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THE BIOAVAILABILITY OF CERTAIN HEAVY METALS IN SELECTED ORGANIC PRODUCTS

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THE BIOAVAILABILITY OF CERTAIN HEAVY METALS IN SELECTED ORGANIC PRODUCTS

By

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DECLARATION

I declare that this thesis is the result of the author's own original work, except where acknowledged, and contains no work accepted for any other degree or diploma at any university.

C E Steyn

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ABSTRACT

A short-term greenhouse pot experiment was conducted to determine the bioavailability of certain trace elements in organic fertilizers. Ni, Co, Cd, Cu, and Zn were selected for study. Lettuce (*Lactuca sativa L.* cv. Golden City), wheat (*Triticum aestivum L.* cv. Inia) and beans (*Phaseolus vulgaris L.* cv. Wintergreen) were used as test crops for metal uptake from 4 kg of soil during a 6 week growth period. A kraal manure and disinfected sewage sludge were applied at 5, 10, and 20 t/ha and a control treatment with no application of an OSA (Organic Soil Ameliorant) was also included. The soil pH was adjusted to just below 5 and above 6,5 by the application of 2 and 11 t/ha $CaCO_3$, respectively. The total trace element content of the above-ground parts of the lettuce and wheat and the pods of the beans were determined while four extractants, NH_4-EDTA, EPA method 3050, DTPA, and AB-DTPA, were used to determine the concentration of the respective elements in the soil. The lettuce plants accumulated the most Co, Cd, and Zn, the wheat plants accumulated the most Cu and the bean pods the most Ni. An increase in soil pH mostly caused the plant concentration (mg/kg) of the trace elements to decrease, although there were some notable exceptions. The soil pH had different influences on the different extractant-metal combinations for a number of reasons. The EPA method 3050 did not predict soil loading and plant bioavailability of trace elements very well. The other extractants, however, predicted soil metal loading well even with very small increases in metal content of the soil. Good predictions of plant bioavailability was also obtained for certain extractant, metal and plant combinations but not in as many cases as expected. Legislation for the maximum permissible Ni content in soil and Cd content in foodstuffs was found to be higher than the average concentrations of these metals in the soil and foodstuffs, respectively. More research is therefore needed to clarify the situation.

UITTREKSEL

’n Korttermyn-poteksperiment om die biobeskikbaarheid van sekere spoorelemente te bepaal, is in die glashuis uitgevoer. Ni, Co, Cd, Cu, en Zn is geselekteer vir die studie. Slaai (*Lactuca sativa L.* cv. Golden City), koring (*Triticum aestivum L.* cv. Inia) en boontjies (*Phaseolus vulgaris L.* cv. Wintergreen) is as toetsgewasse gebruik om die metaalopname uit 4 kg grond gedurende ’n ses-week groeiperiode te bepaal. ’n Kraalmis en ontsmette rioolslyk is teen peile van 5, 10, en 20 t/ha toegedien. ’n Kontrolebehandeling met geen toevoeging van OGV (organiese grondverbeteraar) is ook by die proef ingesluit. Die grond-pH is verstel na net onder 5 en bo 6,5 deur onderskeidelik 2 en 11 t/ha $CaCO_3$ by te voeg. Die totale spoorelement-inhoud van die bogroei van die slaai, koring en die peule van die boontjies is bepaal, terwyl vier ekstraheermiddels, nl. NH_4-EDTA, EPA metode 3050, DTPA en AB-DTPA, gebruik is om die spoorelementkonsentrasie van die grond te bepaal. Die slaai het die meeste Co, Cd, en Zn geakkumuleer, terwyl die koringplante die meeste Cu en die boontjiepeule die meeste Ni geakkumuleer het. ’n Toename in grond-pH het meestal veroorsaak dat die plantkonsentrasie (mg/kg) van spoorelemente afneem, alhoewel daar ’n paar uitsonderings was. Die onderskeie ekstraheermiddel-metaal-kombinasies is verskillend deur die grond-pH beïnvloed, vir verskeie redes. Die EPA metode 3050 het nie die belading van spoorelemente in die grond en hul plantbeskikbaarheid goed voorspel nie. Die ander ekstraheermiddels het wel die byvoeging van spoorelemente aan die grond goed voorspel, selfs met klein byvoegings. Vir sekere kombinasies van ekstraheermiddels, metale en plante kon die plantbeskikbaarheid ook goed voorspel word, maar nie in soveel gevalle as wat verwag is nie. Daar is gevind dat wetgewing vir die maksimum toelaatbare Ni-inhoud vir grond en Cd-inhoud vir plantvoedselsoorte hoër was as onderskeidelik die gemiddelde inhoud van grond en plantvoedselsoorte. Meer navorsing word dus benodig om klaarheid oor die situasie te kry.

CHAPTER 1

INTRODUCTION

1.1 Statement of problem

Many organic soil ameliorants are commercially available in South Africa. For the purpose of this study the term organic soil ameliorant (OSA) encompasses organic fertilizers, organic composts, manures, peats and sewage sludge. Organic fertilizers are composted organic materials with a nutrient value approved by the Fertilizer Society of South Africa and which is enriched to maintain these levels. On the other hand, composted organic materials have no such specifications but are simply organic materials that have been composted. Manures are animal faeces. Peats are defined as dark brown or black residuum produced by the partial decomposition and disintegration of mosses, sedges, trees and other plants that grow in wet places (Van der Watt & Van Rooyen, 1990). Sewage sludge originates from sewage treatment works.

The trace element content of these products can be determined relatively easily. However, the influence of these concentrations on the environment are not generally known. Trace elements are defined as elements occurring in trace amounts in living matter, and include the heavy metals. These elements can be essential or non-essential to organisms but may be toxic in excessive amounts. OSAs potentially have a great pollution danger because of their concentrated use in agriculture. Therefore, the influence of OSAs on the trace element content of soil and plants and the subsequent danger to animals and humans need to be evaluated.

1.2 Motivation

OSAs, usually produced from waste products, have a great pollution potential. The extent of this danger is not well known. The pollution due to OSAs emanates from its inorganic components (heavy metals, nitrogen, etc.), organic components (the most common being pesticides, polychlorinated biphenyls, halogenated aliphatics, ethers, phthalate esters, monocyclic aromatics, phenols, polycyclic aromatic hydrocarbons and nitrosamines) and pathogens (bacteria, protozoa, helminths and viruses) (Korentajer, 1991). One of the major concerns in applying OSAs to agricultural land is their trace element content and the accumulation of these elements in the plant root zone, resulting in a decrease in crop yields and contamination of edible plant parts. The heavy metal content of OSAs varies quite substantially (Table 1). The question therefore is: at which concentrations are heavy metals dangerous to the environment?

As is evident from the report by Bruemmer & van der Merwe (1989), very little research has been carried out on the safe disposal of waste products on agricultural land in South Africa. Guidelines in South Africa are therefore not experimentally well founded. The Department of Health and Population Development has compiled guidelines based on a survey of heavy metal concentrations in sewage sludge from the Cape Province, experimental lysimeter and plot studies (Kitshoff, Engelbrecht & Nell, 1987), and guidelines proposed by Smith and Vasiloudis (1990; 1991) who analyzed sludges from seventy-seven South African sewage works and also consulted overseas guidelines. The great variation in guidelines between countries, and even within South Africa, shows the lack of understanding of the effect of trace elements on the environment due to sludge application.

During preliminary studies it was found that the trace element content of certain commercially available organic materials was higher than the allowable guidelines in South Africa and the average or median of guidelines from many different countries (shown by the shaded areas in Table 2). Due to the uncertainty regarding the correctness of the guidelines, and before harsh regulatory action is taken, the effect of the product on the environment should be evaluated experimentally. The evaluation should take place by way of bioavailability studies, since this is the route by which the trace elements become available to animals and humans.

If the trace element uptake by plants can be predicted for a specific sludge application on a particular soil, long-term loading limits ensuring that additions of trace elements to the food chain are within tolerable limits, can be established (Corey, King, Lue-Hing, Fanning, Street & Walker, 1987).

Table 1. Typical ranges of heavy metal concentrations in sewage sludge, farmyard manure, and composts, as well as a summary of contaminant concentrations found in South African sewage sludge. Taken from Alloway (1990) and Smith & Vasiloudis (1991).

	Sewage sludge	Farmyard manure	Composted refuse	South African sewage sludge		
				Range	Mean	Median
	mg/kg, dry basis					
Ag	<960	-	-	-	-	-
As	3-30	3-25	2-52	<1-34	7	6
B	15-1000	0,3-0,6	-	6-78	31	28
Cd	<1-3410	0,1-0,8	0,01-100	<1-22	12	3
Co	1-260	0,3-24	-	-	-	-
Cr	8-40600	1,1-55	1,8-410	25-10015	551	220
Cu	50-8000	2-172	13-3580	80-17217	654	355
F	-	-	-	32-1260	128	97
Hg	0,1-55	0,01-0,36	0,09-21	<1-22	5	3
Mn	60-3900	30-969	-	-	-	-
Mo	1-40	0,05-3	-	1-24	6	5
Ni	6-5300	2,1-30	0,9-279	6-2660	154	55
Pb	29-3600	1,1-27	1,3-2240	67-10137	452	214
Sb	3-44	-	-	-	-	-
Se	1-10	2,4	-	<1-107	4	2
U	-	-	-	-	-	-
V	20-400	-	-	-	-	-
Zn	91-49000	15-566	82-5894	237-17680	2054	1432

Table 2. A comparison of maximum permissible contaminant concentration in sewage sludge and heavy metal content of some commercially available organic soil ameliorants.

