THE UNIVERSITY OF READING
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# HEAVY METAL AVAILABILITY AND MOBILITY IN SEWAGE SLUDGE-TREATED SOILS

by

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# HEAVY METAL AVAILABILITY AND MOBILITY IN SEWAGE SLUDGE-TREATED SOILS

## ABSTRACT

Sewage sludge is a useful fertiliser for soils, but it also contains heavy metals which may have some potentially harmful effects on crops, animals and possibly humans. Sludge-borne organic matter influences metal mobility and its behaviour may depend on temperature and rainfall.

In a pot experiment, sludge-soil mixtures were incubated at 15 $^{0}$C and 25 $^{0}$C for two years. The availability of Cd, Ni, Pb and Zn was assessed by ryegrass uptake and soil extractions with DTPA and $CaCl_2$ and was found to increase in the higher sludge application. Metal availability at 25 $^{0}$C was greater than that at 15 $^{0}$C. Over time, availability increased in the plants (except Zn which did not change) and $CaCl_2$ extractions, while DTPA did not follow this trend precisely. The more rapid decomposition of organic matter may have caused the increased availability at 25 $^{0}$C.

Field experiments at sites in England and in Greece provided the different temperature conditions simulated in the above pot trial. Metal availability, in the sludge-treated plots, increased with the sludge application. After the first cutting, ryegrass uptake and DTPA extractions did not change. This was probably due to the organic matter content not being changed over time.

The mobility of heavy metals was also assessed by a leaching experiment, where sludge had been put on the top of soil columns. Cadmium, Ni and Zn (but not Pb) moved down to 10 cm depth in the high sludge treatments. In the leachate, metals increased with the sludge as well as with the simulated rainfall rate (450 and 900 mm $a^{-1}$). DOC from the sludge may have facilitated this movement.

In the last pot experiment soil-sludge mixtures were irrigated with solutions containing DOC. The availability of metals increased with the DOC additions, and this was further enhanced in the higher sludge additions.

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# CHAPTER ONE: HEAVY METALS IN THE ENVIRONMENT

## 1.1 INTRODUCTION

Sewage sludge is the residue product from the treatment of waste water. It has been used in agriculture for many years as a fertiliser containing beneficial amounts of organic matter and macro- and micronutrients (Chaney, 1988). However it may contain undesirably high concentrations of heavy metals and persistent organic pollutants, which may have adverse effects on crops and, ultimately, consumers (Solar-Rovira *et al.*, 1996; Petruzzelli *et al.*, 1995). The available fraction of heavy metals is that which can be readily mobilised in the soil environment and taken up by plant roots. Heavy metal mobility is a very important factor relevant to the chemistry in these metals in the soil, because it is related to the risk of movement of these metals down the soil profile and the subsequent contamination of the ground water deposits. The availability of metals to crop plants is also related to the accumulation of metals in the human food chain to potentially hazardous levels. Heavy metal availability to crops after the application of sewage sludge in agriculture has been studied by many researchers in the past, but in some of them unrealistic approaches have been used:

- Reliance on greenhouse studies only without comparing their findings with field data, which sometimes is more accurate. De Vries and Tiller (1978) concluded that pot trials overestimate heavy metal uptake by plants compared with field trials.
- Spiking soils with inorganic salts of heavy metals and then applying the findings to the case of sewage sludge-borne heavy metals. These studies tend to underestimate the adsorption capacity of the sewage sludge.
- Application of unrealistically large quantities of sewage sludge, sometimes of orders of magnitude higher than the common farming practice (8-10 t ha$^{-1}$) and their results cause unnecessary concern.

The role of organic matter (both indigenous and from sewage sludge) in the behaviour of heavy metals in soils has been documented by many workers. However, it is widely admitted that there is a lack of evidence and data for the role of low molecular weight organic compounds in the mobility of heavy metals (Harter and Naidu, 1995). Although a lot of experiments have involved investigations of adsorption and desorption characteristics of Dissolved Organic Carbon (DOC) and heavy metals on the soil constituents, very little of work has been performed with test crops.

Moreover, many workers have investigated the possibility of downward movement of heavy metals in soil profiles and have come up with contradictory findings. Although heavy metals are not thought to move very far in the soil profile, it is generally admitted that there are certain conditions that may facilitate their movement. These conditions may be an acidic soil environment, a sandy soil with a low adsorptive capacity, and when high concentrations of heavy metals (such as when high application rates of sewage sludge are used) are present in the topsoil. However, the extent of this movement is not well defined. In Greece, there was relatively little production of sewage sludge until 1990, but in order to comply with Directive 91/271/EEC of the European Union concerning urban waste water treatment, more than one hundred new waste water treatment plants are under construction around the main urban areas. The fate and behaviour of heavy metals after the application of sewage sludge has so far received very limited consideration in Greece and there is a significant lack of scientific information and data related to local conditions on this issue (Tsadilas *et al*., 1995). Four heavy metals were chosen to be studied: Cd, Ni, Pb and Zn. Cadmium and Zn are very important and mobile elements which have been widely studied, Ni (like Zn) except from being mobile in the soil is essential for plant growth, while Pb (although a relatively immobile metal) is considered as a very serious contaminant. Although Cu is a very environmentally active metal, it was not included in the study because its behaviour is similar to Pb.

In the light of all the above, a series of four experiments was designed which had the following objectives:

- to test the effects of sewage sludge application at three ‘normal’ rates, namely 0, 10 and 50 t ha$^{-1}$, on two different soil types and on plants growing on them under greenhouse conditions and under more realistic field conditions,
- to compare the effect of Cd, Ni, Pb and Zn deposited in these soils at two different incubation temperatures. The temperatures chosen were 15 $^{0}$C and 25 $^{0}$C, which represent conditions typically found in temperate and Mediterranean climates, that is conditions likely to be found in England and Greece, respectively,
- to investigate the leaching of heavy metals down the soil profile in soil columns where sewage sludge was put on the top and where rainfall was applied at two rates. The rates of rainfall chosen were 450 and 900 mm per annum, distributed in a period of one month. These rates are relatively characteristic of the rainfall amounts in Greece and England, respectively.

The hypotheses for this sequence of experiments were the following:

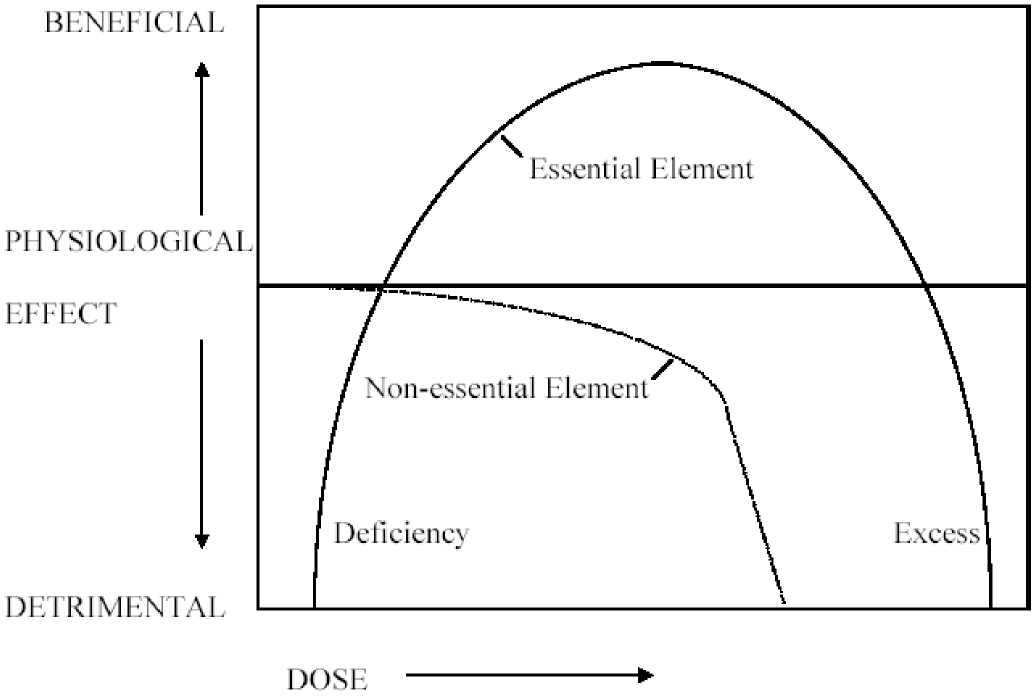
- Higher ambient temperatures lead to higher organic matter decomposition rates. This decomposition may lead to increasing DOC concentrations in the soil-sludge mixtures. This increase in DOC concentration may increase the heavy metal availability, especially in high sewage sludge application rates, compared with soils incubated at lower temperatures.
- Higher rainfall rates, in cases of sewage sludge applied on the soil surface, lead to greater leaching of heavy metals down the soil profile. Their transport may be facilitated by the presence of DOC, which is found in sewage sludge.

- Greenhouse studies can predict the fate and availability of heavy metals likely to be encountered in sludge-treated field soils.

## 1.2 OCCURRENCE OF METALS IN THE ENVIRONMENT

There are approximately 90 elements found in the earth' s crust; of these only 9 account for over 99 % by weight, while the rest account only for 0.14 % and these are called 'trace elements'. Some of these trace elements are considered to be essential for the normal healthy growth of organisms while others are non-essential. Essential elements are those that have been found to have some essential biochemical function in the bodies of organisms. The elements that are universally accepted as essential to all organisms are: C, H, O, Ca, Cu, Cl, F, Fe, Mg, S, Zn, Mn, Mo, N, P, K and Na. Of these C, H, O, Ca, K, P, Mg, S, Cl and N are macro-elements, while the others are essential trace elements or micronutrients (Jones, 1982). Apart from those, there are elements which are essential only to certain organisms. These are: Co (to bacteria and animals), Cr (to animals), Mo (to plants), Ni (to plants), Se (to animals), B (to plants) and I (to animals) (Alloway, 1996). If essential elements are not supplied to the organisms, they can develop deficiency symptoms and possibly die. As the supply of the elements increases organisms increase their production and biochemical functions are restored.

**Figure 1.1:** ***The function of essential and non-essential elements in organisms (From Sadler*** **et al.*****, 1985).***



There is a range of concentration for each of these elements, which is optimum for each organism to grow, after which toxicity symptoms start to develop. For non-essential elements, on the other hand, there is a range of concentration that the organism tolerates, after which toxicity symptoms develop. These typical dose-response functions are shown in Figure 1.1.

Heavy metals are trace elements with a specific density of greater than 6 g cm$^{-3}$. Sadler *et al.* (1985) suggested that under this definition all metals in the periodic table, especially those in Groups I and II should be considered as heavy metals. Alloway (1995a) specifies that rather only metals of industrial significance and natural occurence must be included to this definition. These are Cd, Cr, Co, Cu, Hg, Mn, Mo, Ni, Pb, Se and Zn. A few other researchers have proposed other, but very similar, definitions for the term ‘heavy metals’. Wild (1993) for example included metals with specific density of greater than 5 or 6 g cm$^{-3}$, and specifically those which are discharged by industry. He lists under this definition the elements: As (which is a metalloid), Cd, Cr, Cu, Pb, Hg, Ni, and Zn.

Nowadays, the concentration of heavy metals in soils in technologically advanced countries are frequently greater than their expected background levels (Table 1.1), because of large anthropogenic inputs in the last 150 years. Their extensive uses in industry has created pollution problems, which are sometimes associated with plant toxicity and risks concerning animal and human health. In recent years, research on these elements has received a great deal of interest as they are considered to be potentially highly toxic elements. This may be misleading though, because all elements are harmful when their concentrations in living organisms exceed certain thresholds. After the Industrial Revolution, elevated quantities of heavy metals have been emitted into the atmosphere, changing the natural balance and accelerating, some times dramatically, their biogeochemical cycling.

**Table 1.1:** ***Occurrence of Cd, Ni, Pb and Zn in the environment (μg g$^{-1}$).***

| | Cd | Ni | Pb | Zn |
|---|---|---|---|---|
| Magmatic | <0.3 $^{a}$ | 5-15 $^{a}$ | 10-40 $^{a}$ | 52 $^{e}$ |
| Sedimentary | <0.3 $^{a}$, 0.1 $^{e}$ | 7.9 $^{e}$ | 5.7-10 $^{e}$ | 20-30 $^{e}$ |
| Igneous | 0.13 $^{b,d}$, 0.11 $^{e}$ | 0.5-2,000 $^{e}$ | 3-24 $^{e}$ | 8 $^{d}$, 52-100 $^{e}$ |
| Soil | 0.06-1.1 $^{a}$ | 0.2-450 $^{a}$ | 10-67 $^{a}$ | 17-125 $^{a}$ |
| Soil Solution | 0.2-6 $^{a}$ | 3-25 $^{a}$ | | 4-270 $^{a}$ |
| Ocean (μg kg$^{-1}$) | 0.1 $^{b}$ | | 0.03 $^{b}$ | 2 $^{d}$, 11 $^{b}$ |
| Earth’ s Crust | 0.2 $^{b,c}$, 0.1 $^{e}$ | 75 $^{c}$, 80 $^{e}$ | 13 $^{b}$, 12 $^{c}$, 14 $^{e}$ | 70 $^{b,c}$, 75 $^{e}$ |

*a: Kabata-Pendias and Pendias, 1992; b: O’ Neil, 1993; c: Bunce, 1991; d: Sadler* et al.*, 1985;* e: *Alloway, 1995a*

Moreover, it is important to recognise that heavy metal pollution of soils usually includes several different elements (multi-element pollution) especially in areas of metalliferous mining.

### 1.2.1 Cadmium

The geochemical cycle of Cd and Zn is shown in Figure 1.3. Cadmium belongs to Group II of the Periodic Table. It is found mainly in magmatic and sedimentary rocks with concentrations up to 0.3 $\mu g\ g^{-1}$. In the weathering process of the rock minerals it moves readily into the soil solution, where it is normally found in the form $Cd^{2+}$. This chemical form is the most common form of Cd. Other ionic forms that may be found in the soil solution are: $CdCl^+$, $CdOH^+$, $CdHCO_3^+$, $CdCl_3^-$, $CdCl_4^{2-}$, $Cd(OH)_3^-$ and $Cd(OH)_4^{2-}$ (Kabata-Pendias and Pendias, 1992). Cadmium has had a wide range of uses in industry, including paints and pigments, electroplating, plastic stabilisers and silver-cadmium batteries. Most common Cd industrial uses are shown in Table 1.2.

**Table 1.2:** ***Most common industrial uses of Cd, Ni, Pb and Zn.***

| | Cd | Ni | Pb | Zn |
|---|---|---|---|---|
| Electroplating | b, g | b, g | | |
| Paint pigments | b, e, g | | e | e |
| Plastic stabilisers | b, g | | | |
| Silver-Cd batteries | b, g | b, g | a | |
| Coinage | | b | | |
| Water pipes | | | c, e | |
| Car fuel | | | c, f | |
| Galvanisation | | | | b, g |
| Metal antirust coating | | d | | b, c |
| Roofing | | | d | |
| Absorber of high energy radiation | | | d | |
| Mining | f, a (as by-product) | h | a, c, f | f, a |
| Cable coating | | | a | |

*a: Brooks, 1977; b: O' Neil, 1993; c: Wild, 1993; d: Bunce, 1996; e: Baird, 1995; f: Alloway and Ayres, 1993; g: Volensky, 1990*

Cadmium is a by-product of Zn refining in the Zn mining industry. This process follows the formula:

$$ZnS \xrightarrow[\text{heat}]{O_2} SO_2 + ZnO \xrightarrow[\text{heat}]{C} CO_2 + Zn/Cd \xrightarrow{\text{distil}} Zn + Cd \quad \text{(O' Neil, 1993).}$$

In recent years there has been an elevated input of Cd into the environment through increased use of phosphatic fertilisers, wastes (including sewage sludges) and aerial emissions.

### 1.2.2 Nickel

Nickel is an essential element for plant growth, and it belongs to Group VIIIa of the Periodic Table. Its occurrence in the earth's crust ranges from 1400-2000 μg $g^{-1}$ in the ultramafic rocks to 5-15 μg $g^{-1}$ in rocks with higher silicate contents (acidity), like the granites. The ion $Ni^{2+}$, which is the result of weathering of the rock minerals, is relatively stable in the soil solution. Organic matter has a great influence on this element as Ni can be strongly adsorbed by it (Leeper, 1978). As a result, Ni distribution in the soil profile is related to the organic matter content, and also to oxides and clay minerals. The commonest ionic forms of Ni (except for $Ni^{2+}$) are: $NiOH^{+}$, $HNiO_2^{-}$, $Ni(OH)_3^{-}$ (Kabata-Pendias and Pendias, 1992). Nickel may be a serious airborne contaminant of industries using Ni-carbonyl compounds. Nickel-carbonyl has been reported to be carcinogenic, although this is doubted (O' Neil, 1993). Nickel is widely used in industry, as it is a metal which does not corrode as much as Fe. It is, therefore, used in the production of alloys, on which it confers them stain and corrosion protection (Bunce, 1993). Table 1.2 summarises Ni industrial uses.

### 1.2.3 Lead

The geochemical cycle of Pb is shown in Figure 1.2. Lead is considered to be the most serious pollutant of all heavy metals. It occurs naturally in the acidic series of magmatic rocks. Its natural state is PbS (galena). When rocks weather, Pb very slowly enters the soil solution system as $Pb^{2+}$, but the oxidising state of 4+ may also occur. When the pH rises, Pb changes its ionic form to $Pb(OH)^{+}$ and $Pb(OH)_2^{0}$. In alkaline pH conditions it precipitates as $Pb(OH)_2$, $PbCO_3$ or $PbSO_4$ (Wild, 1993). Illite clay minerals seem to have a higher affinity for Pb than any other clay mineral. Lead is strongly adsorbed to organic matter in soils, more than any other heavy metal, and therefore organic matter is a very important sink for Pb in polluted soils. (Kabata-Pendias and Pendias, 1992).

The anthropogenic emissions of Pb were 22 x $10^{6}$ kg $y^{-1}$ in 1950, while earlier in the 1990s they have been as high as 450 x $10^{6}$ kg $y^{-1}$ (O' Neil, 1993). Industrial sources of Pb include primary smelters, where the processing of the ore material takes place, and secondary

**Figure 1.2:** ***The geochemical cycle of lead (From O' Neil, 1993).***

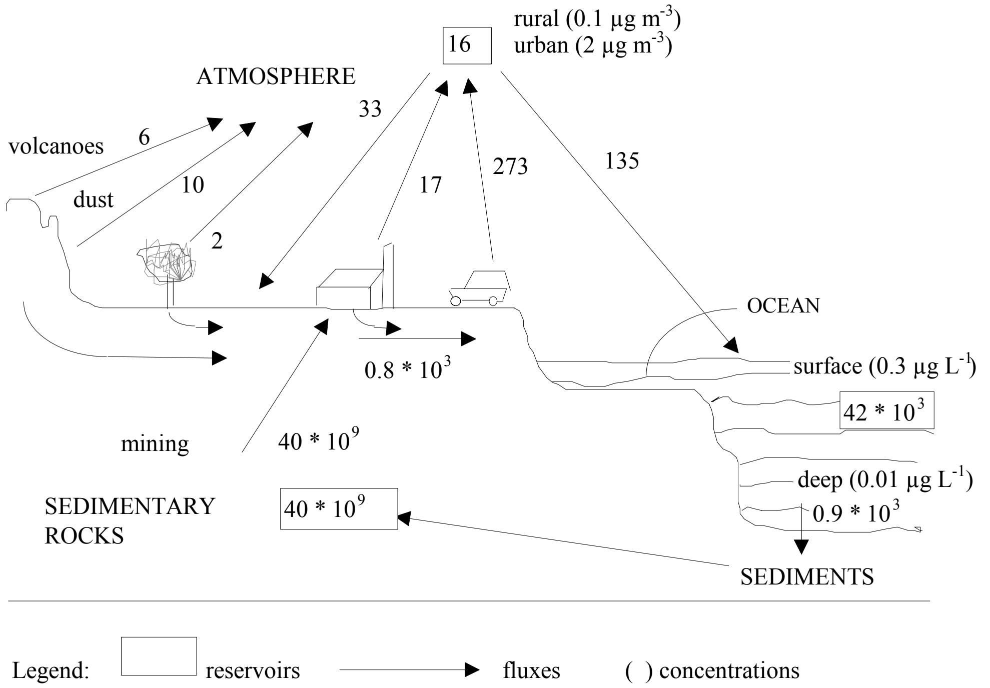
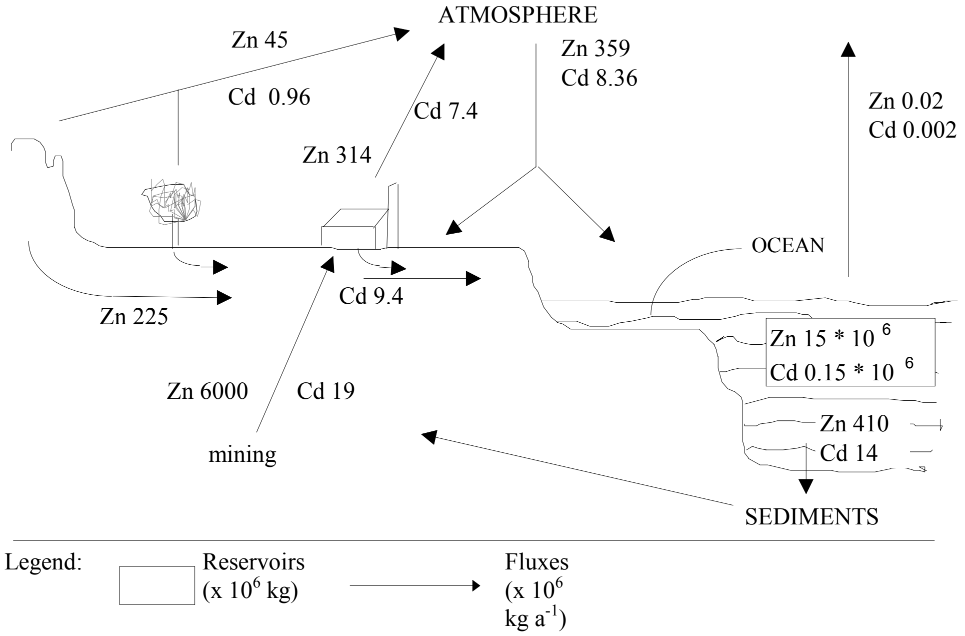


**Figure 1.3:** ***The geochemical cycle of cadmium and zinc (From O' Neil, 1993).***



smelters, where the recovery of Pb from scrap batteries, cable sheathing and other materials takes place. Lead has been used since the Roman times for water pipes, but this has now stopped, because water with $pH < 5$ solubilises Pb into the water supply (O' Neil, 1993). However some old houses may still have Pb pipes. Other common uses of Pb include: $PbCrO_4$ which is a yellow pigment used for road stripping and school buses, $Pb_3O_4$ which is a corrosion resistant red pigment, and $Pb_3(CO_3)_2(OH)_2$ which is a white indoor pigment. The greatest and most commonly known use of Pb is as fuel additives ($Pb(CH_3)_4$ and $Pb(C_2H_5)_4$) which are 'antiknocking' agents (Baird, 1995). Lead is also used as a absorber of high energy X and $\gamma$ rays and in roofing, while PbO is used in crystal glass because it dispenses light spectrally (Bunce, 1993). Table 1.2 summarises Pb industrial uses.

### 1.2.4 Zinc

Zinc is an essential element for the plant growth. It occurs naturally in ore minerals especially sphalerite (ZnS). Weathering produces $Zn^{2+}$ which can substitute for $Mg^{2+}$ in silicate minerals in the soil. The hydroxide $Zn(OH)^+$ is also adsorbed on Fe and Mn oxides and clay minerals containing Al and Si lattices (Lindsay, 1979). Other ionic forms in the soil solution are: $Zn(OH)_2$ and $ZnHPO_4$. Zinc is adsorbed strongly onto organic matter and clay particles in the soil and this adsorption is related to the Cation Exchange Capacity of the system in acidic media and is influenced by organic ligands in alkaline media. Industrial uses of Zn include corrosion protecting coating and manufacture of brass and other alloys. Zinc has a very similar environmental chemical behaviour to Cd, as both elements normally occur together. The geochemical cycle of Zn, along with that of Cd, is shown in Figure 1.3.

## 1.3 HEAVY METAL POLLUTION

Heavy metals have a wide range of uses in industry and as a result their disposal may be a very serious issue. Industries dispose of these metals in a multimedia way; water (through the sewage network of the cities), air (through aerial emission of fine particles and gases) and solid (as sewage sludge). Some of the pollution sources include agrochemicals (Jinadasa *et al.*,1997), mining and smelting and fossil fuel combustion (Alloway, 1995a).

### 1.3.1 Sewage Sludge

Sewage sludge is the residue product from the treatment of wastewater and contains organic matter macronutrients, such as N and P and essential trace elements (Campbell and Beckett, 1988), either in a watery suspension with 2-5 % solids or as a drier 'cake' with 20-30 % solids. In the UK, around $1.1 \times 10^6$ t of dry solids are produced annually ($6.5 \times 10^6$ in the European Union), but this is expected to increase by 50-60 % at the beginning of the 21st century in the UK and by 50 % in the EU (Smith, 1995). Cadmium, Ni, Pb, and Zn are among the heavy metals most commonly found in sewage sludges and their concentrations, along with other pollutants, depend on the origins of the waste water (domestic or industrial), and the level of treatment of the sludge in the cleansing plant. Of the metals mentioned above, only Ni and Zn are essential for the plant growth, while Cd and Pb are not involved in any essential biological function. Although sewage sludge contributes only a low percentage of the overall inputs of heavy metals to soils overall (O‘ Neil, 1993), where it is applied in high rates there is always the risk of their accumulation in the human food chain (Chang *et al.*, 1982). The residues of Cd , Pb, Ni and Zn after the sewage sludge application remain in the soil for a long period of time since only small amounts are leached (Alloway and Jackson, 1991). This is why care must be exercised in the management of sewage sludge when it is applied as a fertiliser in agricultural practice. Therefore, the European Union and other countries recycling sewage sludge on agricultural land have set limits for the maximum concentrations of heavy metals borne in them and in soils (Table 1.3 and 1.4).

#### 1.3.1.1 Treatment of Sewage Sludge

The first sewerage system was used in ancient Rome, where it was able to support a population of up to 1 million people (Gerba, 1996). In England and Wales, the sewage flow is 275 L per capita per day and there are currently 5,000 waste water treatment works nation-wide.

Sewage treatment plants tend to be broadly similar world-wide, but there may be differences concerning the input of technology, climatic conditions of the area and the forms of the final products, which are the sewage effluent and sewage sludge. Waste water treatment works include Preliminary sedimentation, Primary sedimentation, and Secondary treatment.

**Table 1.3:** ***Concentrations of Cd, Ni, Pb and Zn in sewage sludges (μg g$^{-1}$).***

| | Cd | Ni | Pb | Zn |
|---|---|---|---|---|
| Range [a] | <1-3,410 | 6-5,300 | 29-3,600 | 91-49,000 |
| Typical concentrations [b] | 3.2 | 37 | 217 | 889 |
| Permissible limits in the EU [a, c] | 20-40 | 300-400 | 750-1,200 | 2,500-4,000 |
| Permissible limits in the USA [a] | 85 | 420 | 840 | 7,500 |

*a: Alloway, 1995b; b: Smith, 1995; c: EU Directive No 86/278/EEC*

**Table 1.4:** ***Typical and permissible concentrations of Cd, Ni, Pb and Zn in soils $\mu g\ g^{-1}$) and permissible application rate of these metals in soils (kg $ha^{-1}$ $year^{-1}$).***

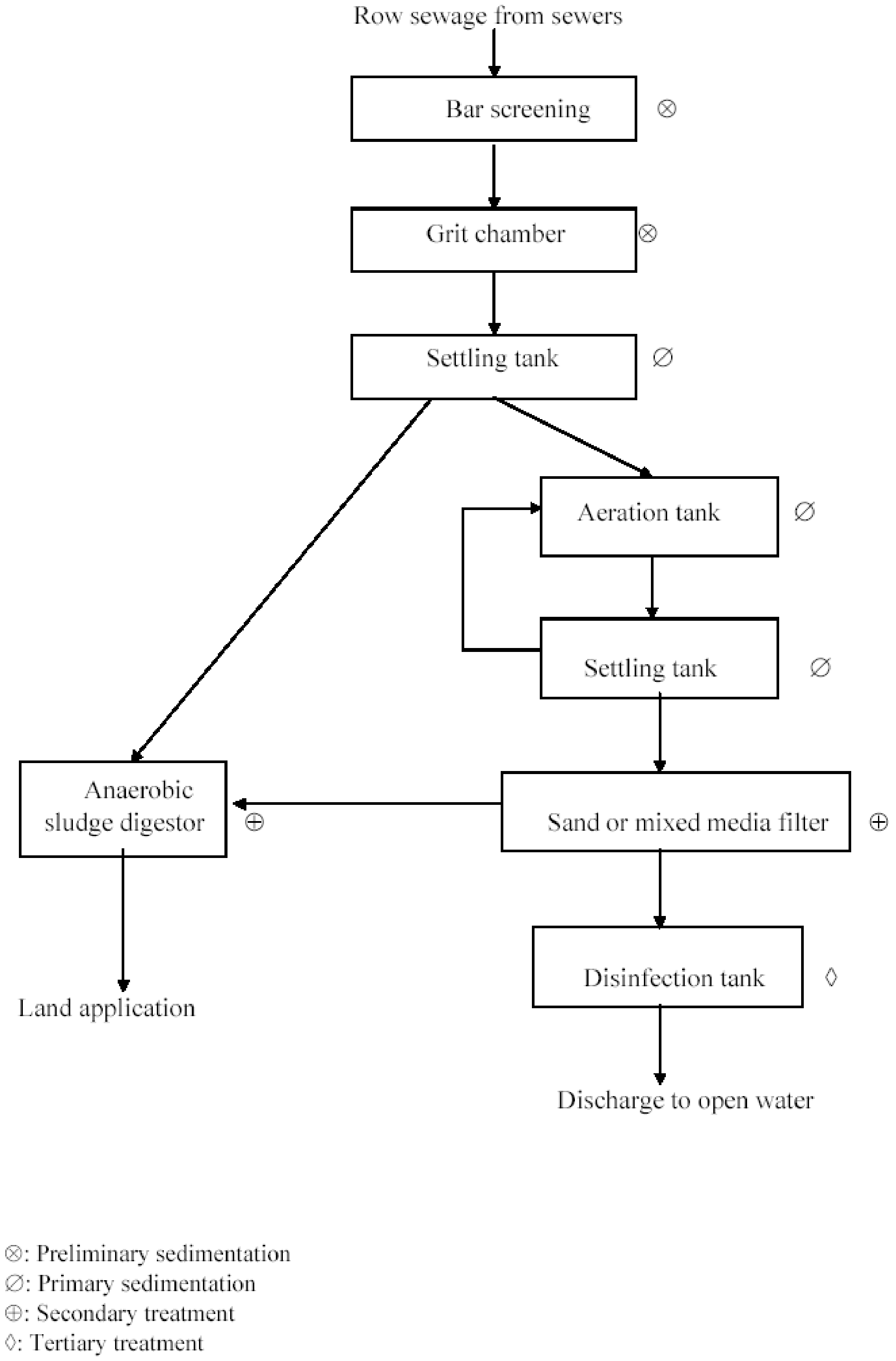
| | Cd | Ni | Pb | Zn |
|---|---|---|---|---|
| Permissible concentrations [a, b] | 1-3 | 30-75 | 50-300 | 150-300 |
| Typical concentrations [c] | 0.7 | 22.6 | 40 | 82 |
| Permissible application [a, b] | 0.15 | 3 | 15 | 30 |

*a: Wild, 1993; b: EU Directive No 86/278/EEC, c: McGrath and Loveland, 1992*

Some works may also have Tertiary treatment. In the first stage, the Preliminary sedimentation, the raw waste water passes through coarse screens, where floating materials are separated. The collected material is buried, incinerated or burnt. After that, stones and grit settle by the use of constant velocity (0.3 m $s^{-1}$) channels. Alternatively, this takes place with the use of small sedimentation tanks, from which the water overflows in such a rate that only grit can settle out (Lester, 1996). In the Primary sedimentation, the retention time of the waste water is 2-6 hours. The waste water flows through circular, rectangular or conical tanks (radial, horizontal or vertical flow, respectively), which have mechanical sludge scraping devices at the bottom. The settling of particles in this stage depends on the particle characteristics (density, size) and the surface loading ($m^3$ $m^{-2}$ $day^{-1}$) with a typical value of 30-45 $m^3$ $m^{-2}$ $day^{-1}$. In this phase, 55 % of the suspended solids are removed (from 400 to 150 mg $L^{-1}$, approximately) and BOD (Biochemical Oxygen Demand) is reduced by 35 % (from 300 to 200 mg $L^{-1}$, approximately). The solids are then concentrated into the primary sludge, and the effluent may be acceptable to be discharged to a watercourse.

The Secondary treatment stage has the objective of reducing the BOD of the effluent, and there are two different techniques for doing this. The first is the trickling filter, where the waste water drips from a rotating ‘shower’ into a gravel bed. Oxygen is diffused in the water drops from the air and the bed is quickly colonised by micro-organisms; the BOD of the waste water is thus reduced. The fact that this system needs a lot of space and that is less active during the winter is a disadvantage. The second technique is the activated sludge reactor, where the waste water is agitated and aerated in large tanks. The aim of this technique is both for the micro-organisms to form aggregates and settle, and for the carbonaceous matter to be oxidised, so that the BOD may be reduced. After that, the oxidised effluent settles again in tanks similar to the ones used in the Primary sedimentation, with the only difference being that the loading is now 40 $m^3$ $m^{-2}$ $day^{-1}$. Sludge treatment, which comes from the Primary

**Figure 1.4:** ***Schematic of the process of a wastewater treatment plant (From Gerba, 1996).***



sedimentation and from the solid by-product of the Secondary treatment is done either aerobically or anaerobically. Prior to treatment, sludge has 1-7 % solids and it is highly offensive. After the treatment, it has 8-30 % solids and is not so offensive (Horan, 1993). The insoluble material is the sludge which can now be used as a fertiliser.

The Tertiary treatment has the objective of further improving the purity of the sewage effluent. Pathogens are killed by disinfecting the final effluent with chlorine (Bunce, 1991). Other aims of the Tertiary treatment would be to increase the pH value of the sludge, in order to decrease the mobility of heavy metals (Qiao *et al.*, 1996; Qiao *et al.*, 1997; McBride, 1998). Figure 1.4 shows schematically the treatment of a biological cleansing plant.

### 1.3.1.2 Fertiliser Value of Sewage Sludge

Sewage sludge is increasingly used as a fertiliser, as it carries some advantages, including the fact that it is often supplied free or cheaply and it contains nutrients and organic matter (Table 1.5). Land disposal is generally considered more environmentally sound than all other disposal options (Chaney *et al.*, 1977; Webber *et al.*, 1996, Schultz and Romheld, 1997). However, sludge treatment and disposal strategies must be carefully evaluated both environmentally (disposal outlets available, governmental legislation) and economically (operating costs of waste water treatment plants) (Davis, 1994). Other disposal options, except for land application, may include incineration, land reclamation, use in forestry and application on dedicated sacrificed land (Smith, 1995). Currently in the UK, 44 % of the sewage

**Table 1.5:** ***Typical concentrations of nutrients and other substances in sewage sludge (From Sommers, 1977; Baker* et al.*, 1977:O' Riordan* et al.*, 1986a; O'Riordan* et al.*, 1986b; O' Riordan* et al.*, 1986c; Ardern, 1977; Palazzo, 1977; Imhoff* et al.*, 1971 and Gerba, 1996).***

| | Org. Matter [a] | Org. Carbon [a] | N[a] | $NH_4$-N [b] | $NO_3$-N [b] | P[a] | S [a] |
|---|---|---|---|---|---|---|---|
| Range | 19-60 | 16-40 | 0.5-17.6 | 800-5600 | 79-160 | 0.5-14.3 | 0.6-1.5 |
| No of sludges | na [d] | >300 | >300 | >300 | >300 | >300 | 28 |
| | K [a] | Mg [a] | Ca [a] | Na [a] | Solids [b] | Diss. Solids [b] | BOD [b] |
| Range | 0.2-7 | 0.18-0.27 | 0.5-3 | 0.23 [c] | 720 [c] | 500 [c] | 220 [c] |
| No of sludges | >200 | 200 | >200 | 200 | na | na | na |

*a: in %, b: in μg g$^{-1}$, c: median value, d: Not Available* sludge is used in agricultural land (20 % of which in long-term pasture), 30 % sea disposal, 8 % landfill, 7 % incineration and the rest goes to other outlets (Smith, 1995). However, sea disposal is no longer possible. Sewage sludge is currently applied to around 60,000 ha, which accounts for less than 1% of the agricultural land in the UK, but after the end of 1998, when its disposal in the sea stops, this percentage will have to increase (Chambers, 1997). Nowadays, sewage sludge can be produced for agricultural use as novel dry granular, pelletised, or fortified organic-based materials, which have more specialised uses in agriculture and horticulture compared with the conventional forms (Smith and Hadley, 1992). The technology for applying sludge is advanced and includes surface spreading and injection of the material into the soil, a practice which helps reduce the odour problems and helps the sludge to be more properly incorporated into the soil. The optimum dose of application is difficult to determine, because there are restrictions depending on soil parameters, such as pH, clay content and the contaminant and nitrogen contents in the sludge (Davis, 1996; Higgins, 1984), but in the UK the application is usually 8-10 t DM ha$^{-1}$, which is equivalent to 250 kg N ha$^{-1}$ year$^{-1}$. Hansen and Chaney (1984) recognised the fact that only sludges low in heavy metal concentrations should be used as fertilisers, but this is not applicable in cases of non-food crops, like ornamental plants and forest species.

Nevertheless, it is evident from much research that sewage sludge increases crop yields, compared with control treatments. Palazzo (1977) found an increase, and this was attributed to the N content of the material. The same was reported by more recent works, including those of Rao and Shantaram (1995a), Rao and Shantaram (1995b) and Sajwan *et al.* (1995).

# CHAPTER TWO: BEHAVIOUR OF HEAVY METALS IN SEWAGE SLUDGE-TREATED SOILS

## 2.1 EFFECTS OF SOIL PROPERTIES ON THE AVAILABILITY OF HEAVY METALS BORNE IN SEWAGE SLUDGE

'Available' heavy metals is the fraction of the total concentration of these metals in the soil, which is soluble in the soil solution or easily exchangeable from the soil surfaces. The availability of metals in soils will depend on their form and concentration in the parent material and their input through fertilisers, sewage sludge and aerial deposition. It will also be affected by leaching processes, adsorption and desorption from the solid phase and mineralisation of the organic matter (Figure 2.1). The fate of heavy metals will be, subsequently, controlled by certain soil properties, which regulate the above processes. Some of these properties are discussed in the following section.

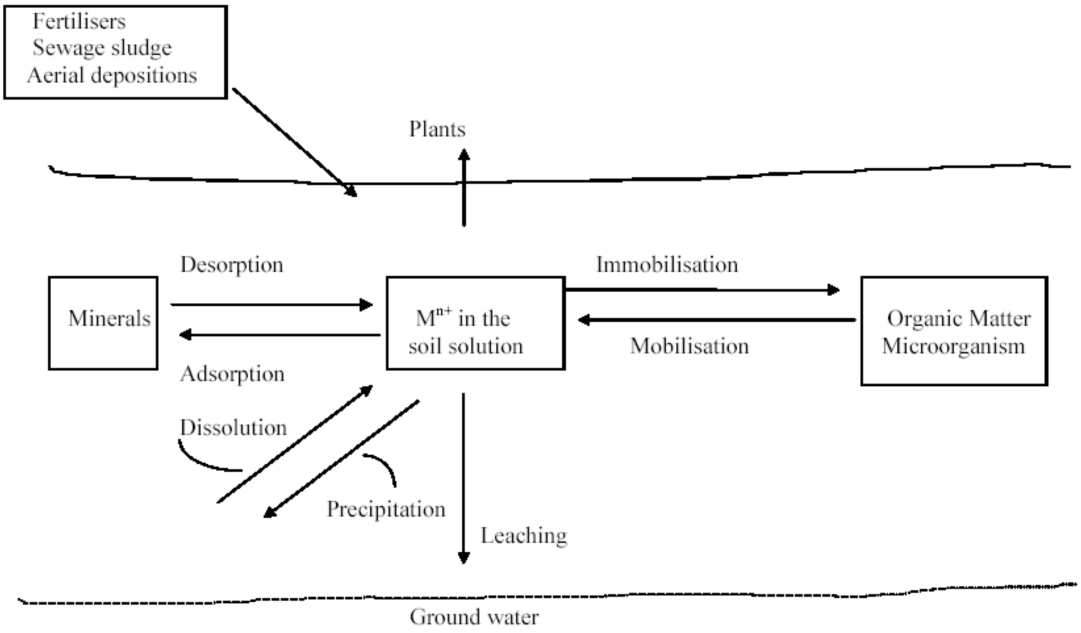
### 2.1.1 pH

pH is the negative logarithm (base 10) of the activity of $H^+$ in a solution:

$$pH = -\log(H^+)$$

It would not be exaggerating to state that this parameter is the most important of all in governing the chemistry of metals in the soil environment. The effect of pH on heavy

**Figure 2.1:** ***Chemical interactions in the soil solution (From Harter and Naidu, 1995).***



metal availability to plants has been reported by many researchers and it is accepted that as pH decreases the solubility of cationic forms of metals in the soil solution increases and, therefore, they become more readily available to plants (Gray *et al*., 1998; Salam and Helmke, 1998; Oliver *et al*., 1998; Li *et al*., 1996; Singh *et al*., 1995; Evans *et al*., 1995; Filius *et al*., 1998; Mann and Ritchie, 1995; Chlopecka *et al*., 1996; Cieslinski *et al*., 1996; Vigerust and Selmer-Olsen, 1985). A number of chemical processes have been suggested in order to explain this behaviour. Harmsen (1977) in his early work, suggested two mechanisms for the increased adsorption of heavy metals as pH increases, which are: 1) Selective adsorption of metals on sites previously occupied by $H^+$ (M/H exchange, M = metal), and 2) Selective adsorption of hydrolysis products of metals to sites previously occupied by $H^+$ (M(OH)/H exchange). In a recent review, Naidu *et al.* (1997) agreed with the significance of the above mechanisms and they added that this process is related to the increase of the adsorption density of the margins of clay minerals. Evans (1989), in his review, explained that pH has a major effect on metal dynamics because it controls adsorption and precipitation, which are the main mechanisms of metal retention to soils. Metal solubility in the solution depends on the solubility product of the solid phase (precipitate) containing the metal. The most important precipitation reaction is hydrolysis, which may be described by the reaction:

$$M(OH)_m + mH^+ \leftrightarrow M^{m+} + mH_2O$$

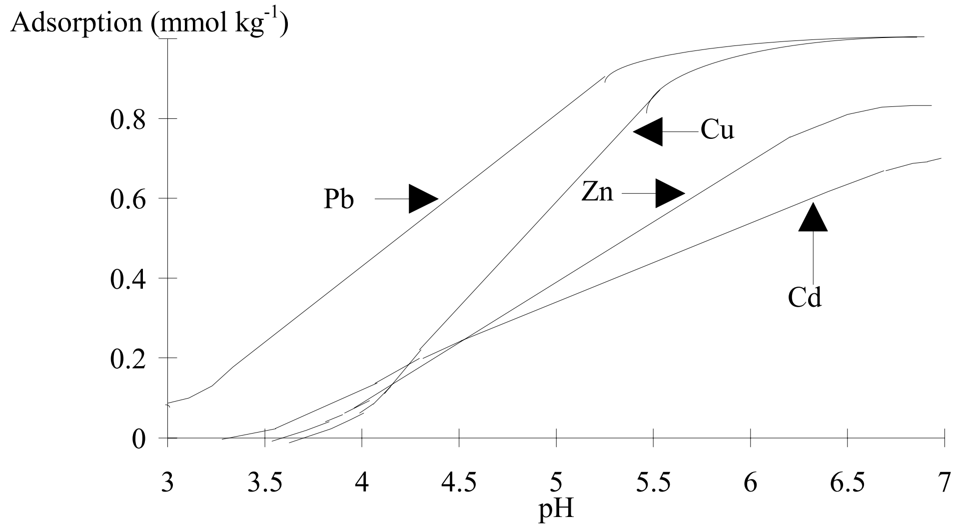
The solubility product (K) of which is equal to:

$$K = \frac{(M^{m+})}{(H^+)^m} \rightarrow pM^{m+} = m\,pH + pK$$

where: M is the metal and m+ the positive valency of the metal.

That means that with an increase of pH , $pM^{m+}$ also increases, and $(M^{m+})$ decreases as a result of precipitation. Hydrolysis increases with pH and, subsequently, metal retention by hydroxides increases with pH. That is, metal solubility in the solution depends on the solubility product of hydroxides. Other findings, from Christensen and Tjell (1983), Christensen (1989) and Christensen (1984) add to the complexity of the picture by saying that under acid pH conditions Cd solubility will increase with the addition of Cd in the soil; at higher pH, however, this equilibrium is governed by the solubility product of compounds other than hydroxides. At pH > 7.5, $CdCO_3$ may be formed; this compound, along with others which are formed at high pH, like $Cd(PO_4)_2$ may control the Cd mobility in the solution. Above a certain pH value, further addition of Cd in the soil will not affect the metal solubility in the solution. Figure 2.2 shows the adsorption of heavy metals plotted against the pH of the soil solution. There is strong evidence that the elevated solubility of heavy metals reflects

Figure 2.2: ***Effect of pH in adsorption of heavy metals (From Elliott* et al.*, 1986).***



their increasing bioavailability. Oliver *et al.* (1996) found a dramatic drop in Cd concentration in wheat grain by a factor of 2, when soil pH increased from 4 to 6.

pH also affects Cation Exchange Capacity (CEC) of soils. For the variable charged soil constituents (such as Fe and Al oxides, organic matter and edges of clay minerals, whose charge depends on pH (as opposed to permanently charged soil constituents that do not change with pH)), there is a pH value at which the net surface charge is zero, which is called the Point of Zero Charge (PZC). The pH-dependent solid matter of soils has a positive charge below PZC and a negative charge above PZC. This is very significant for metal adsorption - and subsequently for metal availability to plants- because above a certain pH value CEC of soil increases. This can partly explain the fact that metal adsorption increases with a rise in soil pH.

### 2.1.2 Redox Potential

Reducing conditions generally cause a reduction in heavy metal mobility (Gonsior *et al.*, 1997). Kabata-Pendias and Pendias (1992) supported this by saying that when the redox potential drops to -0.14 V the solubility of Cd decreases corresponding to the reduction of S:

$$SO_4^{2-} \rightarrow SO_3^{-} \rightarrow H_2S$$

$H_2S$ precipitates with Cd in the form of CdS, which is an insoluble compound. Evans (1989) explained further these processes by saying that both $SO_4^{2-}$ and $HS^-$ affect metal mobility: if $SO_4^{2-}$ is predominant (aerobic conditions) there are acidic pH values in soil which increase the metal mobility, and if $HS^-$ is produced (anaerobic conditions) metal mobility decreases, due to MS precipitation. The same author gave the following formulae:

(1) $SO_4^{2-} + 9H^+ + 8e^- \leftrightarrow HS^- + 4H_2O$, with $K_{redox}$ and

$$(2) \qquad E_h = \frac{RT}{8F} \ln K_{redox} + \frac{RT}{8F} \ln \frac{(SO_4^{2-})}{(HS^-)} + \frac{9RT}{8F}$$

From formulae (1) and (2) we get:

$$E_h = 0.249 + 0.0074 \log \frac{(SO_4^{2-})}{(HS^-)} - 0.066 \text{ pH}$$

where: $E_h$ is the redox potential.

According to the latter formula, $HS^-$ will be predominant at low $E_h$ values (under anaerobic conditions in flooded soils), due to the activity of microbes in the soil. Under these conditions, mineral sulphides will be formed, which will control the solubility of these metals in the soil solution. Metals expected to precipitate as sulphides include $Ag^+$, $Ni^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Hg^{2+}$ and $Fe^{3+}$.

On the other hand, although Xiong and Lu (1993) found that the availability of Cd decreased in flooded soils, this was attributed not to CdS formation (because the $E_h$ value was not appropriate), but to the transfer of Cd to other forms.

However, Alloway (1995a) reported that reducing conditions may not always cause a decrease in metal solubility. He said that reducing conditions may cause the dissolution of Mn, Al and Fe oxides, and this may release the co-precipitated metals into the soil solution.

### 2.1.3 Effect of Other Ions

The competition or synergism between heavy metals and/or with other ions present in the soil system has a very significant effect on metal mobility in solution and in the root zone. An overview of antagonistic and synergistic behaviour of the elements is presented in Figure 2.3. A lot of research effort has been put into the investigation of the interactions of Cd and Zn. The findings appear to be contradictory. Work supporting the synergism suggests a mechanism, by which as Zn concentration increases it exchanges with Cd from the solid phase and thus it increases the solubility and mobility of Cd (Wallace *et al.*, 1980). However, most works suggest an antagonism. This is related to the favourable uptake of one metal, when in higher concentrations, over the other (McLaughlin *et al.*, 1996; Alloway, 1997).

A well recognised competition occurs between macronutrients, like Ca, P and S and heavy metals. Alloway (1995b) reported that when the $Ca^{2+}$ concentration in the solution increases from $10^{-3}$ to $10^{-2}$ M the Cd concentration decreases by 67%. Lead mimics the physiological behaviour of Ca, and thus it may inhibit the function of several enzymes in plants. With high P contents, at neutral to alkaline pH, a precipitation of $Cd(PO_4)_2$ takes place

**Figure 2.3:** ***Interactions of trace elements within plants and at the root surface (From Kabata-Pendias and Pendias, 1992).***

Adjacent to roots

| | Li | Rb | Cu | Zn | Cd | B | Al | Si | Pb | V | As | Se | Cr | Mo | W | Mn | Fe | Co | Ni |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Li | ■ | | | | | | | | | | | | | | | | | | |
| Rb | α | ■ | | | | | | | | | | | | | | | | | |
| Cu | | | ■ | | γ | | α | | | | | | | α | | α | | | |
| Zn | δ | δ | α | ■ | β | | | | | | | | | | | α | α | | |
| Cd | | | | α | ■ | | | | | | | | | | | | | | |
| B | | α | | | | ■ | | α | | | | | | | | | | | |
| Al | | | | | α | | ■ | | | | | | | | | | | | |
| Si | | | | | | α | | ■ | | | | | | | | | | | |
| Pb | | | | δ | β | | | | ■ | | | | | | | | | | |
| V | | | | | | | | | | ■ | | | | | | α | | | |
| As | | | | δ | | | | | | | ■ | | | | | α | | | |
| Se | | | α | α | α | | | | | β | | ■ | | | α | | | | |
| Cr | | | δ | | | δ | | | | δ | | | ■ | | | α | α | | |
| Mo | | | α | | | | | β | | | | | | ■ | α | α | α | | |
| W | | | | | | | | | | | | | δ | | ■ | | | | |
| Mn | β | α | γ | α | γ | δ | | γ | | | α | α | α | γ | | ■ | | | |
| Fe | β | | α | α | γ | γ | | α | | | | | α | α | | α | ■ | α | |
| Co | | | | | | | | | | | | | | | | α | α | ■ | |
| Ni | | | γ | γ | γ | | | | | | | | | | | δ | | | ■ |

Plants

α*: Antagonism,* β*: Synergism,* γ*: Antagonism and/or synergism,* δ*: Possible antagonism*

(Jing and Logan, 1992; Jing and Logan, 1991). Kirkham (1977) found that metals are not translocated from roots to shoots in plants at high P contents. In contrast, Villaroel *et al.* (1993) reported that an addition of P up to 400 kg ha$^{-1}$ encouraged Cd and Zn uptake by Swiss chard in sewage sludge-amended soils. Sulfur, another macronutrient, inhibits the transport of Pb in the plants from roots to shoots and it was found that S-deficiency increased Pb movement to the tops (Kabata-Pendias and Pendias, 1992).

### 2.1.4 Organic Matter

#### 2.1.4.1 Introduction

Once sewage sludge has been applied to the soil, the organic matter of sludge origin is rapidly acted upon by soil microorganisms and the more easily decomposable groups are transformed. These microbial processes are responsible for the effects of sludge on soil properties, both physical and chemical. In physical terms organic matter improves the stability of soil aggregates resulting in better aeration of the soil and better water retention. It also improves the hydraulic conductivity as a result of the increasing percentage of soil macropores. In chemical terms, some of the sludge-borne organic matter is quickly humified and it increases the solid phase of the soil, due to its large specific surface (Stevenson, 1982; Saar and Weber, 1982). Thus, it contributes to the increase of the adsorption sites. Terry *et al.* (1979) found that only after 168 days of sludge application 67 % of the sludge organic matter had been humified. It can thus be said that sewage sludge acts both as a source of heavy metals and also as a sink for their retention. Moreover, humified organic matter in the soil is extremely complex, as it contains functional groups which support a multi-ligand association with metals (Figures 2.4 and 2.5). Table 2.1 shows a typical composition of humified organic matter.

#### 2.1.4.2 Background Theory

Humic acid has a pH-dependent behaviour. Spark *et al.* (1997a) reported that it is insoluble in the soil solution at acidic pH. As the pH increases it dissolves in the solution and the deprotonation of the functional groups increases the repulsion forces amongst the humic acid molecules. This leads to a more open and linear form at high pH, compared with the 'spherocolloidal' form at lower pH values. The same was suggested by Taylor and Theng (1995). Organic ligands on humic and fulvic acids behave as soft Lewis bases, while metals are soft acids and this is why they tend to form complexes.

**Table 2.1:** ***Functional group composition (mmol$_c$ 100g$^{-1}$) of humic acids (From McCarthy, 1989).***

| | Cool, Temperate | | Subtropical |
|---|---|---|---|
| Functional groups | Acid soils | Neutral soils | |
| Total acidity | 570-890 | 620-660 | 630-770 |
| COOH | 150-570 | 390-450 | 420-520 |
| Acidic OH | 320-570 | 210-250 | 210-250 |
| Weakly acidic and Alcoholic OH | 270-350 | 240-320 | 290 |
| Quinone and Ketone C=O | 10-180 | 450-560 | 80-150 |
| $OCH_3$ | 40 | 30 | 30-50 |

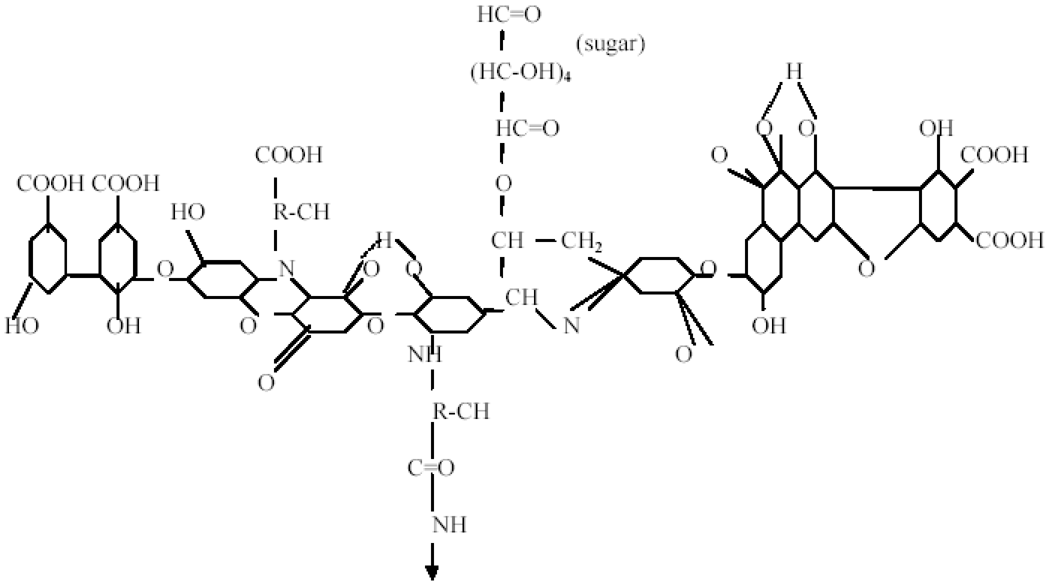
The complexation reaction follows the formula:

$$R\text{-}L^{l-} + M^{m+} \leftrightarrow R\text{-}L\text{-}M^{m-l}$$

where: R is the C-chain, L the active group which actually binds, M the metal and m and l are the valencies of metal and ligand, respectively.

The main complexing functional groups in the organic matter are carboxyls, phenols and alcohols (Tamakatsu and Yoshida, 1977). Bonding may be either covalent, where the metal and counter ion contribute one electron each, or coordinate, where the ligand provides both electrons. Theoretically, any organic molecule containing trivalent N or P or divalent O or S can act as a ligand (Livens, 1991).

**Figure 2.4:** ***A hypothetical humic acid molecule (From Livens, 1991).***

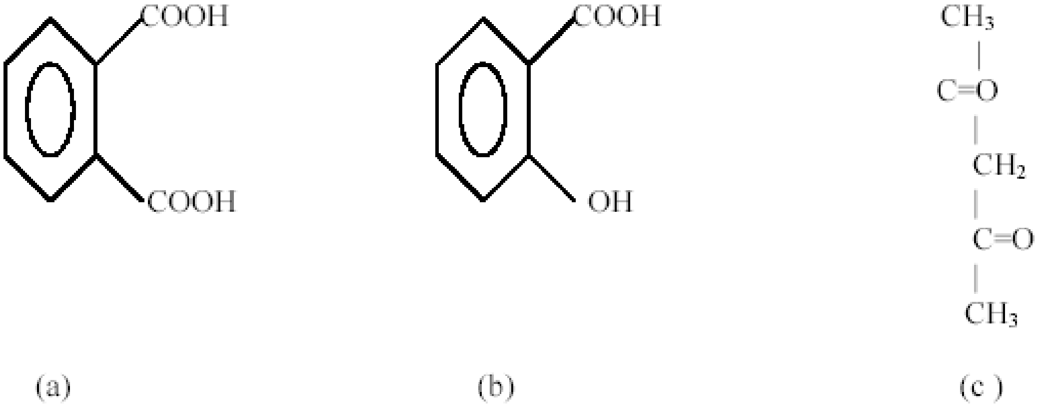


There are two main approaches to explain the binding of metals by organic matter. The first is the discrete ligand model (DLM) and the second the continuous distribution model (CDM). The DLM approach suggests that only a few ligands on the humic substance are required to fit experimental data. Given the complexity of the humic acids, these ligands (or 'ligand classes') must be less than the actual total number of all ligands present on the humic acid. These ligand classes are characteristic of the chemical behaviour of all ligands. This approach gives a way to mimic the binding capacity of humic acids (Dzombak *et al.*, 1986). For an individual adsorption site $v_i$ we have the following formula:

$$v_i = \frac{Ki\,[M]}{1 + Ki\,[M]}$$

where: Ki is the stability constant of the binding of metal M in the site (or ligand class), $v_i$ and [ ] are the concentrations (Dzombak *et al.*, 1986).

**Figure 2.5**: ***Important functional groups for the complexation of metals: a) Phthalate, b) Salicylate and c) Acetylacenate (From Livens, 1991).***



The CDM approach allows a large number of sites to be involved in binding metals. In this model, there is no a discrete Ki defined, but rather a continuum of K values. This model assumes an irregular frequency distribution of the functional groups. The formula describing the macroscopic free lingand concentration, $L_T^f$ is:

$$[L_T^f] = \int_0^\infty \frac{L(K)\,dK}{1+K[M]}$$

which is the analogue of the equation for DLM (Evans, 1989; Dzombak *et al.*, 1986).

### 2.1.4.3 Decomposition of Organic Matter

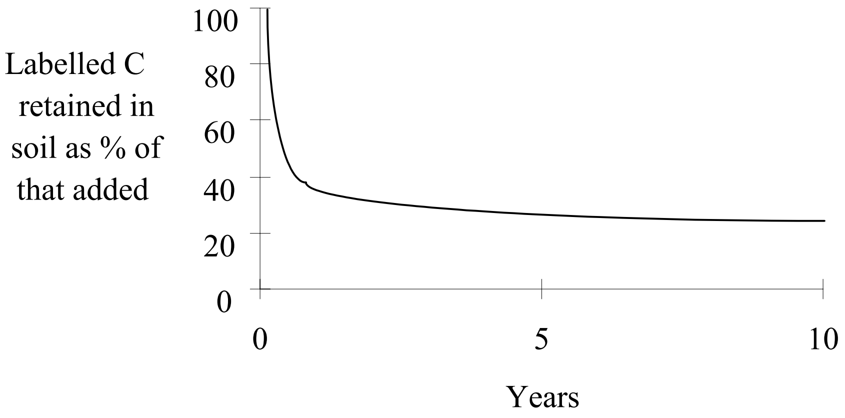
The decomposition of the organic matter in the soil generally follows the trend described in Figure 2.6. Gregorich *et al.* (1998) and Logan *et al.* (1997) reported that the organic matter content of sewage sludge treated soils had decreased significantly even 6 months after sludge application. It has been reported that the total acidity of the organic matter, after application of sewage sludge, increased along with the concentration of phenolic groups (Senesi *et al.*, 1989; Gigliotti *et al.*, 1997).

However, different compounds are decomposed in different ways; a portion of organic matter decomposes very rapidly, while others are very resistant. Linehan (1985) found that compounds like glucose, amino acids, pyruvate and uridine are quickly decomposed, while lignin-like compounds are very slow to decompose.

Decomposition also depends on specific environmental factors, such as temperature, pH, moisture, oxygen availability and inorganic nutrients (Jenkinson, 1988). This researcher further explained that decomposition is slower under water logged conditions, because the microbial activity ceases. This was also found by Campbell (1978). The soil clay content may be another factor affecting the decomposition.

Boddy (1983) found that clay protects organic matter from being decomposed and montmorillonite is more effective than illite or kaolinite in this protective role. Temperature is another well recognised factor. At higher temperatures the activity of the micro-organisms is more intense and the decomposition accelerated. An increase in temperature from 5 to 25 $^0$C increased the $CO_2$ evolution from the soil (Boddy, 1983). This occurs, provided there are no other limiting factors. The same worker found that at high moisture contents decomposition was slower, due to lack of sufficient $O_2$. In cases where $O_2$ supply is good, the 'temperature' effect is even accelerated. Ramunhi *et al.* (1987) reported that high $O_2$ availability in the soil leads to higher decomposition rates, and Knipers (1991) agreed by saying that ploughing accelerated the organic matter decomposition rate.

**Figure 2.6:** ***Decomposition of organic matter (From Jenkinson and Rayner, 1977).***



It has been reported that the organic-N status in the soil organic matter is an indication of the mineralisation of the organic matter (Stevenson, 1982). Nakas and Klein (1980) gave a formula to describe this:

$$\text{Sludge mineralised \%} = 6.37 * \text{organic-N \%} + 4.63$$

#### 2.1.4.4 Interactions of Metals with Organic Matter and their Role on Metal Bioavailability

According to Livens (1991) organo-metallic interactions can be divided into two groups:

1) Ionic interactions (cation exchange). This includes alkali metals (K, Na, Li) and Group II elements (Be, Mg, Ca). These elements are very electropositive, have low ionisation potentials and tend to interact with acidic groups, such as carboxylic and phenolic-groups.
2) Non ionic bonds, which can be either covalent (each element shares one electron) or coordinate (one element gives both electrons). This includes elements like heavy metals; they tend to interact especially with groups containing trivalent elements, like N and P.

It is, thus, evident that heavy metals are influenced by the presence of humic acids, as many times heavy metals have been reported that they are organically bound (Flores *et al.*, 1997; Zhang *et al.*, 1997). Especially for Cu and Pb, speciation depends on organic matter (Campbell and Evans, 1987; Temminghoff *et al.*, 1997). Humic acids bind metals and reduce their availability to plants (Piccolo, 1989) and, thus, metal retention by the soil increases when sewage sludge is applied. He and Singh (1993) found that application of sludge increased the CEC value of the soil (that is the ability of the soil to retain metals). This was further supported by Hargitai (1989), who showed that Pb, Ni, Zn and Cd did not increase their available forms proportionally with sewage sludge application. He also quantified this, by developing an Environmental Protection Capacity (EPC) factor, according to the formula:

$$EPC = D_xH^2K$$

where: $D_x$ is the thickness of the soil layer in cm, H the soil humus content and K is a constant depending on the humus quality.

The higher the EPC value, the less metals are available. When the humus content increases with sewage sludge application, the EPC factor increases and the heavy metal availability is depressed. The effectiveness of humic acids in reducing metal availability is related to its ionisation state, which in turn depends on pH. As stated, humic acids are very insoluble at low pH, having a spherical form, but as pH increases they are ionised, become deprotonated (that is, they provide more adsorption sites) and develop a greater surface area, as they become more linear. This happens up to a pH of 6-8. According to Spark *et al.* (1997a), the solubility of all heavy metals is associated with the solubility of the humic acid; as humic solubility increases (with an increase at pH), metal sorption on it increases. The same workers concluded that humic acids have a greater affinity for metals than for inorganic metal-hydroxide forms, and, thus, they may control metal solubility into the solution. Moreover, Taylor and Theng (1995) found an increased sorption of Cd on the humic-kaolinite solid phase at pH 5-6, which increased with increasing levels of humic acids added to the system, up to 350 $\mu g\ C\ g^{-1}$. They also found that the higher the ionisation of the carboxyl groups, which are highly responsible for the humic activity, the higher the negative charge of humic acids, and, subsequently, the higher the adsorption of metals up to a pH value of 7.6. This pH value, according to PZC measurements (Point of Zero Charge) gave the maximum electronegativity for humic

**Table 2.2:** ***The order of relative affinity for metals of humic and fulvic acids.*.**

| Material | Relative affinity | Reference |
|---|---|---|
| FA (pH 5) | Cu > Pb > Zn | Elliott *et al.* (1986) |
| HA (pH 4.5) | Pb > Cu > Zn > Ni > Cd = Cr | Gao *et al.* (1997) |
| HA (pH 6.5) | Pb > Cu = Zn > Cd > Ni > Cr | Gao *et al.* (1997) |
| HA (pH 4-7) | Zn > Cu > Pb | Elliott *et al.* (1986) |
| HA (pH 4-8) | Cu > Pb >> Cd > Zn | Elliott *et al.* (1986) |
| HA | Pb > Cu > Ni > Zn > Cd | Schnitzer and Khan (1972) |
| HA pH(3.5) | Cu > Ni > Co > Pb > Zn | Linehan (1985) |
| HA (pH 5) | Ni > Co > Cu > Pb > Zn | Linehan (1985) |
| HA (pH 4-7) | Hg = Pb = Cr > Zn > Ni = Zn | Evans (1989) |
| HA (pH 5.8) | Hg = Pb = Cr = Cu > Cd > Zn > Ni | Evans (1989) |

substances. Heavy metals differ in their ability to form stable complexes with organic matter, or, put another way, organic substances exhibit an order of selectivity in adsorbing metals. Table 2.2 shows the relative affinity sequences that have been reported by various workers. Although there is an agreement on the sequences, some metals show inconsistent behaviour. This is especially evident in case of Cd and Zn. Elliott *et al.* (1986) also discussed this, and concluded that this inconsistency seems to depend on differences in the experimental conditions.

#### 2.1.4.5 The Role of DOC (Dissolved Organic Carbon) on Heavy Metal Availability

Soil organic matter can be fractionalised according to its solubility characteristics. Soil organic matter is treated with NaOH and the reaction gives humin (which precipitates) and fulvic and humic acids (which are dissolved). Then the solution is treated with HCl and the reaction allows humic acid to precipitate, while fulvic acid remains dissolved. Thus, organic matter consists of humin (which is an insoluble and chemically inactive material), humic acid (soluble in bases, that is at alkaline pH values) and fulvic acids (soluble in a wider range of pH) (Chefetz *et al.*, 1998). Fulvic acids are organic substances, which predominantly have lower molecular weights than humic acids, usually not more than 5,000 g mol$^{-1}$, and are often called Dissolved Organic Carbon (DOC). DOC comprises amino acids, aliphatic acids, peptide and sugars and their polymers (Table 2.3). These low molecular weight materials have a different behaviour from humic acids in many ways, two of the most important differences being that they are dissolved in aqueous media in the range of pH normally found in soils and that they can be taken up by plant roots directly, along with the metals that they have bound (Hamon *et al.*, 1995; Alloway, 1997). There is evidence that fulvic acids are present in sewage sludge, but also that decomposition of larger organic molecules may lead to the increase of the quantities of fulvic acids present in the soil (Linehan, 1985).

DOC is often reported to reduce metal adsorption on solid state soil constituents by either competing more effectively for the free metal ion and forming organo-metalic complexes or being preferentially adsorbed onto the solid phase (Guisquiani *et al.*, 1998). DOC forms bonds with heavy metals controlling their solubility and reducing their adsorption on soil surfaces. This behaviour is more effective at near neutral pH values than at other pH values (Goody *et al.*, 1995). This is probably because, organo-metallic complexes are predominant at neutral pH, while at higher pH values, hydroxide, carbonate and phosphate associations control metal solubility and at acidic pH values free metal ion are predominant (Lebourg *et al.*, 1998; Reddy *et al.*, 1995). Metal-ligand complex stability generally decreases with a reduction in pH, reflecting the role of $RCOO^-$ in metal complexation (Naidu and Harter, 1998). However, the same authors reported that at very low pH values (below 2), when soil surfaces are positively charged, organic ligands are adsorbed and alter the surface charge; in this way they attract positively charged heavy metals, increasing their adsorption.

**Table 2.3:** ***Organic C, N and P distribution in the DOC (From Harter and Naidu, 1995).***

| Analysis | Concentration, mol $m^{-3}$ | %, Total soluble |
|---|---|---|
| | C | |
| Total | 130 ± 2 | 100.0 |
| Polysaccharide | 15.6 | 12.6 |
| Amino acid | 33.2 | 25.5 |
| Hexosamine | 11.7 | 9.0 |
| Stream distillable | 11.1 | 8.5 |
| Not identified | 5.8 | 45.0 |
| | N | |
| Total | 18.4 ± 0.7 | 100.0 |
| Amino acid | 8.62 | 46.8 |
| Hexasamine | 1.67 | 9.1 |
| Ammonium | 0.52 | 2.8 |
| Not identified | 8.75 | 30.3 |
| Hydrolyseable | 16.2 | 89.0 |
| Non-hydrolyseable | 2.71 | 11.0 |
| | P | |
| Total | $4.84 \pm 10^{-3}$ | 100.0 |

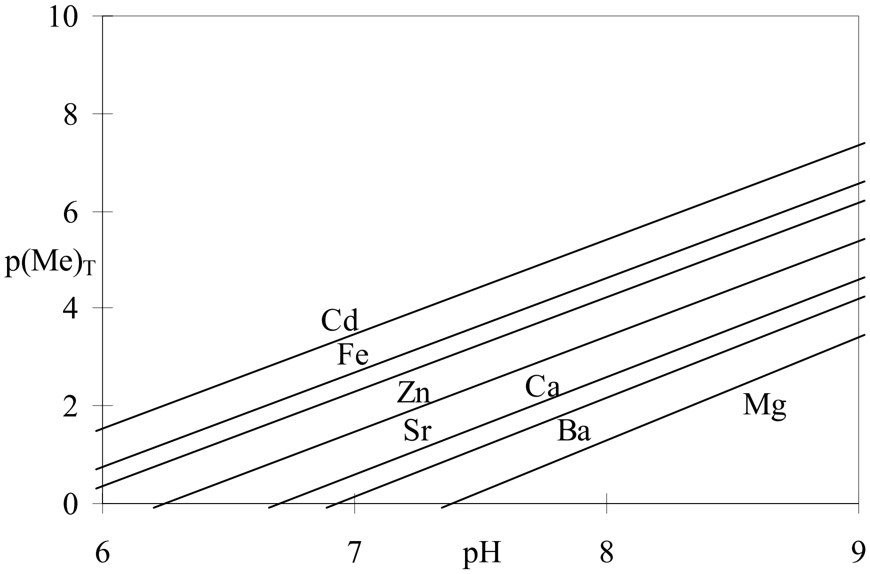
They also reported that, although at low pH ligands increase metal adsorption, at neutral pH values only ligands which form stable complexes (with low pK metal-ligand association values) will be effective in reducing metal adsorption. It is very important to note that aliphatic acids with low pK values (which form more stable bonds with metals) are predominant in mature sludges, while aliphatic acids with higher pK values are mostly predominant in fresh sludges (Baziramakenga and Simard, 1998). This may have environmental implications, because months after the sewage sludge application to a field the solubility of metals may be significantly altered.

Although most researchers reported that DOC increases metal solubility, some findings rather suggest that DOC increased metal adsorption. Chubin and Street (1981) studied the effect of EDTA on Cd adsorption on kaolinite and montmorillonite and they found that EDTA helped Cd to be adsorbed on the solid phase. This was explained by either the organo-clay complex, which has higher affinity for $Cd^{2+}$ than clay alone, or the formation of a clay-$Cd^{2+}$ organo-$Cd^{2+}$ bridge that increased the adsorption capacity of the system.

### 2.1.5 $CaCO_3$

At high pH values calcite ($CaCO_3$) sorbs Cd, as $CdCO_3$, and diminishes its availability. The activity of $CdCO_3$ does not only depend on its solubility product, but on the partial pressure of $CO_2$ ($pCO_2$) in the soil. This, in turn, effects the solubility of

**Figure 2.7:** ***Dissolution of some metal carbonates as a function of pH (From Evans, 1989).***



$CO_2$ in the soil solution. The increasing solubility of $CO_2$ enhances the activity of $CdCO_3$ and thus encourages the decrease in Cd availability (Evans, 1989). Moreover, Papadopoulos and Rowell (1988) found that in a system where calcite is predominant, as Cd concentration increases, $CdCO_3$ precipitates and controls Cd solubility. Precipitation decreases the pH value of the system, as explained from the following formula:

$$HCO^{3-} + Cd^{2+} \leftrightarrow CdCO_{3\,(s)} + H^{+}$$

Calcium is then under-saturated, because Ca is excluded from $CaCO_3$ surface as a result of $CaCO_3$ being covered with $CdCO_3$. Evans (1989) stated that other metals likely to precipitate as carbonates at high $pCO_2$ and pH values, along with $Cd^{2+}$, are: $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$, $Fe^{2+}$, $Zn^{2+}$ and $Pb^{2+}$ (Figure 2.7). Above pH 7.3 the order of carbonates precipitated are:

$$Mg > Ba > Ca > Sr > Zn > Fe > Cd$$

McBride (1980) and Papadopoulos and Rowell (1989) provided an explanation for this sequence by saying the affinity of calcite for metals is related with their ionic radius. This is more evident in case of $Cd^{2+}$, which at high pH substitutes for $Ca^{2+}$, by chemisorption, because they have a very similar ionic radius (0.97 and 0.99 Å, respectively) and the radius of Cd is more appropriate for the sorption.

### 2.1.6 Other Factors

Apart from those mentioned above, there is a range of other factors that affect the adsorption of heavy metals on solid phases or control their solubility. Oxides, especially Fe and Al, are found to affect metal solubility. Bell *et al.* (1991) found that Cd can be associated with polycrystalline Fe oxides, which diminish Cd availability. Harmsen (1977) stated that in freely drained soils and especially under dry conditions the associations between metals and Fe and Al oxides are much stronger, due to the loss of the hydration water of the metals.

Another factor affecting metal availability is the chloride ions; high concentrations of $Cl^-$ may increase the availability of Cd to plants (Sommers and McLaughlin, 1996a; Sommers and McLaughlin, 1996b). At high salinity levels $Cl^-$ may complex with Cd and form $CdCl_2^0$ and $CdCl^-$. The lower the charge of the Cd ion after complexing with $Cl^-$, the greater the concentrations of $CdCl_n^{2-n}$ species ($CdCl^+$, $CdCl_2^0$) in the soil solution (McLaughlin *et al.*, 1997). These complexes are very stable and can prevent Cd from being re-adsorbed on the solid phases and, thus, increase Cd availability (Smolders *et al.*, 1998). Schirado *et al.* (1986) found 10.3 % of Cd was complexed with Cl in saline soils. The presence of $Cl^-$ in the soil solution can be attributed to either salinity in the soil (McLaughlin *et al.*, 1994), application of sewage sludge or the presence of other contaminants such as landfill leachate. Epstein *et al.* (1976), in their early work, found that $Cl^-$ increased in the soil solution with increasing application of sewage sludge. However, a few months after the termination of application $Cl^-$ approached the control values, due to leaching.

The soil physical state may also affect heavy metal availability. The soil physical state is connected with good or poor aeration (freely or poorly drained soils), which affects the production of sulphides. In addition, good aeration leads to accelerated organic matter decomposition, which in turn affects metal availability, as discussed earlier. The clay content provides, along with the organic matter, the adsorptive colloidal complex for the adsorption of heavy metals. It is found that organic matter mineralisation is slower in clayey soils, and there is evidence that the reason for that is that clay protects organic compounds from being decomposed (Jenkinson, 1988). This protection further affects heavy metal availability.

## 2.2 HEAVY METAL MOVEMENT DOWN THE SOIL PROFILE

The movement of heavy metals down the soil profile is of a great importance, because it involves the risk of groundwater contamination and deterioration of drinking water quality. The literature provides examples of cases of massive heavy metal movement up to 3m depth (Lund *et al.*, 1976). Movement is often evident in high applications of heavy metals, usually in a sewage sludge, in soils with low organic matter and clay contents, acidic conditions, and when high rainfall or irrigation water rates have been applied. The movement occurs through soil macropores or cracks which is also referred to as preferential flow (Dowdy and Volk, 1983). There is, however, disagreement about whether the metals move significantly under normal conditions or not. El-Hassanin *et al.* (1993) found that Cd, Zn and Pb only moved in the surface soil layer and, similarly, Higgins (1984) found that no heavy metal movement to the B horizon was evident. Chang *et al.* (1982) reported that four years after the termination of sewage sludge application heavy metals were deposited only in the 0-15 cm upper soil layer, and Harmsen (1977) found that Cd and Pb did not accumulate below 40 cm of the surface in a heavily contaminated Zn smelter area. Dowdy and Volk (1991), likewise, found that heavy metals did not move significantly below the incorporation zone (or plough layer). The same was concluded by Williams *et al.* (1980), Emmerich *et al.* (1982) and Miner *et al.* (1997).

In contrast to the above, work has also been published which suggests that metals moved below the incorporation zone, under certain conditions. Schirado *et al.* (1986) at a site where raw sewage wastewater had been applied for 5 decades found a rather uniform distribution of Cd, Zn and Ni in the soil profile up to a depth of 150 cm, and this was attributed to the movement of the metals. In another field experiment, Li and Shuman (1996a) found movement of Zn down to a depth of 75 cm and of Cd and Pb to a depth of 30 cm. Although Pb is a relatively immobile element, very strongly bound on the solid phase, Stevenson and Welch (1979) applied it as an inorganic salt in extreme quantities of up to 3.2 t ha$^{-1}$ and found movement to a depth of 75 cm.

In addition to these factors, several workers have hypothesised that DOC is also important in the movement of metals down the profile of sewage sludge-amended soils. Well decomposed, low molecular weight organic compounds may facilitate the transport of heavy metals down into the soil profile (Jardine *et al.,* 1992). Guisquiani *et al.* (1992) tested this hypothesis and found some DOC effect, but not with a very substantial contribution. Li and Shuman (1997) investigated this in 40 cm long columns leached with EDTA, $CaCl_2$ and effluent from poultry litter containing 2340 mg DOC L$^{-1}$. This effluent was found to accelerate Cd and Zn movement in contaminated soil, while it increased the adsorption of Zn in the uncontaminated soil columns.

Although the picture is not clear whether there is movement, the existing evidence should be considered seriously and certain soil parameters should be studied before sewage sludge is applied in order to avoid any risk of groundwater contamination.

## 2.3 UPTAKE AND ACCUMULATION OF HEAVY METALS INTO CROP PLANTS

### 2.3.1 Uptake and Accumulation

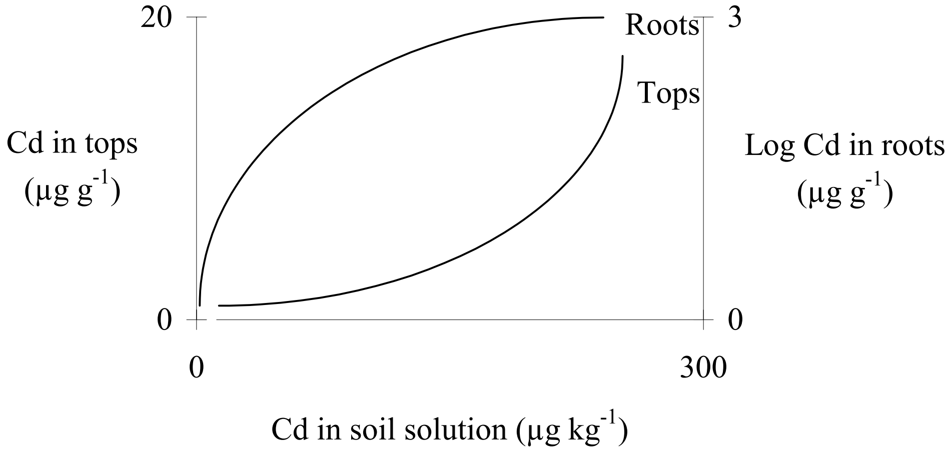
All the above factors reviewed in section 2.1 may affect the uptake of heavy metals in plants. Soil pH, redox potential, organic matter, Fe and Al oxides, soil physical conditions, $CaCO_3$ and $Cl^-$ are factors that determine much of what is potentially available for uptake, but it should be stressed that other parameters not studied here (such as plant genotype and root exudates) are important as well. One of the greatest concerns is that after sewage sludge has been applied to soils, the heavy metals carried in it may accumulate in plants and, subsequently, enter the human food chain or have toxic effects on plants and/or on animals grazing on them.

Heavy metal concentrations in plants depend very much on the concentrations (both total and available) of the metals in the soil in which the plants are grown. Some researchers, however, have found no relationship between metal concentration in soil and in the plant. Kuo (1990) and Del Castilho and Chardon (1995) found no apparent relationship between DTPA-extractable Cd and plant Cd in 5 soils (that differed in chemical properties) with a pH of 5-6. The same was also found by Barbarick and Workman (1987) and O' Connor (1988).

While Cd and Pb are non-essential elements with no biological function within the plant, Ni and Zn are essential micronutrients for plant growth. However, both Ni and Zn can cause toxicities when they are present in plants in excess. Zinc can be relatively easily translocated from roots to shoots in a plant, and in high concentrations it tends to accumulate in mature leaves. Other heavy metals, however, stay in the roots and move slowly, if at all, upwards (Wang *et al.*,1997) (Figure 2.8).

Several ways by which plants can tolerate heavy metals have been reported. Kabata-Pendias and Pendias (1992) reported the following: 1) Selective uptake of ions, 2) Decreased permeability of membranes, 3) Immobilisation of ions in roots, foliage or seeds, 4) Removal of ions from metabolism by deposition in insoluble forms, 5) Alteration in metabolic patterns: Increased enzyme system that is inhibited, or increased antagonistic metabolite, or reduced metabolic pathway by-passing an inhibited site, 6) Adaptation to toxic metal replacement of a physiological metal in an enzyme, 7) Release of ions to plants by leaching from foliage and excretion from roots.

**Figure 2.8:** ***Cd in soil solution, roots and shoots (From Kabata-Pendias and Pendias, 1992).***



However, it is evident that different plant species and different cultivars in the same species have different capabilities in tolerating heavy metals (Young *et al*, 1995). That is, different plants, grown in the same soil contaminated with heavy metals, may accumulate variable quantities of these metals. The response of some plant species to heavy metal exposure is shown in Table 2.4.

### 2.3.2 Residual Effect of Heavy Metals

After sludge has been applied to soils, the heavy metals introduced to the soil environment interact with other soil components for a long time. Alloway and Jackson (1991) in their review stated that it is difficult to assess whether heavy metals remain highly available to plants or not many years after sewage sludge application has ceased. Most of the research works report that metal availability tends to decrease after the termination of sludge application. Chaney and Ryan (1993) found that metal availability decreased after the termination of sewage sludge application. They argued that metal availability is more likely to be kept to a minimum in sludge-amended soils due to specific adsorption capacity of soils after the addition of sludge-borne organic matter. However, McBride (1995), in his review paper, criticised this work by saying that soil organic matter will not possess the same ability to sorb heavy metal over time; this capacity is likely to decrease with maturing, probably due to organic matter decomposition. He also stated that research works tend to underestimate metal dynamics with sludge application; metal availability is measured by plant shoots uptake, but 'uptake may be suppressed by changes in the translocating efficiency of the plant', which may change over time. Chang *et al.* (1997) discussed two hypotheses likely to be valid after the termination of sewage sludge application. The first is the 'Plateau effect', in which heavy metal accumulation in plants reaches a maximum and will not substantially increase, even if heavy metal loadings in sludge increase. The second is the 'Time bomb', in which heavy metal concentrations of plant tissues remain unchanged or rise slowly soon after the termination of sewage sludge, but as organic matter is decomposed in the soil the heavy metal concentrations of the plant tissue rise rapidly. They concluded that data point to the first hypothesis, but they admitted that this conclusion could not be definite, as more data would be needed.

