake of atmospheric cadmium can be considered to be a constant for a given plant species in a given area. The solute cadmium concentration, S, is said to be governed by two main processes, adsorption onto the solid phase and precipitation. This model was demonstrated to describe the behaviour of cadmium in sewage sludge amended soils, but the tested data was restricted in size.

A wide variety of physico-chemical soil parameters have been found to be related to the uptake of cadmium from soils; these include pH (Andersson and Nilsson, 1974; Street et al., 1978; Tyler and McBride, 1982; Gerriste and van Driel, 1984; Sanders et al., 1986), cation exchange capacity (Korcak and Fanning, 1985), organic matter content (Strickland et al., 1979; Gerriste and van Driel, 1984; Neal and Sposito, 1986), soil temperature (Siriratpuriya et al., 1985), soil sorptive capacity (Jarvis and Jones, 1980), concentrations of particular organic acids (Tyler and McBride, 1982), antagonistic, synergistic or additive reactions between elements (Elliot et al., 1986) and many more (McKenzie, 1980; Bingham et al., 1983; Kuo, 1986; Chammugathas and Bollag, 1987a, b). All have been shown to exhibit a significant relationship with the plant uptake of a particular element from a specific soil or soil type, but few

have been shown to be important for a broad spectrum of soils contaminated from a variety of sources. This paper describes the empirical models developed from the data for four crops grown on a diverse range of soils contaminated from several types of sources.

SAMPLES, MATERIALS AND METHODS

Bulk samples of topsoils (usually 0–15 cm) were collected from a wide range of polluted and control sites in England and Wales with additional samples from France, Japan and Norway. A set of 48 soils was used, comprising: 20 samples from sites which had received heavy applications of sewage sludge; nine from industrially polluted sites; a group comprising four soils polluted by lead–zinc mining; two soils naturally (geochemically) enriched in lead from Norway; a soil developed on a metal-rich marine black shale; seven from urban domestic gardens and five control soils.

In addition to these polluted soils, four uncontaminated soils differing markedly in physico-chemical properties were spiked with metals. An acid upland soil, a calcareous soil (rendzina), a calcareous clay loam and a peat were spread out in thin layers and sprayed with nitrate salts of metals. These spiked soils were placed in large plant pots and left to equilibrate in a greenhouse with occasional watering for a minimum of 6 months.

The soils collected in the field were mixed thoroughly and 3 kg was placed in polyethylene plant pots for greenhouse experiments. An additional 1 kg was placed in a small polyethylene plant pot and kept in a "fresh" condition in a greenhouse with occasional watering for the extraction of the soil solution. About 500 g of soil was air-dried, lightly ground and passed through a 2 mm nylon seive. This < 2 mm fraction was used for the determination of all soil chemical and physical parameters.

Samples of the soil solution were obtained by centrifugation of soil, which had been equilibrated at field capacity moisture status, at 3500 rpm for 30 min. The centrifuged extract was then passed through a 0.45 μm membrane filter before analysis by electrothermal atomization atomic absorption spectrometry (ETA–AAS) using background correction.

Total metal concentrations in the soils were extracted by refluxing the sample with concentrated AnalaR grade nitric acid, drying and taking up the soluble fraction in dilute AnalaR grade hydrochloric acid. These solutions together with those obtained by the other soil extractants (EDTA, DTPA, etc.) were analyzed by flame atomic absorption spectrometry (FAAS) using background correction.

The easily reducible iron and hydrous manganese oxide concentrations of the soils were determined after extraction with reducing agents. Sodium dithionate was used for iron (Avery and Bascomb, 1974) and hydroxylamine hydrochloride for manganese (Chao, 1972). The iron and manganese concentrations in the filtered extracts were then determined by FAAS.

The soil variables measured included: pH in distilled water and 0.01 M calcium chloride (1:2.5 w/v), organic matter content by percentage loss in

weight after ignition at 450°C, calcium carbonate equivalence (Avery and Bascomb, 1974) and cation exchange capacity (Hesse, 1971).