	Maximum permissible contaminant concentration in sewage sludge			Contaminant concentration in organic soil ameliorants[4]			
	Mean[1]	Median[2]	South Africa[3]	OSA 10	OSA 20	OSA 3	OSA 33
	——mg/kg——			——mg/kg——			
V	-	-	-	52,80	32,33	32,28	1,59
Cr	891	1000	1750	103,10	294,20	56,74	2,65
Ni	233	200	200	199,20	577,50	113,70	23,20
Co	100	100	100	19,25	7,13	6,68	1,30
Cu	1332	1000	750	25,44	1477,00	46,86	48,90
Zn	3367	2775	2750	228,20	1077,00	154,20	333,00
As	30	15	15	3,82	16,19	3,59	1,32
Mo	20	19	25	1,70	13,34	1,74	1,80
Cd	19	20	20	-	3,82	-	-
Hg	11	10	10	-	3,17	-	-
Pb	588	500	400	52,04	594,70	45,16	12,60

[1] Mean of recommended maximum permissible contaminant concentration in sewage sludge for different countries (Appendix 1, Table 11).

[2] Median of recommended maximum permissible contaminant concentration in sewage sludge for different countries (Appendix 1, Table 11).

[3] Maximum permissible metal content in type D sewage sludge in South Africa (Dept. of National Health and Population Development, 1991) (Appendix 1, Table 1).

[4] Digested by EPA Method 3050 and analyzed by ICP-MS (Appendix 2).

OSA 10: Culterra kraal manure
OSA 20: Growman disinfected sewage sludge
OSA 3: Braaks Moo Poo
OSA 33: Chicken manure

1.3 Objectives

In view of the problems outlined above, the objectives of this study were:

(i) to analyze selected commercially available South African organic soil ameliorants (OSAs)

(ii) to evaluate the bioavailability of selected trace elements as influenced by:

  (a) plant species

  (b) soil pH

  (c) organic matter type

  (d) OSA application levels

(iii) to compare selected extractants as indicators of bioavailability

(iv) to investigate experimental procedures dealing with very low levels of trace elements.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The soil acts as a sink for the collection of pollutants. Metals in particular may accumulate rapidly but are depleted slowly by leaching, plant uptake, erosion or deflation (Davies, 1980). It is this persistence, as well as the fact that, unlike air and water, we are unable to clean soil (Bruemmer & van der Merwe, 1989), that makes soil pollution a far more serious matter than air or water pollution (Davies, 1980). The problem is aggravated by the gradual nature of the soil deterioration and therefore often is undetected until too late.

One of the consequences of an increase in human population is the increase in waste products, including organic waste products e.g. sludge, composts, manure, etc. Considerable research on the effects of the application of organic wastes to agricultural land has been conducted over the past fifteen years. Research reports have produced many conflicting results which are, in part, probably due to plant and soil variables (MacLean, Robinson & MacConnell, 1987). However, there is a growing concern with the quality of compost, especially with regard to its content of heavy metals (Lustenhouwer, Hin, Maessen & Den Boef, 1990). In general, it has been shown that the application of an OSA to agricultural land increases crop yields. On the other hand, heavy metal accumulation in the plant tissue also occurs. The fertility benefits must therefore be weighed against the potential hazards of metal contamination through the application of OSAs to agricultural land.

2.2 The use of organic soil ameliorants

2.2.1 Production of organic wastes

Accurate estimates of the quantities of the various solid wastes produced are difficult to obtain for a variety of reasons. These include lack of control, lack of co-operation from industry, poor record keeping, absence of standard measuring methods and a lack of funds, especially at local government level (Korentajer, 1992).

The total amount of agricultural wastes (including the timber industry) produced in the RSA is estimated to be 20 million tons per year. Agricultural wastes include manure from cattle feedlots (10 million tons per year), non-toxic wastes (e.g. tree felling-trimmings and sawdust, maize stalks, chaff, straw and hay, and sugar cane bagasse), reject produce (reject fruits and vegetables), and hazardous wastes consisting mostly of pesticides and fertilizers contained in run-off, resulting from agricultural operations (Korentajer, 1992).

A pollution problem may also arise from the disposal of sewage sludge on land. Due to the rapid population growth and increasing industrialisation, municipalities and industries in South Africa generate increasing amounts of sludge (Korentajer, 1991). At present South Africa has a population of about 33 million people (Directorate Agricultural Economic Trends, 1994). According to a survey of 77 sewage treatment works serving about a third of the country's people, every person produces 16,5 kg of after-treatment dry sludge a year (Smith & Vasiloudis, 1990). If this is extrapolated to the country as a whole, about 540 000 tons dry sewage sludge may be produced annually.

2.2.2 Composition of organic wastes

Increased yields from the application of OSAs to agricultural land are mainly due to the nutrients nitrogen and phosphorus contained therein. Other inorganic chemical components of OSAs, such as potassium, magnesium, calcium and certain trace elements are also beneficial. In an OSA to which lime has been added as a stabiliser or conditioner, the calcium present will help in ameliorating soils of low pH (Smith & Vasiloudis, 1991). The concentrations of these nutrients found in South African and UK sewage sludges are shown in Table 3. The organic matter content will vary substantially depending on the source of the OSA. Nevertheless, OSAs can be utilized as soil amendments to increase the organic matter content of soil in order to improve its physical condition and to increase its water holding capacity.

However, OSAs may also contain toxic substances in concentrations higher than those found in typical soils. Their continued application could therefore lead to adverse crop and food chain effects. These effects can be due to plant uptake of certain metals and other contaminants in organic and inorganic chemical forms, and

Table 3. Summary of macronutrient concentrations (g/kg, dry basis) found in South African and UK sewage sludges (mainly digested) (Smith & Vasiloudis, 1991).

Nutrient	**Range**	
	South Africa	**UK**
	— g/kg, dry basis —	
Nitrogen	17-58	15-25
Phosphorus	4-41	5-18
Potassium	1-11	1-3
Calcium	11-79	16-25
Magnesium	2-13	1-5

pathogens in the OSA (Smith & Vasiloudis, 1991). The range of concentrations of heavy metals found in OSAs commonly used in South Africa and in South African sewage sludge is given in Table 2.

The role of toxic organic compounds (the most common being pesticides, polychlorinated biphenyls, halogenated aliphatics, ethers, phthalate esters, monocyclic aromatics, phenols, polycyclic aromatic hydrocarbons and nitrosamines) and pathogens (the most common being bacteria, protozoa, helminths an viruses) in soil pollution must not be underestimated (Korentajer, 1991). This aspect of soil pollution is outside the scope of this study but is discussed by Kitshoff *et al,* (1987).

2.2.3 Disposal options

Organic wastes are disposed of mainly in five ways:

(a) Application, in raw or composted form, to agricultural land.

(b) Surface disposal to designated land or so-called "sacrificial land"

(c) Burial in sanitary landfill sites.

(d) Disposal at sea.

(e) Incineration.

These are not the only disposal methods. Some of the wastes can be used as feed for livestock (e.g. chicken manure for cattle feed). In certain areas organic wastes are frequently used as building materials and can even be used in bricks. The manufacture of fuel from organic wastes is also being researched.

If we consider the disposal of sewage sludge more closely we find that less than 30 % of the sewage sludge produced in South Africa is used beneficially. This is calculated from the work of Smith and Vasiloudis (1990) who studied 77 sewage treatment plants in South Africa. Of these treatment plants only 24 supply some of their sewage sludge for beneficial use. This equates to less than 30 % of the sewage sludge produced. In the USA a third of the sewage sludge is used effectively on land or is sold or given away for use in gardens; this practice is being expanded. In Japan 42 % of the sewage sludge is used for coastal reclamation, gardens and farming. In the United Kingdom 51 % of the sewage sludge is used on land (Environmental Protection Agency, 1993). Sewage sludge can therefore be used to a far greater extent in South Africa, but precautions have to be taken to prevent pollution.

2.2.4 Legislation

Legislation for the use of OSAs in agriculture is very difficult to compile because there are so many factors which may influence the effect that OSAs may have on the environment. Furthermore, the legislation must be developed from limited and contradicting information. Various countries therefore have different legislations (Oberholster, 1983; Bruemmer and Van der Merwe, 1989; Schachtschabel, Blume, Bruemmer, Hartge and Schwertmann, 1989; Omex Environmental Ltd, 1990; Department of National Health and Population Development, 1991; Smith and Vasiloudis, 1991; Töpfer, 1992; Environmental Protection Agency, 1993) and some of these are summarized in Appendix 1.

2.3 Trace element bioavailability from OSAs

Assessment of the risk to humans and animals from toxic constituents in OSAs, requires a knowledge of their potential to be transferred from the amended soil to crops and to animals which ingest these crops (Chaney, Bruins, Baker, Korcak, Smith & Cole, 1987). This potential and the attempts to determine it are influenced by many factors.