**Table 2.4** ***Relative accumulation of heavy metals by different crops (From Kloke*** **et al.*****, 1984).***

| High Uptake | Moderate uptake | Low uptake | Very low uptake |
|---|---|---|---|
| Lettuce | Kale | Cabbage | Snapbean family |
| Spinach | Collards | Sweet corn | Peas |
| Chard | Beet | Broccoli | Melon family |
| Escarole | Turnip roots | Cauliflower | Tomato |
| Endive | Radish globes | Brussel sprouts | Pepper |
| Turnip green | Mustard | Celery | Aubergine |
| Carrot | Potato | Berry fruits | Tree fruits |

### 2.3.3 Toxicity Symptoms in Plants

Heavy metals, as well as other elements, when present in excess in plant tissues can cause toxic effects to plants. Cadmium and Pb can change the permeability of cell membranes. All heavy metals have an ability to react with phosphatic groups in plant cells, like ATP and ADP, and reduce their activity. Especially Pb mimics P and can replace it from ATP and ADP; this can jeopardise the biochemical functions of these compounds. Lead also causes the development of dark green leaves, wilting in older leaves, stunted foliage and brown short roots. Cadmium, when in excess, can cause brown margins on leaves, chlorosis, reddish veins and petioles, curled leaves and brown stunted shoots. In rice, severe reduction of root and top growth have been reported. Cadmium also inhibits photosynthesis and $CO_2$ fixation. Zinc depresses nutrient uptake, when in excess, and, thus, reduces plant growth. It also causes chlorosis to new leaves. Nickel causes chlorosis, which is Fe-induced, because Ni inhibits the translocation of Fe from roots to shoots (Kabata-Pendias and Pendias, 1992; Alloway, 1995b).

However, the toxicity symptoms, when heavy metals are in excess in the plant tissues, depend on many other factors, such as plant genotype. As mentioned above (Table 2.4) different plant cultivars are more susceptible than others to heavy metals.

**Table 2.5:** ***Soil tests significantly correlated with plant heavy metal concentrations (From Alloway and Jackson, 1991).***

| Reagent | Elements | Test crop |
|---|---|---|
| 0.005 M DTPA | Zn, Cd, Cu, Mn | Tabacco |
| 0.005 M DTPA | Ni | Lettuce |
| 0.005 M DTPA | Cu | Soya beans |
| 0.005 M DTPA | Cu, Cd | |
| 0.005 M DTPA | Cd | Wheat |
| 0.005 M DTPA | Zn, Cu, Mn, Ni, Cd | Tabacco |
| 0.005 M DTPA | Cd | Potato |
| Ammonium bicarbonate DTPA | Cd, Cu, Ni, Pb, Zn | Swiss Chard |
| Ammonium bicarbonate DTPA | Mo | |
| DTPA, EDTA, $CaCl_2$ | Zn, Cu, Ni | Ryegrass |
| EDTA | Cd, Zn | |
| 0.43 M acetic acid | Ni | Ryegrass, clover |
| 0.01 M $CaCl_2$ | Zn, Cu, Ni | |
| 0.01 M $CaCl_2$ | Cd, Zn | |
| 0.01 M $CaCl_2$ | Cd | Lettuce, radish, carrot, cabbage |
| 0.1 M $NaNO_3$ | Cd | |
| 1 M $NH_4NO_3$ | Cd | Lettuce, radish, carrot, cabbage |

### 2.3.4 Effects of Heavy Metals on Microbial Biomass

Heavy metal deposition with sewage sludge can affect the population of microbes in soil very severely. It has been reported that $N_2$-fixing symbiotic bacteria *Rhizobium leguminosarum* bv. *Trifolii* are especially affected by heavy metals. Alloway (1995b) reported that Zn appears to be a serious hazard affecting *Rhizobium leguminosarum* bv. *Trifolii* even when present at concentrations within the limits set by the EU (300 mg $kg^{-1}$ soil). Chaudri *et al.* (1993), however, found that clover plants only at very heavy applications of sewage sludge (300 t $ha^{-1}$) had a low N-content and showed chrorosis symptoms due to N deficiency resulting from insufficient $N_2$-fixation (due to slow and delayed nodulation of *Rhizobium leguminosarum*). McGrath *et al.* (1988) reported that although heavy metals reduce the rates of $N_2$-fixation by clover due to *Rhizobium leguminosarum* toxicity, the bacteria population in soil does not necessarily decrease significantly. Smith and Giller (1992), on the other hand, did not agree with that and reported that in sludge-treated soils this family of bacteria can not survive.

### 2.3.5 Bioavailability Tests

Various extractants have been employed by many workers to predict the plant uptake of heavy metals. However, it was stressed by Beckett *et al.* (1983) that metal extractability and availability to plants are not necessarily the same, Beckett (1989) reported that the soil tests extracting ‘available’ forms fall into 3 categories:

- Displacing ion solution (such as $NH_4NO_3$, $CaCl_2$)
- Chelating agents (EDTA and DTPA)
- Dilute acids (such as 0.1 M HCl, 1 M $NHO_3$)

DTPA and EDTA are the most widely used extractants (Singh *et al.*, 1996; Xiu *et al.*, 1991). However, dilute salt solutions have been found to give a better prediction of available forms of heavy metals (Krishnamurti *et al.*, 1995; Symeonides and McRae, 1977) (Table 2.5). Extractions with 0.05 or 0.01 M $CaCl_2$ have recently been used by many researchers and, although they extract very low concentrations of metals, they have been found to give the best correlations with the heavy metal accumulation in plant tissues, among many other availability tests (Morgan and Alloway, 1984; Gupta, 1989).

## 2.4 CONCLUSIONS

- Heavy metals occur naturally in the earth' s crust, but their anthropogenic release into the environment causes the contamination problems related with them. From the heavy metals studied, Cd and Pb are not essential for plant growth, while Zn and Ni are. Excess of any of them can be toxic. The main anthropogenic sources of contamination may be the mining industry, aerial emissions by industries and cars, agrochemicals and sewage sludge application.
- Sewage sludge has been found to be a very useful fertiliser containing macro- and micronutrients and organic matter. The main limiting factor for its use is the load of heavy metals that it contains. If the application is not well managed, this may lead to a risk of heavy metal accumulation in the human food chain or movement down the soil profile, which in turn may cause the contamination of ground water resources.
- The main soil factors affecting heavy metal availability to living organisms include pH, organic matter, redox potential, certain hydroxides and $CaCO_3$.
- Under certain conditions, heavy metals may move down the soil profile. These conditions may be high rainfall or irrigation rate water application, soils low in organic matter and clay content and acidic pH soil environments.
- Heavy metals affect plants and microorganisms in the soil. Heavy metal uptake depends on soil availability factors, as well as the plant species' or cultivars' physiology.

# CHAPTER THREE: MATERIALS AND METHODS

This chapter outlines the general materials used and the methods followed in this project. Special methodologies used in individual experiments are described in the relevant chapters. It should be noted that all glassware and plasticware used were acid washed in 3 % HCl overnight and were then washed with ultra pure water 3 times, in order to avoid any contamination risk.

## 3.1 SOIL ANALYSIS

### 3.1.1 Sample Collection and Preparation

In the field, bulk soil samples were taken from the plough layer (< 20 cm) and several subsamples were obtained, in order to represent a reliable cross-section of the area. The samples were taken with a stainless steel spade. In the case of periodically taken samples from the field and the greenhouse, samples were obtained from three different points, in order to ensure representative sampling. The same practice was followed at the different sampling times, that is, the same sampling depth and the same equipment (auger for the field and spatula for the greenhouse pots, both made from stainless steel). In all cases, soil samples were put in clean plastic bags and were homogenised. They were, then, air-dried and were passed through a mesh sieve with 2 mm openings, after they had been disaggregated with a porcelain pestle and mortar. The samples were, then, put in clean plastic bags and sealed. The samples from the greenhouse and field experiments were stored at 4 $^0$C, while all the others were kept at room temperature. All analyses used the < 2 mm ‘fine earth’ fraction.

### 3.1.2 Soil pH

The method for measuring the pH in the soils was taken from Bascomb (1974). pH was measured in 1 : 2.5 w/v soil : water suspension and 0.01 M $CaCl_2$. 10 g of soil were weighed into a 50 mL plastic centrifuge tube and 25 mL of ultra pure water were added. The suspensions were shaken in an end-to-end shaker at 20 rpm at 21 $^0$C for 15 minutes. The samples were, then, left to stand for 1 hour and the pH value determined using a pH metre previously calibrated using 4.0, 7.0 and 9.2 buffer solutions.

After that, 2 mL of 0.125 M $CaCl_2$ was added to the suspensions. The samples were then shaken for another 15 minutes in the same shaker, left stand for 1 hour and measured for pH in the same calibrated pH meter.

### 3.1.3 Soil Organic Matter

The Weight Loss-On-Ignition method (WLOI) was employed in this project to estimate the organic matter content of the soils. The WLOI is an approximation to the organic matter (as any other method is), used by many researchers in recent years (Schulte and

Hopkins, 1996). Table 3.1 shows an overview on the usage of this method. It has been found that the WLOI tends to overestimate the organic matter, when the clay minerals present in the soil have expandable characteristics. In this case lattice water and water of hydration around exchangeable cations is lost at only 150 $^0$C to 250 $^0$C (Schulte and Hopkins, 1996). In this study, the WLOI method was backed-up by the most commonly used, the Walkley and Black (wet oxidation) method for some samples of the long-term pot experiment. The $R^2$ of the correlation between these two methods was 0.970, for number of samples, n = 65, which falls in an acceptable range of other researchers findings.

The WLOI procedure was taken from Rowell (1994). 2 g of soil were weighed into a preweighed porcelain crucible (weight A), which was put in an oven at 105 $^0$C overnight. Next day the crucible was weighed again (weight B), after being cooled down in a desiccator and then put into a 400 $^0$C furnace overnight. Next the crucible was cooled in a desiccator and weighed again (weight C). The WLOI value was determined according to the formula:

$$\text{WLOI, \%} = \frac{B-C}{B-A} * 100$$

The Wet Oxidation method was taken from Rowell (1994). The reagents used were 0.4 M $Fe_2(SO_4)_3$, 66.6 mM $K_2Cr_2O_7$ and Barium diphenilamine sulphonate indicator. The first was produced by dissolving 320 g of $(NH_4)_2SO_4FeSO_4.6H_2O$ and diluting it to 2 L of ultra pure water. The second was produced by weighing 39.23 g of $K_2Cr_2O_7$ and diluting it to 700 mL of ultra pure water. Then 800 mL of concentrated $H_2SO_4$ and 400 mL of concentrated $H_3PO_4$ were added slowly while stirring and the solution was diluted to 2 L with ultra pure water. Soil weighing 0.5 - 1 g was placed into a 100 mL digestion tube, and 40 mL $K_2Cr_2O_7$ were added. The tube was digested at 130 $^0$C for 2 hours (a digestion block, make *Tecator 2040 Digestor*, with capacity of 40 tubes was used for the digestions), was then transferred into a 100 mL conical flask and was titrated against the $Fe_2(SO_4)_3$ solution, after adding a few drops from the indicator. The titration was considered complete when the colour changed from dark brown-red to bright green. If more than 15 mL of the $Fe_2(SO_4)_3$ solution were consumed the digestion was repeated with less amount of soil. The soil organic matter content was then calculated from the following formulae:

$$\text{Org. C, \%} = \frac{4.8\,(1-x/y)}{\text{oven dry soil (g)}} * 100$$

**Table 3.1:** ***Correlation of the Loss-On-Ignition method with Wet Oxidation (From Schulte and Hopkins, 1996).***

| | | y=ax+b | | | |
|---|---|---|---|---|---|
| Temperature | Time | a | b | $R^2$ | n |
| $^0$C | h | | | | |
| 360 | 2 | 0.73 | -0.08 | 0.93 | 63 |
| 360 | 2 | 0.66 | -0.04 | 0.84 | 60 |
| 360 | 2 | 1.04 | -0.36 | 0.97 | 356 |
| 360 | 2 | 0.97 | -0.33 | 0.90 | 316 |
| 360 | 1 | 0.68 | -0.5 | 0.90 | 217 |
| 375 | 16 | 0.79 | -0.7 | - | 65 |
| 400 | 8 | 0.84 | -0.32 | 0.97 | 91 |
| 400 | 6 | 0.57 | - | 0.98 | 55 |
| 430 | 24 | 0.85 | 0.56 | 0.99 | 17 |
| 450 | 12 | 0.9 | -0.02 | 0.92 | 174 |
| 450 | 12 | - | - | 0.89 | 164 |
| 450 | 16 | 0.78 | -0.2 | 0.99 | 38 |
| 500 | 4 | 0.81 | -0.47 | 0.98 | 215 |
| 500 | 4 | 0.6 | -0.33 | 0.87 | 210 |
| 600 | 6 | 0.7 | -1.24 | 0.86 | 60 |
| 600 | 6 | 0.72 | -4.29 | 0.89 | 12 |
| 400 | 16 | 1.41 | 0.64 | 0.68 | 65* |

**: The present project, a: Angle of straight line, b: Intercept of straight line, n: Number of samples*

$$\text{Org. Matter, \%} = \frac{\text{\% org. C}}{0.58}$$

where: x is the actual titrate and y the blank titrate.

### 3.1.4 Cation Exchange Capacity (CEC)

The principle of the measurement of the CEC of a soil is to saturate the soil solution with a cation and then to measure the concentration of this cation on the Atomic Absorption Spectrophotometer (AAS). The cation used in this method to saturate the soil solution is Na. The method was taken from Rowell (1994).

5 g of soil were weighed into a 50 mL plastic centrifuge tube and 30 mL of 1 M NaOAc pH 8.2 were added. The sample was shaken at an end-to-and shaker at 21 $^0$C for 5 minutes and was then centrifuged for 10 minutes at 4000 rpm. The supernatant was discarded new lot of 30 mL of 1 M NaOAc pH 8.2 was added the sample was resuspended and the procedure was repeated for another 2 times. After the supernatant was discarded for the third time 30 mL of 95 % ethanol solution were added, the sample was resuspended and another 3 cycles were conducted. At the end of the third cycle, 30 mL of $NH_4OAc$ pH 7 were added, the sample was resuspended and a new phase of 3 cycles was commenced. This time the supernatants were filtered through a filter paper, Whatman No 42, and collected into a 100 mL volumetric flask. At the end this, the flask was taken to volume with $NH_4OAc$ pH 7 solution. The samples were kept at 4 $^0$C until Na was measured on the FAAS according to the settings presented in Table 3.2. CEC value was then determined by the formula:

$$\text{CEC, cmol}_c\ \text{kg}^{-1}\ \text{soil} = \frac{10 * \text{Na concentration in meq L}^{-1}}{\text{mass of sample (g)}}$$

**3.1.5 $CaCO_3$**

The method employed for measuring the $CaCO_3$ content of the soils was the calcimeter method, taken from Rowell (1994). This method is quick, simple and accurate. The calcimeter was used to measure the $CO_2$ evolved from the soil samples when their $CaCO_3$ content reacts with 4 N HCl. The calcimeter had previously been calibrated with standard amounts of $CaCO_3$, and thus the amount of $CO_2$ which corresponds to the $CaCO_3$ content of the soil samples was known. After the amount mg of $CaCO_3$ were read from the calibration graph, this value was corrected to pressure and temperature reference points, which are 760 mm Hg and 273.15 $^0$K, using the formula:

$$\text{Corrected value} = \text{Read-out value} * \frac{273.15}{273.15 + T} * \frac{P}{760}$$

where: T is the observed temperature in $^0$K and P is the observed pressure in mm Hg.

Then, the corrected value was used to calculate the percentage of the $CaCO_3$ content of the sample using the formula:

$$CaCO_3, \% = \frac{\text{mg } CaCO_3}{\text{mg of sample used}} * 100$$

### 3.1.6 Bulk Density

The bulk density of the soil measures the density of the solids and the pore space per unit volume. The method followed was outlined by Hooda (1992) and it measures this value in a ‘disaggregated’ state of sample. This was determined by taking the average weight of five 10 mL scoops of the oven dry (at 105 $^0$C) sample and expressed in g cm$^{-3}$.

### 3.1.7 Particle Size Distribution

The particle size distribution of the soils was determined by the mechanical analysis using the Bouyoucos hydrometer, as outlined by Sheldrick and Wang (1993). Sand consists of particles >2 mm, silt of particles from 2 mm down to 2 μm, and clay particles < 2μm. 40 g of soil were put into a 600 mL beaker where 250 mL of ultra pure water and 100 mL of 0.5 % sodium hexametaphosphate were added. The latter reagent helped the sample be dispersed chemically. The suspension in the beaker was kept overnight. Next day, the content was transferred, helping with ultra pure water, to a dispersing cup and was mixed for 5 minutes with an electric stirrer. The sample was thus dispersed mechanically too. The suspension was then transferred, helping with ultra pure water, to a 1 L sedimentation cylinder and ultra pure water was added to take volume to 1 L. Some time was then allowed for the mixture to equilibrate thermally and a plunger was inserted to mix thoroughly. If there were foams some drops of amyl alcohol were added. As soon as the mixing was complete the hydrometer was lowered into the suspension and a reading was taken after 40 seconds. The mixture was again thoroughly mixed with the plunger and another reading was taken after 7 hours. The hydrometer was also lowered into a cylinder with ultra pure water only, in order to take the blank reading. The sand, clay and silt distribution was calculated with the formulae:

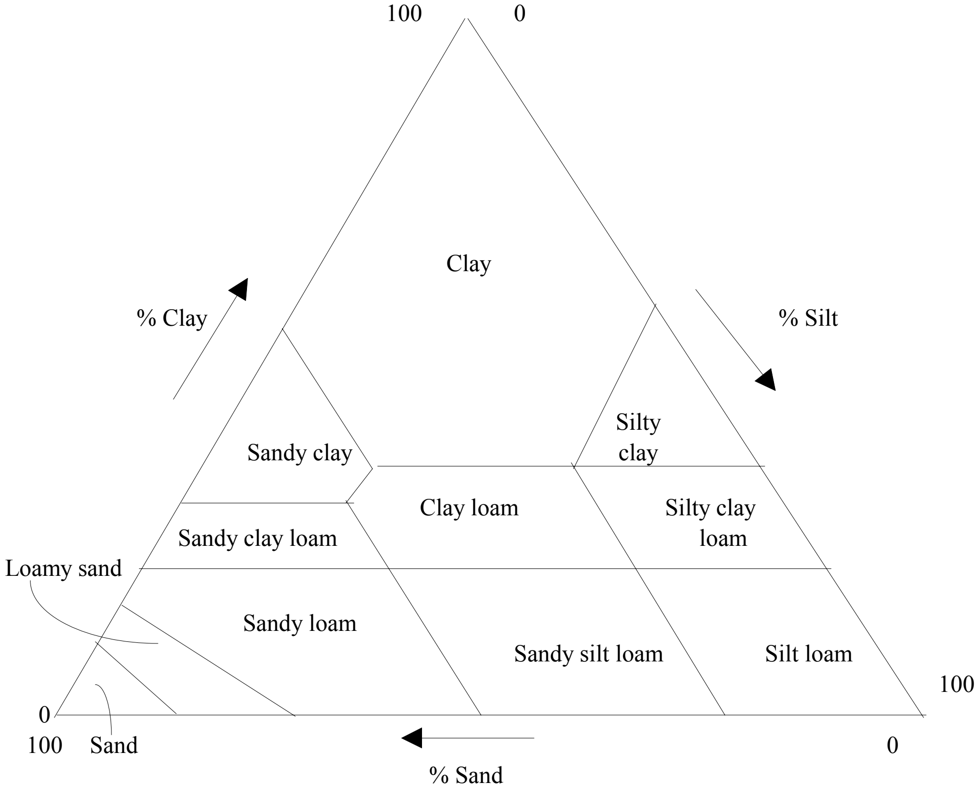
$$\text{Sand, \%} = 100 - (R_{40} - R_L) * \frac{100}{\text{oven dry soil (g)}}$$

$$\text{Clay, \%} = (R_{7h} - R_L) * \frac{100}{\text{oven dry soil (g)}}$$

$$\text{Silt, \%} = 100 - (\text{\% Sand} + \text{\% Clay})$$

where: $R_{40}$ is the reading after 40 seconds, $R_{7h}$ the reading after 7 hours and $R_L$ is the blank reading. The percentages of sand, silt and clay so obtained were then used to assign a textural class to the soil using the textural triangle shown in Figure 3.1.

**Figure 3.1:** ***The particle size distribution triangle (From Soil Survey of England and Wales, 1974).***



### 3.1.8 Free Fe and Al Oxides

The method followed here was taken from Bascomb (1974). 1 g of soil was weighed into a 200 ml screw cap plastic bottle, where 100 mL of a buffer solution were added. This buffer solution was prepared by mixing 400 mL 1 M NaOAc and 156 mL of glacial HAc and making the volume to 1 L with ultra pure water. In the bottle were also added 4 g of sodium dithionite. The sample was shaken for 16 hours in an end-to-end shaker at 20 rpm, and then filtered through a slow filter paper. The solution was determined in the FAAS for Fe and Al according to the settings presented in the Table 3.2.

### 3.1.9 Water Soluble Cations

The water soluble cations represent the soil solution state. Theoretically, the most proper way of determining the water soluble cations would be to extract the soil solution and measure the contents on the FAAS. However, very difficult, and thus other alternatives have been used. The most commonly known is the saturated paste, which, however, has been criticised that it does not represent the real conditions of the soil solution. Thus, the method described by Rhoades (1982) has been employed, according to which 1 : 5 w/v soil : water suspension may be used.

5 g of soil were weighed into a 50 mL plastic bottle, where 25 mL of ultra pure water were added. The mixture was shaken in an end-to-end shaker for 2 hours at 21 $^0$C at 20 rpm and was filtered through a Whatman No 42 filter paper. The clear solution was kept at 4 $^0$C until the analysis. The cations analysed were $Ca^{2+}$, $K^+$, $Mg^{2+}$ and $Na^+$ on the FAAS according to the settings in Table 3.2.

### 3.1.10 Bioavailable Heavy Metals

Two tests were employed to assess the availability of heavy metals, DTPA and 0.05 M $CaCl_2$. The filtrates were determined for Cd, Ni, Pb and Zn using either ICP-OES or FAAS, according to the settings described in Table 3.2 and using standards with matching matrices.

#### 3.1.10.1 Diethylenetriaminepentaacetic (DTPA)

Lindsay and Norvell (1978) developed this test for assessing the bioavailabilty of macronutrients, such as Zn, Mn, Fe and Cu. However, the method has also been used by many researchers for testing the availability of potentially toxic heavy metals. In this project, 10 g of soil were weighed into a 50 mL plastic centrifuge tube, where 20 mL of the DTPA solution were added. This solution was prepared by mixing 9.835 g of 0.005 M DTPA, 7.4 g of 0.01 M $CaCl_2$ and 74.5 g of 0.1 M triethanolamine (TEA) and taking the solution to 5 L volume, after the pH of the solution was adjusted to 7.3 with a few drops of concentrated HCl. The mixture was shaken in an end-to-end shaker for 2 hours at 21 $^0$C at 20 rpm and was filtered through a Whatman No 42 filter paper. The clear solution was kept at 4 $^0$C until analysis according to the settings reported in Table 3.2.

#### 3.1.10.2 0.05 M $CaCl_2$

The extraction of soils with $CaCl_2$ have been used by many workers and many variations, and the results have been highly correlated with the test crop uptake. This is because these neutral dilute reagents do not alter the soil pH and, thus, do not change the retention characteristics of metals. The method used in this project was taken from Alloway and Morgan (1986).

2.5 g of soil were weighed into a 50 mL plastic centrifuge tube, where 25 mL of 0.05 M $CaCl_2$ were added. The tube was then mixed for 16 hours in an end-to-end shaker at 21 $^0$C at 20 rpm. The suspension was filtered through a slow filter paper and the clear solution was kept at 4 $^0$C until analysis according to the settings reported in Table 3.2

### 3.1.11 Total Heavy Metals Digested with *Aqua regia*

The total analysis of heavy metals in soils provides very valuable information about the pollution state of a site. A ‘true’ total method should dissolve all solid phases which bind heavy metals, irrespective of how strong they bind them. The reagents which give the desirable result are concentrated HF and $HClO_4$, but laboratories tend to avoid using them, due to their explosive characteristics. Instead, some alternative *pseudo*-total methods have been suggested, that is digestion of soil with concentrated acids with milder reactions. Among those suggested are $HNO_3$, $H_2SO_4$, HCl or combination of them. *Aqua regia* is a combination of concentrated $HNO_3$ and HCl and has been found to recover 85 to nearly 100 % of the total amounts of metals in soils (McGrath and Cuncliffe, 1985). The method followed in this project was taken from Ure (1995).

1.5 g of soil were placed into a 100 mL digestion tube, where 10.5 mL of concentrated HCl and 3.5 mL of concentrated $HNO_3$ were added with care. The mixtures were left to stand overnight at room temperature, in order to facilitate a more proper dissolution of soil minerals. Next day, the tube was heated to 50 $^0$C until all the reaction had been subsided and then heated to 140 $^0$C for 2 to 2.5 hours (a digestion block, make *Tecator 2040 Digestor*, with capacity of 40 tubes was used for the digestions). At the end of that time, after the tube was left to cool, the content was filtered through a Whatman No 521 filter paper into a 50 mL volumetric flask, helping with a few mL of 0.5 M $HNO_3$. After all the tube contents were transferred, the filter paper itself was washed with a few mL of 0.5 M $HNO_3$, and the volumetric flask was taken to volume with 0.5 M $HNO_3$. The sample was then kept at room temperature until the time of analysis by either FAAS or ICP-OES, according to the settings reported in Table 3.2.

## 3.2 PLANT ANALYSIS

### 3.2.1 Sample Collection and Preparation

Samples from the pot experiments were cut with a clean and (prewashed with ultra pure water) pair of stainless steel scissors, then washed by agitation with ultra pure water to rid all soil and dust particles and were put into a clean paper bag. Field experiment samples were obtained with an electric lawn mower. The plant mass of each plot was put into large black ‘rubbish’ bags, weighed, a small sub-sample was weighed, and the sub-sample was put into small paper bags. From that point only this sub-sample was processed. The paper bags, in both the pot and field experiments, were put into a forced drought oven at 75 $^0$C until no further loss in weight was observed. The time usually needed was 72 hours. The samples were then chopped with a stainless steel pair of scissors and finely ground in a Tema mill. The powder produced from the mill was now used for the analysis of the heavy metal contents of the vegetation samples.

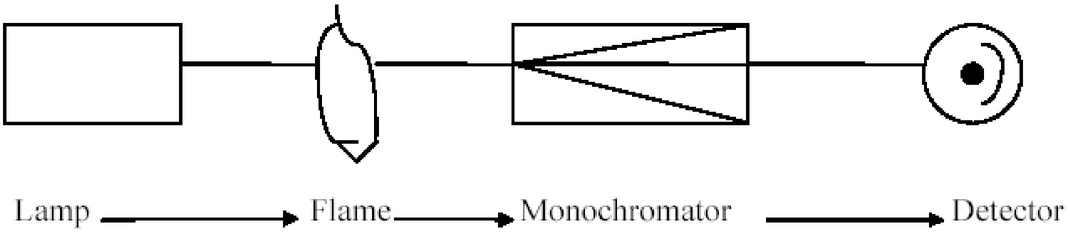
### 3.2.2 Digestion of Plants

The method used for the digestion of plant samples was the 1 : 10 plant : acid digestion, as outlined in Hooda (1992). 0.5 g of plant material were weighed into a 100 mL digestion tube, where 10 mL of concentrated $HNO_3$ were carefully added. The content was stirred in a mechanical stirrer, in order to assure a thorough mix of the plant material with the acid. The mixture was left at room temperature overnight. Next day the tube was carefully heated to 90 $^0$C for 2 hours and then to 140 $^0$C for 6 hours (a digestion block, make *Tecator 2040 Digestor*, with capacity of 40 tubes was used for the digestions). After that the tube was left to cool and the contents were filtered into a 50 mL volumetric flask through a Whatman No 521 filter paper, helping with ultra pure water. After the transfer was complete, the filter paper itself was washed with ultra pure water and the volume was taken to the mark with ultra pure water. The samples were left at room temperature until analysis on either the FAAS or the ICP-OES, where the settings presented in Table 3.2 were followed.

## 3.3 ANALYTICAL PROCEDURES FOR MEASURING HEAVY METAL CONCENTRATIONS

Several techniques for quantifying the metal concentrations in soils and plant samples have been suggested, used and developed over the past few decades. Techniques used have included atomic absorption spectrophotometry (AAS), inductively coupled plasma (ICP) and ion chromatography (IC).

**Figure 3.2:** ***Simplified schematic of a basic AA system (From Perkin Elmer, 1995).***



All analytical instruments need to be calibrated before they are used. The calibration is done when several solutions of a known concentration ('standard solutions') are analysed on the instrument. The known solutions must contain the same element which is about to be measured (analyte) and must be of the same matrix with the 'unknown' samples. For each standard solution a characteristic absorption or emission is given by the instrument, and a calibration curve can be produced, which is usually a straight line. This line gives the relation between the instrument' s absorption or emission with the element' s concentration. Then, when a sample of unknown concentration is introduced in the instrument, through this calibration graph it is possible to calculate what absorption or emission corresponds to what concentration. This calculation is usually done automatically by the instrument, in which case the sample concentration is displayed in the instrument' s monitor when the analysis of each sample is complete.

### 3.3.1 Principles of Instrument Functions

#### 3.3.1.1 Flame Atomic Absorption Spectrophotometer (FAAS)

Atomic absorption occurs when an atom absorbs energy in form of light of a specific wavelength, λ, and is elevated to an excited state. The basic instrumentation would include a source of light, an atom source, a monochromator to isolate the desirable wavelength of light, a detector to measure the light and a data processor and display device (Figure 3.2). The source of light is usually a Hollow Cathode Lamp (HCL). The samples are injected in the gas flame (acetylene or nitrogen gas flame), and atoms taken from the introduced sample are freed with the heat of the gas. Then, the freed atoms come in contact with the light beam from the HCL, because the burner of the gas is aligned with the light beam (Perkin Elmer, 1995). Detection limits of the elements of interest in this project and the settings for their measurements are shown in Tables 3.2 and 3.4. The FAAS used in this project was Perkin Elmer, model 3030.

#### 3.3.1.2 Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS)

In the graphite furnace, instead of injecting the sample in the flame, the sample is placed in a graphite tube, which is aligned with the light beam. Thus, the atoms are retained in the tube rather than passing out in the flame. The tube is heated to very high temperatures, the sample matrix evaporates and, thus, the sample is completely atomised. With this technique all of the sample is atomised and the period of time that this remains in contact with the light beam is long. This improves the sensitivity and accuracy of the measurement markedly compared with the conventional FAAS technique, where not all of the sample is atomised and not for a long time. There steps according to individual needs for each element, which are programmed in the instrument prior to measurement (Perkin Elmer, 1995). The step programmes used in this project are shown in Table 3.3. The GFAAS used in this project was Perkin Elmer, model HGA-500.

**Table 3.2:** ***Setting up of the Atomic absorption spectrophotometer (From Perkin Elmer, 1995).***

| Element | λ (nm) | $SW^e$ (nm) | Flame gases | Sensitivity check [a] |
|---|---|---|---|---|
| Cd | 228.8 | 0.7 | A-Ac [b] | 1.5 |
| Ni | 232 | 0.2 | A-Ac | 7 |
| Pb | 283.3 | 0.7 | A-Ac | 20 |
| Zn | 213.9 | 0.7 | A-Ac | 1 |
| Na [c] | 589 | 0.2 | A-Ac | 0.5 |
| K [c] | 766.5 | 0.7 | A-Ac | 2 |
| Mg | 285.2 | 0.7 | A-Ac | 0.3 |
| Ca | 422.7 | 0.7 | A-Ac | 4 |
| Fe | 248.3 | 0.2 | A-Ac | 60 |
| Al | 309.3 | 0.7 | N-Ac [d] | 50 |

*a: Metal concentration (mg $L^{-1}$) in aqueous solution which will give a reading of approximately 0.2 absorbance units., b: Air-acetylene , c: Determined with emission, d: Nitrogen-acetylene*, *e: Slit width*

**Table 3.3:** ***Settings of the Graphite Furnace for measuring Cd in plant digests (10% $HNO_3$) and 0.05 M $CaCl_2$ (0.05 M $CaCl_2$ in parenthesis) (From Perkin Elmer, 1978).***

| Step | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Temp $^0$C | 120 (120) | 300 (800) | 170 (2300) | 2300 (1800) | 30 (30) |
| Ramp (sec) | 10 (15) | 5 (10) | 0 (0) | 1 (0) | 1 (0) |
| Hold (sec) | 30 (30) | 10 (45) | 4 (7) | 3 (5) | 10 (10) |
| Read | | | -1 (-1) | | |
| Int. Flow (mL $min^{-1}$) | | | 50 (50) | | |

### 3.3.1.3 Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES)

In atomic emission spectrometry the light beam passes through an electron, which is then excited. When the outer orbital electron returns to its lower energy state position it releases energy, whose wavelength is characteristic for the individual element. In ICP, argon gas passes through a copper coil, in which radio frequency (RF) energy has been applied. Argon atoms are accelerated from the magnetic and electric field which the RF creates and, thus, electrons are stripped their atoms. In this environment the liquid sample is injected in droplet form, it is ionised and emits characteristic wavelength energy for each element measured. A polychromator receives this radiation and converts it to electronic signals, which are used for the calculation of the concentration of the element in the sample. ICP is suitable for multi-element analysis, and is very quick and convenient, but its accuracy is not as good as that of the GFAAS for several elements (Perkin Elmer, 1995; Boss and Fredeen, 1989). A simplified diagram of the use of ICP is shown in Figure 3.3. The ICP-OES used in this project was Perkin Elmer, model Optima-3000.

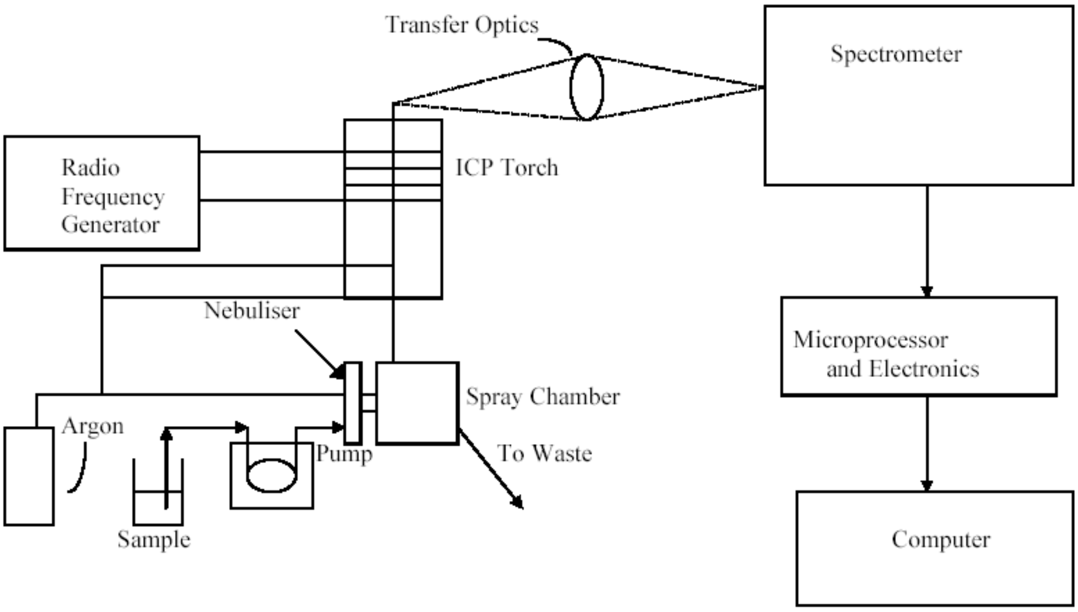
### 3.3.2 Interferences

There are certain types of interferences that may occur in the analytical instruments. In order to perform an accurate measurement these interferences have to be overcome.

- *Chemical interference*

This takes place when the element of interest combines with other elements and the absorbance is changed. Two ways to overcome it are the use of higher flame temperature and the addition of a releasing agent. Example of this interference is the use of lanthanium or EDTA as a releasing agent to calcium solutions to overcome the effects of phosphate from the air-acetylene.

**Figure 3.3:** ***Components of an ICP-OES (From Boss and Fredeen, 1989).***



- *Matrix interference*

This happens when the physical properties (viscosity, surface tension) between standards and samples are considerably different. A way to overcome this interference is the dilution of the sample solution until any difference becomes negligible. Example of this interference is the determination of rubidium in seawater. Standards are made to contain approximately the dissolved salts as are normally found in seawater.

- *Ionisation interference*

This happens when the ionised electron absorbs energy which is different to the energy which is characteristic for the specific element. This can be controlled by the addition to both sample and standards of a large excess of an easily ionised element. Example of this is that barium atomic absorption is strongest at 540 nm resonance line, while barium ionic absorption is strongest at 455 nm resonance line.

- *Spectral interference*

This takes place when the element of interest is in the same wavelength range of another element, which is not determined. The results given will, thus, be erroneously high. For example at 228 nm As interferes with Cd when the ratio As/Cd is greater than 5.

- *Background absorption (molecular interference)*

This happens when there is absorbance or scattering of light, due to the presence of gaseous molecular species, salt particles or smoke. There two ways of overcoming it, namely, use of non-absorbing near-by wavelength and use of deuterium background correction. Example of this interference is barium, which in high Ca matrix will be erroneously high in an air acetylene flame, due to absorption by undissociated Ca molecular species (CaOH) at 554 nm barium wavelength. With nitrous oxide-acetylene flame this is solved (Perkin Elmer, 1976; Perkin Elmer, 1995; Cresser and Marr, 1991).

### 3.3.3 Detection Limits

For defining the term ‘detection limit’, the American Society for Testing and Materials has suggested a standard procedure. In that procedure, several samples containing near zero concentrations of the analyte are analysed and the Standard Deviation (SD) is calculated. Detection limit is then the SD multiplied by a number which is between 3 and 4. For inorganic analyses this number is 3.29 (Black, 1991).

From the atomic absorption or emission techniques, GFAAS is the most sensitive, followed usually by ICP-OES, with the FAAS being the least sensitive. Table 3.4 shows the detection limits of the 4 heavy metals, which have been analysed with these techniques. It should be noted that FAAS was mostly used for the measurement of Na, K, Mg, Ca, Fe and Al for the characterisation analysis of the soil samples. ICP-OES was used for all the ‘routine’ heavy metal analysis in both soil and plant samples. GFAAS was only employed when heavy metal levels were too low to determine on the ICP.

**Table 3.4:** ***Detection limits of Cd, Ni, Pb and Zn (μg $L^{-1}$) (From Perkin Elmer, 1995).***

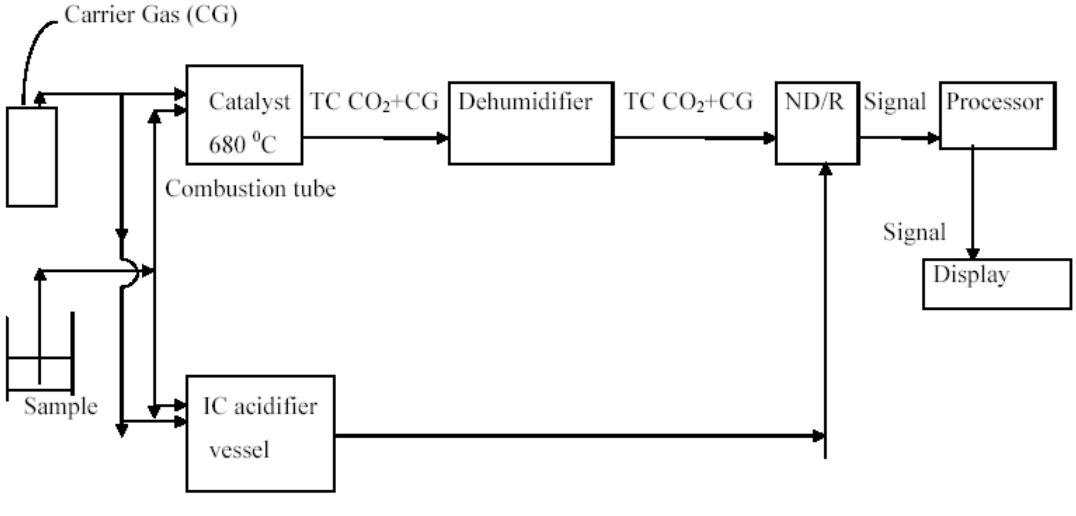
| | FAAS | GFAAS | ICP-OES [a] |
|---|---|---|---|
| Cd | 0.8 | 0.008 | 0.1 |
| Ni | 6 | 0.3 | 0.5 |
| Pb | 1.5 | 0.06 | 1 |
| Zn | 1.5 | 0.1 | 0.1 |

*a: With ultra-sonic nebuliser*

### 3.3.4 Measurement of Dissolved Organic Carbon in a TOC Analyser

In a TOC (Total Organic Carbon) Analyser (the one used in this project was Shimadsu, model TOC-5000) a liquid sample is introduced to the instrument and the carbon, which is dissolved in it, is measured. First, the Total Carbon (TC) is measured and then the Inorganic Carbon (IC) from the same sample. The Organic Carbon (OC) value is the difference between the TC and IC, and represents the Dissolved Organic Carbon (DOC) content of the sample. This value is expressed in ppm (μg $mL^{-1}$). The principle of use for the TC measurement includes a combustion tube, which is filled with an oxidation catalyst which is heated to 680 $^{0}$C. A carrier gas (high purity air) is also supplied in the tube. Then, the sample is introduced to this tube and is combusted to become $CO_2$. The carrier gas, containing the combustion product, is then sent to a reaction vessel to be dried by a dehumidifier and then in a sample cell containing non-dispersive infrared gas analyser (NDIR), where $CO_2$ is detected. The signal is then sent to a processor which displays the $CO_2$ reading as peak area. According to previous calibration with standard samples the TC content is automatically calculated. After that the IC is measured. An aliquot of the same sample passes through an IC vessel, which has been acidified. Only the IC is decomposed to evolve $CO_2$. which is again detected by the NDIR. The IC comes from carbonates and hydrogen carbonates (Shimadzu, 1992). These principles are shown in Figure 3.4.

**Figure 3.4:** ***Schematic of the function of TOC analyser***



## 3.4 DATA QUALITY CONTROL

There are two parameters concerning the quality control of the data. These are accuracy and precision. Accuracy is the closeness of the measurements to a 'real' certified value, while precision is the reproducibility of the observed values of replicates. Precision is determined by the CV (Coefficient of Variation) value, which is expressed in percentage. High CV means low precision. Accuracy is determined as the percentage difference between the

**Table 3.5:** ***Concentrations of Cd, Ni, Pb and Zn in the In-house Reference Materials and the Certified Reference Material (μg g $^{-1}$).***

| | | Cd | | Pb | | Ni | | Zn | |
|---|---|---|---|---|---|---|---|---|---|
| | n[f] | Mean[g] | CV, %[h] | Mean | CV, % | Mean | CV, % | Mean | CV, % |
| AR [a] | 10 | 7.76 | 8.5 | 68.93 | 9.3 | 99.92 | 8.9 | 360.9 | 9.7 |
| DTPA [b] | 30 | 4.72 | 5.0 | 12.06 | 9.3 | 15.21 | 7.6 | 98.45 | 5.7 |
| $CaCl_2$ [c] | 20 | 0.38 | 5.4 | - | - | 0.83 | 9.9 | 4.2 | 9.2 |
| Plant [d] | 12 | 0.015 | 10.2 | 0.55 | 9.5 | 0.16 | 8.5 | 30.9 | 5.1 |
| CRMa [e] | 6 | 72 | 1.8 [i] | 296 | 4 [i] | 174 | 5 [i] | 1063 | 16 [i] |
| CRMb [j] | 6 | 72.13 | 0.7 [i] | 296.12 | 2.72 [i] | 175.2 | 1.93 [i] | 1069.8 | 8.26 [i] |

*a:* Aqua regia-*digested, b: DTPA-extracted, c:* $CaCl_2$*-extracted, d: Plant In-house Reference Material, e: Certified Reference Material (the certified value), f: Number of samples/replicates , g: in μg g* $^{-1}$*, h: Coefficient of variation, j: Certified Reference Material (the measured value), i: Standard error* measured and the certified value. In all analytical techniques both of these values fall when the levels of elements are approaching the detection limit of the instrument. In order to have a quality control in the data produced in this project, both ‘in-house’ and certified reference materials were used, the first referring to precision and the second to accuracy. The in-house reference materials were a sludged soil and barley leaves for soil and plant samples, respectively. These materials were included in every batch of extraction and digestion performed, in order to check the reproducibility of the results over the course of the laboratory work. The results from the in-house reference materials were recorded and compared with the results from previous batches. If the results from these reference materials had a CV value of greater than 10 %, then the last batch of samples would be analysed again, until the limit of 10 % would be achieved. Blank samples (samples which only contain the matrix -not the analyte) were also included and the values measured were subtracted from the rest of the samples of the same batch. The certified reference material, code number CRM 143R, was a sludge soil and was analysed against the in-house reference material. The values found were very close to the reported certified values. Table 3.5 shows comprehensively the results from the analyses of both the in-house and the certified reference material.

## 3.5 STATISTICAL ANALYSIS

Data for each measurement (such as heavy metal concentrations in plants, heavy metals extracted by DTPA *etc.*; these are called dependent variables) were processed for Analysis of Variance (ANOVA) for the determination of the statistically significant differences among various treatments. The SAS package was employed for this analysis. From each of the ANOVA tables produced, the P values are presented in all four experiments, for each treatment in the following way: p value less than 0.05 is indicated with one asterisk, P value less than 0.01 with two asterisks and p value less than 0.001 with three asterisks (*i.e.* probability levels of 95, 99 and 99.9 %, respectively). When asterisks are not presented, this means that that particular treatment was not found to have any significant interaction.

For each treatment, in all four experiments, the LSD (Least Significant Difference) was calculated as well, using the following formula:

$$LSD = t\sqrt{\frac{MSE}{N}}$$

where: t is the Student-t value, taken from appropriate Statistics tables, MSE is the Mean Square Error, taken from the ANOVA table of each dependant variable and N is the number of replicates of each treatment, which is equal to the total number of degrees of freedom in an experiment divided by the number of degrees of freedom of the particular treatment for which the LSD is calculated.

In most of the figures in Chapters 4, 5 and 6 there are error bars for each data point. The error bars in these figures represent the standard error (SE) for each value, which was calculated by the following formula:

$$SE = \frac{SD}{\sqrt{N}}$$

where: SD is the Standard Deviation of the replicates from which the mean value was calculated and N is the number of replicates (which was equal to 4 for the experiments in Chapters 4 and 5 and equal to 2 for the experiment in Chapter 6).

# CHAPTER FOUR: HEAVY METAL AVAILABILITY IN SEWAGE SLUDGE-TREATED SOILS (POT EXPERIMENT).

## 4.1 INTRODUCTION

The availability of metals to plants will depend on various chemical mechanisms in the soil, which are ruled greatly by pH and the soil' s organic matter content (Towers and Paterson, 1997; McBride, 1997a). Organic matter is a dynamic factor; it changes in composition over time, due to the fact that it is decomposed by microbial activity in the soil. It is known that temperature affects the decomposition of organic matter: increasing temperature accelerates the decomposition process, thus, in warmer environments one should expect more rapid decomposition of the soil organic matter. Moreover, Dissolved Organic Carbon (DOC), which may be a product of the degradation of the humic macromolecules, may be found in elevated concentrations in sludge-treated soils and this may lead to higher availability of metals (Lamy *et al.*, 1991). Another important factor ruling heavy metal availability to plants is time. Various workers have investigated the availability of heavy metals over time (Hooda and Alloway, 1993; Obrador *et al.*, 1997; Hyun *et al.*, 1998) and the general conclusions are:

- The mineralisation of organic matter in sewage sludge treated soils could release metals into more labile forms, which could then be more available for plant absorption, or that
- The decomposition of organic matter increases the specific solid surface of soils, and thus increases the adsorption of metal onto soil constituents, which would lead to a decrease in plant availability.

It is difficult to assess which way the equilibrium will go over time because weather onditions, and subsequently temperatures, will be changing due to the ‘Greenhouse Effect’ (Alloway and Jackson, 1991). This project was, thus, initiated to investigate the behaviour of heavy metals in soils treated with sewage sludge under 2 different ambient temperatures, in order to compare the metal availability over a period of 2 years. The specific objectives of this part of the project were:

- To test the effects of the sewage sludge application on two different soil types and on plants grown on them with regard to heavy metal dynamics.
- To compare the effects of Cd, Ni, Pb and Zn added to these soils in the sewage sludge application under two different temperature regimes, which represent the conditions most commonly found in temperate and Mediterranean climates (namely 15 $^0$C and 25 $^0$C, respectively).

- To assess the value of extractions with 0.005 M DTPA and 0.05 M $CaCl_2$ as indices of the bioavailability of heavy metals for the prediction of heavy metal uptake into the tissues of crops.
- To assess the key chemical properties, such as organic matter content and pH on the bioavailability of Cd, Ni, Pb and Zn in soil-sludge mixtures at 3 rates of sewage sludge application over a residual time of 2 years.

The hypotheses tested in this experiment were:

- Mixing of metal-rich sewage sludge with soil will lead to an increase in the concentrations of plant available metals.
- The mineralisation of the organic matter in sewage sludge-treated soils will release metals in more mobile forms, which would then be more available for plant uptake.
- The metal availabilty to plants will be enhanced by a more rapid decomposition of organic matter, caused by higher ambient temperatures.
- Organic matter decomposition, which is faster at higher temperatures, will lead to increasing DOC levels in the soil-sludge mixtures. This may facilitate the increase of heavy metal availability over time.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Experimental Design

Two bulk soil samples were collected from the University of Reading Sonning Farm. These soils represented a wide range of the main chemical and physical properties. One was a loamy sand (LS) which had not been recently cultivated. The other was a clay loam (CL) which was obtained from a grassland area. These soils were air dried, passed through a 10 mm mesh plastic sieve and thoroughly homogenised. A bulk sample of liquid sewage sludge was obtained from a sewage treatment plant near Heathrow Airport. A sub-sample of this was freeze-dried to determine its solids content, which was found to be 8 %. The soils and the sewage sludge mixtures were put in 4 L plastic pots and were thoroughly mixed at rates equivalent to 0, 10 and 50 t $ha^{-1}$ of dry matter on an area basis. Each treatment was replicated eight times; half of the pots in each treatment were put in a greenhouse, where a temperature of 25 $^0$C was maintained and the remaining pots were transferred to a constant temperature room, where the temperature was maintained at 15 $^0$C, three months after the beginning of the experiment. The pots at 25 $^0$C were under normal daylight, while pots at 15 $^0$C received lighting of at least 1.5 lux for 12 hours per 24-hour day.

Ryegrass (*Lolium perenne* L.) was chosen as the test crop. Ryegrass seeds were sown in a growth media and after they had emerged they were transplanted to the pots. The same number of seedlings were transplanted in each pot. The plants were frequently watered with de-ionised water, according to their needs; the cool room pots were watered twice a week and the greenhouse pots 3-4 times a week. The watering was done from underneath (subirrigation), in order to maintain the soils at near to Field Capacity moisture status.

### 4.2.2 Methodology

Sub-samples of the bulk soils and of the sewage sludge, were passed through a 2 mm mesh sieve and their key physical and chemical properties determined. The results are shown in Table 4.1. Representative soil samples were taken from the pots at regular intervals for 2 years. These sampling times were: Weeks 0 (the day the experiment commenced), 2, 4, 8, 16, 32, 48, 72 and 100. From each pot, 3 sub-samples were taken from 0-15 cm depth at random points and bulked, in order to ensure that the sample represented the whole pot. Each sample was air-dried, homogenised and passed through a 2 mm mesh sieve, after which it was analysed for pH, organic matter with the Weight Loss-On-Ignition (WLOI) and Wet Oxidation methods, DTPA and 0.05 M $CaCl_2$ extractable metals (Cd, Ni, Pb and Zn).

**Table 4.1:** ***Selected physical and chemical properties of the soils and sludge used in the experiment.***

| | $CaCO_3$ | Al | Fe | $Ca^{2+}$ | $K^+$ | $Na^+$ | $Mg^{2+}$ |
|---|---|---|---|---|---|---|---|
| | % | Dithionite extracted % | | Water soluble (µg $g^{-1}$) | | | |
| CL Soil | 3.00 | 0.13 | 1.32 | 265.00 | 12.90 | 37.15 | 7.65 |
| LS Soil | 1.10 | 0.09 | 0.93 | 46.80 | 7.65 | 16.80 | 2.65 |
| Sludge | - | - | - | - | - | - | - |
| | Sand | Silt | Clay | | pH | | LOI[a] |
| | % | | | Category | $H_2O$ | $CaCl_2$ | % |
| CL Soil | 19.87 | 40.07 | 40.06 | CL | 7.65 | 7.19 | 10.41 |
| LS Soil | 78.37 | 14.64 | 6.99 | LS | 7.21 | 6.68 | 2.37 |
| Sludge | - | - | - | - | 8.15 | 7.45 | 62.42 |
| | EC[b] | CEC[c] | Bd[d] | *Aqua regia*-digested (µg $g^{-1}$) | | | |
| | dS $m^{-1}$ | $cmol_c$ $kg^{-1}$ | g $cm^{-3}$ | Cd | Ni | Pb | Zn |
| CL Soil | 0.91 | 49.03 | 0.92 | 0.52 | 20.88 | 25.58 | 48.69 |
| LS Soil | 0.17 | 8.47 | 1.21 | 0.34 | 13.93 | 17.82 | 34.69 |
| Sludge | - | - | 0.75 | 7.31 | 39.82 | 230.7 | 532.8 |

*a: Loss-On-Ignition, b: Electrical conductivity, c: Cation exchange capacity, d: Bulk density*

**Table 4.2.1:** ***Total concentrations of Cd, Ni, Pb and Zn in the sludge-soil mixtures (μg $g^{-1}$).***

LS Soil

| | Cd | Ni | Pb | Zn |
|---|---|---|---|---|
| Control | 0.34 | 13.93 | 17.82 | 34.69 |
| 10 t $ha^{-1}$ | 0.38 | 14.38 | 20.54 | 40.32 |
| 50 t $ha^{-1}$ | 0.42 | 15.61 | 29.12 | 58.76 |

CL Soil

| | Cd | Ni | Pb | Zn |
|---|---|---|---|---|
| Control | 0.52 | 20.88 | 25.58 | 48.69 |
| 10 t $ha^{-1}$ | 0.57 | 22.21 | 26.27 | 53.27 |
| 50 t $ha^{-1}$ | 0.84 | 22.83 | 36.22 | 80.55 |

**Table 4.2.2:** ***Added sewage sludge and expected concentrations of Cd, Ni, Pb and Zn in the soil- sludge mixtures.***

LS Soil

| | Sludge added (g sludge $pot^{-1}$) | Concentrations of metals expected (μg $g^{-1}$) | | | |
|---|---|---|---|---|---|
| | | Cd | Ni | Pb | Zn |
| Control | - | 0.34 | 13.93 | 17.82 | 34.69 |
| 10 t $ha^{-1}$ | 24.79 | 0.40 | 14.03 | 19.71 | 39.60 |
| 50 t $ha^{-1}$ | 123.96 | 0.64 | 15.35 | 27.35 | 57.20 |

CL Soil

| | Sludge added (g sludge $pot^{-1}$) | Concentrations of metals expected (μg $g^{-1}$) | | | |
|---|---|---|---|---|---|
| | | Cd | Ni | Pb | Zn |
| Control | - | 0.52 | 20.88 | 25.58 | 48.69 |
| 10 t $ha^{-1}$ | 32.61 | 0.60 | 21.33 | 27.98 | 54.34 |
| 50 t $ha^{-1}$ | 163.05 | 0.92 | 23.05 | 37.90 | 77.30 |

The total heavy metals concentrations were determined by *Aqua regia* digestion on the samples taken in Week 0. DOC was analysed in the samples from Week 4 and Week 100, in order to assess the state of DOC in the soils at the beginning and the end of the experiment.

The plant samples were harvested in Weeks 7, 14, 21, 28, 35, 44 and 51 following their initial transplanting. The material from every harvest was first washed thoroughly in ultra pure water and dried in a forced drought oven at 75 $^{0}$C until no further loss on their weight was observed. They usually needed 72 hours to reach this state. They were ground in a Tema mill and stored in glass jars. Their total heavy metals concentration was assessed by digestion in concentrated $HNO_3$. All these procedures and methods were performed as described in Chapter 3.

All soil extracts and digests and the plant material digests were analysed either on the Atomic Absorption Spectrophotometer (AAS) or on the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). The Cd levels extracted with the $CaCl_2$ solution were below the ICP detection limit (1 μg $L^{-1}$) and were analysed on the Graphite Furnace AAS, where the detection limit for this element is 0.008 μg $L^{-1}$.

All data were statistically analysed in an Analysis of Variance (ANOVA) using the SAS package. The LSD test was, then, employed. Treatments were considered as significantly different for a probability level of confidence of greater than 95 % ($p < 0.05$).

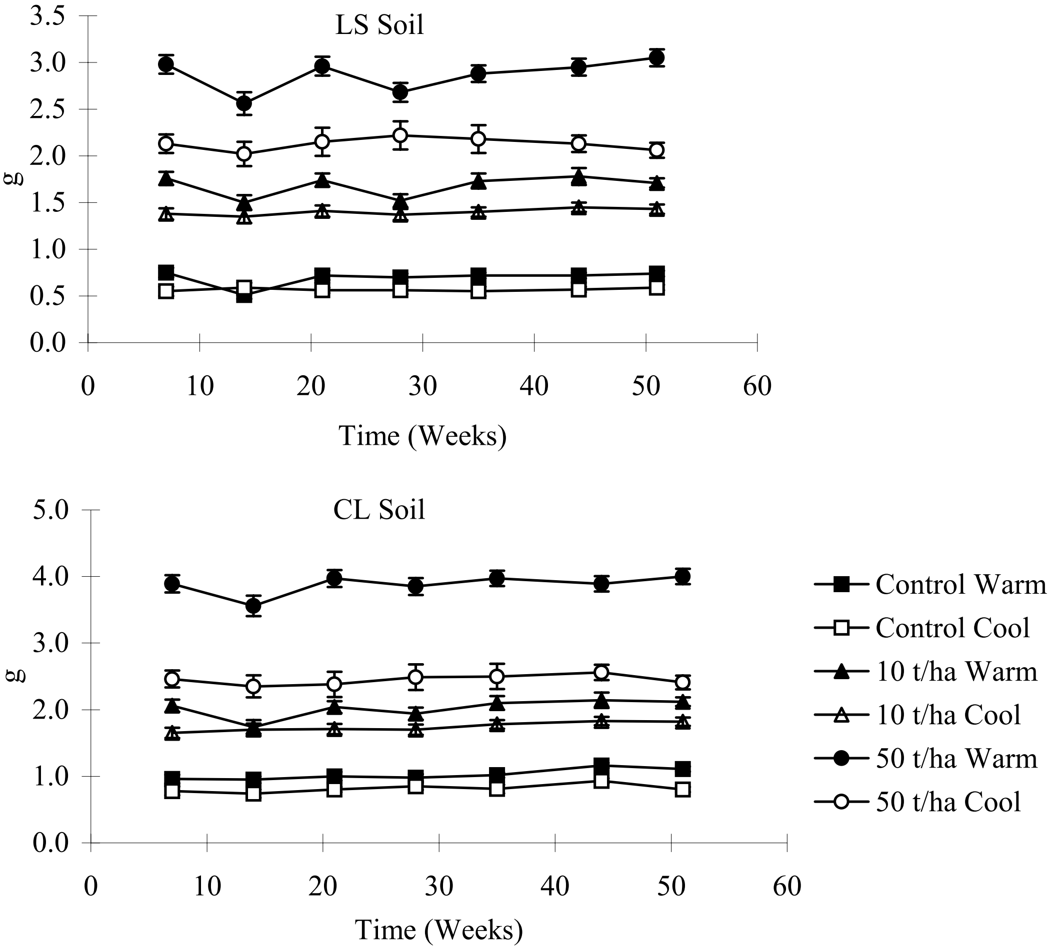
## 4.3 RESULTS

The application of sewage sludge increased the heavy metal concentrations significantly. Table 4.2.1 shows the total concentrations of all four metals in the various treatments. For quality control reasons, Table 4.2.2 shows a 'budget', that is, the amount of sludge added to the soils (in g sludge per 3 kg pot and not in t $ha^{-1}$) and the expected concentrations of the metals in each sludge treatment.

### 4.3.1 Plant Dry Matter Yields

The results for the plant dry matter yields for all four metals are shown in the Figure 4.1 and in Appendix I (Table A3). The ambient temperature had a very important effect on the plant yield. The DM yields in the each treatment remained essentially the same during the 1 year period, but marked differences occurred between the 15 $^0$C and the 25 $^0$C treatments. The plants grown in the cool environment had significantly lower production of material. This trend was only observed after the third harvest (Week 21). This delay may be due to the fact that a reliable 'cool' environment was only found about four months after commencing of this study and from then onwards the cool temperature effects became more obvious. At 25 $^0$C, there was a marked enhancement of plant production, especially in the pots which had received sewage sludge at the highest rate (50 t $ha^{-1}$). The sludge application in both cool and warm temperatures increased the production significantly. However, it increased the heavy metal uptake as well, with the highest increase observed at the 50 t $ha^{-1}$ rate. In all cases, no phytotoxicity symptoms were apparent, although the highest rate of sludge was at least 5 times greater than the levels normally applied in the common agricultural practices. It was observed that as sewage sludge application increased the effect of temperature was further enhanced. In both soils, differences were not observed at 0 t $ha^{-1}$, while at 10 t $ha^{-1}$ between 15

**Figure 4.1:** ***Ryegrass yield (g DM).***



and 25 $^{0}$C there was 0.5 g DM difference, and at 50 t ha$^{-1}$ between 2 temperatures there was a difference of 1 g pot$^{-1}$ for the LS and 1.5 g for the CL soils. In treatments in the warm environment, in the second harvest (Week 14), a significant decrease of the production was observed, which was accompanied by a decrease in the metals uptake. This is probably due to an insect infestation that took place between harvests at Weeks 7 and 14. The plants were sprayed with an insecticide not containing any of the metals under study and the production increased again. In the fourth harvest (Week 28), another steep decrease in the metals uptake happened in the warm temperature plants, which does not follow the general trend. This occurred when the heating device in the greenhouse was out of order and took a week to be fixed. Thus, it is likely that the plants were stressed by this condition.

### 4.3.2 Concentrations of Heavy Metals in Ryegrass

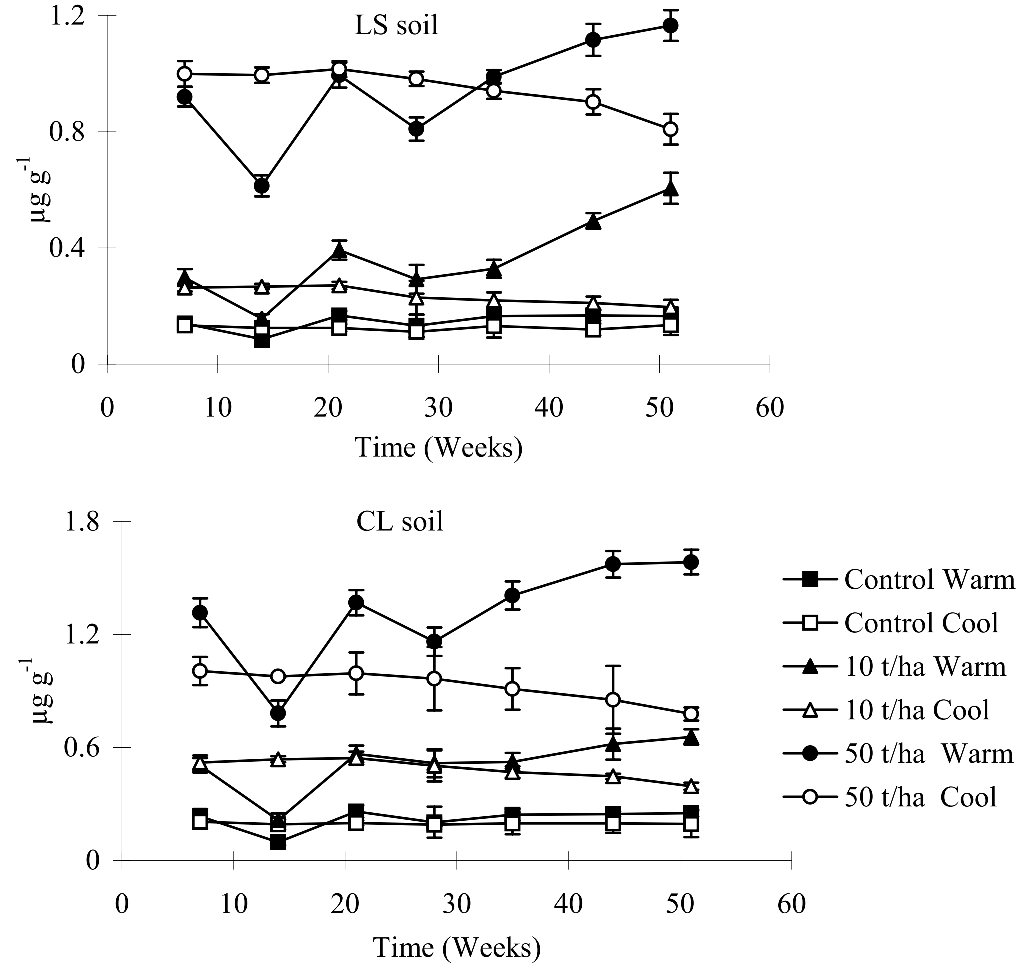
The plants grown in the CL soil had higher levels of all metals in all treatments than those grown in the LS soil. This can be explained by the fact that the CL soil had a greater initial concentration in total and bioavailable forms of metals, as is apparent from the DTPA and $CaCl_2$ extractions.

#### 4.3.2.1 Cadmium

Cadmium concentrations in the plant mass (DM) are shown in Figure 4.2.1 and in Appendix I -Table A1.1. Cadmium concentrations in the plants grown in the CL soil were up to 1.58 μg $g^{-1}$, while in the LS soil they were up to 1.17 μg $g^{-1}$ at the 50 t $ha^{-1}$ rate. The sewage sludge rate had a significant effect on the Cd concentrations in the ryegrass. As sludge rate increased plant concentrations increased, as well for both soils and at both temperatures. In the LS soil, the difference between 10 and 50 t $ha^{-1}$, for both temperatures, if the control is subtracted, was around 5 times, which was also the rate of increase in sludge addition. In the CL soils, however, the rate of increase was lower, probably due to greater adsorption of Cd on the soil constituents and lower availability of this metal in this soil.

Temperature played a vital role in the concentrations of Cd in the plant. At the higher temperature, plant concentrations were higher than in the lower temperature with an LSD of 0.08 (Figure 4.2.2). Figure 4.2.2 was extracted from Figure 4.2.1, by taking the mean of the values over time for the all three sludge treatments in both temperatures and soils and then subtracting the control sludge treatment (*ie*, there is no time factor in Figure 4.2.2). The same sort of graph is displayed for the rest of metals as well (where Figures 4.2.4, 4.2.6 and 4.2.8 are extracted from Figures 4.2.3, 4.2.5 and 4.2.7, respectively). The same graphs are done for $CaCl_2$ and DTPA extractions as well. The graphs between the $CaCl_2$ extractions and plant concentration are very similar and this indicates that $CaCl_2$ was the extractant that predicted heavy metal availability better than DTPA. In the LS soil, ambient temperature had the same effect at both 10 and 50 t $ha^{-1}$ rates of sludge, where the decrease from 25 to 15 $^{0}$C was of around 0.1 μg $g^{-1}$. In the CL soils, however, the rate of decrease in concentration was affected by the sludge rate as well as the temperature. At 10 t $ha^{-1}$, Cd concentrations in the plants in both temperatures were not significantly different, while at 50 $ha^{-1}$ the difference between the two temperatures was around 0.6 μg $g^{-1}$. Temperature had a more dramatic effect in the plants grown in the soils with the greater CEC (in the CL soil), where the availability of Cd was better controlled by sorption on the solid phase of the soil, probably because more Cd was exchangeable per unit mass of soil and thus more Cd became readily available to plants when organic matter decomposed more rapidly at 25 $^{0}$C than at 15 $^{0}$C. Over the one year residual period, Cd taken up by the ryegrass increased significantly in the warm environment treatments, except the controls, with an LSD of 0.08, especially at the higher sludge rate. However, in the LS soil this trend was only observed after week 28. Until that time, if the second and fourth cuttings are not taken into account, there was no significant difference

**Figure 4.2.1:** ***Concentration of Cd in ryegrass ($\mu g\ g^{-1}$).***



*LSD Test*

*Soil 0.03* *Rate x Temp 0.06* *Temp x Time 0.12****

*Rate 0.04* *Soil x Rate x Temp 0.08* *Soil x Rate x Time 0.23***

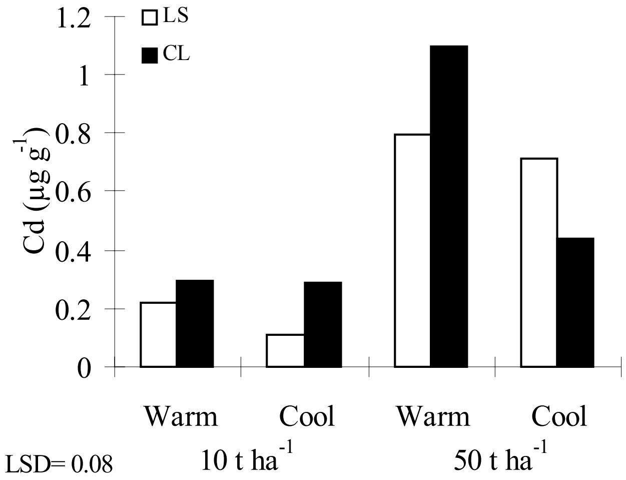
*Soil x Rate 0.06**** *Time 0.08** *Soil x Temp x Time 0.18*

*Temp 0.03** *Soil x Time 0.12* *Rate x Temp x Time 0.23****

*Soil x Temp 0.05* *Rate x Time 0.10****

*Soil x Rate x Temp x Time 0.40****

**Figure 4.2.2:** ***Cd concentration in ryegrass (control treatments are subtracted) (μg g$^{-1}$).***

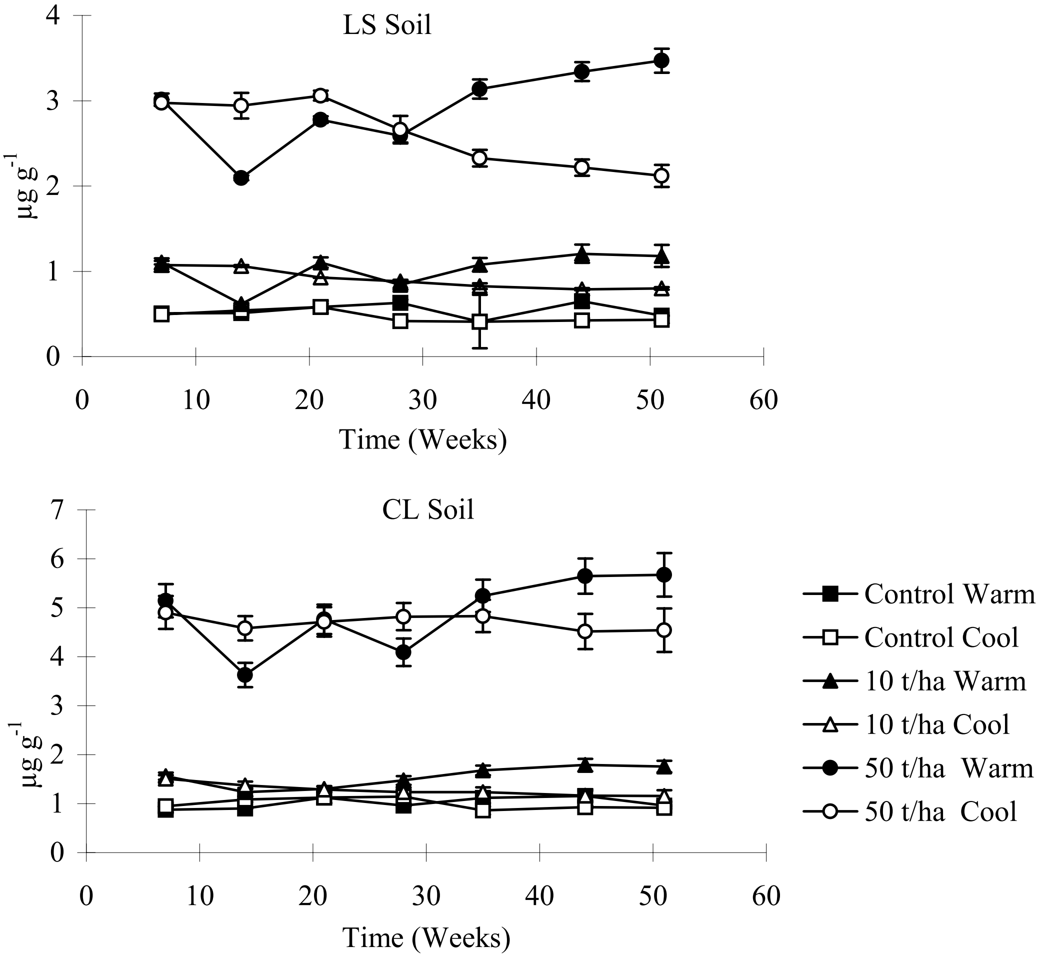


between temperatures at all sludge application rates. After that time, the differences between temperatures became significant. In the CL soil, the difference between temperatures was apparent even from the first harvesting at 50 t ha$^{-1}$. Despite the fact that there was an increase over time in the warm environment, in the plants grown at 15 $^{0}$C, a decrease over time may be observed, especially in the high sludge rates. This behaviour of the warm and the cold environments may be explained by the fact that the rapid decomposition of the organic matter may have caused the increase in uptake in the warm environment, because metals formerly bound on the organic matter may have been released and become more bioavailable to the plants.

### 4.3.2.2 Nickel

Nickel concentrations in the plant mass are shown in Figure 4.2.3 and in Appendix I - Table A1.2. Nickel concentrations in the plants grown in the CL soil were up to 5.68 μg g$^{-1}$, while in the LS soil it was up to 3.47 μg g$^{-1}$ at the 50 t ha$^{-1}$ rate. As the sludge rate increased, plant concentrations increased in both soils and at both temperatures. In the LS soil the difference between 10 and 50 t ha$^{-1}$ (if the control is subtracted) was of a factor of around 5 times (from 0.5 to 2.5 μg g$^{-1}$ ), and this was also the case of Cd, which was the rate of increase of sludge addition and in the CL soil slightly greater than 5 (from 0.5 to 3.5 μg g$^{-1}$). At the higher temperature, plant concentrations were higher than in the lower temperature in both soils and at both 10 and 50 t ha$^{-1}$, but this was not significant (LSD of 0.10) (Figure 4.2.4).

**Figure 4.2.3:** ***Concentration of Ni in ryegrass (μg $g^{-1}$).***



*LSD Test*

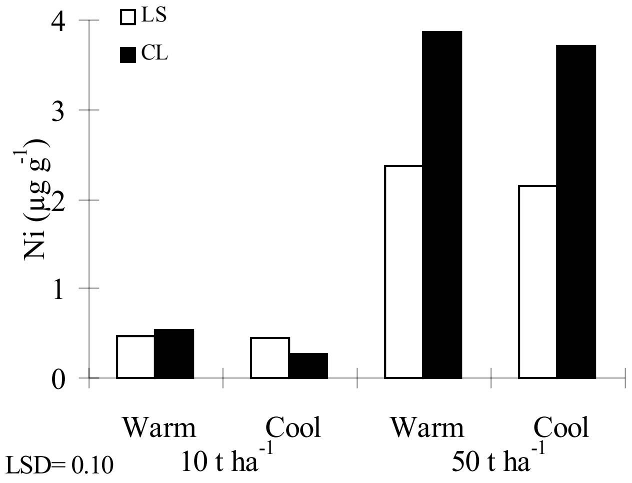
*Soil 0.18*
*Rate 0.14*
*Soil x Rate 0.16***
*Temp 0.18*
*Soil x Temp 0.23*

*Rate x Temp 0.26*
*Soil x Rate x Temp 0.10*
*Time 0.08**
*Soil x Time 0.12*
*Rate x Time 0.10****

*Temp x Time 0.12*
*Soil x Rate x Time 0.24*
*Soil x Temp x Time 0.19*
*Rate x Temp x Time 0.24*

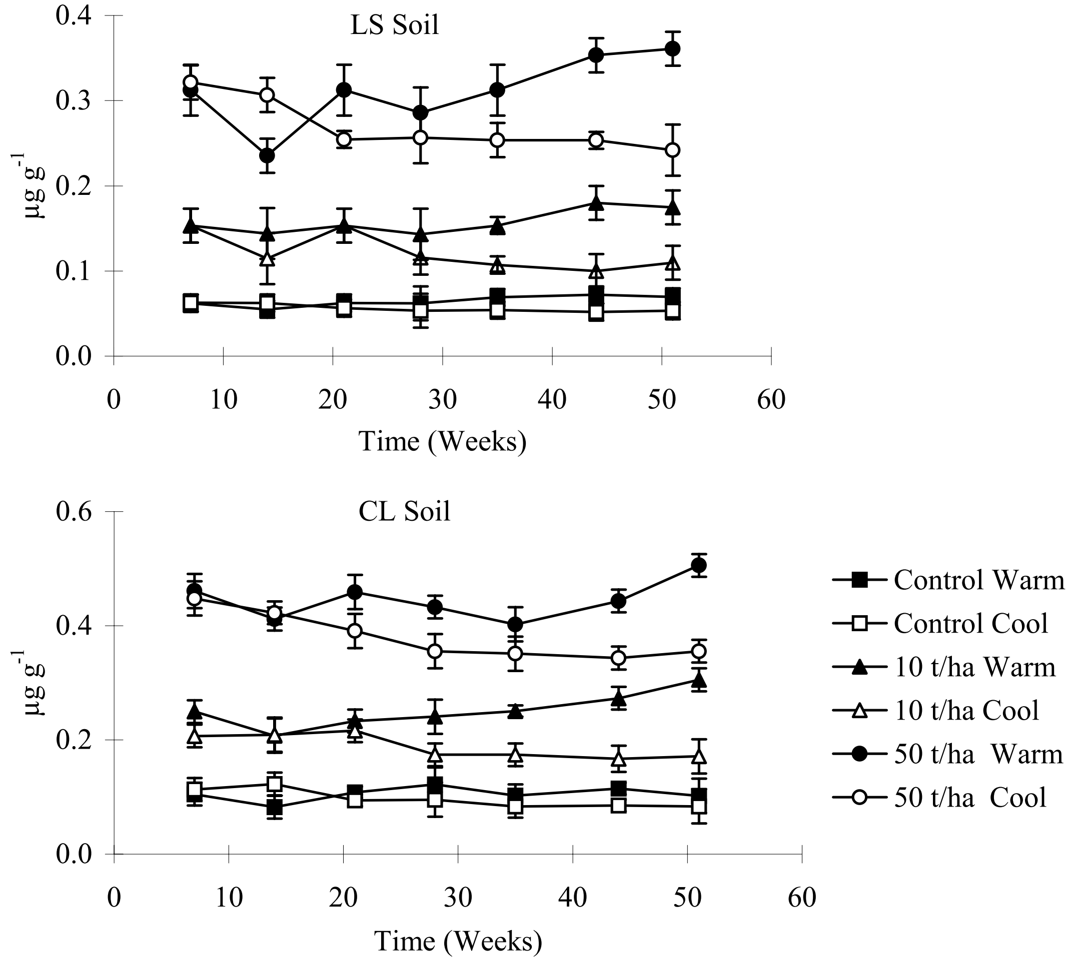
*Soil x Rate x Temp x Time 0.42*

**Figure 4.2.4:** ***Ni concentration in ryegrass (control treatments are subtracted) (µg g$^{-1}$).***



In the course of the one year residual period, there was a significant trend of increasing Ni being taken up by the test crop in the 25 $^{0}$C treatments, except the controls (LSD of 0.08), especially at the high sludge rate. However, in the LS soil, this trend could only be observed after week 28. Until that time, if no account was taken of the second and fourth cuttings, there was no significant difference between

**Figure 4.2.5:** ***Concentration of Pb in ryegrass (μg $g^{-1}$).***



*LSD Test*

*Soil 0.01* *Rate x Temp 0.02* *Temp x Time 0.03*

*Rate 0.02* *Soil x Rate x Temp 0.03** *Soil x Rate x Time 0.06****

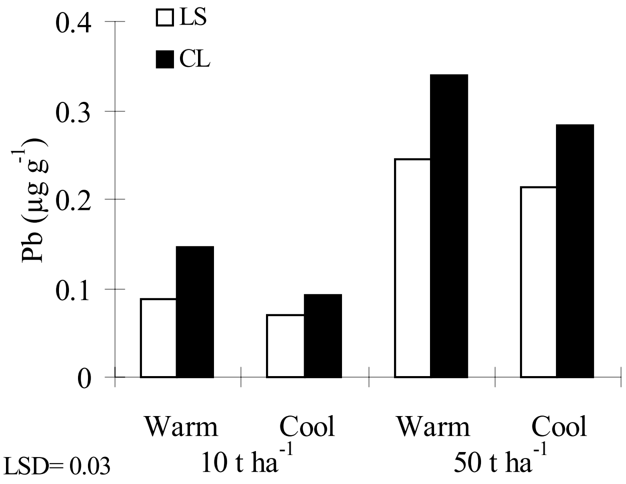
*Soil x Rate 0.01**** *Time 0.03* *Soil x Temp x Time 0.05*

*Temp 0.01** *Soil x Time 0.03**** *Rate x Temp x Time 0.06****

*Soil x Temp 0.02* *Rate x Time 0.03****

*Soil x Rate x Temp x Time 0.10****

**Figure 4.2.6:** ***Pb concentration in ryegrass (control treatments are subtracted) (μg g$^{-1}$).***



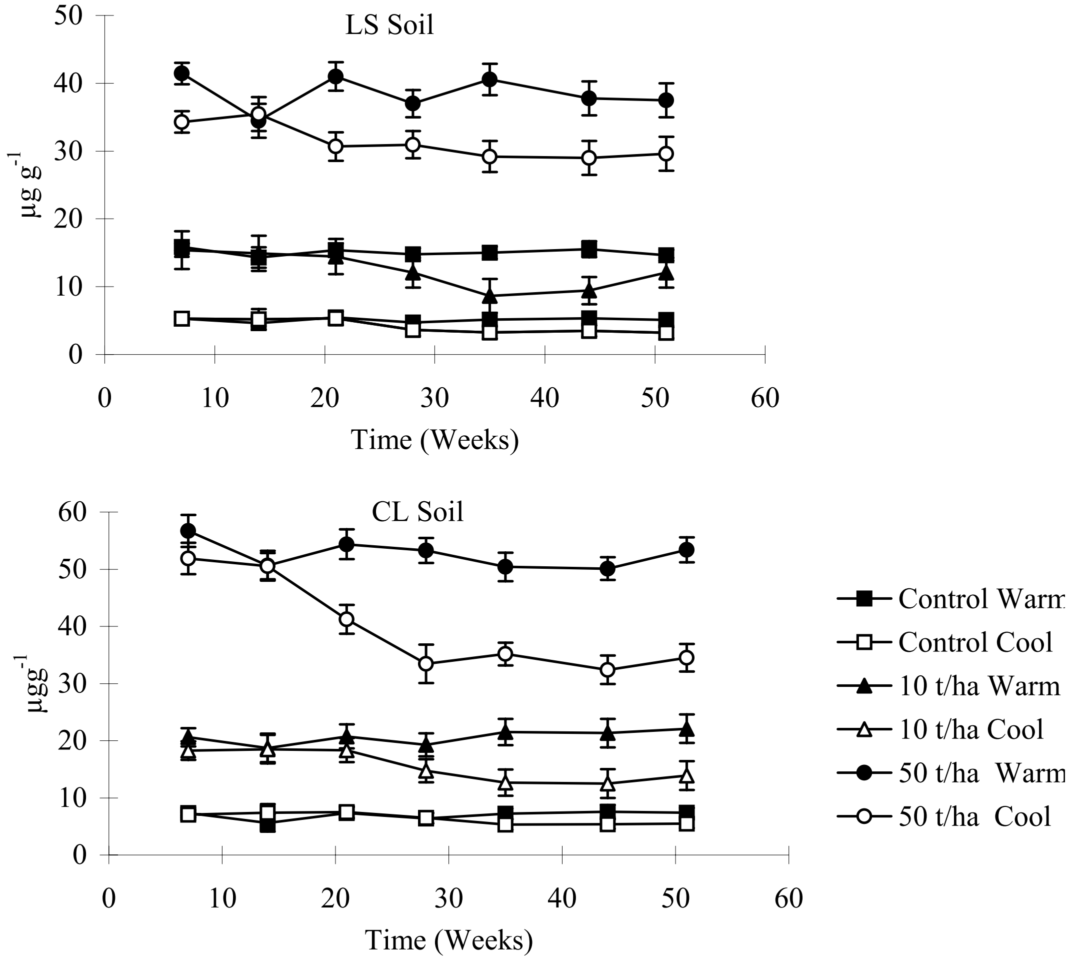
temperatures at all sludge application rates. After that time, the differences between temperatures became significant in both the sludge treatments. In the CL soil the difference between temperatures was again not apparent until the fourth cutting. Nickel concentration in the plants grown at 15 $^0$C decreased over time, especially in the high sludge rates, as in the case of Cd.

### 4.3.2.3 Lead

Lead concentrations in the plant mass are shown in Figure 4.2.5 and in Appendix I - Table A1.3. Lead concentrations in the plants grown in both soils were very low. In the CL soil these were up to 0.51 μg g$^{-1}$, while in the LS soil these were up to 0.36 μg g$^{-1}$ at the 50 t ha$^{-1}$ rate. The sewage sludge rate also had a significant effect on the Pb concentrations in the plant. As the sludge rate increased, plant concentrations increased in both soils and at both temperatures. In the LS soil the difference between 10 and 50 t ha$^{-1}$ was very small. This small increase in plant concentration compared with Cd and Ni was probably due to the fact that Pb was the least mobile heavy metal in this study and is widely recognised as having a low soil-plant transfer coefficient (Kloke *et al.*, 1984). The sewage sludge organic matter held Pb strongly, so that the availability was effectively decreased as the Pb was supplied to soils in the sludge. Temperature played a vital role in the concentration of Pb in the ryegrass only in the CL soil (LSD 0.03), where at 50 t ha$^{-1}$ it increased from 0.28 to 0.33 μg g$^{-1}$ between 15 and 25 $^0$C (Figure 4.2.6).