The vegetables grown comprised: cabbages (cv Winter Monarch), carrots (cv James' Scarlet Intermediate), lettuce (cv Webbs Wonder) and radish (cv French Breakfast). These were grown consecutively and all received a small application of NPK fertilizer. The plants were harvested when most of the crop had reached an appropriate stage of maturity. However, some plants growing on the most heavily polluted soils were stunted and/or showed marked symptoms of toxicity on the leaves. After harvest, the edible parts of all plants were thoroughly scrubbed and washed in distilled water to remove all traces of soil, oven-dried at 105°C and milled ready for analysis.

The vegetable samples were refluxed in concentrated AnalaR grade nitric acid, taken to dryness and the residue redissolved in dilute AnalaR grade hydrochloric acid. This solution was then filtered and made up to a known volume in distilled deionized water. Metal concentrations in the final solutions were determined by FAAS for the relatively high concentrations and by ETA–AAS for the lower concentrations. As with the soil analysis, Smith–Hiefjte background correction was used in all these determinations.

DATA ANALYSIS

The data were initially analyzed by univariate statistical methods such as correlation and linear regression analysis. Table 1 lists the variables studied. In order to investigate the data set more rigorously, some of these primary variables, such as the concentrations of cadmium extracted by the various reagents, were expressed as percentages of the total metal concentration in the soil.

Plant accumulation of cadmium and lead was expressed as a percentage of the total soil concentration, referred to as the 'accumulation ratio' or transfer coefficient, and as the total mass of metal accumulated. Many of the data were expressed as logarithms to the base ten in order to improve the linearity of significant relationships and to normalize the frequency distributions.

The data set was divided into two subsets on the basis of the sources of the metal contamination, as shown in Fig. 1.

The data from each of these two subsets were then combined in turn with data for control soils and analyzed by the univariate statistical techniques stated above. However, these analyses were found to be statistically insignificant and so stepwise multiple regression analysis was used to develop multivariate models.

RESULTS AND DISCUSSION

The data for the 23 soil variables listed in Table 1 were tested against the values for the cadmium concentrations in the edible portions of the four vegetables by stepwise multiple regression analysis. It was found that only eight of these variables were significantly correlated with crop cadmium con-

TABLE 1

Soil and plant physico-chemical variables studied

pH (H_2O)
pH ($CaCl_2$)
Soil solution pH
Organic matter content (percentage loss on ignition)
Cation exchange capacity
Percentage calcium carbonate equivalence
Percentage sand, silt and clay
Total Cd
Total Pb
Total Zn
Total Ni
Total Cu
Soil solution Cd
Soil solution Pb
EDTA extractable Cd and Pb
DTPA extractable Cd and Pb
NH_4OAc extractable Cd and Pb
HAc extractable Cd and Pb
HCl extractable Cd and Pb
NH_4OAc + HAc extractable Cd and Pb
NH_4NO_3 extractable Cd and Pb
NH_4OAc + EDTA extractable Cd and Pb
$CaCl_2$ extractable Cd
Cabbage Cd and Pb concentration
Carrot Cd and Pb concentration
Radish Cd and Pb concentration
Lettuce Cd and Pb concentration

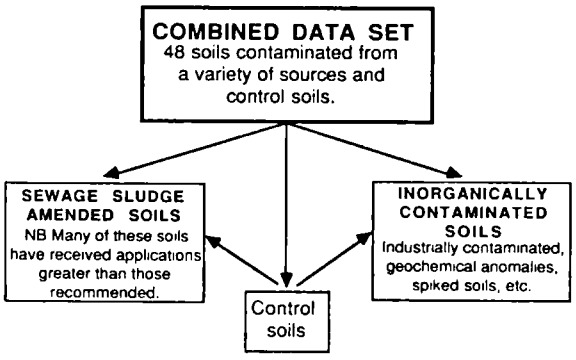

Fig. 1. Grouping scheme for statistical analyses.

centrations. The descriptive statistics for these soil parameters and for the cadmium contents of the cabbage, carrot, lettuce and radish samples are shown in Table 2 and Fig. 2.