2.3.1 The effect of soil properties on bioavailability of trace elements

For plant uptake of trace elements from sludge-amended soil, some researchers confirm the importance of soil type while others found no significant correlation with soil type . However, today it is commonly accepted that soil type plays an important role in heavy metal availability and therefore toxicity (MacLean *et al*, 1987).

Hue, Silva & Afirin (1988) showed that soils differed in their ability to tolerate high metal loading rates when the metals are added in sludges. Andepts could tolerate the highest loading rates, more so than Vertisols, while Oxisols could only tolerate low loading rates.

Soil consists of a heterogeneous mixture of different organic and organomineral substances, clay minerals, oxides of iron, aluminum, and manganese, and other solid components as well as a variety of soluble substances. Therefore there are many binding mechanisms for heavy metals in soil which vary with the composition of the soils, the soil reaction, and redox conditions. Thus, an element may form different species according to whether it is bound to various soil compounds, reacting surfaces, or external or internal binding sites with different bonding energies (Bruemmer, 1986). Some of these forms are:

(1) Simple or complex ions in soil solution

(2) exchangeable ions

(3) ions linked to organic substances

(4) ions occluded in or co-precipitated with oxides, carbonates and phosphates, or other secondary minerals

(5) ions in the crystalline lattices of the primary minerals (Viets, 1962; MacLaren & Crawford, 1973; Soon & Bates, 1982).

The ecological effects of heavy metals in soils are closely related to their concentration and species distribution in the solid and liquid phases of soils (Bruemmer, 1986). Addition of organic wastes changes the composition of the soil and organic material, subsequently affecting metal species distribution.

Soil properties that affect the reactions and resultant plant uptake of sewage sludge constituents include pH, organic matter content, cation exchange capacity, iron and aluminum oxides, texture, aeration, specific sorption sites and water availability (Sommers, Van Volk, Giordano, Sopper & Bastian, 1987).

Soil pH is probably the soil property that affects the uptake of heavy metals the most. It is commonly recommended that soil pH be maintained above 6,5 for sludge-amended soils, although some reports indicated adequate control of metal uptake at pH 6,0 (Sommers *et al*, 1987; Mulchi, Adamu, Bell & Chaney, 1992). In South Africa the Department of National Health and Population Development presently recommends that for agricultural purposes sewage sludge should not be used at a soil pH(water) below 6, but there is presently a strong move to increase this to 6,5. At the higher soil pH the metals are generally less available, with the exception of As, Mo, Se, V and some valence states of Cr that are more available under alkaline or calcareous soil conditions (Adriano, 1986).

Hinesly, Redborg, Ziegler and Alexander (1982) found that the soil CEC inversely affected the uptake of Cd by maize when Cd was supplied as a soluble salt, but not when it was supplied as a constituent of a municipal sewage sludge. Korcak and Fanning (1985) confirmed this when they found that the more reactive the soil material (the higher the CEC), the higher the Zn and Cd uptake in sludge-amended soils but the lower the Zn and Cd uptake when added as metal salts.

Soil texture, structure and depth are important in determining porosity and soil hydraulic properties such as permeability and drainage rates. These properties also influence soil water content and aeration/respiration rates which influence the type and rates of soil microbial activity, soil chemical reactions, and plant root development.

Therefore, soil physical properties are related to trace element uptake by plants but current knowledge do not allow quantification of these relationships (Sommers *et al*, 1987).

Fe and Mn oxides have a high sorption capacity, particularly for trace elements of which large amounts can be accumulated in nodules and at Fe- and Mn-rich surfaces. The mechanisms of sorption involve the isomorphic substitution of divalent or trivalent cations for Fe and Mn ions, cation exchange reactions, and oxidation effects at the surface of the oxide precipitates. The amount of a particular ion that is adsorbed depends mainly on the pH of the equilibrium solution (Kabata-Pendias & Pendias, 1992).

2.3.2 The effect of the properties of organic soil ameliorants on trace element bioavailability

The composition of organic soil ameliorants differs considerably from that of soil. When added to soil it may increase or decrease element availability in the soil depending on its composition. For example, Narwal, Singh & Panhwar (1983) found that Zn, Cd and Ni in fodder rape were increased while Mn, Cu and Co decreased upon addition of sludge. The composition of OSAs may vary considerably (Table 1) due to the source of material and preparation methods. Therefore, there may be great variation in trace element content between different organic soil ameliorants. Furthermore, the trace element content may vary with time in a specific organic soil ameliorant due to the variation in the source of the product (e.g. variation in industrial effluent). Sommers, Nelson & Yost (1976) described this variation in sludge composition and found that the largest variations between the mean and median were found for Cd, Zn, Cu, Ni and Pb. Since it appears that metal levels in sludges may be one of the main factors determining acceptable rates of organic material application to land, good environmental management will require the identification and regulation of major sources of metals in the material.

The treatment of organic soil ameliorants before application may help to lessen the risk of toxic element levels occurring in the soil. Garcia, Hernandez & Costa (1990) found that composting and maturation increased the concentration of heavy metals in the materials due to the loss of weight of the material during these processes.

The metals also became more insoluble, and the extractants employed removed larger amounts of metals from the raw compost than from the mature compost.

The long-term effects of adding organic material to land must also be taken into account when considering the environmental impact of organic materials. This is emphasised by Wallace & Wallace (1994) who also suggest that studies should last at least 40 or more years. It is quite obvious that there will probably be a build-up of toxic elements in the soil if successive additions of organic wastes are made. This has been shown by Levine, Hall, Barrett & Taylor (1989), Kiemnec, Hemphill, Hickey, Jackson & Volk (1990) and MacGrath & Cegarra (1992).

A further cause for concern is the residual effect of toxic elements after sludge application has been terminated. For instance Heckman, Angle & Chaney (1987) indicated that sludge composition and soil pH can have a substantial influence on soybean metal uptake for at least 9 years after the initial sludge application. Conflicting results have however been obtained, probably due to the variety of conditions under which the experiments were conducted. The whole spectrum of results have been obtained, viz. an increase in availability (Schauer, Wright & Pelchat, 1980; Korcak & Fanning, 1985; Soon & Bates, 1985), a decrease (Hinesly, Ziegler & Barrett, 1979; Sommers, Nelson & Silviera, 1979; Bidwell & Dowdy, 1987), no change (Dowdy, Larson, Titrud & Latterell, 1978; Chang, Page & Bingham, 1982; MacGrath & Cegarra, 1992) while still others found that the residual effect is very specific for the element , organic material type and soil type (Silviera & Sommers, 1977). It was also found that there are fluctuations in availability with time. Kelling, Keeney, Walsh & Ryan (1977) found an initial increase (after 10 months) of extractable Cu, Zn, Cd and Ni in some treatment levels in a silt loam soil of pH 6; after 26 months, however, the extractability of all four metals had decreased. Wollan & Beckett (1979) found that extractable Cu decreases slightly in the shorter term and then returns to near its initial value after 90-100 days. Zn extractability on the other hand rises sharply after 2-4 days and then drops to near the initial value after 50- 100 days. The residual effect of organic waste pollution is very uncertain and should thus be considered anew for every set of conditions.

2.3.3 The effect of plant type on bioavailability of trace elements

Plant species and even cultivars vary significantly in their sensitivity as well as their ability to accumulate sludge-applied trace elements. This must therefore be considered when assessing the pollution danger of OSAs. Food plants which tolerate relatively high concentrations of potentially hazardous elements are likely to create a greater health risk than those which are more sensitive and show definite symptoms of toxicity (Alloway, 1990). Sauerbeck (1991) came to some very important conclusions regarding the uptake and availability of heavy metals derived from sewage sludge. It was found that the availability of sludge-derived heavy metals decreased in the order (Cd, Zn) > (Ni,Cu) > (Pb, Cr). Further, the simplified assumption that dicotyledons absorb more heavy metals than do monocotyledons, is not completely true. This rather depends on the species of plant and on the metal concerned. The vegetative plant parts are usually richer in heavy metals than the grains and the storage organs. However, this does not always hold, especially not for Cu and Ni. For example, Keefer, Singh & Horvath (1986) and Sauerbeck (1991) found that green beans showed an increase in Ni concentration in the edible portion following sludge application. Therefore, generalisation regarding crop sensitivities and ability to accumulate trace elements cannot easily be made. The sensitivity and accumulation ability of a plant depends on the specific element, the plant cultivar, plant part and environmental conditions (e.g. climate, soil pH, etc.).

Interactions between metals and other elements may also modify results and must be taken into account. Antagonistic and synergistic interactions can occur between heavy metals and major nutrient elements (Alloway, 1990).

2.3.4 The effect of experimental procedure on the determination of the bioavailability of trace elements

There are a few experimental procedures that may influence plant uptake of trace elements and should therefore be taken into account when interpreting results.

Incubation of sludge with the soil prior to planting may change the availability and therefore the uptake of metals by plants. According to Lagerwerff, Biersdorf, Milberg & Brower (1977), incubation considerably diminished Cu and Pb uptake. It is not clear what the effect of incubation is on the extractability of metals due to conflicting findings (Bloomfield & Pruden, 1975; Silviera & Sommers, 1977; Wollan & Beckett, 1979).