In the course of the one year residual period there was a trend of increasing Pb taken up by the test crop in the warm environment treatments, except the controls. However, this increase was not very significant (LSD of 0.03). Despite the fact that there was an increase

**Figure 4.2.7:** ***Concentration of Zn in ryegrass (μg g$^{-1}$).***



*LSD Test*

*Soil 3.89**

*Rate 4.76***

*Soil x Rate 6.74**

*Temp 3.89**

*Soil x Temp 5.49**

*Rate x Temp 6.74***

*Soil x Rate x Temp 6.86****

*Time 8.45****

*Soil x Time 13.02****

*Rate x Time 10.97****

*Temp x Time 13.02****

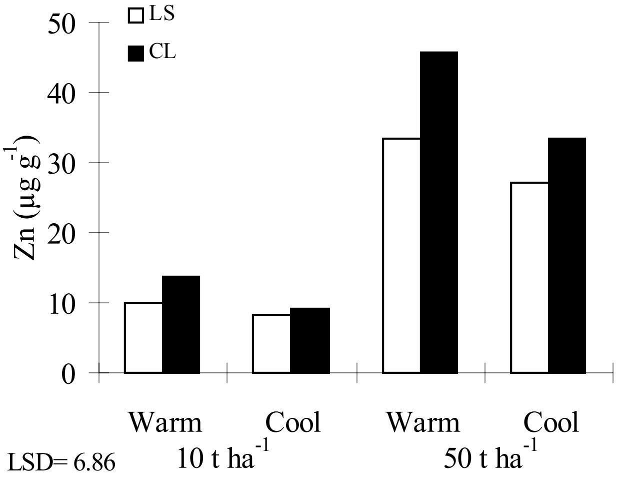
*Soil x Rate x Time 25.67****

*Soil x Temp x Time 19.74****

*Rate x Temp x Time 25.67****

*Soil x Rate x Temp x Time 43.56****

**Figure 4.2.8:** ***Zn concentration in ryegrass (control treatments are subtracted) (μg g$^{-1}$).***



over time in the warm environment, in the plants grown at 15 $^0$C a decrease over time was observed, although this was not very significant. The fact that Pb was not significantly affected by the time factor is probably due to the fact that this element is the least mobile metal considered in this study and the temperature had a minimal effect on it.

**4.3.2.4 Zinc**

Zinc concentrations in the plant mass are shown in Figure 4.2.7 and in Appendix I -Table A1.4. Zinc concentrations in the plants grown in the CL soil were up to 53.40 μg g$^{-1}$, while in the LS soil they were up to 37.50 μg g$^{-1}$ at the 50 t ha$^{-1}$ rate, the highest of all the metals investigated, probably because the concentration of Zn in the sewage sludge was the highest among the other metals. Soil played a significant role on Zn concentrations in plant (LSD value of 3.89). The sewage sludge rate also had a significant effect on the Zn concentrations in the plant. As sludge rate increased, plant concentrations increased in both soils and at both temperatures. In the LS soil the difference between 10 and 50 t ha$^{-1}$ (when the control was subtracted), was the same as the CL soil.

Temperature played a vital role in the concentration of Zn in the plant. At 25 $^0$C the plant concentrations were higher than at 15 $^0$C (LSD of 3.89) (Figure 4.2.8), and these differences became greater with increasing sludge application rate. In the CL soil at 10 t ha$^{-1}$, Zn concentration in ryegrass was lower by 4.5 μg g$^{-1}$ from 25 $^0$C to 15 $^0$C, while at 50 t ha$^{-1}$ the difference was of around 12.2 μg g$^{-1}$. In the LS soils, however, the decrease in concentration was much less than in the CL soil. At 10 t ha$^{-1}$ the ryegrass at both temperatures had statistically the same concentration of Zn, while at 50 ha$^{-1}$ the difference between the two temperatures was around 6.4 μg g$^{-1}$. Temperature had a more dramatic effect in the plants grown in soils with greater CEC, where the availability of Zn was better controlled by the solid phase of the soil. This behaviour was very similar to that observed with Cd, and was probably due to the fact that both elements have very similar chemical behaviour in the soil environment under the same conditions (Bailey *et al.*, 1995).

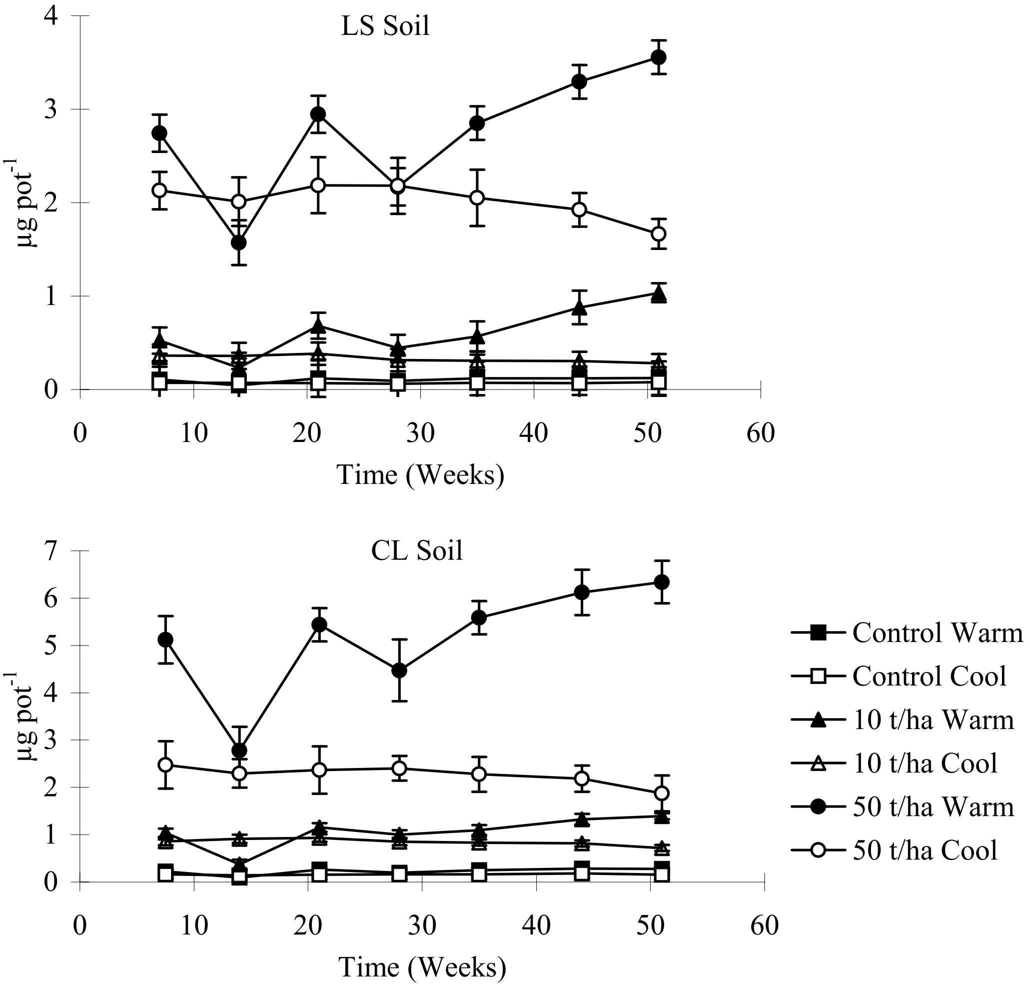
In the course of the one year residual period, there was no trend of increasing Zn, unlike what was found for Cd and Ni. However, throughout this residual period, plants grown at the higher temperature had greater Zn concentrations. Despite the fact that there was an increase over time in the warm environment, the plants grown at 15 $^{0}$C showed a decrease over time, especially at high sludge rates, in the CL soil.

The amounts of metals taken up by the ryegrass in declining order were : Zn > Ni > Cd > Pb when all treatments, in both soils were taken into consideration.

### 4.3.3 Uptake of Heavy Metals by Ryegrass

Cadmium, Ni, Pb and Zn uptake by the plant mass in this experiment is shown in Figures 4.3.1-4.3.4 and in Appendix I (Tables A2.1-A2.4). Plant uptake data confirmed the plant concentration findings for all 4 metals. The soil type was statistically important in affecting the plant uptake only in case of Zn (LSD of 4.48). As sewage sludge additions increased, uptake increased as well. This pattern was very significant for all elements, with an LSD of 0.71 for Cd, 2.57 for Ni, 0.15 for Pb and 6.73 for Zn.

**Figure 4.3.1:** ***Uptake of Cd by ryegrass (µg pot$^{1}$).***



*LSD Test*

*Soil 0.41*

*Rate 0.50*

*Soil x Rate 0.71**

*Temp 0.41****

*Soil x Temp 0.66**

*Rate x Temp 0.80*

*Soil x Rate x Temp 0.11*

*Time 0.80***

*Soil x Time 1.30*

*Rate x Time 1.10****

*Temp x Time 1.30****

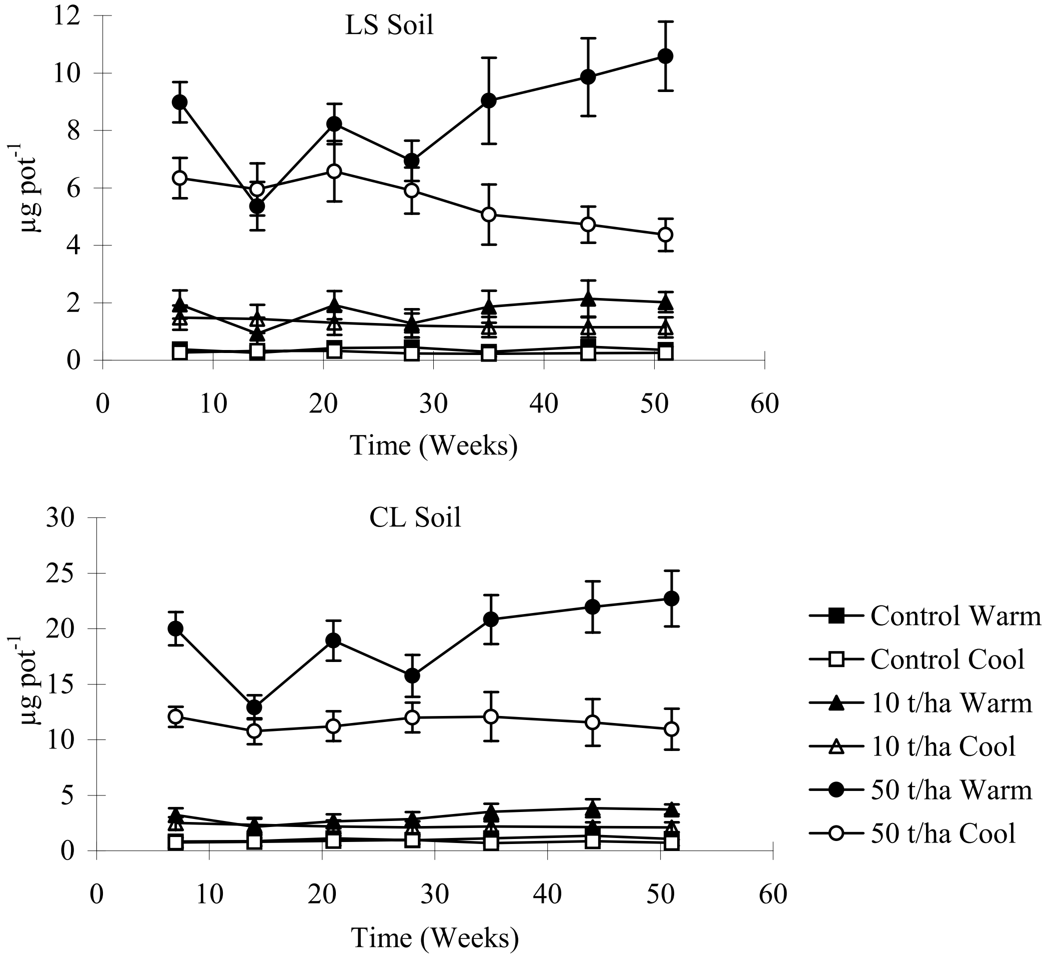
*Soil x Rate x Time 2.60****

*Soil x Temp x Time 1.84*

*Rate x Temp x Time 2.55****

*Soil x Rate x Temp x Time 4.59****

**Figure 4.3.2:** ***Uptake of Ni by ryegrass (μg pot$^{-1}$).***



*LSD Test*

*Soil 1.36*

*Rate 1.34*

*Soil x Rate 2.57***

*Temp 1.36*

*Soil x Temp 1.16*

*Soil x Rate x Temp x Time 20.53*

*Rate x Temp 1.57*

*Soil x Rate x Temp 1.20*

*Time 2.50**

*Soil x Time 5.63*

*Rate x Time 6.29****

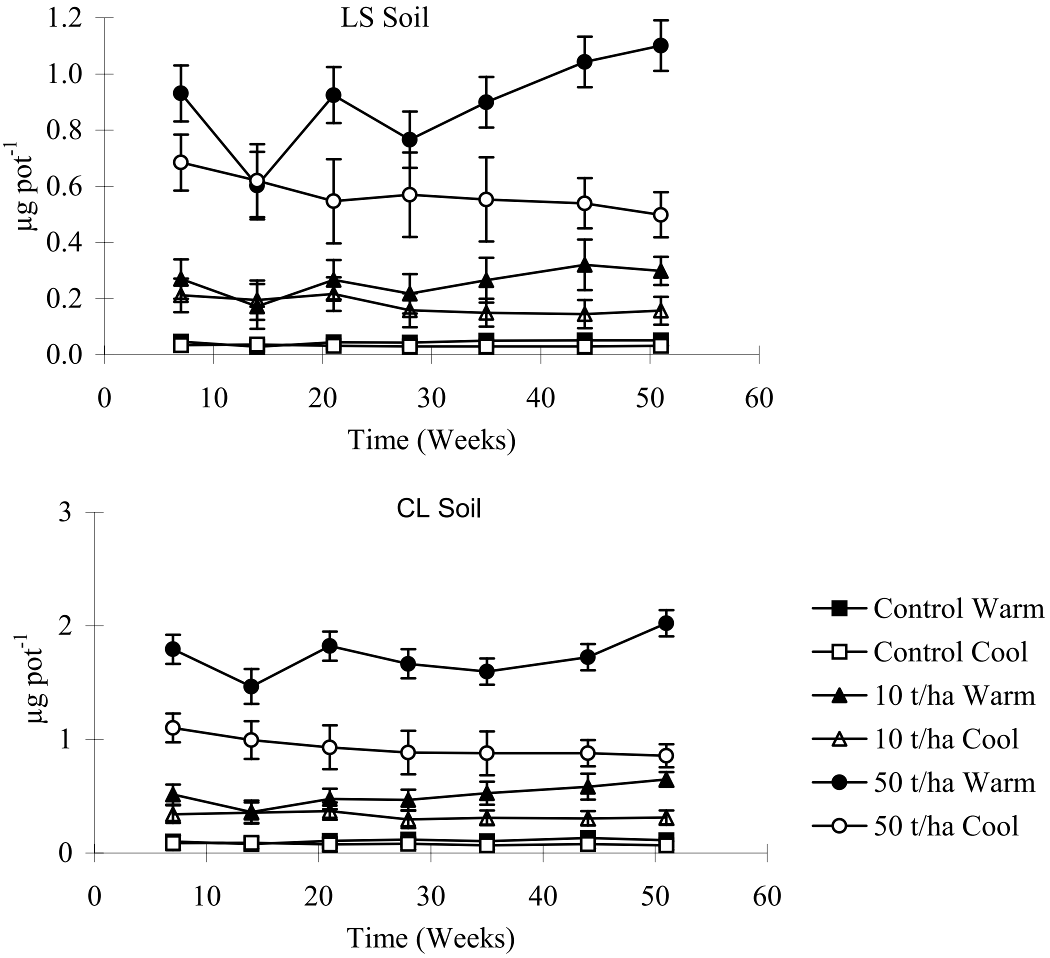
*Temp x Time 7.50*

*Soil x Rate x Time 10.13*

*Soil x Temp x Time 9.38*

*Rate x Temp x Time 12.13*

**Figure 4.3.3:** ***Uptake of Pb by ryegrass (µg pot$^{-1}$).***



*LSD Test*

*Soil 0.09*

*Rate 0.11*

*Soil x Rate 0.15****

*Temp 0.09**

*Soil x Temp 0.12*

*Rate x Temp 0.87*

*Soil x Rate x Temp 1.49*

*Time 0.18*

*Soil x Time 0.28****

*Rate x Time 0.24****

*Temp x Time 0.28****

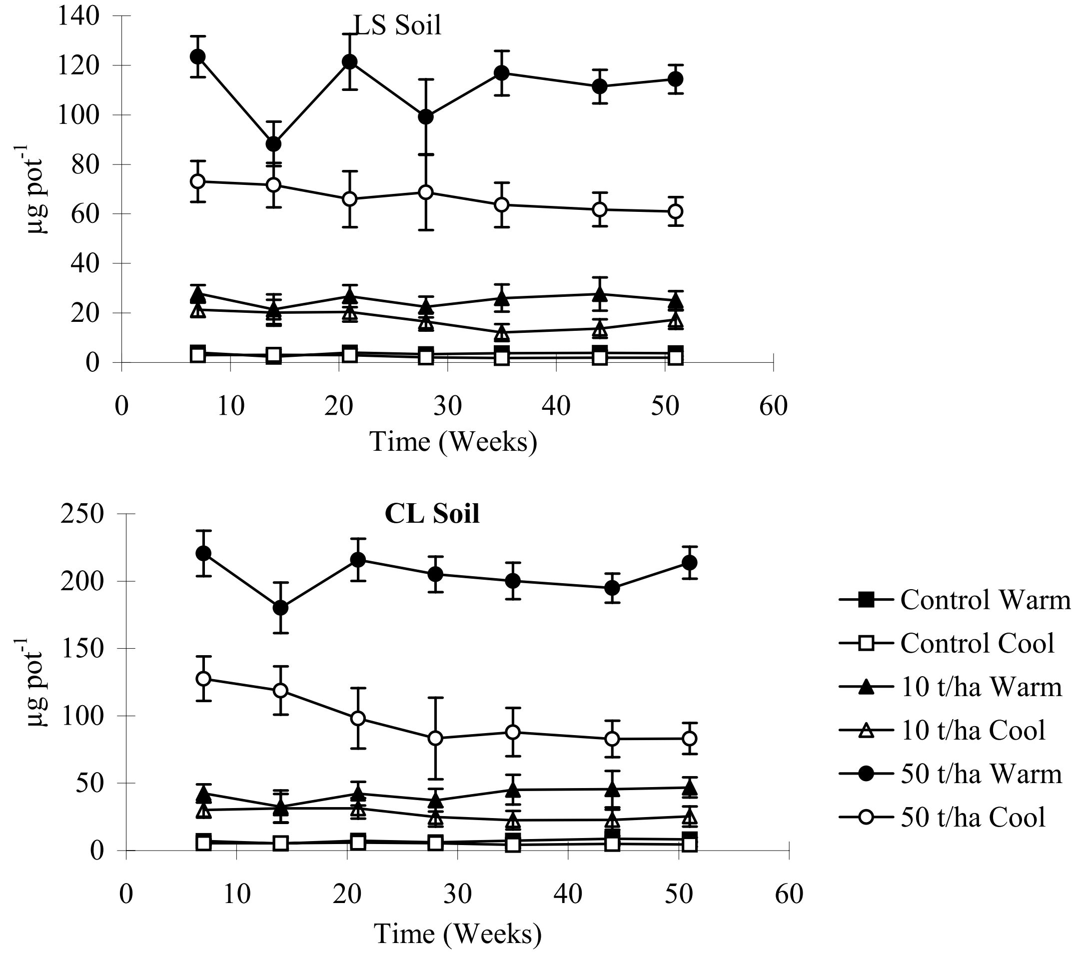
*Soil x Rate x Time 0.55*

*Soil x Temp x Time 0.41*

*Rate x Temp x Time 0.55****

*Soil xRate x Temp x Time 0.92****

**Figure 4.3.4:** ***Uptake of Zn by ryegrass (μg pot$^{-1}$).***



*LSD Test*

*Soil 4.48** *Rate x Temp 6.23*** *Temp x Time 10.12****

*Rate 5.32*** *Soil x Rate x Temp 6.86**** *Soil x Rate x Time 22.28****

*Soil x Rate 6.73** *Time 7.81* *Soil x Temp x Time 11.61****

*Temp 4.48* *Soil x Time 10.13**** *Rate x Temp x Time 22.25****

*Soil x Temp 5.61** *Rate x Time 9.08****

*Soil x Rate x Temp x Time 33.71****

**Figure 4.3.5:** ***Cd uptake by ryegrass (control treatments are subtracted) (μg pot$^{-1}$).***

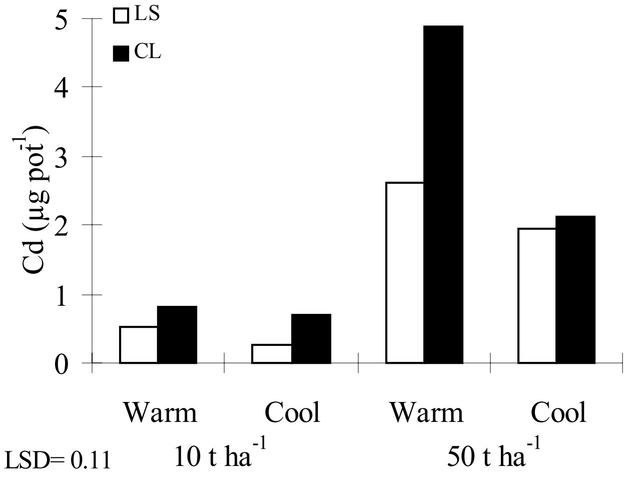


**Figure 4.3.6:** ***Ni uptake by ryegrass (control treatments are subtracted) (μg pot$^{-1}$).***

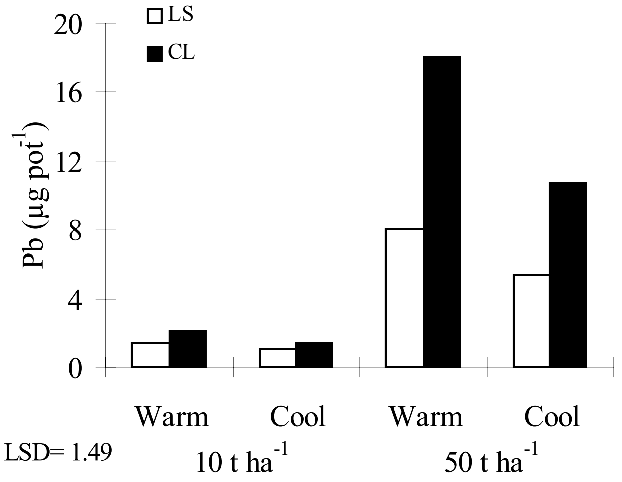


**Figure 4.3.7:** ***Pb uptake by ryegrass (control treatments are subtracted) (μg pot$^{-1}$).***

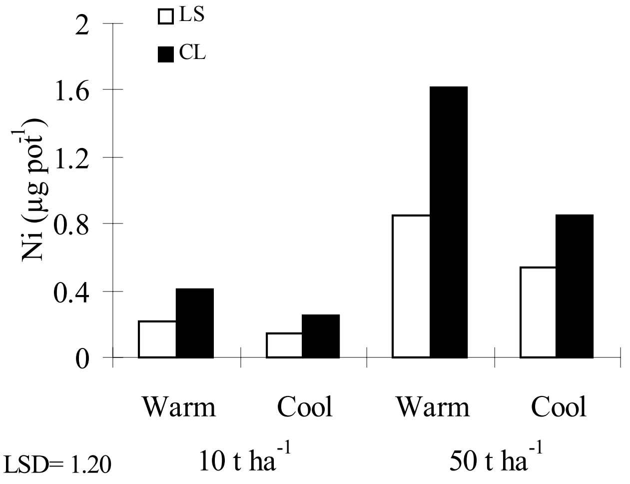
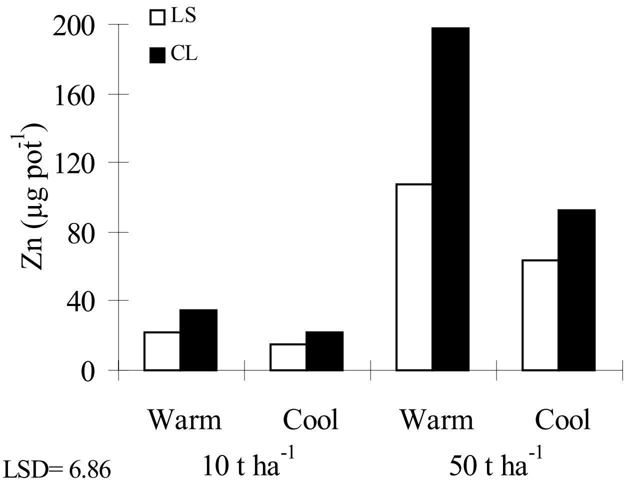


**Figure 4.3.8:** ***Zn uptake by ryegrass (control treatments are subtracted) (μg pot$^{-1}$).***



Temperature was a factor that affected plant uptake very significantly, especially at the higher sludge application rate in the CL soil (Figures 4.3.5, 4.3.6, 4.3.7 and 4.3.8; these were extracted from Figures 4.3.1, 4.3.2, 4.3.3 and 4.3.4, respectively in the same way as Figure 4.2.2 was extracted from 4.2.1). In case of Cd in the CL soil at 10 t ha$^{-1}$, uptake was statistically the same at both temperatures, but at 50 t ha$^{-1}$ plants grown at 15 $^{0}$C took up only half the amounts taken up by the plants at 25 $^{0}$C (4.8 and 2.1 μg Cd pot$^{-1}$ for 25 and 15 $^{0}$C respectively) (LSD 0.11). In the SL soil, differences for the two temperatures were again a lot greater at 50 t ha$^{-1}$. At 10 t ha$^{-1}$ the Cd uptake was 0.52 μg pot$^{-1}$ for the warm environment and 0.26 μg pot$^{-1}$ for the cool, while at 50 t ha$^{-1}$ it was 2.62 μg pot$^{-1}$ and 1.94 μg pot$^{-1}$ for the warm and the cool ambient temperatures, respectively.

The same pattern was also followed by Ni. In the CL soil at 10 t ha$^{-1}$, uptake was statistically the same, but at 50 t ha$^{-1}$ plants grown at 15 $^{0}$C took up 10.70 μg Ni pot$^{-1}$ and at 25 $^{0}$C 17.97 μg Ni pot$^{-1}$ (LSD 1.49). In the LS soil, the differences for 2 temperatures were again a lot greater in the 50 t ha$^{-1}$ treatment. At 10 t ha$^{-1}$ there was not any difference between the 2 temperatures, while at 50 t ha$^{-1}$ the uptake was 8.05 and 5.29 μg Ni pot$^{-1}$ for the warm and the cool ambient temperature respectively.

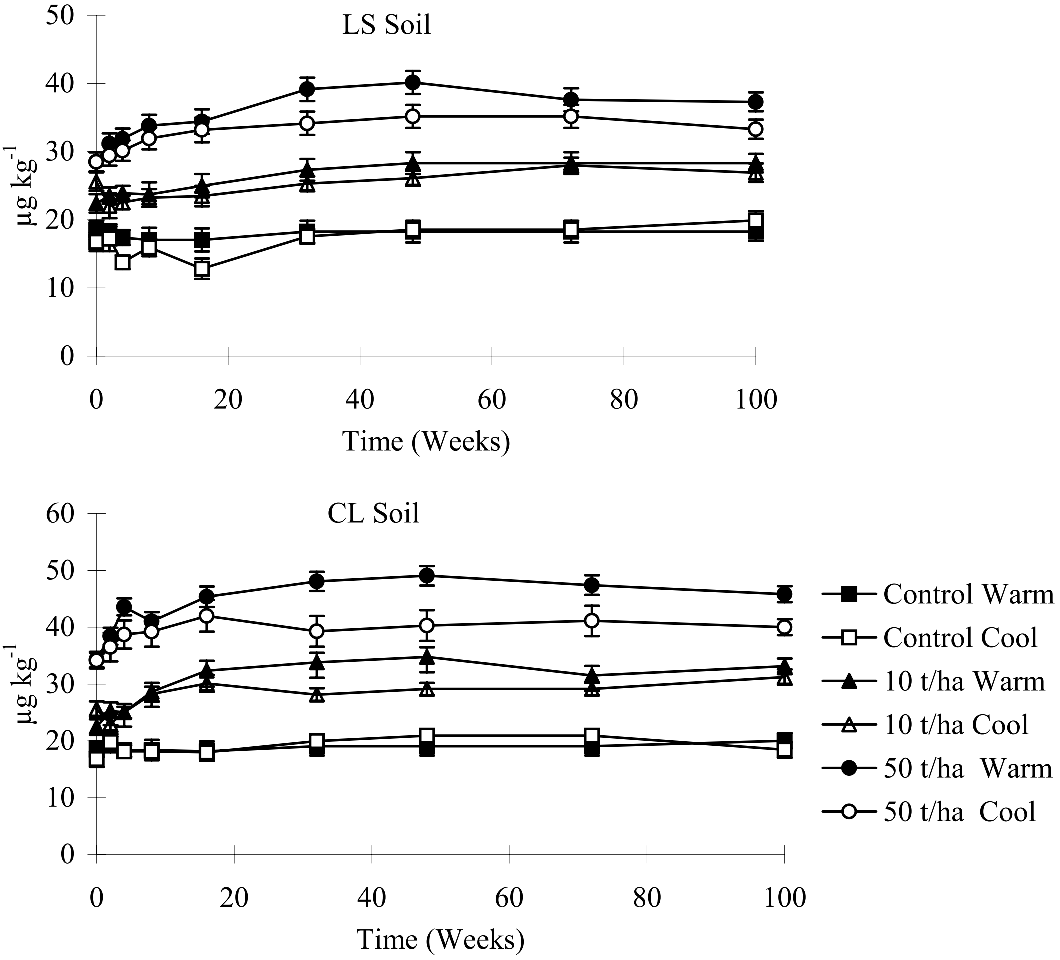
In case of Pb, in the CL soil at 10 t ha$^{-1}$ the uptake was statistically the same between the two temperatures, but at 50 t ha$^{-1}$ plants at 15 $^{0}$C took up 0.85 μg Pb pot$^{-1}$ and in 25 $^{0}$C they amounted 1.61 μg Pb pot$^{-1}$ (LSD 1.2). In the LS soil differences for 2 temperatures were again much greater at 50 t ha$^{-1}$. At 10 t ha$^{-1}$ there was not any difference between the 2 temperatures, while at 50 t ha$^{-1}$ the uptake of Pb was 0.85 and 0.54 μg pot$^{-1}$ for the warm and the cool environment respectively. In the case of Zn, at 10 t ha$^{-1}$, neither soils showed any differences between the two temperatures, but at 50 t ha$^{-1}$, the differences were significant with an LSD of 6.86. At this rate of sludge, in the CL soil, differences for the two temperatures were a lot greater than in the LS soil, as with all other metals. At 50 t ha$^{-1}$, in the LS soil, the values were 64.13 and 107.12 μg Zn pot$^{-1}$ for the cool and warm temperatures and in the CL soil the values were 92.21 and 197.13 μg Zn pot$^{-1}$ (almost double the uptake, like in case of Cd) for cool and warm ambient temperatures respectively (Figure 4.3.5-4.3.8).

Over time, uptake of Cd and Ni increased significantly in the plants grown at 25 $^{0}$C (LSD of 0.8 for Cd and 2.50 Ni), while Pb increased only in the LS soil. In the case of Pb, in the CL soil and for Zn in both soils, no increase was observed. In the cool environment, as in case of the plant concentrations, there was a decrease over time.

### 4.3.4 Heavy metals extracted with $CaCl_2$

The Cd, Ni and Zn concentrations extracted with $CaCl_2$ are shown in Figures 4.4.1-4.4.3 and in Appendix I (Tables A4.1-A4.3). The $CaCl_2$ solution in all cases did not

**Figure 4.4.1:** ***$CaCl_2$- extractable Cd (μg $kg^{-1}$).***



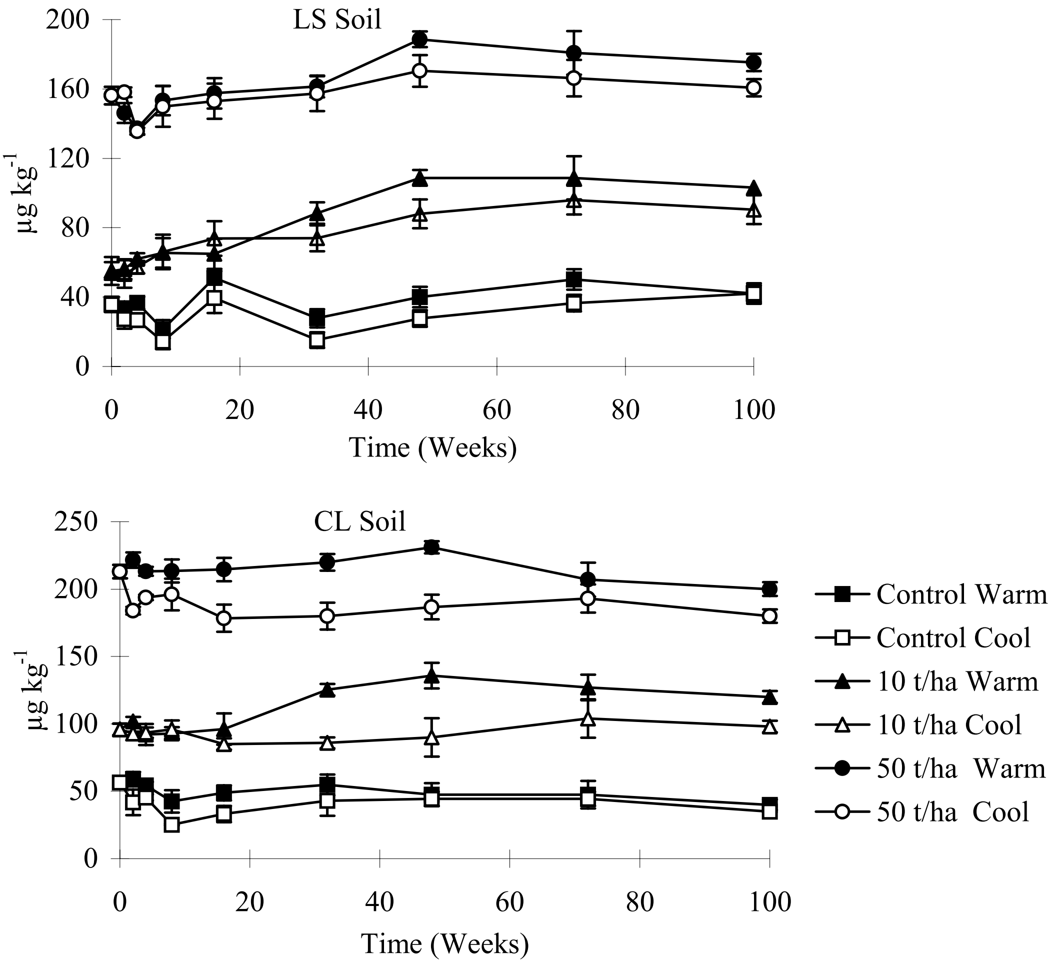
*LSD Test*

Soil 0.53
Rate 0.64
Soil x Rate 0.90***
Temp 0.53***
Soil x Temp 0.74***
Soil x Rate x Temp x Time 5.66***

Rate x Temp 0.90***
Soil x Rate x Temp 1.31***
Time 1.12***
Soil x Time 1.72
Rate x Time 1.45***

Temp x Time 1.72**
Soil x Rate x Time 3.34***
Soil x Temp x Time 2.56***
Rate x Temp x Time 3.34

**Figure 4.4.2:** ***$CaCl_2$-extractable Ni (µg $kg^{-1}$).***



*LSD Test*

*Soil 1.04*

*Rate 1.27**

*Soil x Rate 1.79*

*Temp 1.04*

*Soil x Temp 1.47*

*Soil x Rate x Temp x Time 11.31*

*Rate x Temp 1.79*

*Soil x Rate x Temp 2.61*

*Time 2.23***

*Soil x Time 3.43**

*Rate x Time 2.89**

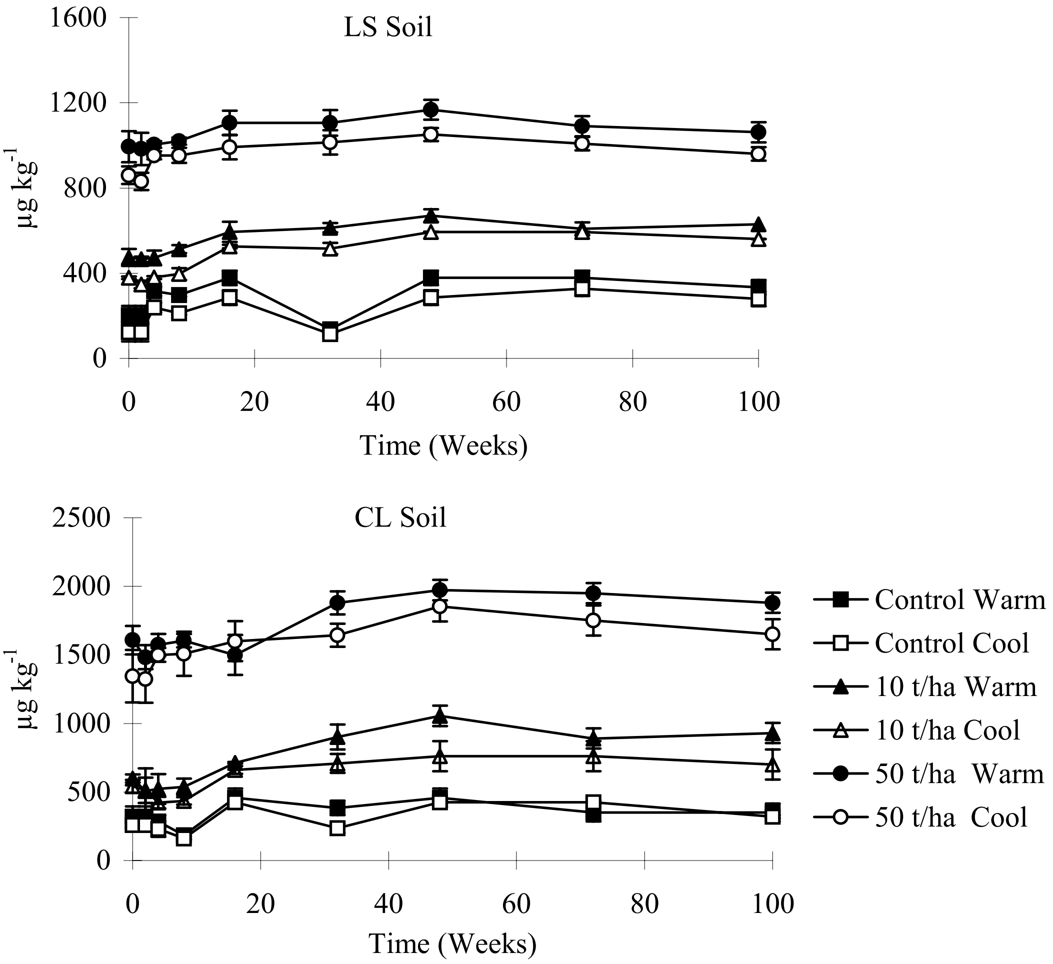
*Temp x Time 3.43*

*Soil x Rate x Time 6.67*

*Soil x Temp x Time 5.13*

*Rate x Temp x Time 6.67*

**Figure 4.4.3:** ***$CaCl_2$- extractable Zn (μg $kg^{-1}$).***



*LSD Test*

*Soil 24.02*
*Rate 29.43*
*Soil x Rate 41.70**
*Temp 24.02*
*Soil x Temp 33.95*
*Soil x Rate x Temp x Time 266.37*

*Rate x Temp 41.70*
*Soil x Rate x Temp 61.01*
*Time 52.34****
*Soil x Time 80.69*
*Rate x Time 68.03**

*Temp x Time 80.69*
*Soil x Rate x Time 156.96**
*Soil x Temp x Time 120.73*
*Rate x Temp x Time 156.96*

**Figure 4.4.4:** ***CaCl$_2$- extractable Cd (control treatments are subtracted) (μg kg$^{-1}$).***

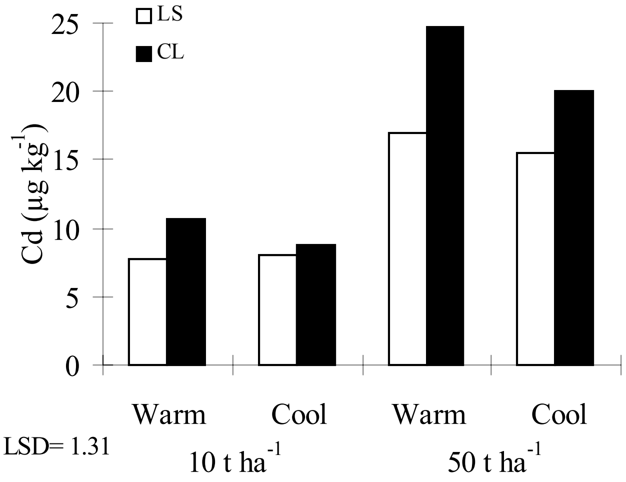


**Figure 4.4.5:** ***CaCl$_2$- extractable Ni (control treatments are subtracted) (μg kg$^{-1}$).***

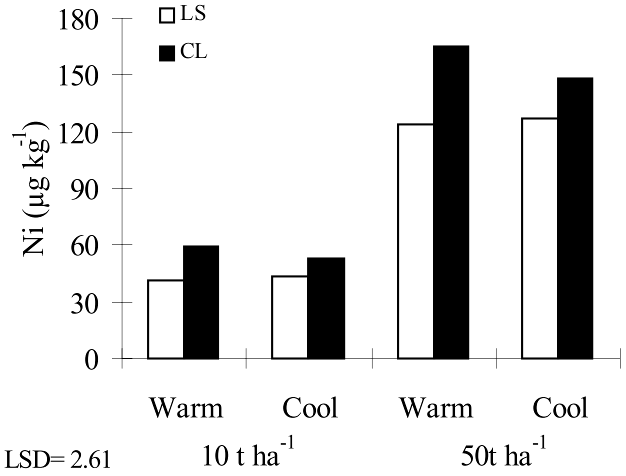
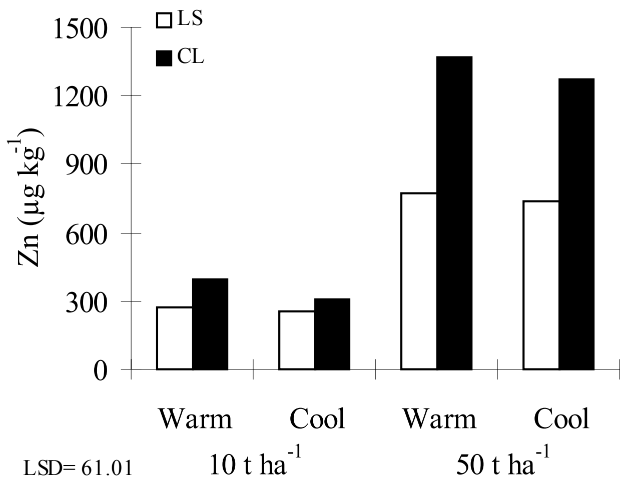


**Figure 4.4.6:** ***CaCl$_2$-extractable Zn (control treatments are subtracted) (µg kg$^{-1}$).***



extract detectable concentrations of Pb. As Pb is a very immobile element (Hooda and Alloway, 1993), it is very probable that it was strongly bound and not exchanged sufficiently and was present at concentrations in the extracts below the analytical detection limits (0.06 µg L$^{-1}$ is the detection limit of GFAAS). As expected, the CL soil showed greater extractable concentrations of all metals than the LS soil, although the soil factor did not significantly affect metal extractability with CaCl$_2$. As shown in Figures 4.4.1-4.4.3 the application of sewage sludge significantly increased the CaCl$_2$ extractable metal fractions, with the high application rate giving rise to the greatest concentrations (LSD values of 0.90 for Cd, 1.79 for Ni and 41.70 for Zn). In all treatments, samples taken from the greenhouse (25 $^0$C) had greater extractable heavy metals concentrations than those from the cool environment (15 $^0$C) (Figures 4.4.4, 4.4.5 and 4.4.6; these were extracted from Figures 4.4.1, 4.4.2 and 4.4.3, respectively in the same way as Figure 4.2.2 was taken from Figure 4.2.1. This effect was particularly significant in the CL soil and the difference was even greater at the higher sludge application rates, as in the plant concentrations. For all metals in the 10 t ha$^{-1}$ treatment, the temperature did not have a very significant effect, although at 50 t ha$^{-1}$ the warm environment gave rise to greater concentrations of metals than in the cool: 20.04 to 24.77 µg kg$^{-1}$for Cd, 148.42 to 164.69 µg kg$^{-1}$ for Ni, and 1270 to 1370 µg kg$^{-1}$ for Zn at 15 $^0$C and 25 $^0$C, respectively. The LSD values were 1.31, 2.61 and 61.01 for Cd, Ni and Zn, respectively (Figures 4.4.4-4.4.6). In almost all cases, the levels of the metals were significantly higher at the end of the period of 2 years of incubation than at the beginning. This trend was significant with LSD values of 1.12 for Cd, 2.23 for Ni and 52.34 for Zn. This may be due to the decomposition of the sludge-borne organic matter which occurred during that time. Some heavy metals would have been released into the soil solution and/or would have became readily exchangeable and thus extractable by $CaCl_2$. This decomposition was faster in the greenhouse (25 $^0$C) and this is why the concentrations extracted from the greenhouse samples were greater.

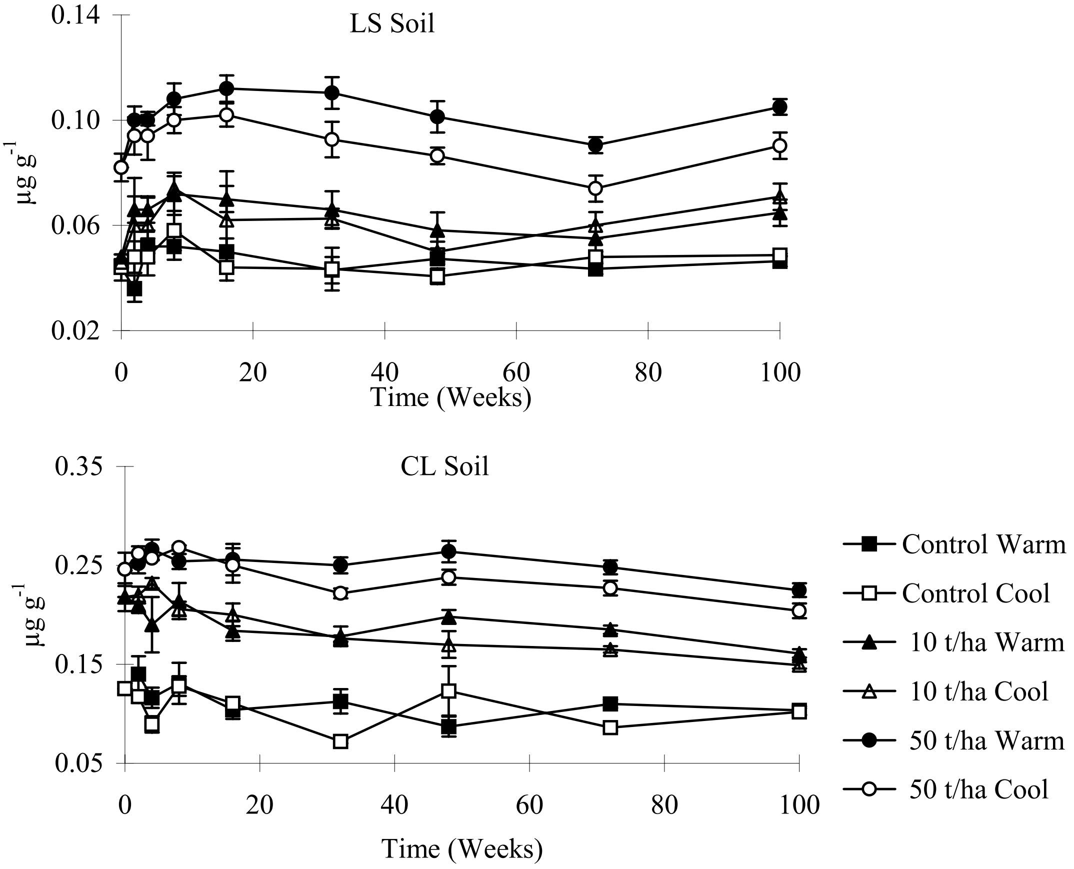
The order in which the metals were extracted by $CaCl_2$ in all the treatments was: Zn > Ni > Cd.

### 4.3.5 Heavy metals extracted with DTPA

The Cd, Ni, Pb and Zn concentrations extracted with DTPA are shown in Figures 4.5.1-4.5.4 and in Appendix I (Tables A5.1-A5.4). As was found for $CaCl_2$, DTPA extracted significantly greater concentrations of heavy metals from the CL soil rather than from the LS soil. However, the soil factor was not statistically significant, if all other factors (rate of sludge, time and temperature) are combined. The heavy metal concentrations extracted with DTPA were affected by the application rate of the sewage sludge. Pots that received the higher sludge application rate gave significantly higher concentrations of metals extracted with DTPA (LSD values of 0.01 for Cd, 0.09 for Ni, 0.51 for Pb and 0.69 for Zn). Unlike the findings for plant concentrations, plant uptake and $CaCl_2$ extractions, the temperature did not significantly affect the levels of the DTPA-extractable metals, except for Ni in the CL soil in the 50 t ha$^{-1}$ treatment (Figures 4.5.5, 4.5.6, 4.5.7 and 4.5.8; these were extracted from Figures 4.5.1, 4.5.2, 4.5.3 and 4.5.4, respectively in the same way as Figure 4.2.2 was extracted from 4.2.1). DTPA-extractable metals showed a significant decline in all treatments over the residual time of 2 years, especially in the high sewage sludge application rates (LSD value of 0.01 for Cd, 0.14 for Ni, 0.90 For Pb and 1.33 for Zn). This emphasises the finding that $CaCl_2$ followed the plant concentration and uptake trends much more closely than DTPA did. Jackson and Alloway (1991) found that $CaCl_2$ was correlated more closely than DTPA with plant uptake, as $CaCl_2$ was a lot more sensitive to the soil physico-chemical conditions, such as pH and could therefore predict availability of metals to plants more reliably.

The order in which the metals were extracted by DTPA, taking into consideration all the treatments was: Zn > Pb > Ni > Cd.

**Figure 4.5.1:** ***DTPA-extractable Cd (µg g$^{-1}$).***



*LSD Test*

*Soil 0.01*

*Rate 0.01**

*Soil x Rate 0.01*

*Temp 0.01*

*Soil x Temp 0.01**

*Rate x Temp 0.01*

*Soil x Rate x Temp 0.01*

*Time 0.01**

*Soil x Time 0.01***

*Rate x Time 0.01*

*Temp x Time 0.01*

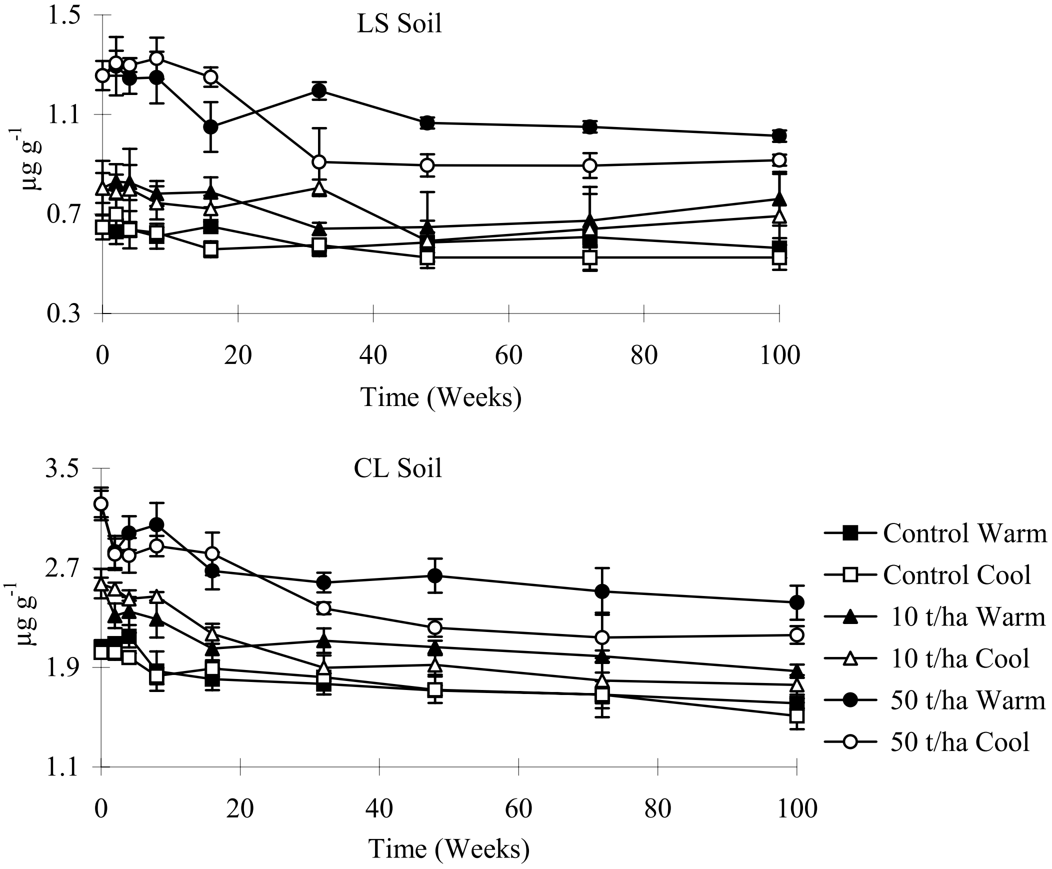
*Soil x Rate x Time 0.02**

*Soil x Temp x Time 0.01***

*Rate x Temp x Time 0.02*

*Soil x Rate x Temp x Time 0.03*

**Figure 4.5.2:** ***DTPA-extractable Ni (µg $g^{-1}$).***



*LSD Test*

*Soil 0.08*

*Rate 0.09**

*Soil x Rate 0.12*

*Temp 0.08*

*Soil xTemp 0.11*

*Rate x Temp 0.12*

*Soil x Rate x Temp 0.17*

*Time 0.14***

*Soil x Time 0.21****

*Rate x Time 0.17****

*Temp x Time 0.21****

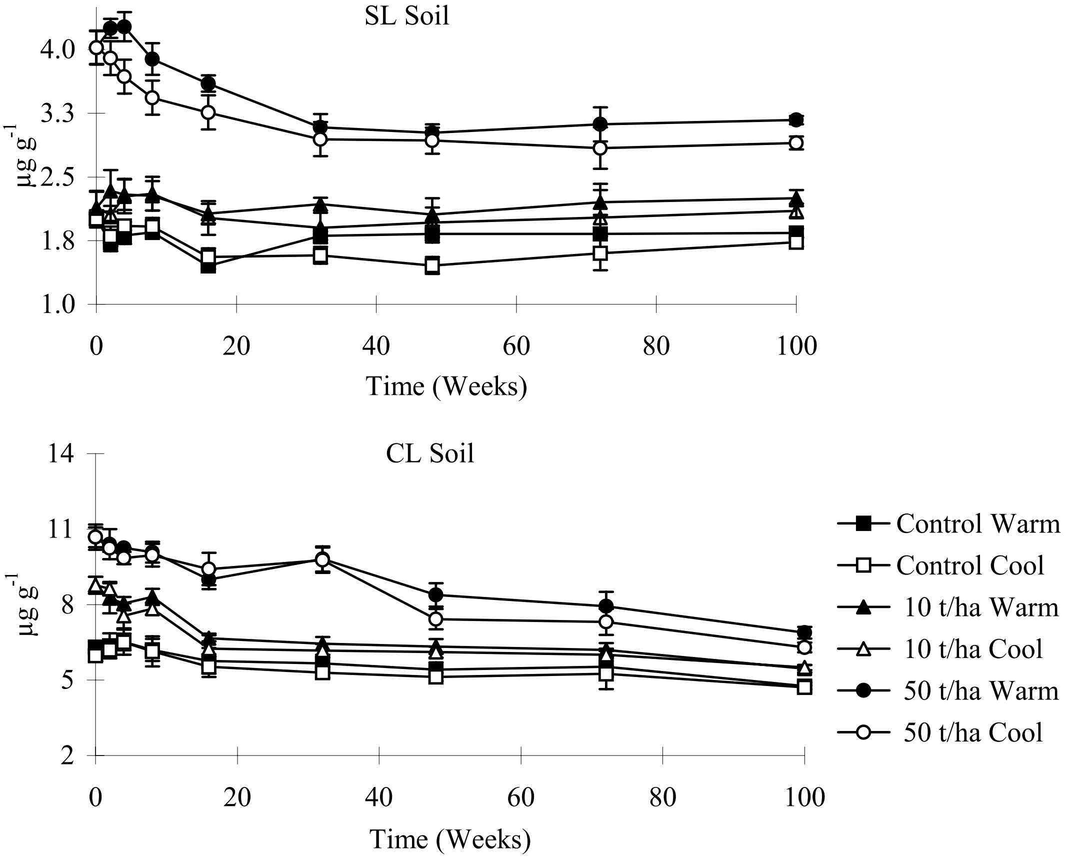
*Soil x Rate x Time 0.40**

*Soil x Temp x Time 0.30*

*Rate x Temp x Time 0.40*

*Soil x Rate x Temp x Time 0.67*

**Figure 4.5.3:** ***DTPA-extractable Pb (μg $g^{-1}$).***

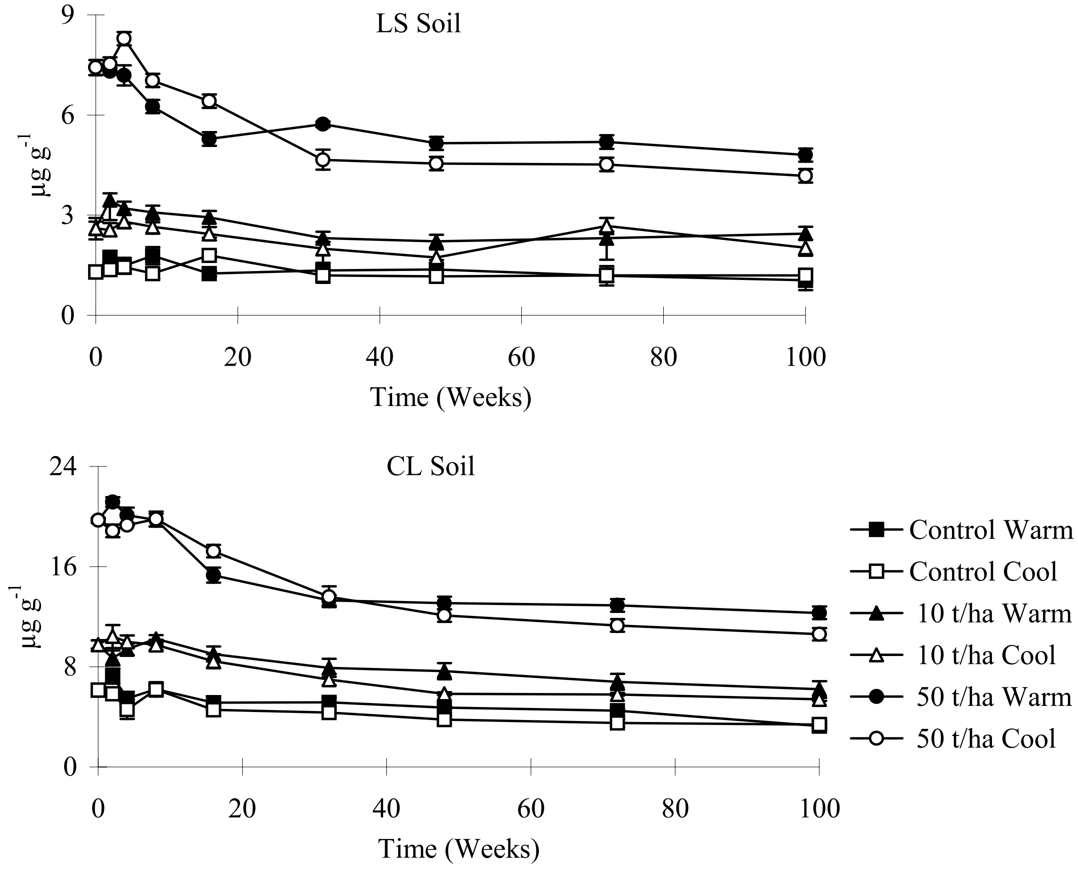


*LSD Test*

| | | |
|---|---|---|
| *Soil 0.42* | *Rate x Temp 0.72* | *Temp x Time 1.38* |
| *Rate 0.51** | *Soil x Rate x Temp 1.05* | *Soil x Rate x Time 2.68* |
| *Soil x Rate 0.72* | *Time 0.90* | *Soil x Temp x Time 2.06* |
| *Temp 0.42* | *Soil x Time 1.38*** | *Rate x Temp x Time 2.68** |
| *Soil x Temp 0.59* | *Rate x Time 1.16** | |

*Soil x Rate x Temp x Time 4.54*

**Figure 4.5.4:** ***DTPA-extractable Zn (µg g$^{-1}$).***



*LSD Test*

*Soil 0.57*

*Rate 0.69****

*Soil x Rate 0.97*

*Temp 0.97*

*Soil x Temp 0.57*

*Rate x Temp 0.80*

*Soil x Rate x Temp 0.97*

*Time 1.33**

*Soil x Time 2.05****

*Rate x Time 1.72****

*Temp x Time 2.05*

*Soil x Rate x Time 3.98****

*Soil x Temp x Time 3.06**

*Rate x Temp x Time 3.98*

*Soil x Rate x Temp x Time 6.75**

**Figure 4.5.5: DTPA-extractable *Cd (control treatments are subtracted) (µg g$^{-1}$).***

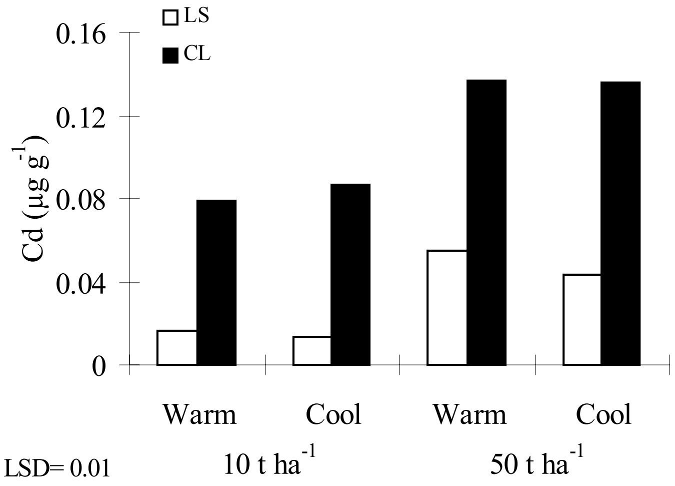


**Figure 4.5.6: *DTPA-extractable Ni (control treatments are subtracted) (µg g$^{-1}$).***

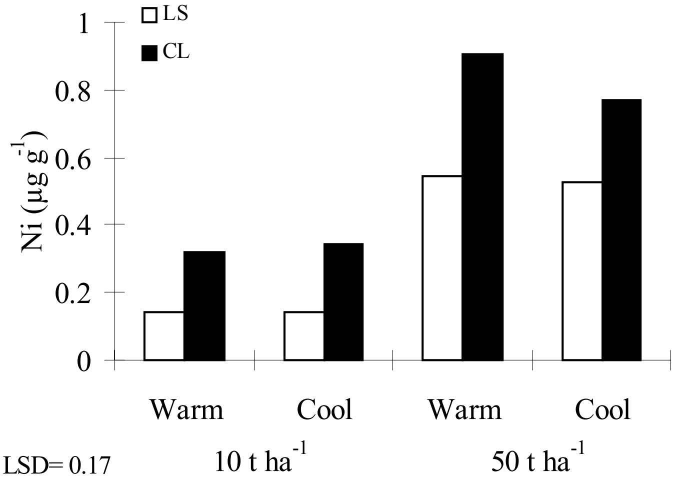


**Figure 4.5.7:** ***DTPA-extractable Pb (control treatments are subtracted) (μg g$^{-1}$).***

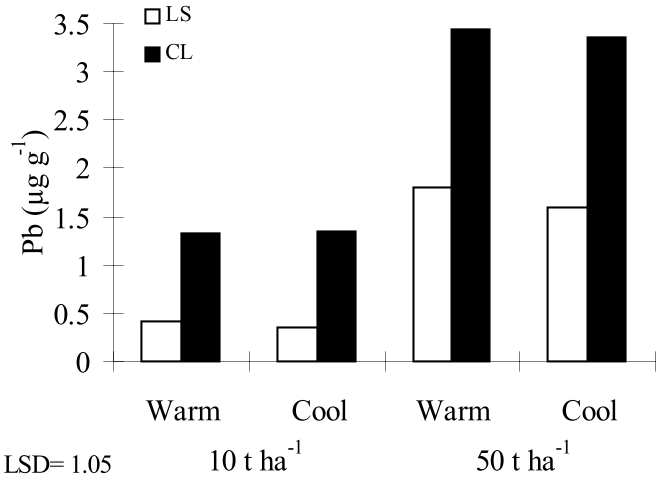
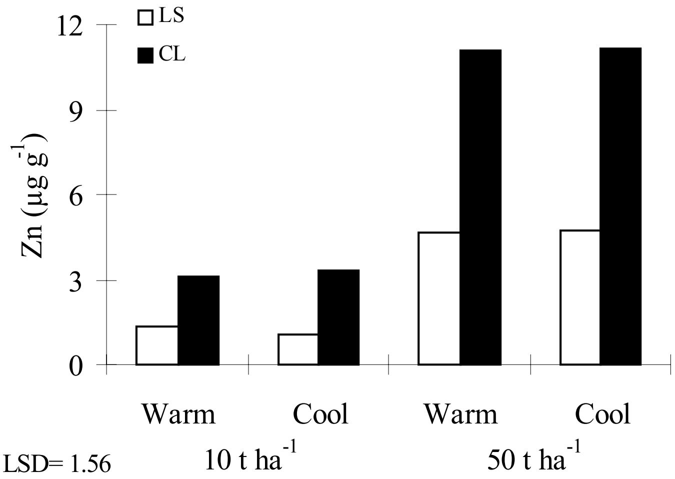


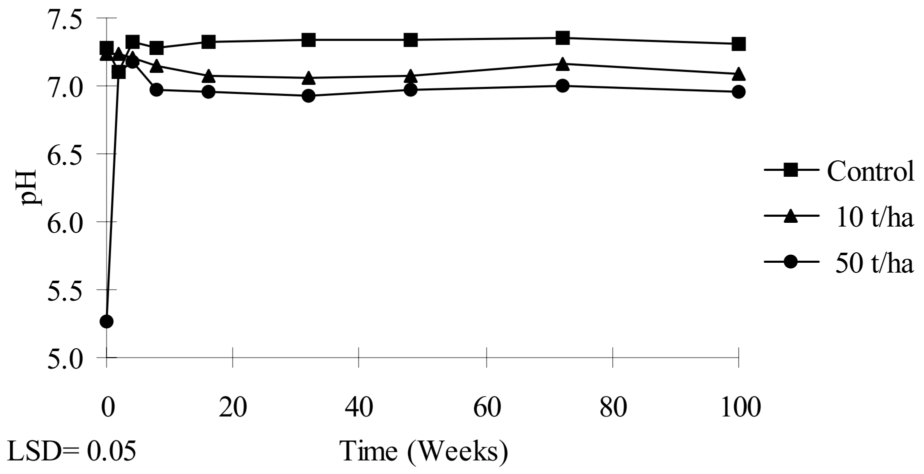
**Figure 4.5.8:** ***DTPA-extractable Zn (control treatments are subtracted) (μg g$^{-1}$).***



### 4.3.6 Soil pH Conditions

Soil pH values were measured in water and 0.01 M $CaCl_2$ and are shown in Figures 4.6.1 and 4.6.2 (where both soils and both temperatures are combined) and in Appendix I (Tables A6.1 and A6.2). Differences between temperatures were not significant. This is important, because it shows that any differences in heavy metal availability between temperatures were not because of any pH differences. The pH values did not differ significantly between the two soils and in the samples taken from the two temperature treatments. The application of sludge tended to decrease the pH, especially at the higher rates. It must be stressed that the control samples were neutral to slightly alkaline. The loamy sand (LS) soil had a pH value of 7.21 and the clay loam (CL) soil a pH value of 7.34 in the sampling of week 0 (measured in the 1 : 2.5 soil : water suspension). The 10 t $ha^{-1}$ treatment caused a decrease in pH to 6.9 in the LS and to 7.29 to the CL. The 50 t $ha^{-1}$ further lowered the pH values of the two soils to 6.71 and 7.21, respectively, two years after the application of

**Figure 4.6.1:** ***pH measured in 1: 2.5 soil : $H_2O$ suspension.***



*LSD Test*

*Soil 0.03*

*Rate 0.03****

*Soil x Rate 0.04****

*Temp 0.03*

*Soil x Temp 0.04*

*Rate x Temp 0.04*

*Soil x Rate x Temp 0.05*

*Time 0.04****

*Soil x Time 0.06****

*Rate x Time 0.05****

*Temp x Time 0.06****

*Soil x Rate x Time 0.11*

*Soil x Temp x Time 0.08*

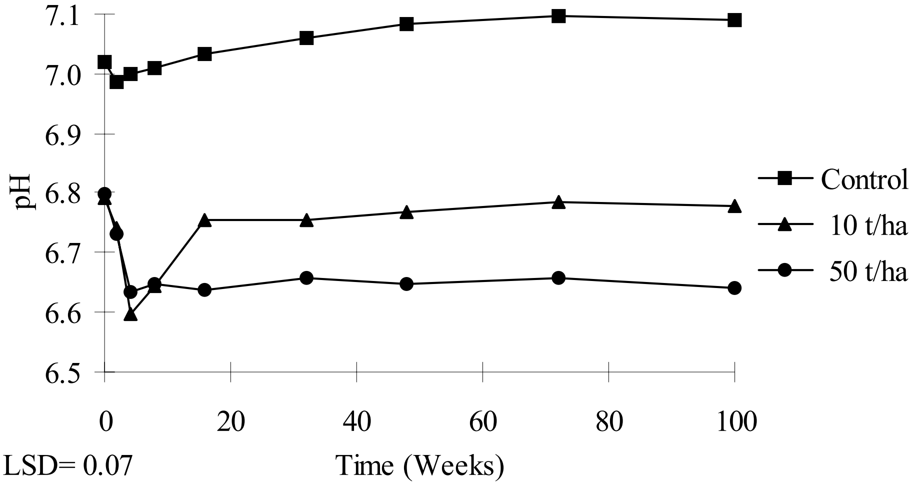
*Rate x Temp x Time 0.11****

*Soil x Rate x Temp x Time 0.18**

sewage sludge (LSD value of 0.03). Tsadilas *et al.* (1995) found the same decline in the pH in the same order (greater decline with elevated sludge applications). The LSD value for the sludge rate for the pH measured with 0.01 M $CaCl_2$ was 0.04. The pH value measured in the 0.01 M $CaCl_2$ followed the same trends. The greater decline in the pH was observed in the LS soil rather than in the CL and this was because of the more effective buffer capacity of the latter soil.

It is apparent that it took around two months for the pH to reach a normal trend; after Week 8 the pH in each treatment remained statistically the same. These fluctuations in the early stages of the experiment may be explained by the fact that when a sewage sludge is applied in a soil very intensive microbial activity takes place, during which the pH was unstable. It may have taken a period of two months for this activity to reach a plateau and this plateau was observed in the pH measurements in the samples after Week 8.

**Figure 4.6.2:** ***pH measured in 0.01 M $CaCl_2$.***



*LSD Test*

*Soil 0.04*
*Rate 0.04*
*Soil x Rate 0.05**
*Temp 0.04*
*Soil x Temp 0.05*
*Soil x Rate x Temp x Time 0.28*
*Rate x Temp 0.05*
*Soil x Rate x Temp 0.07*
*Time 0.06****
*Soil x Time 0.09****
*Rate x Time 0.07*
*Temp x Time 0.09****
*Soil x Rate x Time 0.17*
*Soil x Temp x Time 0.13**
*Rate x Temp x Time 0.17*

### 4.3.7 Organic Matter

Loss-On-Ignition is shown in Figure 4.7.2 and Loss-On-Ignition and organic matter (with the Wet Oxidation method) results are shown in the Appendix, in Tables A7.1 and

A7.2. The application of the sludge significantly increased the organic matter content of the soils in all treatments. The organic matter content of the LS soil was increased from 2.37 % to 3.2 % and that of the CL from 10.4 % to 13.7 % as sludge rate increased from control to 50 t ha$^{-1}$. The organic matter content of the soil-sludge mixtures was dependent on the temperature. As expected, the decomposition of the organic matter was more rapid in the warm environment, due to more intensive microbial activity. The difference between temperatures was significant with an LSD value of 0.02. The results shown in Figure 4.7.2 (where both soils were combined) suggest that this decline occurred mostly in the first 4 months of the experiment; after that point the organic matter seemed to decline very slowly. The same was observed by Hooda and Alloway (1993).The Weight Loss-On-Ignition method (WLOI) at 400 $^0$C was employed in this study to estimate the organic matter contents of the soils. In this study the WLOI method was backed-up by Walkley and Black (wet oxidation)

**Figure 4.7.1:** ***Relationship between Loss-On-Ignition and Organic Matter for the CL soil.***

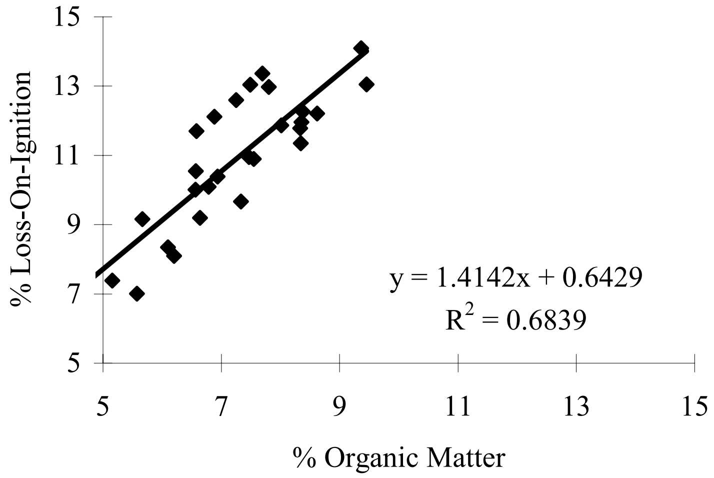
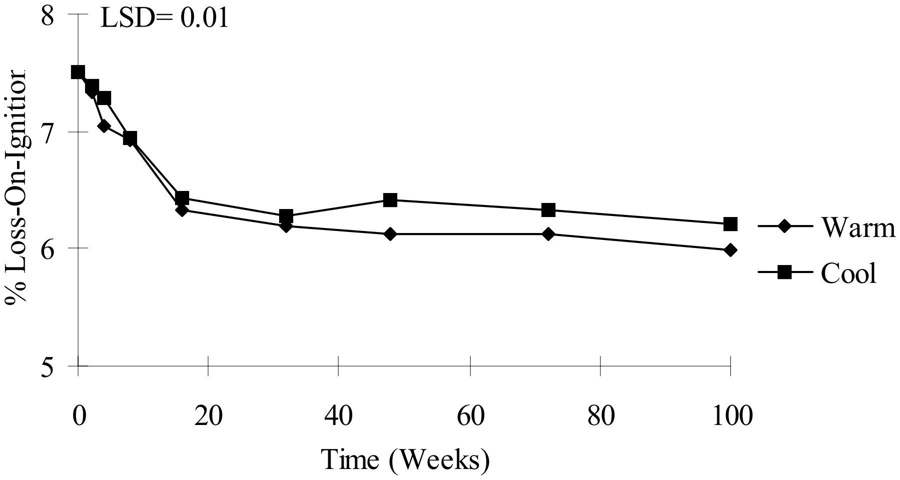


**Figure 4.7.2:** ***% Loss-On-Ignition.***



*LSD Test*

*Soil 0.02****
*Rate 0.03**
*Soil x Rate 0.04*
*Temp 0.02*
*Soil x Temp 0.03*
*Rate x Temp 0.04*
*Soil x Rate x Temp 0.01*
*Time 0.05*
*Soil x Time 0.08****
*Rate x Time 0.07*
*Temp x Time 0.08**
*Soil x Rate x Time 0.16**
*Soil x Temp x Time 0.12**
*Rate x Temp x Time 0.16*
*Soil x Rate x Temp x Time 0.28*

method for the samples of Week 0 and Week 32. The correlation was done for each one soil, in order to assess whether LOI was an appropriate measurement of the soil organic matter. Results in Figure 4.7.1 are for the CL soil and the $R^2$ of the correlation between these two methods was 0.68 (the $R^2$ of the SL soil was similar), which falls within an acceptable range of other researchers findings (Schulte and Hopkins, 1996) (see also Table 3.1).

### 4.3.8 Soil-Plant Transfer Coefficients

Transfer Coefficient (TC) is described by the following formula:

$$TC = \frac{[M]_{plant}}{[M]_{soil}}$$

where: $[M]_{plant}$ is the concentration of an element in the test plant tissues and $[M]_{soil}$ is the total concentration of the same element in the soil where this plant is grown.

**Table 4.3:** ***Transfer coefficients of  Cd, Ni, Pb and Zn.***

LS Soil

| | Warm | | | | Cool | | | |
|---|---|---|---|---|---|---|---|---|
| | Cd | Ni | Pb | Zn | Cd | Ni | Pb | Zn |
| Control | 0.56 | 0.04 | 0.004 | 0.15 | 0.43 | 0.03 | 0.003 | 0.08 |
| 10 tha$^{-1}$ | 1.56 | 0.09 | 0.007 | 0.36 | 0.48 | 0.05 | 0.004 | 0.29 |
| 50 tha$^{-1}$ | 2.74 | 0.21 | 0.010 | 0.65 | 1.75 | 0.12 | 0.008 | 0.48 |

CL Soil

| | Warm | | | | Cool | | | |
|---|---|---|---|---|---|---|---|---|
| | Cd | Ni | Pb | Zn | Cd | Ni | Pb | Zn |
| Control | 0.45 | 0.04 | 0.005 | 0.15 | 0.40 | 0.04 | 0.004 | 0.13 |
| 10 tha$^{-1}$ | 1.16 | 0.08 | 0.010 | 0.45 | 0.65 | 0.04 | 0.005 | 0.24 |
| 50 tha$^{-1}$ | 1.72 | 0.21 | 0.010 | 0.68 | 1.00 | 0.20 | 0.010 | 0.42 |

The TC values give an indication of the mobility of the elements in the soils and this is particularly important for the heavy metals, because high TC at high sewage sludge application rates may involve a risk of human food chain accumulation. The results in Table 4.3 show that Cd was the most mobile heavy metal out of the group Zn, Ni, Pb and Cd. Cadmium was 150-350 times more mobile than Pb, which tended to be taken up by the plant to the least extent. This was also reported by Kloke *et al.* (1984). The order of reduced mobility was: Cd > Zn > Ni > Pb. The elevated sewage sludge application rates led to greater TC values for all elements in both soils, because the quantities of the heavy metals introduced with the sludge were substantially higher in the higher applications. This may also indicate that these metals were less strongly bound than the indigenous metals in the soil before adding the sludge. This order of metal mobility was in accordance with other published findings as well. Moreno *et al.* (1996) found that Cd and Zn were the most mobile elements, while Ni and Cu were not absorbed by the test crops as much. Li and Shuman (1996b) also found that the order of mobility in their experiments was Cd > Zn > Ni. The CL soil gave only slightly higher TC values than the LS soil and this should be expected, as the CL soil contained higher heavy metal quantities initially. However, this initial difference is closer in terms of TC values (in case of Cd, the CL soil had even lower TC values than the LS soil) and this shows that a lighter textured soil may result in the heavy metals being more mobile and bioavailable (Singh *et al.*, 1995). In the cool environment the TC values were consistently lower than in the warm and this may be due to the slower decomposition of the organic matter (which makes the heavy metals less accessible to the plant), the slower growth of the test plants themselves and lesser evapotranspiration rates.

## 4.4 DISCUSSION

Heavy metal extractability and availability was clearly shown to be affected by the additions of sewage sludge to soils. These parameters increased with the increase in application rate of sludge. In the CL soil, higher plant concentrations were seen than in the LS soil, probably due to the fact that the total metal concentrations in the CL soil were higher than that in the LS soil. However, the increase in plant uptake with the sludge application was in most cases more conservative than in the soil which had a lower CEC value. The same has been found by many other researchers in both pot and field experiments with sludge (Del Castilho *et al.*, 1993; Sposito *et al.*, 1983; Bell *et al.*, 1992).

In this experiment, pots were maintained under two different ambient temperatures (15 and 25 $^0$C) during a two-year period. It should be stressed that the different light regimes at 15 and 25 $^0$C, could have had some effect on the physiology and growth of the plants. This difference should especially be demonstrated at 25 $^0$C where daylight in different seasons was very different in length. However, even at 25 $^0$C this effect was not significant because the yields did not change considerably over time (Figure 4.1). The temperature factor had a marked effect on plant concentration and uptake of heavy metals and on the extractability of these metals with $CaCl_2$. As for the plant measurements, Cd, Ni and Zn concentrations increased significantly at 25 $^0$C, and this increase was further enhanced in the higher sludge application rate. Lead, being the least mobile heavy metal in this study, was not significantly affected by temperature. Organic matter decomposition may well have been the main factor that affected the enhancement of the plant availability of metals with the increased temperature. At the higher temperature, organic matter would have decomposed faster than at 15 $^0$C. This may have released heavy metals into more bio-available forms faster in the 25 $^0$C room than at 15 $^0$C. Hani *et al.* (1996) found that the solubility of heavy metals can increase with the degradation of sewage sludge-borne organic matter. Hooda and Alloway (1993) also found that increasing temperature may increase the plant uptake of heavy metals. However, they argued that this was probably caused by a weaker plant growth at lower temperatures. Although this sort of explanation cannot be ruled out, under the experimental conditions in this project, there was strong evidence that this behaviour in plant uptake was also caused by the chemical reactions in the soil system described above. Information concerning this evidence can be obtained by the $CaCl_2$ extractions. According to this, temperature significantly affected heavy metal extractability, especially in the CL soil and at the higher sludge application rates. It should be noted that plant parameters were affected by temperature more significantly in the same soil (CL) and at the same sludge rate (50 t ha$^{-1}$). Since $CaCl_2$

extractions showed the same trends in metal availability (compare Figures 4.2.2, 4.2.4 and 4.2.8 for plant concentrations and Figures 4.4.4.-4.4.6 for $CaCl_2$ extractions), the increase in metal uptake cannot only have happened due to higher plant transpiration rates at 25 $^0$C. The reason must be the decomposition of the organic matter, otherwise $CaCl_2$ would not follow the plant trends. Extraction with $CaCl_2$ has been recognised as being useful for predicting metal availability closely and precisely (Andrewes *et al.*, 1996) and it seems like this is what happened in this experiment too. Extraction with DTPA on the other hand, although it predicted the increase in plant concentration and plant uptake, failed to assess closely enough the effect of temperature. In the data for DTPA-extractable metals, temperature did not differentiate significantly the values of metals extracted. Merkel (1996) also found that the concentrations of Cd in wheat grain were better correlated with $CaCl_2$ extractions compared with DTPA, but he also concluded that DTPA was a suitable extractant for heavy metals.

Over time, there was an increase in plant concentration and uptake and $CaCl_2$ extractable metals. This increase, however, could only be observed in the higher temperature treatments for Cd, Ni and Pb, but not for Zn. The data from the lower temperature showed that plant parameters and $CaCl_2$ extractable metals were relatively unaffected.

Several researchers have studied the time factor in relation to availability of heavy metal to plants after sludge application to soils. It is commonly agreed that it is difficult to predict plant availability months or years after the application of sludge, and this is evident from the fact that research findings differ considerably (Alloway and Jackson, 1991). The literature provides many examples of research findings that suggest that heavy metal availability to plants in sewage sludge treated soils either decreased or did not change over time. Chang *et al.* (1997) studied a residual period of 10 years and concluded that metal availability to plants tended to reach a plateau after some time; this seems to be an equilibrium value, after which heavy metal concentrations in plant tissues would not increase even if sludge applications increase further. However, they admitted that their results may not provide a definite answer. Hyun *et al.* (1998) assessed the hypothesis that organic matter may affect the availability of Cd, using a residual time of 10 years. They found that the organic matter content declined by 43 % over that period of time, but the soluble Cd was not found to have increased. However, soluble Cd did not decline over that time either. Canet *et al.* (1998) applied sewage sludge in equivalent doses of 400, 800 and 1200 kg N ha$^{-1}$ (approximately 13, 26 and 39 t ha$^{-1}$ DM sludge) and 7 years later no significant increase in the metal uptake by Swiss chard and lettuce was observed. Krauss and Diez (1998) 12 years after the ceasing of intense sewage sludge application (40 mg Cd and 808 mg Pb kg$^{-1}$ had been applied to soil) found that ryegrass, barley, maize and potato crops were unaffected. On the same lines, Bidwell and Dowdy (1987) found that immediately after the termination of sewage sludge applications there was a dramatic decrease in metal availability to plants.