The values for the coefficients of variation shown in Table 2 indicate that the

TABLE 2

Descriptive statistics for selected variables from the combined data set

Variable	*n*	Mean	Range	Coefficient of variation	Median
A: pH ($CaCl_2$)	47	5.98	3.17–7.60	18.88	6.20
B: Percentage loss on ignition	47	12.89	1.80–72.00	96.52	9.72
C: $CaCO_3$ equivalence (%)	47	7.47	0.02–62.67	240.47	0.37
D: Total Cd ($\mu g\,g^{-1}$)	47	29.84	< LOD–364.5	201.96	9.45
E: Cation exchange capacity (meq g^{-1})	40	3.74	0.17–13.87	77.65	3.09
F: Free manganese oxides ($\mu g\,g^{-1}$)	47	434.36	23.00–3379	153.62	222
G: 1 *M* NH_4NO_3 extractable Cd ($\mu g\,g^{-1}$)	47	2.81	0.10–70.60	370.63	0.30
H: 0.05 *M* $CaCl_2$ extractable Cd ($\mu g\,g^{-1}$)	47	8.48	0.20–91.80	216.25	2.00
Cabbage Cd ($\mu g\,g^{-1}$)	37	15.17	0.50–79.50	136.46	6.20
Carrot Cd ($\mu g\,g^{-1}$)	23	3.71	0.30–8.60	82.83	1.50
Lettuce Cd ($\mu g\,g^{-1}$)	36	18.04	< LOD–100	118.65	9.03
Radish Cd ($\mu g\,g^{-1}$)	36	5.51	0.40–54.00	174.44	2.50

Notes: Values which were below the limits of detection for the methods used are indicated by < LOD. *A* – *H* are used in the regression equations to denote the particular independent variable.

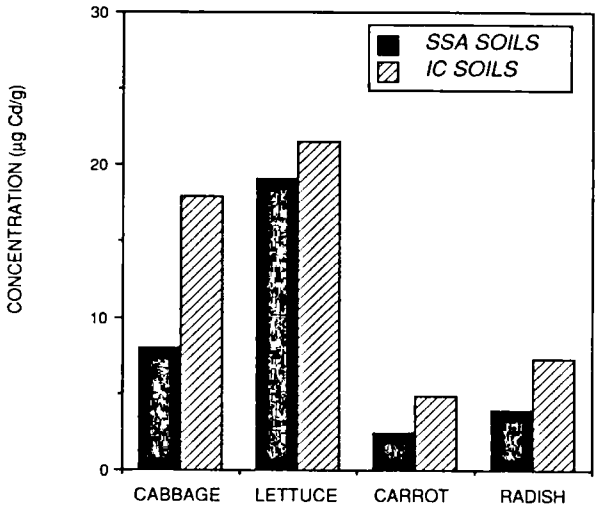

Fig. 2. Mean values of soil variables from the sewage sludge amended (SSA) and inorganically contaminated (IC) soil data sets.

combined data for all 48 soils are very heterogeneous for all of the variables except pH. When the data sets for sewage sludge amended and inorganically polluted soils are considered separately, as shown in Fig. 3, the most marked differences are in the total cadmium concentrations, which are much higher in the inorganic data set due to the inclusion of soil samples from lead–zinc mining contaminated land in Shipham ($< 365\,\mu g\,Cd\,g^{-1}$). The mean calcium