Considerable research on micro-element uptake by plants has been conducted using metal salts. However, metals applied to a soil as salt, commonly a sulphate, chloride, or nitrate salt, accumulates in plants more readily than the same quantity of metal added in sludge (Cunningham, Keeney & Ryan, 1975; Dijkshoorn, Lampe & Van Broekhaven, 1981; Hooda & Alloway, 1993). Metals in sludges are often associated with the insoluble inorganic components and are not readily plant available (Soon, 1981). Therefore, results obtained using metal salts cannot be extrapolated to metals added by sludge, when assessing their plant uptake and potential toxicity to plants and animals.

Greenhouse or pot experiments tend to enhance uptake of heavy metals over that of field experimentation (de Vries & Tiller, 1978). The enhanced uptake of trace elements in pot experiments generally results from four factors (Sommers *et al*, 1987):

(1) the use of acid forming fertilizers,
(2) increased soluble salt content from fertilizers in a smaller soil volume than in the field,
(3) root confinement, and
(4) unnatural watering patterns.

It must therefore be realized that plant concentrations found in greenhouse trials may differ from those found in field studies and therefore probably overestimates the environmental impact of heavy metal application by sludge.

Overseas guidelines or legislation cannot be used in South Africa because the soils and climate differ. Rainfall, temperature and sunlight (energy) play an important role in plant growth and soil reactions and thus the availability of metals for leaching and uptake are also influenced. It has been demonstrated that higher temperatures increase Zn, Cd and Pb uptake as well as that of some other macronutrients (Schmid, Haag &

Epstein, 1965; Wallace, Romney, Hale & Hoover, 1969; Cutler & Rains, 1974; Hooda & Alloway, 1993). Also, cool, wet climates stimulate Zn deficiencies (Bauer & Lindsay, 1965). Higher Cd concentrations in soybean shoots have been reported with an increase in soil temperature (Haghiri, 1974) but other researchers basically found no effect (Sheaffer, Decker, Chaney & Douglass, 1979).

Management practices used during cultivation of crops may also influence bioavailability. Some of these practices are irrigation, drainage, liming, fertilization and addition of amendments (Sommers *et al*, 1987). They should be carefully considered when projects are started and results interpreted.

When studying the impact of trace elements on the environment the interactive effect of a combination of trace elements must also be considered (Wallace & Wallace, 1994). Results can be vastly different when mixtures of metals are applied (Wallace, 1982; Wallace & Berry, 1983; Wallace & Berry, 1989).

2.4 Analytical techniques

2.4.1 ICP-MS in soil and plant analysis

Inductively coupled plasma-mass spectrometry (ICP-MS) is still a relatively new technique, which is only entering its second decade of development. Due to this, much of the applications that have been developed are preliminary and have been presented at conferences and workshops (Riddle, Van der Voet & Doherty, 1988). This is especially true for complex matrices such as soil (Yamasaki & Tamura, 1990), although the technique was originally developed for use in geoanalysis (Date, Cheung & Stuart, 1987). Since the first papers on ICP-MS were published (Houk, Fassel, Flesch, Svec, Gray & Taylor, 1980; Date & Gray, 1981; Date & Gray, 1983(a); Date & Gray, 1983(b); Gray & Date, 1983), the technique developed extremely rapidly and its performance is improving quite remarkably, taking advantage of progress made in the design of sample introduction systems for inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Marshall, 1990). Several reviews and reports, exclusively about the ICP-MS, on the development history and instrumentation of the ICP-MS in general have been published to date (Gray, 1986; Houk, 1986; Kawaguchi, 1988; Riddle *et al*, 1988; Douglas, 1989; Hieftje & Vickers, 1989; Date, 1991). Two very good books on the ICP-MS have also been published (Date & Gray, 1989; Jarvis, Gray & Houk, 1992).

The current capabilities of the instrument can be summarised as follows (from Cantle, 1987; Riddle *et al*, 1988):

(1) A wide range of elements can be determined with sub $\mu g\ kg^{-1}$ limits of detection
(2) Automatic operation
(3) Rapid, multi-element capability
(4) Wide dynamic range with linearity over six orders of magnitude
(5) Minimal matrix effects
(6) Fast sample throughput - typically a few minutes
(7) Direct analysis of solutions
(8) Simple spectra, even for complex matrices
(9) Rapid isotope ratio determination
(10) Isotope dilution capability for high accuracy determinations
(11) Low and uniform background.

The principal advantages of ICP-MS are the sensitivity of the technique and the relative simplicity of the mass spectrum (Riddle *et al*, 1988). Detection limits for most elements are far below $\mu g\ dm^{-3}$ levels and are improving almost daily as the technique improves.

2.4.2 Sample preparation

The high sensitivity of ICP-MS and the wide range of trace elements which are incorporated into most analytical protocols require that great care must be taken to avoid contamination during sample preparation. Contamination may arise from three main sources: (1) equipment used to grind, sieve and homogenise samples; (2) the laboratory environment and digestion apparatus and (3) analytical reagents used during sample preparation (Jarvis, 1992).

Care must be taken with laboratory cleanliness and safety. If this is not done all other precautions are useless and results will not be reliable. Where possible samples should be collected and stored in acid washed polyethylene containers or their equivalent. Prior to analysis, samples and standards should be presented to the instrument in disposable polystyrene containers. All volumetric glassware and plastic ware should be acid washed and then equilibrated with de-ionised water (VG PlasmaQuad System Manual, 1989).

Samples are usually acidified (e.g. natural waters) or digested (e.g. biological materials, soils) with a variety of inorganic acids prior to analysis. The choice of acid at this stage can have far-reaching effects with respect to the generation of polyatomic ion interferences. The polyatomic ion peaks in both H_2O_2 and HNO_3 are identical to those identified in deionised water and these media are therefore considered ideal matrices. When other acids are used, for example HCl or H_2SO_4, matrices which are far more complex are introduced and interferences should be anticipated.

The water used should have a resistance between 10^{10} to 10^{18} ohm and the acids should be at least Aristar grade or preferably freshly sub-boiled in a quartz still. Stock solutions of 1000 $\mu g\ ml^{-1}$ should be prepared. These can be Spex, BDH spectrosol grade reagent or equivalents. Stock solutions should be analyzed semi-quantitatively for impurities and this data logged with the reagents (VG PlasmaQuad System Manual, 1989). Care should be taken regarding blank contamination. Some elements are considerably more prone to cause memory by contamination of the ICP and interface components(e.g. Hg and Pb).

During preparation, the sample also needs to be subjected to certain constraints, to assure good results. Limitation of orifice geometry restricts the maximum dissolved solids tolerance to around 1% w/v. Such levels are easily handled by the V-groove nebuliser. However, for continuous operation, it is advised that this should be kept to around 0,2% maximum. Further, it is preferred that the acid concentration of the sample should be less than 5% v/v for HNO_3 and less than 1% v/v for HF, H_2SO_4 and H_3PO_4. The use of high acid concentrations can cause sample and skimmer cone damage and hence shorten their lifetimes (VG PlasmaQuad System Manual, 1989).

Despite considerable new research on sample introduction techniques, solution nebulisation remains the preferred method for most ICP-MS applications. Therefore, some form of dissolution is a prerequisite for routine analysis (Jarvis, 1992). For soil and plant analysis many digestion and extraction methods have been suggested. This is quite evident from the literature, e.g. those on soils (Haq, Bates & Skoon, 1980; Pickering, 1981; Beckett, 1989; Calvet, Bourgeois & Msaky, 1990) and in general (Boch, 1979).

2.4.2.1 Soil extractants

It is important to obtain information on the possible behaviour of toxic elements from studies which should be as simple as possible for use in routine analysis. Chemical procedures have been proposed that allow the determination of amounts of toxic elements assumed to occur as different species in the soil. These procedures are attractive because the manner in which an element species is influenced by different factors can easily be determined. However, the interpretation of the element concentration in the extract is not straight-forward because there are many external factors that may influence it. For example, the extraction may change the soil properties which changes the species distribution of the element in the soil.

Many soil extractants were developed when the main problems were nutrient deficiencies. Therefore, there is some doubt as to the suitability of the extractants in determining toxic levels in soils polluted by amendments, waste dumping or atmospheric deposition. Reliable soil extractants which can be used to determine the content of plant available heavy metals in a range of metal-contaminated soils are needed (Haq *et al*, 1980; Beckett, 1989).

Basically extraction methods can be divided into two groups: single chemical extraction and sequential extraction. Sequential extractions distinguish between the different states or species of the elements. They are supposed to be able to extract successively the fractions of elements occurring in different states (Calvet *et al*, 1990). In this study single chemical extractions were used. The extractants are considered to extract bioavailable elements. They could in principle, allow determination of amounts which are likely to be absorbed by plants, but the correlation between the quantity extracted and the bioavailability of the element in the soil to the plants must be determined for every element-soil-plant system.

Due to the fact that extractants were developed for a very specific use, the extractants are apparently unsuccessful under certain conditions that deviate from the conditions for which they were developed. The DTPA soil test is an example. In predicting metal availability in sludge-amended soils, the test was reportedly successful in certain cases (Bingham, Page, Mahler & Ganje, 1975; Street, Lindsay & Sabey, 1977; Barbarick & Workman, 1987; Bidwell & Dowdy, 1987) but unsuccessful in others (Baxter, Aguilar & Brown, 1983; Barbarick & Workman, 1987; Bidwell & Dowdy, 1987). The failure of the extractant can be ascribed to the misuse of the test. The test was developed for specific conditions and chemically can only function successfully under those conditions. Further, any alteration to the method may cause failure of the method to predict bioavailability successfully.