On the other hand, Petruzzelli *et al.* (1989) found that Zn and Cd concentrations in plants grown in sludge-treated soils were higher 4 years after sludge application. Sloan *et al.* (1997) found that 15 years after sludge application Cd was still highly recovered from the soil, while McBride *et al.* (1997b) found that Cd and Zn were still more available to plants in sludge-treated plots than in the control plots after 15 years of sludge application. Obrador *et al.* (1997) studied metal effect on crops for a residual time of one year and found that there was an increase in the potential mobility of metals with time, although the DTPA extractable metal concentrations declined with time. On the same lines, Heckman *et al.* (1987) in their field experiment, applied up to 112 t ha$^{-1}$ of sludge and found that after a period of 5 to 9 years the concentrations of Cd and Zn in soybean plants were still greatly enhanced. White *et al.* (1997) applied sludge at rates of 45 and 90 t ha$^{-1}$ in a land with warm temperatures and measured the extractability of metals with DTPA over a period of 9 years. They found that DTPA-extractable Cd, Pb and Zn increased very significantly (Cd and Zn had nearly a tenfold increase) until the fourth year, and than they subsequently decreased. However, the metal concentrations were significantly higher at the end of the experiment that at the beginning. Although they failed to explain the reasons for the peaks in metal concentrations in the fourth year, their work provided evidence that heavy metal extractability may be significantly increased in warm environments. Gardiner *et al.* 1995) applied sewage sludge for 5 years at a rate of 20 t ha$^{-1}$ y$^{-1}$ and found an impressive 6 fold increase in Cd concentration in Swiss chard. After that time the increase was slower. However, apart from Cd, other metals including Zn, Cu and Ni did not increase as much. Other research works that reported increased heavy metal availability to plants in sewage sludge-treated soils include Mulchi *et al.* (1992), who found that after nearly 10 years of continuous sludge application to soil, concentrations of Zn, Cu, Mn, Ni and Cd correlated very strongly in tabacco with ‘total’ metal concentrations in the soil ($R^2$ values of 0.65-0.95) and Chang *et al.* (1987), who found that Cd concentrations in plant tissues (radish and Swiss chard) tended to increase yearly with each sludge application.

In this project, plant concentrations and $CaCl_2$ soil extractions in the warm environment showed a slight increase over time. The hypothesis suggested in the papers referred to above may provide the explanation for this behaviour. The decomposition of the organic matter may have released metals in more available forms in the first year, until a plateau was reached. According to $CaCl_2$ extractions in the second year, things seem to be maintained without any significant increase or decrease. Extractions with DTPA, on the other hand, showed a slow decrease in all treatments except from the control, especially at the higher sludge application rates. That means that $CaCl_2$ predicted more closely the plant concentration than DTPA in its trends over time.

The difference in decomposition characteristics of the organic matter at the different ambient temperatures may have also caused the production of low molecular weight organic acids (DOC). It is known that the role of DOC may be important in understanding the behaviour of organic matter, as low molecular weight organic compounds may modify the bioavailability of heavy metals. As DOC can be a product of microbial decomposition of organic matter (Alloway, 1997) the state of DOC in the soil system was checked in order to assess whether there was an increase in DOC levels as a result of the organic matter decrease. Dissolved organic compounds were measured at two time-points: Week 4 and Week 100. Week 4 was preferred over Week 0, because from the pH data there was evidence that intense microbial activity was going on in the soils for the first month, which suggest unstable DOC production. The results are presented in Table 4.4. It should be noted that DOC increased over time in the CL soil which initially contained the higher amount of organic matter. DOC increased even in the control treatment, but this increase was a lot lower than with 50 t ha$^{-1}$ sludge treatment. The DOC concentration was generally unaffected by the different temperature treatments. These results suggest that the presence of DOC, which increased over time with the decomposition of organic matter, may have facilitated the increase in metal plant concentration and uptake. On the other hand, the DOC status in the LS soil did not have the same pattern as that of the CL soil. In the LS soil, DOC showed a small decline in both the control and the 50 t ha$^{-1}$ treatments. This suggested that the decomposition of the organic matter in that soil did not significantly contribute to organic acids of the particular molecular weight that characterises the DOC compounds. This also showed that the low molecular weight organic compounds in a more sandy soil were not as well adsorbed and thus not as protected from leaching as they were in the CL soil. This behaviour of DOC had an effect on

**Table 4.4:** ***DOC status over time (mg L$^{-1}$).***

| | | LS Soil | |
|---|---|---|---|
| | W4 | W100 | |
| | | Warm | Cool |
| Control | 26.48 | 18.84 | 19.22 |
| 50 t ha$^{-1}$ | 36.92 | 33.79 | 33.36 |
| | | CL Soil | |
| | W4 | W100 | |
| | | Warm | Cool |
| Control | 54.86 | 57.86 | 58.47 |
| 50 t ha$^{-1}$ | 60.24 | 66.22 | 67.75 |

the rate of increase that took place over time in the $CaCl_2$ extractions in the two soils. Combining the 10 and 50 t ha$^{-1}$ treatments and both temperatures for Cd and Zn, which are the most mobile elements and would be expected to exhibit the most marked differences in availability, it was found that for Cd the increase from Week 4 to Week 100 was 23 % for the LS soil and 29 % for the CL soil. Similarly, for Zn the increase from Week 4 to Week 100 was 16 % for the LS soil and 25 % for the CL soil. Cadmium and Zn increased more in the CL soil (where DOC increased) than in the LS soil (where DOC showed a slight decrease) over time. This showed that the difference in the DOC status between the two soils may have affected the $CaCl_2$ extractability of the most mobile heavy metals, as $CaCl_2$ is a very sensitive index for predicting the availability of heavy metals to plants. Hamon *et al.* (1995) studied the changes in the soil solution of growing raddish in a pot experiment, which lasted only for 30 days, and concluded that the speciation of Cd and Zn could be explained by the presence of DOC in the soil solution. They found that the changes in DOC were closely followed by similar changes in Cd and Zn, and that at the end of the experiment only 28 % of Cd and 13 % of Zn was uncomplexed.

The behaviour of heavy metals in the pots at the two different temperatures was much more enhanced at the higher sludge application rates. In the 50 t ha$^{-1}$ treatment, $CaCl_2$ extractable metal concentrations and plant availability were affected by temperature a to a greater extend than at 10 t ha$^{-1}$, enhancing the levels of all metals at that treatment. Chang *et al.* (1983) studied the availability of Cd and Zn in barley leaves over a 3-year time period after the termination of sewage sludge applications and found that in the last year there was an increase in plant uptake, which was more evident at the higher sludge application rate (15 t ha$^{-1}$).

The soil type was a very important factor in this study. The CL soil contained greater quantities of heavy metals at the beginning of the experiment, and greater contents of organic matter and clay. As a result, all extractants recovered greater concentrations of heavy metals from the CL soil than from the LS soil. In addition, plant availability of metals was always greater in the CL soil than in the LS soil. However, it can be seen from the Transfer Coefficients that the difference between the two soils was a lot less in terms of relative mobility, and this seems to suggest that the more sandy soil mobilises relatively greater quantities of heavy metals. On the other hand, it should be noted that the effect of temperature was much more important in the CL soil than in the LS soil. This may be due to the fact that the limiting factor of lower temperature mostly affected the soil where the adsorption of heavy metals and nutrients is greater due to more available adsorption sites and where the plants are more likely to be stressed from the less optimum (lower) temperature conditions.

The Transfer Coefficient, which expresses the relative mobility of metals, increased with the increase in sludge application, and with temperature. However, the increase with sludge application was a lot smaller than the factor of 5 times between 10 and 50 t ha$^{-1}$ application rates, probably because sewage sludge acts both as a source and as a sink of heavy metals, due to the fact that it contributed to the adsorptive sites in the soil (Heckman *et al.*, 1987). Bubb and Lester (1991) found that the free ion metals were more available than the organically bound, and this also supports the evidence that the application of heavy metals to soils with sewage sludge does not necessarily increase metal availability to plants linearly. The fact that the warm temperature treatments had greater Transfer Coefficient values is indicative of the importance of this factor in affecting heavy metal availability in sludge-treated soils.

**Table 4.5:** ***Percentage increase in plant availability and soil extractability of Cd, Ni, Pb and Zn between 15 $^0$C and 25 $^0$C in the 50 t ha$^{-1}$ treatment.***

| | Plant Concentration | | Plant Uptake | | $CaCl_2$ | | DTPA | |
|---|---|---|---|---|---|---|---|---|
| | CL [a] | LS [b] | CL | LS | CL | LS | CL | LS |
| Cd | 60 | 10 | 56 | 27 | 17 | 6 | NS [c] | NS |
| Ni | NS | NS | 50 | 37 | 10 | NS | NS | NS |
| Pb | 12 | 12 | 41 | 37 | - | - | NS | NS |
| Zn | 26 | 8 | 53 | 43 | 7 | 4 | NS | NS |

*a: CL soil, b: LS soil, c: Not Significant*

However, it can be partly explained from the fact that the growth of ryegrass was slower at the cool temperature (15 $^0$C). The order of relevant mobility was: Cd > Zn > Ni > Pb, which was the one found by other researchers, such as Sloan *et al.* (1997) and Hooda and Alloway (1993).

Cadmium in the plant concentration data was the element that was most affected by temperature (Table 4.5). In the 50 t ha$^{-1}$ treatment, Cd increased 60 % in the CL soil between 15 and 25 $^0$C, which was by far the largest increase. Similarly, Pb increased 12 % in both soils between the two temperatures. Zinc, on the other hand, increased 26 % for the CL soil and 8% for the LS soil. Nickel was not affected significantly by the temperature.

However, Ni showed a significant increase between the two temperatures in the uptake data. At the 50 t ha$^{-1}$, Ni increased 50 % in the CL soil and 37 % in the LS soil. Cadmium was again the element that was mostly affected by temperature, but only in the CL soil, where it had an increase of 56 %, while in the LS soil it had an increase of only 27 %. Lead uptake was higher than Pb concentration. This element had 41 % increase in the CL and

37 % increase in the LS soil for plant uptake, while for plant concentration the increase was 12 % for both soils. Zinc increased its uptake in the CL soil nearly as much as Cd (by 53 %), while Zn increase in the LS soil was the biggest of all other metals (43 %).

In the $CaCl_2$ extraction data, the plant concentration trends were followed, with Cd having the greatest increase in the 50 t $ha^{-1}$ treatment between 15 and 25 $^0$C in both soils and Ni not having a significant increase in the LS soil. For Cd there was an increase of 17 % and 6 % for the CL and LS soils respectively, for Zn 7 % and 4 % in the CL and LS soils respectively and for Ni there was an increase of 10 % in the CL soil. On the other hand, DTPA extractions were rather unaffected by temperature. However, over the residual time of two years, Cd was the metal that increased the most, as it is evident from the plant concentration and uptake and in the $CaCl_2$ data (Table 4.6). These numbers were calculated taking into consideration the warm temperature (25 $^0$C) and the 10 and 50 t $ha^{-1}$ treatments and including both soils. Cadmium increased by 26 % in the plant concentration, 19 % in the plant uptake and 25 % in the $CaCl_2$ extractions. Nickel increased by 10 %, 14 % and 18 % in the plant concentration, plant uptake and $CaCl_2$ extractions respectively, while Pb increased 13 % and 11 % in the plant concentration and uptake respectively. Zinc exhibited very different behaviour; it did increase over time in the plant data, but showed an increase of 20 % in the $CaCl_2$ extractions.

**Table 4.6:** ***Percentage of increase for Cd, Ni, Pb and Zn between Week 0 and 100 for both soils and excluding 0 t ha$^{-1}$ and 15 $^0$C treatments.***

| | Plant Concentration | Plant Uptake | $CaCl_2$ |
|---|---|---|---|
| Cd | 26 | 29 | 25 |
| Ni | 10 | 14 | 18 |
| Pb | 13 | 11 | - |
| Zn | -4 | -3 | 20 |

## 4.5 CONCLUSIONS

Organic matter played a key role in heavy metal availability and plant uptake. Fast decomposition rates led to greater quantities of heavy metals being taken up (as was evident in the warm temperature treatments). Treatments, where organic matter decomposed faster, gave greater Transfer Coefficients at high sewage sludge application rates. Increases in DOC in the soil-sludge mixtures (as a result of organic matter decomposition in the CL soil for both temperatures) may have helped enhance heavy metal availability, especially at high sludge application rates. However, DOC did not seem to have an important effect in the LS soil, probably because it was leached from the soil-sludge mixtures.

Over the residual time of 2 years, the availability of heavy metals increased at 25 $^0$C in the frst year and reached a plateau in the second year (as it was evident from the data of $CaCl_2$ and plant uptake). At 15 $^0$C, the availability of metals decreased, probably due to the fact that the plants grown at that temperature showed poorer growth than those at 25 $^0$C and thus were less able to absorb greater concentrations of heavy metals.

$CaCl_2$ and DTPA proved reliable soil test reagents for predicting the heavy metals bioavailability, but $CaCl_2$ followed plant concentration and uptake trends more closely than DTPA.

Temperature had a marked effect on the heavy metals enhancing their bioavailability, especially at the higher sewage sludge application rates.

Cadmium was the most mobile heavy metal in this study; the order of mobility of the metals in this study was: Cd > Zn > Ni > Pb. The availability of Cd, Ni, Pb and Zn depended on the ambient temperature (it increased with the temperature), sewage sludge application rates and soil type.

# CHAPTER FIVE: AVAILABILITY OF HEAVY METALS IN SEWAGE SLUDGE TREATED SOILS (FIELD EXPERIMENT)

## 5.1 INTRODUCTION

Results regarding heavy metal availability from pot experiments generally tend to overestimate the real (field) situations (De Vries and Tiller, 1978). However, when data from pot experiments are combined with field trials, they provide the right balance for assessing results, because then a clearer picture of heavy metal behaviour and mobility may be obtained. In the previous pot experiment two types of soils were placed in two different temperature regimes and chemical behaviour of heavy metals regarding their bio-availability was determined. These temperatures were 15 and 25 $^0$C, representing temperature conditions typical in Mediterranean and temperate climates. In this field experiment the same rates of sewage sludge were applied in plots set up in South East England (Reading) and in Central Greece (Vardates). Along with the application of ‘low’ rate of 10 t ha$^{-1}$, the rate of 5 t ha$^{-1}$ of DM sewage sludge was also applied in this field trial, as it was close to the 8-10 t ha$^{-1}$ dose which is commonly used in agricultural practice. Although it was not feasible to use the same sludge in these two sites, the main objective was to compare findings from the pot experiment and assess them in the light of data from more realistic open-air field conditions.

Thus, it was decided to set up a field experiment, in order to verify results from the previous pot experiment. The objectives of this experiment were:

- to test the effects of the sewage sludge applications on two soil types and on plants grown on them with regard to the dynamics of heavy metal added to these soils.
- To compare the effects of Cd, Ni, Pb and Zn added to these soils in sewage sludge application under two different climatic conditions, the first being in Greece and the second in England.
- To assess the value of extractions with 0.05 M DTPA as indices of the bio-availability of heavy metals for the prediction of their uptake into plants.
- To assess the key chemical properties such as organic matter content and pH on the bio-availability of Cd, Ni, Pb and Zn in soil-sludge mixtures at 4 rates of sewage sludge application over a residual time of 1 year.

The hypotheses tested in this study were:

- The mineralisation of the organic matter in sewage sludge-treated soils releases metals in more mobile forms, which is then more available for plant uptake.
- The availability of metals to plants is enhanced by the more rapid decomposition of organic matter, caused by higher ambient temperatures.

- Organic matter decomposition, which is faster at higher temperatures, leads to increasing DOC levels in soil-sludge mixtures; this facilitates the increase of heavy metal availability over time.
- The studies of heavy metals in a growth experiment in pots in a greenhouse can predict the behaviour of heavy metals in an out-door larger-scale experiment.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Experimental Design

The site in England, where the field trial was set up, was at the University of Reading' s Sonning farm. Sewage sludge with 23 % total solids content was obtained from a waste water treatment plant near Heathrow Airport. The sludge was the same as the one used in the leaching experiment (Chapter 6). The sewage sludge was applied at 4 rates, equivalent to 0, 5, 10 and 50 t DM ha$^{-1}$ in 16 plots of 2 x 4 m$^2$ and the treatments were replicated 4 times. The plots were placed in 4 rows of 11, that is, in the site there were 44 plots in total. The 16 plots (4 rates of sludge * 4 replicates = 16) of this study were randomly distributed at the site. The other 28 plots were used for a comparative experiment with cattle manure and poultry litter.

The sewage sludge was applied in December 1996. The appropriate amounts of sludge were spread by hand evenly on each of the 16 plots. A few days later the sludge was more properly incorporated into the soil up to a depth of 5 cm, using a mechanical rotator. The plots were sampled with an auger to a depth of 10 cm in Week 0 (the beginning of the experiment), and then Weeks 2, 4, 8, 16, 32 and 48. Because of the freezing conditions in early January 1997, the sampling of Week 0 was delayed until the week commencing on 13 January 1997. The last samples were obtained in the week commencing 15 December 1997.

The plots were sown with ryegrass (*Lolium perenne* L.). The crop was sown when the weather permitted it, which was in April 1997. From that time onwards, 6 cuttings were taken with a rotary 52 cm lawn mower. The cuttings of the crop were taken on 5 June, 20 June, 16 July, 12 August, 4 September and 31 October 1997.

The site in Greece was in the experimental station of NAGREF (National Agricultural Research Foundation) in the village of Vardates, near Lamia, in Central Greece. Sewage sludge was obtained from the waste water treatment plant in the town of Lamia (of 50,000 inhabitants) and had a 9 % total solid content. The sewage sludge was applied at 4 rates, equivalent to 0, 4, 10 and 50 t DM ha$^{-1}$ in 12 plots of 2.5 x 5 m$^2$ and the treatments were replicated 3 times. The plots were arranged in 3 rows of 5 (15 plots in total).

At Vardates, the sewage sludge was applied in November 1996. The appropriate amounts of sludge were spread evenly on each of the 12 plots and incorporated into the soil to a depth of 5 cm. The plots were sampled with an auger to a depth of 10 cm in Week 0 (the beginning of the experiment), and then Weeks 3, 6, 9, 17, 33 and 48. Samples of Week 0 were taken on 13 November 1996.

The plots were sown with ryegrass (*Lolium perenne* L.). The crop was in November 1996. From that time onwards, 3 cuttings were taken. The cuttings were taken in Weeks 33, 44 and 48 from the commencement of the experiment.

This project was part of a British Council/NAGREF collaboration scheme.

### 5.2.2 Analyses

Samples of the sewage sludge used at both sites were oven dried to assess their total solid content and passed through a 2 mm sieve. They were then analysed for their total heavy metal concentration by digestion in *Aqua regia,* pH (in 1 : 2.5 w/v sludge : water ratio) and organic matter (Loss-On-Ignition). Soil samples from the control plots at both sites were used for the characterisation of the selected chemical and physical properties, reported in Tables 5.1.1 and 5.1.2. The soil samples were air dried, and passed through a 2 mm sieve. The samples were then analysed for pH, with 1 : 2.5 w/v soil : water ratio and organic matter (Loss-On-Ignition). Soil samples from Sonning were also analysed for pH with 0.01 M $CaCl_2$, Dissolved Organic Carbon concentration and 'available' heavy metal concentrations with 0.05 M DTPA. At Sonning, the heavy metals assessed in the soil samples were Cd, Ni,

**Table 5.1.1:** ***Selected physical and chemical properties of the soil used in the Sonning trial.***

| | $CaCO_3$ | Al | Fe | $Ca^{2+}$ | $K^+$ | $Na^+$ | $Mg^{2+}$ |
|---|---|---|---|---|---|---|---|
| | % | Dithionite extracted % | | Water soluble (μg $g^{-1}$) | | | |
| Soil | 0.28 | 1.75 | 0.75 | 153.25 | 1.70 | 36.25 | 5.28 |
| Sludge | - | - | - | - | - | - | - |
| | Sand | Silt | Clay | Category | pH | | CEC [a] |
| | % | | | | $H_2O$ | $CaCl_2$ | $cmol_c\ kg^{-1}$ |
| Soil | 74.61 | 5.09 | 20.30 | SCL | 7.19 | 6.46 | 15.21 |
| Sludge | - | - | - | | 6.46 | 6.35 | - |
| | *Aqua regia*-digested (μg $g^{-1}$) | | | | LOI [b] | Bd [c] | EC [d] |
| | Cd | Ni | Pb | Zn | % | g $cm^{-3}$ | dS $m^{-1}$ |
| Soil | 0.10 | 9.09 | 30.44 | 42.67 | 4.40 | 1.08 | 0.48 |
| Sludge | 2.41 | 38.08 | 317.21 | 645.50 | 41.83 | 0.85 | - |

*a: Cation Exchange Capacity, b: Loss-On-Ignition, c: Bulk Density, d: Electrical Conductivity*

Pb and Zn, while at Vardates the only metals determined were Pb and Zn. After cutting, plant samples were washed in destilled water, oven dried and analysed for their Cd, Ni, Pb and Zn concentration with concentrated $HNO_3$ digestion (Pb and Zn were analysed in the plant samples from Vardates). All the data from the trial in Vardates were provided by Papadopoulos, Akrivos and Dimirkou. Soil and plant samples were processed and analysed as described in Chapter 3 of Materials and Methods.

**Table 5.1.2:** ***Selected physical and chemical properties of the soil used in the Vardates trial (Courtesy of Papadopoulos* et al*)* .**

| | pH | EC [a] | LOI [b] | OM [c] | *Aqua regia*-digested, µg $g^{-1}$ | |
|---|---|---|---|---|---|---|
| | | µS $cm^{-1}$ | % | % | Pb | Zn |
| Soil | 8.44 | 134.33 | 3.84 | 1.93 | 7.91 | 75.12 |
| Sludge | 7.54 | 279 | 72.30 | - | 130.10 | 756.30 |

*a: Electrical Conductivity, b: Loss-On-Ignition, c: Organic Matter (Walkley and Black method)*

## 5.3 RESULTS

The concentrations of total heavy metals in both the Sonning and Vardates trial are presented in Tables 5.2.1 and 5.2.2, respectively. It can be seen that as in the pot experiment, the application of sewage sludge increased the total concentrations of heavy metals in both trials.

**Table 5.2.1:** ***Total concentrations of Cd, Ni, Pb and Zn in the Sonning trial (µg $g^{-1}$).***

| | Cd | Ni | Pb | Zn |
|---|---|---|---|---|
| Control | 0.10 | 9.48 | 27.13 | 48.20 |
| 5 t $ha^{-1}$ | 0.11 | 9.88 | 28.38 | 50.74 |
| 10 t $ha^{-1}$ | 0.12 | 10.45 | 29.38 | 55.85 |
| 50 t $ha^{-1}$ | 0.19 | 13.48 | 38.05 | 86.49 |

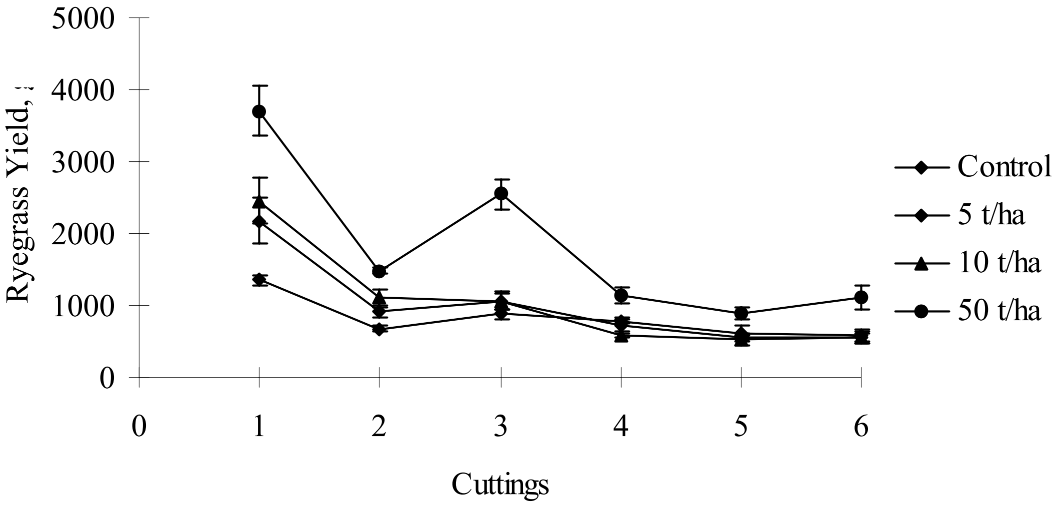
**Table 5.2.2:** ***Total concentrations of Pb and Zn in the Vardates trial (µg $g^{-1}$) (Courtesy of Papadopoulos* et al.*).***

| | Pb | Zn |
|---|---|---|
| Control | 7.91 | 75.12 |
| 4 t $ha^{-1}$ | 10.50 | 66.84 |
| 10 t $ha^{-1}$ | 11.33 | 74.26 |
| 50 t $ha^{-1}$ | 12.35 | 89.10 |

### 5.3.1 Ryegrass Yield

The yield of ryegrass (dry matter) increased significantly with the increase in the rate of sewage sludge application (LSD value of 123) (Figure 5.1 and Appendix II-Table A1). In all 6 cuttings, the 50 t ha$^{-1}$ treatment had a significantly higher yield than the other 3 treatments. This showed that there were no obvious toxic affects as a result of the sludge application to the field. Even at application rates as high as 50 t ha$^{-1}$ (which is 5 times higher application than in the common agricultural practice), the use of sludge was beneficial to plants, probably due to its N and P contents and its contribution to soil water holding capacity. In a field experiment, Businelli *et al.* (1996) found that sludge applications up to a rate of 90 t ha$^{-1}$ increased the plant yields significantly. Other researchers who found a beneficial effect of sludge application to plant yields include: Wong *et al.* (1996), Pedreno *et al.* (1996), Balik *et al.* (1998), Ayuso *et al.* (1996), Weir and Allen (1997) and Nogales *et al.* (1997). The latter workers applied sludge up to 200 t ha$^{-1}$ in their pot experiment, without the yield of the plants being adversely affected, although this would have provided an excesive amount of N. However, in cuttings 4, 5 and 6 in this project, the 5 and 10 t ha$^{-1}$ treatments had lower yields than the control. The 50 t ha$^{-1}$ treatments in these cuttings were always substantially higher than the others.

**Figure 5.1:** ***Yield of ryegrass in the Sonning trial (g).***



LSD test

*Sludge 123****

*Time 158****

*Sludge x Time 408****

Time had a very important effect on the yield of ryegrass grown in the plots of the field trial. The first cuttings had a significantly higher yield than the following cuttings (LSD value of 158). This may be due to the fact that the first cutting was carried out 2 months after the establishment of the crop. The remaining 5 cuttings were carried out in the space of 4 months. It may be noted in Figure 5.1 that after the first cutting, yields in all treatments reached a point after which they remained in these values up to the end of the trial without changing very significantly (except the peak in the 50 t ha$^{-1}$ treatment in the third cutting). The control treatment had in the first cutting 1348 g, 673 g in the second cutting and 574 g in the last cutting. The 50 t ha$^{-1}$ treatment had 3704, 1475 and 1117 g in the first, second and last cutting respectively, while the 5 and 10 t ha$^{-1}$ treatments had very similar yields.

### 5.3.2 Plant Concentrations of Heavy Metals in Reading and Greece

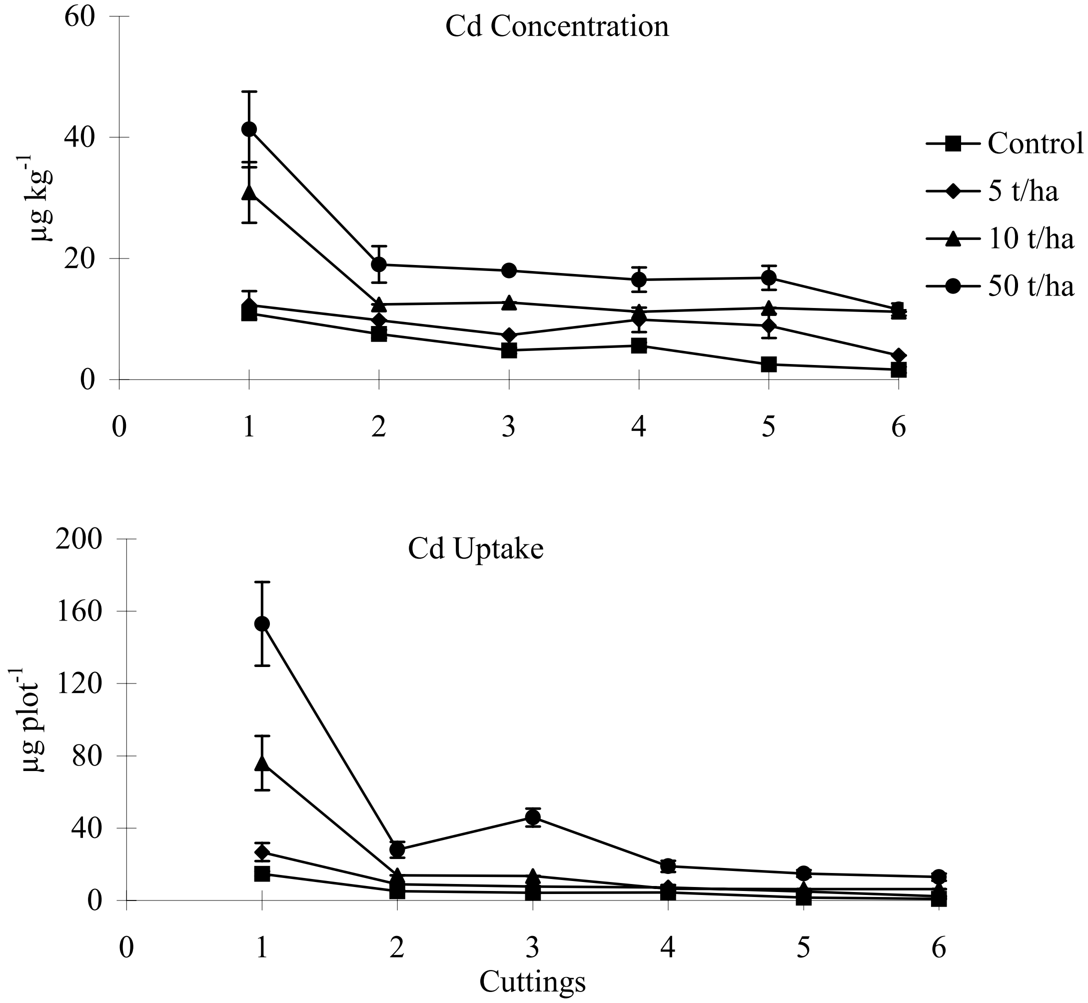
In the Sonning farm trial Cd, Ni, Pb and Zn were measured in the plants, while in Vardates trial only Pb and Zn were measured.

#### 5.3.2.1 Cadmium

Cadmium concentrations in ryegrass were measured in μg kg$^{-1}$ DM and uptake was measured in μg plot$^{-1}$ (Figure 5.2 and Appendix II-Tables A2.1.1-A2.1.2). Cadmium concentrations in ryegrass increased significantly with the increase in sewage sludge application rate from 0 to 50 t ha$^{-1}$ (LSD value of 0.80). In the first cutting, 50 t ha$^{-1}$ had a 4 times greater concentration of Cd than the control treatment (41.3 and 10.9 μg kg$^{-1}$, respectively). However, this important increase in Cd did not have any apparent toxic effects in the growth of the crop. In the first cutting, the 10 t ha$^{-1}$ treatment had 30.9 μg Cd kg$^{-1}$. This meant that from 10 to 50 t ha$^{-1}$ there was only a 1.5 times increase relative to the control. This increase from 10 to 50 t ha$^{-1}$ was still observed in the following 5 cuttings. This showed that not all of the Cd added to the soil with sewage sludge application was available to plants. The concentration of Cd was restricted at the higher sludge application rate of 50 t ha$^{-1}$. This was contrary to what was found in the pot experiment, where the difference in the concentrations of Cd in the ryegrass between 10 and 50 t ha$^{-1}$ was almost of a factor of 5. This was probably due to the fact that in this field trial there are many limiting factors that have an antagonistic affect on the availability of metals.

Time had an important effect on Cd availability to ryegrass. From the first cutting to the last, Cd decreased from 41.3 to 11.5 μg kg$^{-1}$ in the 50 t ha$^{-1}$ treatment. The same trend was observed in the other treatments as well. In the controls, Cd decreased from 10.9 to 1.6 μg kg$^{-1}$, in the 5 t ha$^{-1}$ treatment Cd decreased from 12.3 to 4.0 μg kg$^{-1}$ and in the 10 t ha$^{-1}$ treatment Cd decreased from 30.9 to 11.2 μg kg$^{-1}$ from the first to the last cutting. It can be noted in Figure 5.2 that, as in the data of ryegrass yield, after the first cutting, where the concentrations of Cd at all treatments were high, the concentrations decreased significantly (LSD 1.05) and reached a point after which it did not decrease further. Concentrations of Cd remained statistically unchanged at that level until the end of the experiment in all treatments, except for 50 t ha$^{-1}$, which was further reduced in the last cutting. This behaviour may have been influenced by the dry matter production (yield) of the crop. It can be noted that the yields at all sludge application rates were high in the first cutting and decreased in the subsequent cuttings. The same was observed with the Cd concentrations in the plants, where concentrations were high in the first cutting at all sludge application rates, and decreased in

**Figure 5.2:** ***Ryegrass concentration (μg kg$^{-1}$) and uptake (μg plot$^{-1}$) of Cd in the Sonning trial.***

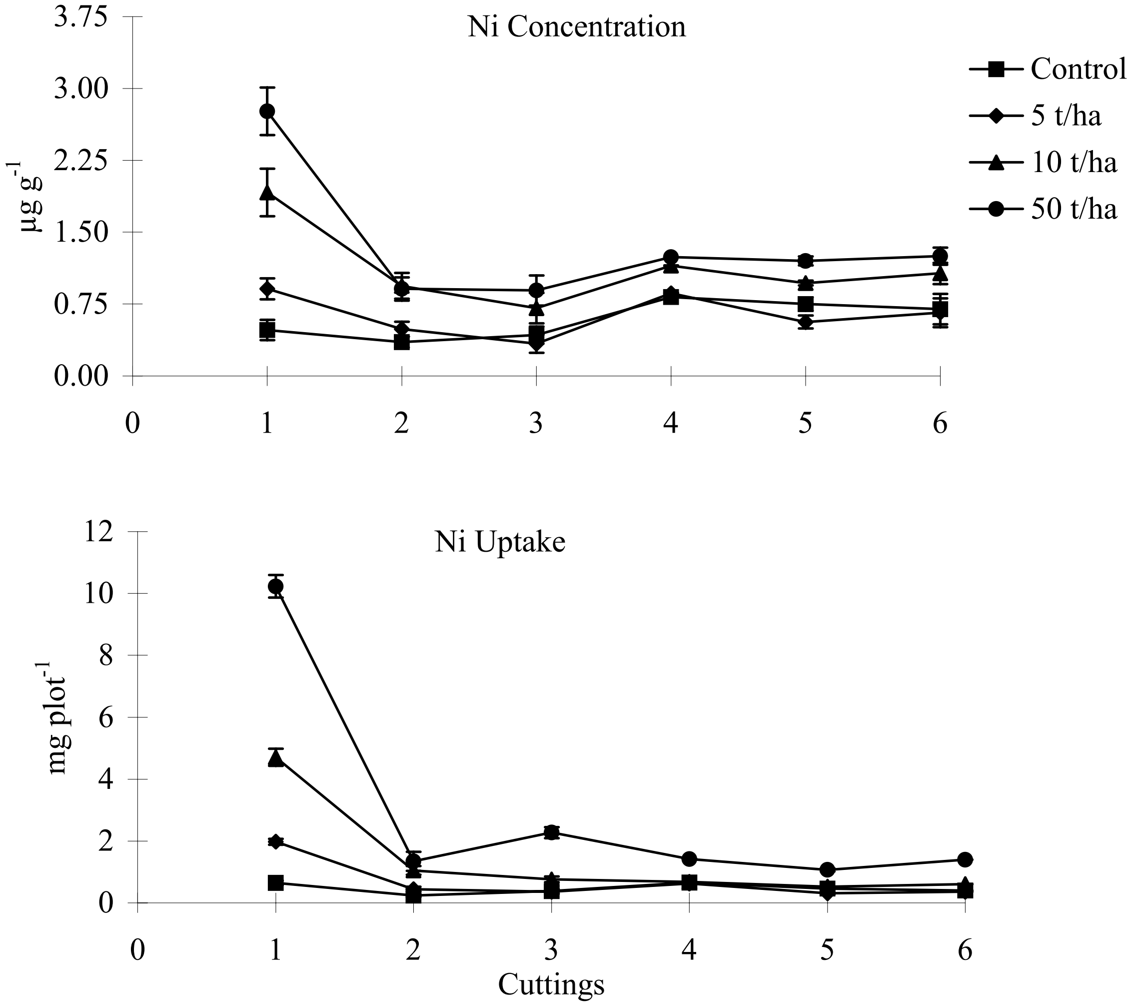


LSD test (on the left for concentration and on the right for uptake)

| | | | |
|---|---|---|---|
| *Sludge* | *0.80* | *Sludge* | *1.84* |
| *Time* | *1.05*** | *Time* | *2.37**** |
| *Sludge x Time* | *2.72* | *Sludge x Time* | *6.12** |

the subsequent cuttings. This may be due to the fact that plants with higher yields may have stimulated Cd uptake. This was very much in accordance with the findings from the pot experiment, where the plant concentration of heavy metals was dependent on the plant yield. The same behaviour was found by Cd uptake by ryegrass. Cadmium uptake increased significantly (LSD 1.84) in some cases with the increase in sewage sludge application rate. Over time, uptake decreased significantly (LSD 2.37) after the first cutting.

**Figure 5.3:** ***Ryegrass concentration (μg g$^{-1}$) and uptake (mg plot$^{-1}$) of Ni in the Sonning trial.***



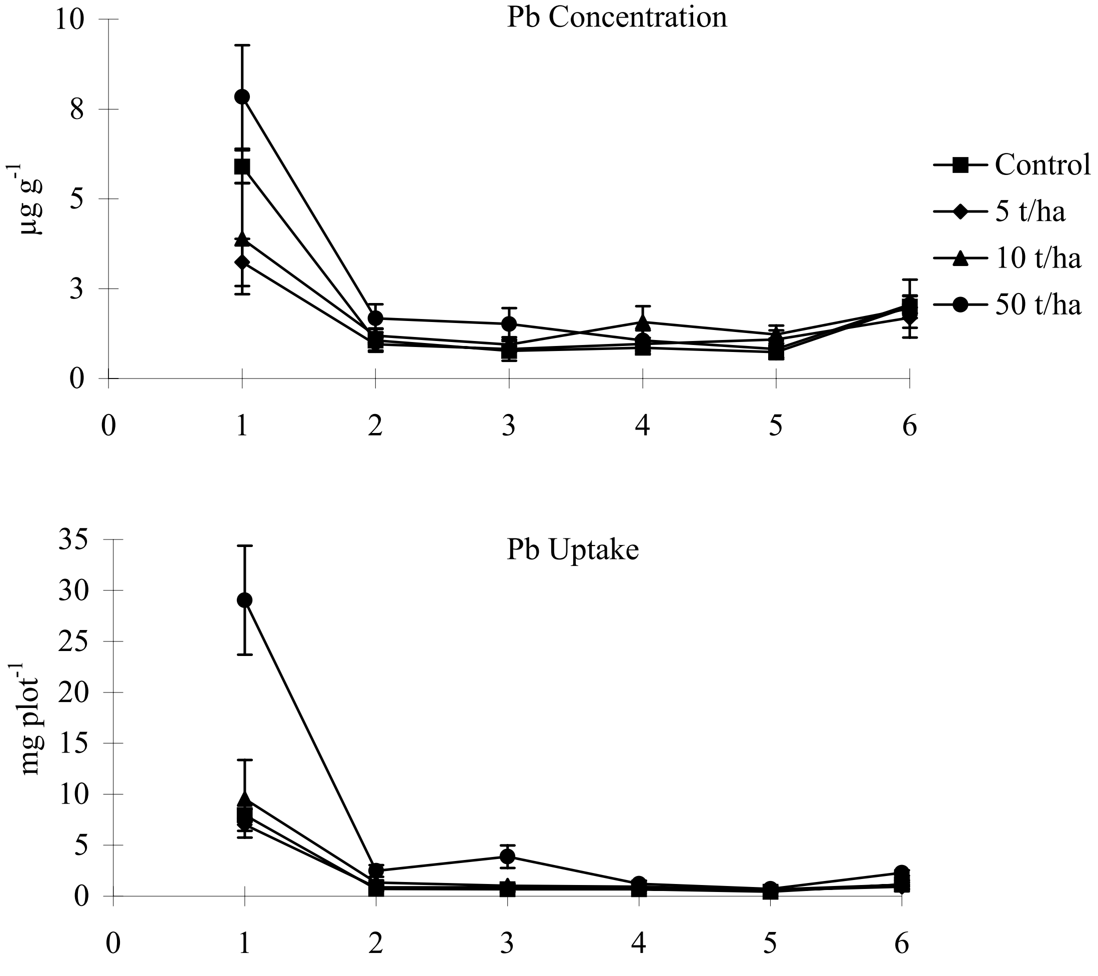
LSD test (on the left for concentration and on the right for uptake)

| | |
|---|---|
| *Sludge 0.40** | *Sludge 0.97**** |
| *Time 0.52**** | *Time 1.25**** |
| *Sludge x Time 1.34* | *Sludge x Time 3.22*** |

### 5.3.2.2 Nickel

Nickel concentrations in ryegrass were measured in μg $g^{-1}$ DM and Ni uptake was measured in mg $plot^{-1}$ (Figure 5.3 and Appendix II-Tables A2.2.1-A2.2.2). Nickel concentrations in ryegrass increased significantly with the increase in sewage sludge application rate. (LSD value of 0.40). In the first cutting, the 50 t $ha^{-1}$ treatment had a 5 times greater concentration in Ni than the control treatment (2.76 and 0.48 μg $g^{-1}$ respectively). On the other hand, from the 10 to the 50 t $ha^{-1}$ treatments, the difference was only of a factor of 1.3, which, however, was not observed in the next 5 cuttings, where differences between 10

**Figure 5.4:** ***Ryegrass concentration (μg $g^{-1}$) and uptake (mg $plot^{-1}$) of Pb in the Sonning trial.***



LSD test (on the left for concentration and on the right for uptake)

| | | | |
|---|---|---|---|
| *Sludge* | *0.64** | *Sludge* | *2.03**** |
| *Time* | *0.82**** | *Time* | *2.61**** |
| *Sludge x Time* | *2.11** | *Sludge x Time* | *6.71**** |

and 50 t $ha^{-1}$ were not significant.

Time affected significantly Ni availability. As in the case of Cd, Ni decreased from 2.76 to 1.25 μg $kg^{-1}$ in the 50 t $ha^{-1}$ treatment between the first and the last cuttings. The same decrease was observed in the other treatments as well, except from the control. In the control treatment, Ni increased from 0.48 to 0.69 μg $g^{-1}$. However, this difference was not significant (LSD of 0.52). It should be noted in Figure 5.3, that after the first cutting, where the concentrations of Ni at all treatments were high, the concentrations decreased significantly (LSD 0.52) and reach a point in the second cutting, after which they did not decrease further significantly. Concentrations of Ni remained statistically unchanged at that level until the end of the experiment in all treatments.

The same behaviour with Ni uptake was found as with Ni concentrations. Nickel uptake was significantly higher (LSD 0.97) with the higher sewage sludge application rate. Over time, uptake decreased significantly (LSD 1.25) after the first cutting.

#### 5.3.2.3 Lead

Lead concentrations in ryegrass were measured in μg $g^{-1}$ DM and Pb uptake was measured in mg $plot^{-1}$ (Figures 5.4 and Appendix II-Tables A2.3.1-A2.3.2) in the Sonning farm trial. In the Vardates site, Pb was measured in the plant mass, but it was found to be below the detection limit of the ICP-OES instrument used by NAGREF. Lead concentrations in ryegrass increased significantly with the higher sewage sludge application rate (LSD value of 0.64). However, as with Ni, this was evident only in the first cutting. After the first cutting, concentrations of Pb in ryegrass were not affected by the elevated rates of sewage sludge, because there were no significant differences between the of control, 5, 10 and 50 t $ha^{-1}$ treatments.

Time was an important factor affecting Pb concentrations in the ryegrass. As with Cd and Ni, Pb decreased from 7.83 to 2.05 μg $g^{-1}$ in the 50 t $ha^{-1}$ treatment between the first and the last cutting. The same decrease was observed in the other treatments as well. The concentrations of Pb decreased significantly (LSD 0.82) and reach a point after which it did not decrease furhter until the end of the experiment.

Lead uptake by ryegrass had the same behaviour with Pb concentrations. Lead uptake increased significantly (LSD 2.03) with the higher sewage sludge application rate, but only in the first cutting. Over time, uptake decreased significantly (LSD 2.61) after the first cutting.

### 5.3.2.4 Zinc

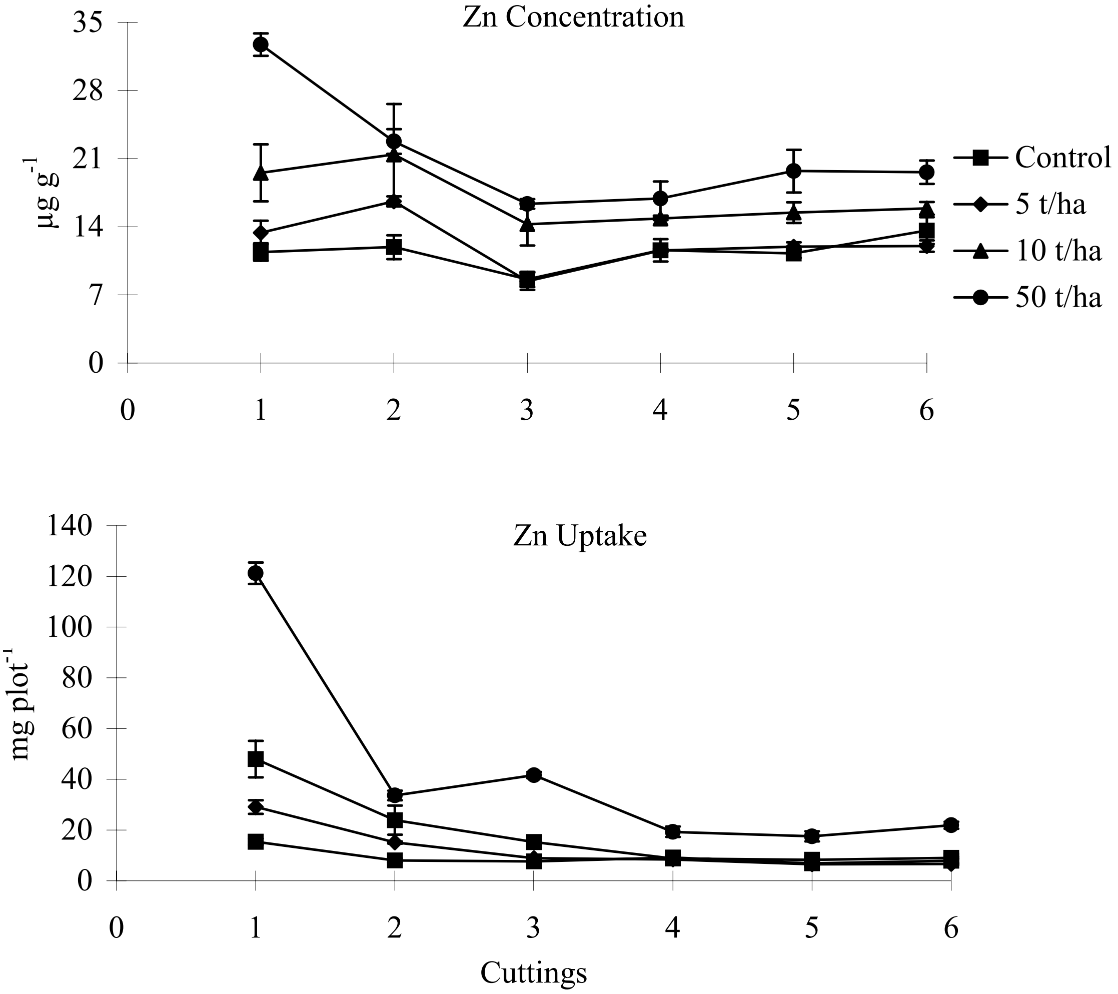
Zinc concentrations in ryegrass were measured in μg $g^{-1}$ DM and Zn uptake was measured in mg $plot^{-1}$ (Figures 5.5 and Appendix II-Tables A2.4.1-A2.4.2) in the Sonning farm field trial. In the Vardates trial, Zn concentrations in the plant mass were measured in μg $g^{-1}$. Zinc concentration in ryegrass increased significantly with the higher sewage sludge application rate (LSD value of 2.17). It is, however, evident that in the low sludge treatments (5 and 10 t $ha^{-1}$) the differences from the control were not very significant. Gasiunas *et al.* (1995) commented that at low sewage sludge applications (8 t $ha^{-1}$), which represent the normal rates of sludge additions in common agricultural practice, the load of heavy metals did not have any significant effect on the crops. From 10 to 50 t $ha^{-1}$ there was only an increase of 2.6 times (19.5 and 32.7 μg Zn $g^{-1}$, respectively). This showed that the higher the sludge application, the lower the relative availability of Zn. This increase from 10 to 50 t $ha^{-1}$ was not observed in the next 5 cuttings.

Time had an important effect on Zn availability to ryegrass. The effect of time was the same as in the case of Cd, Ni and Pb. Zinc decreased between the first and the last cutting (LSD of 2.79) in all the treatments. However, this was only significant in the 50 t $ha^{-1}$ treatment. This behaviour of Zn over time was different from what was observed for Cd, Ni and Pb, where the concentrations of these metals in ryegrass after the first cutting decreased over time significantly.

Zinc uptake by ryegrass followed the same trends with Zn concentrations. Zinc uptake increased significantly (LSD 4.00) with the higher sewage sludge application rate. However, uptake decreased significantly (LSD 5.15) after the first cutting in all sludge treatments, unlike Zn plant concentration, in which only the 50 t $ha^{-1}$ treatment uptake decreased significantly over time.

At Vardates, Zn concentration increased with the increase in sewage sludge application rate by as much as 20 % from the control to the 50 t $ha^{-1}$ treatment in the first cutting. It can be noted in Table 5.3 that Zn concentrations in the 4 t $ha^{-1}$ treatment were the same as those in the 50 t $ha^{-1}$ treatment,

**Figure 5.5:** ***Ryegrass concentration (µg g$^{-1}$) and uptake (mg plot$^{-1}$) of Zn in the Sonning trial.***



LSD test (on the left for concentration and on the right for uptake)

*Sludge 2.17**** *Sludge 4.00****

*Time 2.79**** *Time 5.15****

*Sludge x Time 7.19*** *Sludge x Time 13.26****

**Table 5.3:** ***Concentrations of Zn in ryegrass in the Vardates trial (µg g$^{-1}$) (Courtesy of Papadopoulos* et al.*).***

| | Cutting 1 | Cutting 2 | Cutting 3 |
|---|---|---|---|
| Control | 12.68 | 25.71 | 16.73 |
| 4 t ha$^{-1}$ | 15.87 | 26.12 | 17.08 |
| 10 t ha$^{-1}$ | 13.46 | 29.37 | 18.64 |
| 50 t ha$^{-1}$ | 15.84 | 29.04 | 30.51 |

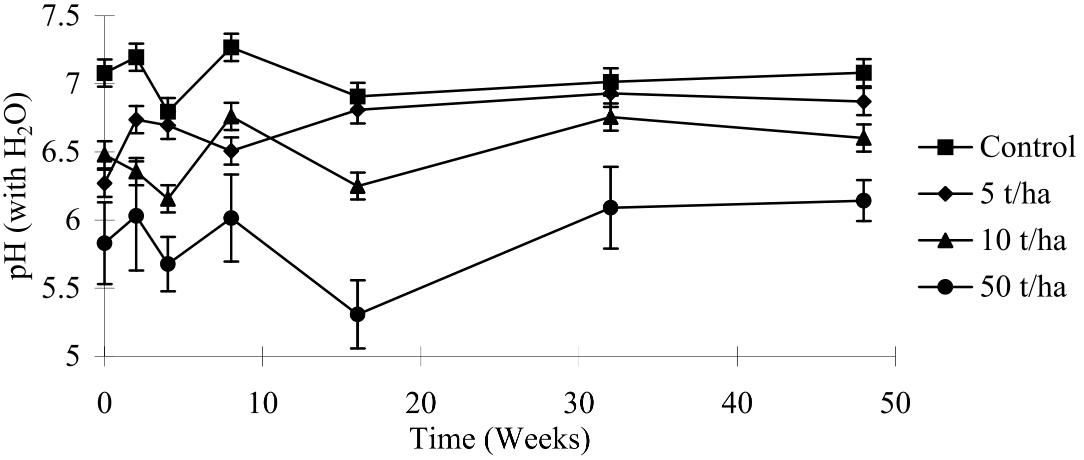
probably due to the 'dilution effect'. In the next cutting, Zn concentrations in all sludge treatments increased substantially, compared with the first cutting. Control Zn concentrations increased from 12.68 to 25.71 μg $g^{-1}$, and the 50 t $ha^{-1}$ treatment increased from 15.84 to 29.04 μg $g^{-1}$. The other sludge treatments had the same steep increase. In the last (third) cutting the Zn concentrations in the control, 4 and 10 t $ha^{-1}$ treatments decreased again and nearly equalised with the values in the first cutting, while Zn in the 50 t $ha^{-1}$ treatment remained as high as in the second cutting.

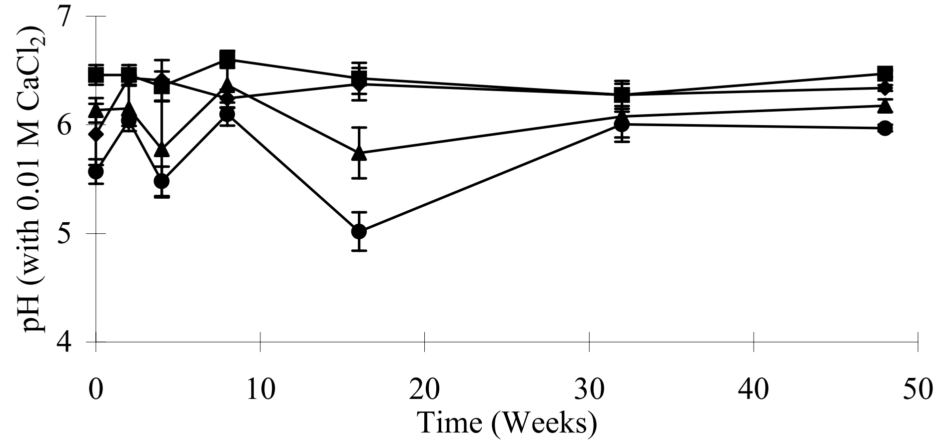
### 5.3.3 Measurement of Soil pH in the Experiments in Reading and Greece

Soil pH values in the Sonning trial were measured both in $H_2O$ and in $CaCl_2$ suspension, while the soil pH in the Vardates trial were only measured in $H_2O$ (Figures 5.6.1 - 5.6.2 and Appendix II-Tables A3.1.1, A3.1.2 and A3.2). In the Sonning farm plots, pH values were significantly affected by the application of sewage sludge (LSD 0.19 for $H_2O$ and LSD 0.16 for $CaCl_2$). As sewage sludge rates increased, pH values decreased. In the literature there are a few workers who reported the same effect of sludge on soil pH values. Brallier *et al.* (1996) found that as sludge application increased (up to 500 t $ha^{-1}$ in a pot experiment) pH values decreased. They had the opinion that this was caused by the nitrification that took place in the sludge shortly after it was applied in the soil. The same effect was found by Hooda and Alloway (1993), who again emphasised that this behaviour was probably due to nitrification process in the sludge. It should be noted that in this project, in the earlier pot experiment the same effect of sludge application on the soil pH values was also found (Chapter 4).

It can be seen that pH values in the soils needed some time to equilibrate, after sludge was applied to the soils. Especially in the higher sludge applications there were fluctuations in the pH values up to Week 16. It was evident that these were caused by the application of sewage sludge, because in the control and the 5 t $ha^{-1}$ treatments there were much smaller effects. In Week 16, the 50 t $ha^{-1}$ treatment

**Figure 5.6.1:** ***Soil pH (in $H_2O$ and $CaCl_2$ suspension) in the Sonning trial.***



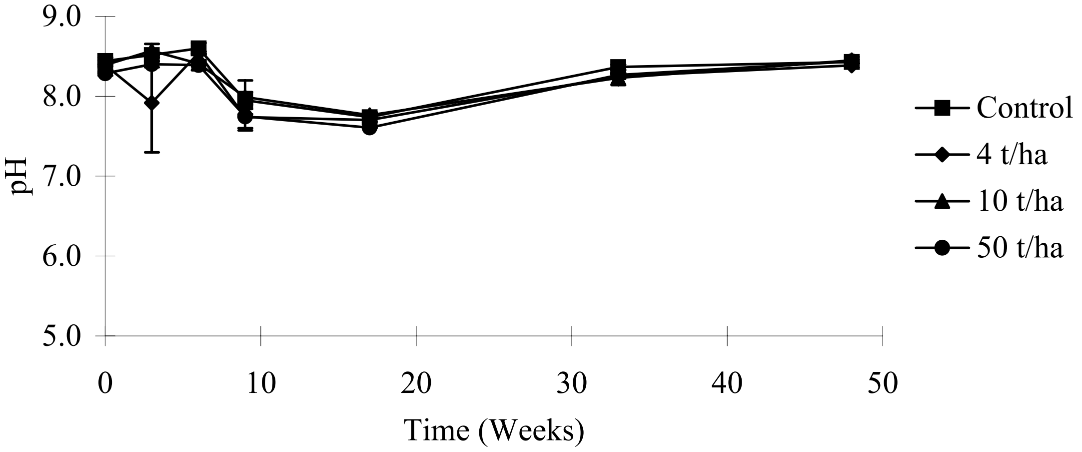


LSD test (on the left for $H_2O$ suspension and on the right for $CaCl_2$ suspension)

| | |
|---|---|
| *Sludge 0.19**** | *Sludge 0.16**** |
| *Time 0.28* | *Time 0.23* |
| *Sludge x Time 0.73* | *Sludge x Time 0.31* |

showed its lowest pH. In the next sampling (Week 32) pH had increased again to the value of Week 8. It seemed that after Week 32, pH even in the 50 t $ha^{-1}$ treatment was in equilibrium and kept relatively constant over time. In the Greek trial, pH values decreased as sewage sludge application increased, but not very substantially. It is important that over time, it took 4 months for the pH values to reach an equilibrium, after which it seemed that pH was unaffected with time. In Week 16, a minimum pH value was reached, just like in case of the Sonning trial. These fluctuations in the early stages after sewage sludge was applied, may be due to intense microbial activity, which was stimulated with the application of sludge in the soil.

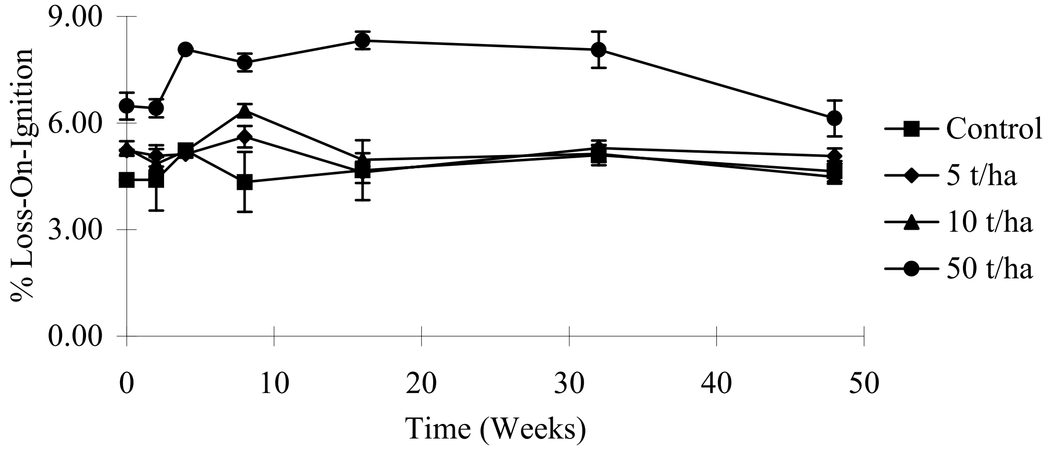
**Figure 5.6.2:** ***Soil pH (in $H_2O$) in the Vardates trial.***



### 5.3.4 Measurement of Loss-On-Ignition in the Experiments in Reading and Greece

Organic matter contents in the soils measured by Loss-On-Ignition is presented in Figures 5.7.1-5.7.2 and in Appendix II-Table A4.1-A4.2. In the Sonning farm trial, organic matter was significantly affected by the application of sewage sludge. It increased with the increase in sludge application to soil (LSD of 0.42). However, the control, 5 and 10 t $ha^{-1}$ treatments

**Figure 5.7.1: Loss-On-Ignition in the Sonning trial (%).**



LSD test

*Sludge* *0.42****

*Time* *0.62*

*Sludge x Time* *1.59*

were not significantly different, that is organic matter did not increase significantly as sludge application increased from 0 to 10 t ha$^{-1}$. The only significant difference was that between these 3 treatments and 50 t ha$^{-1}$. Smith (1995) stated that in field experiments it needs very large applications of sludge (sometimes greater than those commonly applied in agricultural practice) in order to increase significantly the organic matter content of a soil.

Over time, the organic matter content did not show the same behaviour as in the earlier pot experiment, where there was a significant decrease in the first 4 months and after that a very slow decline up to the end of the experiment. At Sonning, in the control, 5 and 10 t ha$^{-1}$ treatments there was only a slight (and not significant) decrease over time. In the 50 t ha$^{-1}$ treatment, there was a significant decrease (LSD of 0.62) after 8 months from the commencement of the experiment, but before this, there was no sign of any decrease. In Vardates, the picture was very much in accordance with Sonning, with no important decrease of the organic matter content until Week 32 (a decrease was observed in the 50 t ha$^{-1}$ treatment after Week 3, but this may have been due to analytical or other errors, since the initial content of organic matter was recovered again in Week 32). This delayed decrease of the soil organic matter content in the Sonning experiment was caused by the fact that this experiment was affected by the weather (temperature fluctuation and moisture availability). The experiment started in January, when there were very low temperatures. The first sign of any significant decrease in organic matter was given only after Week 32 (in the 7th month,

**Figure 5.7.2:** ***Loss-On-Ignition in the Vardates trial (%).***

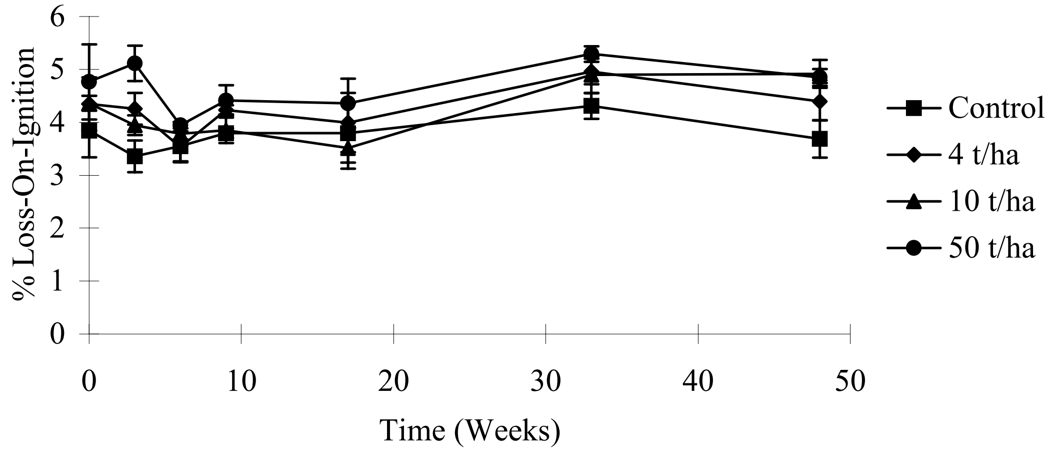
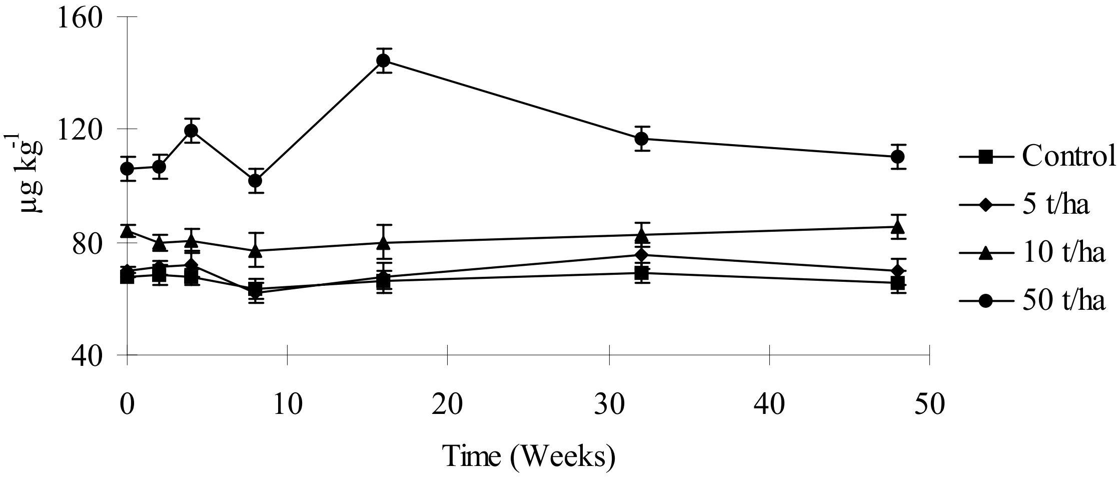


**Figure 5.8.1:** ***DTPA-extractable Cd in the Sonning trial (µg kg$^{-1}$).***



LSD test

*Sludge* *6.59****

*Time* *9.62*

*Sludge x Time* *24.76*

that is after July) when the temperatures were more likely to be conducive to the decomposition of the organic matter.

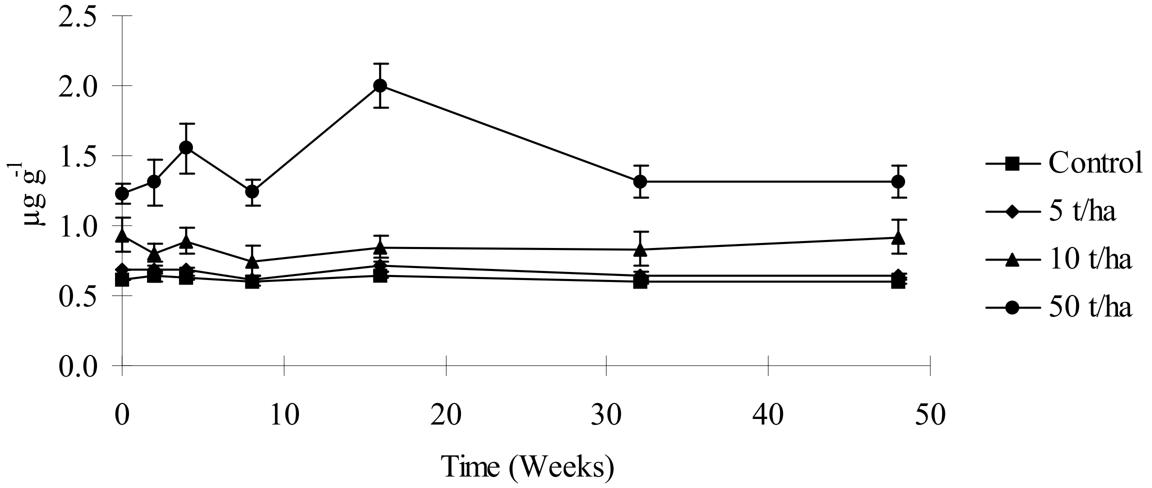
### 5.3.5 Heavy Metals Extracted with DTPA

Soil samples from Sonning were extracted with DTPA in order to assess the suitability of this reagent in predicting the bioavailability of heavy metals to plants in the yield.

#### 5.3.5.1 Cadmium

DTPA-extractable Cd is presented in Figure 5.8.1 and in Appendix II-Table A5.1. DTPA-extractable Cd was significantly affected by the addition of sewage sludge (LSD of 6.59). The levels of Cd increased with the increase in sewage sludge application rate. However, the control treatment did not significantly differ from the 5 t ha$^{-1}$ treatment. Between the 5 and 10 t ha$^{-1}$ treatments there was an increase of 14 %, and from 10 to 50 t ha$^{-1}$ a further increase of 29 %, when all treatments are combined. Over the period of one year, Cd extracted from the control, 5 and 10 t ha$^{-1}$ treatments did not show any significant difference (LSD of 9.62).

**Figure 5.8.2:** ***DTPA-extractable Ni in the Sonning trial (μg g$^{-1}$).***



LSD test

*Sludge 0.11****

*Time 0.17*

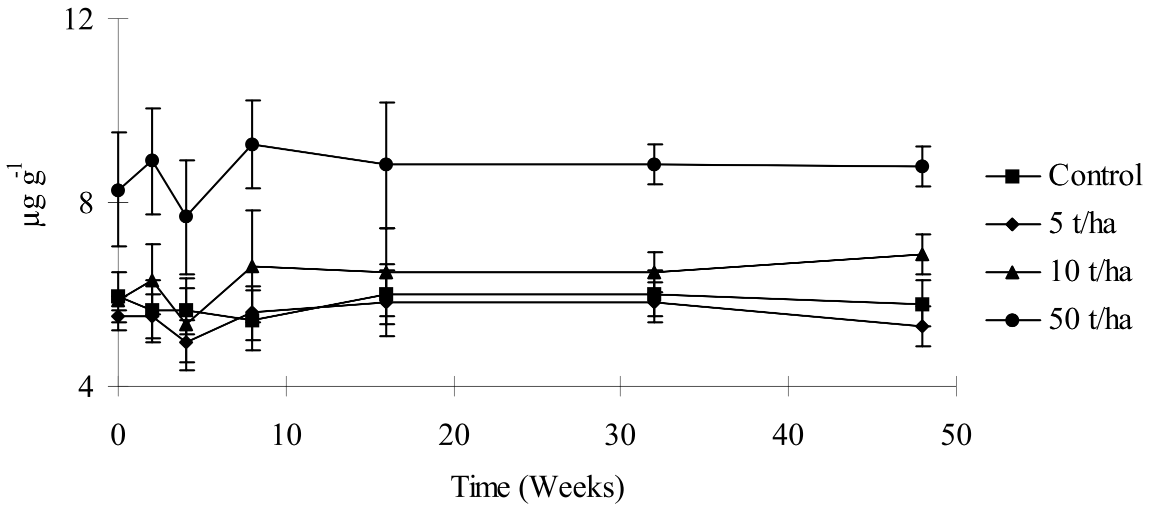
*Sludge x Time 0.44*

However, Cd from the 50 t ha$^{-1}$ treatment showed large variations in the first 2 months (until Week 8). Cadmium increased significantly (LSD of 9.62) from 107 to 120 μg kg$^{-1}$, from Week 2 to Week 4, and then fell again to 102 μg kg$^{-1}$. These variations were also reflected in the fluctuations of the soil pH values. In Week 16 Cd increased very substantially to 145 μg kg$^{-1}$ (30 % increase from the previous sampling of week 8). It is important to note that at the same time pH values in the plots of the 50 t ha$^{-1}$ treatment fell to their lowest level. The reason for the peak in Cd extractability by DTPA from the soil in the 50 t ha$^{-1}$ treatment may be explained in the significant drop of the pH value at that sampling time. In Week 16 (which was in April 1997) the weather conditions may have encouraged a rapid rate of nitrification in the sewage sludge added to the soil. The rise in temperature (which would be the first after the winter) may have accelerated the nitrification processes caused by bacteria like *Nitrozomonas*, *Azotobacter* and *Rhizombium* in the soil. After Week 16, Cd extracted by DTPA declined again to initial Cd concentrations (110 μg kg$^{-1}$).

### 5.3.5.2 Nickel

DTPA-extractable Ni is presented in Figure 5.8.2 and in Appendix II-Table A5.2. DTPA-extractable Ni was significantly affected by the addition of sewage sludge (LSD of

**Figure 5.8.3:** ***DTPA-extractable Pb in the Sonning trial (µg g$^{-1}$).***



LSD test

*Sludge 0.68****

*Time 1.00*

*Sludge x Time 2.59*

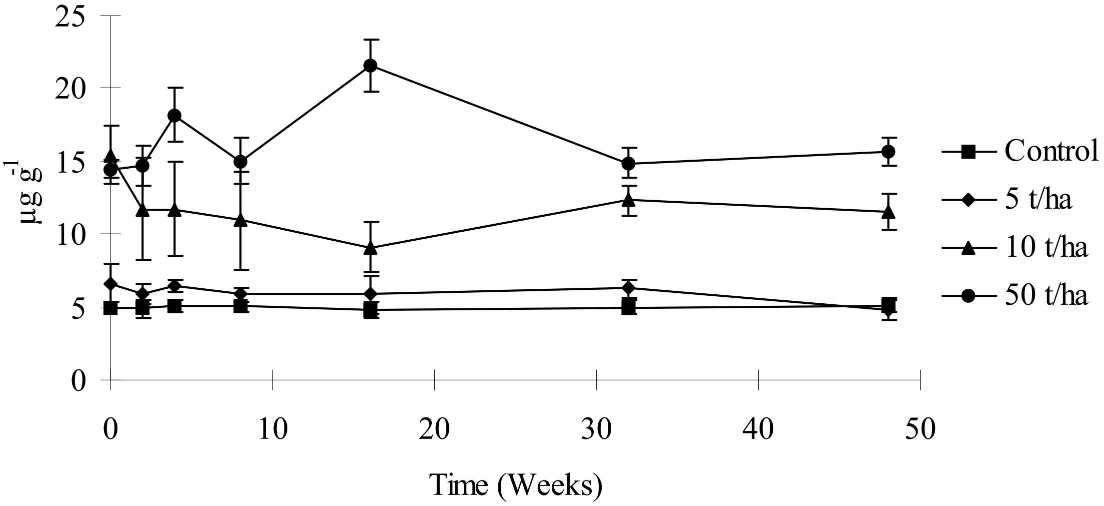
0.11). The levels of Ni increased with the higher sewage sludge application rate. However, the increase was only significant between 5 to 10 t ha$^{-1}$ treatments (21 % increase) and between the 10 and 50 t ha$^{-1}$ treatments (40 % increase), when all treatments are combined. The difference between the control and 5 t ha$^{-1}$ was not significant.

Over the period of one year, Ni extracted from the control, the 5 and the 10 t ha$^{-1}$ treatments did not show any significant difference (LSD of 0.17). However, Ni from the 50 t ha$^{-1}$ treatment showed fluctuations in the first 2 months (until Week 8), like Cd. These fluctuations were also reflected in the variations in the soil pH values. In Week 16, extractable-Ni increased very substantially to 2.00 µg g$^{-1}$ (38 % increase from the previous sampling of Week 8), as in case of Cd. The explanation for this behaviour may again be due to the rapid nitrification process in the sewage sludge added in higher quantities in these plots. This nitrification probably caused the decrease in pH value in the 50 t ha$^{-1}$ treatments, which subsequently led to the increased the extractability of Ni. After Week 16, Ni extracted by DTPA declined again to around the initial Ni concentration (1.31 µg g$^{-1}$).

### 5.3.5.3 Lead

DTPA-extractable Pb is presented in Figure 5.8.3 and in Appendix II-Table A5.3. As in the case of Cd and Ni, DTPA-extracted Pb was significantly affected by the addition of sewage sludge (LSD of 0.68). The levels of Pb increased with the higher sewage sludge application rate. However, the control treatment did not significantly differ from the 5 and the 10 t ha$^{-1}$ treatments. Over the period of one year, Pb extracted from all sludge treatments (control, 5 and 10 and 50 t ha$^{-1}$) did not show any significant increase or decrease (LSD of 1.00). However, Pb extracted  in the 50 t ha$^{-1}$ treatment showed fluctuations during the first 2 months (until Week 8), which were probably reflections of the pH fluctuations in that time. In Week 16, Pb did not increase as other heavy metals in this study did. As a result of Pb being a very immobile element, compared with Cd, Ni and Zn, the substantial decrease in pH in Week 16 did not have any significant effect on extractable Pb concentrations. It should noted that after Week 8, Pb did not show any further significant changes in the 50 t ha$^{-1}$ treatment.

**Figure 5.8.4:** ***DTPA-extractable Zn in the Sonning trial (μg g$^{-1}$).***



LSD test

*Sludge 1.93****

*Time 2.82*

*Sludge x Time 7.26*

### 5.3.5.4 Zinc

DTPA-extractable Zn is presented in Figure 5.8.4 and in Appendix II-Table A5.4. As with all the metals studied, DTPA-extracted Zn increased with the increase in sewage sludge application rate (LSD of 1.93). However, the control treatment did not significantly differ from the 5 t ha$^{-1}$. The same was observed by Bragato *et al.* (1998), who found that sludge application rates up to 15 t ha$^{-1}$ could not significantly increase the DTPA extractability of Cd, Zn, Pb and Ni. The explanation may lie on the fact that organic matter binds heavy metals very efficiently at low metal concentrations. Over the period of one year, Zn extracted from the control, 5 and 10 t ha$^{-1}$ did not show any significant differences (LSD of 2.82), as with all the heavy metals discussed previously. However, Zn in the 50 t ha$^{-1}$ treatment showed fluctuations in the first 2 months (until Week 8), like Cd and Ni. In Week 16, Zn increased very substantially to 21.51 μg g$^{-1}$ (30 % increase from the previous sampling of Week 8, similar to that of Cd and Ni). The explanation for this behaviour may again be due to the rapid nitrification process in the sewage sludge added in higher quantities in that plots. After Week 16, Zn extracted by DTPA declined again to around the initial Zn concentrations (15.66 μg g$^{-1}$).

### 5.3.6 Measurements of Dissolved Organic Carbon

Dissolved Organic Carbon concentrations in the various treatments are presented in Table 5.4. From the results of the previous pot experiment (Chapter 4) it was suggested that DOC can play a very important role in solubilising heavy metals and increasing their availability to plants. From the data presented in Table 5.4 it can be noted that DOC increased substantially during the experiment in both the control (by 34 %) and 50 t ha$^{-1}$ (by 43 %) treatments.

However, this increase in DOC concentration in soils was not great enough to influence the availability of heavy metals in the sludge-treated plots. In a field experiment there are many limiting factors that could prevent the availability of heavy metals from increasing. The weather conditions (temperature, humidity and rainfall) play an important and often unpredictable role on the chemical processes of metals. Chang *et al.* (1992) found no apparent correlation between soil extractable heavy metals and heavy metal concentrations in plants in their field trial. It was assumed that this happened due to uncontrolled factors that were predominant in their experiment. Plant concentration and uptake of heavy metals depended on the plant yield, which in turn depended on the season of the year and on the timing of the

**Table 5.4:** ***Dissolved Organic Carbon concentration in the Sonning trial (μg mL$^{-1}$).***

| | Week 4 | Week 48 |
|---|---|---|
| Control | 34.61 | 52.62 |
| 50 t ha$^{-1}$ | 39.33 | 69.67 |

cutting (plants left to grow for a longer time had higher concentrations of metals). This makes it difficult to decide whether the availability was affected by DOC, since the above mentioned factors had a much greater effect on the plant yield, and thus on plant uptake.

Extractable concentrations of heavy metals in the soil were affected by unpredictable things, such as the rapid nitrification (which again depended on the weather conditions), which affected the extractability of Cd, Ni and Zn very significantly. It may, thus, be the case that the increase in DOC concentrations in the soil did not affect the heavy metal extractability, since this was governed by a variety of external factors. I addition, DTPA is an organic extractant solubilising mostly 'organically bound' metals (Tessier *et al.*, 1979). Since the organic matter content of the soil did not significantly change until 48 weeks from the commencement of the experiment it may be said that the DTPA extractable concentrations of heavy metals did not increase or decrease over time because of the behaviour of the organic matter.

**Table 5.5:** ***Transfer Coefficient values for Cd, Ni, Pb and Zn in the Sonning trial and for Zn in the Vardates trial.***

| | Cd | Ni | Pb | Zn (Sonning) | Zn (Vardates) |
|---|---|---|---|---|---|
| Control | 0.07 | 0.06 | 0.07 | 0.24 | 0.24 |
| 5 t ha$^{-1}$ | 0.10 | 0.06 | 0.05 | 0.24 | 0.29 [a] |
| 10 t ha$^{-1}$ | 0.15 | 0.11 | 0.06 | 0.30 | 0.28 |
| 50 t ha$^{-1}$ | 0.14 | 0.10 | 0.07 | 0.25 | 0.28 |
| Mean | 0.11 | 0.08 | 0.06 | 0.26 | 0.27 |

*a: 4 t ha$^{-1}$*

### 5.3.7 Soil-Plant Transfer Coefficients

As discussed in the previous pot experiment (Chapter 4), the higher the TC value of a metal, the more mobile/available this is under the specific experimental conditions. Results for the TC of Cd, Ni, Pb and Zn in this field experiment are presented in Table 5.5. For all 4 metals, except Pb, TC values were affected by the additions of sewage sludge. For Cd, Ni and Zn, the order of increased mobility in the sludge treatments was:

$$\text{Control} > 5 \text{ t ha}^{-1} > 50 \text{ t ha}^{-1} > 10 \text{ t ha}^{-1}$$

This meant that TC values increased from the control treatment to 10 t ha$^{-1}$, but they subsequently decreased in the 50 t ha$^{-1}$ treatment. This seems to suggest that the relative mobility of Cd, Ni and Zn decreased with the higher sludge application rate, because of the adsorption of these metals on the solid phase of the soils, whose specific surface increased with the addition of elevated quantities of sewage sludge. Lead was not affected by the additions of sewage sludge, as there were no differences between TC values for Pb in the different sludge treatments. In accordance with this, Gerritse *et al.* (1982) and Sloan *et al.* (1997) found that there was no relationship between plant uptake of Pb and soil concentration of Pb. Scora and Chang (1997) also found no increase in Pb levels in peppermint plants, although Pb was up to 3 times higher in the sludged soil than in the control treatment.

For all 4 sludge treatments the order of reducing mobility among metals was:
Zn in Vardates (0.27) > Zn in the Sonning (0.25) > Cd (0.11) > Ni (0.08) > Pb (0.06)

Numbers in the parentheses indicate TC values. Zinc and Cd were the most mobile elements in this study. These findings are in accordance with these of many other workers. Moreno *et al.* (1997) found that Zn and Cd were much more bioavailable than Ni and Cu, while Hamon *et al.* (1997) predicted that, in their pot experiment, 12 % of Zn and 20-36 % of Cd would be available for uptake. On the same lines, Berti and Jacobs (1996) said that Cd, Zn and Ni were of great environmental concern, due to their high relatively mobility, while Cr, Cu and Pb were likely to be less of a problem.

Zinc was shown to be the most mobile element in this study, unlike the findings in the previous pot experiment, where the decreasing order was: Cd, Zn, Ni and Pb. The reason for this difference between the two experiments may be due to the fact that the sludge used for the pot experiment had substantially greater concentration in Cd (7.31 and 2.41 $\mu g\ g^{-1}$ for the pot and field experiments respectively). This meant that in the field trials, the load of Cd was significantly less than in the pot experiment, and the Zn/Cd ratio was much greater in the field experiment. This may have led to much greater competition of the two elements in favour of Zn, which kept the mobility of Cd lower than that of Zn. This was also supported by Gerritse *et al.* (1983a) and Gerritse *et al.* (1983b), who performed a pot experiment with 33 soils and found that an increase of available Zn in soil led to decreased concentrations of Cd in plants, when Cd concentrations were low in the soils.

Compared with the TC values found in the pot experiment, Zn had almost the same value in both experiments (0.25 in the pot experiment, and 0.24 in the Sonning) and 0.27 in the Vardates field experiments), while Cd had a TC value of 1.26 in the pot experiment and 0.11 in the field experiment. One would expect that TC values would be lower in the field trial than in the pot experiment, due to various limiting factors being operative in ‘open air’ conditions (McBride, 1995). This was probably the reason why TC for Cd was almost 10 times lower in the field trial. An additional reason for this difference may be the one discussed above: the competition of Cd and Zn, due to a significantly lower load of Cd in the sewage sludge, which was beneficial for Zn. McLaughlin *et al.* (1995), in their field experiment, found a similar competition effect between Cd and Zn. When Zn concentrations increased in the soil up to 100 kg $ha^{-1}$ (equal to 83 $\mu g\ g^{-1}$ soil, which is very similar to the concentration of Zn in this experiment) Cd concentration in potato plants decreased by 25-50 %. In terms of TC, this decrease would have halved the TC value for Cd due to competition with Zn. This favourable competition of Zn over Cd may be the reason that the TC value for Zn remained nearly unchanged in both pot and field experiments.

The TC values for Zn were higher in Vardates than in Sonning (0.25 and 0.27 respectively), although this difference was very marginal. However, this may point to the fact that under warmer conditions mobile heavy metals like Zn may be more soluble in the soil solution and thus more available to plants. The same thing was predicted from the pot experiment, where it was found that TC values for heavy metals in the warmer temperature were higher than TC values in the lower temperature treatments.