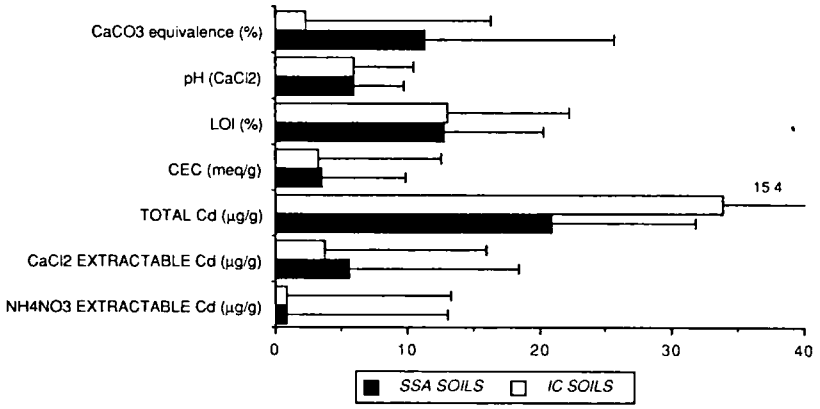

Fig. 3. Mean cadmium concentrations in crops from the sewage sludge amended (SSA) and inorganically contaminated (IC) soil data sets.

equivalence value is higher in the sludge amended soils as a result of including soil samples from a long-term field trial on shallow soils over chalk.

In general, both groups of samples comprised a wide range of soil types differing in mineralogy and a range of physico-chemical variables as well as different sources of metal contamination. Although it might be expected that sewage sludge amended soils would have the higher organic matter contents (percent loss on ignition), the inclusion of inorganically polluted soils from upland sites with relatively high organic matter contents resulted in the two groups of soil samples having similar mean levels of organic matter.

The data for the cadmium concentrations in the edible portions of crops show a marked difference between leafy vegetables and root crops. The order of decreasing mean cadmium concentration was: lettuce > cabbage $\gg$ radish > carrot. In all crops the mean cadmium concentrations in the plants grown on sludge amended soils were lower than those in plants grown on the inorganically contaminated soils. The differences in cadmium concentrations in the edible portions were around 50% lower for radish and carrot, 44% for cabbage and 11% for lettuce grown on the sludge amended soils.

The multiple regression equations for the accumulation of cadmium in the four crops are shown in Table 3. For each crop three equations are given; firstly, an equation for all of the soil data, secondly for the inorganically contaminated group ('IC') of soils and, thirdly, the sewage sludge amended ('SSA') group of soils. The splitting of the data into the separate groups gives an improved fit for the multiple regression equations, especially for lettuces and carrots. In all cases the sewage sludge amended soils show a better fit (higher R^2 value) than the inorganically contaminated soils, as can be seen in Fig. 4. This is due, in part, to the reduced variance of the data for the sludge amended soils. The finding that the respective equations for the sludge amended and inorganically contaminated soils include some different

TABLE 3

Multiple regression equations for each crop

Crop	Multiple regression equation	R^2 value
Cabbage	ALL: $Y = 6.12 - 4.92\log A - 1.16\log B - 0.49\log F + 0.23\log G$	0.66***
	IC: $Y = 4.19 - 5.01\log A - 0.32\log D$	0.66***
	SSA: $Y = -0.27 - 0.82\log D + 1.48\log E$	0.71***
Lettuce	ALL: $Y = 2.72 - 2.74\log A - 0.53\log D$	0.59***
	IC: $Y = 2.93 - 3.16\log A - 0.43\log D$	0.65***
	SSA: $Y = 4.04 - 4.25\log A - 0.61\log D$	0.77***
Carrot	ALL: $Y = -1.50 - 0.64\log B + 0.90\log H$[a]	0.79***
	IC: $Y = 0.13 - 0.79\log D + 0.59\log E$	0.89***
	SSA: $Y = -4.75\log A - 1.63\log D + 3.16\log E$	0.92***
Radish	ALL: $Y = 0.24 - 0.81\log D$	0.55***
	IC: $Y = 0.12 - 0.70\log D$	0.47**
	SSA: $Y = -0.45 + 0.85\log B - 0.95\log D$	0.83***

Y = log cadmium accumulation ratio.
For the identification of the independent variables, see Table 2.
***p = 0.1%; **p = 1%.
ALL = Combined data set (see Fig. 1).
IC = Inorganically contaminated soils.
SSA = Sewage sludge amended soils.
[a]The value for H is expressed as a percentage of the total soil cadmium concentration.