The pH of the extracting solution may play a very important role in the suitability of a specific extractant. Both the solubility of elements in the soil and the stability of element-chelate complexes can be highly pH dependent. Thus when using chelating agents to extract elements from soils of differing pH, it is not clear whether one should attempt to perform all extractions at the natural soil pH or alternatively buffer the extractions to a constant pH (Haynes & Swift, 1983). To determine plant bioavailability it will probably be best to keep the extractant at the soil's natural pH, but this approach would have to be tested experimentally.

2.4.2.1.1 Di-ammonium ethylenediaminetetraacetic acid (NH_4-EDTA)

The NH_4-EDTA soil test was developed by Trierweiler and Lindsay (1969) for Zn and later modified by Beyers and Coetzer (1971). This method is now described in the Handbook of Standard Soil Testing Methods for Advisory Purposes (The Non-Affiliated Soil Analysis Work Committee, 1990) for Cu, Mn, Zn and Co. It is therefore used for routine analysis of certain micro-elements in South Africa. The method uses 0,02 mol dm^{-3} NH_4-EDTA which, according to Beyers and Coetzer (1971), is sufficient for effective extraction of the elements. The solution pH is between 4,5 and 4,8.

This method seems to be very efficient in determining micro-element bioavailability from soils and is recommended by Bruemmer & van der Merwe (1989) as the method to be used in predicting the pollution danger of heavy metals in soils. It must be kept in mind that the buffer action of the NH_4-EDTA is inadequate when the soil contains free lime (The Non-Affiliated Soil Analysis Work Committee, 1990).

2.4.2.1.2 Diethylenetriaminepentaacetic Acid (DTPA)

The DTPA soil test was originally developed to identify near-neutral and calcareous soils with insufficient available Zn, Fe, Mn or Cu for maximum yields of crops (Lindsay & Norvell, 1978). The soil test extractant is a mixture of 0,005 mol dm^{-3} DTPA, 0,1 mol dm^{-3} triethanolamine (TEA), and 0,01 mol dm^{-3} $CaCl_2$ adjusted to pH 7,30. The extractant was designed specifically to avoid excessive dissolution of $CaCO_3$ with the concomitant release of occluded micronutrients, which are normally not available for absorption by roots (O'Connor, 1988). Therefore, if the soil test is applied to acid soils or artificially acidified soils (by for example sludge or acid), the buffering capacity of the soil test solution may be exceeded. Metal-chelate equilibria will be disrupted and dramatic shifts in individual metal chelating tendencies can result (O'Connor, 1988).

According to O'Connor (1988) the DTPA soil test only predicts bioavailability well when the micro-elements are deficient, but at high element concentrations increases in extractable micro-elements are not well correlated to plant content. According to O'Connor (1988) the test can therefore only be used qualitatively to characterize soil metal loading when the metals are present in high concentrations in the soil.

When studying metals other than Fe, Zn, Mn and Cu with the DTPA soil test (e.g. Cd, Ni, Pb) results may not be very reliable, as found by Barbarick and Workman (1987) for Ni and Pb. The DTPA soil test may therefore not be very reliable when conditions deviate from the specific conditions for which the test was developed.

2.4.2.1.3 Ammonium Bicarbonate-Diethylenetriaminepentaacetic Acid (AB-DTPA)

The AB-DTPA soil test was developed by Soltanpour and Schwab (1977) to extract simultaneously labile NO_3-N, P, K, Zn, Fe, Mn, and Cu from neutral and calcareous soils. Later, it was used for the extraction of potentially toxic elements from soils and mine spoils and soils treated with sewage sludge. The list of elements whose bioavailability can be determined with AB-DTPA also include Pb, Cd, Ni, Se, As, B, Mo, and S (Soltanpour, 1991). The AB-DTPA extraction solution is a 1 mol dm^{-3} NH_4HCO_3 and 0,005 mol dm^{-3} DTPA adjusted to a pH of 7,6. The AB-DTPA solution acts as a relatively good buffer but the relevance of such extraction systems to bioavailability from acid soils seems questionable (Haynes & Swift, 1983).

Barbarick and Workman (1987) indicated that AB-DTPA is as valid as, if not superior to, DTPA for metal extraction and offers the additional advantage in allowing nutrient analysis if one wants to assess both plant nutrients and trace metals.

2.4.2.1.4 EPA method 3050

This method is an acid digestion procedure used to digest sediments, sludges, and soil samples. It is attractive due to the fact that only HNO_3 and H_2O_2 are used. These acids cause the least interferences during analysis and the method is therefore well suited for most instruments. This method has been adopted by the U.S. EPA as a standard method and recovers almost 100% of the metal from samples (Sims, Igo & Skeans, 1991). The following elements can be analyzed: Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Pb, K, Mg, Mn, Mo, Na, Ni, Os, Se, Tl, V, Zn.

Once again the experimental procedure must be followed strictly to obtain reliable results. The method is basically a total digestion of metals and does not predict bioavailability.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

OSAs may contain trace element concentrations that can cause pollution of the environment if applied indiscriminately to soil. Therefore, the influence of OSAs on the trace element content of soil and plants and the subsequent danger to animals and humans need to be evaluated.

To fulfil the objectives stated in paragraph 3.1, a pot experiment (20 cm diameter plastic pots) was conducted in a glasshouse. Three of the treatments were also repeated in a growth cabinet in order to determine whether any contamination of plant material occurred in the glasshouse.

3.2 Materials

3.2.1 The soil

It is commonly accepted that soil type plays an important role in heavy metal availability and therefore toxicity (MacLean *et al*, 1987). However, in this study only one soil type was used in order to keep experimental size down. The many different soil types will best be studied using one well chosen indicator plant while varying the soil type.

The soil was taken from an artificially acidified field plot on the University of Pretoria's experimental farm. This acidic soil was chosen so that the required pH differences could be created simply by adding $CaCO_3$ to the soil.

The soil is a red sandy clay soil with a particle size analysis as given in Table 4 and chemical properties as given in Table 5.

Table 4. Particle size analysis of the soil used.

Particle size class	**Size range**	**Percentage in range**
	— mm—	— % —
Coarse sand	>0,25	19,24
Fine sand	0,25-0,1	22,86
Very fine sand	0,1-0,05	10,99
Total sand	>0,05	53,09
Silt	0,05-0,002	6,27
Clay	<0,002	44,15
Total	-	103,51

Table 5. Some chemical characteristics of the soil.

Soil characteristic	Value
$pH(H_2O)$	4,50
CEC ($pH7,NH_4^+$)	2,20 $cmol_c/kg$
P (Bray II)	8,58 mg/kg
Saturated paste water extract:	— mg/kg —
Ca	21,39
Mg	12,20
K	15,79
Na	1,53
Ammonium acetate extractable:	— mg/kg —
Ca	311,5
Mg	99,2
K	182,8
Na	5,0

3.2.2 Organic soil ameliorants

Following a preliminary study (results in Table 2), two materials from different origins were chosen, viz. a kraal manure (OSA 10) and a disinfected sewage sludge (OSA 20). Thus the effect of OSA composition on the bioavailability of trace elements could also be studied. These two OSAs were furthermore chosen because they have Ni, Cu, As, and Pb concentrations (Table 6) in excess of the maximum permissible concentrations according to South African guidelines(Appendix 1, Table 1) and/or the mean and median of overseas guidelines (Appendix 1, Table 11).

Table 6. Trace element concentrations of the organic soil ameliorants used in this study.

Element	Culterra kraal manure (OSA 10)	Growman disinfected sewage sludge (OSA 20)
	mg/kg	
V	52,80	32,33
Cr	103,10	294,20
Ni	199,20	577,50
Co	19,25	7,13
Cu	25,44	1477,00
Zn	228,20	1077,00
As	3,82	16,19
Mo	1,70	13,34
Cd	ND	3,82
Hg	ND	3,17
Pb	52,04	594,70

ND = not detected

Four application rates of each OSA were used in order to determine the effect of increased metal concentrations in the soil, on the bioavailability to plants and the possibility of predicting the plant uptake of trace elements by soil extractants.

3.2.3 Crops

Three crops were used viz. wheat (*Triticum aestivum L.* cv. Inia), lettuce (*Lactuca sativa L.* cv. Golden City) and bush beans(*Phaseolus vulgaris L.* cv. Wintergreen).

Wheat was chosen because it is a monocotyledon while bush beans is a dicotyledon. Lettuce was chosen because it is generally considered to be a good accumulator of trace elements and is often used as an indicator plant.

3.2.3 Calcium carbonate addition

The soil pH was altered by adding $CaCO_3$ at a rate of 2 and 11 tons/ha which, respectively, resulted in a pH of just below 5,0 and above 6,5. Analytical grade $CaCO_3$ was used to prevent any contamination of the soil and to ensure that equilibrium is reached quickly. The pH(water) of 6,5 was chosen because it is generally considered that above this soil pH most trace elements become less bioavailable.

3.3 Methods

3.3.1 Experimental procedure

Soil was collected from the field site and sieved through a 3 mm sieve. A 16 kg sample of the soil was mixed on a plastic sheet with the chemicals necessary for each treatment. The OSAs were added on a dry mass basis (Table 7). The mixed soil was divided into 4 kg per pot and placed on a rotating table in the glass house.