## 5.4 DISCUSSION

Several workers have reported that pot experiment findings concerning heavy metal availability tend to overestimate the risks from the application of sewage sludge. De Vries and Tiller (1978), in their early work, conducted experiments both inside and outside of a greenhouse and came to this conclusion. Their main conclusions were that the difference in results are due to more favourable conditions normally found in a greenhouse, such as temperature, humidity and light. McBride (1995) agreed with this statement, but added that differences in rooting pattern are more critical than the above three factors. Most of the crops' root systems go well beyond the sludge incorporation zone in soil and thus the heavy metal uptake is more conservative. In pot experiments, plant roots are 'forced' to stay where sludge is thoroughly incorporated with the soil. He added that these hypotheses provide an explanation for the fact that most of the times field experiments underestimate heavy metal availability to plants. Logan and Chaney (1983) found that pot experiments can overestimate heavy metal mobility by a factor of 1.5-5, compared with a counterpart field trial. They provided two reasons for this effect: (i) pots have lower pH values due to more intense use of $NH_4$-N fertilisers and (ii) in pot experiments there are abnormal watering patterns. Chaney *et al.* (1987) said that in order to have pot experiments that simulate field conditions in a better way, pots should be as big as possible. Carlton-Smith and Stark (1987) investigated heavy metal mobility in 40 plots on areas with long history in sludge application and found no phytotoxicity in the test crops. Sauerbeck and Hein (1991) compared Ni availability to 13 plant species in a pot and in a field experiment and found that Ni availability in the pot experiment was higher.

Dry matter yields increased significantly with the addition of sewage sludge. No toxicity symptoms were apparent even at the highest sludge application treatments. In fact, sludge additions stimulated ryegrass growth. Some workers have found a decline in the yield of test crops in sludge-treated soils, but this was always caused by toxicity effects of heavy metals (Singh *et al.*, 1997; Singh *et al.*, 1998; Jarausch-Wehrheim *et al.*, 1996). Yields in this project were also affected by conditions like the timing of the cutting and the season (earlier or later in the Summer).

The accumulation of heavy metals in ryegrass increased with the increase in sewage sludge application rates. The same was found by Krebs *et al.*(1998) in their field experiment, and Heckman *et al.* (1987), who even found that under field conditions heavy metal concentration in plants increased linearly with the increase of sewage sludge up to a rate of 448 t ha$^{-1}$ (which is an unrealistically high application rate for sludge). However, in the present experiment this increase was not linear up to the high rates of 50 t ha$^{-1}$. Cadmium, Ni and Zn concentrations in 10 and 50 t ha$^{-1}$ treatments showed only a 1.5 times difference, if the control was subtracted. This showed that increases in sludge application, which by definition increases the concentration of heavy metals in soils, did not increase the available form of metal proportionally. Sposito *et al.* (1982) found that the application of sewage sludge increased the organically bound and exchangeable metals, although the exchangeable did not increase linearly. Similar conclusions were drawn by Hooda and Alloway (1994), Sims and Kline (1991) and Luo and Christie (1998). O' Connor *et al.* (1983) also found that most of the Ni and Zn in soil were not available for plant uptake.

It was also evident that plant dry matter yields affected the heavy metal accumulation in the plant. In cuttings where yield was higher, heavy metal concentrations in the plant mass were also higher. This is something that was also evident in the pot experiment, where plants at 15 $^0$C had both lower yields and lower heavy metal uptake, while plants at 25 $^0$C had both higher yields and higher heavy metal uptake. However, as it was discussed earlier (Chapter 4), the difference in metal uptake between different temperature treatments in the pot experiment may well have been caused by the decomposition of organic matter and its complexation of metals in mobile forms. This, in turn, may have increased the availability of heavy metals to plants over time but to a greater extent at 25 $^0$C than at 15 $^0$C treatment.

Concentrations of all heavy metals in the plant mass followed the same trend over time. In the first cutting they were very high and subsequently they decreased to a point, after which they did not decreased significantly until the end of the experiment. If the concentration in the first cutting is excluded, then it can be noted that heavy metal concentration in ryegrass did not decrease in the course of one year. Exceptions to that pattern were the 50 t ha$^{-1}$ treatment for Cd, which was further reduced slightly in the last cutting and Zn, which reached the lowest point after the second cutting. McBride (1995) reported that in many field experiments heavy metal availability to plants does not change quickly after sewage sludge application for two main reasons. Firstly, because microbial biomass is likely to become less effective in decomposing the organic matter due to toxic effects of the heavy metals. If organic matter content stays the same, it is likely that metal availability will be buffered. Secondly, because in field trials sludge is not completely mixed with soil and 'sludge particles may reach equilibrium with the soil matrix very slowly' so that the toxic impact of sludge to plants may need some time to become more obvious. In this project, an important factor that may be related to this behaviour of heavy metals in ryegrass was the fact that the organic matter content of the soil did not decrease over the first 32 weeks of the experiment. Organic matter only showed a significant decrease in the last sampling at Week 48. This points to the importance of organic matter in affecting the availability of metals to plants, which was also stressed by Miner *et al.* (1997). The organic matter content did not decrease, as it had in the pot experiment, and this buffered the heavy metal uptake by ryegrass, which did not seem to change after the first cutting significantly until the end of the experiment. However, this rapid decrease in plant uptake after the first cutting may indicate immobilisation of metals in the soil, probably after a rapid degradation of the sludge organic matter has stabilised. Although this possibility cannot be ruled out, it is again indicative of the important role of the organic matter in the availability of heavy metals.

The values of soil pH in both England and Greece followed an identical pattern with time. In the early stages of the experiments there was an instability in the values, especially at the highest application rates of sewage sludge, something that was predicted by the pot experiment as well. In Week 16 there was a dramatic drop in pH, which affected all treatments in Vardates, but only the 50 t ha$^{-1}$ treatment in Sonning. As discussed above, this may have been a result of nitrification of the sewage sludge, which took place when the weather was more appropriate. That must have been in April in Sonning, and 3-4 weeks earlier in Vardates. These occasions were reflected in the samplings of Weeks 16 and 17 for Sonning and Vardates, respectively.

The DTPA-extractable Cd, Ni and Zn concentrations showed a corresponding peak in the sampling of Week 16. Singh *et al.* (1995) also reported that pH values have an important effect on the extractability of metals with DTPA. Nevertheless, DTPA predicted heavy metal uptake well, as it followed the trends in the plants. Apart from the peak in Week 16, DTPA-extractable metals did not significantly change over time and neither did the metal concentration in ryegrass after the first cutting.

## 5.5 CONCLUSIONS

Yield was affected by various external factors such as the timing of the cutting and the weather conditions. These factors played an important role in the heavy metal uptake in the first cutting. The fact that yield may affect metal uptake was predicted in the pot experiment, where plants grown at 15 $^0$C had lower yields and lower metal uptake also. However, there is evidence that metal uptake in the pot experiment also depended on the differences in temperature, which caused a different pattern of decomposition of the sludge-borne soil organic matter, which, in turn, affected metal uptake.

Organic matter was a key factor for metal availability, as predicted from the pot experiment. The fact that metal concentrations in the test crop did not change greatly after the first cutting may be due to the fact that organic matter did not decompose significantly until Week 48. Low molecular weight organics, increased over time, as they did in the CL soil of the pot experiment, both in the control and in the 50 t ha$^{-1}$ treatments. However, unlike the evidence from the pot experiment about the importance of DOC in heavy metal availability, DOC did not significantly influence the uptake of heavy metals by ryegrass in the field experiment.

Soil pH decreased with the increase in rate of sludge application, as predicted from the pot experiment. Over time, pH values showed significant fluctuations in the first 4-8 Weeks, as in case of the pot experiment, especially at the highest sludge treatment. This was also reflected in the DTPA extractions of the field experiment soils. In Week 16, both in Sonning and in Vardates, there was a significant drop in pH values, which was probably due to rapid nitrification at that time of year.

Extractions of heavy metals with DTPA gave an accurate prediction of the plant uptake. DTPA increased significantly with the increase in sewage sludge and did not change with time, apart from a peak in Week 16 at 50 t ha$^{-1}$, which was probably a result of pH drop in that sludge treatment at that time.

Transfer coefficient values gave the following order of availability and mobility of heavy metals:

$$Zn > Cd > Ni > Pb$$

Zinc and Cd were the most mobile elements in the study and Pb was the least. This was in accordance with findings of many researchers. The reason for Zn having greater TC values than Cd was probably due to the Cd concentration in sewage sludge being much lower than in the pot experiment. This increased the competition between the two metals, which was beneficial for Zn. This is probably the explanation for the fact that the TC values for Zn were almost the same as in the pot experiment, although it would be expected that TC would be greater in a pot rather than in a field experiment (McBride, 1995). Zinc had greater TC values under the conditions in Vardates than in Sonning, although the difference was marginal (0.27 and 0.25 respectively). This, however, gives some evidence that warmer weather conditions may enhance heavy metal mobility, as predicted in the pot experiment.

# CHAPTER SIX: LEACHING OF HEAVY METALS DOWN THE PROFILE OF A SEWAGE SLUDGE-TREATED SOIL

## 6.1 INTRODUCTION

Heavy metal contamination and its role in plant uptake in agricultural soils has received a lot of attention from researchers. However, because it is assumed that these metals are strongly sorbed on the solid phase of the soils, it is believed that downward movement is not a very important process. There is, however, some work which suggests that there is a risk of heavy metal movement down the soil profile even in sludge-treated soils. This is more likely to occur in cases of heavy loading of metals onto soils, and in cases where metals have been applied to soils with low sorptive capacities. This generally implies low contents of organic matter and clay and low pH values (Stevenson and Welch, 1979). Moreover, there is evidence that there may be a risk of movement of heavy metals in the soil profile when the concentration of DOC in soil increases, as a result of the production of root exudates or the decomposition of higher molecular weight humic substances of the soil organic matter (Moolenaar and Beltrami,1998). Likewise, a preliminary study in the course of this study indicated that in a heavily sludged site (sewage farm) the movement of all heavy metals was below 80 cm depth, and that this behaviour of metals was explained by the presence of mobile forms of organic matter.

Since a previous experiment (Chapter 4) exhibited the evidence that Dissolved Organic Carbon (DOC) may have a significant role in heavy metal mobility in soils treated with sewage sludge, it was decided that an experiment should be performed with the objective of investigating the role of DOC. The soil used in this experiment was the same as the LS soil used in the pot experiment (Chapter 4), in order to compare data and assess the mechanisms more easily. Moreover, in the pot experiment different temperature conditions had been used to assess their role in affecting the availability of heavy metals to plants. In this experiment, another climatic variable (rainfall rate) was also tested, because it may increase the solubility and mobility of heavy metals. Higher rainfall and irrigation rates may increase the risk of metal contamination and movement, compared with areas that receive low levels of precipitation.

The objectives of this experiment were to:

- study the leaching of the heavy metals Cd, Ni, Pb and Zn in a sandy soil, which had received 0, 10 and 50 t ha$^{-1}$ of sewage sludge,
- compare the leaching characteristics of heavy metals under 2 rainfall regimes, one typical of Eastern Greece and the other of South Eastern England, namely 450 and 900 mm per year,

- apply amounts of water for leaching the metals equivalent to 1, 2 and 3 years after the application of sewage sludge.

The hypotheses which were tested in this experiment were that:

- Higher rainfall rates in sewage sludge treated soils lead to greater leaching of heavy metals.
- Applications of higher amounts of sewage sludge will increase the depth down to which heavy metals will be transported.
- The leachability of heavy metals increases with time.
- The transport of heavy metals down the soil profile is facilitated by the presence of DOC, which is found in or formed by the degradation of the sewage sludge.

## 6.2 GREAT BILLING INVESTIGATION

### 6.2.1 Material and Methods of the Great Billing Investigation

In this project, a preliminary study was firstly conducted using heavily sludged soils from Great Billing, Northamptonshire. In this preliminary study, soil samples were obtained from Great Billing Sewage Farm, down to a depth of 80 cm in 20 cm intervals. Three sites were sampled: the first (S1) was a heavily sludged area, where sewage sludge had been deposited from an adjacent waste water treatment plant for up to a century. The second (S2)

**Table 6.1:** ***Some characteristic chemical and physical properties of soils in the Great Billing studies.***

| | Al | Fe | $Ca^{2+}$ | $K^{+}$ | $Na^{+}$ | $Mg^{2+}$ | CEC[c] |
|---|---|---|---|---|---|---|---|
| | Dithionite extracted % | | Water soluble (μg $g^{-1}$) | | | | $cmol_c\ kg^{-1}$ |
| S2[a] | 3.64 | 1.54 | 100.75 | 40.25 | 11.75 | 2.25 | 12.50 |
| S3[b] | 3.75 | 1.25 | 81.00 | 8.40 | 8.50 | 1.95 | 15.11 |
| | Sand | Silt | Clay | | pH | | EC[d] |
| | % | | | Category | $H_2O$ | $CaCl_2$ | dS $m^{-1}$ |
| S2 | 46.19 | 34.60 | 19.21 | SSL | 6.85 | 6.15 | 0.39 |
| S3 | 59.01 | 24.35 | 16.64 | SL | 6.72 | 6.03 | 0.24 |
| | *Aqua regia*-digested (μg $g^{-1}$) | | | | LOI[f] | $CaCO_3$ | Bd[e] |
| | Cd | Ni | Pb | Zn | % | % | g $cm^{-3}$ |
| S2 | 0.80 | 15.78 | 37.25 | 87.14 | 5.18 | 0.33 | 1.02 |
| S3 | 0.93 | 18.52 | 67.80 | 119.05 | 5.06 | - | 1.16 |

*a: Site 2 from the Great Billing preliminary investigation, b: Site 3 from the Great Billing preliminary investigation, c: Cation Exchange Capacity, d: Electrical Conductivity, e: Bulk Density, f: Loss-On-Ignition* was an unsludged site (the control site of the first), while the third site (S3) was a farm that had been applying sewage sludge as a fertiliser (at normal agricultural rates of 8 t ha$^{-1}$) for many decades. The samples were obtained with a steel auger and put in clean plastic bags. They were then taken to the laboratory, air dried, lightly ground in a pestle and mortar and passed through a 2 mm sieve. For the preliminary studies, the soil samples were analysed for the Total concentration of heavy metals with *Aqua regia*. Organic matter was determined by the Loss-On-Ignition method. The surface layers of the 3 sites were analysed for some characteristic chemical and physical parameters, including pH (1: 2.5 soil : water and 0.01 M $CaCl_2$), cation exchange capacity, electrical conductivity, soluble salts ($Na^+$, $Ca^{2+}$, $Mg^{2+}$ and $K^+$), $CaCO_3$, bulk density, particle size analysis and Al and Fe oxides (Table 6.1). All analyses described above were performed with the standard methods outlined in Chapter 3 (Materials and Methods).

### 6.2.2 Results of the Great Billing Investigation

The first site (S1) had a CEC value of 64.08 cmol$_c$ kg$^{-1}$, mainly attributed to its organic matter content, since the associated control (S2) had a CEC of only 12.50 cmol$_c$ kg$^{-1}$. The application of sewage sludge had caused the pH of S1 to decrease by more than 1 unit, from 6.85 to 5.45.

Results of heavy metal movement and organic matter are presented in Figures 6.1.1-6.1.5 and in Table A1 (Appendix III). For the movement of metals, it may be observed that in S1 the depth 20-40 cm had greater total concentrations of all metals. A concentration of 40.71 μg Cd g$^{-1}$ was found in the first layer, and this increased to 46.08 μg g$^{-1}$ in the second layer. Similarly, Pb increased from 667.97 to 717.66 μg g$^{-1}$ and Zn from 1311 to 1384 μg g$^{-1}$ in 0-20 and 20-40 cm depths, respectively. Nickel was an exception; it decreased from 376.03 to 335.68 μg g$^{-1}$ in the first two depths. Below this depth, all metals decreased to nearly half of the concentrations in which they occurred in the top layer. In S2, the heavy metals had either an even distribution down the soil profile to the measured depth of 80 cm, or a very slow decrease. It should be pointed that in the site S1, heavy loadings of sewage sludge were applied on the top of the soil and, thus, the mineral soil was ‘buried’ and that probably the top 50 cm of the site was the added sludge itself. It is, however, characteristic that metal concentrations in the 60-80 cm layer (mineral soil) in S1 were many times greater than those in the topsoil of the control site S2. The Cd concentration in the 60-80 cm layer was 23.28 μg g$^{-1}$, while that in the control site was 0.85 μg g$^{-1}$. Nickel had a concentration of 215.28 μg g$^{-1}$ in the bottom layer in S1, compared to 12.13 μg g$^{-1}$ in the S2. Lead had a concentration of 408.35 μg g$^{-1}$ in the 60-80 cm layer in S1 compared to 37.25 μg g$^{-1}$ in the

**Figure 6.1.1:** ***Cd distribution in the soil profile (μg $g^{-1}$).***

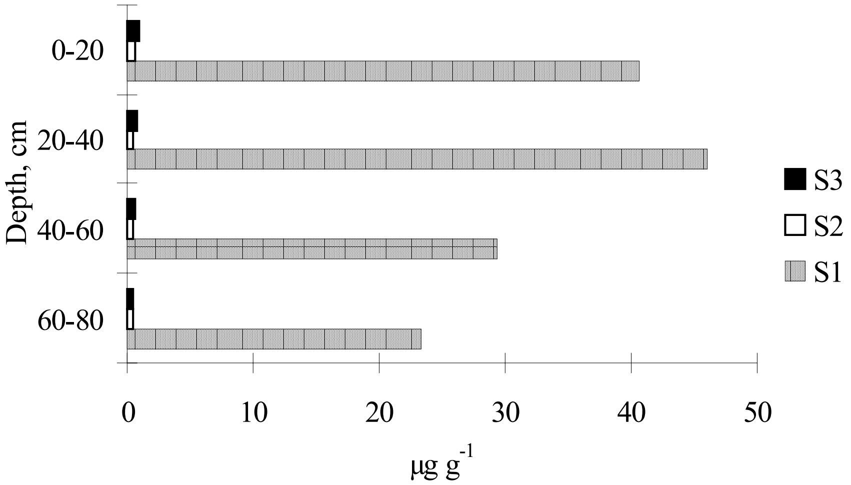


**Figure 6.1.2:** ***Ni distribution in the soil profile (μg $g^{-1}$).***

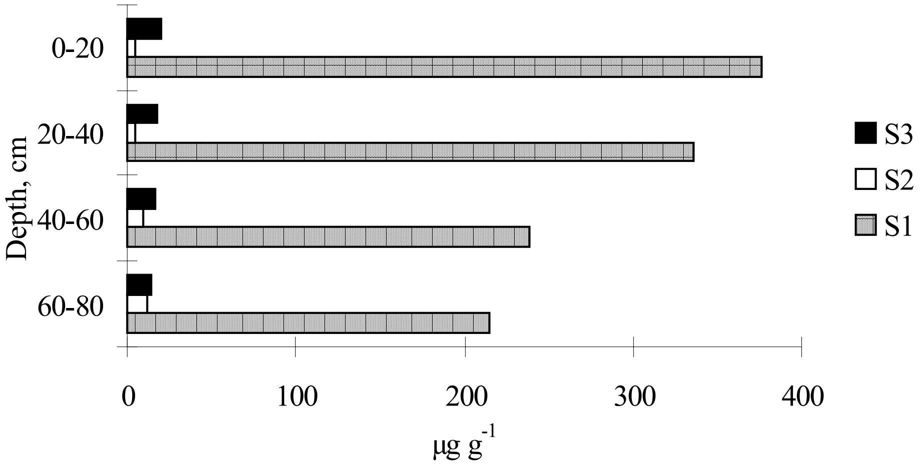


**Figure 6.1.3:** ***Pb distribution in the soil profile (μg g$^{-1}$).***

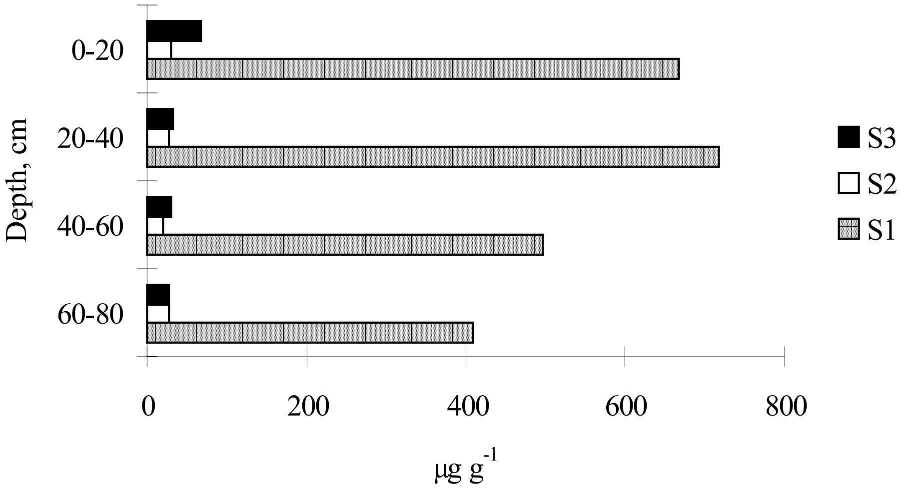


**Figure 6.1.4:** ***Zn distribution in the soil profile (μg g$^{-1}$).***

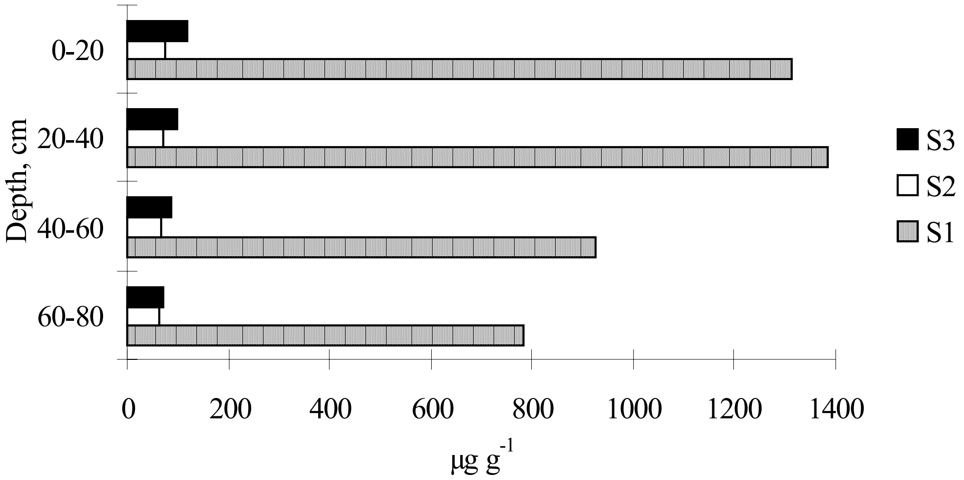
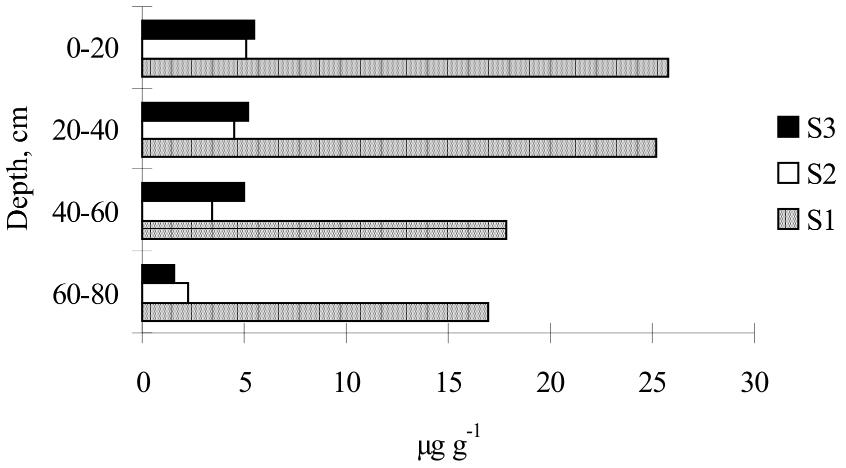


**Figure 6.1.5:** ***Loss-On-Ignition in the soil profile (%).***



S2, while Zn exhibited again an impressive increase from 87.14 μg $g^{-1}$ to 784.06 μg $g^{-1}$ between the bottom layer in S1 and the top layer in S2. This is an indication of a drastic movement of all heavy metals in the soil profile, which was caused by the heavy application of sewage sludge for a long time.

In S3, the concentrations of all heavy metals were also elevated compared with the control unsludged site, but the difference was not greater than 2 times from S2. The concentrations of metals decreased with depth and became very similar to those in S2, indicating that the movement of heavy metals in S3 was not greater than 60 cm depth.

Loss-On-Ignition was 25 % in the first two depths in S1 and it decreased to 17 % in the 60-80 cm depth. In the control site, S2, the distribution of organic matter was uniform (3 %) down to a depth of 60 cm. In the 60-80 cm depth it was 1.2 %.

## 6.3 MATERIALS AND METHODS

### 6.3.1 Experimental Design

From the preliminary study, evidence of heavy metal movement down the soil profile was exhibited and in the light of this indication a greenhouse-based study was designed. In this greenhouse-based leaching experiment, a sandy loam soil, the same as the one used in the previous pot experiment, was used. The soil was collected with an auger from the plough layer (0-20 cm), passed through a 9 mm plastic sieve and homogenised. After that it was packed loosely into PVC cylindrical tubes of 450 mm height and 150 mm diameter. The bulk density of the soil after it was packed was 1.3 g $cm^{-3}$, very similar to the value measured in the laboratory.

The bases of the PVC tubes were fitted with perforated perspex plates to allow free drainage and prevent anaerobic conditions developing. At the bottom of the soil columns 2 layers of a plastic net were fitted in order to avoid the movement of soil particles into the leachate. The PVC tubes were fitted with plastic funnels underneath, which helped the leachate to be collected in plastic bottles. The bottles were of 2 L capacity with narrow necks to avoid evaporation of the collected leachate. In the middle of the experiment it was noticed that algae were growing on the surfaces of the plastic bottles and funnels and so black plastic bags were placed around them to prevent further algal growth.

Sewage sludge (23 % DM) (the same sludge as that used in the field experiment) from a waste water treatment plant near Heathrow Airport was applied to the tops of the columns. The rates of sludge application were 0, 10 and 50 t ha$^{-1}$, the same as those used in the earlier pot experiment.

Two water application rates were applied to the soil columns, equivalent to 450 and 900 mm per year. The amount of water of 1 year was applied in 1 month. The water was applied manually every day, with a gardener’ s watering can, in order to ensure an even distribution of the applied water on the surface of the soil column. 265 mL of de-ionised water were applied daily on the 450 mm annual rainfall rate columns, while 530 mL of de-ionised water were applied daily for the 900 mm annual rainfall rate columns. The 12 treatments (2 water rates * 3 sludge rates * 2 replicates = 12) were replicated 3 times. In the first month water was applied to all 36 columns and in the next month, 12 of them were excluded and only 24 were watered, while in the third month another 12 soil columns were excluded and only the remaining 12 were watered. In this way one third of the columns were applied with 1 year’ s rainfall, another third with 2 years’ rainfall and the remaining third with 3 years’ rainfall.

**Table 6.2:** ***The volume of water eluted from the soil columns (mL).***

| 450 mm Annual Equivalent Rainfall | | | |
|---|---|---|---|
| | Year 1 | Year 2 | Year 3 |
| Control | 6672 | 8729 | 9184 |
| 10 t ha$^{-1}$ | 7360 | 8733 | 9369 |
| 50 t ha$^{-1}$ | 7760 | 8539 | 9194 |

| 900 mm Annual Equivalent Rainfall | | | |
|---|---|---|---|
| Control | 15632 | 15842 | 13877 |
| 10 t ha$^{-1}$ | 15988 | 15601 | 13959 |
| 50 t ha$^{-1}$ | 15528 | 16380 | 13501 |

The leachates were collected when bottles beneath the columns were nearly full. Their weight was recorded and it was assumed that their weight (in g), after the bottle' s weight was subtracted, was equal to their volume (in mL). A small aliquot of 20 mL from the leachate of each column was kept every week of the 3 months' duration of this experiment. All the aliquots were kept in cold room at 4 $^0$C, while the rest of the leachate was discarded. The leachate subsamples were measured for their concentrations of heavy metals and their DOC concentration. Although they were collected once in a week throughout the 3-month period, only the results from weeks 2, 6, 7, 8, 10 and 12 are presented. The results from the ICP-OES were in μg L$^{-1}$ and those from the TOC Analyser were in μg mL$^{-1}$. These values were multiplied by the volume of leachate eluted (Table 6.2).At the end of the experiment the columns were left for one month to dry and then the plastic tubes were carefully cut longitudinally with a mechanical saw. After the columns of soil were removed from the PVC tubes, they were cut into 2 cm slices in the top 0-10 cm. Below this, they were cut into 5 cm slices. The soil 'slices' were then left to air dry and were subsequently ground, sieved through a 2 mm sieve and stored until the analysis.

**Table 6.3:** ***Some characteristic chemical and physical properties of soils and sewage sludge.***

| | Al | Fe | $Ca^{2+}$ | $K^+$ | $Na^+$ | $Mg^{2+}$ | CEC [b] |
|---|---|---|---|---|---|---|---|
| | Dithionite extracted % | | Water soluble, μg g$^{-1}$ | | | | cmol$_c$ kg$^{-1}$ |
| LS Soil [a] | 0.09 | 0.93 | 46.80 | 7.65 | 16.80 | 2.65 | 8.47 |
| Sludge | - | - | - | - | - | - | - |
| | Sand | Silt | Clay | | pH | | EC [c] |
| | % | | | Category | $H_2O$ | $CaCl_2$ | dS m$^{-1}$ |
| LS Soil | 78.37 | 14.64 | 6.99 | LS | 7.21 | 6.68 | 0.17 |
| Sludge | - | - | - | - | 6.46 | 6.35 | - |
| | *Aqua regia*-digested, μg g$^{-1}$ | | | | LOI [e] | $CaCO_3$ | Bd [d] |
| | Cd | Ni | Pb | Zn | % | % | g cm$^{-3}$ |
| LS Soil | 0.34 | 13.93 | 17.82 | 34.69 | 2.37 | 1.10 | 1.21 |
| Sludge | 2.41 | 38.08 | 317.21 | 645.50 | 41.83 | - | 0.85 |

*a: LS Soil, used in the leaching experiment, b: Cation Exchange Capacity, c: Electrical Conductivity, d: Bulk Density, e: Loss-On-Ignition*

### 6.3.2 Analyses

A soil subsample was analysed for some characteristic chemical and physical parameters, including pH (1: 2.5 soil : water and 0.01 M $CaCl_2$), Loss-On-Ignition, cation exchange capacity, electrical conductivity, soluble salts ($Na^+$, $Ca^{2+}$, $Mg^{2+}$ and $K^+$), $CaCO_3$, bulk density, particle size analysis and Al and Fe oxides (Table 6.3). The movement of heavy metals in the soil columns was assessed by the measurement of the total concentration of these metals in the soil columns in the soil slices down to a depth of 15 cm after the termination of the leaching by *Aqua regia* digestion.

As for the liquid eluted underneath the soil columns, samples were filtered through a slow filter paper (Whatman No 42) and analysed by ICP-OES. Lead was not detectable, while Cd was near to its detection limit. Although pre-concentration techniques could have been used to determine measurable concentrations, it was not considered necessary. Aliquots were also measured for their DOC concentration on a Shimadzu TOC Analyser. All analyses described above were performed with the standard methods outlined in Chapter 3 (Materials and Methods).

## 6.4 RESULTS

### 6.4.1 The Soil Columns

The results of the measurements in the soil columns are presented in Table A3.1 and Table A3.2 (Appendix III). The total concentrations of Cd, Ni, Pb and Zn were measured in the soil columns down to a depth of 15 cm. It should be noted that the sewage sludge was not incorporated in the soil. Thus, concentrations of metals greater than the controls are entirely due to movement of these metals in the soil profile even in the first depth, 0-2 cm. The results for each metal are presented in different subdivisions.

#### 6.4.1.1 Cadmium

Cadmium moved significantly down to the soil profile under both rates of water application to a depth of 10 cm (Figures 6.2.1-6.2.2). In the columns where the load of Cd was greater (50 t $ha^{-1}$ treatments), Cd moved to a greater depth compared with the 10 t $ha^{-1}$ treatment. Thus, sludge application had a significant effect with an LSD value of 0.01 (Table 6.4). While the 10 t $ha^{-1}$ treatment showed significant movement down to 4 cm, the 50 t $ha^{-1}$ was found to have moved significantly further down to a depth of 8 cm. Combining all 3 Years and both water application rates, in the first depth (0-2 cm), 0.32 $\mu g\ g^{-1}$ were present in the control, 0.42 $\mu g\ g^{-1}$ in the 10 t $ha^{-1}$ and 0.50 $\mu g\ g^{-1}$ in the 50 t $ha^{-1}$ treatments. The columns with the 50 t $ha^{-1}$ application had significantly greater concentrations of Cd to a depth of 8 cm, where 50 t $ha^{-1}$ had 0.36 $\mu g\ g^{-1}$ and the control had 0.29 $\mu g\ g^{-1}$. In the next depth (8-10 cm) the differences were not significantly different, that was 0.31 $\mu g\ g^{-1}$ for the

**Figure 6.2.1:** ***Concentration of Cd in the soil profile in a 3-year period under 450 mm per year rainfall rate (x-axis: µg g$^{-1}$).***

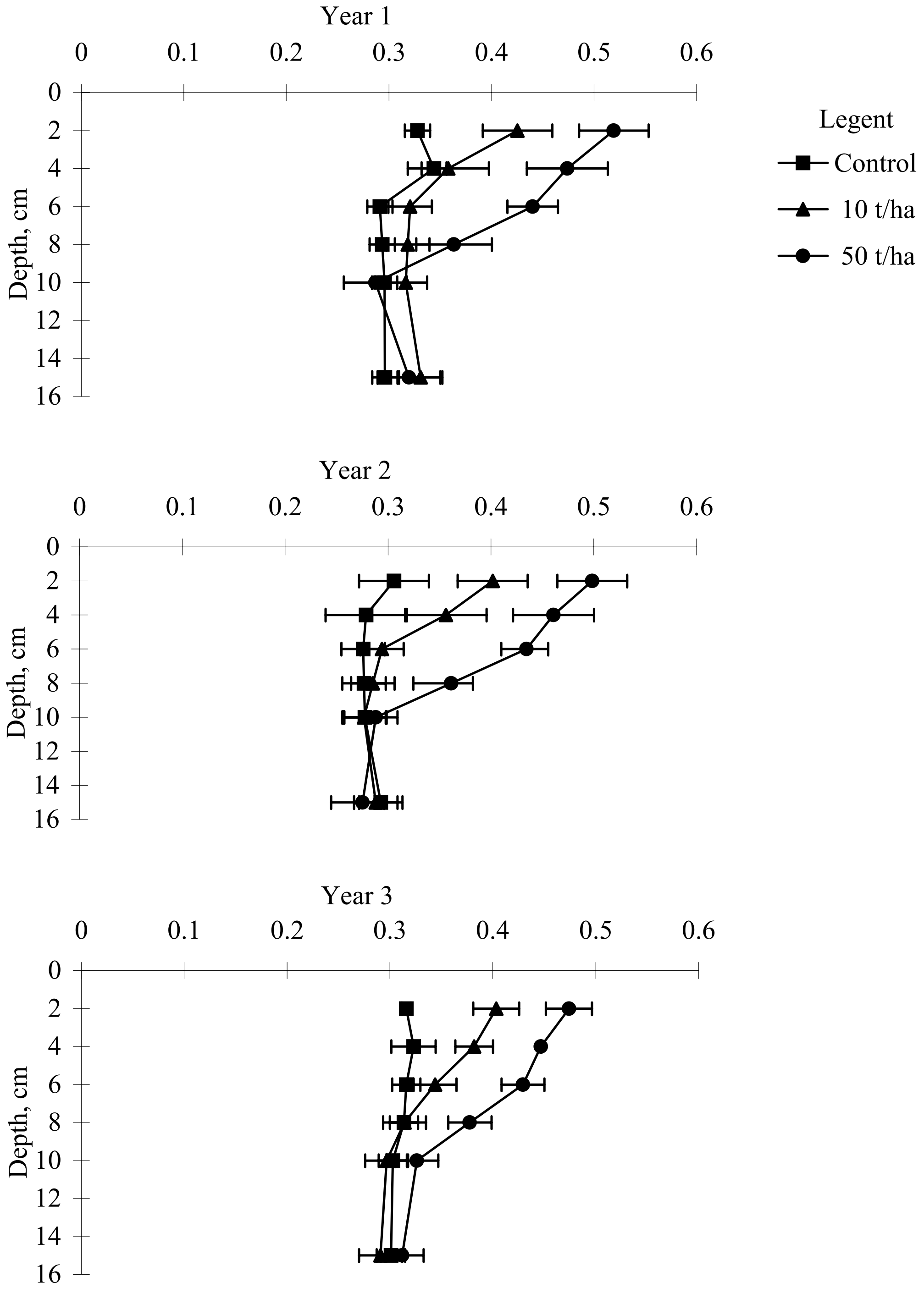


**Figure 6.2.2:** ***Concentration of Cd in the soil profile in a 3-year period under 900 mm per year rainfall rate (x-axis: µg g$^{-1}$).***

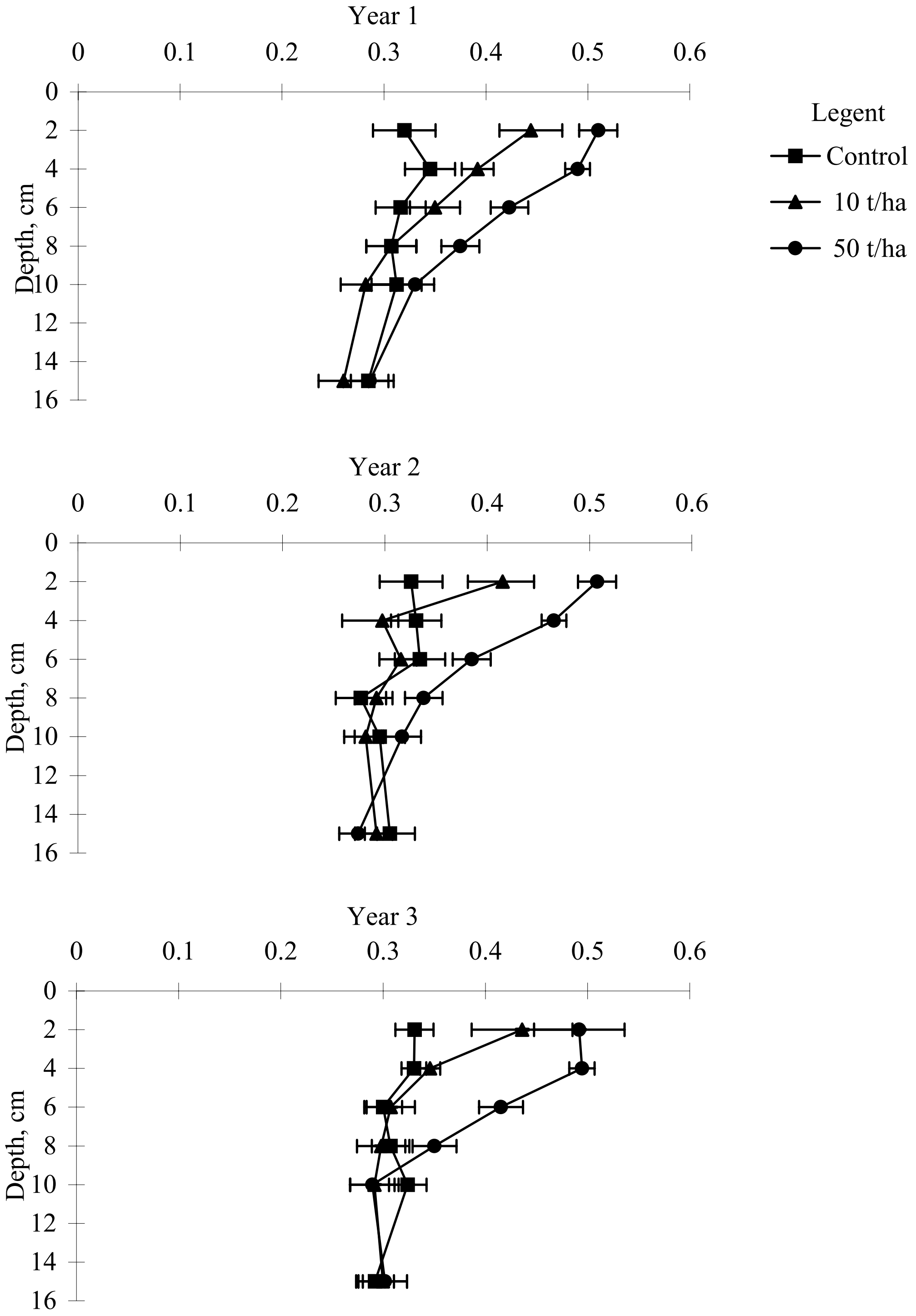


**Table 6.4:** _**LSD test for the concentration of Cd in the profile of the soil columns.**_

_Water 0.01_
_Sludge 0.01*_
_Water x Sludge 0.02*_
_Time 0.01_
_Water x Time 0.02*_
_Sludge x Time 0.02***_
_Water x Sludge x Time 0.04_
_Depth 0.02***_
_Water x Depth 0.03_
_Sludge x Depth 0.04**_
_Water x Sludge x Depth 0.08***_
_Time x Depth 0.06*_
_Water x Time x Depth 0.08_
_Sludge x Time x Depth 0.11***_
_Water x Sludge x Time x Depth 0.49*_

50 t ha$^{-1}$ treatment and 0.29 µg g$^{-1}$ for the control, with an LSD of 0.04. In the course of 3 years' equivalent rainfall application, there were no significant changes in the rate of movement down the profile. This was evident with both the rainfall application rates. For example at 900 mm, having an LSD value of 0.04, at 50 t ha$^{-1}$ for the first depth, there were 0.51 µg Cd g$^{-1}$ in Year 1 (the first month in which 1 year' s equivalent rainfall was applied), 0.51 µg Cd g$^{-1}$ in Year 2 and 0.49 µg Cd g$^{-1}$ in the Year 3. At 10 t ha$^{-1}$ the values were of similar order, that is 0.44, 0.42 and 0.44 µg Cd g$^{-1}$ from Year 1 to Year 3.

The different loadings of leaching water (rainfall rates) proved not to have very significant effect on the leaching of Cd down the profile. At both 450 mm and 900 mm the movement of Cd was down to the same depth (10 cm). At both these rainfall rates there was no significant movement of Cd from Year 1 to Year 3, that is the movement of Cd did not increase significantly with time between the two different water rates.

### 6.4.1.2 Nickel

Nickel moved significantly down the soil profile under both water application rates to a depth of 10 cm (Figures 6.2.3-6.2.4). Sludge application had a significant effect with an LSD value of 1.20 (Table 6.5). Combining all 3 Years and both water application rates, in the first depth (0-2 cm) the control contained 15.19 µg Ni g$^{-1}$, the 10 t ha$^{-1}$ treatment had 24.90 µg Ni g$^{-1}$ and the 50 t ha$^{-1}$ treatment contained 35.14 µg Ni g$^{-1}$. The columns with the 50 t ha$^{-1}$ application had significantly greater concentrations of Ni down to a depth of 15 cm. In the depth of 6-8 cm the 50 t ha$^{-1}$ treatment had 22.25 µg Ni g$^{-1}$ and the control contained 15.06 µg g$^{-1}$, while in the next depth (8-10 cm), the differences were only slightly significantly different, with 18.04 µg Ni g$^{-1}$ in the 50 t ha$^{-1}$ treatment and 14.38 µg Ni g$^{-1}$ in control (LSD of 3.65). In the next depth (10-15 cm) differences were not significant (12.92 and 14.81 µg Ni g$^{-1}$ for the control and the 50 t ha$^{-1}$ treatment, respectively). In the 10 t ha$^{-1}$ treatment, Ni moved to 4 cm, as in the case of Cd.

In the course of 3 years' equivalent rainfall application, there was a significant increase in the rate of Ni leached down the soil profile to a depth of 10 cm. In the 450 mm rainfall application rate, this was especially observed below 6 cm depth. At 6-8 cm depth, at

**Figure 6.2.3:** ***Concentration of Ni in the soil profile in a 3-year period under 450 mm per year rainfall rate (x-axis: µg g$^{-1}$).***

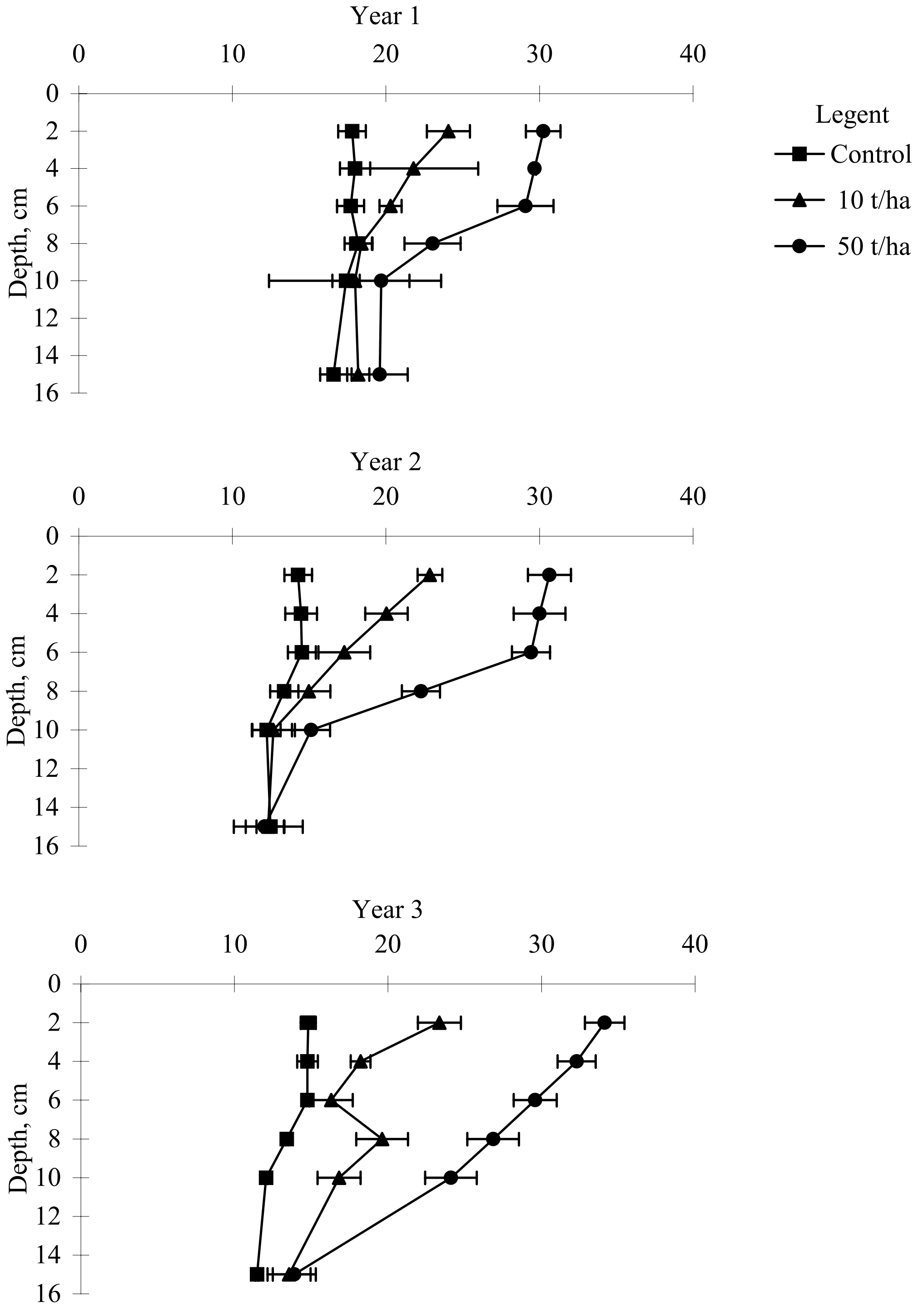
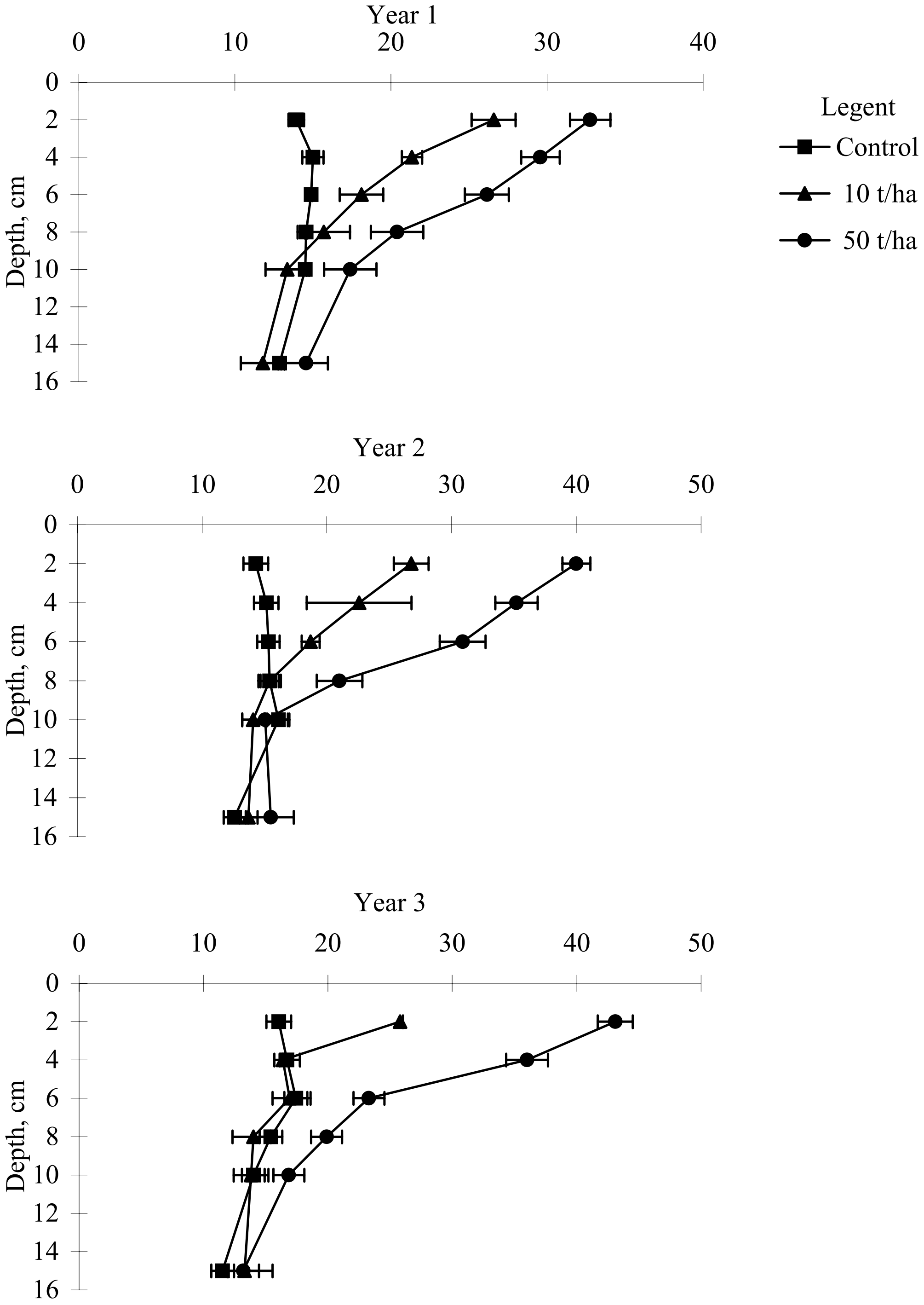


**Figure 6.2.4:** ***Concentration of Ni in the soil profile in a 3-year period under 900 mm per year rainfall rate (x-axis: μg g$^{-1}$).***


Year 1

Year 2

Year 3

**Table 6.5:** ***LSD test for the concentration of Ni in the profile of the soil columns.***

*Water 0.90* *Sludge x Time 2.45**** *Water x Sludge x Depth 6.32****
*Sludge 1.20*** *Water x Sludge x Time 3.95* *Time x Depth 3.65***
*Water x Sludge 1.81** *Depth 1.65**** *Water x Time x Depth 6.32*
*Time 1.20* *Water x Depth 2.76* *Sludge x Time x Depth 10.81****
*Water x Time 1.81* *Sludge x Depth 3.65****
*Water x Sludge x Time x Depth 45.62***

50 t ha$^{-1}$ 23.04 and 26.85 µg Ni g$^{-1}$ were found between Years 1 and 3, respectively. At 8-10 cm depth concentrations of 19.70 and 24.10 µg Ni g$^{-1}$ were found between Years 1 and 3. In the 900 mm water rate, the differences between years were important in the first 3 soil slices (from 0 to 6 cm) at 50 t ha$^{-1}$. However, in the 4-6 cm depth the significant increase was only between Year 1 and Year 2, while in Year 3 there was a slight decrease compared with Year 2. At 0-2 cm depth, concentrations of 32.76 and 43.10 µg Ni g$^{-1}$ were found between Year 1 and 3, while at 2-4 cm depth, concentrations of 29.57 and 36.00 µg Ni g$^{-1}$ were found between Year 1 and 3. At 4-6 cm depth, the difference between Year 1 and 2 was from 26.15 to 30.90 µg g$^{-1}$, which was very significant. All the above differences had an LSD value of 3.65.

The different applications of leaching water was proved not to be very significant in the leaching of Ni down the profile. At both 450 mm and 900 mm the movement of Ni was down to the same depth (10 cm). At both these water rates there was no significant difference in the movement of Ni from Year 1 to Year 3. However, there was an exception in this effect of water rate: that was in the concentration of Ni in Year 2 and Year 3 at the 50 t ha$^{-1}$ treatment in the first 2 soil depths. In Year 2, the 900 mm water rate gave 40.00 and 35.20 µg Ni g$^{-1}$, respectively for the 2 depths, while 450 mm gave 30.64 and 30.00 µg Ni g$^{-1}$, respectively. In Year 3, the 900 mm gave 43.10 and 36.00 µg g$^{-1}$ in the first two depths, while 450 mm gave 34.12 and 32.29 µg g$^{-1}$ in the first two depths, respectively. These differences were significant with an LSD value of 3.95. These differences suggest that the greater rainfall rate facilitated the movement of Ni in the soil profile, although it was only over 4 cm.

### 6.4.1.3 Lead

Lead is the least mobile element among those included in this study and it is reported in the literature that its movement in the soil profile is very limited (Sawhney *et al.*, 1994). Lead was only found in significantly greater concentrations compared with the control in the first depth, 0-2 cm (Figures 6.2.5-6.2.6). Combining all Year and water treatments, there was an increase from 17.66 to 25.21 µg Pb g$^{-1}$ between the control and the 50 t ha$^{-1}$ treatment

**Figure 6.2.5:** ***Concentration of Pb in the soil profile) in a 3-year period under 450 mm per year rainfall rate (x-axis: µg g$^{-1}$).***

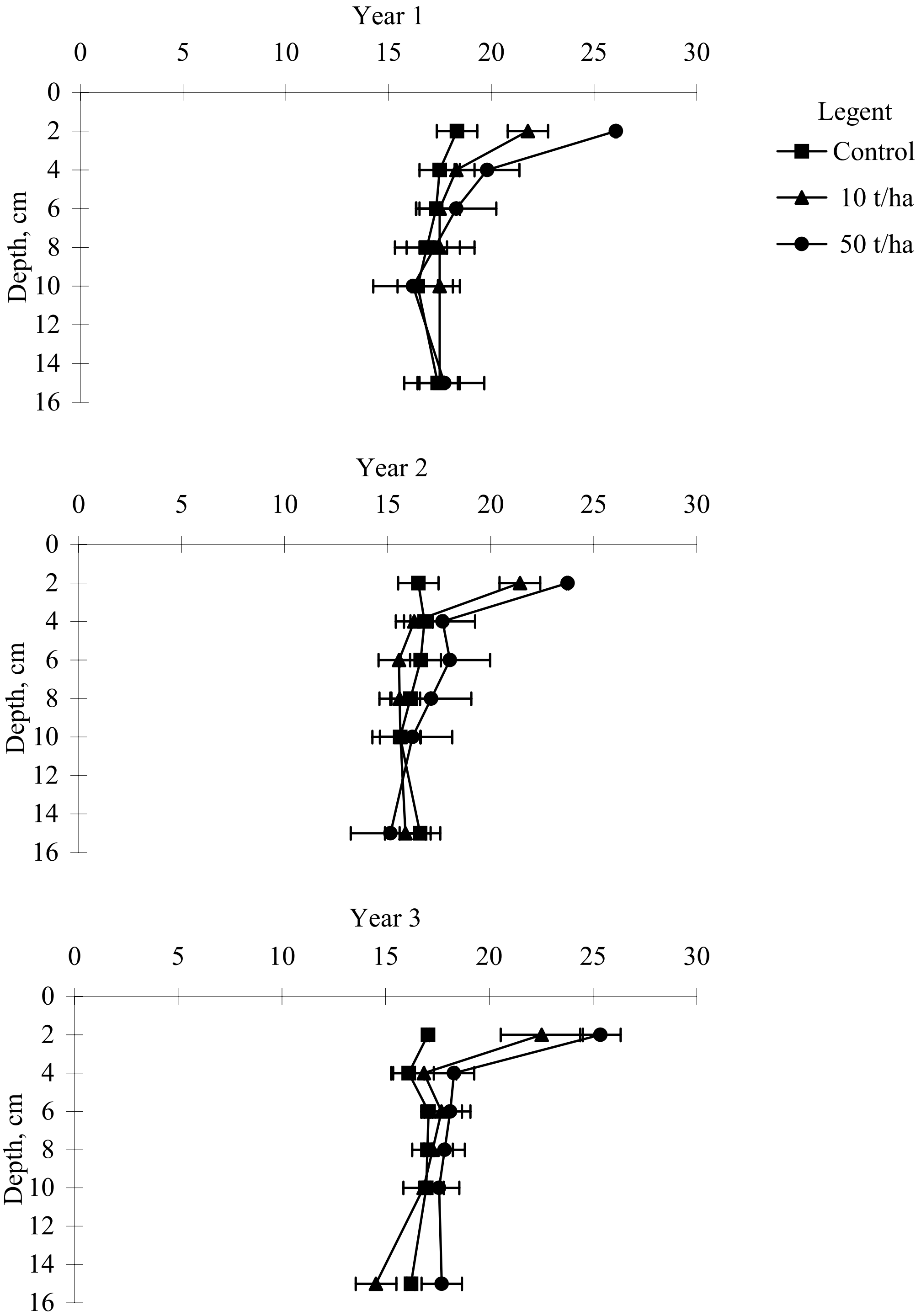


**Figure 6.2.6:** ***Concentration of Pb in the soil profile in a 3-year period under 900 mm per year rainfall rate (x-axis: µg g$^{-1}$).***

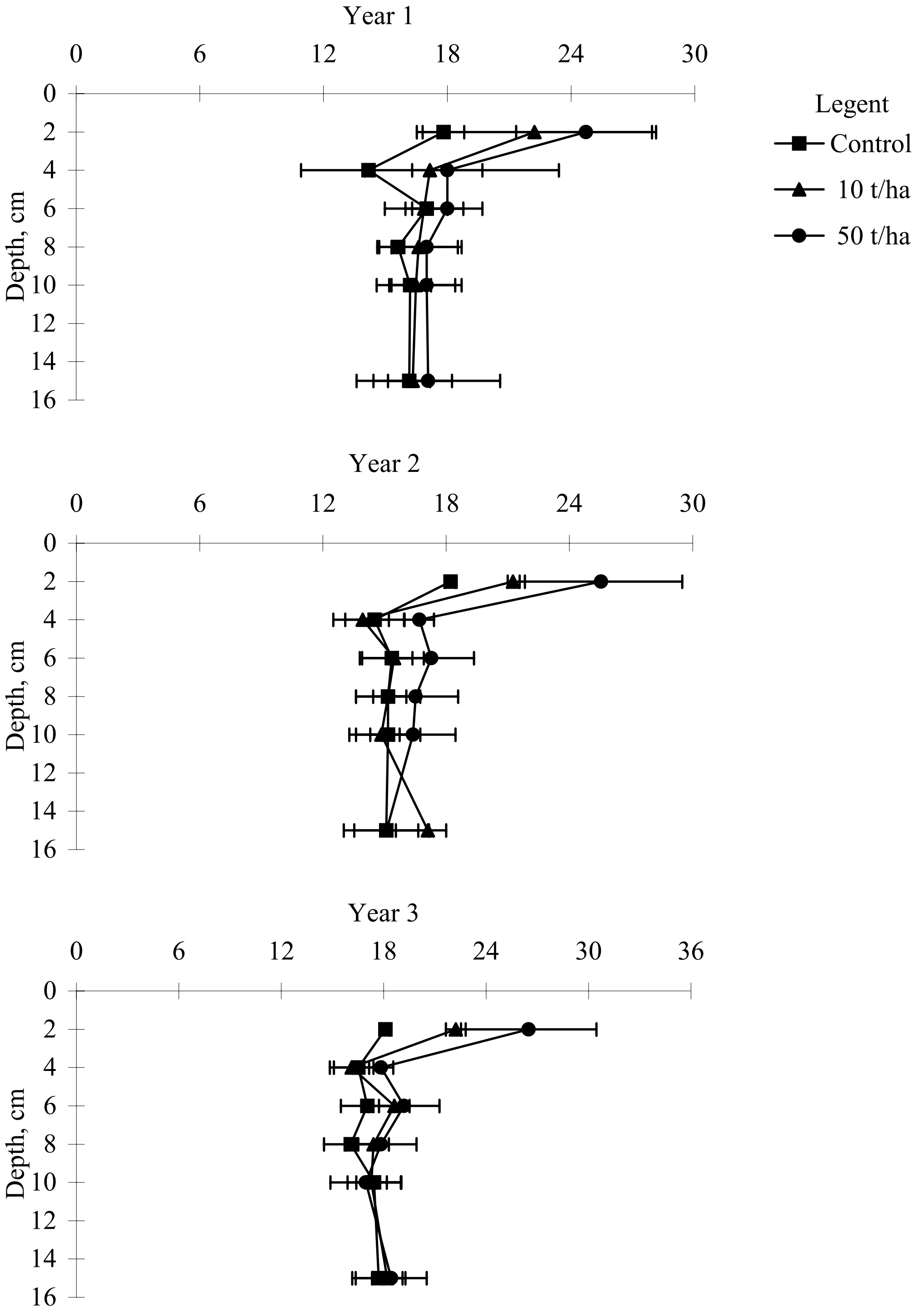


**Figure 6.2.7:** ***Concentration of Zn in the soil profile in a 3-year period under 450 mm per year rainfall rate (x-axis: µg g$^{-1}$).***

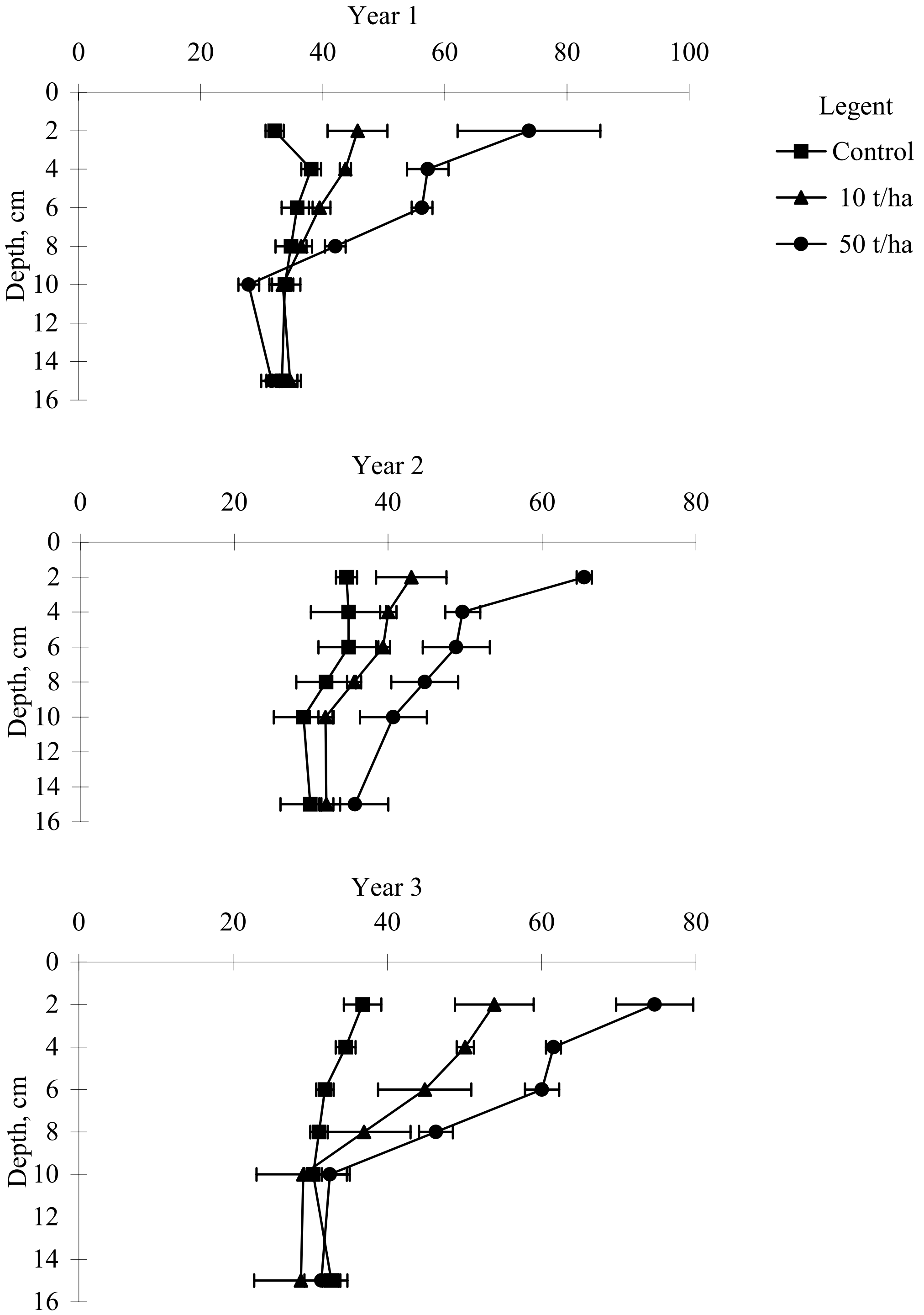


**Figure 6.2.8:** ***Concentration of Zn in the soil profile in a 3-year period under 900 mm per year rainfall rate (x-axis: µg $g^{-1}$).***

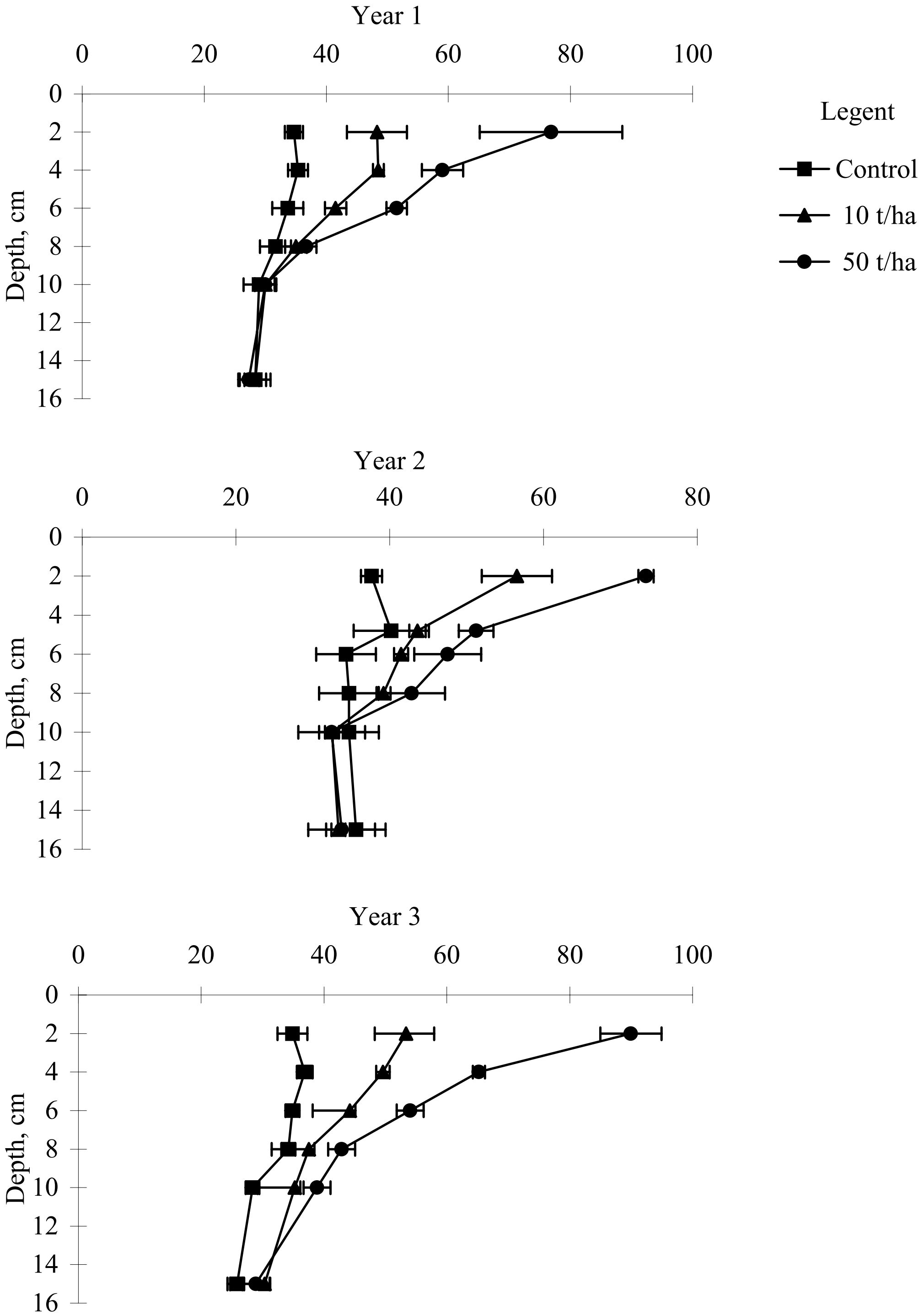


**Table 6.6:** ***LSD test for the concentration of Pb in the profile of the soil columns.***

| | |
|---|---|
| Water | 1.62 |
| Sludge | 1.98 |
| Water x Sludge | 2.97 |
| Time | 1.98 |
| Water x Time | 2.97 |
| Sludge x Time | 3.78 |
| Water x Sludge x Time | 2.61 |
| Depth | 4.05*** |
| Water x Depth | 6.05 |
| Sludge x Depth | 5.49 |
| Water x Sludge x Depth | 9.81 |
| Time x Depth | 5.49 |
| Water x Time x Depth | 9.81 |
| Sludge x Time x Depth | 16.65 |
| Water x Sludge x Time x Depth | 69.66 |

(significant with an LSD value of 1.98 (Table 6.6)). After that depth the treatments were without any statistical differences. Although no other factor had an important effect on the mobility of Pb, the time factor played a significant role in facilitating the movement of Pb in the first depth. In the 900 mm treatment, with 50 t ha$^{-1}$, Pb increased from 24.73 to 26.49 µg g$^{-1}$ between Year 1 and Year 3 in the 0-2 cm depth, which was slightly significant for an LSD value of 1.98, but this was not observed in the 450 mm treatment. The higher water rate leached more Pb levels at the higher sludge application rate in the course of 3 'years'. This suggests that there may be a potential risk of Pb movement when soil conditions are favourable. In this experiment, packed columns were used, but in the field, macropores will have developed which could lead to more pronounced downward transport of heavy metals.

#### 6.4.1.4 Zinc

Zinc moved significantly down the soil profile under both water application rates up to a depth of 10 cm (Figures 6.2.7-6.2.8). In the 10 t ha$^{-1}$ treatment, Zn significantly moved down to 6 cm which was the deepest movement compared with Cd and Ni. The columns with the 50 t ha$^{-1}$ application had significantly greater concentrations of Zn to a depth of 8 cm, where 50 t ha$^{-1}$ had 42.59 µg Zn g$^{-1}$ and control 33.07 µg Zn g$^{-1}$. In the next depth (8-10 cm) the differences were less significant, with 33.74 µg Zn g$^{-1}$ in the 50 t ha$^{-1}$ treatment and 30.87 µg Zn g$^{-1}$ in the control, (LSD of 5.52) (Table 6.7). In the course of 3 years' equivalent rainfall application, at 900 mm, there was a significant increase in the rates of Zn being leached down the soil profile, while there was no time effect in the 450 mm water rate. At 900 mm, at 50 t ha$^{-1}$ the Zn concentration was 76.80 µg g$^{-1}$ for the first depth in Year 1, 73.96

**Table 6.7:** ***LSD test for the concentration of Zn in the profile of the soil columns.***

*Water 4.45* *Sludge x Time 10.57* *Water x Sludge x Depth 27.41*
*Sludge 5.52* *Water x Sludge x Time 17.23* *Time x Depth 15.02*
*Water x Sludge 8.21* *Depth 7.36*** *Water x Time x Depth 27.45*
*Time 5.52* *Water x Depth 11.28* *Sludge x Time x Depth 45.62*
*Water x Time 8.21* *Sludge x Depth 15.02*
*Water x Sludge x Time x Depth 190.85**

$\mu$g Zn g$^{-1}$ in Year 2 (not significantly different from Year 1) and 89.32 $\mu$g Zn g$^{-1}$ in the Year 3 (LSD value of 8.21). At 10 t ha$^{-1}$, the values were of similar order (48.30, 56.53 and 53.36 $\mu$g Zn g$^{-1}$ for Years 1, 2 and 3, respectively). In the subsequent depths the differences remained significant down to 4 cm, where at 50 t ha$^{-1}$, the Zn concentrations between Years 1 and 3 were 56.04 and 65.21 $\mu$g g$^{-1}$. Below this soil depth the differences between years were not important.

The different applications of leaching water proved to be a significant factor in the leaching of Zn in the profile only down to the second depth (0-2 cm) in the 50 t ha$^{-1}$ treatment. However, at both 450 mm and 900 mm the movement of Zn was down to the same depth (10 cm). The increase in the water rate by a factor of 2 did not seem to solubilise and cause the movement of Zn to a greater depth than the lower water rate. This behaviour was apparent in case of Cd as well.

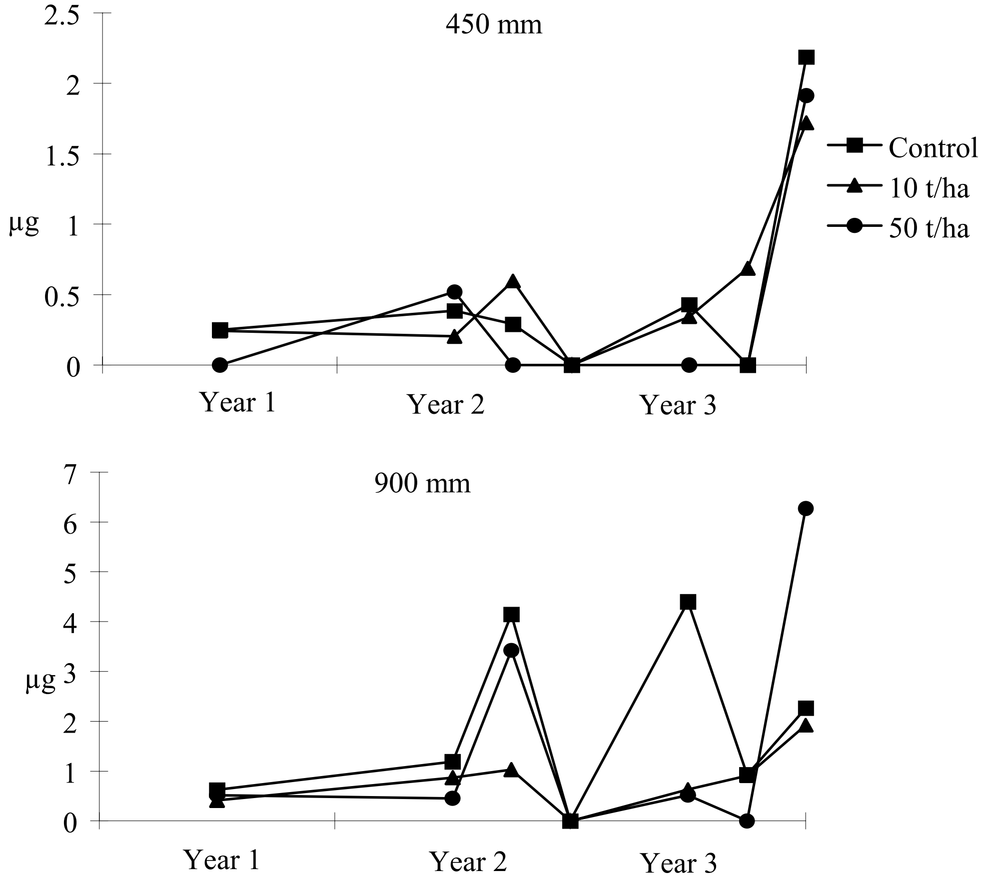
### 6.4.2 Leachates

The concentrations of Cd, Ni and Zn and DOC in the leachates collected underneath the soil columns, were multiplied by the volume of water eluted from the soil columns (Table 6.2). The numbers obtained by this multiplication were the absolute amounts of these metals in the leachates, expressed in $\mu$g (in mg for DOC). The results are presented in two modes: individually (as they were measured after the weekly sampling) and cumulatively (where, for each water and sludge treatment, the value of the previous week was added to the value of the next week). The results from the analyses of the leachates for heavy metals and DOC are presented in Table A2.1 for Cd, in Table A2.2 for Ni, in Table A2.3 for Zn and in Table A2.4 for DOC, in the Appendix III. The fact that heavy metals were detected in the leachates, while the measured movement in the soil columns was only as low as 15 cm depth, indicates that there was preferential flow, which caused the metals to move even beyond the layer of accumulation.

### 6.4.2.1 Cadmium

As mentioned above, Cd was close to its detection limit on the ICP-OES which is 0.1 μg $L^{-1}$. The results are in μg (Figures 6.3.1-6.3.2). The LSD test for Cd in the leachate is presented in Table 6.8. The characteristic of the Cd concentration in the leachate was that it did not increase with the higher sludge application, but it rather decreased. There was a small increase from the control to 10 t $ha^{-1}$ treatment, but a decrease from 10 to 50 t $ha^{-1}$ treatment in the 450 mm rate. It can be seen that in many sampling times, the concentration of Cd in the leachate was below the ICP-OES detection limit. This may mean that the adsorption of Cd increased in the columns at 50 t $ha^{-1}$. In the higher sludge rate, as water was flowing through

**Figure 6.3.1:** ***Cd in the leachate of the soil columns (μg).***



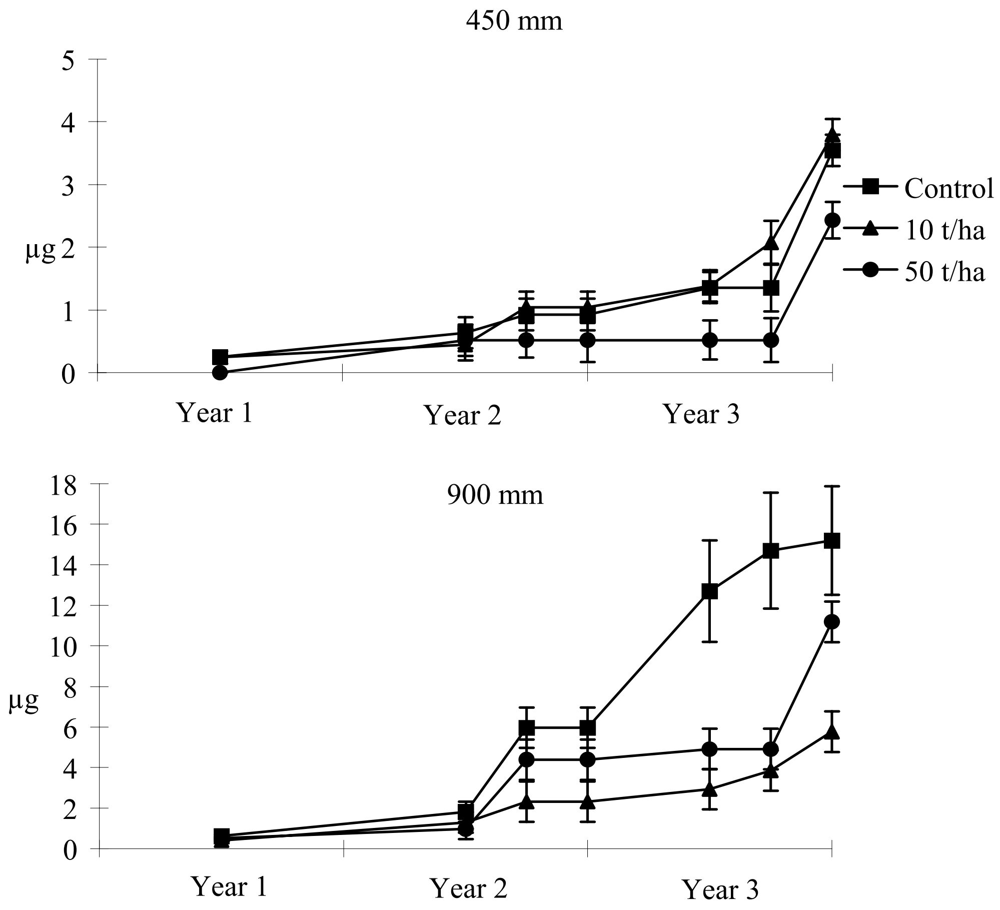
the soil, the Cd was rather more sorbed onto the solid surfaces of the soil due to the fact that the soil had a greater adsorption capacity due to the addition of sludge-borne organic matter.

### 6.4.2.2 Nickel

Nickel increased in the leachates with the increase in sludge application, especially in the 450 mm rate (Figures 6.3.3-6.3.4). This increase was significant for an LSD of 0.86 (Table 6.9). It is characteristic that the 900 mm data were lower in Ni concentration than 450 mm in the first half of the experiment, despite the fact that the leachate volume at 900 mm was nearly double than that at 450 mm. This indicates the fact that from columns of the same sludge treatment the same amount of Ni was leached, and that this amount was diluted twice in the 900 mm water rate.

The concentration of Ni in the leachate of the 450 mm rainfall was less at the end of the experiment than at the beginning. This difference was significant with an LSD of 4.89 and this suggests that the Ni was washed down the profile more rapidly in the early stages of the experiment; once the first more soluble forms of Ni were leached the process was slower. In

**Figure 6.3.2:** ***Cd in the leachate of the soil columns (cumulative values) (μg).***


450 mm

900 mm

**Table 6.8:** ***LSD test for Cd in the leachate of the soil columns.***

*Water 0.33* *Water x Sludge 1.58* *Water x Time 4.15*

*Sludge 0.57* *Time 1.91* *Sludge x Time 7.84*

*Water x Sludge x Time 35.95*

**Figure 6.3.3:** ***Ni in the leachate of the soil columns (μg).***

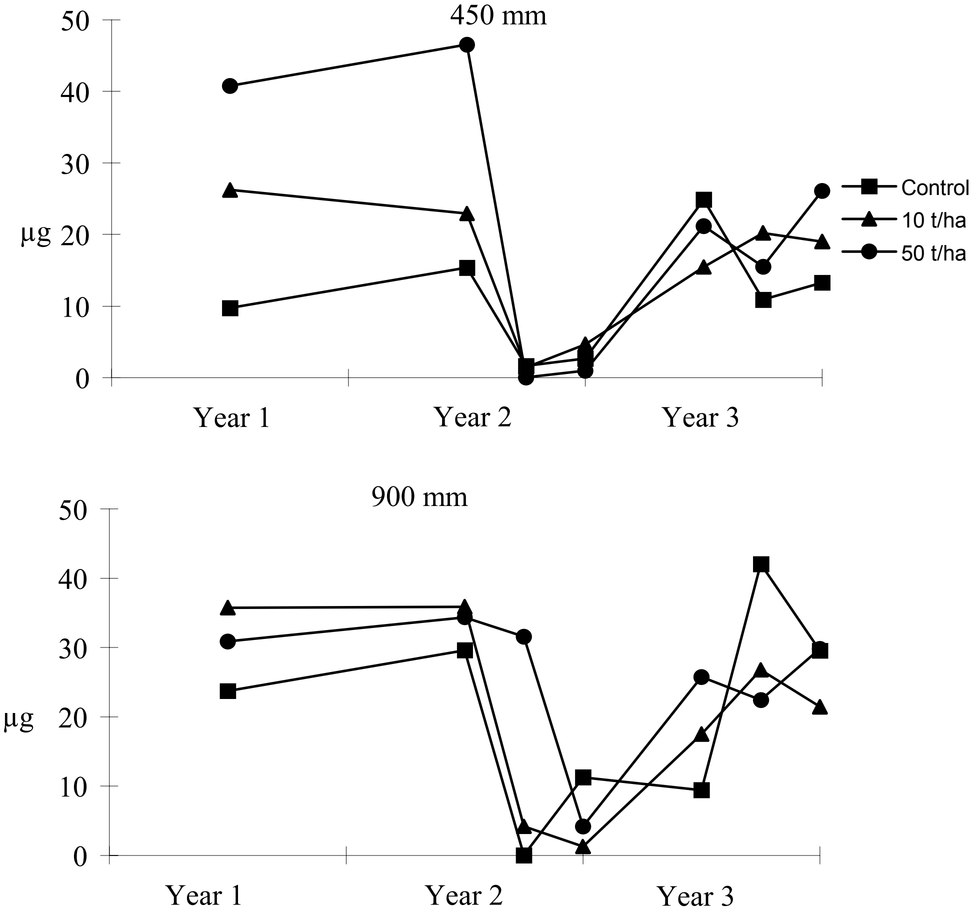


**Figure 6.3.4:** ***Ni in the leachate of the soil columns (cumulative values) (µg).***

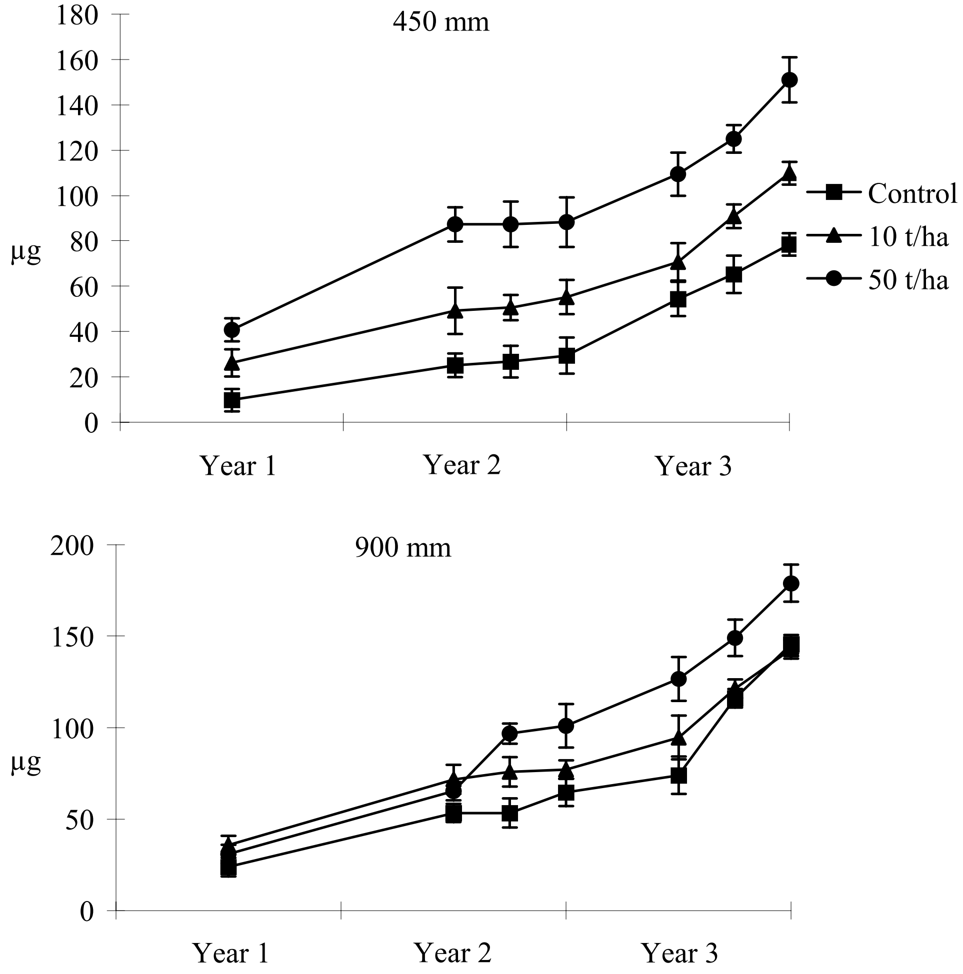


**Table 6.9:** ***LSD test for Ni in the leachate of the soil columns.***

*Water 0.74* *Water x Sludge 1.43* *Water x Time 2.84*

*Sludge 0.86** *Time 1.60* *Sludge x Time 4.89**

*Water x Sludge x Time 20.43*

the 900 mm water rate, however, this is not as obvious and the rates of Ni leaching were not different at the beginning and end of this study. This was probably so, because of the greater volume of water that helped to leach Ni down to a greater extent. The greater quantity of water washing the soil column made Ni always as mobile as in the beginning over the period of 3 years equivalent rainfall. It should be noted that during weeks 7 and 8 there was a steep decrease in Ni concentration, which was probably caused by the microbial growth which may have sequestered some of the soluble Ni. After this period things seem to have followed the earlier pattern.