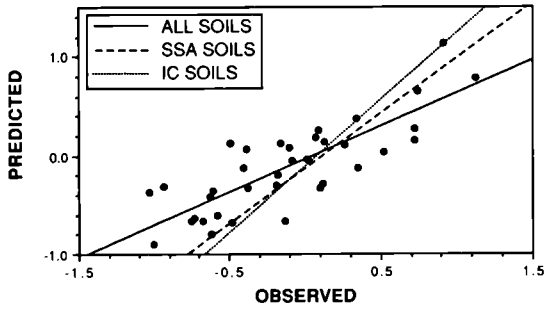

Fig. 4. Relationship between observed and predicted cadmium accumulation ratios for cabbages.

parameters in the cases of cabbage, carrot and radish suggests that differences probably occur in the speciation of the cadmium in the two types of contaminated soil. Tills and Alloway (1983) found that cationic cadmium species, probably Cd^{2+}, predominated in several types of polluted soils, which agrees with the predictions by Mahler et al. (1980) and others using the model GEOCHEM. Nevertheless, Tills and Alloway (1983) showed that sludge amended soil did contain a higher proportion of soluble organically bound cadmium than the inorganically contaminated soils, but the amounts did not appear to be large (< 13.2%). However, the fractionation procedure which they used may have

underestimated the size of the organic fraction. Neal and Sposito (1986) have shown that even a small concentration of sewage sludge-derived organic ligands can inhibit the adsorption of cadmium on soils and may affect its uptake by plants.

The most frequently occurring variable in the multiple regression equations is the total cadmium content, followed by the pH (measured in 0.01 M $CaCl_2$), hydrous manganese oxide and organic matter contents (percentage loss on ignition). The amounts of cadmium extracted by the soil test reagents rarely appear in the equations, which probably indicates the unsuitability of soil partial extractions for use on a wide range of soils with markedly different properties. Cation exchange capacity (CEC) was only shown to be a significant variable in two of the nine equations and in each case it was for the sludge amended soils. Other workers, such as King (1988), have also failed to find a correlation between cadmium uptake and CEC.

It is interesting to note that in the eight models in which total cadmium is included, it is always present as a negative coefficient, indicating an inverse relationship with the cadmium accumulation in the plant tissue. This implies that the proportion of the total soil cadmium accumulated in the edible portion of the crops is lowest in the soils with the highest cadmium contents. This is largely due to the highly contaminated soils having relatively high adsorptive capacities for cadmium. In the inorganically contaminated group, the soils from Shipham with very high total cadmium contents had higher adsorptive capacities than any other soils due mainly to their relatively high pH and calcium carbonate contents, which caused the replacement of calcium in calcite with cadmium and precipitation of $CdCO_3$ (Alloway et al., 1988; Papadopoulos and Rowell, 1988). The heavily sludged soils with high cadmium contents would have a greater adsorptive capacity, due to the organic matter and other sludge-derived adsorptive materials, than soils which had received smaller applications of sewage sludges. In addition to the higher sorptive capacity of the most heavily polluted soils, in the case of the soils from near lead–zinc mines the presence of discrete ore particles could also help to account for this inverse relationship between relative accumulation in the crop and total cadmium in the soil. These ore particles would be dissolved in the concentrated acid digestion procedure, but not all of their metal content would be in a form available for uptake into plant roots. It is important to stress that this negative function of total cadmium may not occur in all soils. In this study it is coincidental that the soils with the highest cadmium concentrations are also highly calcareous and have the highest adsorptive capacity for cadmium. In contrast, the negative function of pH with plant accumulation of cadmium is expected, since it is recognized that most trace metals are more mobile under acid conditions than they are at higher pH. Surprisingly, the concentration of cadmium in the soil solution was not found to be a significant variable in the regression analysis.