Table 7. Additions of $CaCO_3$ and OSAs for the different treatments.

Material added	t/ha[1]	g/pot
$CaCO_3$	2	2,66
	11	14,66
Organic soil ameliorant	5	6,67
	10	13,33
	20	26,67

[1] The t/ha was calculated using a 20 cm soil depth and an average soil density of 1500 kg/m^3

Nutrients were added to all the pots to ensure that no nutrient deficiencies occurred (Table 8).

Table 8. Standard chemical addition made to each pot.

Element	mg/kg	kg/ha-20cm	Chemical	g chemical/pot
N	160	481	$(NH_4)_2SO_4$ + $(NH_4)H_2PO_4$	2,52 0,89
P	60	180	$(NH_4)H_2PO_4$	0,89*
K	100	300	KCl	0,76
Mg	200	600	$MgSO_4.7H_2O$	8,11

* Only one application of 0,89 g/pot $(NH_4)H_2PO_4$ was made but since it contains both N and P it is shown under both N and P.

The wheat and lettuce were germinated in vermiculite and transplanted into the pots. The beans were sown directly into the pots. After planting the soil was saturated with de-ionized water. The pots were then maintained at 15%(mass/mass) water content by weighing. No leaching was permitted. At this water content the matric potential is approximately -10 kPa. After the plants were established the extra plants were removed so that only

2 lettuce plants, 5 wheat plants and 2 bean plants remained. The yields (g/plant) and biomass (μg/plant) of each element is given per plant in the results to standardize the results. The amount per pot can be calculated using the above-mentioned number of plants per pot. Plants were treated according to standard recommendations for pests and diseases. The above-ground parts of the lettuce and the pods of the beans were harvested and weighed because they are the edible plant parts. The wheat was considered to be a forage crop and therefore its above-ground parts was harvested and weighed. Harvesting took place at 6 weeks and all the plants were first rinsed off in de-ionized water before being dried at 65 °C. A representative soil sample was taken from each pot at the end of the experiment.

3.3.2 Growth cabinet

Three of the treatments,replicated four times, were repeated with wheat in a growth cabinet to determine if any contamination takes place in the glasshouse. The treatments were:

0 t/ha Organic Soil Ameliorant + 2 t/ha $CaCO_3$
20 t/ha Disinfected Sewage Sludge (OSA 20)+ 2 t/ha $CaCO_3$
20 t/ha Disinfected Sewage Sludge (OSA 20)+ 11 t/ha $CaCO_3$

These pots were also maintained at 15% (mass/mass) water content. The growth cabinet was kept at the following conditions:

Day length: 13,5 hours: Night length: 10,5 hours
Day temperature: 25 °C for 12 hours
Night temperature: 18 °C for 12 hours

The plants from these treatments were also harvested at 6 weeks and treated as described before. A representative soil sample was taken from each pot at the end of the experiment.

3.3.3 Analytical procedures

The plant material had to be ground before analysis thereof. Several grinding apparatus were tested for ease of operation, effectiveness of grinding, efficiency in handling large quantities of materials, and most importantly for possible contamination of the sample by trace elements. No suitable apparatus could be found and the plant material was subsequently ground by hand using a porcelain mortar and pestle.

The plant samples were digested with a 7:3 HNO_3:$HClO_4$ mixture on a Technicon digestion block (Appendix 2). The digested samples were analyzed for V, As, Pb, Cr, Se, Ni, Mo, Co, Cd, Cu, Zn, and Hg. All the elements were analyzed by ICP-MS excluding Zn which was analyzed by Atomic Absorption Spectrometry (AAS). The soil was extracted with NH_4-EDTA (Appendix 2) and the extract analyzed by ICP-MS for all the elements as mentioned above. Soil from two replicates of each treatment, excluding the 5 t/ha OSA application, were also extracted by DTPA, AB-DTPA, and EPA method 3050 (Appendix 2). For each of these methods most of the elements were analyzed by ICP-MS except Cu and Co in AB-DTPA, Ni and Cr in EPA method 3050 and Cu in DTPA, all of which were analyzed by AAS.

3.3.4 Statistical analysis

An ANOVA (StatSoft, 1986) analysis of variance was conducted for the plant yield of the three crops separately, the soil pH and Ni, Co, Cd, Cu, and Zn uptake by the plants (mg/kg and μg/plant) as well as the soil content as extracted by NH_4-EDTA, EPA method 3050, DTPA and AB-DTPA. Duncan's multiple range tests (StatSoft, 1986) were also conducted to determine more specific differences, for certain cases.

Multiple linear regression analyses with the plant concentration (mg/kg or μg/plant) of each element as dependant variable and the soil concentration (mg/kg), as determined by the different extraction methods, of the same element as independent variable, were conducted. Multiple linear regression analyses were further also conducted to compare the relationship between the soil concentrations as determined by the different extractants (StatSoft, 1986).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

The plants and soil were analyzed for twelve trace elements: V, As, Pb, Cr, Se, Ni, Mo, Co, Cd, Cu, Zn, Hg. Of these, only Ni, Co, Cd, Cu, and Zn will be discussed. The results of the other elements (also Pb and As which occurred at high concentrations in OSA 20) either varied too much or were not suitably analyzed by the methods used.

Each element will be discussed separately but a general discussion of all the elements will be given at the end. The ANOVA analyses of variance are given in Appendix 3 and will be referred to in the discussion when necessary. The mean values of the various parameters measured will be given in tables with an indication of significant differences according to Duncan's multiple range test. Significant differences are indicated by different letters following the mean value.

4.2 Plant yield

The yield per plant for the various treatments are given in Table 9. The most significant and puzzling difference (Table 9; Appendix 3: Table 1) in the yield is the decrease of lettuce and bean pod yield due to the increase in $CaCO_3$ application rate from 2 t/ha to 11 t/ha (representing a soil pH of below 5 and above 6,5, respectively). This was unexpected but could be due to some essential element in the soil becoming unavailable to the plants due to the application of $CaCO_3$. A deficiency in phosphate is unlikely because phosphate was added to the soil as an inorganic salt which is readily available to plants. Wheat yields were not influenced by the application of $CaCO_3$ probably because wheat has a more extensive root system than beans and lettuce and is therefore able to obtain sufficient elements to sustain normal growth. Conclusions drawn from the plant uptake of trace elements at the 11 t/ha $CaCO_3$ application rate must therefore be made with caution.

Table 9. Mean plant yields as influenced by $CaCO_3$ application rate, OSA type and OSA application rate.

Plant	$CaCO_3$ rate (t/ha)	Control 0 t/ha OSA	OSA type and application rate (t/ha)					
			OSA 10			OSA 20		
			5	10	20	5	10	20
		Plant yield (g dry matter/plant)[2]						
Lettuce	2	1,49 a[1]	1,96 b	2,59 c	4,30 d	2,60 bc	2,78 bc	4,02 d
	11	0,10 a	0,55 a	0,48 a	0,37 a	0,10 a	0,25 a	0,30 a
Wheat	2	2,50 a	2,81 a	2,82 a	2,70 a	2,30 a	2,70 a	2,96 a
	11	2,91 a	2,61 a	2,63 a	2,36 a	2,46 a	2,54 a	2,54 a
Bean pods	2	2,72 a	3,42 a	2,32 a	3,28 a	4,36 a	5,93 a	5,68 a
	11	0,34 a	0,74 a	0,63 a	0,74 a	0,22 a	0,82 a	2,59 b

[1] Values in each row followed by the same symbol do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 4 replicates

There is a significant increase in lettuce yield with an increase in OSA application rate for both OSA types at the 2 t/ha $CaCO_3$ addition, but there is no significant difference in yield between the OSA types (see also Appendix 3: Table 1).

The wheat yields do not show any yield differences due to the OSA application rate, OSA type or $CaCO_3$ application rate.

The bean pods however show a significant difference in yield between the two OSA types at 11 t/ha $CaCO_3$ added. The OSA 20 applications resulted in increased yields with increased application rates while with OSA 10 there were no yield differences.

From these results it is clear that the addition of the OSAs to already fertilized soil generally resulted in a yield increase which, however, was significant only in some cases. No yield decreases due to possible toxicities at high OSA application rates were observed.

4.3 Soil pH after the experiment

The soil pH was only measured at the end of the experiment and the change in pH during the experiment was not taken into account. Van der Watt, Sumner & Cabrera (1994) found, in a 21 day experiment with chicken manure, that there was an initial increase in soil pH after which the soil pH decreased. The soil pH may therefore change during the duration of the experiment, and this should be taken into account. Nevertheless, a few significant changes in the final soil pH were observed (Table 10; Appendix 3: Table 2).

The soil pH was influenced significantly by the plant type, OSA type, OSA application rate and $CaCO_3$ application rate (Appendix 3: Table 2). As expected there is a dramatic rise in soil pH with increased $CaCO_3$ application rate. The 11 t/ha $CaCO_3$ application rate resulted in a soil pH greater than 6,5, above which heavy metals are generally accepted to be far less available than at lower soil pH's.

Table 10. Mean final soil pH(H_2O) values as influenced by $CaCO_3$ application rate, OSA type and OSA application rate.