In the accumulative data, the amount of Ni leached down was greater at 900 mm than that at 450 mm, but this was not proportional to the increase in the volume of water. The concentration of Ni at 50 t ha$^{-1}$ in the 450 mm rate was 151 ng, while at 900 mm it was 178 ng. In the accumulative data it was more obvious that the Ni found in the leachates was significantly affected by the rate of sewage sludge application, with the columns with higher application of sludge having greater concentrations of Ni in the leachates.

### 6.4.2.3 Zinc

The Zn concentration increased in the leachate significantly with the increase in the sludge application rate (Figures 6.3.5-6.3.6) with an LSD value of 88 (Table 6.10) when data from all 12 weeks are combined. The order was only altered in the 900 mm rate between the control and the 10 t ha$^{-1}$ treatments, where the 10 t ha$^{-1}$ contained less Zn than the control. This was probably due to an increase in adsorption at 10 t ha$^{-1}$, an effect that was observed in the case of Cd as well. At 50 t ha$^{-1}$ the load of Zn was sufficiently large so that the adsorption capacity of the soil could not sorb all of it and no further decrease was observed, but rather a significant increase in Zn concentration in the leachate.

Over time, Zn increased in the leachates at both water rates from week 2 to week 6 and 7. Zinc concentrations started from a very minimal value to reach nearly the maximum 4 and 5 weeks later. After that period, there was a steep decrease, which was only recovered in the end of the 12-week period of this study. This decrease was very similar to Ni, and it may have been due to the same reason (sequestration by the microbial activity). After that period, Zn reached nearly its maximum concentration at both water rates, which indicates that the period of 12 weeks was not long enough to show any significant difference in its solubility. This indicates the fact that Zn is much more soluble and readily mobile than Ni in the soil environment (Forstner, 1993).

Unlike Ni, the amounts of Zn leached down was greater at the 900 mm than that in the 450 mm, by a factor of nearly 2 (Figure 6.3.6). The amounts of Zn at 50 t ha$^{-1}$ in the 450 mm rate was 940 ng, while at 900 mm it was 1970 ng. This provides the evidence that Zn

**Figure 6.3.5:** ***Zn in the leachate of the soil columns (μg).***

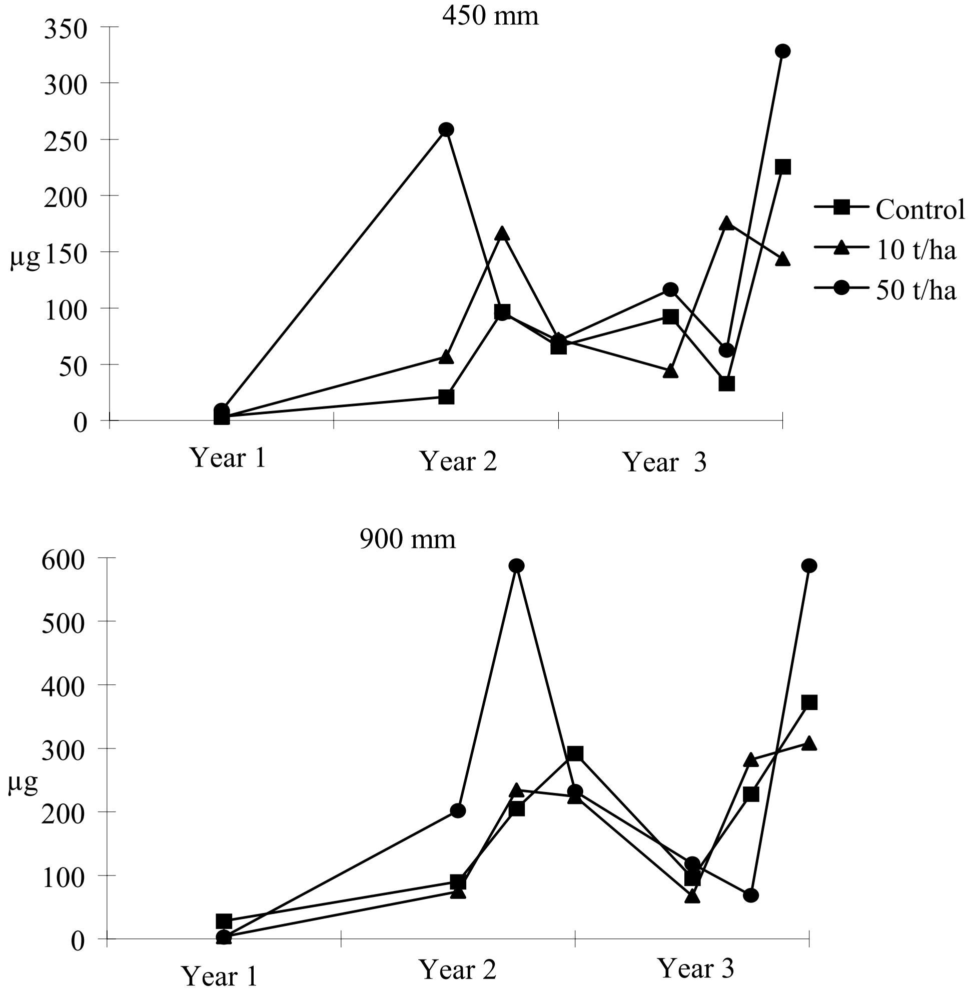


**Figure 6.3.6:** ***Zn in the leachate of the soil columns (cumulative values) (µg).***

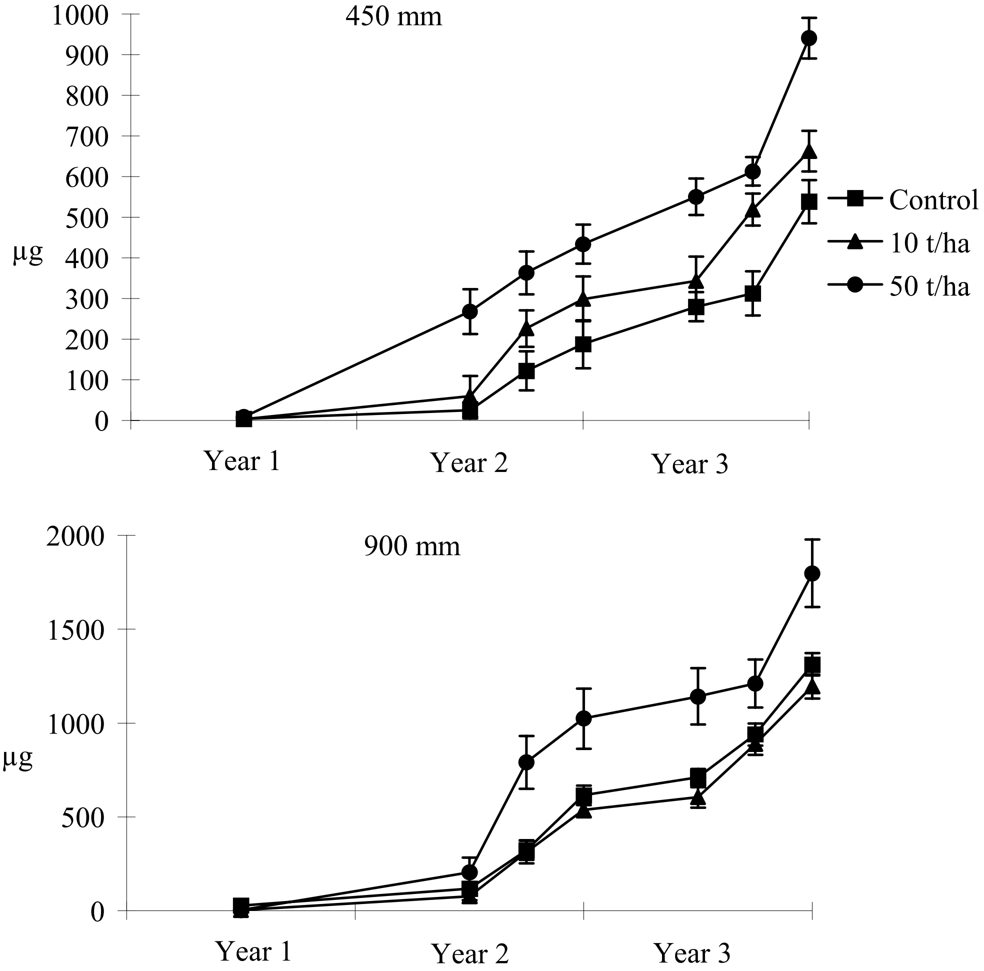


**Table 6.10:** ***LSD test for Zn in the leachate of the soil columns.***

*Water 72* *Water x Sludge 178* *Water x Time 355*

*Sludge 88** *Time 201** *Sludge x Time 611*

*Water x Sludge x Time 2552*

**Figure 6.3.7:** ***DOC in the leachate of the soil columns (mg).***

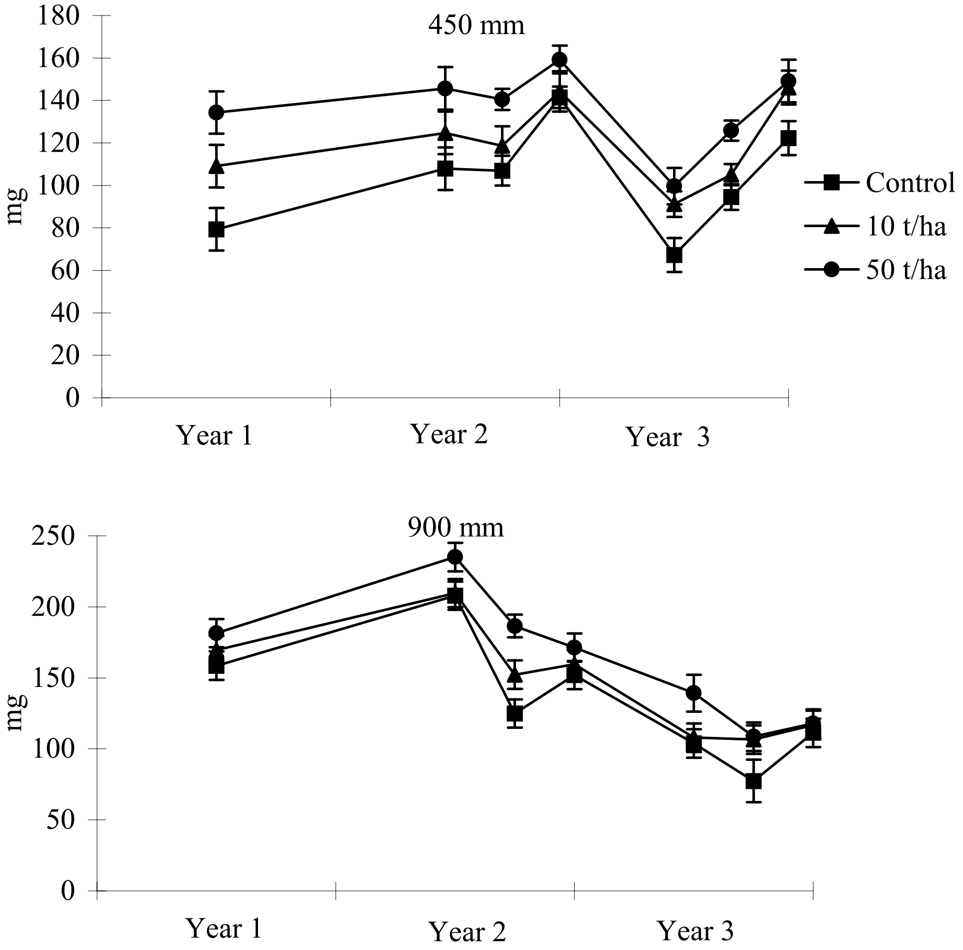


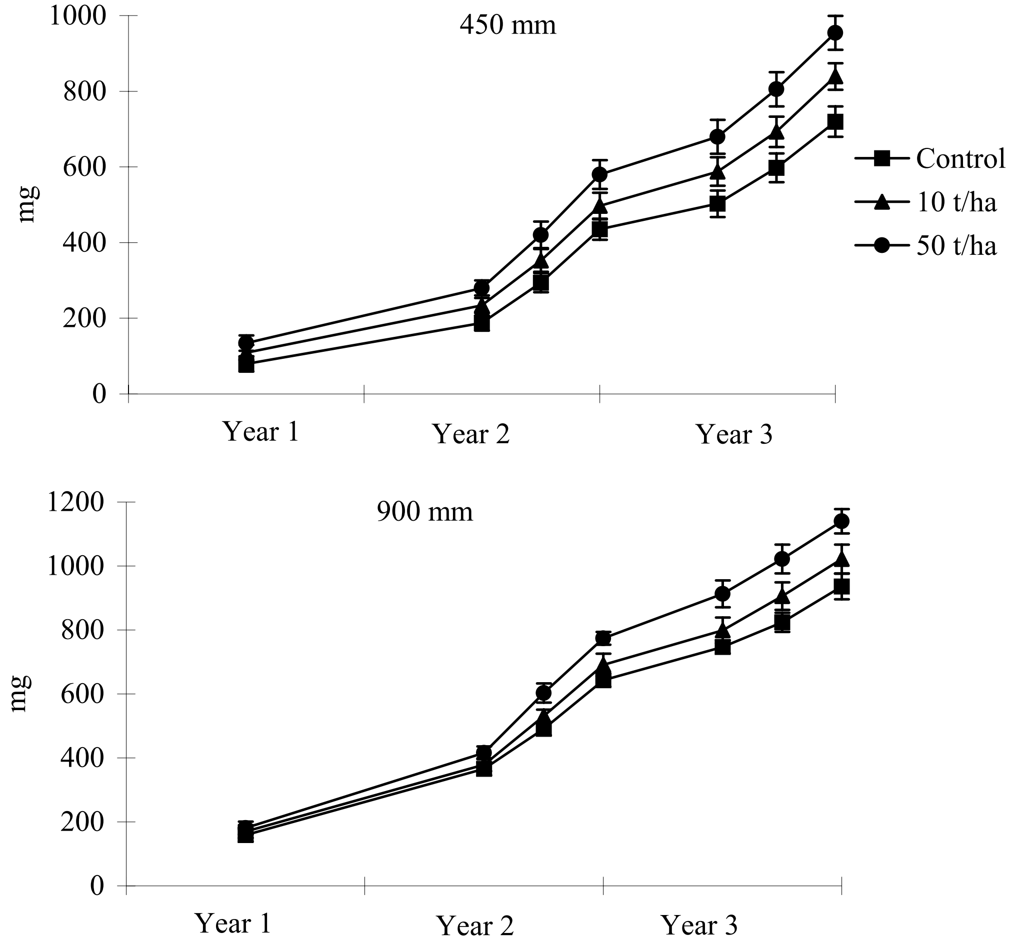
**Table 6.11:** ***LSD test for DOC in the leachate of the soil columns.***

*Water 8.77** *Water x Sludge 16.98**** *Water x Time 33.77****

*Sludge 10.30**** *Time 19.08**** *Sludge x Time 58.00****

*Water x Sludge x Time 242.31*

**Figure 6.3.8:** ***DOC in the leachate of the soil columns (cumulative values) (mg).***



was a lot more readily mobile and soluble than Ni, since an increasing amount of water in the leaching process also increased the levels of Zn being leached down in the soil profile.

### 6.4.2.4 Dissolved Organic Carbon

Dissolved Organic Carbon (DOC) was found in significant quantities in the leachates. Its concentration increased significantly with the increase in sludge application (Figures 6.3.7-6.3.8), with an LSD value of 10.30 (Table 6.11). This indicated that the excess DOC came from the sewage sludge which was applied on the top of the soil columns.

Levels of DOC decreased significantly with time (LSD 19.08) in the 900 mm water rate, but did not differ significantly at the 450 mm water rate. Since DOC can create stable complexes with heavy metals, which can remain dissolved in an aqueous solution over a wide range of pH, the application of higher water rates may cause it to be leached down the profile complexed with metals. DOC was shown to be readily leached through a soil; the rate of water application had an important role on this leaching, because at 900 mm the DOC was at a higher concentration than at 450 mm, with a significant LSD value of 8.77. However, this difference was not proportional to the difference in water volume applied to the soil column. Nevertheless, this showed that DOC was not sorbed onto the soil constituents, but was carried dissolved in the water all the way through the soil column. This provides an indication of the importance of DOC in a column of 40 cm and suggests that DOC may be responsible for the leaching of heavy metals, with the rate of leaching increasing with the rate of water application (rainfall).

The fact that a more mobile element like Zn was leached in amounts almost twice as high at 900 mm than at 450 mm, which was similar to the behaviour of DOC, indicates that this movement may have been facilitated by the presence of DOC compounds in the sludge-amended soil The movement of Cd was also significant since its concentrations were very low in the source, the sewage sludge (2.41 $\mu g\ g^{-1}$), but was still present in the leachate and this may provide an indication that this movement may have been facilitated by the presence of DOC.

## 6.5 DISCUSSION

Since the pH value of the soils in this experiment was around neutrality and the pH of the sewage sludge added to the soils was at the same value, it can be assumed that the movement of heavy metals and DOC that was observed in the soil columns was not a result of solubilisation due to pH effects. pH did not affect the adsorptive properties of the soils, so that it may be concluded that the mobility of the metals was due to solubilisation by other mechanisms.

Camobreco *et al.* (1996) discussed the difference between disturbed and undisturbed soil columns when studying metal leaching and concluded that since a lot of the movement occurs because of preferential flow and because a disturbed soil column will have its macro-pores destroyed, it would be expected that in undisturbed soils the movement of metals should be substantially greater. This is why the findings in this study are very important. Although repacked soil columns were used, it was found that the movement of metals was significantly increased in soils where sewage sludge was applied and that metals including Cd, Ni and Zn along with DOC were transported as deep as 40 cm. They were found in significant concentrations in the leachates and this may indicate the significance of DOC in facilitating the movement and solubility of heavy metals applied to soils with sewage sludge.

In the soil columns firstly, when studying the movement of metals down to a depth of 15 cm, it was found that Cd, Ni and Zn moved significantly at least down to 10 cm. This mobility was always significantly affected by the addition of sewage sludge on the top of the soil columns, with the greater movement occuring at the higher sludge application rate (50 t $ha^{-1}$). Lead was only found in significant quantities as far down the column as 2 cm. It should

**Table 6.12:** ***Budget of Cd, Ni and Zn in the soil columns: The amounts of Cd, Ni and Zn in the sludge, the leachate (in µg) and the percentage difference between the sludge and leachate.***

| | | 900 mm Equivalent Rainfall | | 450 mm Equivalent Rainfall | |
|---|---|---|---|---|---|
| | | 10 t ha$^{-1}$ | 50 t ha$^{-1}$ | 10 t ha$^{-1}$ | 50 t ha$^{-1}$ |
| Cd | Sludge | 42.41 | 212.08 | 42.41 | 212.08 |
| | Leachate | 5.77 | 11.19 | 3.79 | 2.43 |
| | % of Difference | 13 | 5 | 9 | 1 |
| Ni | Sludge | 670 | 3,351 | 670 | 3,351 |
| | Leachate | 142 | 178 | 109 | 151 |
| | % of Difference | 21 | 5 | 16 | 4 |
| Zn | Sludge | 11,360 | 56,804 | 11,360 | 56,804 |
| | Leachate | 1195 | 1797 | 662 | 940 |
| | % of Difference | 10 | 3 | 6 | 2 |

be noted here that the sewage sludge was only applied on the top of the columns and was not incorporated, so that even the increased concentrations of a metal in the 2 cm soil depth indicates that it has undergone some movement.

Cadmium was affected by the application of sewage sludge. In the 10 t ha$^{-1}$ treatment Cd moved only 4 cm, while at 50 t ha$^{-1}$ it moved significantly down to 10 cm. The time factor and the water rate factor were found to give no significant difference in the Cd behaviour. This may have happened because the load of this metal was very low in the sewage sludge. The fact that Cd appeared in the leachates, but with the control treatments having greater concentrations than the 50 t ha$^{-1}$ treatment, seems to suggest that the adsorption of Cd on the soil constituents reduced its mobility, since the load of Cd was not sufficient to saturate the adsorptive sites.

Nickel increased its mobility with the increase in sewage sludge application. The time factor had a significant effect on Ni mobility. In the 450 mm water rate, Ni increased in the depth 6-15 cm, while at the 900 mm water rate it increased in 0-6 cm. Another significant effect that the rainfall rate had on Ni mobility was that at 900 mm Ni was increased compared to the 450 mm at Year 3 in the higher sludge application rate (50 t ha$^{-1}$) in the first 2 depths. This indicates the fact that the increase in the volume of leaching water may have increased the leaching of metals present in soils which are mobile and in relatively high concentrations. Nickel increased in the leachate with the increase in sludge application and it also increased in the leachate under the 900 mm water rate compared with the 450 mm water rate. Over time, Ni did not decrease in the leachate under the 900 mm water rate, unlike 450 mm, where it decreased. This may mean that the higher rainfall rate kept Ni always more soluble and caused the continuous leaching of this element into the leachate. Zinc in the soil columns moved deeper with the increase in sludge application. In the 10 t ha$^{-1}$ treatments Zn was found to have moved 6 cm, while at the 50 t ha$^{-1}$ it moved 10 cm. In the leachate, Zn concentration increased with the sewage sludge application, as in case of Ni. The rainfall rate increased the concentration of Zn present in the leachate. The 900 mm treatment had nearly twice as much Zn than the 450 mm treatment, which showed that Zn is more mobile and more influenced by the increased water application than Ni. This was also evident from the fact that Zn did not decrease over time in the leachates, as Ni did. This indicated that the pools of the soluble Zn could sufficiently supply the leachate at the end of the period of 3 Years equivalent rainfall. A useful way of assessing the extent of leaching of metals that were eluted in the leachate (Cd, Ni and Zn) is to calculate the absolute amounts of these metals in the in the sewage sludge (which was applied on the top of the soil columns) and the amounts of the same metals in the leachate (which was eluted at the bottom of the soil columns). The amounts of the metals were expressed in μg. For the sludge, these were calculated by multiplying the concentration of the metals (in μg g$^{-1}$) by the amount of sludge applied on the top of the columns (in g). For the leachate, these amounts were calculated by multiplying the sum of the concentrations of the metals in the leachates (in μg L$^{-1}$) by the volume of eluted water (in mL).

It can be seen in Table 6.12 that the increase in the rate of rainfall increased the amounts of metals eluted in the leachate. It is important to note that even the proportional amounts (the percentage of difference between the amounts of the studied metals in the sludge and in the leachate) of heavy metals increased with the increase in rainfall rate from 450 mm to 900 mm. This is evidence that the increase in the volume of applied water solubilised greater amounts of heavy metals from the sewage sludge applied on the top of the columns, probably with the assistance of dissolved organic compounds. It must be pointed out that the movement of the metals in the soil columns was not found below 10 cm depth. However, the amounts of metals found in the leachate were transported further than this depth, because the columns were 40 cm deep. Richards *et al.* (1998) found that the preferential flow and metal complexation with soluble organics may allow leaching of these metals without easily detectable readsorption in the subsoil. This may be of important environmental implication, because it was exhibited that the risk of increasing heavy metal movement down the soil profile in sludge-treated soils is greater when larger elevated amounts of water are applied in that soil, either as a rainfall or as irrigation.

It is also important to note that as sewage sludge application rate increased, the amount of metals eluted in the leachate also increased. It was, however, evident that proportionally, greater amounts of Cd, Ni and Zn were eluted in the leachate in the 10 t ha$^{-1}$ than in the 50 t ha$^{-1}$ treatment. This is evidence that the sewage sludge increased the adsorptive capacity of the soil with the sludge-borne organic matter that was added in the soil and provided the means for sorbing the heavy metals as well as being their source. However, since greater quantities of metals were eluted underneath the columns with the higher sludge rate, the necessity of a wise management in applying sewage sludge has to be stressed: the risk of metals moving well below the incorporation zone dissolved in the water may be greater at high sludge application rates, especially in places receiving larger amounts of rain or irrigation.

Dissolved Organic Carbon (DOC) was found in the leachates and its concentration increased with the increase in sludge application rate, which shows that sewage sludge supplied low molecular weight organic acids while the sludge was washed by the applied water. DOC also increased with the increase in the water rate from 450 mm to 900 mm. This may be of significant environmental consequences, because it was shown that higher rates of applied water (either rainfall or irrigation) may increase the amounts of DOC leached from the sludge in soils applied with sewage sludge, and this may influence the solubility and availability of heavy metals. DOC decreased with time in the 900 mm water rate treatment, while it did not significantly change in the 450 mm water rate treatment. It is evident that in the higher water rate, the soluble organic acids were transported faster down through the soil profile into the leachate at the beginning of the experiment. This seems to suggest that the leaching of the DOC from the soil columns was approaching a minimum point at 900 mm, probably because the most soluble compounds of DOC had been washed out. At 450 mm, this did not happen, probably because more time was needed for this plateau to be reached.

Evidence that heavy metals may move in the soil profile was provided by Lund *et al.* (1976). In their field experiment the researchers used sludge with a high content of heavy metals and found that Zn had moved down to 50 cm, Cd to 17 cm while Ni to 75 cm. Also Schirado *et al.* (1986) reported that heavy metals had a uniform distribution in the soil profile to a depth of 1 m, due to their movement. Results such as these tend to have been obtained by the use of high applications of sewage sludge or sewage effluents. In experiments conducted with normal applications of sludge and water leaching, heavy metal movement was more limited, but was some times found to be very significant. Davis *et al.* (1988) measured the metal distribution in the soil profile in a field experiment where sludge had been applied at a rate of 40 t ha$^{-1}$ and rainfall rate was around 560 mm per annum over a period of 4 years (for comparison, in this experiment, the rainfall rate was 450 mm and 900 mm per annum over a period of 3 years). They found a significant movement of Cd, Ni, Pb and Zn to a depth of 10 cm, but it was argued that this was caused by infiltration of sludge particles, and no metal solubilisation could be justified. Barbarick *et al.* (1998) applied sewage sludge for 11 years at a rate of 27 t $ha^{-1}$ $yr^{-1}$. Nine years after the commencement of their experiment, they found a movement of Zn down to 125 cm depth. Other heavy metals (such as Pb, Cd and Ni), however, did not move significantly below the incorporation zone.

Since organic matter plays an important role in metal binding, some researchers have tested whether DOC compounds influence metal leaching. Fotovat *et al.* (1996) reported that metals such as Cd, Ni and Zn may be influenced in their solubility characteristics from the presence of DOC. LaBauve *et al.* (1988) applied synthetic waste water to soils and measured the leachate for soluble metals. It was found that the synthetic material increased the solubility of metals, especially Cd and Ni, and this was particularly attributed to the soluble organic matter of the waste. Guisquiani *et al.* (1992), on the other hand, used 50 cm soil columns treated with 90 t $ha^{-1}$ of sludge and applied half of the amount of water that was used for the lower rainfall rate (225 mm $yr^{-1}$) in this experiment for leaching. They found an increase in the concentrations of metals in the leachate from the amended soils. This was ascribed both to the soluble metal content of the sewage sludge and the soluble organic matter. They concluded that DOC had little affect on the metal leaching, since only a small percentage of it was transported down the soil profile. Li and Shuman (1996a), in their field experiment where soils had been contaminated with flue dust for 6 years, found that water-soluble Zn was elevated in the subsoil, and they concluded that this was caused by leaching of organic-Zn soluble compounds.

Sawhney *et al.* (1994) mixed soil and compost containing heavy metals at rates of 0, 15, 50 and 100 % compost. They measured the leachate after applying 20 mm of water per day for 70 days (for comparison, it should be reminded that in this experiment 16 mm and 2 mm water were applied per day for 84 days). They found that all the heavy metals studied (Cd, Pb, Cr, Cu and Zn) had been moved in the leachate. They also found that in the first half of their experiment the concentrations of metals in the leachate were higher while in the second half they were retained in a minimum point, indicating that the soluble pools of the metals were 'exhausted' in the first half of their course of experiment. Under the conditions of this experiment, such a decline was not always evident. The concentration in the leachate was only lower at the end than in the beginning in the case of Ni at the 450 mm water treatment and in the case of DOC at the 900 mm treatment. This also indicated that Ni and Zn were still very mobile even after the application of 3 years' equivalent rainfall, and their mobility did not decline even at the end of the experiment, that is their source (sewage sludge) was not exhausted. It may also mean that they did not have enough time to appear in the leachate (as these metals are slower in moving in the soil compared with other cations; also the columns were longer than those described in the experiment by Sawhey *et al.* (1994)). This enhanced solubility may be connected with the increased concentration of DOC in the leachate even after 3 years' water application. The fact that Cd was present in the leachate, although in very low concentrations, suggests that this movement may have also been facilitated by the DOC. Moreover, Christensen *et al.* (1996) also found that DOC increased heavy metal mobility in sludge-treated soils, while Zhu and Alva (1993) found a positive correlation between DOC and Cu and Zn in the leachates in their leaching experiment. They found that when the concentration of DOC decreased the transport of these metals also decreased. Bolton and Evans (1991) measured sludge landfill leachates for heavy metals and applied a computer model to calculate the speciation of these elements. It was found that metals were predominantly complexed with $Cl^-$ anions and fulvic acids (similar in chemical characteristics as DOC). In a recent work, Kalbitz and Wennrich (1998) studied an industrial site highly contaminated with heavy metals. Although they found no significant movement of Cd and Zn in the soil profile, their results confirmed that Cd and Zn were translocated in the leachates underneath 1.5 m lysimeters and that movement was influenced by DOC.

In this study, DOC was found to have been transported 40 cm in the soil column and subsequently appeared in the leachate and although the metals were not affected by the increase of applied water in their movement in the soil profile, the elution of these metals in the leachate was affected. The reason for this may be the more intensive leaching conditions that were followed in this experiment. Thus, under these conditions it may be possible that DOC played some role in increasing the solubility of heavy metals and that DOC is responsible for the fact that heavy metals were transported all the way through the 45 cm soil column into the leachate. The fact that the amounts of Cd, Ni and Zn eluted in the leachate were enhanced with the increase in water rate may indicate that water dissolved organic acids were involved in this transport.

## 6.6 CONCLUSIONS

Cadmium, Ni and Zn moved significantly in the soil profile down to a depth of 10 cm. This movement was affected by the sewage sludge application rate and in the case of Ni and Zn by the water application rate and the duration of the leaching. This indicated that these two elements had water soluble pools greater than that of Cd, since greater quantities of Ni and Zn were moved with 900 mm water rate than with the 450 mm rate and was also supported from the leachate findings. Lead, being a relatively immobile element (Evans, 1989), only moved to the first depth in the soil columns.

These data suggest that DOC increased the mobility of these heavy metals by acting as a ligand in the soil with the application of sewage sludge. Although repacked soil columns were used, which did not contain any natural macro-pores, the DOC and heavy metals moved beyond the layer of their deposition. DOC may have carried the metal down the soil profile even in case of Cd, which was applied with the sludge in low concentrations. Jardine *et al.* (1989) reported that preferential flow may be very significant under certain conditions.

Results from this study showed that these conditions may be the elevated water rate application, since Ni, Zn and DOC were influenced by this factor and increased their concentration in the leachate significantly with the increase of leaching water.

Nickel and Zn and DOC (especially Zn) were present in the leachate in concentrations that did not decline over the course of application of 3 years' equivalent rainfall and this suggested that the water soluble organic matter may have been responsible for the continuous high solubility of metals in the leachate.

# CHAPTER SEVEN: THE ROLE OF DISSOLVED ORGANIC CARBON IN HEAVY METAL MOBILITY

## 7.1 INTRODUCTION

Dissolved organic carbon (DOC) consists of several low molecular weight organic compounds, such as polyphenols, simple aliphatic acids, amino acids and sugar acids (Fox and Comerfield, 1990). DOC stays dissolved in the soil solution under natural conditions and it has been found that it may be responsible for the dissolution equilibria of metals in the soil solution (Reddy *et al.*, 1995). Although recent research has indicated a wide influence of DOC on heavy metal solubility, it is admitted that little is known about its role in metal chemistry in the soil solution (Jardine *et al.*, 1989). Acidic compounds in DOC are short-lived in soils, as they are used by the microbial population for their biological functions and it is, thus, sometimes assumed that these compounds are of little importance in 'real life' conditions. This may be the reason for the lack of scientific interest in the investigation of DOC, resulting in a paucity of information on its role on metal mobility in the soil systems (Harter and Naidu, 1995).

Although many workers have dealt with the adsorption characteristics of DOC on soil constituents and its effect in metal adsorption and solubility in the soil solution (Kaiser and Zech, 1997; Krishnamurti *et al.*, 1997; Spark *et al.*, 1997a; Spark *et al.*, 1997b) there is a lack of information concerning the influence of DOC in the availability of heavy metals to plants. Because DOC compounds are relatively short-lived, many researchers use synthetic compounds in their experiments. The conclusions drawn from such experiments are difficult to generalise and, thus, more evidence on the role of natural DOC is necessary. Moreover, earlier experiments in the course of this study, namely the pot and the leaching experiments, exhibited some evidence of the positive role of DOC in enhancing the availability and mobility of heavy metals in the soil environment. Thus, the need to investigate this role of natural DOC was further supported.

A pot experiment was set up with the aim of irrigating plants sown in mixtures of soil and sewage sludge in pots with solutions containing elevated concentrations of natural DOC and to measure plant and soil parameters in order to assess the role of DOC in heavy metal availability to plants. The hypothesis tested was that the presence of extra DOC in the soil solution will enhance the availability of heavy metals to plants in sewage sludge-treated soils.

## 7.2 MATERIALS AND METHODS

### 7.2.1 Sample Collection

Two different bulk soils were used in the experiment and were collected from the Sonning Farm of the University of Reading. The first soil was obtained from the same area as the loamy sand (LS soil), which was used in the earlier pot experiment (Chapter 4). The second soil was obtained from the site where the field experiment (Chapter 5) was established (SCL soil). The collection method was the same as those outlined in section 3.1.1. Liquid sewage sludge was collected from a waste water treatment plant near Heathrow Airport. It was an anaerobically digested sludge with 6 % total solids content.

### 7.2.2 Experimental Design

The air dry soils were mixed with sludge at equivalent rates of 0, 10 and 50 t $ha^{-1}$ DM on an area basis. The mixtures were placed in 100 mL plastic pots and the treatments were replicated 3 times. One third of them were to be irrigated with ultra pure water, the second third with a solution containing 285 mg DOC $L^{-1}$ and the remaining third was watered with a solution containing 470 mg DOC $L^{-1}$. Each of these 18 treatments (*i.e.*, 2 soils * 3 rates of sludge * 3 DOC levels = 18) was duplicated in order to obtain statistically reliable results and this resulted in a total of 36 pots. The 100 mL pots were used because if bigger capacity pots were chosen, far greater quantities of DOC solution would be necessary to be produced, and this would be technically very difficult.

Ryegrass (*Lolium perenne* L.) was the test crop, as in all the previous experiments where a test crop was used. Ryegrass seeds were sown in a growth medium to ensure a good rate of germination. After germinating, 10 seedlings were transplanted into each of the 36 pots. The experiment was left to run for 6 weeks after the transplanting, which allowed sufficient time for the plants to grow. During this period the pots were in a greenhouse at a temperature of around 22 $^{0}$C and under normal daytime light. The pots were watered from underneath (subirrigation) to maintain the field capacity of the soils. The pots were moved around to compensate for light differences.

### 7.2.3 Extraction of DOC to Use as a Watering Solution

DOC was extracted from the clay loam (CL) soil, which was used in the earlier pot experiment (Chapter 4), and which contained about 5 % of organic carbon. To assist the extraction of greater quantities of DOC from the soil, a dilute solution of $10^{-3}$ M NaOH was used. 300 g of air dry soil were weighed into a 1 L plastic centrifuge bottle, where 300 mL of $10^{-3}$ M NaOH were placed. The bottle was shaken overnight in a mechanical horizontal shaker of 100 strokes per minute. Next day the bottle was centrifuged for 1h at 3600 rpm and the supernatant was collected into 50 mL plastic centrifuge tubes, which were again centrifuged for 1 h at 3600 rpm, in order to remove some of the suspended solids. The supernatant was then filtered through a medium filter paper followed by a 0.45 μm membrane filter under vacuum. This was done in order to remove any large colloids from the solution and retain only the dissolved solid matter. The organic compounds in this dissolved solid matter would represent DOC. One third of this clear solution was then diluted 2 times with ultra pure water. Both of the clear solutions (the diluted and the undiluted) were kept in a refridgerator at 4 $^0$C and used for watering the pots. Each extraction batch was replicated once in a week in order to make sure that the DOC solutions were fresh, and the organic compounds were not decomposed by microbes. A small aliquot was kept from each extraction batch in the freezer in order to measure the concentrations of DOC in each weekly extraction and these were analysed at the end of the experiment in the Shimadzu TOC Analyser. The weekly extraction procedure resulted in 750 mL of 470 mg L$^{-1}$ DOC solution (undiluted) and 750 mL of 285 mg L$^{-1}$ DOC solution (the diluted). Table 7.1 shows the concentrations of DOC extracted in the weekly extractions, while Table 7.2 presents some characteristic physical and chemical properties of the soils and sewage sludge used in this experiment.

Although Na may have an effect on the chemistry of the soil-sludge mixtures in this experiment (it may cause dispersion of the organic matter), it is believed that no such effect was evident or significant. The concentration of Na in the DOC extracting solution was very low and could not alter significantly the chemistry of the mixtures.

**Table 7.1:** ***Concentrations of DOC extracted to be used as a watering solution (mg L$^{-1}$).***

| | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Mean |
|---|---|---|---|---|---|---|---|
| DOC | 470.53 | 470.83 | 468.53 | 475.37 | 465.86 | 470.31 | 470.24 |

### 7.2.4 Analyses Performed

At the end of the 6 week period, the plants were harvested, processed and digested according to the procedures described in sections 3.2.1 and 3.2.2. The soils were taken out of the pots, air dried, sieved and measured according to the procedures described at the section 3.1.1. The ryegrass plants were analysed for the heavy metals Cd, Ni, Pb and Zn using the ICP-OES. For Cd and Ni, the samples were below the instrument' s detection limit and so it

**Table 7.2***: Some physical and chemical properties of the soils and sewage sludge.*

| | $CaCO_3$ | Al | Fe | $Ca^{2+}$ | $K^+$ | $Na^+$ | $Mg^{2+}$ |
|---|---|---|---|---|---|---|---|
| | % | Dithionite extracted, % | | Water soluble (µg $g^{-1}$) | | | |
| LS Soil | 1.10 | 0.09 | 0.93 | 46.80 | 7.65 | 16.80 | 2.65 |
| SCL Soil | 0.28 | 1.75 | 0.75 | 153.25 | 1.70 | 36.25 | 5.28 |
| Sludge | - | - | - | - | - | - | - |

| | Sand | Silt | Clay | Category | pH | | CEC |
|---|---|---|---|---|---|---|---|
| | % | | | | $H_2O$ | $CaCl_2$ | $cmol_c$ $kg^{-1}$ |
| LS Soil | 78.37 | 14.64 | 6.99 | LS | 7.21 | 6.68 | 8.47 |
| SCL Soil | 74.61 | 5.09 | 20.30 | SCL | 7.19 | 6.46 | 15.21 |
| Sludge | - | - | - | - | 6.89 | 6.67 | - |

| | LOI | Bd | EC | *Aqua regia*-digested (µg $g^{-1}$) | | | |
|---|---|---|---|---|---|---|---|
| | % | g $cm^{-3}$ | dS $m^{-1}$ | Cd | Ni | Pb | Zn |
| LS Soil | 2.37 | 1.21 | 0.17 | 0.34 | 13.93 | 17.82 | 34.69 |
| SCL Soil | 4.40 | 1.08 | 0.48 | 0.10 | 9.09 | 30.44 | 42.67 |
| Sludge | 60.67 | 0.81 | - | 3.31 | 30.56 | 200.15 | 512.28 |

| | DOC |
|---|---|
| | mg $L^{-1}$ |
| LS Soil | 22.95 |
| SCL Soil | 41.94 |
| Sludge | 2574.53 |

was necessary to analyse these elements by GFAAS. The soils were measured for pH both in water (1 : 2.5 ratio) and 0.01 M $CaCl_2$ suspensions and organic matter by the Loss-On-Ignition method. The metals Cd, Ni, Pb and Zn were extracted in the soils with DTPA and 0.05 M $CaCl_2$ and digested with *Aqua regia*. All these procedures were performed according to the methods outlined in the relevant sections in Chapter 3. Along with these methods, an extraction of DOC was performed on all the soil samples. The method adopted was the one used by Baham and Sposito (1983). 2 g of soil were weighed into a 50 mL plastic centrifuge tube, where 20 mL of ultra pure water were added. The tube was shaken in an 'end-over-end' shaker at 20 rpm at 21 $^0$C overnight. On the next day, the tube was centrifuged for 15 minutes at 3600 rpm. The supernatant was then taken into a 20 mL plastic syringe. In the opening of the syringe a 0.45 µm polysulfon membrane filter was put and the solution was forced through the filter into a 20 mL plastic tube. The clear solution was kept in the freezer until the analysis, which was performed on a Shimadzu TOC-5000 Analyser.

All results were analysed statistically using the Analysis of Variance (ANOVA) and the LSD (Least Square Difference) test was employed to determine the significance of the differences between treatments. The statistical package used was SAS and the confidence limit was 95 % ($P < 0.05$).

For the sake of simplicity, the treatment where plants were watered with ultra pure water, was called DOC-0, the treatment where plants were watered with a solution containing 285 mg DOC $L^{-1}$ was called DOC-1 and the treatment where plants were watered with a solution containing 470 mg DOC $L^{-1}$ was called DOC-2. In addition, the 0 t $ha^{-1}$ treatment was called the control, while the other 2 sewage sludge application rates remain as they are, 10 t $ha^{-1}$ and 50 t $ha^{-1}$.

**Table 7.3:** ***Total concentration of Cd, Ni, Pb and Zn in soil samples digested with*** **Aqua regia** ***(μg $g^{-1}$).***

| | LS Soil | | | |
|---|---|---|---|---|
| | Cd | Ni | Pb | Zn |
| Control | 0.37 | 12.47 | 15.36 | 32.08 |
| 10 t $ha^{-1}$ | 0.44 | 13.01 | 17.97 | 39.15 |
| 50 t $ha^{-1}$ | 0.69 | 15.05 | 28.67 | 66.75 |

| | SCL Soil | | | |
|---|---|---|---|---|
| | Cd | Ni | Pb | Zn |
| Control | 0.10 | 9.09 | 30.45 | 42.68 |
| 10 t $ha^{-1}$ | 0.12 | 9.62 | 31.24 | 48.30 |
| 50 t $ha^{-1}$ | 0.19 | 11.62 | 34.40 | 72.46 |

## 7.3 RESULTS

The total concentrations of Cd, Ni, Pb and Zn are shown in Table 7.3. It can be seen that the concentration of these metals in the soil-sludge mixtures increased with the increase in the sewage sludge application rate. It should be noted that the two soils used in this experiment varied in their physical and chemical properties. The SCL soil contained more clay than the LS soil (20 % for the SCL soil, while only 7 % for the LS soil). The SCL soil also had a greater organic matter content of 4.4 %, while the LS soil had 2.5 %. Consequently, the SCL had a greater CEC (Cation Exchange Capacity) value than the LS soil (15.21 and 8.47 $cmol_c$ $kg^{-1}$ for the SCL and the LS soil respectively). These characteristics were important in explaining the chemical processes which occurred in these soils.

### 7.3.1 Plant Yield

Plant yields were much lower than that in the pot experiment in Chapter 4, probably because in that experiment much larger pots were used. The dry matter yields of ryegrass plants are presented in Table 7.4. Table A1 (Appendix IV) shows the replicates of the yields, as an indication of variability. Plants grown in the SCL soil had greater dry matter mass than those in the LS soil in the control sewage sludge treatments. In DOC-0 the LS control (0 t ha$^{-1}$) had only 0.01 g, while the SCL control had 0.07 g of production. It was evident that the ryegrass yield increased dramatically with increasing the rate of sewage sludge application, especially in the DOC-0 treatments. This increase can be explained by the fact that nutrient input increased in soils as the sewage sludge rate increased. Thus, in DOC-0 in both the LS and SCL soils there was an increase in plant production by a factor of 2 between 10 t ha$^{-1}$ and 50 t ha$^{-1}$.

**Table 7.4:** ***Yield of ryegrass (g DM).***

| | LS Soil | | |
|---|---|---|---|
| | DOC-0 | DOC-1 | DOC-2 |
| Control | 0.010 | 0.030 | 0.055 |
| 10 t ha$^{-1}$ | 0.225 | 0.275 | 0.275 |
| 50 t ha$^{-1}$ | 0.420 | 0.420 | 0.435 |

| | SCL Soil | | |
|---|---|---|---|
| Control | 0.070 | 0.135 | 0.075 |
| 10 t ha$^{-1}$ | 0.290 | 0.320 | 0.350 |
| 50 t ha$^{-1}$ | 0.510 | 0.500 | 0.515 |

However, the difference of 7 times that was found between the controls of the two soils had been narrowed down as sewage sludge rate increased. At 10 t ha$^{-1}$ this difference was only 1.29 times, while at 50 t ha$^{-1}$ it is 1.21 times. This is probably due to the fact that nutrients in the LS soil became more available for plants due to fewer adsorbing sites in this soil, as the CEC value for this soils was only half of that in the SCL soil. On the same lines Balik *et al.* (1998) found that plant yield increased more to a greater extent in the least fertile soil as sewage sludge application increased.

Moreover, as the rate of DOC application increased, the plant yield increased as well. That was more evident in the control in the LS soil. Yield increased 5 fold from DOC-0 to DOC-2, from 0.01 g to 0.05 g. This was probably due to the nutrients that the DOC solutions provided to plants. This increase from DOC-0 to DOC-2 was, however, slower as the sewage sludge rate increased. At 10 t ha$^{-1}$ the plant yield only increased from 0.25 g at DOC-0 to 0.275 g at DOC-2; at 50 t ha$^{-1}$ from 0.42 g at DOC-0 to 0.435 g at DOC-2. In the SCL soil, which was relatively richer in nutrients, the increase in yield as DOC levels increased was not observed so clearly but the yields of the plants grown in this soil were still greater than those in the LS soil. Plants in the control treatments did not show significant yield increases, being maintained at around 0.07 g between DOC-0 and DOC-2, and plants at 50 t ha$^{-1}$ at around 0.5. At 10 t ha$^{-1}$, however, there was an increase from 0.29 g at DOC-0 to 0.35 g at DOC-2, which seems to suggest that at that sewage sludge rate the nutrient input from the combination of sewage sludge and DOC solution was optimal. In the control treatments, the nutrients added from the DOC solution were not sufficient to increase the yield of the plants.

The yield increase with the addition of sludge shows the valuable fertilising effect of sewage sludge, which has been pointed out by many researchers. Yield increase may have a positive effect on heavy metal uptake from soils, as biochemical activities are accelerated as plants become ‘healthier’.

### 7.3.2 Organic Matter

The organic matter content of the soil-sludge mixtures was measured by the Loss-On-Ignition method (Table 7.5). Organic matter increased with the increase in sewage sludge application rate significantly. In the LS at DOC-0 levels the organic matter content increased from 2.42 % in control to 4.8 % in 50 t ha$^{-1}$, showing a 2 fold increase. The SCL soil with a significantly greater content in organic matter in the control, that is 4.12 %, increased also to 6.15 % at 50 t ha$^{-1}$. This increase, however, was smaller than that in the LS soil. As DOC levels increased in almost all sewage sludge rates and in both soils the organic matter content also increased, though not significantly according to the LSD test. That means that there was a trend of increase, but this was not of statistical importance. Watering with DOC solutions meant that low molecular weight and non-colloidal compounds were introduced into the soil-sludge mixtures.

**Table 7.5:** ***Loss-On-Ignition (%).***

LS Soil

| | DOC-0 | DOC-1 | DOC-2 |
|---|---|---|---|
| Control | 2.42 | 2.56 | 2.55 |
| 10 t ha$^{-1}$ | 2.96 | 2.79 | 3.06 |
| 50 t ha$^{-1}$ | 4.80 | 4.85 | 5.11 |

SCL Soil

| | DOC-0 | DOC-1 | DOC-2 |
|---|---|---|---|
| Control | 4.12 | 4.31 | 4.48 |
| 10 t ha$^{-1}$ | 4.65 | 4.65 | 4.60 |
| 50 t ha$^{-1}$ | 6.15 | 6.78 | 6.29 |

*LSD test*

*Soil 0.23**** *Soil x Sludge rate 0.47*

*Sludge rate 0.28**** *Soil x DOC watering 0.47*

*DOC watering 0.29* *Sludge rate x DOC watering 0.65*

*Soil x Sludge rate x DOC watering 1.42*

### 7.3.3 pH Measurement in Water

pH measured in the soil-water suspensions (Table 7.6) showed a strong dependence on the loading rates of sewage sludge and additions of DOC as a watering solution. There was a significant trend of decreasing pH values of the mixtures as sludge application increased. At DOC-0, controls had pH 7.95, which became 7.66 at 10 t ha$^{-1}$ and 7.09 at 50 t ha$^{-1}$ with an LSD value of 0.16 in the LS soil. In the SCL soil, the control had a value of 7.47, which dropped to 6.90 at 10 t ha$^{-1}$ and to 6.81 at 50 t ha$^{-1}$. This decrease was more dramatic in the LS soil due to its lower pH buffering capacity, which was evident from its lower CEC value than that of the SCL soil. The same decreasing order was maintained with the higher levels of DOC, with the most dramatic pH drop being observed in the sample from the pots watered with the DOC-1 solution in both soils (the difference between control and 50 t ha$^{-1}$ was greater at DOC-1 compared with either DOC-0 or DOC-2). In the LS soil there was a pH decrease in DOC-1 from control to 50 t ha$^{-1}$. In the SCL soil, the decrease was of a similar value, and also in DOC-2 the same was observed. The other treatment which had a significant effect on pH was watering with the DOC additions. As the addition of DOC increased, the pH increased as well in both soils and with all 3 rates of sewage sludge. This was probably

**Table 7.6:** ***pH measured with 1: 2.5 water suspension.***

LS Soil

| | DOC-0 | DOC-1 | DOC-2 |
|---|---|---|---|
| Control | 7.95 | 8.22 | 8.37 |
| 10 t ha$^{-1}$ | 7.66 | 7.84 | 8.15 |
| 50 t ha$^{-1}$ | 7.09 | 7.18 | 7.59 |

SCL Soil

| | DOC-0 | DOC-1 | DOC-2 |
|---|---|---|---|
| Control | 7.47 | 7.77 | 7.75 |
| 10 t ha$^{-1}$ | 6.90 | 7.26 | 7.51 |
| 50 t ha$^{-1}$ | 6.81 | 6.67 | 6.90 |

_LSD test_

_Soil 0.07***_

_Sludge rate 0.09***_

_DOC watering 0.10***_

_Soil x Sludge rate x DOC watering 0.48_

_Soil x Sludge rate 0.16_

_Soil x DOC watering 0.16_

_Sludge rate x DOC watering 0.22_

**Table 7.7:** ***pH measured in 0.01 M $CaCl_2$ suspension.***

LS Soil

| | DOC-0 | DOC-1 | DOC-2 |
|---|---|---|---|
| Control | 7.42 | 7.53 | 7.56 |
| 10 t ha$^{-1}$ | 7.21 | 7.35 | 7.59 |
| 50 t ha$^{-1}$ | 6.81 | 6.90 | 6.90 |

SCL Soil

| | DOC-0 | DOC-1 | DOC-2 |
|---|---|---|---|
| Control | 7.04 | 7.14 | 7.26 |
| 10 t ha$^{-1}$ | 6.68 | 6.89 | 7.08 |
| 50 t ha$^{-1}$ | 6.54 | 6.52 | 6.66 |

_LSD test_

_Soil 0.01***_

_Sludge rate 0.02***_

_DOC watering 0.02***_

_Soil x Sludge rate x DOC watering 0.12_

_Soil x Sludge rate 0.04_

_Soil x DOC watering 0.04_

_Sludge rate x DOC watering 0.05_

due to the effect of Na in the mixture of soil, sludge and DOC. Again this pH increase was less in the SCL soil, due to its high pH buffering capacity. As can be seen, in the LS soil the differences between DOC-2 and DOC-0 tended to increase from 0.42 in the control to 0.55 in the 50 t ha$^{-1}$ treatment. In the SCL soil, however, although there was an increase in the differences between DOC-2 and DOC-0 from control to 10 t ha$^{-1}$ (from 0.38 to 0.61), at 50 t ha$^{-1}$ there was a steep decrease and the difference was only 0.09. This showed that the organic matter added to the soil with the high CEC value resulted in an increased buffer capacity and this stopped the pH value from increasing further.

### 7.3.4 pH Measured in 0.01 M $CaCl_2$

The pH measured in the soil samples with 0.1 M $CaCl_2$ soil suspensions (Table 7.7) showed much the same trend as the pH measured in water. The differences, however, among treatments were a lot more statistically significant. The drop in pH with the increase in sewage sludge rate agrees with the finding of several other workers, including Tsadilas *et al.* (1995). The same drop of pH was observed in the earlier pot experiment (Chapter 4) in both soils and in both temperature treatments.

### 7.3.5 DOC

The soil samples were analysed for their DOC content and the results showed the expected pattern (Table 7.8). DOC increased significantly in both soils as the rate of sewage sludge increased, due to the input of sludge-borne organic matter. The difference between 50 t ha$^{-1}$ and 10 t ha$^{-1}$, if the control is subtracted by both of them, was always of around 5 times, which is the value that would have been be expected if there were no other DOC inputs except from the sewage sludge. As the concentration of DOC solution increased from DOC-0 to DOC-2 there was a significant increase in the DOC concentrations extracted from both the soils. DOC-2 was twice as high as DOC-1, if DOC-0 is subtracted, which is what should be expected, because the input of DOC increased by a factor of 2. These results suggest that the level of DOC depended on the state of organic matter in the soil, and this is illustrated by the fact that the SCL soil had a significantly greater content in DOC than the LS soil for all sewage sludge and DOC treatments. The results also indicate that the DOC added in the pots as a watering solution contributed to the increase of the DOC levels of the soils accordingly.

**Table 7.8***: DOC extractions in 1: 10 soil: water suspension (w/v) (mg $L^{-1}$).*

LS Soil

| | DOC-0 | DOC-1 | DOC-2 |
|---|---|---|---|
| Control | 22.95 | 34.75 | 47.85 |
| 10 t $ha^{-1}$ | 28.74 | 40.43 | 56.70 |
| 50 t $ha^{-1}$ | 47.19 | 72.69 | 93.60 |

SCL Soil

| | DOC-0 | DOC-1 | DOC-2 |
|---|---|---|---|
| Control | 41.94 | 60.17 | 66.77 |
| 10 t $ha^{-1}$ | 45.67 | 62.83 | 78.37 |
| 50 t $ha^{-1}$ | 64.69 | 82.17 | 103.95 |

*LSD test*

| | | | |
|---|---|---|---|
| *Soil* | *2.30**** | *Soil x Sludge rate* | *4.72* |
| *Sludge rate* | *2.86**** | *Soil x DOC watering* | *4.72* |
| *DOC watering* | *2.96**** | *Sludge rate x DOC watering* | *6.54** |
| *Soil x Sludge rate x DOC watering* | *14.37* | | |

### 7.3.6 Plant concentration and uptake

It should be noted that there were no significant differences between the 2 soils in the concentration and uptake by plants of the 4 elements, Cd, Ni, Pb and Zn.

#### 7.3.6.1 Cadmium

Cadmium is a very toxic and mobile element in the soil environment. Cadmium was measured in the plant mass both as concentration, in μg Cd per kg of plant DM (Table 7.9 and Figure 7.1) and as uptake, in ng Cd per total dry plant mass in a pot (Table 7.10). The concentration of Cd increased in the plants as the rate of sewage sludge increased in both soils from control to 10 t $ha^{-1}$ in all DOC treatments. In most cases, however, the concentration from 10 to 50 t $ha^{-1}$ decreased slightly, probably due to a ‘dilution effect’. This means that the plant dry matter production at 50 t $ha^{-1}$ was sufficiently high that the quantities of Cd absorbed from the soil were less than in the 10 t $ha^{-1}$ treatment in terms of μg Cd per g of

**Table 7.9**: ***Concentrations of Cd, Ni, Pb and Zn in ryegrass (μg g$^{-1}$, Cd in μg kg$^{-1}$).***

| | LS Soil | | | SCL Soil | | |
|---|---|---|---|---|---|---|
| | Cd | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 30 | 20 | 23 | 15 | 14 | 15 |
| 10 t ha$^{-1}$ | 75 | 78 | 98 | 21 | 26 | 28 |
| 50 t ha$^{-1}$ | 73 | 83 | 113 | 26 | 26 | 27 |
| | Ni | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 2.18 | 1.95 | 2.14 | 2.80 | 3.33 | 2.25 |
| 10 t ha$^{-1}$ | 6.52 | 6.34 | 6.01 | 3.95 | 3.20 | 3.73 |
| 50 t ha$^{-1}$ | 6.60 | 6.77 | 8.18 | 5.49 | 4.39 | 6.64 |
| | Pb | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 0.21 | 0.32 | 0.40 | 0.20 | 0.31 | 0.46 |
| 10 t ha$^{-1}$ | 0.36 | 0.55 | 0.57 | 0.49 | 0.36 | 0.40 |
| 50 t ha$^{-1}$ | 0.57 | 0.40 | 0.67 | 0.56 | 0.49 | 0.50 |
| | Zn | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 2.64 | 2.23 | 2.11 | 2.88 | 2.37 | 2.21 |
| 10 t ha$^{-1}$ | 7.72 | 8.65 | 9.75 | 9.41 | 11.20 | 12.73 |
| 50 t ha$^{-1}$ | 18.05 | 22.40 | 26.32 | 23.28 | 27.03 | 25.97 |

*LSD test for Cd*

*Soil 20.33* *Soil x Sludge rate 41.65*
*Sludge rate 28.21* *Soil x DOC watering 41.65*
*DOC watering 26.13* *Sludge rate x DOC watering 57.67*
*Soil x Sludge rate x DOC watering 126.50*

*LSD test for Ni*

*Soil 1.38* *Soil x Sludge rate 2.83*
*Sludge rate 1.91** *Soil x DOC watering 2.83*
*DOC watering 1.77* *Sludge rate x DOC watering 3.91*
*Soil x Sludge rate x DOC watering 8.59*

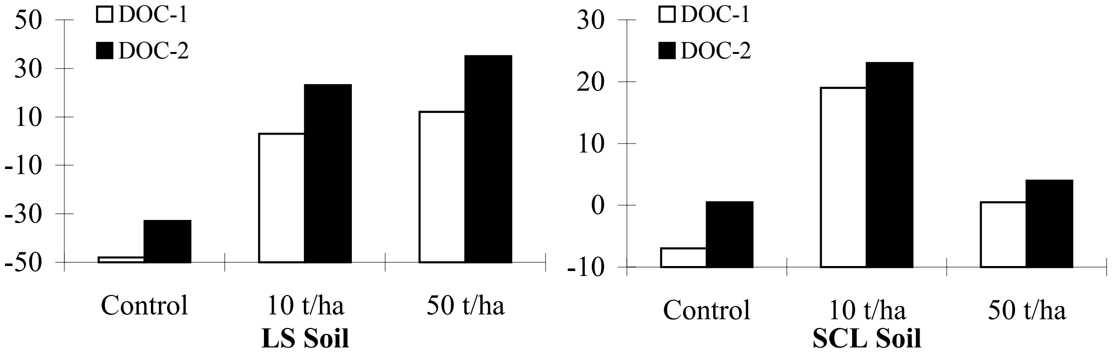
*LSD test for Pb*

*Soil 0.10* *Soil x Sludge rate 0.21*
*Sludge rate 0.06** *Soil x DOC watering 0.21*
*DOC watering 0.13*** *Sludge rate x DOC watering 0.29*
*Soil x Sludge rate x DOC watering 0.63*

*LSD test for Zn*

*Soil 1.90* *Soil x Sludge rate 3.90*
*Sludge rate 1.29** *Soil x DOC watering 3.90*
*DOC watering 2.45*** *Sludge rate x DOC watering 5.40*
*Soil x Sludge rate x DOC watering 11.86*

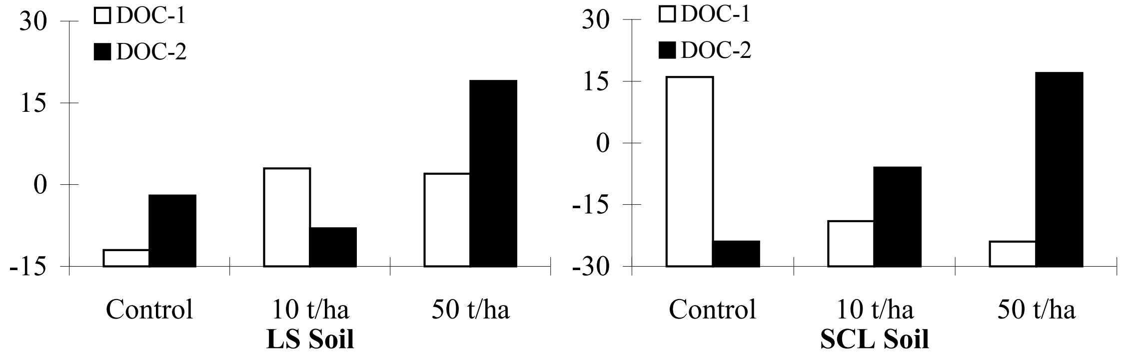
**Figure 7.1:** ***Percentage increase of Cd plant concentration with DOC additions (y-axis: % increase).***



plant. This 'dilution effect' was apparent in the DOC-0 in the LS soil and in the DOC-1 and DOC-2 in the SCL soil. It can be seen that as the DOC additions increased, the concentrations of Cd increased as well in both soils and at all sewage sludge rates, except in the control. In the sandy soil at 10 t ha$^{-1}$, the Cd concentration was 75 μg kg$^{-1}$ at DOC-0, and increased to 78 μg kg$^{-1}$ in DOC-1 and to 98 μg kg$^{-1}$ in DOC-2. At 50 t ha$^{-1}$ where the Cd present in the soil was 5 times more, this increase was even more impressive. The concentration of 73 μg kg$^{-1}$ in DOC-0, increased to 83 μg kg$^{-1}$ in DOC-1 and to 113 μg kg$^{-1}$ in DOC-2. In the SCL soil these trends remained the same, showing an increase in Cd concentration as DOC additions increased at 10 t ha$^{-1}$ and 50 t ha$^{-1}$ from DOC-0 to DOC-2. This increase did not happen in the control treatments. At 10 t ha$^{-1}$, Cd concentration was 21 μg kg$^{-1}$ at DOC-0 and became 26 μg kg$^{-1}$ at DOC-1 and 28 μg kg$^{-1}$ at DOC-2, which is similar to that between the same treatments in the LS soil. At 50 t ha$^{-1}$, however, the difference between DOC-0 and DOC-2 was much smaller. This much smaller increase in Cd concentration may be explained by the fact that the plant could not absorb more Cd from the soil, possibly because the adsorption capacity of the soil increased with the addition of organic matter borne in sewage sludge. In the pots, where 50 t ha$^{-1}$ had been applied, the high addition of DOC did not have any significant effect on the availability of Cd.

As for the uptake of Cd by the ryegrass, it can be seen that as sewage sludge application increased uptake increased as well. This increase was significant with an LSD of 3.87, for both soils. As DOC additions increased at each sludge rate, Cd uptake increased as well, even in the control sludge treatments. However, the increase was not significant for the control (LSD of 3.59). In the LS soil, at 10 t ha$^{-1}$ the increase from DOC-0 to DOC-2 was from 16.8 to 26.8 ng pot$^{-1}$, while in the SCL soil, at 10 t ha$^{-1}$ the increase from DOC-0 to DOC-2 was significantly different. At 50 t ha$^{-1}$ the increase from DOC-0 to DOC-2 in the LS

**Figure 7.2**: ***Percentage increase of Ni plant concentration with DOC additions (y-axis: % increase).***



soil was more marked, from 30.5 to 56.5 ng pot$^{-1}$. However, in the SCL soil the increase was very small and not significant, as it happened for the same increase in the Cd concentration.

### 7.3.6.2 Nickel

Nickel was measured in the plant in μg g$^{-1}$ of plant DM mass (Table 7.9 and Figure 7.2) and in μg pot$^{-1}$ (Table 7.10). As the sewage sludge rate increased, both the Ni concentration and uptake per pot increased. There was no 'dilution effect' apparent in the Ni concentration data, as there was in the case of Cd. This increase was significant at an LSD value of 1.91 for concentration and 0.51 for uptake. As the addition of DOC increased, the Ni concentration increased only in the 50 t ha$^{-1}$ treatments. In the control and the 10 t ha$^{-1}$ treatments, in the LS soil, there was a decrease from DOC-0 to DOC-2, for the control and the 10 t ha$^{-1}$ treatments, and the same was evident for the SCL soil. At 50 t ha$^{-1}$, however, there was an increase in Ni concentration from DOC-0 to DOC-2 for the LS soil (from 6.60 to 8.18 μg g$^{-1}$) and for the SCL soil (from 5.49 to 6.64 μg g$^{-1}$). The same trend can be observed in the Ni uptake, where at 50 t ha$^{-1}$ there was an increase (2.77 to 3.56 μg) from DOC-0 to DOC-2 in the LS soil and in the SCL soil an increase (2.80 to 3.42 μg), which were very similar to the Ni concentration data. In the control and 10 t ha$^{-1}$ treatments for Ni uptake, however, there was no decrease from DOC-0 to DOC-2, but instead a small increase. This was due to the greater plant production (yield) as DOC levels increased which balanced up the concentration decrease and gave a small net increase. The fact that in the control and 10 t ha$^{-1}$ treatments there was a small decrease, while at 50 t ha$^{-1}$ an increase as DOC levels increase can be attributed to the fact that DOC did not have a great effect on the behaviour of Ni when the Ni total concentration was not very high in the soil system. Nickel was still dependent on surface

**Table 7.10:** ***Uptake of Cd, Ni, Pb and Zn from ryegrass ($\mu$g pot$^{-1}$, Cd in ng pot$^{-1}$).***

| | LS Soil | | | SCL Soil | | |
|---|---|---|---|---|---|---|
| | Cd | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 0.3 | 0.6 | 1.2 | 1.1 | 1.9 | 2.5 |
| 10 t ha$^{-1}$ | 16.8 | 21.3 | 26.8 | 6.1 | 8.3 | 9.8 |
| 50 t ha$^{-1}$ | 30.5 | 34.7 | 56.5 | 13.3 | 13.0 | 13.9 |
| | Ni | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 0.02 | 0.06 | 0.17 | 0.20 | 0.45 | 0.17 |
| 10 t ha$^{-1}$ | 1.47 | 1.74 | 1.65 | 1.14 | 1.02 | 1.30 |
| 50 t ha$^{-1}$ | 2.77 | 2.84 | 3.56 | 2.80 | 2.20 | 3.42 |
| | Pb | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 0.00 | 0.01 | 0.02 | 0.01 | 0.04 | 0.03 |
| 10 t ha$^{-1}$ | 0.08 | 0.15 | 0.16 | 0.14 | 0.11 | 0.14 |
| 50 t ha$^{-1}$ | 0.24 | 0.17 | 0.29 | 0.28 | 0.24 | 0.26 |
| | Zn | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 0.03 | 0.06 | 0.11 | 0.20 | 0.32 | 0.17 |
| 10 t ha$^{-1}$ | 1.74 | 2.38 | 2.20 | 2.73 | 3.58 | 4.46 |
| 50 t ha$^{-1}$ | 7.58 | 9.41 | 11.45 | 11.87 | 13.52 | 13.37 |

*LSD test for Cd*

*Soil 2.79* *Soil x Sludge rate 5.72*
*Sludge rate 3.87** *Soil x DOC watering 5.72*
*DOC watering 3.59* *Sludge rate x DOC watering 7.92*
*Soil x Sludge rate x DOC watering 17.37*

*LSD test for Ni*

*Soil 0.36* *Soil x Sludge rate 0.75*
*Sludge rate 0.51** *Soil x DOC watering 0.75*
*DOC watering 0.47* *Sludge rate x DOC watering 1.04*
*Soil x Sludge rate x DOC watering 2.28*

*LSD test for Pb*

*Soil 0.02* *Soil x Sludge rate 0.06*
*Sludge rate 0.03** *Soil x DOC watering 0.06*
*DOC watering 0.03** *Sludge rate x DOC watering 0.08*
*Soil x Sludge rate x DOC watering 0.17*

*LSD test for Zn*

*Soil 1.46* *Soil x Sludge rate 3.00*
*Sludge rate 2.03*** *Soil x DOC watering 3.00*
*DOC watering 1.88*** *Sludge rate x DOC watering 4.15*
*Soil x Sludge rate x DOC watering 9.12*

adsorption for its solubility in the soil solution and the addition of 285 and 470 mg $L^{-1}$ of soluble carbon did not affect its affinity characteristics. At 50 t $ha^{-1}$, however, the quantities of Ni were increased by 5 times compared to 10 t $ha^{-1}$. This may mean that some of the newly introduced Ni was affected by the presence of increased amounts of DOC as the competition between DOC in the solution and the flocculated solid particles was less important. Thus, some of the Ni may have been dependent on DOC. Nickel solubility in the soil solution was increased, due to the greater affinity it showed for dissolved organic compounds and this was reflected in the plant uptake in both soils, especially in the LS soil where the solid phase was less important and did not compete for Ni adsorption as well as the SCL soil.

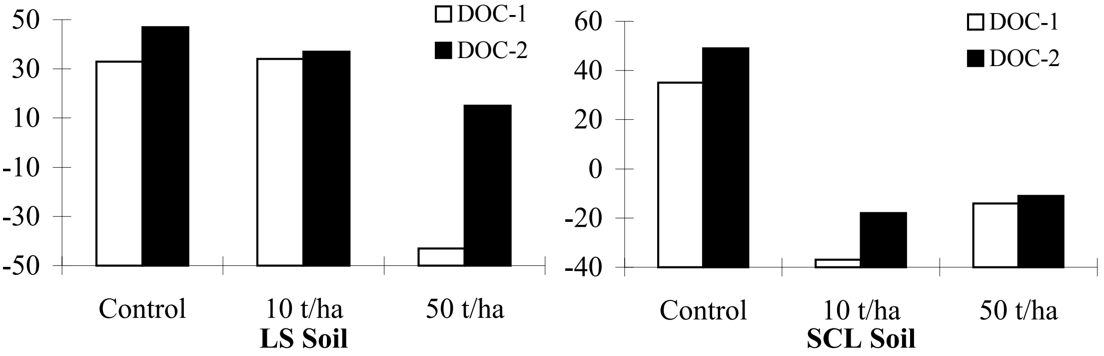
The same increase was also observed for Cd uptake in the LS soil, which was probably due to the same competition effect. Senesi *et al.* (1989) reported that in sewage sludge-treated soils, organics showed a well defined selectivity in binding metal ions added to soils with sewage sludge. As the metal loadings increased, the metal adsorption-desorption equilibria shifted to preferential binding of metals such as Cu, Fe, Ni and Cr, which form stable complexes with the organic ligands. However, this increase was only half of that of Cd at 50 t $ha^{-1}$, and this is due to Cd being a more mobile element than Ni (Evans, 1989). Neil and Sposito (1986), in their experiment with low Cd concentrations in the soil solution, concluded that the solid phase and the organic matter in particular was very important in controlling the solubility of Cd. The Cd concentrations in the solutions were 4-6 μmol $kg_{solid}^{-1}$ (0.45-0.65 μg $g^{-1}$), which approached the range of Cd used in this experiment. They found that Cd solubility increased with the increase in sewage sludge application, due to the presence of DOC. As Cd was added to the system, it showed a high affinity for DOC, which kept Cd soluble and mobile. Cadmium affinity was shifted from the solid phase to DOC compounds. This was evident up to an application rate of 150 t $ha^{-1}$ of liquid sludge. Only at sewage sludge rates of 500 t $ha^{-1}$ of liquid sludge this was trend altered. This alteration, in the experiment reported, took place for Cd at 50 t $ha^{-1}$ for the SCL soil, where CEC values were much greater than in the LS soil. In addition, the solubility of Ni increased at 0 t $ha^{-1}$ because the pH was depressed at this sewage rate from 7.47 to 6.81. This pH value was a lot closer to 6, which has been reported to give maximum effectiveness to DOC in solubilising metals like Ni (Spark *et al.*, 1997a).

#### 7.3.6.3 Lead

Lead was shown to be the least mobile of all 4 elements under study (Cd, Ni, Pb and Zn). This was supported by the fact that plant concentrations (Table 7.9 and Figure 7.3) were very small compared to the total Pb soil concentrations. However, both sewage sludge rates and DOC additions as watering solutions had a significant positive effect on Pb concentration and uptake (Pb increased as both sludge rate and DOC increased). As sludge rates increased, Pb concentrations showed a significant increase in both soils and at all DOC levels. The LSD for the concentration was 0.06, while for the uptake it was 0.03. The only exception was in the LS soil, at DOC-1 level between 10 t $ha^{-1}$ and 50 t $ha^{-1}$, where there was a decrease, due to the 'dilution effect'.

DOC had a very important effect on Pb availability to plants as it did in the case of Cd and Zn. As DOC increased from DOC-0 to DOC-2, the Pb concentration and uptake also increased in both soils and at all 3 sludge rates. The LSD was 0.13 for concentration and 0.03 for uptake. The difference in the effect on Pb compared with Cd and Ni was that there was an impressive increase of Pb from DOC-0 to DOC-2 in the control treatments. This increase was from 0.21 to 0.40 μg $g^{-1}$ in the LS and 0.20 to 0.41 μg $g^{-1}$ in the SCL soil. The same marked increase was observed in the 10 t $ha^{-1}$ treatment in the LS, while the Pb values seem to reach a plateau in the SCL soil. The marked increase in the control may be put down to the relatively low growth rate of the plants. In the control pots, the plants were physically weaker than those that received sludge, probably due to a lack of nutrients. This affected the biochemistry of metal absorption from roots, but not equally for all 4 elements. Those elements which were more immobile, and less readily solubilised in the solution, like Pb, were affected to a greater extent. The fact that healthier (more strongly growing) plants absorbed greater quantities of heavy metals was pointed out in the discussion of the earlier pot experiment and the field experiment. There, the plant uptake showed a dependence on the plant dry matter yield. As DOC was added, the plants received nutrients which improved productivity as shown by the yield data. This also helped Pb uptake and, thus, Pb concentration in the plants increased. The same thing also happened at 10 t $ha^{-1}$ in the LS soil, where Pb concentration followed the yield trend closely but not in the SCL, where the quantities of Pb supplied in the soil seemed to be immobilised from the solid phase, which was being built up with the addition of sewage sludge. In these pots, the elevated DOC did not appear to have an important effect. At 50 t

**Figure 7.3:** ***Percentage increase of Pb plant concentration with DOC additions (y-axis: % increase).***

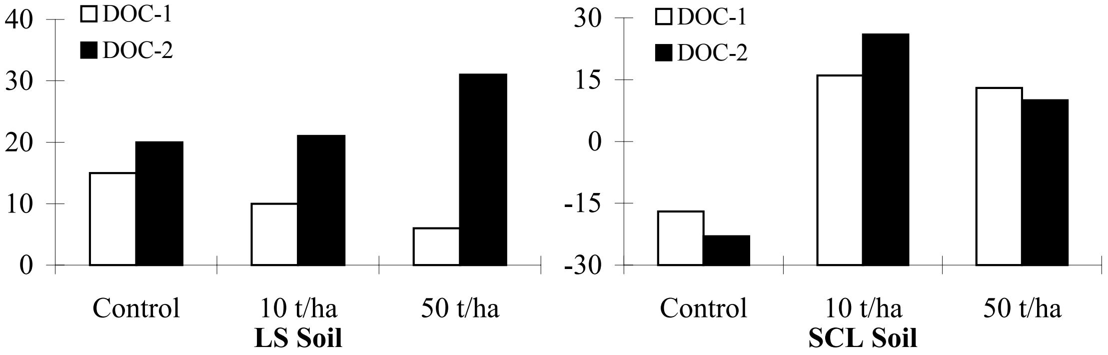


ha$^{-1}$ in the LS soil, there was an increase between DOC-0 and DOC-2, which was smaller than that in Cd and Ni. In the soil with the lower CEC (the LS soil), DOC addition still had a significant role in increasing Pb availability. In the SCL soil, it can be seen that again DOC addition had no effect in Pb availability, due to the strong adsorption of Pb on the solid surfaces. The same trends were followed by the Pb uptake, a very similar picture about Pb can be drawn availability to ryegrass.

### 7.3.6.4 Zinc

Zinc concentration (Table 7.9 and Figure 7.4) in ryegrasss and uptake per pot (Table 7.10) was significantly affected by both the sewage sludge and DOC treatments. The LSD for the sludge rate was 1.29 and that for DOC was 2.45. As the sludge rate increased, the Zn concentration increased as well in both soils and in all DOC treatments. Similarly, as DOC levels increased availability of Zn increased as well and this was evident in the 10 and the 50 t ha$^{-1}$ treatments, but in the control there was a small decrease which was not significant. At 10 t ha$^{-1}$ from DOC-0 to DOC-2 there was a similar increase in both soils. In the LS soil DOC-0 had 7.72 μg g$^{-1}$and DOC-2 had 9.75 μg g$^{-1}$. In the SCL soil, between the same treatments, the values had an increase from 9.41 to 12.73 μg g$^{-1}$. At 50 t ha$^{-1}$, in the LS soil between the same treatments, there was an increase from 18.05 to 26.32 μg g$^{-1}$. In the SCL, however, the increase between DOC-0 and DOC-2 was a lot smaller, that is from 23.28 to 25.97 μg g$^{-1}$. This behaviour was similar to that of Cd, where in both soils, at 10 t ha$^{-1}$, the differences between DOC-0 and DOC-2 were similar, while at 50 t ha$^{-1}$, in the LS soil, the difference was greater than that at 10 t ha$^{-1}$. Another similarity between Zn and Cd was that in the SCL soil the increases with the addition of DOC were a lot smaller compared with the LS soil. That was also the observed trend for Ni, but the similarities between Cd and Zn were more evident. The reason for these similarities is probably the fact that Zn has a very similar chemical activity and behaviour to Cd in the soil environment (Bailey *et al.*, 1995). The data for Zn uptake supported the same evidence for the effect of DOC in Zn availability. The treatments were significant with an LSD for the rate of sewage sludge of 2.03 and for the DOC additions of 1.88.

**Figure 7.4:** ***Percentage increase of Zn plant concentration with DOC additions (y-axis: % increase).***



### 7.3.7 Soil Extractions

The response of the metals was measured in the soil-sludge mixtures with solutions that extract the available forms of them. These solutions were the same as those used in the earlier pot experiment, DTPA and 0.05 M $CaCl_2$.