The better fit of the regression equations for the sewage sludge amended soils is probably due to their greater homogeneity compared with the inorgan-

ically contaminated group. Although sewage sludges from any particular wastewater treatment works show variations in metal concentrations due to differences in effluents and dilution, the matrix is relatively constant over a fixed range and the soils referred to here had all been farmed for centuries. The amendment of soils with sewage sludge, where a relatively uniform amount is applied from a tanker to the surface, also results in greater homogeneity than occurs in the more random contamination from mining and other inorganic sources. The inorganically polluted soils covered a wide range of soil types, including acid mountain soils from Norway and Wales, a paddy soil from Japan, limestone, sandy and clayey soils, and the sources of contamination also varied considerably.

CONCLUSIONS

Stepwise multiple regression analysis to determine the soil variables having the greatest effect on the accumulation of cadmium in cabbages, carrots, lettuces and radishes grown in soils respectively contaminated by sewage sludge and inorganic forms of metals from various sources showed the following.

(i) Only eight out of the 23 soil variables tested were found to be significantly related, in various combinations, to the concentrations of cadmium accumulated by the crops. These included: pH (in $CaCl_2$), percentage loss on ignition, total cadmium, hydrous manganese oxide content, 1 *M* ammonium nitrate extractable cadmium, 0.01 *M* calcium chloride extractable cadmium and cation exchange capacity.

(ii) When the data were divided into two sets, for the sewage sludge amended and inorganically contaminated soils, respectively, it was found that, in most cases, the multiple regression equations gave a better fit than for those for the combined data. Nevertheless, the variables in the equation for cabbages on all the soils included a wider range of soil properties expected to be involved in metal accumulation by crops (pH, organic matter, hydrous manganese oxides and ammonium nitrate extractable cadmium) than some of the equations for one of the other crops on either group of soils, such as radish on inorganically contaminated soil where only total cadmium (as a negative coefficient) and constants were included.

(iii) Total cadmium occurred in all equations as a negative coefficient, thus indicating that the accumulation of cadmium by crops was greatest on the soils with the lower total cadmium contents. A high calcium carbonate content in the mining contaminated soils from Shipham and some of the sludged soils, plus the high organic matter in the soils with the heaviest sewage sludge applications helped to account for this.

(iv) In most cases different combinations of soil factors were included in the equations for the sludged and inorganically contaminated groups. For example, the equation for cabbages on sludged soils included pH and total cadmium, whereas that for cabbages on inorganically contaminated soils

included total cadmium and cation exchange capacity. However, for lettuces, all equations involved pH and total cadmium. Differences in the speciation of cadmium in the soil possibly account for some of these variations. Different metal species in the soil solution may be more or less readily absorbed by the roots of different plant species. The speciation of cadmium in plant tissues is also likely to be of toxicological importance (Fox, 1983).

(v) The only soil test reagents shown to be relevant to the equations were 0.01 *M* calcium chloride and 1 *M* ammonium nitrate, both of which were only included in one of the nine equations, respectively. Commonly used partial extractants such as EDTA and DTPA were not found to be significant, neither was cadmium in the soil solution (obtained by centrifugation of pore water).

Although stepwise multiple regression analysis of soil and crop data enables the relative accumulation of cadmium in crops to be described, it does not always reveal the major soil factors involved. The inorganically contaminated group in particular were very heterogeneous with regard to both soil properties and sources of contamination. It is most unlikely that this range of diversity would be encountered in any particular region where the bioavailability of cadmium in polluted soils is being assessed.

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