$CaCO_3$ rate (t/ha)	**Control 0 t/ha OSA**	**OSA type and application rate (t/ha)**					
		OSA 10			**OSA 20**		
		5	**10**	**20**	**5**	**10**	**20**
	Soil pH(H_2O)[2]						
2	4,59 a[1]	4,58 a	4,67 a	4,74 a	4,58 a	4,61 a	4,71 a
11	6,88 ab	6,96 ab	6,93 ab	7,00 a	6,80 ab	6,79 ab	6,74 b

[1] Values in the row followed by the same symbol do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 12 replicates.

The OSA type at the 11 t/ha $CaCO_3$ application rate resulted in different trends with increased OSA application rate. OSA 10 (kraal manure) caused the soil pH to increase slightly while OSA 20 (disinfected sewage sludge) caused the soil pH to decrease slightly. This could be due to a difference in pH of the two OSA types. These differences are however very small and probably have very little influence on the bioavailability of the trace elements.

Although the plant type significantly influenced the soil pH the differences were very small and probably not of practical value. The differences could be a result of physiological acidity/alkalinity effects and/or different water regimes caused by the differences in the plants' water needs. The different water regimes influence the soil microbiology and soil chemistry, therefore causing differences in soil pH. As these differences are not one of the objectives of the study the soil pH of the different plant types are grouped to see the more important influence of the OSA types and application rate.

4.4 Nickel

4.4.1 Ni in plants

The Ni uptake by the different plant types differ significantly (Appendix 3: Table 3). The bean pods, in this experiment, had higher Ni concentrations than lettuce and wheat, which is in agreement with the data of Sauerbeck (1991) (Table 11). Kabata-Pendias & Pendias (1992) also state that Ni is very mobile in plants and that berries and grains are reported to contain elevated Ni concentrations. This is contrary to the generalization that plant seeds, relative to the vegetative parts, do not accumulate large quantities of trace elements.

The Ni concentrations of the plants, for the 2 t/ha $CaCO_3$ application, seem to be high but not phytotoxic if compared with the Ni concentrations of plants found by other authors (Table 11). These concentrations also do not seem to be detrimental to human and animal health. Dietary nickel is, apparently, non-toxic to man. Nickel is poorly absorbed from food and beverages and only between 3 and 6 percent of dietary intake is believed to be retained in the body (Rielly, 1991). The Ni levels in the plant therefore do not seem to be an environmental problem.

It is important to note that, for the control where no OSA application was made, high Ni contents in the plants were found (Tables 11 and 12). This is an indication that the soil used has high background values and that the repeated application of more Ni could result in toxic levels of Ni being attained.

Table 11. Average and critical concentrations of Ni in plants as reported by different authors.

Source of data	Lettuce	Wheat	Bean pods	Plants in general
	——Ni (mg/kg dry matter)——			
Range for this experiment at low soil pH	4,95-8,31	1,88-5,19	11,23-19,66	
Range for this experiment at high soil pH	0,00-1,51	0,89-1,50	1,06-6,03	
Kabata-Pendias & Pendias (1992): Average plant concentration	1-1,8	0,17-0,7	1,7-3,7	0,06-3,7
Kabata-Pendias & Pendias (1992): Plants grown in contaminated sites	1,8-84			0,1-32 000
Kabata-Pendias & Pendias (1992): Excessive or toxic levels				10-100
Kabata-Pendias & Pendias (1992): Tolerable in agronomic crops				50
MacNicol & Beckett (1985): Upper critical levels	20-45	8-46	10-83 (leaves)	8-220
Reilly (1991): Average concentration				<0,05-0,30
Keefer *et al*, (1986): Maximum application of 180 tons/ha sludge containing 12 700mg/kg Ni			5,00-11,0	
Sauerbeck (1991): Average concentration	ca. 1-2	ca. 1-2	ca. 8	
Barbarick & Workman (1987): Maximum application of 80,4 tons/ha sludge containing an average of 92,5 mg/kg Ni		0,2-2,3		
Hue *et al*, (1988): Maximum application of 180 tons/ha sludge containing 29 mg/kg Ni.	1,8-17,2			
Schauer *et al*, (1980): Maximum loading of 33 kg/ha Ni	5,2-9,6			
Risser & Baker (1990): Suggested tolerance levels of Ni in plants				3
Roca & Pomares (1991): Calcareous soil with a maximum application of 57,30 kg/ha Ni.	0,48-0,90			

Table 12. Mean Ni concentration (mg/kg dry matter) and total Ni uptake (μg/plant) of plants as influenced by $CaCO_3$ application rate, OSA type and OSA application rate.

Plant	$CaCO_3$ rate (t/ha)	Control 0 t/ha OSA	OSA type and application rate (t/ha)					
			OSA 10			OSA 20		
			5	10	20	5	10	20
		Ni concentration (mg/kg dry matter)[2]						
Lettuce	2	8,31 a[1]	5,91 a	5,39 a	4,95 a	7,84 a	6,97 a	6,17 a
	11	ND a	0,06 a	0,35 a	ND a	0,10 a	1,51 a	0,17 a
Wheat	2	4,48 a	3,45 a	3,20 a	1,88 a	2,97 a	5,36 a	5,19 a
	11	0,89 a	0,90 a	1,50 a	1,06 a	1,33 a	1,27 a	1,12 a
Bean pods	2	19,66 a	16,69 a	19,55 a	11,23 a	13,88 a	13,73 a	14,33 a
	11	1,89 a	6,03 a	2,05 a	2,19 a	1,06 a	3,17 a	3,37 a
		Ni uptake (μg/plant)						
Lettuce	2	12,20 a	11,49 a	13,32 a	21,52 a	20,10 a	18,86 a	25,46 a
	11	ND a	0,05 a	0,14 a	ND a	ND a	0,63 a	0,10 a
Wheat	2	10,95 a	9,66 a	8,96 a	5,05 a	6,98 a	13,86 a	15,05 a
	11	2,49 a	2,46 a	3,78 a	2,47 a	3,28 a	3,16 a	3,01 a
Bean pods	2	55,54 a	54,22 a	49,95 a	31,82 a	60,25 a	83,54 a	78,16 a
	11	1,05 ab	4,75 a	1,83 a	1,78 a	0,32 a	2,94 a	8,41 b

ND = not detected

[1] Values in the row followed by the same symbol do not differ significantly at P<0,05 according to Duncan's multiple range test.

[2] Mean of 4 replicates.

Although there are no significant differences in nickel concentrations (mg/kg dry material) due to the different types of OSAs or their application rates, OSA type and application rate do cause significant differences in total nickel uptake (μg/plant) because of their influence on plant yield (Appendix 3: Tables 3 and 4). According to Duncan's multiple range test there is a significant increase in Ni uptake per plant by bean pods only for the 20 t/ha OSA 20 at 11 t/ha $CaCO_3$ applied (Table 12), possibly due to the better growth obtained in this particular treatment (Table 9). Although not significant, there is a decrease in plant Ni concentration (mg/kg dry matter) with the application of OSA 10 at all application rates, and of OSA 20 at the lowest application rate. This could be due to a dilution effect as a result of increased plant growth, as shown by the fact that the total uptake (μg/plant) of Ni by lettuce increased slightly due to the application of OSA 10. However, the bean pod and wheat uptake of Ni (μg/plant) decreased which probably indicates that OSA 10 also decreased the total Ni uptake per plant slightly but not significantly. OSA 20 on the other hand has a higher Ni concentration and causes slight but not significant increases in total Ni uptake (μg/plant) in the plants. The Ni concentration of the bean pods and lettuce decreased which can be attributed to the dilution effect of improved plant growth.

The most dramatic result is the significant decrease in Ni concentration and uptake due to the increase in pH following the application of 11 t/ha of $CaCO_3$ (Table 12 and Appendix 3: Tables 3 and 4).

4.4.2 Ni in soil

The plant types had a significant influence on the extraction of Ni by NH_4-EDTA, DTPA and AB-DTPA. The reason for this should be very much the same as the influence on soil pH. These influences are however not one of the objectives of the study and the results for the different plant types are therefore grouped for the different extractants and will not be discussed further.

The extractable soil Ni contents exceeded the maximum permissible concentrations in soil as given for South Africa by the Dept. of National Health and Population Development (1991) and Risser & Baker (1990) (Table 13). The Ni in the control treatments is already higher than these levels and they are far below the other permissible levels set. The question therefore arises whether the South African standard based on EPA method 3050 is not too low. There is a significant increase in Ni extracted by NH_4-EDTA due to the application of OSA 20 (Table 14). If each application of 20 t/ha of OSA 20 causes the same increase in Ni extracted by NH_4-EDTA it would take approximately 30 applications of OSA 20 at 20 t/ha to reach the threshold value. However, according to the results of Roca & Pomares (1991), each successive application gave higher levels of extractable metals but a lower proportion than the first application. It should therefore take more than the 30 applications to reach the threshold value.

Table 13. Average and maximum permissible Ni concentration (mg/kg) in the soil as extracted by different extractants and reported by different authors.