#### 7.3.7.1 Cadmium

Cadmium extracted from the soil with both extractants (Tables 7.11-7.12 and Figures 7.5-7.6) was positively affected by both the sludge rate and the DOC additions. As the sludge rate increased, the DTPA-extractable Cd increased significantly (LSD of 6.38), for both soils and for all 3 DOC levels. The increases between 10 and 50 t $ha^{-1}$ were appreciable, which seem to represent the difference of 5 times of the total Cd addition with sludge. The same can be observed with $CaCl_2$-extractable Cd, where differences between 10 and 50 t $ha^{-1}$, if the control is subtracted, approached a factor of 5 times. The LSD value for the sludge rates in $CaCl_2$ was 2.4. This trend was more marked than the response in plant concentration and uptake, where the relevant differences were almost always less than 2. Neither DTPA nor $CaCl_2$ could precisely predict Cd availability, in sludge-soil mixtures. Extractable Cd concentrations increased significantly as DOC levels increased for each sludge rate in both soils. This increase was significant, with LSD values of 2.91 for DTPA and 0.5 for $CaCl_2$. With $CaCl_2$ the differences between DOC-0 and DOC-2 in the control sludge treatments were not significant, but they were significant at 10 and 50 t $ha^{-1}$. The behaviour of $CaCl_2$-extractable Cd also showed a similarity to the Cd response in the plant concentration, having similar rate of increase. At 50 t $ha^{-1}$ it increased from 7.8 to 10.9 μg $kg^{-1}$, which was greater

**Table 7.11:** ***0.05 M $CaCl_2$-extractable Cd, Ni, Pb and Zn (μg $kg^{-1}$).***

| | LS Soil | | | SCL Soil | | |
|---|---|---|---|---|---|---|
| | Cd | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 2.0 | 2.4 | 2.4 | 2.4 | 2.7 | 2.0 |
| 10 t $ha^{-1}$ | 3.8 | 4.3 | 4.8 | 3.2 | 3.4 | 3.9 |
| 50 t $ha^{-1}$ | 7.8 | 9.6 | 10.9 | 9.5 | 9.5 | 9.8 |
| | Ni | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 22.0 | 20.0 | 24.8 | 26.9 | 29.8 | 33.1 |
| 10 t $ha^{-1}$ | 40.7 | 40.9 | 45.3 | 32.4 | 45.1 | 56.3 |
| 50 t $ha^{-1}$ | 141.1 | 132.8 | 168.5 | 100.4 | 116.9 | 120.1 |
| | Pb | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 123.8 | 104.1 | 111.7 | 110.1 | 112.4 | 111.5 |
| 10 t $ha^{-1}$ | 109.9 | 102.7 | 100.3 | 109.1 | 105.2 | 110.2 |
| 50 t $ha^{-1}$ | 108.2 | 102.1 | 110.6 | 98.9 | 105.9 | 106.8 |
| | Zn | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 159.3 | 142.4 | 184.6 | 174.1 | 177.6 | 171.5 |
| 10 t $ha^{-1}$ | 206.3 | 174.1 | 294.5 | 183.9 | 232.5 | 266.7 |
| 50 t $ha^{-1}$ | 277.6 | 357.6 | 416.0 | 503.0 | 522.2 | 572.4 |

*LSD test for Cd*

*Soil 6.0* *Soil x Sludge rate 12.4*
*Sludge rate 2.4**** *Soil x DOC watering 12.4***
*DOC watering 0.5*** *Sludge rate x DOC watering 17.2**
*Soil x Sludge rate x DOC watering 37.8*

*LSD test for Ni*

*Soil 69.1* *Soil x Sludge rate 141.6*
*Sludge rate 46.9** *Soil x DOC watering 141.6**
*DOC watering 18.8*** *Sludge rate x DOC watering 196.1*
*Soil x Sludge rate x DOC watering 430.9*

*LSD test for Pb*

*Soil 0.9* *Soil x Sludge rate 2.0*
*Sludge rate 1.3* *Soil x DOC watering 2.0*
*DOC watering 1.6* *Sludge rate x DOC watering 2.7*
*Soil x Sludge rate x DOC watering 5.9*

*LSD test for Zn*

*Soil 9.3* *Soil x Sludge rate 1625.9*
*Sludge rate 44.8*** *Soil x DOC watering 1625.9**
*DOC watering 10.2*** *Sludge rate x DOC watering 2251.1*
*Soil x Sludge rate x DOC watering 4947.1*

**Figure 7.5**: ***Percentage increase in Cd extractability with CaCl$_2$ with DOC additions (y-axis: % increase).***

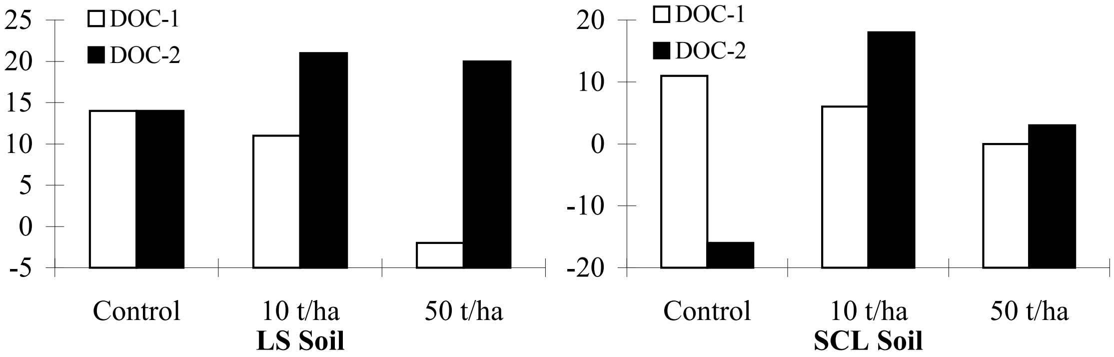


**Figure 7.6**: ***Percentage increase in Cd extractability with DTPA with DOC additions (y-axis: % increase).***

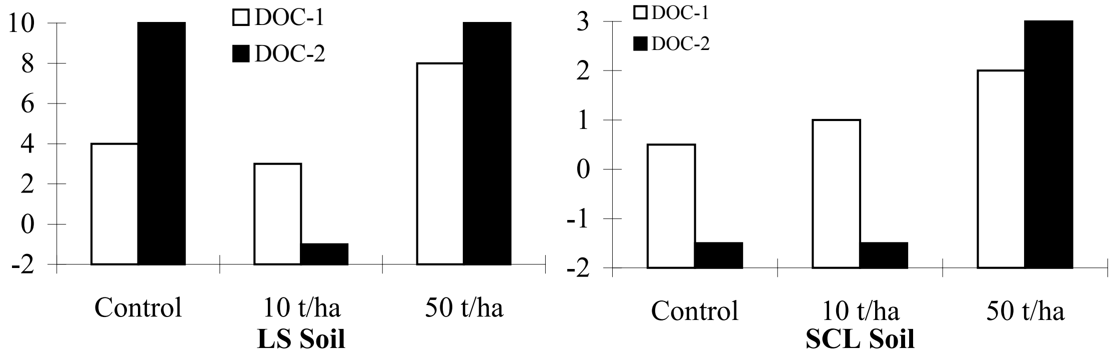


**Figure 7.7**: ***Percentage increase in Ni extractability with $CaCl_2$ with DOC additions (y-axis: % increase).***

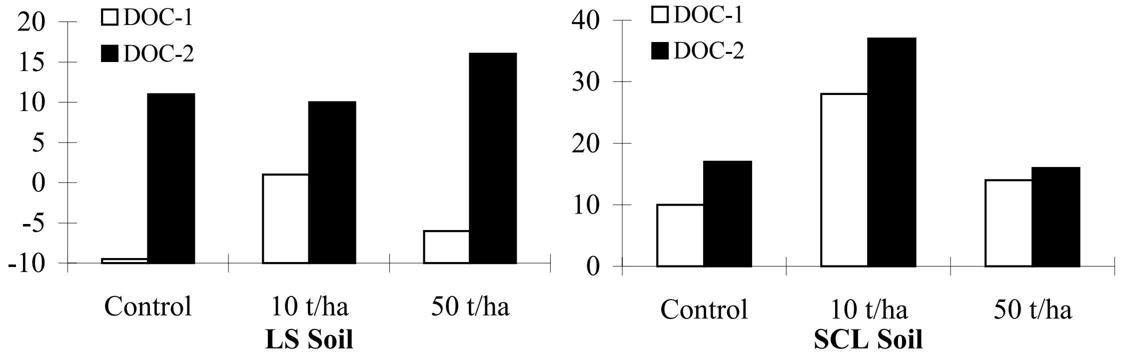
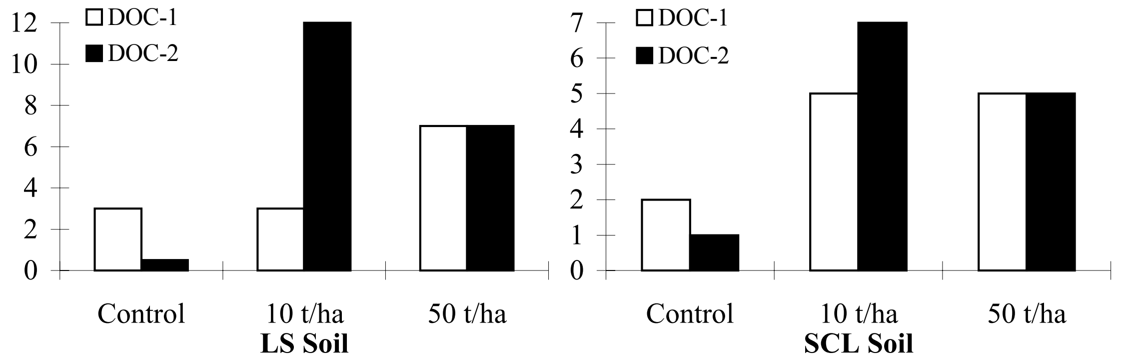


**Figure 7.8**: ***Percentage increase in Ni extractability with DTPA with DOC additions (y-axis: % increase).***



than the increase at 10 t ha$^{-1}$. In the SCL soil, the same similarities persisted; at 10 t ha$^{-1}$ between DOC-0 and DOC-2 there was an increase from 3.2 to 3.9 μg kg$^{-1}$, which was similar to that in the LS soil. At 50 t ha$^{-1}$, however, the increase between DOC-0 and DOC-2 was a lot smaller, that is from 9.5 to 9.8 μg kg$^{-1}$, which was not statistically important. The plant response in the SCL soil was again very similar.

In contrast, the DTPA-extractable Cd showed differences between DOC-0 and DOC-2 which were neither significant in the control sludge treatment in both soils, nor in the 10 t ha$^{-1}$. At 50 t ha$^{-1}$, however, in the LS soil there was an increase of 10 % which was much greater than the increase in the SCL soil from 142 to 146 μg kg$^{-1}$. This was very similar to $CaCl_2$-extractable Cd and plant Cd concentration. $CaCl_2$ extractions predicted very closely the Cd behaviour and availability as affected by the elevated quantities of DOC, in the soil systems. For Cd, $CaCl_2$ was more sensitive in following the plant trends than DTPA.

#### 7.3.7.2 Nickel

Nickel extracted with DTPA and $CaCl_2$ (Tables 7.11-7.12 and Figures 7.7-7.8) was significantly affected by the addition of sewage sludge and the loading of DOC. As the rate of sludge increased, the DTPA and $CaCl_2$-extractable Ni increased as well (LSD of 0.01 for DTPA and 46.9 for $CaCl_2$). As in the case of Cd, the differences between 10 and 50 t ha$^{-1}$, if the controls are subtracted, for both DTPA and $CaCl_2$ approached a factor of 5 times, which is the difference that would be expected for the total concentration of Ni. Again, a difference of 5 was not reflected in the plant response, where the differences between 10 and 50 t ha$^{-1}$ were of a factor of around 2 or less, for both soils and all DOC treatments. Thus, both $CaCl_2$ and DTPA did not seem to predict precisely enough the concentration and uptake of Ni in plants affected by the sludge rates.

Extraction with $CaCl_2$ and DTPA predicted plant responses to the DOC treatments in both soils very well, but DTPA was less efficient. With $CaCl_2$, in both soils between DOC-0 and DOC-2 there were no significant changes in the control treatments, where values were around 22 for the LS and 30 μg kg$^{-1}$ for the SCL soil. However, in the LS soil at 10 t ha$^{-1}$ the differences found between DOC-0 and DOC-2 were from 40.7 to 45.3 μg kg$^{-1}$, but with an LSD of 1.88 they are considered to be only slightly different. At 50 t ha$^{-1}$ the difference was statistically more important with an increase from 141.1 to 168.5 μg kg$^{-1}$ . In the SCL soil, at 10 t ha$^{-1}$ there was an impressive increase with the increase in DOC levels from 32.4 to 56.3

**Table 7.12:** ***DTPA-extractable Cd, Ni, Pb and Zn ($\mu g\ g^{-1}$, Cd in $\mu g\ kg^{-1}$).***

| | LS Soil | | | SCL Soil | | |
|---|---|---|---|---|---|---|
| | Cd | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 49 | 51 | 54 | 56 | 56 | 55 |
| 10 t ha$^{-1}$ | 72 | 74 | 71 | 76 | 77 | 75 |
| 50 t ha$^{-1}$ | 127 | 138 | 141 | 142 | 145 | 146 |
| | Ni | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 0.59 | 0.57 | 0.59 | 0.44 | 0.45 | 0.44 |
| 10 t ha$^{-1}$ | 0.80 | 0.83 | 0.91 | 0.67 | 0.71 | 0.73 |
| 50 t ha$^{-1}$ | 1.85 | 1.98 | 1.99 | 1.48 | 1.57 | 1.55 |
| | Pb | | | | | |
| | DOC- 0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 2.19 | 2.22 | 2.28 | 3.50 | 3.64 | 3.47 |
| 10 t ha$^{-1}$ | 3.19 | 3.64 | 3.57 | 4.77 | 4.57 | 4.64 |
| 50 t ha$^{-1}$ | 7.88 | 8.37 | 8.38 | 9.50 | 9.68 | 9.75 |
| | Zn | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 1.29 | 1.34 | 1.35 | 4.17 | 4.20 | 4.03 |
| 10 t ha$^{-1}$ | 4.00 | 4.42 | 4.31 | 7.42 | 7.46 | 7.11 |
| 50 t ha$^{-1}$ | 14.35 | 15.69 | 15.58 | 17.55 | 18.38 | 18.49 |

*LSD test for Cd*

*Soil 7.39* *Soil x Sludge rate 9.42*
*Sludge rate 6.38**** *Soil x DOC watering 9.42*
*DOC watering 2.91** *Sludge rate x DOC watering 13.05*
*Soil x Sludge rate x DOC watering 28.63*

*LSD test for Ni*

*Soil 0.02* *Soil x Sludge rate 0.05*
*Sludge rate 0.01** *Soil x DOC watering 0.05*
*DOC watering 0.03** *Sludge rate x DOC watering 0.06*
*Soil x Sludge rate x DOC watering 0.13*

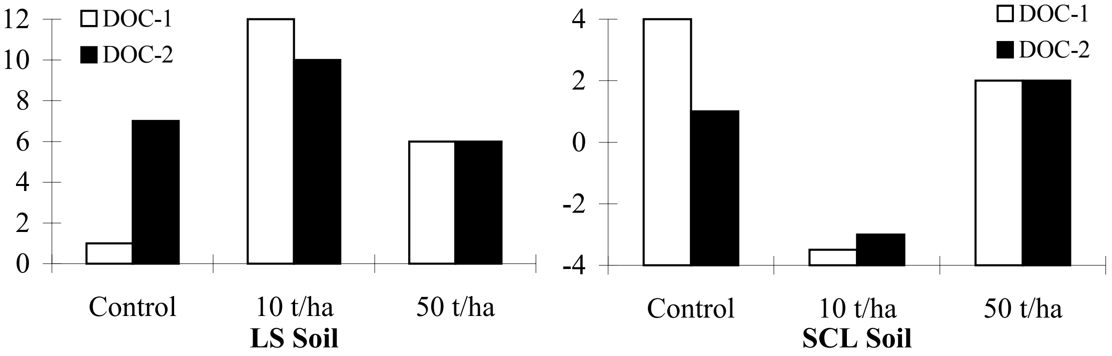
*LSD test for Pb*

*Soil 0.02* *Soil x Sludge rate 0.06*
*Sludge rate 0.03** *Soil x DOC watering 0.06*
*DOC watering 0.03** *Sludge rate x DOC watering 0.08*
*Soil x Sludge rate x DOC watering 0.17*

*LSD test for Zn*

*Soil 0.15* *Soil x Sludge rate 0.32*
*Sludge rate 0.13*** *Soil x DOC watering 0.32*
*DOC watering 0.20*** *Sludge rate x DOC watering 0.44*
*Soil x Sludge rate x DOC watering 0.96*

**Figure 7.9**: ***Percentage increase in Pb extractability with DTPA with DOC additions (y-axis: % increase).***



μg $kg^{-1}$, but this was smaller at 50 t $ha^{-1}$ ( 100.4 to 120.1 μg $kg^{-1}$). This means that in both soils the Ni concentration increased at 50 t $ha^{-1}$ between DOC-0 and DOC-2 in a similar rate to that of the plant response.

With DTPA extractions, as DOC levels increased the Ni concentration increased at all sludge rates except the control. The LSD value for the DOC treatments was 0.03. At 10 t $ha^{-1}$, in the LS soil the values increased from 0.80 to 0.91 μg $g^{-1}$ and at 50 t $ha^{-1}$ from 1.81 to 1.99 μg $g^{-1}$ from DOC-0 to DOC-2. At 10 t $ha^{-1}$ the increase was from 0.67 to 0.73 μg $g^{-1}$, while at 50 t $ha^{-1}$ the increase was from 1.48 to 1.55 μg $g^{-1}$. $CaCl_2$ extractions predicted the plant responses more precisely than DTPA for Ni as they did for Cd as well. In both soils, at 10 t $ha^{-1}$ the differences between DOC-0 and DOC-2 were not significant in $CaCl_2$ and plant concentration, while at 50 t $ha^{-1}$ a similar increase for both $CaCl_2$ and plant concentration was observed. DTPA predicted the increases at 10 t $ha^{-1}$ and 50 t $ha^{-1}$ between DOC-0 and DOC-2 and the quantities of Ni that it extracted were significantly different, but the values did not follow plant concentrations as $CaCl_2$ did. It can be seen that increases in the additions of DOC significantly increased the availability and extractability of Ni, as it did for Cd, especially in the higher sewage sludge application rates. Only in the SCL soil, with the higher CEC value, was the increase in availability smaller at 50 t $ha^{-1}$ than at 10 t $ha^{-1}$ for both metals.

### 7.3.7.3 Lead

As Pb is the least mobile element amongst those under study, and since $CaCl_2$ extracts metals both from the soil solution and from readily desorbable sites, only small quantities of Pb would be expected to be in this position. Thus, Pb extractions by $CaCl_2$ were found to be either too noisy or below the detection limits of the ICP-OES. This also happened in the earlier pot experiment. Lead was only extracted from the soil with DTPA (Table 7.12 and Figure 7.9) in measurable concentrations and the extractions were significantly affected by the sewage sludge application rates and DOC additions in both soils. As sludge increased, Pb extractability increased as well, with an LSD of 0.03. Again, in both soils the differences between 10 and 50 t ha$^{-1}$, if the controls are subtracted, were a lot higher than those observed in the plant concentration and uptake data of Pb. As DOC levels increased, extractable Pb increased as well in most treatments (LSD 0.03). In both soils in the control treatments, Pb did not increase significantly between DOC-0 and DOC-2. This is evidence that the steep increase that took place in the controls for the plant as DOC increased were due to improved plant growth which led to the uptake of increased Pb, which was available in the root zone. In the LS soil at 10 t ha$^{-1}$, the increase between DOC-0 and DOC-2 from 3.19 to 3.57 μg g$^{-1}$, while at 50 t ha$^{-1}$ this increase was smaller, that is from 7.88 to 8.38 μg g$^{-1}$. The explanation for this may be that the sludge rate contributed to the solid phase adsorptive sites and competed for Pb. As a result, increased DOC became less important in controlling the Pb solubility. In the SCL soil the Pb behaviour was different because this soil had a greater CEC and would be expected to sorb Pb more strongly. At 10 t ha$^{-1}$ there was no increase in Pb levels as the DOC increased, in fact there was a small but non statistically significant decrease. At 50 t ha$^{-1}$, however, there was a small increase. This small increase showed that at high Pb levels DOC may be important and may increase Pb mobility, when the adsorption sites of the solid phases are near to their capacity, but this was not necessarily reflected in the plant concentration and uptake.

### 7.3.7.4 Zinc

$CaCl_2$ and DTPA-extractable Zn concentration (Tables 7.11-7.12 and Figures 7.10-7.11) were significantly affected by the increases in sewage sludge rate and DOC levels. As sludge rates increased, both DTPA and $CaCl_2$ concentrations increased in both soils and in all DOC treatments. The LSD value for $CaCl_2$ was 44.8, while for DTPA it was 0.13, leaving only the differences between control and 10 t ha$^{-1}$ at DOC-0 for both soils in the $CaCl_2$ not being significant. As DOC levels increased, Zn concentrations increased significantly as well in all sludge treatments, except the controls. LSD value for $CaCl_2$ was 10.2 and DTPA is 0.20. Zinc has very similar chemical behaviour to Cd in the soil environment and this was exhibited in the soil extractions as well. Zinc concentrations extracted with $CaCl_2$ were affected by DOC in the same way as Cd, and both $CaCl_2$-extractable Cd and Zn correlated closely with Cd and Zn behaviour in plant uptake. In the $CaCl_2$ extractions in the control treatments in the LS soils, there was a slightly significant increase as DOC increased and this showed the importance of DOC in mobilising Zn even without any addition of this element in sludge.

**Figure 7.10:** ***Percentage increase in Zn extractability with $CaCl_2$ with DOC additions (y-axis: % increase).***

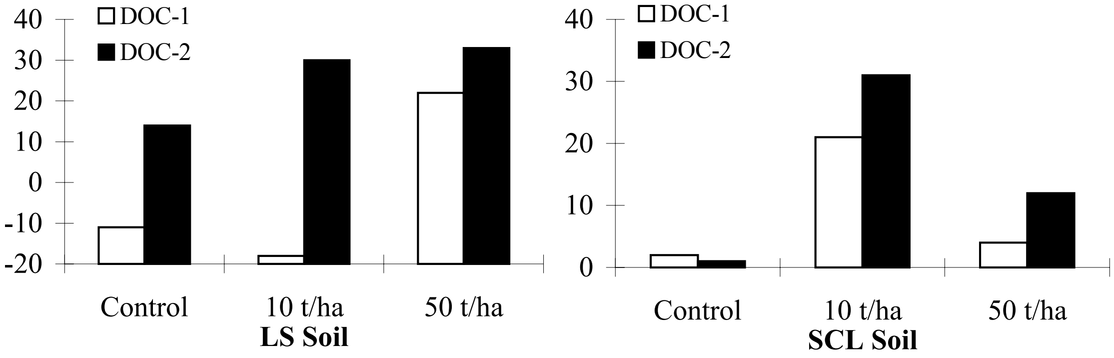
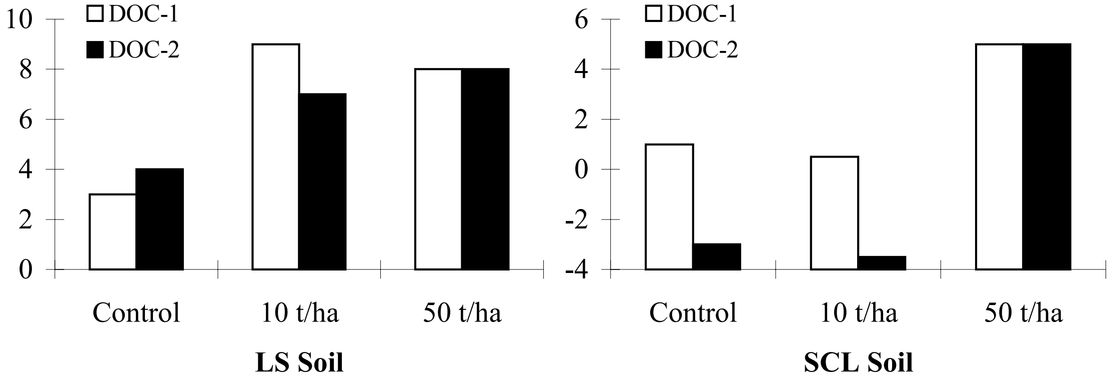


**Figure 7.11:** ***Percentage increase in Zn extractability with DTPA with DOC additions (y-axis: % increase).***



This happened in the SCL soil with low CEC, where elevating concentrations of DOC can be more effective in competing with adsorption sites for Zn. In all the other control treatments extracted by $CaCl_2$ and DTPA, the Zn concentrations did not increase significantly, just like the plant response. This was also evident for Cd in $CaCl_2$, DTPA and plant concentration. Like in the case of Cd and Ni, $CaCl_2$ predicted plant availability more precisely than DTPA. At 10 t ha$^{-1}$ in the LS soil there was an increase between DOC-0 and DOC-2 from 206.3 to 294.5 μg kg$^{-1}$, which was similar to the increase in the SCL soil.

In the SCL soil the increase was of from 183.9 to 266.7 μg kg$^{-1}$. At 50 t ha$^{-1}$, on the other hand, in the LS soil the difference between DOC-0 and DOC-2 increased more than at 10 t ha$^{-1}$ (277.6 to 416.0 μg kg$^{-1}$). This was similar to the increase in the plant concentration at 50 t ha$^{-1}$. In the SCL soil, at 50 t ha$^{-1}$, however, the increase was not greater than that in the 10 ha$^{-1}$ treatment (503.0 to 572.4 μg kg$^{-1}$), between DOC-0 and DOC-2. This smaller increase was similar to the one that took place in the plant Zn concentration. The same trends were observed for Cd as well. Thus, this behaviour may be explained in the same way as the plant concentrations of Zn, that is, due to the effect of the competition between adsorptive sites on organic matter and DOC for the sorbing of Zn. DTPA also followed these trends, but not so precisely. In the LS soil at 10 t ha$^{-1}$ DOC increased the Zn extractability (from 4.00 to 4.31 μg g$^{-1}$), as DOC increased from DOC-0 to DOC-2. At 50 t ha$^{-1}$, as in $CaCl_2$, this increase was greater (from 14.35 to 15.58 μg g$^{-1}$). In the SCL soil, however, at 50 t ha$^{-1}$ the increase was slower, (17.55 to 18.49 μg g$^{-1}$) between DOC-0 and DOC-2.

It can be seen for Zn as with Cd and Ni, that DOC increased the Zn extractability from the soils, especially when the sludge application rate was higher. The presence of greater quantities of metals resulted in higher mobilisation rates caused by the additions of DOC in the soils. Moreover, in the SCL soil, where the situation differed, the solid phase supplied more available sorption sites and competed more effectively with DOC for Zn and other elements. Nevertheless, DOC additions still increased the mobility of Zn even under these circumstances.

### 7.3.8 Soil-Plant Transfer Coefficients

Cadmium availability as indicated by Transfer Coefficients (Table 7.13) increased in all DOC treatments and in both soils with the addition of sewage sludge up to 10 t ha$^{-1}$. From 10 t ha$^{-1}$ to 50 t ha$^{-1}$ there was an increase in mobility in only half of the treatments, while in the other half there was a decrease. A decrease was observed in the DOC-0 treatment in the LS soil and in the DOC-1 and DOC-2 treatments in the SCL soil. Overall, however, there was an increase in uptake with 50 t ha$^{-1}$ giving a slightly higher mobility than 10 t ha$^{-1}$, 50 t

**Table 7.13:** ***Transfer coefficients for Cd, Ni, Pb and Zn.***

| | LS Soil | | | SCL Soil | | |
|---|---|---|---|---|---|---|
| | Cd | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 0.008 | 0.016 | 0.033 | 0.101 | 0.182 | 0.238 |
| 10 t ha$^{-1}$ | 0.533 | 0.480 | 0.604 | 0.505 | 0.729 | 0.986 |
| 50 t ha$^{-1}$ | 0.442 | 0.503 | 0.836 | 0.713 | 0.696 | 0.748 |
| | Ni | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 0.018 | 0.047 | 0.138 | 0.216 | 0.494 | 0.185 |
| 10 t ha$^{-1}$ | 1.128 | 1.888 | 1.271 | 1.189 | 1.888 | 1.355 |
| 50 t ha$^{-1}$ | 1.843 | 1.975 | 2.364 | 2.407 | 2.062 | 2.943 |
| | Pb | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 0.001 | 0.006 | 0.014 | 0.005 | 0.018 | 0.011 |
| 10 t ha$^{-1}$ | 0.045 | 0.058 | 0.087 | 0.046 | 0.037 | 0.056 |
| 50 t ha$^{-1}$ | 0.084 | 0.084 | 0.163 | 0.083 | 0.071 | 0.075 |
| | Zn | | | | | |
| | DOC-0 | DOC-1 | DOC-2 | DOC-0 | DOC-1 | DOC-2 |
| Control | 0.008 | 0.002 | 0.019 | 0.014 | 0.075 | 0.039 |
| 10 t ha$^{-1}$ | 0.444 | 0.608 | 0.544 | 0.565 | 0.742 | 0.923 |
| 50 t ha$^{-1}$ | 1.324 | 1.410 | 1.715 | 1.638 | 1.865 | 1.846 |

ha$^{-1}$ having mean Transfer Coefficient (TC) values of 0.65 and 10 t ha$^{-1}$ 0.63. This shows that the addition of sewage sludge may act both as a source and a sink of Cd, because it supplies Cd to the soil system and it adsorbed it with the organic matter borne in sewage sludge. In the case of Ni, Pb and Zn, however, there was a steady increase as the sludge rate increased. The sum of TC values for all three treatments of DOC for both soils was 1.09 in the control, 8.72 in the 10 t ha$^{-1}$ and 13.59 in the 50 t ha$^{-1}$ treatment for Ni, while for Pb the TC values were 0.05, 0.32, 0.55 respectively and for Zn 0.15, 3.82 and 9.79 for the control, 10 and 50 t ha$^{-1}$ treatments respectively. However, the differences between 10 and 50 t ha$^{-1}$, if the control is subtracted, were always a lot less than a factor of 5, which leads again to the conclusion that organic matter borne in sewage sludge was responsible for strongly sorbing these metals in the soil solid phase. Hooda and Alloway (1993) also recognised this fact.

As DOC levels increased, the availability of all 4 metals increased as well. When the 2 soils were combined, the sums given for all 3 rates of sewage sludge, the TC values were for Cd 2.30 at DOC-0, 2.60 at DOC-1 and 3.44 for DOC-2. For the same levels of DOC, Ni TC values were 6.79, 8.35 and 8.25. For Pb the TC values were 0.26, 0.27 and 0.40 respectively and for Zn the values were 3.99, 4.70 and 5.08. For all 4 elements there was a significant increase as DOC levels increased, which indicates that DOC was responsible for mobilising heavy metals in the soil system, when metals have been applied with sewage sludge. The increase in metal mobility was 49 % for Cd, 21 % for Ni, 53 % for Pb and 27 % for Zn. The steep increase of 53 % which was observed for Pb was at first surprising, because

Pb is considered to be a relatively immobile heavy metal. It should be borne in mind that at DOC-0 and at the control sludge treatments plants took up very small quantities of Pb. As DOC was added, and plants increased their production and they were able to absorb available Pb, which had been inaccessible previously. It was characteristic that the concentrations of Pb increased significantly in the control sludge treatments from DOC-0 to DOC-2 and this increase was reflected in the TC values. In other words, the 53 % increase in Pb mobility was probably not due to Pb being mobilised into the soil solution, but rather due to plants being able to absorb Pb when higher DOC levels were applied at the control sludge treatments.

Although the overall mobility shows that Ni was the most mobile and Cd the least mobile element, we can see that in terms of increased mobility as DOC levels increase, Cd was the most readily mobile and Ni the least, with Zn being in the middle in both cases.

When all DOC and sewage sludge treatments for both soils were combined together, sums of the TC values gave 8.35 for Cd, 23.41 for Ni, 0.94 for Pb and 13.78 for Zn. That is, the order of mobility was Ni > Zn > Cd > Pb. The reason for Cd being only in the third position in this sequence may be put down to weak plant uptake of this element in the control treatments, because the soils themselves had very low concentrations of Cd to begin with (0.37 and 0.10 $\mu g\ g^{-1}$ for the LS and SCL soil respectively). On the other hand, Ni started with 12.47 and 9.09 $\mu g\ g^{-1}$ and Zn with 32.08 and 42.68 $\mu g\ g^{-1}$ for the LS and SCL respectively. Nickel and Zn did not show such a large increase in mobility as Cd, probably because there were greater concentrations of these two metals in the soils and, thus, relatively high uptake levels in the control treatments.

## 7.4 DISCUSSION

Ideally, the DOC extracts should have been dialysed to remove $Na^+$ ions, but it was thought that their concentration was not high enough to have a marked effect on the soil organic matter, nor on the behaviour of the heavy metals.

DOC had a significant effect on metal mobility and availability to plants. Increasing DOC additions in the soils which had previously received 0, 10 and 50 t $ha^{-1}$ DM sewage sludge treatments, enhanced the extractability and uptake of metals significantly. Over a period of 6 weeks the plants were watered with ultra pure water, a solution with 285 mg $L^{-1}$ DOC and a solution with 470 mg $L^{-1}$ DOC. The additions of DOC would have affected the microbial activity and chemical reactions in the soil system and it is probable that metal-organic complexes became predominant. In this experiment, the pH values were around neutrality. Naidu and Harter (1998) found that at neutral pH, water soluble organic acids competed for heavy metals with solid surfaces, because both organic ligands and surfaces bear a negative charge which attracts metal cations. Thus, in systems with high concentrations of DOC, ligands compete more effectively for metals and reduce metal adsorption on soil surfaces

Reddy *et al.* (1995) evaluated the effect of DOC-$M^{2+}$ complexation on $M^{2+}$ activities in the soil solution, in soils which varied in DOC concentration from 11 to 57 mg $L^{-1}$ (which were very similar to the conditions in this experiment, where DOC concentration ranged from 23 to 42 mg $L^{-1}$). They found that Zn and Cu activities were greater in the presence of DOC, with the soils containing high DOC having as much as 95 % of their ionic species complexed with DOC. They also predicted that at neutral pH values, metal-DOC complexes were predominant and responsible for metal solubility, while at acidic pH values metal-ionic forms or metal-ion pairs were predominant. As the pH values were neutral in this study, it is probable that additions of DOC became the main factor affecting metal chemistry. It is known that metal-organic complexes affect metal solubility. Harter and Naidu (1995) state there are 3 options for the solubility of organic ligands :

1. They compete with sorption sites for metals and reduce the availability of the adsorption sites,
2. they interact non-ionically (H-bonding or Van-der-Waals forces), or
3. they create organo-metallic bridges which are fixed on the solid surfaces.

The same authors also stated that the first mechanism leads to increased mobility of the metals, while the other two lead to reduced mobility.

It is likely that under the experimental conditions in the work reported here, the DOC compounds became dominant and affected metal mobility. The data support the first mechanism in which DOC affected the chemistry of metals in the soils. The DOC, therefore, increased metal mobility in the soil solution, especially of those metals which are reported to be more mobile (Cd, Ni and Zn). From the TC data the order of increased mobility of the metals was Cd > Zn > Ni, which is the order of relative affinity of metals for organic substances (Spark *et al.*, 1997a). In this sequence, the TC values of Pb were not included, because, as explained in the relevant section, it is likely that Pb mobility did not increase due to the enhancement of Pb solubility by DOC.

Given that DOC affects metal solubility and mobility in soils, this may have important environmental implications. Lamy *et al.* (1993) explained these implications by saying that DOC may be synthesised under natural conditions, such as from root exudates and decomposition of organic matter. In their experiment they applied 11 t $ha^{-1}$ of sewage sludge in France and found that DOC from the sludge caused a reduction in the retention of Cd in these soils at pH values of 5 to 7. The significance of this was that most of the agricultural soils have a pH range similar to that of this work, and this implies risks for heavy metal accumulation in food crops and possibly to movement down the soil profile.

There is a lot of published work that deals with the way DOC affects the mobility of heavy metals, and most of it suggests that the creation of stable metal-organic ligand complexes keeps the metals in the soil solution. Davis (1984) found that DOC was more effective in reducing metal availability at pH values higher than 6.3, while below that value DOC was found to increase metal availability. Elliott and Denneny (1982) evaluated synthetic DOC compounds, acetic, oxalate and nitrilotriacetate (NTA) acids and EDTA in the complexation of Cd. Cadmium adsorption on soils was reduced and the mechanisms suggested were the same as those discussed here. Neil and Sposito (1986) performed adsorption experiments on 6 different soils where liquid sludge had been applied and where low Cd concentrations were present in solution (around 0.20 μg $L^{-1}$). These were similar to the Cd concentrations in the soil solution in this experiment, where the extractant 0.05 M $CaCl_2$ (which extracts soluble forms of Cd) extracted around 0.20 μg $L^{-1}$ in the control treatments. They found that Cd adsorption was depressed and ascribed it to soluble organics from the sludge. Only at application rates of 500 t $ha^{-1}$ (liquid sludge) did Cd adsorption increase again. The fact that the sewage sludge can cause the increase of DOC in the soils was also recognised by Singh and Pandeya (1998), who found that the complexes of Cd with fulvic acids were positively correlated with the soil organic matter content and the CEC value of the soil. Xu *et al.* (1989) found that fulvic acids up to 50 μg $mL^{-1}$ introduced in the system depressed Cd adsorption on $Al_2O_3$. Baham and Sposito (1994) also found that when DOC concentrations increased, Cu-DOC stable complexes were created and remained soluble. They also found that DOC did not have the same effect on Cd solubility. Moreover, Prasad and Sarangthem (1993) and Jardine *et al.* (1989) also reported that Zn and Co were kept in the solution in the presence of DTPA and EDTA. In all these studies, there were no investigations of metal mobility based on plant availability, thus, there is a lack of data on this topic.

Krishnamurti *et al.* (1997) used 5 synthetic organic acids of low molecular weight, which represent DOC compounds: acetic, citric, fumaric and succinic acids at rather high concentrations of 100 and 1000 mg $L^{-1}$. They also used an availability index (1 M $NH_4Cl$) and evaluated Cd mobility in the A horizons of 3 soils. They found that the $NH_4Cl$-extractable Cd increased after reaction with organic acids, and therefore predicted that chelation of Cd with low molecular weight acids may change the equilibrium between the solid phase and solution and may increase the release of a mobile metal into the soil solution. They also said that it was possible that this may lead to increased plant uptake, which in turn will establish a diffusion gradient to transport more chelated metals towards the root surface. Harter and Naidu (1995) also said that because DOC forms soluble complexes with metals, this may have the effect of increasing the availability of these metals. The enhanced mobility of Cd, Ni, Pb and Zn in this study, affected by DOC, was evident from the soil extractions and the studies on plant concentration and uptake.

Both DTPA and $CaCl_2$ extracted significant increasing quantities of all 4 metals as DOC increased. DTPA is an organic ligand which can extract organically bound metals (Bailey *et al.*, 1995) and has been successfully used along with $CaCl_2$ as an availability index for metals (Jackson and Alloway, 1991). $CaCl_2$, on the other hand, is a lot more sensitive than DTPA, as it extracts soluble or readily exchangeable metals and it gives a clear picture of what happens in the soil solution, which is necessary in order to evaluate the role of DOC. In this study, this prediction was confirmed. Plant concentration and uptake were significantly increased for all metals except Pb. This was mostly evident in the SCL soil, where the high organic matter content was probably the reason that Pb did not increase with DOC treatments. The DOC effect was greater at higher sewage sludge rates for the LS soil for Cd, Ni and Zn, probably because at 50 t $ha^{-1}$ the supply of metals could continue to form stable ligands with DOC and solid phases could not compete so effectively for metals with DOC. In the SCL soil, though, with a greater content of organic matter and clay, and, thus, a higher CEC than the LS soil, the higher application of sewage sludge (50 t $ha^{-1}$) contributed sorptive sites to the solid phase and this depressed the rate of increase of Cd and Zn as DOC additions increased. These processes were reflected in the extractants used to predict plant uptake, with $CaCl_2$ being more precise in assessing the availability than DTPA.

The formation and composition of DOC is probably very complex due to the dynamic nature of microbial decomposition of organic substances in soils. It was not possible to include any microbial investigations in this project, but it is recognised that using solutions containing DOC and an associated population of micro-organisms will have given rise to complex interactions in the treated soils. Nevertheless, consistent trends were observed which can be interpreted as an indication of the importance of the DOC on metal mobility.

## 7.5 CONCLUSIONS

Increasing concentrations of DOC resulted in increasing metal extractability from the soils and elevated metal concentration and uptake from the plants. As sewage sludge application increased, this effect was facilitated and accelerated in the LS soil for Cd, Ni and Zn. In the SCL soil, where the solid phase has a stronger effect on solubility due to higher CEC, the rate of increase in plant availability was less at 50 than at 10 t $ha^{-1}$, for all metals except Ni. Lead, being the least mobile element of all 4, at 10 and 50 t $ha^{-1}$ was not affected by DOC.

Both of the availability indices used, 0.05 M $CaCl_2$ and DTPA, were useful in predicting metal availability to plants, but $CaCl_2$ followed the plant concentration trends more closely.

Elevated concentrations of DOC up to 470 mg $L^{-1}$ resulted in increased metal mobility in the soils . The order of mobility for all treatments and both soils was: Ni > Zn > Cd > Pb. However, in terms of increased mobility from DOC-0 to DOC-2 the sequence was: Cd > Zn > Ni > Pb (excluding TC values of Pb from the control sludge treatments).

As DOC may be the product of a variety of microbial degradation processes, acting upon a variety of substances including root exudates and sewage sludge, the environmental implications are important as sludge is applied to agricultural soils with pH 5-7 (which is the optimum range for DOC activity). Although DOC is short-lived, its dynamics may have an important influence in the chemical reactions in a wide range of sludge-amended agricultural soils, especially at high sewage sludge application rates.

# CHAPTER 8: SUMMARY

## 8.1 General Conclusions

The aim of this project was to investigate the mobility and availability of heavy metals (Cd, Ni, Pb and Zn) to plants in sewage sludge-treated soils. Particular attention was drawn to the role of key soil properties such as pH and organic matter (indigenous and sludge-borne) and to climatic conditions (temperature and rainfall) on the chemical behaviour of heavy metals. Four experiments were carried out, whose broad objective was to study the above effects. Three of them dealt with heavy metal availability to plants, while the fourth studied the mobility of heavy metals down in the soil profile.

The first three experiments comprised a long-term pot experiment, which lasted for two years, a field-based experiment, which lasted for one year, and another -shorter- pot experiment, which investigated the effect of Dissolved Organic Carbon (DOC) on metal availability to plants. In the experiment based in the field, the objective was to compare the results obtained from the greenhouse-based studies to a larger scale ‘open air’ experiment.

With the addition of sewage sludge to the soils, the total heavy metal concentrations increased significantly. As expected, the organic matter content of the soils increased as well, and subsequently the adsorptive capacity of the soils increased as well. This affected the behaviour of heavy metals very significantly. This meant that in the growth experiments the availability of heavy metals to ryegrass did not increase linearly, and this was evident from the TC values in all 3 growth experiments, *ie* the TC values increased much less than a factor of 5 between 10 and 50 t ha$^{-1}$ (heavy metal concentrations increased by a factor of 5 between 10 and 50 t ha$^{-1}$, but their availability in most cases increased only 1-2 times between 10 and 50 t ha$^{-1}$, as it is evident from the TC values). This effect was also evident in the leaching experiment (see Table 6.12 and the subsequent discussion). Mahler *et al.* (1987) spiked with Cd both sludge-amended (with a long history of sludge application of 5 to 30 years) and control soils and found that in the sludged soils Cd availability to plants increased less than in the control soils. The difference was even evident between sludged soils with higher and lower organic matter content (where it was assumed that sludged soils had lower organic matter content due to decomposition). In other words, soils with higher organic matter contents, derived mainly from sewage sludge, caused Cd to be less mobile than soils where organic matter was decomposed. This finding shows the significance of organic matter in reducing the availability of heavy metals. In sludge-treated soils, sludge can be the both the source and the ‘sink’ of heavy metals. In this work, the mobility of each heavy metal was measured with the Transfer Coefficient factor (TC). In all growth experiments, it was found that the TC was higher with the higher application of sludge (50 t ha$^{-1}$) than in the low (10 t ha$^{-1}$) but this increase was much less than 5-fold.

Another finding which indicates the protective role of the organic matter, is the fact that in the growth experiments its decomposition patterns dictated the rate of heavy metal uptake to plants. In the long-term pot experiment, the organic matter was decomposed slightly (but significantly) faster at 25 $^0$C than at 15 $^0$C. As a result, metal availability was higher at 25 $^0$C than at 15 $^0$C. Metal availability was assessed both by measuring the metal concentrations of plant shoots and by extracting the soils with $CaCl_2$ and DTPA solutions (however, DTPA extractions did not follow the treands of plant uptake and $CaCl_2$). Although there was a possibility that the difference in uptake between the two temperatures may have been due to faster water absorption of the plant (due to faster transpiration), there is evidence that this was not the critical factor that affected the differences in metal availability. Soil extractions with $CaCl_2$ indicated that heavy metals were indeed more mobile at 25 $^0$C. In the field experiment, on the other hand, organic matter was not decomposed significantly until Week 48. As a result, heavy metal availability was a lot more stable over time and this was evident both in the metal concentrations accumulated by ryegrass and in the soil extractions with DTPA.

The water soluble part of organic matter, DOC, was also a very important factor affecting the solubility of heavy metals. The short-term pot experiment showed that DOC, when present in soils at elevated concentrations, increases heavy metal availability to plants significantly. This increase was further enhanced at high rates of sewage sludge application. The leaching of heavy metals was also affected significantly by the presence of DOC. Results from the leaching experiment showed that heavy metals moved in the soil columns down to 10 cm. Although beyond 10 cm there was no evidence of metal movement, the leachates were enriched by transported heavy metals. Because DOC was also found in the leachates, and both heavy metal and DOC concentrations in the leachates increased with the higher application of sludge and rainfall rate, it was suggested that the movement of heavy metals was facilitated by the presence of DOC which derived from the sewage sludge. Richards *et al.* (1998) also found that heavy metals moved well beyond the soil incorporation zone, and were found in significant amounts in leachates, without, however, being readsorbed on the surfaces of the subsoil. They also suggested that the latter was strong evidence that water-soluble organo-metallic complexes were responsible for this transport.

Since DOC may be the product of either decomposition of organic matter or root exudates, in both the long-term pot experiment and the field experiment the DOC content was measured at the beginning and at the end of these experiments. In the pot trial, DOC increased in the CL soil, but slightly decreased in the LS soil. This indicated that the decomposition of organic matter contributed to the increase of the DOC content; in the LS soil, however, it was probably leached by the continuous supply of irrigation water, while in the CL soil (with a higher clay and organic matter content) the DOC was retained in the soil more easily and its increased concentration was more evident. This had a profound impact on the availability of heavy metals: availability of Cd and Zn (Cd and Zn extracted with $CaCl_2$) increased over time more in the CL soil than in the LS soil. On the other hand, in the field trial, although the DOC concentration increased over time, this increase did not result in any significant effect on metal availability to the crop. There are two probable reasons that could explain this. Firstly, the organic matter content only decreased significantly in Week 48 and this buffered the metal activity in the soils. Secondly, there were other uncontrolled factors that affected greatly the availability of heavy metals (such as the weather and the season of crop cuttings); these factors seemed to have overshadowed the effect of DOC to heavy metals.

The addition of sewage sludge to soils in the growth experiments not only increased the organic matter content but it also affected the pH of the soils. In all three growth experiments, pH decreased in the higher application of sewage sludge. Tsadilas *et al.* (1995) suggested that sewage sludge tends to buffer the soil pH to around neutrality. This may be the case in this work, since all control soils were slightly alkaline. This shift of pH may have helped in an enhancement in metal availability at 50 t $ha^{-1}$, but it did not have any significant contribution in the changes in availability over time, because the pH remained rather unchanged after an initial equilibrium time of 4 weeks until the end of the experiments.

Climatic conditions played a key role in heavy metal mobility in soils and their availability to plants. In the long-term pot experiment, at 25 $^{0}$C, metal availability was significantly enhanced and this was evident from both the plant uptake and the $CaCl_2$ extractions. Although this may have happened due to the faster growth rates of the plants at 25 $^{0}$C, the increase in heavy metal availability was probably due to the faster decomposition of organic matter in the soil-sludge mixtures at that temperature. In the same experiment, the TC values (Table 4.3) were remarkably higher at 25 $^{0}$C than at 15 $^{0}$C for all four metals and the difference was further enhanced in the higher application rates of sewage sludge. This is very important, since it suggests that the vulnerability of the sludged soils increase in areas with higher annual temperatures. In the field experiment, results from both England and Greece showed that the TC values were very similar. However, the TC value for Zn in Greece was slightly higher than that in England, and this may be an indication of the role of different climatic conditions (and particular temperature) in the availability of heavy metals.

On the other hand, in the leaching experiment, the effect of two rates of annual rainfall was tested. The rate of 900 mm $a^{-1}$ did not significantly increase the movement of Cd, Ni and Zn in all treatments down in the soil profile when compared with the 450 mm $a^{-1}$ rate.

In this project, it was found heavy metal availability can depend on soil characteristics. In the long-term field experiment, a CL soil and a LS soil were used. Although the CL soil had a greater concentration in heavy metals (and both soil extractions and plant concentrations in metals were significantly higher than in the LS soil) the relative mobility of heavy metals was increased in the LS soil more than in the CL soil. In other words, although the TC values for the CL soil were higher than for the LS soil, the difference was much less than the difference in the total heavy metal concentrations in the soils.

Over the residual time of 2 years, the availability of heavy metals (soil extractability and plant concentrations) increased at 25 $^0$C in the first year and reached a plateau in the second year. At 15 $^0$C, the availability of metals decreased, probably due to the fact that the plants grown at that temperature had a slower growth rate than those at 25 $^0$C and thus were unable to absorb greater concentrations of heavy metals.

Both $CaCl_2$ and DTPA proved to be reliable reagents for predicting heavy metal availability in soils, but $CaCl_2$ followed plant uptake data more closely, in both pot experiments. On the other hand, in the field experiment, extractions of heavy metals with DTPA gave a more accurate prediction of the plant uptake.

Cadmium was the most mobile element in the long-term pot experiment. In this experiment, the order of reduced mobility was: Cd > Zn > Ni > Pb. The mobility of Cd, Ni, Pb and Zn depended on the ambient temperature (the mobility increased with temperature), sewage sludge application rates and soil type. In the field based experiment, Zn and Cd were the most mobile elements in the study and Pb was the least (the order of reduced mobility was: Zn > Cd > Ni > Pb). The reason for Zn being more mobile than Cd was probably the fact that Cd concentrations in the sewage sludge was much less than in the pot experiment. This increased the competition between the two metals, which was beneficial for Zn. Zinc was more mobile at Vardates than at Sonning, although the difference was marginal (Transfer Coefficient values of 0.27 and 0.25, respectively). This, however, gives some evidence that warmer weather conditions may enhance heavy metal mobility, as it was predicted in the pot experiment.

No toxicity symptoms were apparent in ryegrass in all three growth experiments, even at the highest application rates of sewage sludge (50 t $ha^{-1}$). Yields increased with the increase in sludge application. In the field experiment, the plant yield increased with the application of sewage sludge and affected heavy metal uptake by the test crop. The fact that yield may affect metal uptake was predicted in the pot experiment, where plants at 15 $^0$C with lower yield had lower metal uptake as well.

Increasing concentrations of DOC resulted in increasing metal extractability from the soils and elevated metal uptake from the plants. As sewage sludge application increased, this effect was further facilitated and accelerated in the LS soil for Cd, Ni and Zn. Lead, being the least mobile element of the four investigated, was not affected by DOC. The environmental implications of increasing concentrations of DOC in sludge-treated soils are very important (DOC may increase in soils with a variety of natural processes, including decomposition of organic matter). Sludge is applied to agricultural soils where pH is around 5-7 (which is the optimum range for DOC activity) and although DOC is short-lived, its dynamics may increase the availability of heavy metals in a wide range of sludge-amended agricultural soils, especially at high sewage sludge application rates. DOC may also increase the risks of heavy metals being solubilised and leached in the soil profile, especially at high sludge application rates and water application regimes. The effect of DOC on metal mobility may be further enhanced in places where the climatic conditions (high rainfall and warm temperatures) encourage such increases.

In the leaching experiment the soil columns were repacked, which means that the macropores of the soil were not present. Although the movement of heavy metals should be expected to be minimal, Cd, Zn and Ni were found in significantly elevated quantities down to a depth of 10 cm. This movement was affected by the sewage sludge application rate and in some treatments in case of Ni and Zn by the water application rate and the duration of the leaching. Cadmium, Ni and Zn were eluted in the leachates, and their concentrations depended on the sludge application rate and the water rate (the greater the sludge and the water application rate, the larger the amounts of metals eluted in the leachates). Lead, being a relatively immobile element, only moved to 2 cm in the soil columns.

The results seem to suggest that DOC was a factor which facilitated the movement of heavy metals in the soil profile. DOC was found in the leachates in elevated amounts, and its elution depended on the same factors as those that affected the elution of Cd, Ni and Zn (namely, the application rate of water and the addition of sewage sludge). Moreover Ni, Zn and DOC were present in the leachate in concentrations that did not decline over the course of 3 years' equivalent rainfall. This further suggests that the water soluble organic matter was responsible for the continuous high solubility of metals in the leachates. It was evident that the added sewage sludge on the top of the soil columns was responsible for the increase in DOC concentration in the leachates. This may be of environmental significance when policies concerning sewage sludge application to land are decided. In areas where sandy soils are predominant (in this experiment an LS soil was used) and high rainfall rates occur, heavy application of sewage sludge may increase the risk of heavy metals contaminating the ground water. The vulnerability of ground water should not only be assessed in terms of the pH status of the soil (in this experiment, pH was slightly alkaline), but it should be based on other environmental factors as well.

## 8.2 Needs for Future Work

DOC was found to greatly influence the mobility of heavy metals in sewage sludge-treated soils. However, closer studies of the characteristics of individual water soluble compounds of low molecular weight (that DOC consists of) are necessary. These compounds have different solubility characteristics and different abilities of binding certain heavy metals, and this may have an important effect in the chemical behaviour of metals in a soil amended with sewage sludge. Chromatography studies may be needed to investigate which organic functional groups evolve from the mineralisation of organic matter, and whether the decomposition patterns in different temperature regimes significantly affect the evolution of different water soluble organic compounds. Moreover, it would be necessary to study the change over time in composition of DOC after sludge has been applied to soils, because this change in composition may, in turn, affect the availability of heavy metals to plants over time.

Many similarities were found between the long-term pot experiment and the field experiment, but there were also differences in heavy metal behaviour. These were probably caused by uncontrolled parameters in the open-air experimental conditions. However, longer-term field studies are needed if more definite conclusions about heavy metal availability are to be drawn. Bearing in mind that there has been an unresolved debate over the past 20 years on whether metal availability in sludged soils decreases or increases over time, more data and evidence would be important in assessing which hypothesis is more valid. This will be a matter of increasing concern in the near future, because greater quantities of sewage sludge will need to be applied on agricultural lands (due to the ceasing of the sludge disposal in the sea at the end of 1998). Therefore, more evidence will be needed to assess the risks from the land application of sludge.

It would be important to investigate whether DOC increases metal movement down the soil profile under field conditions in sewage sludge-amended soils. In sludged soils which are drained with pipes, the drained water could be sampled and analysed for DOC and heavy metal concentrations. The same could be done in soils with different rates of sludge application, including a control (no sludge). This would give evidence on the role of dissolved organic compounds on metal mobility in field conditions, and subsequent risks could be assessed. This would have major effect in drawing conclusions on environmental issues, especially for assessing drinking water regulations imposed by public health authorities.

## REFERENCES

Alloway, B.J. 1995. Soil processes and the behaviour of metals. In: Alloway, B.J. (Ed.) *Heavy Metals in Soils*. Blackie Academic and Professional, London, pp. 38-57.

Antoniadis, V. and Alloway, B.J. 1998. Cd and Zn availability in sewage sludge-treated soils. In: *Proceedings of the 7th Hellenic Soil Science Society Conference*, Agrinio, Greece, pp. 397-407.

Andrewes, P., Town , R.M., Hedley, M.J. and Loganathan, P. 1996. Measurment of plant available cadmium in New Zealand soils. *Australian Journal of Soil Research* **34**, 441-452.

Ardern, D.A. 1977. The agricultural use of the municipal sludge. In: *Land Use as a Waste Management Alternative*. Ann Arbor Science, Michigan, pp. 583-601.

Ayuso, M., Hernadez, T., Garcia, C. and Pascual, J.A. 1996. A comparative study of the effect on barley growth of humic substances extracted from municipal wastes and from traditional organic materials. *Journal of the Science of Food and Agriculture* **72**, 493-500.

Baham, J. and Sposito, G. 1983. Chemistry of water-soluble, metal-complexing ligands extracted from an anaerobically-digested sewage sludge. *Journal of Environmental Quality* **12**, 96-100.

Baham, J. and Sposito, G. 1994. Adsorptiom of dissolved organic carbon extracted from sewage sludge on montmorilonite and kaolinite in the presence of metal ions. *Journal of Environmental Quality* **23**, 147-153.

Bailey, V.L., Grant, C.A., Racz, G.J. and Bailey, L.D. 1995. A practical method for assessing Cd levels in soil using the DTPA extraction technique with Graphite Furnace analysis. *Communications in Soil Science and Plant Analysis* **26**, 961-968.

Baird, C. 1995. *Environmental Chemistry*. W.H. Freeman and Company, New York, pp. 347-391.

Baker, D.E., Amacher, M.C. and Doty, W.T. 1977. Monitoring sewage sludge soils and crops for Zn and Cd. In: *Land as a Waste Management Alternative*. Ann Arbor Science, Michigan, pp.261-281.

Balik, J., Tlustos, P., Szokova, J. and Blahnik, R. 1998. The effect of sewage sludge application on the accumulation of mercury in plants. *Rostlina Vyroba* **44**, 267-274.

Barbarick, K.A. and Workman, S.M. 1987. Ammonium-bicarbonate-DTPA and DTPA extractions of sludge-amended soils. *Journal of Environmental Quality* **16**, 125-130.

Barbarick, K.A., Ippolite, J.A. and Westfall, D.G. 1998. Extractable trace elements in the soil profile after years of biosolids application. *Journal of Environmental Quality* **27**, 801-805.

Bascomb, C.L. 1974. Physical and chemical analysis of <2 mm samples. In: Avery, B.W. and Bascomb, C.L. (Eds.) *Soil Survey Laboratory Methods*. Soil Survey Technical Monograph, No 6, Harpenden, pp. 14-42.

Baziramakenga, R. and Simard, R.R. 1998. Low molecular weight aliphatic acid contents of composted manures. *Journal of Environmental Quality* **27**, 557-561.

Beckett, P.H.T. 1989. The use of extractants in studies on trace metals in soils, sewage sludges and sludge-treated soils. *Advances in Soil Science* **9**, 143-176.

Beckett, P.H.T., Warr, E. and Davis, R.D. 1983. Cu and Zn in soils treated with sewage sludge: Their ‘extractability’ to reagents compared with their ‘availaibility’ to plants. *Plant and Soil* **70**, 3-14.

Bell, P.F., James, B.R. and Chaney, R.L. 1991. Heavy metal extractrability in long-term sewage sludge and metal-salt amended soils. *Journal of Environmental Quality* **20**, 481-486.

Bell, P.F., Mulchi, C.L. and Chaney, R.L. 1992. Microelement concentrations in Maryland air-cured tabacco. *Communications in Soil Science and Plant Analysis* **23**, 1617-1628.

Berti, W.R. and Jacobs, L.W. 1996. Chemistry and phytotoxicity of soil trace elements from repeated sewage sludge applications. *Journal of Environmental Quality* **25**, 1025-1032.

Bidwell, A.M. and Dowdy, R.H. 1987. Cadmium and zinc availability to corn following termination of sewage sludge applications. *Journal of Environmental Quality* **16**, 438-442.

Black, S.C. 1991. Data analysis and presentation. In: Hewitt, C.N. (Ed.) *Instrumental Analysis of Pollutants*. Elsevier Applied Science, London, pp. 335-355.

Boddy, L. 1983. Carbon dioxide release from decomposition wood: effect of water content and temperature. *Soil Biology and Biochemistry* **15**, 501-510.

Bolton, K.A. and Evans, L.J. 1991. Elemental composition and speciation of some landfill leachates with particular reference to cadmium. *Water, Air and Water Pollution* **60**, 43-53.

Boss, C.B. and Fredeen, K.J. 1989. *Concepts, Instrumentation and Techniques in Inductively Coupled Plasma-Atomic Emission Spectrometry*. Perkin-Elmer Corporation, Norwalk, USA.

Bragato, G., Leita, L., Figliolia, A. and de Nobili, M. 1998. Effects of sewage sludge pre-treatment on microbial biomass and bioavailability of heavy metals. *Soil and Tillage Research* **46**, 129-134.

Brallier, S., Harrison, R.B., Hennry, C.L. and Dogsen, X. 1996. Liming effects on availability of Cd, Cu, Ni and Zn in a soil amended with sewage sludge 16 years previously. *Water, Air and Soil Pollution* **86**, 195-206.

Brooks, R.R. 1977. Pollution through trace elements. In: Bockris, J.O' M. (Ed.) *Environmental Chemistry*. Plenum Press, New York, pp. 429-476.

Bubb, J.M.and Lester, J.N. 1991. The impact of heavy metals on lowland rivers and the implication for man and the environment. *Journal of environmental Quality* **100,** 207-233.

Bunce, N.J. 1991. *Environmental Chemistry*. Wherz Publishing Ltd, Winnipeg, pp. 203-211.

Bunce, N.J. 1993. *Introduction to Environmental Chemistry*. Wherz Publishing Ltd, Winnipeg, pp. 341-522.

Businelli, M., Gigliotti, G. and Guisquiani, P.L. 1996. Trace-element fate in soil-profile and corn plant after massive applications of urban compost: A 6-year study. *Agrochimica* **40**, 145-152.

Canet, R., Pomares, F., Tarazona, F. and Estela, M. 1998. Sequential fractionation and plant availability of heavy metals as affected by sewage sludge applications to soils. *Communications in Soil Science and Plant Analysis* **29**, 697-716.

Camobreco, V.J., Richards, B.K., Stenhuis, T., Peverly, J.H. and McBride, M.B. 1996. Movement of heavy metals through undisturbed and homogenised soil columns. *Soil Science* **161** 740-750.

Campbell, C.A. 1978. Soil organic carbon, nitrogen and fertility. In: Schnither, M. and Khan, S.U. (Eds.) *Soil Organic Matter*. Elsevier Scientific Publishing Company, Amsterdam, pp. 201-247.

Campbell, J.H. and Evans, R.D. 1987. Inorganic and organic ligand binding of lead and cadmium and resultant implications for bioavailability. *The Science of the Total Environment* **62**, 219-227.

Campbell, D.J. and Beckett, P.H.T. 1988. The soil solution in a soil treated with digested sewage sludge. *Journal of Soil Science* **39**, 283-298.

Carlton-Smith, C.H. and Stark, J.H. 1987. Sites With a History of Sludge Deposition. Interim Report of Field Trials: April 1983-March 1986 (SDA 9166 SLD). WRc Report No DoE 1376-M. WRc Medmenham, Marlow.

Chambers, B. 1997. Science of sewage sludge spreading. *Environmental Protection* **1**, 2.

Chaney, R.L. 1988. Effective utilisation of sewage sludge on cropland in the United States and toxical considerations for land application. In: *Land Application of Sewage Sludge*. Association for the Utilisation of Sewage Sludge, Tokyo, pp.77-105.

Chaney, R.L. and Ryan, J.A. 1993. Heavy metals and toxic organic pollutants in MSW-compost: Research results on phytoavailability, bioavailability, fate, *etc.* In: Hoitink, H.A.J. and Keener, H.M. (Eds.) *Science and Engineering of Composting: Design,* *Environmental, Microbiological and Utilization Aspects*. Renaissance Publications, Worthington, Ohio, pp. 451-506.

Chaney, R.L., Hornick, S.B. and Simon, P.W. 1977. Heavy metal relationships during land utilisation of sewage sludge in the Northeastern USA. In: *Land as a Waste Management Alternative*. Ann Arbor Science, Michigan, pp.283-314.

Chaney, R.L., Bruins, R.J.F., Baker, D.E., Korcak, R.F., Smith, J.E. and Cole, D. 1987. Transfer of sludge-applied trace elements to the food chain. In: Page, A.L., Logan, T.J. and Ryan, J.A. (Eds.) *Land Application of Sludge Food Chain Implications*. Lewis Publishers Inc., Chelsea, Michigan, pp. 67-99.

Chang, A.C., Page , A.L. and Bingham, F.T. 1982. Heavy metal absorption by winter wheat following termination of cropland sludge applications. *Journal of Environmental Quality* **11**, 705-708.

Chang, A.C., Page, A.L., Warneke, J.E., Resceto, M.R. and Jones, T.E. 1983. Accumulation of cadmium and zinc in barley grown on sludge treated soils: A long-term field study. *Journal of Environmental Quality* **12**, 391-397.

Chang, A.C., Page, A.L. and Wernicke, J.E. 1987. Long-term sludge application on cadmium and zinc accumulation in Swiss chard and radish. *Journal of Environmental Quality* **16**, 217-221.

Chang, A.C., Granato, T.C. and Page, A.L. 1992. A methodology for establishing phytotoxicity criteria for chromium, copper, nickel and zinc in agricultural land application of municipal sewage sludges. *Journal of Environmental Quality* **21**, 521-536.

Chang, A.C., Hyun, H. and Page, A.L. 1997. Cadmium uptake for Swiss chard grown on composted sewage sludge-treated field plots: Plateau or time bomb? *Journal of Environmental Quality* **26**, 11-19.

Chaudri, A.M., McGrath, S.P., Giller, K.E., Rietz, E. and Sauerbeck, D.R. 1993. Enumeration of indigenous *Rhizombium leguminosarum* biovar *Trifolii* in soils previously treated with metal-contaminated sewage sludge. *Soil Biology and Biochemistry* **25**, 301-309.

Chefecz, B., Adani, F., Genevini, P., Tambone, F., Hadar, Y. and Chen, Y. 1998. Humic acid transformation during composting of municipal solid waste. *Journal of Environmental Quality* **27**, 794-800.

Chlopecka, A., Bacon, J.R., Wilson, M.J. and Kay, J. 1996. Forms of cadmium, lead and zinc in contaminated soils in Southwest Poland. *Journal of Environmental Quality* **25**, 69-79.

Christensen, T.H. 1984. Cadmium soil sorption at low concentrations: 1. Effect of time, cadmium load, pH and calcium. *Water, Air and Soil Pollution* **21**, 105-115.

Christensen, T.H. 1989. Cadmium soil sorption at low concentrations: 8. Correlation with soil parameters. *Water, Air and Soil Pollution* **44**, 71-21

Christensen, T.H. and Tjell, T.C. 1983. Interpretation of experimental results on cadmium crop uptake from sewage sludge amended soil. In: *Processing and Use of Sewage Sludge*, Brighton.

Christensen, J.B., Jensen, D.L. and Christensen, T.H. 1996. Effect of dissolved organic carbon on the mobility of cadmium, nickel and zinc in leachate polluted groundwater. *Water Research* **30**, 3037-3049.

Chubin, R.G. and Street, J.J. 1981. Adsorption of cadmium on soil constituents in the presence of complexing ligands. *Journal of Environmental Quality* **10**, 225-228.

Cieslinski, G., Neilsen, G.H.. and Hogue, E. J. 1996. Effect of soil cadmium application and pH on growth and cadmium accumulation in roots, leaves and fruit of strawberry plants (*Fragaria ananassa* Duch.). *Plant and Soil* **180**, 267-276.

Council of European Communities. 1986. Council Directive of 4 July 1986 concerning *'The Protection of the Environment and in Particular of the Soil When Sewage Sludge Is Used in Agriculture'* (86/278/EEC). Official Journal of European Communities, No L181/8.

Council of European Communities. 1991. Council Directive of 21 May 1991 concerning *'Urban Waste Water Treatment'* (91/271/EEC). Official Journal of the European Communities, No L 135/40-52.

Cresser, M.S. and Marr, I.L. 1991. Optical spectrometry in the analysis of pollutants. In: Hewitt, C.N. (Ed.) *Instrumental Analysis of Pollutants*. Elsevier Applied Science, London, pp. 99-145.

Davis, J.A.1984. Complexation of trace metals by adsorbed natural organic matter. *Geochimica et Cosmochimica Acta* **48**, 679-691.

Davis, R.D. 1994. Planning the best strategies for sludge treatment and disposal operations. *Water Science and Technology* **30**, 149-158.

Davis, R.D. 1996. The impact of the EU and UK environmental pressures on the future of sludge treatment and disposal. *Journal of the Chartered Institution of Water and Environmental Management* **10**, 65-69.

Davis, R.D., Charlton-Smith, C.H., Stark, J.H. and Campbell, J.A. 1988. Distribution of metals in grassland soils following surface applications of sewage sludge. *Environmental Pollution* **49**, 99-115.

De Vries, M.P.C. and Tiller, K.G. 1978. Sewage sludge as a soil amendment, with special reference to Cd, Cu, Mn, Pb and Zn- Comparison of result from experiments conducted inside and outside a glasshouse. *Environmetnal Pollution* **16**, 321-240.

Del Castilho, P. and Chardon, W.J. 1995. Uptake of soil cadmium by three field crops and its prediction by a pH-dependent Freundlich sorption model. *Plant and Soil* **171,** 263-266.

Del Castilho, P., Chardon, W.J. and Solomons, W. 1993. Influence of cattle manure slurry application on the solubility of cadmium, copper and zinc in a manured acidic, laomy-sand soil. *Journal of Environmental Quality* **22**, 689-697.

Dowdy, R.H. and Volk, V.V. 1983. Movement of heavy metals in soils. In: *Chemical Mobility and Reactivity in Soil Systems.* Soil Science Society of America and American Society of Agronomy, Madison, pp.229-240.

Dowdy, R.H., Latterel, J.J., Hinesly, T.D., Grossman, R.B. and Sullivan, D.L. 1991. Trace metal movement in an aeric Ochraqualf following 14 years of annual sludge application. *Journal of Environmental Quality* **20,** 119-123.

Dzombak, D.A., Fish, W. and Morel, F.M.M. 1986. Metal-humate interactions: 1. Discrete ligand and continuous distribution models. *Environmental Science and Technology* **20**, 669-675.

El-Hassanin, A.S., Labib, T.M. and Dobal A.T. 1993. Potential Pb, Cd, Zn, and B contamination of sandy soils after different irrigation periods with sewage effluent. *Water, Air and Soil Pollution* **66**, 239-249.

Elliott, H.A. and Denneny, C.M. 1982. Soil adsorption of cadmium from solution containing organic matter. *Journal of Envoronmental Quality* **11**, 658-663.

Elliott, H.A., Liberati, M.R. and Huang, L.P. 1986. Competitive adsorption of heavy metals by soils. *Journal Environmental Quality* **15**, 214-219.

Emmerich, W.E., Lund, L.J., Page, A.L. and Chang, A.C. 1982. Movement of heavy metals in sewage sludge-treated soils. *Journal of Environmental Quality* **11**, 174-178.

Epstein, E., Taylor, J.M. and Chaney, R.L. 1976. Effects of sewage sludge and sludge compost applied to soil on some soil physical and chemical properties. *Journal of Environmental Quality* **5**, 442-426.

Evans, L.J. 1989. Chemistry of metal retention by soils. *Environmental Science and Technology* **23**, 1046-1056

Evans, L.J., Spiers, G.A. and Zhao, G. 1995. Chemical aspects of heavy metal solubility with reference to sewage sludge-amended soils. *International Journal of Environmental and Analytical Chemistry* **59**, 291-302.

Filius, A., Streck, T. and Richter, J. 1998. Cadmium sorption and desorption in limed topsoils as influenced by pH: Isotherms and simulated leaching. *Journal of Environmental Quality* **27**, 12-18.

Flores, L., Blas, G., Hernadez, G. and Alcala, R. 1997. Distribution and sequential extraction of some heavy metals from soils irrigated with waste water from Mexico city. *Water, Air and Soil Pollution* **98**, 105-117.

Forstner, V. 1993. Riverine and estuarine sedimentation of pollutants and leaks from sludges and wastes- Analystical, prognostic and remedial approaches. *Land Degradation and Rehabilitation* **4**, 281-296.

Fotovat, A., Naidu, R. and Oades, J.M. 1996. The effect of major cations and ionic strength on desorption of native heavy metals in acidic and sodic soils. In: 1st International Conference on *'Contaminants in the soil Environment'*, Adelaide, Australia, pp. 193-194.

Fox, T.R. and Comerfield, N.B. 1990. Low molecular weight organic acid in selected forest soils of the South-eastern USA. *Soil Science Society of America Journal* **54**, 1763-1767.

Fresquez, P.R., Francis, R.E. and Dennis, G.L. 1990. Sewage sludge effects on soil and plant quality in a degraded, semi-arid grassland. *Journal of Environmental Quality* **19**, 324-329.

Fujii, R., Henderickson, L.L. and Corey, R.B. 1983. Ionic activities of trace metals in sludge-amended soils. *The Science of the Total Environment* **28**, 179-190.

Gao, S.A., Walker, W.J. Dalgren, R.A. and Bold, J. 1997. Simultaneous sorption of Cd, Cu, Ni, Zn, Pb and Cr on soils treated with sewage sludge supernatant. *Water, Air and Soil Pollution* **93**, 331-345.

Gardiner, D.T., Miller, R.W., Badamchian, B., Azzari, A.S. and Sisson, D.R. 1995. Effects of repeated sewage sludge applications on plant accumulation of heavy metals. *Agricultural Ecosystems and Environment* **55**, 1-6.

Gasiunas, V., Fleckenstein, J. and Schung, E. 1995. Effects of sewage sludge application on heavy metal concentrations of Lithuanian soils. *Landbanforschung Volkenrode* **45**, 1-3.

Gerba, C.P. 1996. Municipal waste and drinking water treatment. In: Pepper, L., Gerba, C.P. and Brussaeu, M.L. (Eds.) *Pollution Science*. Academic Press, San Diego, pp. 301-319.

Gerritse, R.G., Vriesema, R., Daleberg, J.W. and Deroos, H.D. 1982. Effect of sewage sludge on trace element mobility in soils. *Journal of Environmental Quality* **11**, 359-363.

Gerritse, R.G., van Driel, W., Smilde, K.W. and van Luit, B. 1983a. Uptake of heavy metals by crops in relation to their concentrations in the soil solution. In: *Heavy Metals in the Environment*. Proceedings in International Conference, September 1983, Heidelberg. CEP-Consultants, Ediburgh, pp. 1057-1062.

Gerritse, R.G., van Driel, W., Smilde, K.W. and van Luit, B. 1983b. Uptake of heavy metals by crops in relation to their concentrations in the soil solution. *Plant and Soil* **11**, 393-404.

Giglioti, G., Guisquiani, P.L., Businelli, D. and Macchioni, A. 1997. Composition changes of dissolved organic matter in a soil amended with municipal waste compost. *Soil Science* **162**, 919-926.

Gonsior, S.J., Sorci, S.J., Zoellner, M.J. and Landenberger, B.D. 1997. The effects of EDTA on metal solubilization in river sediment/water system. *Journal of Environmental Quality* **26**, 957-966.

Goody, D.C., Shand, P., Kinniburgh, D.G. and van Riemsdijk, W.H. 1995. Field-based partition coefficients for trace elements in soil solutions. *European Journal of Soil Science* **46**, 265-285.

Gray, C.W., McLaren, R.G., Roberts, A.H. and Condron, L.M. 1998 Sorption and desorption of cadmium from some New Zealand soils: Effect of pH and contact time. *Australian Journal of Soil Research* **36**, 199-216.

Gregorich, E.G., Rochette, P., McGuire, S., Liang, B.C. and Lessard, R. 1998. Soluble organic carbon and carbon dioxide fluxes in maize fields receiving spring-applied manure. *Journal of Environmental Quality* **27**, 209-214.

Guisquiani, P.L., Gigliotti, G. and Businelli, D. 1992. Mobility of heavy metals in urban waste-amended soils. *Journal of Environmental Quality* **21**, 330-335.

Guisquiani, P.L., Concezzi, L., Businelli, M and Macchioni, A. 1998. Fate of pig sludge liquid fraction in calcareous soil: Agricultural and environmental implications. *Journal of Environmental Quality* **27**, 364-371.

Gupta, U.C. 1989. Effect of zinc fertilization on a plant zinc concentration of forages and cereals. *Canadian Journal of Soil Science* **69**, 473-479.

Hamon, R.E., Lorenz, S.E., Holm, P.E., Christensen, T.H. and McGrath, S.P. 1995. Changes in trace metal species and other components of the rhizosphere during growth of radish. *Plant and Environment* **18**, 749-756.

Hamon, R., Wundke, J., McLaughlin, M. and Naidu, R. 1997. Availability of zinc and cadmium to different plant species. *Australian Journal of Soil Research* **35**, 1267-1277.

Hani, H., Siegenthaler, A. and Cardinas, T. 1996. Soil effects due to sewage sludge application in agriculture. *Fertilizer Research* **43**, 149-156.

Hansen, L.G. and Chaney, R.L. 1984. Environmental and food chain effects on the agricultural use of sewage sludge. *Reviews in Environmental Toxicology* **1**,103-172.

Hargitai, L. 1989. The role of humus status of soils in binding toxic elements and compounds. *The Science of the Total Environment* **81/82**, 643-652.

Harmsen, K. 1977. *Behaviour of Heavy Metals in Soils*. Centre for Agricultural Publishing and Documentation, Wageningen, pp.5-171.

Harter, R.D. and Naidu, R. 1995. Role of organic-metal complexation in metal sorption by soils. *Advances in Agronomy* **55**, 219-263.

He, Q.B. and Singh, R. 1993. Effect of organic matter on the distribution, extractability and uptake of cadmium in soils. *Journal of Soil Science* **44**, 641-650.

Heckman, J.R., Angle, J.S. and Chaney, R.L. 1987. Residual effects of sewage sludge on soybean: I. Accumulation of heavy metals. *Journal of Environmental Quality* **16**, 113-117.

Higgins, A.J. 1984. Environmental constrains of land application of sewage sludge. *American Society of Agricultural Engineers* **27**, 407-414.

Hooda, P.S. 1992. *The Behaviour of Trace Metals in Sewage Sludge-Amended Soils*. PhD Thesis, University of London, pp 51-52.

Hooda, P.S. and Alloway, B.J. 1993. Effects of time and temperature on the bioavailability of Cd and Pb from sludge-amended soils. *Journal of Soil Science* **44**, 97-110.

Hooda, P.S. and Alloway, B.J. 1994. Changes in operational fractions of trace metals in two soils during two years of reaction time following sewage sludge treatment. *International Journal of Environmental and Analytical Chemistry* **57**, 289-311.

Horan, N.J. 1993. *Biological Wastewater Treatment Systems: Theory and Operation*. John Wiley and Sons, Chichester, UK, pp. 41-52.

Hyun, H., Chang, A.C., Parker, D.R. and Page, A.L. 1998. Cadmium solubility and phytoavailability in sludge-treated soil: Effects of soil organic carbon. *Journal of Environmental Quality* **27**, 329-334.

Imhoff, K., Muller, W.J. and Thristlethwayte, D.K.B. 1971. *Disposal of Sewage Sludge and Other Water-Borne Wastes.* 2nd Edition, Butterworths, London, pp. 102-119.

Jackson, A.P. and Alloway, B.J. 1991. The bioavailabilty of cadmium to lettuce and cabbage in soils previously treated with sewage sludge. *Plant and Soil* **132**, 179-186.

Jarausch-Wehrheim, B., Mocquot, B. and Mench, M. 1996. Uptake and partitioning of sludge-borne copper in field-grown maize (*Zea mays* L.). *European Journal of Agronomy* **5**, 259-271.

Jardine, P.M., Weber, N.L. and McCarthy, J.F. 1989. Mechanisms of dissolved organic carbon adsorption on soil. *Soil Science Society of America Journal* **53**, 1378-1385.

Jardine, P.M., Dunnivant, F.M., Selim, H.M. and McCarthy, J.F. 1992. Comparison of models for describing the transport of dissolved organic carbon in aquifer columns. *Soil Science Society of America Journal* **56**, 393-401.

Jenkinson, D.S. 1988. Soil organic matter and its dynamics. In: Wild, A. (Ed.) *Russell' s Soil Conditions and Plant Growth.* 11th Edition, Longman Scientific and Technical, Essex, pp. 564-607.

Jenkinson, D.S. and Raynes, J.H. 1977. The turnover of soil organic matter in some of the Rothamsted classical experiments. *Soil Science* **123**, 298-305.

Jinadasa, K.B.P.N., Milham, P.J., Hawkins, C.A., Comish, P.S., Williams, P.A., Kaldor, C.J. and Conroy, J.P. 1997. Survey of cadmium levels in vegetables and soils of Greater Sydney, Australia. *Journal of Environmental Quality* **26**, 924-933.

Jing, J. and Logan, T.J. 1991. Chelating resin method for estimation of sludge-cadmium bioavailability. *Communications in Soil Science and Plant Analysis* **22**, 2029-2035.

Jing, J. and Logan, T.J. 1992. Effects of sewage sludge cadmium concentration on chemical extractability and plant uptake. *Journal of Environmental Quality* **21**, 73-81.

Jones, U.S. 1982. *Fertilizers and Soil Fertility*. 2nd Edition. Reston Publishing Company, Reston, Virginia, USA.

Kabata-Pendias, A. and Pendias, H. 1992. *Trace Elements in Soils and Plants*. 2nd Edition, CRC Press, Boca Rator, pp.1-87, 131-141.

Kaizer, K. and Zech, W. 1997. Competitive sorption of dissolved organic matter fractions to soils and related mineral phases. *Soil Science Society of America Journal* **61**, 64-69.

Kalbitz, K. and Wennrich, R. 1998. Mobilisation of heavy metals and arsenic in polluted wetland soils and its dependence on dissolved organic matter. *Science of the Total Environment* **209**, 27-39.

Kirkham, M.B, 1977. Trace elements in sludge on land: effect on plants, soils and ground water. In: *Land as a Waste Management Alternative*. Ann Arbor Science, Michigan, pp. 209-247.

Kloke, A., Sauerbeck, D.R. and Vetter, H. 1984. The contamination of plants and soils with heavy metals in the transpert of metals in terrestrial food chain. In: Nriagu, J.O. (Ed.) *Changing Metal Cycles and Human Health*, pp. 113-141.

Knipers, H. 1991. Agronomic aspects of plowing and non-plowing. *Soil and Tillage Research* **21**, 167-176.

Krauss, M., and Diez, T. 1997. Uptake of heavy metals by plants from highly contaminated soils. *Agribiological Research- Zeitschrift fur Agrarbiologie Agrikulturechemie Okologie* **50**, 340-349.

Krebs, R., Gupta, S.K., Furrer, G. and Schulin, R. 1998. Solubility and plant uptake of metals with and without liming of sludge-amended soils. *Journal of Environmental Quality* **27**, 18-23.

Krishnamurti, G.S.R., Huang, P.M., van Rees, K.C.J., Kozak, L.M. and Rostar, H.P.W. 1995. A new soil test method for the determination of plant available cadmium in soils. *Communications in Soil Science and Plant Analysis* **26**, 2857-2867.

Krishnamurti, G.S.R., Cieslinski, G., Huang, P.M. and van Rees, K.C.J. 1997. Kinetics of cadmium release from soils as influenced by organic acids: Implication in cadmium availability. *Journal of Environmental Quality* **26**, 271-277.

Kuo, S. 1990. Cadmium buffering capacity and accumulation in Swiss chard in some sludge-amended soils. *Journal of Soil Science Society of America* **54**, 86-91.

LaBauve, J.M., Kotuby-Amacher, J. and Gambrell, R.P. 1988. The effect of soil properties and a synthetic municipal landfill leachate on the retention of Cd, Ni, Pb and Zn in soil and sediment materials. *Journal of World Pollution Control Federation*, pp. 379-385.

Lamy, I., Djafer, M. and Terce, M. 1991. Influence of oxalic acid on the adsorption of cadmium at the geothite surface. *Water, Air and Soil Pollution* **57-58**, 457-465.

Lamy, I., Bourgois, S. and Bermond, A. 1993. Soil cadmium mobility as a consequence of sewage sludge disposal. *Journal of Environmental Quality* **22**, 731-737.

Lebourg, A., Sterckman, T., Ciesielski, H. and Proix, N. 1998. Trace metal speciation in three unbuffered salt solutions used to assess their bioavailability in soils. *Journal of Environmental Quality* **27**, 584-590.

Leeper, G.W. 1978. *Managing the Heavy Metals on the Land*. Marcel Dekker Inc., New York.

Lester, J.N. 1996. Sewage and sewage sludge treatment. In: Harrison, R.M. (Ed.) *Pollution: Causes, Effects and Control*. 3rd Edition. The Royal Society of Chemistry, Cambridge, UK, pp. 93-122.

Li, Y.M., Chaney, R.L., Schneiter, A.A. and Johnson, B. 1996. Effect of field limestone applications on cadmium content of sunflower (*Helianthum annuus* L.) leaves and kernels. *Plant and Soil* **180**, 297-302.

Li, Z.B. and Shuman, L.M. 1996a. Heavy metal movement in metal contaminated soil profile. *Soil Science* **161**, 656-666.

Li, Z.B. and Shuman, L.M. 1996b. Redistribution of forms of zinc, cadmium and nickel in soils treated with EDTA. *Science of the Total Environment* **191**, 95-107.

Li, Z.B. and Shuman, L.M. 1997. Mehlich-1 and DTPA extractable lead in soils in relation to soil properties. *Communications in Soil Science and Plant Analysis* **28**, 351-363.

Lindsay, W.L. 1979. *Chemical Equilibrium in Soils*. Wiley Interscience.

Lindsay, W.L. and Norvell, W.A. 1978. Development of DTPA test for zinc, iron, manganese and copper. *Soil Science Society of America Journal* **42**, 421-428.

Lineham, D.J. 1985. Organic matter and trace metals in soils. In: Vaugham, D. and Malcolm, R.E. (Eds.) *Soil Organic Matter and Biological Activity*. Martinus Nijhoff/Dr W. Junk Publishers, Dordrecht, pp. 403-422.

Livens, F.R. 1991. Chemical reactions of metals with humic material. *Environmental Pollution* **70**, 183-208.

Logan, T.J. and Chaney, R.L. 1983. Utilisation of municipal wastewater and sludge on land. In: Page, A.L., Gleason, T.L., Smith Jr J.E., Iskander, I.K. and Sommers, L.E. (Eds.) *Proceedings of the 1983 Workshop on Utilization of Municipal Wastewater and Sludge on Land*. University of California, Riverside, pp. 235-326.

Logan, T.J., Lindsay, B.J., Goins, L.E. and Ryan, J.A. 1997. Field assessment of sludge metal bioavailability to crops: Sludge rate response. *Journal of Environmental Quality* **26**, 534-550.

Lund, L.J., Page, A.L. and Nelson, C.O. 1976. Movement of heavy metals below sewage disposal ponds. *Journal of Environmental Quality* **5**, 330-334.

Luo, Y.M. and Christie, P. 1998. Bioavailability of cadmium and zinc in soils treated with alkaline stabilized sewage sludges. *Journal of Environmental Quality* **27**, 325-342.

Mahler, R.J., Ryan, J.A. and Reed, T. 1987. Cadmium sulfate application to sludge-amended soils: I. Effect on yield and cadmium availability to plants. *Science of the Total Environment* **67**, 117-131.

Mann, S.S. and Ritchie, G.S.P. 1995. Form of cadmium in sandy soils after amendment with soils of higher fixing capacity. *Soil Science and Plant Nutrition* **87**, 23-29.

McBride, M.B. 1980. Chemisorption of $Cd^{2+}$ on calcite surfaces. *Soil Science Society of America Journal* **44**, 26-28.

McBride, M.B. 1995. Toxic metal accumulation from agricultural use of sludge-Are USEPA regulations protective. *Journal of Environmental Quality* **24**, 5-18.

McBride, M.B. 1998. Soluble trace metal in alkaline stabilized sludge products. *Journal of Environmental Quality* **27**, 578-584.

McBride, M.B., Sauve, S. and Hendershot, W. 1997a. Solubility control of Cu, Zn, Cd and Pb in contaminated soils. *European Journal of Soil Science* **48**, 337-346.

McBride, M.B., Richards, B.K., Stenhuis, T., Russo, J.J. and Sauve, S. 1997b. Mobilility and solubility of toxic metals and nutrients in soil fifteen years after sludge application. *Soil Science* **162,** 487-500.

McCarthy, J.F. 1989. Bioavailability and toxicity of metals and hydrophobic organic contaminants. *ACS Symposium Series* **219**, 263-277.

McGrath, S.P. and Cuncliffe, C.H. 1985. A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Cd, Pb, Cr, Co and Mn from soils and sewage sludges. *Journal of Science of Food and Agriculture* **36**, 794-798.

McGrath, S.P. and Loveland, P.J. 1992. *The Soil Geochemical Atlas of England and Wales.* Blackie Academic and Professional, London.

McGrath, S.P., Brookes, P.C. and Giller, K.E. 1992. Effects of potential toxic elements in soil derived from past applications of sewage sludge on Nitrogen fixation by *Trifolium repens* L.. *Soil Biology and Biochemistry* **20**, 415-425.

McLaughlin, M.L., Palmer, L.T., Tiller, K.G., Beech, T.A. and Smart, M.K. 1994. Increased soil salinity caused elevated Cd concentrations in field-grown potato tubers. *Journal of Environmental Quality* **23**, 1013-1018.

McLaughlin, M.J., Maier, N.A., Freeman, K., Tiller, K.G., Williams, C.M.J. and Smart, M.K. 1995. Effect of potassic and phosphatic fertilizer type, fertilizer Cd concentration and zinc rate on cadmium uptake by potatoes. *Fertilizer Research* **40**, 63-70.

McLauhglin, M.J., Tiller, K.G., Naidu, R. and Stevens, D.P. 1996. Review: The behaviour and environmental impact of contaminants in fertilizers. *Australian Journal of Soil Research* **34**, 1-54.

McLaughlin, M.J., Maier, N.A., Rayment, G.E., Sparrow, L.A., Berg, G., Mikay, A., Milham, P., Merry, R.H. and Smart, M.K. 1997. Cadmium in Australia potato tubers and soils. *Journal of Environmental Quality* **26**, 1644-1649.

Merkel, D. 1996. Cd-, Cu-, Pb- and Zn-contents of wheat grain and soils, extracted with $CaCl_2$/DTPA (CAD), $CaCl_2$ and $NH_4NO_3$, respectively. *Agroecological Research-Zeitschrift fur Agrabiologic Agrikulturchemie Okologie* **49**, 30-37.

Miner, G.S., Gutierrez, R. and King, L.D. 1997. Soil factors affecting plant concentrations of cadmium, copper and zinc on sludge-amended soils. *Journal of Environmental Quality* **26**, 989-994.

Moolenaar, S.W. and Beltrami, T. 1998. Heavy metal balances of an Italian soil as affected by sewage sludge and Bordeaux Mixture applications. *Journal of Environmental Quality* **27,** 828-835.

Moreno, J.L., Garcia, C., Hernadez, T. and Pascual, J.A. 1996. Transference of heavy metals from a calcareous soil amended with sewage sludge compost to barley plants. *Bioresource Technology* **55**, 251-258.

Moreno, J.L., Garcia, C., Hernadez, T. and Ayuaso, M. 1997. Application of composted sewage sludges contaminated with heavy metals to an agricultural soil- Effect on lettuce growth. *Soil Science and Plant Nutrition* **43**, 565-573.

Morgan, H. and Alloway, B.J. 1984. The value of chemical extractants for predicting the availability of cadmium to vegetables. In: Hemphill, B.D. (Ed.) *Trace Substances and Environmental Health XVIII*. pp. 539-547.

Mulchi, C.L., Adamu, C.A., Bell, P.F. and Chaney, R.L. 1992. Residual heavy metal concentrations in sludge-amended coastal plain soils: 2. Predicting metal concentrations in tabacco from soil test information. *Communications in Soil Science and Plant Analsis* **23**, 1053-1069.

Naidu, R. and Harter, R.D.1998. Effect of different organic ligands on cadmium sorption by and extractability from soils. *Soil Science Society of America Journal* **62**, 644-650.

Naidu, R., Kookana, R.S., Sumner, M.E., Harter, R.D. and Tiller, K.G. 1997. Cadmium sorption and transport in variable charge soils: A review. *Journal of Environmental Quality* **26**, 602-617.

Nakas, J.P. and Klein, D.A. 1980. Mineralisation capacity of bacteria and fungi from the rhizosphere-rhizoplane of a semiarid grassland. *Applied and Environmental Microbiology* **39**, 113-117.

Neil, R.H. and Sposito, G. 1986. Effects of soluble organic matter and sewage sludge amendment on cadmium sorption by soils at low cadmium concentrations. *Soil Science* **142**, 164-172.

Nogales, R., Gallardo Lara, F. Benitez, E., Soto, J., Hervas, D. and Polo, A. 1997. Metal extractability and availability in a soil after heavy metal application of either nickel or lead in different forms. *Water, Air and Soil Pollution* **94**, 33-44.

O' Connor, G.A. 1988. Use and misuse of the DTPA soil test. *Journal of Environmental Quality* **17**, 715-718.

O' Connor, G.A., Essington, M.E., Elrashidi, M. and Bowman, R.S. 1983. Nickel and zinc sorption in sludge-amended soils. *Soil Science* **135**, 228-235.

O' Neil, P. 1993. *Environmental Chemistry.* 2nd Edition, Chapman and Hall, London, pp. 193-202, 215-221.

O' Riordan, E.G. Podd, V.A. Tunney, H. and Fleming, G.A. 1986a. The chemical composition of Irish sewage sludges: 1. Nitrogen content. *Irish Journal of Agricultural Research* **25**, 223-229.

O' Riordan, E.G. Podd, V.A. Tunney, H. and Fleming, G.A. 1986b. The chemical composition of Irish sewage sludges: 2. Phosphorus, potassium, magnesium, calcium and sodium contents. *Irish Journal of Agricultural Research* **25**, 231-237.

O' Riordan, E.G. Podd, V.A. Tunney, H. and Fleming, G.A. 1986c. The chemical composition of Irish sewage sludges: 3. Trace element contents. *Irish Journal of Agricultural Research* **25**, 239-249.

Obrador, A., Rico, M.I., Mingot, J.I. and Alvarez, J.M. 1997. Metal mobility and potential bioavalability in organic matter-rich soil-sludge mixtures: Effect of soil type and contact time. *The Science of the Total Environment* **206** 117-126.

Oliver, D.P., Tiller, K.G., Connyers, M.K., Sattery, W.J., Alston, A.M. and Merry, R.H. 1996. Effectiveness of liming to minimise uptake of cadmium by wheat and barley grain grown in the field. *Australian Journal of Agricultural Research* **47**, 1181-1193.

Oliver, D.P., Tiller, K.G., Alston, A.M., Cozens, G.D. and Merry, R.H.1998. Effect of soil pH and applied cadmium on cadmium concentration in wheat grain. *Australian Journal of Soil Research* **36**, 571-583.

Palazzo, A.J. 1977. Land application of waste water: Forage growth and utilisation of applied nitrogen, phosphorus and potassium. In: *Land as a Waste Management Alternative*. Ann Arbor Science, Michigan, pp.171-180.

Papadopoulos, P. and Rowel, D.L. 1988. The reactions of cadmium with calcium carbonate surfaces. *Journal of Soil Science* **39**, 23-36.

Papadopoulos, P. and Rowel, D.L. 1989. The reactions of copper and zinc with calcium carbonate surfaces. *Journal of Soil Science* **40**, 39-48.

Pedreno, J.N., Gomez, I., Moral, R. and Mataix, J. 1996. Improving the agricultural value of a semiarid soil by addition of sewage sludge and almond residue. *Agricultural Ecosystems and Environment* **58**, 115-119.

Perkin Elmer. 1976. *Instructions: Model 3030 Atomic Absorption Spectrophotometer*. Norwalk, Connecticut, USA.

Perkin Elmer. 1978. *Instructions: HGA-500 Graphite Furnace Atomic Absoprtion Spectrophotometer*. Norwalk, Connecticut, USA.

Perkin-Elmer. 1995. *The Guide to Techniques and Applications of Atomic Spectrometry*. The Perkin-Elmer Corporation, Norwalk, USA.

Petruzzelli, G., Lubrano, L. and Guidi, G. 1989. Uptake by corn and chemical extractability of heavy metals from a four year compost treated soil. *Plant and Soil* **116**, 23-27.

Petruzzelli, G., Petronio, B.M., Gennaro, M.C., Vanni, A., Liberatory, A. and Barbafieri, M. 1995. Influence of sewage sludge utilisation and disposal on heavy metal mobility in soil-An overview. *Annali di Chimica* **85**, 385-393.

Piccolo, A. 1989. Reactivity of added humic substances towards plant available heavy metals in soils. *The Science of the Total Environment* **81**/**82**, 607-614.

Prasad, B. and Sharangthem, I. 1993. Adsorption of zinc as affected by its sources in calcareous soils. *Journal of Indian Society of Soil Science* **41**, 261-265.

Qiao, L. and Ho, G. 1996. The effect of clay amendment on speciation of heavy metals in sewage sludge. *Water Science and Technology* **34**, 413-420.

Qiao, L. and Ho, G. 1997. The effects of clay amendment and composting on metal speciation in digested sludge. *Water Research* **31**, 951-964.

Ramunhi, A., Scialdone, R., Pignalosa, V. and di Gennaro, A. 1987. Humin susceptibility to microbial decomposition in a mollic vitrandept soil by '*in vitro*' experiments. *The Science of the Total Environment* **62**, 413-417.

Rao, K.J. and Shantaram, M.V. 1995a. Concentrations and relative availability of heavy metals in urban solid wastes of Hyderabad, India. *Bioresource Technology* **53**, 53-55.

Rao, K.J. and Shantaram, M.V. 1995b. Contents of heavy metals in crops treated with urban solid wastes. *Journal of Environmental Biology* **16**, 225-232.

Reddy, K.J., Wang, L. and Gross, S.P. 1995. Solubility and mobility of copper, zinc and lead in acidic environments. *Plant and Soil* **171**, 53-58.

Rhoades, J.D. 1982. Soluble Salts. In: Page, A.L., Miller, R.H. and Keegy, D.R. (Eds.) *Methods of Soil Analysis-Part 2*. 2nd Edition, American Society of Agronomy Inc. and Soil Science Society of America Inc. Publisher, Madison, pp. 181-199.

Richards, B.K., Steenhuis, J.H., Paverly, J.H. and McBride, M.B. 1998. Metal mobility at an old, heavily loaded sludge application site. *Environmental Pollution* **99**, 365-377.

Rowell, D.L. 1994. *Soil Science: Methods and Applications*. Longman, Harlow.

Saar, R.A. and Weber, J.H. 1982. Fulvic acid: Modifier of metal-ion chemistry. *Environmental Science and Technology* **16**, 510-517.

Sadler, P.J., Higham, D.P. and Nickolson, J.K. 1985. The environmental chemistry of metals with examples from studies of the speciation of cadmium. In: Irgolic, K.J. and Martel, A.E. (Eds.) *Environmental Inorganic Chemistry*, VCH Publishers Inc., Florida, pp. 239-271.

Sajwan, K.S., Ornes, W.H. and Youngblood, T. 1995. The effect of fly ash, sewage sludge and application rates on biomass production. *Journal of Environmental Science and Health Part A- Environmental Science and Engineering and Toxic and Hazardous Substance Control* **30**, 1327-1337.

Salam, A.K. and Helmke, P.A. 1998. The pH dependence of free ionic activities and total dissolved concentrations of copper and cadmium in soil solution. *Geoderma* **83**, 281-291.

Sauerbeck, D.R. and Hein, A. 1991. The Nickel uptake from different soils and its prediction by chemical extractions. *Water, Air and Soil Pollution* **57-58**, 861-871.

Sawhney, B.L., Burbee, G.J. and Stilwell, D.E. 1994. Leachability of heavy metals from growth media containing source-separated minicipal solid waste compost. *Journal of Environmental Quality* **23**, 718-722.

Schirado, T., Vergara, I., Schalscha, E.B. and Pratt, P.F. 1986. Evidence for movement of heavy metals in a soil irrigated with untreated wastewater. *Journal of Environmental Quality* **15**, 9-12.

Schnitzer, M. and Khan, S.U. 1972. *Humic Substances in the Environment*. Marcel Dekker Inc., New York, pp. 137-202, 253-254.

Schulz, R. and Rolheld, V. 1997. Recycling of municipal and industrial organic wastes in agriculture: Benefits, limitations and means of improvement. *Soil Science and Plant Nutrition* **43**, 1051-1056.

Scora, R.W. and Chang, A.C. 1997. Essential oil quality and heavy metal concentrations of peppermint grown on a municipal sludge-amended soil. *Journal of Environmental Quality* **26**, 975-979.

Senesi, N., Sposito, G., Holtzclaw, K.M. and Bradford, G.R. 1989. Chemical properties of metal-humic acid fractions of a sewage sludge-amended aridisol. *Journal of Environmental Quality* **18**, 186-194.

Shculte, E.E. and Hopkins, B.G. 1996. Estimation of soil organic matter by weight Loss-On-Ignition. In: Magdoff, F.R., Tabatabai, M.A. and Hanlon, E.A. Jr. (Eds.) *Soil organic matter: Analysis and Interpretation*. Soil Science Society of America, Madison, USA.

Sheldrick, B.H. and Wang, C. 1993. Particle size distribution. In: Carter, M.R. (Ed.) *Soil Sampling and Methods of Analysis*. Canadian Society of Soil Science, Lewis Publishers, pp. 499-509.

Shimadzu. 1992. *Instruction Manual: Total Organic Carbon Analyzer, Model TOC-5000*, Tokyo.

Sims, J.T. and Kline, J.S. 1991. Chemical fractionation and plant uptake of heavy metals in soils amended with co-composted sewage sludge. *Journal of Environmental Quality* **20**, 387-395.

Singh, A.K. and Pandeya, S.B. 1998. Sorption and release of cadmium-fulvic acid complexes in sludge-treated soils. *Bioresourse Technology* **66**, 119-127.

Singh, B.R., Narwai, R.P., Jeng, A.S. and Almas, A. 1995. Crop uptake and extractability of cadmium in soils naturally high in metals at different pH levels. *Communications of Soil Science and Plant Analysis* **26**, 2123-2142.

Singh, S.P., Tack, F.M.G. and Verloo, M.G. 1996. Extractability and bioavailability of heavy metals in surface soils derived from dredged sediments. *Chemical Speciation and Bioavailability* **8**, 105-110.

Singh, S.P., Tack, F.M.G. and Verloo, M.G. 1997. Uptake of metals in maize as affected by irrigation water quality and clay content of the soil. *Agrochimica* **41**, 27-32.

Singh, S.P., Tack, F.M.G. and Verloo, M.G. 1998. Heavy metal fractionation and extractability in dredged sediment surface soils. *Water, Air and Soil Pollution* **102,** 313-328.

Sloan, J.J., Dowdy, R.H., Dolan, M.S. and Linden, D.R. 1997. Long-term effects of biosolids applications on heavy metal bioavailability in agricultural soils. *Journal of Environmental Quality* **26**, 966-974.

Smith, S.R. 1995. *Agricultural Recycling of Sewage Sludge and the Environment*. CAB International, Wallingford, UK.

Smith, S.R. and Giller, K.E. 1992. Effective *Rhizombium leguminosarum* biovar *Trifolii* present in five soils contaminated with heavy metals from long-term applications of sewage sludge or metal mine spoil. *Soil Biology and Biochemistry* **24**, 781-788.

Smith, S.R. and Handley, P. 1992. Nitrogen-fertilizer value of activated sewage derived protein- Effect of environment and nitrification inhibiter on $NO_3$ release, soil microbial activity and yield of summer cabbage. *Fertilizer Research* **33**, 47-57.

Smolders, E., Lambregts, R.M., McLaulin, M.J. and Tiller, K.G. 1998. Effect of soil solution chlorine on cadmium availability to Swiss chard. *Journal of Environmental Quality* **27**, 426-432.

Soil Survey Staff. 1974. *Soil Survey Handbook*. Soil Survey of England and Wales, Harpenden, UK.

Solar-Rovira, P., Soler-Soler, J., Soler-Rovira, J, Polo, A. 1996. Agricultural use of sewage sludge and its regulation. *Fertilizer Research* **43**, 173-177.

Sommers, E. and McLaughlin, M.J. 1996a. Chloride increases cadmium uptake in Swiss chard in a resin-buffered nutrient solution. *Soil Science Society of America Journal* **60,** 1443-1447.

Sommers, E. and McLaughlin, M.J. 1996b. Effect of Cl on Cd uptake by Swiss Chard in nutrient solutions. *Plant and Soil* **179,** 57-64.

Spark, K.M., Wells, J.D. and Johnson, B.B. 1997a. The interaction of a humic acid with heavy metals. *Australian Journal of Soil Research* **35**, 89-101.

Spark, K.M., Wells, J.D. and Johnson, B.B. 1997b. Sorption of heavy metals by mineral-humic acid substrates. *Australian Journal of Soil Research* **35**, 113-122.

Sposito, G., Lung, L.J. and Chang, A.C. 1982. Trace metal chemistry in arid-zone field soils amended with sewage sludge: I. Fractionation of Ni, Cu, Zn, Cd and Pb in solid phases. *Soil Science Society of America Journal* **46**, 260-264.

Sposito, G., LeVesque, C.S., LeClaire, J.P. and Chang, A.C. 1983. Trace metal chemistry in arid zone field soils amended with sewage sludge: III. Effect of time and the extraction of trace metals. *Soil Science Society of America Journal* **47**, 898-902.

Stevenson, F.J. 1982. *Humus Chemistry: Genesis, Composition, Reactions*. Wiley and Sons, Inc., New York.

Stevenson, F.J. and Welch, L.F. 1979. Migration of applied lead in a field soil. *Environmental Science and Technology* **13**, 1255-1259.

Symeonides, C. and McRae, S.G. 1977. The assessment of plant available cadmium in soils. *Journal of Environmental Quality* **6**, 120-123.

Takamatsu, T. and Yoshida, T. 1978. Determination of stability constants of metal-humic acid complexes by potentiometric titration and ion-selective electrodes. *Soil Science* **125**, 337-386.

Taylor, M.D. and Theng, B.K.G. 1995. Sorption of cadmium by complexes of kaolinite with humic acid. *Communications in Soil Science and Plant Analysis* **26**, 767-776.

Temminghoff, E.J.M. van der Zee, S.E.A.T.M. and De Haan, H.A.M. 1997. Copper mobility in a copper-contaminated sandy soil as affected by pH and solid and dissolved organic matter. *Environmental Science and Technolgy* **31**, 1109-1115.

Terry, R.E., Nelson, D.W. and Sommers, L.E. 1979. Carbon cycling during sewage sludge decomposition in soils. *Soil Science Society of America Journal* **43**, 494-499.

Tessier, A., Campbell, P.G.C. and Bissom, M. 1979. Sequential extraction procedure for the speciation of particlulate trace metals. *Analytical Chemistry* **51**, 844-850.

Towers, W. and Paterson, E. 1997. Sewage sludge application tp lands-a preliminary assessment of the sensitivity of Scottish soils to heavy metals inputs. *Soil Use and Management* **13**, 149-155.

Tsadilas, C.D., Matsi, T. , Barbayiannis, N. and Dimoyiannis, D. 1995. Influence of sewage sludge application on soil properties and on distribution and availability of heavy metal fractions. *Communications of Soil Science and Plant Analysis* **26** , 2603-2619.

Ure, A.M. 1995. Methods of analysis for heavy metals in soils. In: Alloway, B.J. (Ed.) *Heavy Metals in the Environment*. Blackie Academic and Professional, London, pp. 58-102.

Vigerust, E. and Selmer-Olsen, A.R. 1985. Basis for metal limits relavant to sludge utilisation. In: *Directorate - General Science, Research and Development, Environmental Research Programme*. Elsier Applied Science Publishers, London and New York: pp.26-41

Villaroel, J.R., Chang, A.C. and Amrhein, C. 1993. Cd and Zn phytoavailability of a field-stabilised sludge-treated soil. *Soil Science* **155**, 197-205.

Volensky, B. 1990. Removal and recovery of heavy metals by biosorption. In: *Biosorption of Heavy Metals*. CEC Press, Boston, pp. 7-45.

Wallace, A., Romney, E.M. and Alexander, G.V. 1980. Zinc-cadmium interactions on the availability of each to bush bean plants grown in solution culture. *Journal of Plant Nutrition* **2**, 38-53.

Wang, P., Qu, E., Li, Z. and Shuman, L.M. 1997. Fractions and availability of Nickel in Loessial soil amended with sewage or sewage sludge. *Journal of Environmental Quality* **26**, 795-801.

Weber, M.D., Rogers, H.R., Watts, C.D., Boxall, A.B.A., Davis, R.D. and Scoffin, R. 1996. Monitoring and prioritisation of organic contaminants in sewage sludges using specific chemical analysis and predictive, non-analytical methods. *Science of the Total Environment* **185**, 27-44.

Weir, C.C. and Allen, J.R. 1997. Effects of using organic wastes as soil amendments in urban horticultural practices in the District of Columbia. *Journal of Environmental Science and Health Part A- Environmental Science and Engineering and Toxic and Hazardous Substance Control* **32**, 323-332.

White, C.S., Lotfin, S.R. and Aguilar, R. 1997. Application of biosolids to degraded semiarid rangeland: Nine-year responses. *Journal of Environmental Quality* **26**, 1663-1671.

Wild, A. 1993. *Soils and the Environment*. Cambridge University Press, pp. 189-211.

Williams, D.E., Vlamis, J., Pukite, A.H. and Corey, J.E. 1980. Trace element accumulation, movement and distribution in the soil profile from massive applications of sewage sludge. *Soil Science* **129**, 119-132

Wong, J.W.C., Li, G.X.X. and Wong, M.H. 1996. The growth of *Brassica chinesis* in heavy metal-contaminated sewage sludge compost from Hong Kong. *Bioresource Technology* **58**, 309-313.

Xiong, L.M. and Lu, R.K. 1993. Effect of liming on plant accumulation of cadmium under upland or flooded conditions. *Environmental Pollution* **79**, 199-203.

Xiu, H., Taylor, R.W., Shuford, J.W. and Tadessee, W. 1991. Comparison of extractants for available sludge-borne metals: a residual study. *Water, Air and Soil Pollution* **57-58**, 913-922.

Xu, H., Ephraim, J., Ledin, A. and Allard, B. 1989. Effects of fulvic acid on the adsorption of Cd(II) on alumina. *The Science of the Total Environment* **81/82**, 653-660.

Young, X., Baligar, V.C., Martens, D.C. and Clark, R.B. 1995. Influx transport and accumulation of cadmium in plant species grown at different $Cd^{2+}$ activities. *Journal of*

*Environmental Science and Health Part B-Pesticides, Food Contamination and Agricultural Wastes* **30**, 569-583.

Zhang, M., Alva, A.K., Li, Y.C. and Calvert, D.V. 1997. Chemical association of Cu, Zn, Mn and Pb in selected sandy citrus soils. *Soil Science* **162**, 181-198.

Zhu, B. and Alva, A.K. 1993. Trace-metal and cation-transport in a sandy soil with various amendments. *Soil Science of America Journal* **57**, 723-727.

# APPENDIX I

**Table A1.1:** ***Cd concentration in ryegrass ($\mu g\ g^{-1}$).***

| | | LS Soil | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.14 | 0.09 | 0.17 | 0.13 | 0.17 | 0.17 | 0.17 |
| | Cool | 0.13 | 0.12 | 0.13 | 0.11 | 0.13 | 0.12 | 0.13 |
| 10 t ha$^{-1}$ | Warm | 0.30 | 0.16 | 0.39 | 0.29 | 0.33 | 0.49 | 0.61 |
| | Cool | 0.26 | 0.27 | 0.27 | 0.23 | 0.22 | 0.21 | 0.20 |
| 50 t ha$^{-1}$ | Warm | 0.92 | 0.61 | 1.00 | 0.81 | 0.99 | 1.12 | 1.17 |
| | Cool | 1.00 | 1.00 | 1.02 | 0.98 | 0.94 | 0.90 | 0.81 |

| | | CL Soil | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.24 | 0.10 | 0.26 | 0.20 | 0.24 | 0.25 | 0.25 |
| | Cool | 0.21 | 0.19 | 0.20 | 0.19 | 0.20 | 0.20 | 0.19 |
| 10 t ha$^{-1}$ | Warm | 0.51 | 0.22 | 0.57 | 0.52 | 0.52 | 0.62 | 0.66 |
| | Cool | 0.52 | 0.54 | 0.54 | 0.50 | 0.47 | 0.45 | 0.40 |
| 50 t ha$^{-1}$ | Warm | 1.32 | 0.78 | 1.37 | 1.16 | 1.41 | 1.57 | 1.58 |
| | Cool | 1.01 | 0.98 | 0.99 | 0.97 | 0.91 | 0.85 | 0.78 |

**Table A1.2:** ***Ni concentration in ryegrass ($\mu g\ g^{-1}$).***

| | | LS Soil | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.51 | 0.51 | 0.58 | 0.63 | 0.41 | 0.65 | 0.48 |
| | Cool | 0.50 | 0.54 | 0.58 | 0.42 | 0.41 | 0.43 | 0.43 |
| 10 t ha$^{-1}$ | Warm | 1.10 | 0.62 | 1.10 | 0.84 | 1.08 | 1.20 | 1.18 |
| | Cool | 1.07 | 1.06 | 0.93 | 0.88 | 0.83 | 0.79 | 0.80 |
| 50 t ha$^{-1}$ | Warm | 3.01 | 2.09 | 2.78 | 2.59 | 3.14 | 3.34 | 3.47 |
| | Cool | 2.98 | 2.94 | 3.06 | 2.66 | 2.33 | 2.22 | 2.12 |

| | | CL Soil | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.88 | 0.90 | 1.13 | 0.96 | 1.11 | 1.16 | 0.96 |
| | Cool | 0.95 | 1.09 | 1.13 | 1.14 | 0.86 | 0.93 | 0.92 |
| 10 t ha$^{-1}$ | Warm | 1.56 | 1.24 | 1.30 | 1.47 | 1.68 | 1.79 | 1.76 |
| | Cool | 1.51 | 1.37 | 1.29 | 1.24 | 1.24 | 1.16 | 1.16 |
| 50 t ha$^{-1}$ | Warm | 5.14 | 3.63 | 4.77 | 4.09 | 5.25 | 5.65 | 5.68 |
| | Cool | 4.90 | 4.58 | 4.71 | 4.82 | 4.83 | 4.52 | 4.54 |

**Table A1.3:** ***Pb concentration in ryegrass (μg $g^{-1}$).***

| | | LS Soil | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.06 | 0.06 | 0.06 | 0.06 | 0.07 | 0.07 | 0.07 |
| | Cool | 0.06 | 0.06 | 0.06 | 0.05 | 0.05 | 0.05 | 0.05 |
| 10 t $ha^{-1}$ | Warm | 0.15 | 0.11 | 0.15 | 0.14 | 0.15 | 0.18 | 0.17 |
| | Cool | 0.15 | 0.14 | 0.15 | 0.12 | 0.11 | 0.10 | 0.11 |
| 50 t $ha^{-1}$ | Warm | 0.31 | 0.24 | 0.31 | 0.29 | 0.31 | 0.35 | 0.36 |
| | Cool | 0.32 | 0.31 | 0.25 | 0.26 | 0.25 | 0.25 | 0.24 |
| | | CL Soil | | | | | | |
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.11 | 0.08 | 0.11 | 0.12 | 0.10 | 0.12 | 0.10 |
| | Cool | 0.11 | 0.12 | 0.09 | 0.10 | 0.08 | 0.09 | 0.08 |
| 10 t $ha^{-1}$ | Warm | 0.25 | 0.21 | 0.23 | 0.24 | 0.25 | 0.27 | 0.31 |
| | Cool | 0.21 | 0.21 | 0.22 | 0.17 | 0.17 | 0.17 | 0.17 |
| 50 t $ha^{-1}$ | Warm | 0.46 | 0.41 | 0.46 | 0.43 | 0.40 | 0.44 | 0.51 |
| | Cool | 0.45 | 0.42 | 0.39 | 0.36 | 0.35 | 0.34 | 0.36 |

**Table A1.4:** ***Zn concentration in ryegrass (μg $g^{-1}$).***

| | | LS Soil | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 5.30 | 4.63 | 5.50 | 4.73 | 5.17 | 5.33 | 5.10 |
| | Cool | 5.30 | 5.20 | 5.33 | 3.63 | 3.27 | 3.50 | 3.20 |
| 10 t $ha^{-1}$ | Warm | 15.87 | 14.30 | 15.40 | 14.77 | 15.03 | 15.53 | 14.63 |
| | Cool | 15.40 | 14.90 | 14.43 | 12.07 | 8.63 | 9.43 | 12.07 |
| 50 t $ha^{-1}$ | Warm | 41.43 | 34.47 | 41.00 | 37.00 | 40.57 | 37.77 | 37.50 |
| | Cool | 34.30 | 35.47 | 30.67 | 30.93 | 29.20 | 29.00 | 29.60 |
| | | CL Soil | | | | | | |
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 7.37 | 5.60 | 7.37 | 6.37 | 7.23 | 7.57 | 7.41 |
| | Cool | 7.03 | 7.40 | 7.50 | 6.50 | 5.30 | 5.40 | 5.50 |
| 10 t $ha^{-1}$ | Warm | 20.60 | 18.73 | 20.73 | 19.27 | 21.53 | 21.33 | 22.10 |
| | Cool | 18.27 | 18.50 | 18.33 | 14.73 | 12.67 | 12.50 | 13.90 |
| 50 t $ha^{-1}$ | Warm | 56.70 | 50.63 | 54.37 | 53.27 | 50.40 | 50.10 | 53.40 |
| | Cool | 51.87 | 50.53 | 41.23 | 33.43 | 35.17 | 32.40 | 34.50 |

**Table A2.1:** ***Uptake of Cd by ryegrass (μg pot$^{-1}$).***

| | | LS Soil | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.10 | 0.04 | 0.12 | 0.09 | 0.12 | 0.12 | 0.12 |
| | Cool | 0.07 | 0.07 | 0.07 | 0.06 | 0.07 | 0.07 | 0.08 |
| 10 t ha$^{-1}$ | Warm | 0.52 | 0.23 | 0.68 | 0.44 | 0.57 | 0.88 | 1.04 |
| | Cool | 0.36 | 0.36 | 0.38 | 0.31 | 0.31 | 0.30 | 0.28 |
| 50 t ha$^{-1}$ | Warm | 2.74 | 1.57 | 2.95 | 2.17 | 2.85 | 3.29 | 3.56 |
| | Cool | 2.13 | 2.01 | 2.19 | 2.18 | 2.05 | 1.92 | 1.67 |
| | | CL Soil | | | | | | |
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.23 | 0.09 | 0.26 | 0.20 | 0.25 | 0.29 | 0.28 |
| | Cool | 0.16 | 0.14 | 0.16 | 0.16 | 0.16 | 0.18 | 0.16 |
| 10 t ha$^{-1}$ | Warm | 1.04 | 0.37 | 1.15 | 1.00 | 1.10 | 1.32 | 1.39 |
| | Cool | 0.86 | 0.91 | 0.93 | 0.86 | 0.83 | 0.82 | 0.72 |
| 50 t ha$^{-1}$ | Warm | 5.12 | 2.78 | 5.44 | 4.47 | 5.59 | 6.12 | 6.34 |
| | Cool | 2.48 | 2.29 | 2.37 | 2.40 | 2.28 | 2.18 | 1.87 |

**Table A2.2:** ***Uptake of Ni by ryegrass (μg pot$^{-1}$).***

| | | LS Soil | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.38 | 0.26 | 0.42 | 0.44 | 0.30 | 0.47 | 0.35 |
| | Cool | 0.27 | 0.32 | 0.33 | 0.23 | 0.22 | 0.24 | 0.25 |
| 10 t ha$^{-1}$ | Warm | 1.94 | 0.92 | 1.92 | 1.28 | 1.86 | 2.14 | 2.02 |
| | Cool | 1.48 | 1.44 | 1.30 | 1.21 | 1.16 | 1.14 | 1.14 |
| 50 t ha$^{-1}$ | Warm | 8.98 | 5.36 | 8.22 | 6.94 | 9.03 | 9.86 | 10.58 |
| | Cool | 6.34 | 5.94 | 6.58 | 5.91 | 5.07 | 4.72 | 4.36 |
| | | CL Soil | | | | | | |
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.84 | 0.86 | 1.13 | 0.94 | 1.14 | 1.34 | 1.06 |
| | Cool | 0.74 | 0.80 | 0.90 | 0.97 | 0.70 | 0.87 | 0.73 |
| 10 t ha$^{-1}$ | Warm | 3.22 | 2.15 | 2.65 | 2.86 | 3.53 | 3.83 | 3.73 |
| | Cool | 2.49 | 2.33 | 2.20 | 2.10 | 2.20 | 2.13 | 2.11 |
| 50 t ha$^{-1}$ | Warm | 20.01 | 12.92 | 18.93 | 15.76 | 20.82 | 21.97 | 22.70 |
| | Cool | 12.06 | 10.77 | 11.22 | 12.00 | 12.08 | 11.57 | 10.95 |

**Table A2.3:** ***Uptake of Pb by ryegrass (μg pot$^{-1}$).***

| | | LS Soil | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.05 | 0.03 | 0.04 | 0.04 | 0.05 | 0.05 | 0.05 |
| | Cool | 0.03 | 0.04 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| 10 t ha$^{-1}$ | Warm | 0.27 | 0.17 | 0.27 | 0.22 | 0.27 | 0.32 | 0.30 |
| | Cool | 0.21 | 0.19 | 0.22 | 0.16 | 0.15 | 0.15 | 0.16 |
| 50 t ha$^{-1}$ | Warm | 0.93 | 0.60 | 0.92 | 0.77 | 0.90 | 1.04 | 1.10 |
| | Cool | 0.68 | 0.62 | 0.55 | 0.57 | 0.55 | 0.54 | 0.50 |
| | | CL Soil | | | | | | |
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.10 | 0.08 | 0.11 | 0.12 | 0.10 | 0.13 | 0.11 |
| | Cool | 0.09 | 0.09 | 0.08 | 0.08 | 0.07 | 0.08 | 0.07 |
| 10 t ha$^{-1}$ | Warm | 0.51 | 0.36 | 0.48 | 0.47 | 0.53 | 0.58 | 0.65 |
| | Cool | 0.34 | 0.36 | 0.37 | 0.30 | 0.31 | 0.31 | 0.31 |
| 50 t ha$^{-1}$ | Warm | 1.79 | 1.47 | 1.82 | 1.67 | 1.60 | 1.72 | 2.02 |
| | Cool | 1.10 | 0.99 | 0.93 | 0.88 | 0.88 | 0.88 | 0.86 |

**Table A2.4:** ***Uptake of Zn by ryegrass (μg pot$^{-1}$).***

| | | LS Soil | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 3.98 | 2.36 | 3.96 | 3.31 | 3.72 | 3.84 | 3.77 |
| | Cool | 2.92 | 3.07 | 2.99 | 2.03 | 1.80 | 2.00 | 1.89 |
| 10 t ha$^{-1}$ | Warm | 27.93 | 21.45 | 26.80 | 22.45 | 26.01 | 27.65 | 25.02 |
| | Cool | 21.25 | 20.12 | 20.35 | 16.53 | 12.09 | 13.68 | 17.26 |
| 50 t ha$^{-1}$ | Warm | 123.47 | 88.23 | 121.36 | 99.16 | 116.83 | 111.41 | 114.38 |
| | Cool | 73.06 | 71.64 | 65.93 | 68.67 | 63.66 | 61.77 | 60.98 |
| | | CL Soil | | | | | | |
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 7.07 | 5.32 | 7.37 | 6.24 | 7.38 | 8.78 | 8.23 |
| | Cool | 5.49 | 5.48 | 6.00 | 5.53 | 4.29 | 5.02 | 4.40 |
| 10 t ha$^{-1}$ | Warm | 42.44 | 32.60 | 42.30 | 37.38 | 45.22 | 45.65 | 46.85 |
| | Cool | 30.14 | 31.45 | 31.35 | 25.05 | 22.55 | 22.88 | 25.30 |
| 50 t ha$^{-1}$ | Warm | 220.56 | 180.25 | 215.84 | 205.08 | 200.09 | 194.89 | 213.60 |
| | Cool | 127.59 | 118.75 | 98.14 | 83.25 | 87.92 | 82.94 | 83.15 |

**Table A3:** ***Ryegrass yield (g).***

| | | LS Soil | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.75 | 0.51 | 0.72 | 0.70 | 0.72 | 0.72 | 0.74 |
| | Cool | 0.55 | 0.59 | 0.56 | 0.56 | 0.55 | 0.57 | 0.59 |
| 10 t ha$^{-1}$ | Warm | 1.76 | 1.50 | 1.74 | 1.52 | 1.73 | 1.78 | 1.71 |
| | Cool | 1.38 | 1.35 | 1.41 | 1.37 | 1.40 | 1.45 | 1.43 |
| 50 t ha$^{-1}$ | Warm | 2.98 | 2.56 | 2.96 | 2.68 | 2.88 | 2.95 | 3.05 |
| | Cool | 2.13 | 2.02 | 2.15 | 2.22 | 2.18 | 2.13 | 2.06 |

| | | CL Soil | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Week 7 | Week 14 | Week 21 | Week 28 | Week 35 | Week 44 | Week 51 |
| Control | Warm | 0.96 | 0.95 | 1.00 | 0.98 | 1.02 | 1.16 | 1.11 |
| | Cool | 0.78 | 0.74 | 0.80 | 0.85 | 0.81 | 0.93 | 0.80 |
| 10 t ha$^{-1}$ | Warm | 2.06 | 1.74 | 2.04 | 1.94 | 2.10 | 2.14 | 2.12 |
| | Cool | 1.65 | 1.70 | 1.71 | 1.70 | 1.78 | 1.83 | 1.82 |
| 50 t ha$^{-1}$ | Warm | 3.89 | 3.56 | 3.97 | 3.85 | 3.97 | 3.89 | 4.00 |
| | Cool | 2.46 | 2.35 | 2.38 | 2.49 | 2.50 | 2.56 | 2.41 |

**Table A4.1:** *Cd extracted with* $CaCl_2$ *(µg kg$^{-1}$).*

| | | LS Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 19 | 18 | 17 | 17 | 17 | 18 | 18 | 18 | 18 |
| | Cool | 17 | 17 | 14 | 16 | 13 | 18 | 19 | 19 | 20 |
| 10 t ha$^{-1}$ | Warm | 22 | 24 | 24 | 24 | 25 | 27 | 28 | 28 | 28 |
| | Cool | 26 | 22 | 23 | 23 | 23 | 25 | 26 | 28 | 27 |
| 50 t ha$^{-1}$ | Warm | 28 | 31 | 32 | 34 | 34 | 39 | 40 | 38 | 37 |
| | Cool | 28 | 29 | 30 | 32 | 33 | 34 | 35 | 35 | 33 |

| | | CL Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 19 | 20 | 18 | 18 | 18 | 19 | 19 | 19 | 20 |
| | Cool | 17 | 20 | 18 | 18 | 18 | 20 | 21 | 21 | 18 |
| 10 t ha$^{-1}$ | Warm | 22 | 25 | 25 | 29 | 32 | 34 | 35 | 32 | 33 |
| | Cool | 26 | 23 | 25 | 28 | 30 | 28 | 29 | 29 | 31 |
| 50 t ha$^{-1}$ | Warm | 34 | 38 | 44 | 41 | 45 | 48 | 49 | 47 | 46 |
| | Cool | 34 | 37 | 39 | 39 | 42 | 39 | 40 | 41 | 40 |

**Table A4.2:** ***Ni extracted with $CaCl_2$ ($\mu g\ kg^{-1}$).***

| | | LS Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 36 | 32 | 37 | 21 | 51 | 28 | 40 | 50 | 42 |
| | Cool | 36 | 27 | 27 | 14 | 39 | 15 | 28 | 37 | 42 |
| 10 t ha$^{-1}$ | Warm | 55 | 56 | 62 | 66 | 65 | 88 | 109 | 109 | 103 |
| | Cool | 55 | 53 | 57 | 66 | 74 | 74 | 88 | 96 | 90 |
| 50 t ha$^{-1}$ | Warm | 156 | 146 | 137 | 153 | 157 | 161 | 189 | 181 | 175 |
| | Cool | 156 | 158 | 136 | 150 | 153 | 157 | 170 | 166 | 161 |

| | | CL Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 57 | 59 | 54 | 43 | 49 | 55 | 47 | 47 | 40 |
| | Cool | 57 | 42 | 45 | 25 | 33 | 43 | 44 | 44 | 35 |
| 10 t ha$^{-1}$ | Warm | 96 | 102 | 92 | 93 | 96 | 125 | 136 | 127 | 120 |
| | Cool | 96 | 93 | 94 | 96 | 85 | 86 | 90 | 104 | 98 |
| 50 t ha$^{-1}$ | Warm | 213 | 221 | 213 | 214 | 215 | 220 | 231 | 207 | 200 |
| | Cool | 213 | 184 | 194 | 196 | 178 | 180 | 187 | 193 | 180 |

**Table A4.3:** ***Zn extracted with $CaCl_2$ (µg $kg^{-1}$).***

| | | LS Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 202 | 202 | 315 | 298 | 378 | 137 | 378 | 378 | 333 |
| | Cool | 124 | 124 | 239 | 212 | 285 | 114 | 285 | 328 | 280 |
| 10 t $ha^{-1}$ | Warm | 474 | 465 | 472 | 513 | 594 | 614 | 670 | 609 | 630 |
| | Cool | 379 | 347 | 379 | 396 | 525 | 516 | 594 | 594 | 560 |
| 50 t $ha^{-1}$ | Warm | 994 | 984 | 1004 | 1020 | 1106 | 1106 | 1167 | 1090 | 1062 |
| | Cool | 860 | 831 | 953 | 953 | 991 | 1014 | 1051 | 1008 | 960 |

| | | CL Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 321 | 321 | 285 | 185 | 459 | 383 | 459 | 350 | 350 |
| | Cool | 259 | 259 | 230 | 161 | 424 | 237 | 424 | 424 | 320 |
| 10 t $ha^{-1}$ | Warm | 598 | 506 | 522 | 538 | 713 | 901 | 1055 | 890 | 930 |
| | Cool | 542 | 513 | 423 | 435 | 661 | 708 | 762 | 762 | 700 |
| 50 t $ha^{-1}$ | Warm | 1607 | 1483 | 1573 | 1604 | 1499 | 1880 | 1972 | 1950 | 1880 |
| | Cool | 1344 | 1323 | 1499 | 1509 | 1600 | 1644 | 1854 | 1750 | 1650 |

**Table A5.1:** ***Cd extracted with DTPA (μg $g^{-1}$).***

| | | LS Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 0.04 | 0.04 | 0.05 | 0.05 | 0.05 | 0.04 | 0.05 | 0.04 | 0.05 |
| | Cool | 0.04 | 0.05 | 0.05 | 0.06 | 0.04 | 0.04 | 0.04 | 0.05 | 0.05 |
| 10 t $ha^{-1}$ | Warm | 0.05 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.06 | 0.06 | 0.06 |
| | Cool | 0.05 | 0.06 | 0.06 | 0.07 | 0.06 | 0.06 | 0.05 | 0.06 | 0.07 |
| 50 t $ha^{-1}$ | Warm | 0.08 | 0.10 | 0.10 | 0.11 | 0.11 | 0.11 | 0.10 | 0.09 | 0.11 |
| | Cool | 0.08 | 0.09 | 0.09 | 0.10 | 0.10 | 0.09 | 0.09 | 0.07 | 0.09 |

| | | CL Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 0.13 | 0.14 | 0.12 | 0.13 | 0.10 | 0.11 | 0.09 | 0.11 | 0.10 |
| | Cool | 0.13 | 0.12 | 0.09 | 0.13 | 0.11 | 0.07 | 0.12 | 0.09 | 0.10 |
| 10 t $ha^{-1}$ | Warm | 0.22 | 0.21 | 0.19 | 0.21 | 0.18 | 0.18 | 0.20 | 0.19 | 0.16 |
| | Cool | 0.22 | 0.22 | 0.23 | 0.21 | 0.20 | 0.18 | 0.17 | 0.17 | 0.15 |
| 50 t $ha^{-1}$ | Warm | 0.25 | 0.25 | 0.27 | 0.25 | 0.26 | 0.25 | 0.26 | 0.25 | 0.23 |
| | Cool | 0.25 | 0.26 | 0.26 | 0.27 | 0.25 | 0.22 | 0.24 | 0.23 | 0.20 |

**Table A5.2:** ***Ni extracted with DTPA (μg $g^{-1}$).***

| | | LS Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 0.65 | 0.63 | 0.64 | 0.61 | 0.65 | 0.56 | 0.59 | 0.61 | 0.56 |
| | Cool | 0.65 | 0.70 | 0.64 | 0.62 | 0.56 | 0.58 | 0.52 | 0.53 | 0.53 |
| 10 t $ha^{-1}$ | Warm | 0.80 | 0.83 | 0.83 | 0.78 | 0.79 | 0.64 | 0.65 | 0.67 | 0.76 |
| | Cool | 0.80 | 0.79 | 0.80 | 0.75 | 0.72 | 0.81 | 0.59 | 0.64 | 0.69 |
| 50 t $ha^{-1}$ | Warm | 1.26 | 1.29 | 1.25 | 1.25 | 1.05 | 1.19 | 1.07 | 1.05 | 1.01 |
| | Cool | 1.26 | 1.31 | 1.30 | 1.33 | 1.25 | 0.91 | 0.90 | 0.89 | 0.92 |

| | | CL Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 2.06 | 2.09 | 2.15 | 1.87 | 1.81 | 1.77 | 1.71 | 1.68 | 1.61 |
| | Cool | 2.02 | 2.02 | 1.98 | 1.83 | 1.89 | 1.82 | 1.72 | 1.68 | 1.51 |
| 10 t $ha^{-1}$ | Warm | 2.57 | 2.31 | 2.35 | 2.29 | 2.05 | 2.12 | 2.06 | 1.99 | 1.87 |
| | Cool | 2.57 | 2.53 | 2.45 | 2.47 | 2.17 | 1.90 | 1.92 | 1.79 | 1.76 |
| 50 t $ha^{-1}$ | Warm | 3.21 | 2.83 | 2.98 | 3.05 | 2.68 | 2.58 | 2.64 | 2.51 | 2.42 |
| | Cool | 3.21 | 2.81 | 2.80 | 2.87 | 2.81 | 2.38 | 2.22 | 2.14 | 2.16 |

**Table A5.3:** ***Pb extracted with DTPA (μg g$^{-1}$).***

| | | LS Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 2.01 | 1.73 | 1.81 | 1.85 | 1.45 | 1.81 | 1.83 | 1.83 | 1.84 |
| | Cool | 2.01 | 1.80 | 1.92 | 1.92 | 1.56 | 1.58 | 1.46 | 1.60 | 1.73 |
| 10 t ha$^{-1}$ | Warm | 2.13 | 2.34 | 2.29 | 2.28 | 2.07 | 2.18 | 2.06 | 2.20 | 2.25 |
| | Cool | 2.13 | 2.06 | 2.27 | 2.30 | 2.02 | 1.90 | 1.96 | 2.02 | 2.10 |
| 50 t ha$^{-1}$ | Warm | 4.02 | 4.25 | 4.27 | 3.89 | 3.60 | 3.09 | 3.02 | 3.12 | 3.17 |
| | Cool | 4.02 | 3.90 | 3.68 | 3.43 | 3.26 | 2.95 | 2.93 | 2.84 | 2.90 |

| | | CL Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 6.31 | 6.36 | 6.52 | 6.20 | 5.76 | 5.66 | 5.42 | 5.53 | 4.76 |
| | Cool | 5.97 | 6.20 | 6.51 | 6.14 | 5.53 | 5.30 | 5.13 | 5.25 | 4.71 |
| 10 t ha$^{-1}$ | Warm | 8.77 | 8.25 | 8.04 | 8.31 | 6.66 | 6.45 | 6.33 | 6.20 | 5.45 |
| | Cool | 8.77 | 8.63 | 7.55 | 7.83 | 6.25 | 6.17 | 6.11 | 6.00 | 5.50 |
| 50 t ha$^{-1}$ | Warm | 10.67 | 10.40 | 10.25 | 10.08 | 9.00 | 9.82 | 8.38 | 7.93 | 6.88 |
| | Cool | 10.67 | 10.24 | 9.85 | 9.96 | 9.42 | 9.76 | 7.42 | 7.31 | 6.30 |

**Table A5.4:** ***Zn extracted with DTPA ($\mu g\ g^{-1}$).***

| | | LS Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 1.30 | 1.73 | 1.48 | 1.79 | 1.25 | 1.34 | 1.37 | 1.19 | 1.05 |
| | Cool | 1.30 | 1.37 | 1.44 | 1.25 | 1.79 | 1.19 | 1.17 | 1.20 | 1.20 |
| 10 t ha$^{-1}$ | Warm | 2.61 | 3.45 | 3.21 | 3.09 | 2.93 | 2.31 | 2.21 | 2.31 | 2.45 |
| | Cool | 2.61 | 2.56 | 2.81 | 2.65 | 2.44 | 1.99 | 1.73 | 2.67 | 2.03 |
| 50 t ha$^{-1}$ | Warm | 7.43 | 7.31 | 7.19 | 6.25 | 5.28 | 5.72 | 5.15 | 5.19 | 4.80 |
| | Cool | 7.43 | 7.53 | 8.29 | 7.03 | 6.41 | 4.66 | 4.55 | 4.52 | 4.18 |

| | | CL Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 6.14 | 7.25 | 5.47 | 6.21 | 5.12 | 5.15 | 4.73 | 4.50 | 3.27 |
| | Cool | 6.14 | 5.84 | 4.60 | 6.19 | 4.56 | 4.34 | 3.79 | 3.50 | 3.39 |
| 10 t ha$^{-1}$ | Warm | 9.78 | 8.70 | 9.39 | 10.22 | 9.01 | 7.91 | 7.65 | 6.80 | 6.20 |
| | Cool | 9.78 | 10.42 | 10.00 | 9.75 | 8.44 | 6.98 | 5.84 | 5.80 | 5.40 |
| 50 t ha$^{-1}$ | Warm | 19.70 | 21.15 | 20.10 | 19.72 | 15.32 | 13.31 | 13.09 | 12.90 | 12.30 |
| | Cool | 19.70 | 18.85 | 19.31 | 19.80 | 17.23 | 13.60 | 12.09 | 11.30 | 10.60 |

**Table A6.1:** ***pH in 1 : 2.5 soil : water suspension.***

| | | LS Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 7.21 | 6.94 | 7.24 | 7.11 | 7.23 | 7.33 | 7.32 | 7.32 | 7.23 |
| | Cool | 7.21 | 6.97 | 7.13 | 7.32 | 7.31 | 7.23 | 7.23 | 7.25 | 7.25 |
| 10 t ha$^{-1}$ | Warm | 7.14 | 7.18 | 7.10 | 7.17 | 6.90 | 6.82 | 6.88 | 7.08 | 6.90 |
| | Cool | 7.14 | 7.14 | 7.19 | 7.07 | 6.94 | 6.91 | 6.95 | 6.97 | 6.88 |
| 50 t ha$^{-1}$ | Warm | 6.91 | 7.00 | 7.02 | 6.77 | 6.68 | 6.68 | 6.77 | 6.83 | 6.71 |
| | Cool | 6.91 | 6.81 | 7.06 | 6.73 | 6.83 | 6.63 | 6.72 | 6.76 | 6.70 |

| | | CL Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 7.34 | 7.21 | 7.39 | 7.40 | 7.40 | 7.42 | 7.41 | 7.42 | 7.37 |
| | Cool | 7.35 | 7.28 | 7.54 | 7.33 | 7.37 | 7.37 | 7.38 | 7.41 | 7.38 |
| 10 t ha$^{-1}$ | Warm | 7.30 | 7.34 | 7.26 | 7.20 | 7.25 | 7.23 | 7.25 | 7.31 | 7.29 |
| | Cool | 7.30 | 7.31 | 7.29 | 7.18 | 7.21 | 7.25 | 7.23 | 7.29 | 7.28 |
| 50 t ha$^{-1}$ | Warm | 7.25 | 7.24 | 7.24 | 7.17 | 7.16 | 7.19 | 7.19 | 7.22 | 7.21 |
| | Cool | 7.25 | 7.37 | 7.42 | 7.22 | 7.15 | 7.18 | 7.21 | 7.18 | 7.19 |

**Table A6.2:** ***pH in 0.01 M $CaCl_2$..***

| | | LS Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 6.84 | 6.86 | 6.90 | 6.91 | 6.90 | 6.94 | 6.96 | 6.99 | 6.95 |
| | Cool | 6.84 | 6.89 | 6.87 | 6.88 | 6.85 | 6.90 | 6.93 | 6.93 | 6.89 |
| 10 t ha$^{-1}$ | Warm | 6.48 | 6.48 | 6.48 | 6.48 | 6.49 | 6.42 | 6.51 | 6.49 | 6.45 |
| | Cool | 6.48 | 6.28 | 6.39 | 6.46 | 6.47 | 6.47 | 6.46 | 6.50 | 6.48 |
| 50 t ha$^{-1}$ | Warm | 6.40 | 6.45 | 6.37 | 6.28 | 6.35 | 6.35 | 6.34 | 6.39 | 6.34 |
| | Cool | 6.39 | 6.54 | 6.31 | 6.33 | 6.32 | 6.37 | 6.36 | 6.34 | 6.31 |

CL Soil

| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | Warm | 7.19 | 6.79 | 7.08 | 7.10 | 7.20 | 7.21 | 7.22 | 7.23 | 7.22 |
| | Cool | 7.19 | 6.83 | 7.15 | 7.16 | 7.18 | 7.19 | 7.23 | 7.24 | 7.29 |
| 10 t ha$^{-1}$ | Warm | 7.10 | 6.76 | 6.83 | 7.04 | 7.05 | 7.04 | 7.08 | 7.07 | 7.14 |
| | Cool | 7.11 | 6.76 | 6.81 | 7.02 | 7.09 | 7.07 | 7.07 | 7.11 | 7.06 |
| 50 t ha$^{-1}$ | Warm | 7.20 | 6.77 | 6.84 | 6.99 | 6.93 | 6.94 | 6.93 | 6.94 | 6.95 |
| | Cool | 7.20 | 7.17 | 7.01 | 7.00 | 6.95 | 6.97 | 6.96 | 6.97 | 6.96 |

**Table A7.1:** ***Loss-On-Ignition (%).***

| | | LS Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 2.37 | 2.30 | 2.21 | 2.10 | 1.54 | 1.46 | 1.55 | 1.57 | 1.69 |
| | Cool | 2.37 | 2.36 | 2.39 | 2.31 | 1.65 | 1.59 | 1.67 | 1.78 | 1.86 |
| 10 t ha$^{-1}$ | Warm | 2.68 | 2.76 | 2.80 | 2.57 | 1.81 | 1.73 | 1.85 | 2.00 | 1.93 |
| | Cool | 2.68 | 2.37 | 2.50 | 2.48 | 1.88 | 1.82 | 1.97 | 1.97 | 2.00 |
| 50 t ha$^{-1}$ | Warm | 3.20 | 3.22 | 3.22 | 3.18 | 2.66 | 2.51 | 2.45 | 2.30 | 2.35 |
| | Cool | 3.20 | 3.50 | 3.51 | 3.45 | 2.72 | 2.69 | 2.63 | 2.64 | 2.63 |

| | | CL Soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 | Week 72 | Week 100 |
| Control | Warm | 10.41 | 9.74 | 9.29 | 9.26 | 8.95 | 8.93 | 9.04 | 9.14 | 8.80 |
| | Cool | 10.41 | 10.47 | 10.02 | 9.65 | 9.65 | 9.23 | 9.54 | 9.20 | 9.09 |
| 10 t ha$^{-1}$ | Warm | 12.61 | 12.58 | 11.58 | 11.35 | 10.82 | 10.57 | 10.56 | 10.56 | 10.26 |
| | Cool | 12.61 | 12.45 | 12.49 | 11.62 | 11.17 | 10.47 | 10.80 | 10.75 | 10.30 |
| 50 t ha$^{-1}$ | Warm | 13.78 | 13.43 | 13.12 | 13.11 | 12.15 | 11.94 | 11.34 | 11.17 | 10.87 |
| | Cool | 13.78 | 13.20 | 12.76 | 12.16 | 11.56 | 11.87 | 11.87 | 11.67 | 11.36 |

**Table A7.2:** ***Organic matter (by Wet Oxidation) (%).***

| | | LS Soil | | CL Soil | |
|---|---|---|---|---|---|
| | | Week 0 | Week 32 | Week 0 | Week 32 |
| Control | Warm | 1.22 | 1.15 | 7.20 | 6.08 |
| | Cool | 1.22 | 1.15 | 7.20 | 6.10 |
| 10 t ha$^{-1}$ | Warm | 1.52 | 1.24 | 7.65 | 6.66 |
| | Cool | 1.52 | 1.26 | 7.65 | 6.79 |
| 50 t ha$^{-1}$ | Warm | 2.37 | 1.59 | 9.06 | 7.31 |
| | Cool | 2.37 | 1.97 | 9.06 | 7.73 |

## APPENDIX II

**Table A1:** ***Ryegrass yield from the site in Sonning (g).***

| | Cutting 1 | Cutting 2 | Cutting 3 | Cutting 4 | Cutting 5 | Cutting 6 |
|---|---|---|---|---|---|---|
| Control | 1348 | 673 | 880 | 790 | 613 | 574 |
| 5 t ha$^{-1}$ | 2171 | 907 | 1057 | 720 | 548 | 548 |
| 10 t ha$^{-1}$ | 2456 | 1116 | 1068 | 585 | 535 | 567 |
| 50 t ha$^{-1}$ | 3704 | 1475 | 2547 | 1142 | 887 | 1117 |

**Table A2.1.1:** ***Cadmium concentration in ryegrass (μg kg$^{-1}$) in the Sonning trial.***

| | Cutting 1 | Cutting 2 | Cutting 3 | Cutting 4 | Cutting 5 | Cutting 6 |
|---|---|---|---|---|---|---|
| Control | 10.95 | 7.54 | 4.80 | 5.62 | 2.50 | 1.60 |
| 5 t ha$^{-1}$ | 12.30 | 9.80 | 7.30 | 9.87 | 8.87 | 4.00 |
| 10 t ha$^{-1}$ | 30.90 | 12.40 | 12.70 | 11.20 | 11.80 | 11.20 |
| 50 t ha$^{-1}$ | 41.30 | 19.00 | 18.00 | 16.50 | 16.80 | 11.58 |

**Table A2.1.2:** ***Cadmium uptake in ryegrass (μg plot$^{-1}$) in the Sonning trial.***

| | Cutting 1 | Cutting 2 | Cutting 3 | Cutting 4 | Cutting 5 | Cutting 6 |
|---|---|---|---|---|---|---|
| Control | 14.77 | 5.07 | 4.23 | 4.44 | 1.53 | 0.92 |
| 5 t ha$^{-1}$ | 26.71 | 8.89 | 7.72 | 7.11 | 4.87 | 2.20 |
| 10 t ha$^{-1}$ | 75.92 | 13.84 | 13.57 | 6.56 | 6.32 | 6.36 |
| 50 t ha$^{-1}$ | 153.01 | 28.03 | 45.85 | 18.85 | 14.91 | 12.94 |

**Table A2.2.1:** ***Nickel concentration in ryegrass (μg g$^{-1}$) in the Sonning trial.***

| | Cutting 1 | Cutting 2 | Cutting 3 | Cutting 4 | Cutting 5 | Cutting 6 |
|---|---|---|---|---|---|---|
| Control | 0.48 | 0.35 | 0.42 | 0.82 | 0.75 | 0.69 |
| 5 t ha$^{-1}$ | 0.90 | 0.48 | 0.33 | 0.86 | 0.56 | 0.66 |
| 10 t ha$^{-1}$ | 1.91 | 0.94 | 0.70 | 1.15 | 0.97 | 1.07 |
| 50 t ha$^{-1}$ | 2.76 | 0.90 | 0.89 | 1.24 | 1.20 | 1.25 |

**Table A2.2.2:** ***Nickel uptake in ryegrass (mg plot$^{-1}$) in the Sonning trial.***

| | Cutting 1 | Cutting 2 | Cutting 3 | Cutting 4 | Cutting 5 | Cutting 6 |
|---|---|---|---|---|---|---|
| Control | 0.65 | 0.24 | 0.38 | 0.65 | 0.46 | 0.40 |
| 5 t ha$^{-1}$ | 1.97 | 0.44 | 0.36 | 0.62 | 0.31 | 0.36 |
| 10 t ha$^{-1}$ | 4.70 | 1.05 | 0.76 | 0.67 | 0.52 | 0.61 |
| 50 t ha$^{-1}$ | 10.23 | 1.34 | 2.27 | 1.42 | 1.06 | 1.40 |

**Table A2.3.1:** ***Lead concentration in ryegrass (μg g$^{-1}$) in the Sonning trial.***

| | Cutting 1 | Cutting 2 | Cutting 3 | Cutting 4 | Cutting 5 | Cutting 6 |
|---|---|---|---|---|---|---|
| Control | 5.89 | 1.05 | 0.76 | 0.85 | 0.73 | 1.99 |
| 5 t ha$^{-1}$ | 3.23 | 0.95 | 0.82 | 0.96 | 1.08 | 1.69 |
| 10 t ha$^{-1}$ | 3.88 | 1.19 | 0.94 | 1.57 | 1.22 | 1.94 |
| 50 t ha$^{-1}$ | 7.83 | 1.67 | 1.51 | 1.05 | 0.82 | 2.05 |

**Table A2.3.2:** ***Lead uptake in ryegrass (mg plot$^{-1}$) in the Sonning trial.***

| | Cutting 1 | Cutting 2 | Cutting 3 | Cutting 4 | Cutting 5 | Cutting 6 |
|---|---|---|---|---|---|---|
| Control | 7.95 | 0.71 | 0.67 | 0.68 | 0.45 | 1.15 |
| 5 t ha$^{-1}$ | 7.02 | 0.86 | 0.87 | 0.69 | 0.60 | 0.93 |
| 10 t ha$^{-1}$ | 9.55 | 1.33 | 1.01 | 0.92 | 0.66 | 1.10 |
| 50 t ha$^{-1}$ | 29.04 | 2.47 | 3.87 | 1.21 | 0.73 | 2.30 |

**Table A2.4.1:** ***Zinc concentration in ryegrass (μg g$^{-1}$) in the Sonning trial.***

| | Cutting 1 | Cutting 2 | Cutting 3 | Cutting 4 | Cutting 5 | Cutting 6 |
|---|---|---|---|---|---|---|
| Control | 11.40 | 11.90 | 8.59 | 11.57 | 11.25 | 13.61 |
| 5 t ha$^{-1}$ | 13.39 | 16.61 | 8.40 | 11.55 | 11.94 | 12.02 |
| 10 t ha$^{-1}$ | 19.52 | 21.42 | 14.24 | 14.84 | 15.45 | 15.90 |
| 50 t ha$^{-1}$ | 32.71 | 22.75 | 16.34 | 16.89 | 19.72 | 19.60 |

**Table A2.4.2:** ***Zinc uptake in ryegrass (mg plot$^{-1}$) in the Sonning trial.***

| | Cutting 1 | Cutting 2 | Cutting 3 | Cutting 4 | Cutting 5 | Cutting 6 |
|---|---|---|---|---|---|---|
| Control | 15.37 | 8.01 | 7.57 | 9.15 | 6.91 | 7.82 |
| 5 t ha$^{-1}$ | 29.09 | 15.08 | 8.89 | 8.32 | 6.55 | 6.60 |
| 10 t ha$^{-1}$ | 47.97 | 23.91 | 15.22 | 8.69 | 8.28 | 9.03 |
| 50 t ha$^{-1}$ | 121.22 | 33.58 | 41.64 | 19.30 | 17.50 | 21.90 |

**Table A3.1.1:** ***Soil pH measured in $H_2O$ suspension in the Sonning trial.***

| | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 |
|---|---|---|---|---|---|---|---|
| Control | 7.08 | 7.20 | 6.80 | 7.27 | 6.91 | 7.02 | 7.08 |
| 5 t ha$^{-1}$ | 6.27 | 6.74 | 6.70 | 6.51 | 6.81 | 6.93 | 6.87 |
| 10 t ha$^{-1}$ | 6.48 | 6.36 | 6.16 | 6.76 | 6.25 | 6.76 | 6.60 |
| 50 t ha$^{-1}$ | 5.83 | 6.03 | 5.68 | 6.02 | 5.31 | 6.09 | 6.14 |

**Table A3.1.2:** ***Soil pH measured in $CaCl_2$ suspension in the Sonning trial.***

| | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 |
|---|---|---|---|---|---|---|---|
| Control | 6.46 | 6.46 | 6.36 | 6.60 | 6.43 | 6.28 | 6.47 |
| 5 t ha$^{-1}$ | 5.91 | 6.43 | 6.41 | 6.24 | 6.38 | 6.28 | 6.34 |
| 10 t ha$^{-1}$ | 6.14 | 6.15 | 5.78 | 6.37 | 5.74 | 6.08 | 6.18 |
| 50 t ha$^{-1}$ | 5.57 | 6.04 | 5.48 | 6.10 | 5.02 | 6.00 | 5.97 |

**Table A3.2:** ***Soil pH measured in $H_2O$ suspension in the Vardates trial (Courtesy of Papadopoulos* et al.*).***

| | Week 0 | Week 3 | Week 6 | Week 9 | Week 17 | Week 33 | Week 48 |
|---|---|---|---|---|---|---|---|
| Control | 8.44 | 8.52 | 8.60 | 7.95 | 7.74 | 8.36 | 8.43 |
| 4 t ha$^{-1}$ | 8.39 | 7.92 | 8.54 | 7.74 | 7.70 | 8.25 | 8.39 |
| 10 t ha$^{-1}$ | 8.39 | 8.57 | 8.41 | 7.99 | 7.76 | 8.23 | 8.45 |
| 50 t ha$^{-1}$ | 8.29 | 8.40 | 8.39 | 7.75 | 7.61 | 8.27 | 8.44 |

**Table A4.1:** ***Loss-On-Ignition (%) in the Sonning trial.***

| | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 |
|---|---|---|---|---|---|---|---|
| Control | 4.43 | 4.40 | 5.23 | 4.34 | 4.67 | 5.10 | 4.65 |
| 5 t ha$^{-1}$ | 5.23 | 5.08 | 5.13 | 5.62 | 4.61 | 5.30 | 5.07 |
| 10 t ha$^{-1}$ | 5.29 | 4.85 | 5.21 | 6.35 | 4.97 | 5.13 | 4.48 |
| 50 t ha$^{-1}$ | 6.48 | 6.42 | 8.07 | 7.71 | 8.32 | 8.06 | 6.13 |

**Table A4.2:** ***Loss-On-Ignition (%) in the Vardates trial (Courtesy of Papadopoulos*** **et al.*).***

| | Week 0 | Week 3 | Week 6 | Week 9 | Week 17 | Week 33 | Week 48 |
|---|---|---|---|---|---|---|---|
| Control | 3.84 | 3.36 | 3.55 | 3.79 | 3.80 | 4.31 | 3.68 |
| 4 t $ha^{-1}$ | 4.35 | 4.26 | 3.53 | 4.23 | 3.99 | 4.96 | 4.39 |
| 10 t $ha^{-1}$ | 4.36 | 3.94 | 3.78 | 3.85 | 3.51 | 4.90 | 4.91 |
| 50 t $ha^{-1}$ | 4.76 | 5.11 | 3.94 | 4.41 | 4.36 | 5.29 | 4.85 |

**Table A5.1:** ***DTPA-extractable Cd (μg $kg^{-1}$) in the Sonning trial.***

| | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 |
|---|---|---|---|---|---|---|---|
| Control | 68 | 69 | 68 | 64 | 66 | 69 | 66 |
| 5 t $ha^{-1}$ | 70 | 71 | 72 | 62 | 68 | 75 | 70 |
| 10 t $ha^{-1}$ | 84 | 80 | 81 | 77 | 80 | 83 | 85 |
| 50 t $ha^{-1}$ | 106 | 107 | 120 | 102 | 145 | 117 | 110 |

**Table A5.2:** ***DTPA-extractable Ni (μg $g^{-1}$) in the Sonning trial.***

| | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 |
|---|---|---|---|---|---|---|---|
| Control | 0.61 | 0.64 | 0.63 | 0.60 | 0.65 | 0.60 | 0.61 |
| 5 t $ha^{-1}$ | 0.69 | 0.69 | 0.69 | 0.62 | 0.71 | 0.65 | 0.64 |
| 10 t $ha^{-1}$ | 0.93 | 0.81 | 0.89 | 0.75 | 0.85 | 0.83 | 0.92 |
| 50 t $ha^{-1}$ | 1.23 | 1.31 | 1.55 | 1.24 | 2.00 | 1.31 | 1.31 |

**Table A5.3:** ***DTPA-extractable Pb (μg $g^{-1}$) in the Sonning trial.***

| | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 |
|---|---|---|---|---|---|---|---|
| Control | 5.95 | 5.64 | 5.64 | 5.43 | 6.02 | 6.02 | 5.80 |
| 5 t $ha^{-1}$ | 5.53 | 5.51 | 4.97 | 5.59 | 5.81 | 5.81 | 5.31 |
| 10 t $ha^{-1}$ | 5.86 | 6.32 | 5.37 | 6.61 | 6.46 | 6.46 | 6.86 |
| 50 t $ha^{-1}$ | 8.27 | 8.90 | 7.68 | 9.25 | 8.81 | 8.81 | 8.78 |

**Table A5.4:** ***DTPA-extractable Zn (μg $g^{-1}$) in the Sonning trial.***

| | Week 0 | Week 2 | Week 4 | Week 8 | Week 16 | Week 32 | Week 48 |
|---|---|---|---|---|---|---|---|
| Control | 5.00 | 4.88 | 5.10 | 5.04 | 4.79 | 4.99 | 5.10 |
| 5 t $ha^{-1}$ | 6.64 | 5.89 | 6.43 | 5.86 | 5.84 | 6.30 | 4.81 |
| 10 t $ha^{-1}$ | 15.43 | 11.74 | 11.72 | 10.92 | 9.13 | 12.29 | 11.56 |
| 50 t $ha^{-1}$ | 14.48 | 14.72 | 18.19 | 15.01 | 21.51 | 14.90 | 15.66 |

## APPENDIX III

**Table A1:** ***Loss-On-Ignition and Cd, Ni, Pb and Zn digested with*** **Aqua regia** ***(μg $g^{-1}$). Soils from Great Billing.***

| | | LOI | Cd | Ni | Pb | Zn |
|---|---|---|---|---|---|---|
| Depth (cm) | | % | μg $g^{-1}$ | | | |
| 0-20 | | 25.78 | 40.71 | 335.68 | 667.97 | 1311.59 |
| 20-40 | Site 1 | 25.19 | 46.08 | 376.03 | 717.66 | 1384.1 |
| 40-60 | | 17.89 | 29.35 | 238.71 | 496.32 | 924.95 |
| 60-80 | | 16.95 | 23.28 | 215.28 | 408.35 | 784.06 |
| 0-20 | | 4.47 | 0.85 | 12.13 | 37.25 | 87.14 |
| 20-40 | Site 2 | 3.76 | 0.77 | 10.02 | 30.38 | 83.48 |
| 40-60 | | 2.87 | 0.64 | 5.31 | 21.96 | 84.46 |
| 60-80 | | 1.26 | 0.55 | 4.29 | 26.81 | 77.64 |
| 0-20 | | 5.06 | 0.93 | 20.33 | 67.8 | 119 |
| 20-40 | Site 3 | 4.52 | 0.82 | 18.23 | 30.35 | 100.47 |
| 40-60 | | 3.43 | 0.64 | 16.08 | 33.42 | 86.17 |
| 60-80 | | 2.25 | 0.52 | 14.55 | 28.64 | 72.58 |

**Table A2.1:** ***Cd in the leachate (μg) (1 year' s rainfall was applied in 4 Weeks).***

| | 450 mm Rainfall Rate per year | | | | | | |
|---|---|---|---|---|---|---|---|
| | Week 2 | Week 6 | Week 7 | Week 8 | Week 10 | Week 11 | Week 12 |
| Control | 0.25 | 0.39 | 0.29 | nd[a] | 0.43 | nd | 2.19 |
| 10 t $ha^{-1}$ | 0.24 | 0.20 | 0.60 | nd | 0.34 | 0.69 | 1.72 |
| 50 t $ha^{-1}$ | nd | 0.52 | nd | nd | nd | nd | 1.91 |
| | 900 mm Rainfall Rate per year | | | | | | |
| | Week 2 | Week 6 | Week 7 | Week 8 | Week 10 | Week 11 | Week 12 |
| Control | 0.63 | 1.19 | 4.15 | nd | 4.40 | 0.93 | 2.27 |
| 10 t $ha^{-1}$ | 0.41 | 0.87 | 1.03 | nd | 0.63 | 0.91 | 1.92 |
| 50 t $ha^{-1}$ | 0.52 | 0.45 | 3.43 | nd | 0.52 | nd | 6.27 |

*a: not detectable*

**Table A2.2:** ***Ni in the leachate (μg) (1 year' s rainfall was applied in 4 Weeks).***

| | 450 mm Rainfall rate per year | | | | | | |
|---|---|---|---|---|---|---|---|
| | Week 2 | Week 6 | Week 7 | Week 8 | Week 10 | Week 11 | Week 12 |
| Control | 9.73 | 15.38 | 1.64 | 2.66 | 24.87 | 10.90 | 13.27 |
| 10 t $ha^{-1}$ | 26.20 | 22.93 | 1.43 | 4.63 | 15.44 | 20.21 | 19.00 |
| 50 t $ha^{-1}$ | 40.76 | 46.54 | nd[a] | 0.97 | 21.19 | 15.48 | 26.07 |
| | 900 mm Rainfall Rate per year | | | | | | |
| | Week 2 | Week 6 | Week 7 | Week 8 | Week 10 | Week 11 | Week 12 |
| Control | 23.73 | 29.59 | nd | 11.26 | 9.40 | 42.05 | 29.56 |
| 10 t $ha^{-1}$ | 35.72 | 35.88 | 4.16 | 1.24 | 17.50 | 26.78 | 21.44 |
| 50 t $ha^{-1}$ | 30.85 | 34.34 | 31.56 | 4.15 | 25.74 | 22.43 | 29.83 |

*a: not detectable*

**Table A2.3:** ***Zn in the leachate (μg) (1 year' s rainfall was applied in 4 Weeks).***

| 450 mm Rainfall Rate per year | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Week 2 | Week 6 | Week 7 | Week 8 | Week 10 | Week 11 | Week 12 |
| Control | 3.55 | 21.17 | 96.77 | 65.70 | 92.38 | 32.90 | 225.76 |
| 10 t ha$^{-1}$ | 2.75 | 56.87 | 166.58 | 72.43 | 44.41 | 175.86 | 143.86 |
| 50 t ha$^{-1}$ | 8.94 | 258.66 | 95.38 | 70.84 | 116.30 | 62.53 | 328.26 |
| 900 mm Rainfall Rate per year | | | | | | | |
| | Week 2 | Week 6 | Week 7 | Week 8 | Week 10 | Week 11 | Week 12 |
| Control | 28.01 | 90.37 | 205.01 | 292.23 | 95.75 | 228.25 | 372.54 |
| 10 t ha$^{-1}$ | 3.72 | 74.57 | 234.61 | 224.18 | 67.79 | 282.09 | 308.33 |
| 50 t ha$^{-1}$ | 2.58 | 201.65 | 587.39 | 232.09 | 118.18 | 68.77 | 587.21 |

**Table A2.4:** ***Dissolved Organic Carbon in the leachate (mg) (1 year' s rainfall was applied in 4 Weeks).***

| 450 mm Rainfall Rate per year | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Week 2 | Week 6 | Week 7 | Week 8 | Week 10 | Week 11 | Week 12 |
| Control | 79.36 | 107.89 | 106.91 | 141.46 | 67.18 | 94.52 | 122.21 |
| 10 t ha$^{-1}$ | 109.09 | 124.72 | 118.70 | 144.21 | 91.18 | 105.06 | 146.10 |
| 50 t ha$^{-1}$ | 134.34 | 145.68 | 140.50 | 159.26 | 99.66 | 125.80 | 149.14 |
| 900 mm Rainfall Rate per year | | | | | | | |
| | Week 2 | Week 6 | Week 7 | Week 8 | Week 10 | Week 11 | Week 12 |
| Control | 158.53 | 207.82 | 124.90 | 151.95 | 103.89 | 77.34 | 111.27 |
| 10 t ha$^{-1}$ | 169.57 | 209.67 | 152.22 | 159.59 | 107.93 | 106.53 | 116.84 |
| 50 t ha$^{-1}$ | 181.47 | 235.03 | 186.46 | 171.29 | 139.22 | 108.57 | 117.87 |

**Table A3.1:** ***Distribution of Cd, Ni, Pb and Zn in the soil columns at 450 mm rainfall rate per year (*****Aqua regia*****-digested, µg g$^{-1}$).***

| | | Cd | | | | | | Ni | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0-2 cm | 2-4 cm | 4-6 cm | 6-8 cm | 8-10 cm | 10-15 cm | 0-2 cm | 2-4 cm | 4-6 cm | 6-8 cm | 8-10 cm | 10-15 cm |
| Year 1 | Control | 0.33 | 0.34 | 0.29 | 0.29 | 0.30 | 0.30 | 17.80 | 18.00 | 17.70 | 18.20 | 17.40 | 16.60 |
| | 10 t ha$^{-1}$ | 0.43 | 0.36 | 0.32 | 0.32 | 0.32 | 0.33 | 24.07 | 21.80 | 20.30 | 18.40 | 18.00 | 18.20 |
| | 50 t ha$^{-1}$ | 0.52 | 0.47 | 0.44 | 0.36 | 0.29 | 0.32 | 30.24 | 29.67 | 29.08 | 23.04 | 19.70 | 19.60 |
| Year 2 | Control | 0.31 | 0.28 | 0.28 | 0.28 | 0.28 | 0.29 | 14.29 | 14.48 | 14.53 | 13.38 | 12.23 | 12.48 |
| | 10 t ha$^{-1}$ | 0.40 | 0.36 | 0.29 | 0.29 | 0.28 | 0.29 | 22.87 | 20.03 | 17.30 | 14.98 | 12.67 | 12.33 |
| | 50 t ha$^{-1}$ | 0.50 | 0.46 | 0.43 | 0.36 | 0.29 | 0.28 | 30.64 | 30.00 | 29.45 | 22.29 | 15.13 | 12.11 |
| Year 3 | Control | 0.32 | 0.32 | 0.32 | 0.31 | 0.30 | 0.30 | 14.81 | 14.76 | 14.76 | 13.42 | 12.07 | 11.47 |
| | 10 t ha$^{-1}$ | 0.40 | 0.38 | 0.34 | 0.31 | 0.30 | 0.29 | 23.35 | 18.22 | 16.30 | 19.62 | 16.82 | 13.57 |
| | 50 t ha$^{-1}$ | 0.47 | 0.45 | 0.43 | 0.38 | 0.33 | 0.31 | 34.12 | 32.29 | 29.59 | 26.85 | 24.10 | 13.89 |

| | | Pb | | | | | | Zn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0-2 cm | 2-4 cm | 4-6 cm | 6-8 cm | 8-10 cm | 10-15 cm | 0-2 cm | 2-4 cm | 4-6 cm | 6-8 cm | 8-10 cm | 10-15 cm |
| Year 1 | Control | 18.34 | 17.50 | 17.32 | 16.87 | 16.42 | 17.39 | 32.14 | 38.11 | 35.79 | 34.80 | 33.82 | 33.33 |
| | 10 t ha$^{-1}$ | 21.78 | 18.30 | 17.50 | 17.50 | 17.50 | 17.50 | 45.70 | 43.71 | 39.50 | 36.47 | 33.45 | 34.66 |
| | 50 t ha$^{-1}$ | 26.07 | 19.80 | 18.30 | 17.25 | 16.20 | 17.72 | 73.78 | 57.21 | 56.25 | 42.06 | 27.87 | 31.63 |
| Year 2 | Control | 16.49 | 16.78 | 16.60 | 16.11 | 15.61 | 16.57 | 34.60 | 34.86 | 34.86 | 31.95 | 29.04 | 29.90 |
| | 10 t ha$^{-1}$ | 21.73 | 16.68 | 15.03 | 15.12 | 16.20 | 15.14 | 43.03 | 40.03 | 39.36 | 35.61 | 31.85 | 32.00 |
| | 50 t ha$^{-1}$ | 23.42 | 17.30 | 18.55 | 17.59 | 15.62 | 15.86 | 65.49 | 49.70 | 48.87 | 44.78 | 40.69 | 35.70 |
| Year 3 | Control | 17.05 | 16.11 | 17.07 | 17.01 | 16.96 | 16.22 | 36.80 | 34.60 | 31.91 | 31.15 | 30.40 | 32.76 |
| | 10 t ha$^{-1}$ | 22.53 | 16.85 | 17.70 | 17.27 | 16.83 | 14.54 | 53.87 | 50.10 | 44.86 | 36.98 | 29.10 | 28.79 |
| | 50 t ha$^{-1}$ | 25.35 | 18.30 | 18.12 | 17.85 | 17.59 | 17.71 | 74.66 | 61.54 | 60.04 | 46.30 | 32.56 | 31.47 |

**Table A3.2:** ***Distribution of Cd, Ni, Pb and Zn in the soil columns at 900 mm rainfall rate per year (*Aqua regia*-digested, µg g$^{-1}$).***

| | | Cd | | | | | | Ni | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0-2 cm | 2-4 cm | 4-6 cm | 6-8 cm | 8-10 cm | 10-15 cm | 0-2 cm | 2-4 cm | 4-6 cm | 6-8 cm | 8-10 cm | 10-15 cm |
| Year 1 | Control | 0.32 | 0.35 | 0.32 | 0.31 | 0.31 | 0.28 | 13.93 | 15.00 | 14.88 | 14.57 | 14.51 | 12.86 |
| | 10 t ha$^{-1}$ | 0.44 | 0.39 | 0.35 | 0.31 | 0.28 | 0.26 | 26.58 | 21.35 | 18.12 | 15.70 | 13.36 | 11.79 |
| | 50 t ha$^{-1}$ | 0.51 | 0.49 | 0.42 | 0.37 | 0.33 | 0.29 | 32.76 | 29.57 | 26.15 | 20.40 | 17.40 | 14.56 |
| Year 2 | Control | 0.33 | 0.33 | 0.33 | 0.28 | 0.30 | 0.31 | 14.30 | 15.15 | 15.32 | 15.41 | 16.11 | 12.61 |
| | 10 t ha$^{-1}$ | 0.42 | 0.30 | 0.32 | 0.29 | 0.28 | 0.29 | 26.77 | 22.60 | 18.70 | 15.41 | 14.10 | 13.73 |
| | 50 t ha$^{-1}$ | 0.51 | 0.47 | 0.38 | 0.34 | 0.32 | 0.27 | 40.00 | 35.20 | 30.90 | 21.02 | 15.06 | 15.51 |
| Year 3 | Control | 0.33 | 0.33 | 0.30 | 0.31 | 0.32 | 0.29 | 16.05 | 16.72 | 17.42 | 15.41 | 14.01 | 11.54 |
| | 10 t ha$^{-1}$ | 0.44 | 0.35 | 0.31 | 0.30 | 0.29 | 0.30 | 25.80 | 16.37 | 16.94 | 14.01 | 13.83 | 13.31 |
| | 50 t ha$^{-1}$ | 0.49 | 0.49 | 0.42 | 0.35 | 0.29 | 0.30 | 43.10 | 36.00 | 23.31 | 19.90 | 16.86 | 13.22 |

| | | Pb | | | | | | Zn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0-2 cm | 2-4 cm | 4-6 cm | 6-8 cm | 8-10 cm | 10-15 cm | 0-2 cm | 2-4 cm | 4-6 cm | 6-8 cm | 8-10 cm | 10-15 cm |
| Year 1 | Control | 17.82 | 14.19 | 17.00 | 15.62 | 16.20 | 16.15 | 34.69 | 35.36 | 33.69 | 31.69 | 28.98 | 28.34 |
| | 10 t ha$^{-1}$ | 22.23 | 17.16 | 16.87 | 16.60 | 16.49 | 16.33 | 48.30 | 48.53 | 41.53 | 35.02 | 30.03 | 28.35 |
| | 50 t ha$^{-1}$ | 24.73 | 18.00 | 18.00 | 17.00 | 17.00 | 17.08 | 76.80 | 59.04 | 51.53 | 36.69 | 30.00 | 27.30 |
| Year 2 | Control | 18.21 | 14.51 | 15.35 | 15.18 | 15.18 | 15.08 | 37.63 | 40.20 | 34.33 | 34.69 | 34.69 | 35.60 |
| | 10 t ha$^{-1}$ | 21.53 | 13.69 | 15.27 | 15.52 | 14.38 | 15.10 | 56.54 | 43.62 | 41.50 | 39.19 | 32.49 | 33.33 |
| | 50 t ha$^{-1}$ | 25.25 | 16.93 | 17.47 | 16.18 | 15.84 | 17.11 | 73.33 | 51.23 | 47.53 | 42.86 | 32.47 | 33.75 |
| Year 3 | Control | 18.10 | 16.52 | 17.05 | 16.07 | 17.44 | 17.71 | 34.86 | 36.86 | 34.86 | 34.19 | 28.35 | 25.83 |
| | 10 t ha$^{-1}$ | 22.23 | 16.13 | 18.62 | 17.41 | 17.29 | 18.21 | 53.37 | 49.61 | 44.21 | 37.53 | 35.24 | 30.32 |
| | 50 t ha$^{-1}$ | 26.49 | 17.85 | 19.19 | 17.85 | 16.96 | 18.44 | 89.96 | 65.21 | 54.04 | 42.86 | 38.86 | 28.92 |

## Appendix IV

**Table A1:** ***Replicates of ryegrass yields (g pot$^{-1}$).***

| | LS Soil | | | | | |
|---|---|---|---|---|---|---|
| | DOC-0 | | DOC-1 | | DOC-2 | |
| | Replicate 1 | Replicate 2 | Replicate 1 | Replicate 2 | Replicate 1 | Replicate 2 |
| Control | 0.01 | 0.01 | 0.03 | 0.03 | 0.06 | 0.05 |
| 10 t ha$^{-1}$ | 0.25 | 0.20 | 0.31 | 0.24 | 0.25 | 0.30 |
| 50 t ha$^{-1}$ | 0.44 | 0.40 | 0.42 | 0.42 | 0.47 | 0.40 |
| | SCL Soil | | | | | |
| Control | 0.06 | 0.08 | 0.14 | 0.13 | 0.07 | 0.08 |
| 10 t ha$^{-1}$ | 0.26 | 0.32 | 0.33 | 0.31 | 0.32 | 0.38 |
| 50 t ha$^{-1}$ | 0.52 | 0.5 | 0.39 | 0.61 | 0.62 | 0.41 |