Source of data	**NH_4-EDTA**	**EPA 3050**	**DTPA**	**AB-DTPA**
	——mg/kg soil——			
Range for the mean concentration at low soil pH in this experiment	2,01-2,69	38,58-42,69	1,63-1,95	3,15-4,79
Range for the mean concentration at high soil pH in this experiment	1,50-2,11	37,99-40,93	0,65-0,87	1,76-2,52
Bruemmer & van der Merwe (1989): Maximum permissible concentration with soil pH($CaCl_2$) above 5,5	20			
Kabata-Pendias & Pendias (1992): Mean content of surface soils		1,3-3240		
Kabata-Pendias & Pendias (1992): Ni contamination in surface soils		2-26000		
Maximum permissible concentration in soil for: South Africa Germany United Kingdom: pH 6,0-7,0 pH 5,0<5,5		 15 50 75 50		
Risser & Baker (1990): Suggested maximum cumulative Ni levels depending on soil texture		11,2-67,2		
Roca & Pomares (1991): Range with maximum application of 27,60 kg/ha Ni over 3 years	1,9-4,4		0,5-1,3	0,3-1,5
Korcak & Fanning (1978): Sludge treated soils with a maximum of 27,65 mg/kg Ni applied			0-13,6	
Sims *et al,* (1991): Sludged sites with a total loading rate of 140 kg/ha Ni		3,5-21,0	<0,1-2,4	
Barbarick & Workman (1987): Maximum application of 80,4 tons/ha of sludge containing an average of 92,5 mg/kg Ni			0,4-3,7	0,5-3,4

Table 14. Soil extractable Ni found with the various extractants as influenced by $CaCO_3$ application rate, OSA type and OSA application rate.

Extractant	$CaCO_3$ rate (t/ha)	Control 0 t/ha OSA	OSA type and application rate (t/ha)					
			OSA 10			OSA 20		
			5	10	20	5	10	20
		——Ni in soil (mg/kg soil)——						
NH_4-EDTA[2]	2	2,09 ab[1]	2,02 ab	2,01 ab	2,03 a	2,16 ab	2,34 b	2,69 c
	11	1,50 a	1,61 ab	1,67 ab	1,77 b	1,58 ab	1,75 b	2,11 c
EPA method 3050[3]	2	39,16 a	NA	42,10 a	40,73 a	NA	38,58 a	42,69 a
	11	39,56 a	NA	40,93 a	37,99 a	NA	39,56 a	39,75 a
DTPA[3]	2	1,66 a	NA	1,63 a	1,78 a	NA	1,84 a	1,95 a
	11	0,65 a	NA	0,77 a	0,78 a	NA	0,78 a	0,87 a
AB-DTPA[3]	2	4,20 a	NA	3,15 a	3,64 a	NA	4,79 a	4,60 a
	11	1,76 a	NA	1,89 a	2,52 a	NA	2,03 a	2,19 a

NA = Not analyzed

[1] Values in the row followed by the same symbol do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 12 replicates.

[3] Mean of 6 replicates.

The maximum amount of Ni added to the soil is 3,87 mg per kg of soil in the case of OSA 20 at an application rate of 20 t/ha. In spite of this no significant differences in soil Ni content determined with EPA method 3050 were found (Appendix 3 : Table 6). This may be due to the large variation in results obtained by this method. The other extractants however did show significant differences using the ANOVA analysis of variance (Appendix 3). Using Duncan's multiple range test to determine the differences between the OSA types and application rate there are only significant differences with the NH_4-EDTA extractable Ni. The mean Ni values for DTPA and AB-DTPA extractions (Table 14) are comprised of 6 replicates while for NH_4-EDTA there are 12 replicates. In spite of this it still seems as if NH_4-EDTA predicts the Ni loading of the soil, by the OSAs at both soil pH levels, the best. The NH_4-EDTA extractant seems to yield the most significant differences and is able to detect the differences in Ni loading due to OAS application rate and OSA type.

The OSA 20 caused a significant increase in Ni as compared to OSA 10, where there is no significant increase in Ni. This result can be explained if the Ni contents of OSA 10 and OSA 20 are compared (Table 6).

The most prominent difference caused by the treatments in NH_4-EDTA, DTPA and AB-DTPA extractable Ni is the significant decrease in Ni extracted with increased $CaCO_3$ application rate from 2 t/ha to 11 t/ha (Appendix 3: Tables 5, 7 and 8). Mitchell, Bingham & Page (1978) found a similar decrease in DTPA extractable Ni with an increase in soil pH. This decrease could be due to one of three factors or a combination of factors: (1) The increased Ca^{2+} content of the soil results in competition for the complexing sites of the extractant and consequently less Ni is extracted; (2) the buffer capacity of the extractant could be exceeded and the pH of the extracting solution may be increased or decreased causing changes in the stability constants of Ni complexes; (3) the Ni may be precipitated or may be sorbed more by the soil components at the higher soil pH (Harter, 1983; Bruemmer, Gerth & Tiller, 1988). A discussion relating to the most probable reason for the decrease will be given in the general discussion.

4.4.3 Relationship between soil Ni and plant Ni

If the results of the 2 t/ha and 11 t/ha $CaCO_3$ rates are pooled there are significant correlations with all three plant types for NH_4-EDTA, DTPA and AB-DTPA extractable Ni and plant concentration and uptake (Table 15). These good correlations are obtained because the $CaCO_3$ application rates influence the plant concentration and uptake and soil extraction similarly. If the 2 t/ha and 11 t/ha $CaCO_3$ application rates are studied independently only the Ni uptake by bean pods show some correlation with NH_4-EDTA extracted Ni in the soil at both $CaCO_3$ application rates (Table 14). This indicates that the plant bioavailability as predicted by the soil concentrations as determined by the different extractants is not that good. However, the influence of soil pH on the bioavailability is predicted well by the different extractants. Mitchell *et al*, (1978) also obtained good correlations for DTPA extracted Ni with lettuce ($r=0,80^{***}$) and wheat ($r=0,87^{***}$) concentration using two

Table 15. The significant ($P < 0,05$) multiple linear regression equations for Ni concentration (mg/kg dry matter) and uptake (µg/plant) in the plants as related to the extractable Ni in the soil when determined by the different soil extractants.

Extractant	Plant type	$CaCO_3$ rate (t/ha)	Plant concentration (mg/kg) or uptake (µg/plant)	Regression equation[1]	R^2 value
NH_4-EDTA	Lettuce	2 & 11	Concentration	NimgL=-8,15+6,22NH_4Ni	0,37***
			Uptake	NiµgL=-27,44+19,16NH_4Ni	0,44***
	Wheat	2 & 11	Concentration	NimgW=-2,53+2,63NH_4Ni	0,19**
			Uptake	NiµgW=-9,27+8,20NH_4Ni	0,25***
	Bean pods	2	Uptake	NiµgB2=-11,15+30,05NH_4Ni	0,31**
		11	Uptake	NiµgB11=-7,11+5,69NH_4Ni	0,18*
		2 & 11	Concentration	NimgB=-4,32+6,61NH_4Ni	0,17**
			Uptake	NiµgB=100,57+23,30NH_4Ni-20,47pH	0,72***
DTPA	Lettuce	2 & 11	Concentration	NimgL=-3,49+5,68DTPANi	0,56***
			Uptake	NiµgL=-13,29+19,99DTPANi	0,71***
	Wheat	2 & 11	Concentration	NimgW=-0,53+2,55DTPANi	0,53***
			Uptake	NiµgW=-2,03+7,30DTPANi	0,60***
	Bean pods	2 & 11	Concentration	NimgB=-4,42+9,46DTPANi	0,55***
			Uptake	NiµgB=-32,05+43,20DTPANi	0,65***
AB-DTPA	Lettuce	2 & 11	Concentration	NimgL=-3,30+1,98ABNi	0,58***
			Uptake	NiµgL=-10,74+6,38ABNi	0,61***
	Wheat	2 & 11	Concentration	NimgW=-1,04+1,35ABNi	0,51***
			Uptake	NiµgW=-3,02+3,70ABNi	0,53***
	Bean pods	2 & 11	Concentration	NimgB=-1,28+3,05ABNi	0,34**
			Uptake	NiµgB=-29,24+17,31ABNi	0,62***

* Significant at $P < 0,05$

** Significant at $P < 0,01$

*** Significant at $P < 0,001$

[1] NimgL/NimgW/NimgB = Ni concentration (mg/kg dry matter) in lettuce, wheat and bean pods; NiµgL/NiµgW/NiµgB = Total Ni uptake (µg/plant) by lettuce, wheat and bean pods; NH_4Ni = Ni in soil (mg/kg) determined by using the NH_4-EDTA extraction method; DTPANi = Ni in the soil (mg/kg) determined by using the DTPA extraction method; ABNi = Ni in the soil (mg/kg) determined by using the AB-DTPA extraction method; pH = Soil pH after the experiment.

soils, one with a high pH (7,5) and the other with a low pH (5,7), with large increases in Ni application. Barbarick & Workman (1987) found significant correlations with Ni extracted by DTPA and AB-DTPA with Ni concentration in swiss chard for calcareous and acid soils separately in a pot trial. Conversely, in a field trial poor correlations were found because the soil pH was reasonably uniform and the Ni applications were far lower than for the pot study. Korcak & Fanning (1978) found, for Ni in maize, that when combining limed and unlimed soils better correlations than for the limed soil, but poorer correlations than for the unlimed soil were obtained. High and low soil pH therefore cause different slopes in the regression equations due to increases in Ni content of the soil. Soil pH must therefore be taken into account when predicting Ni uptake. Significant correlations will therefore be obtained when Ni loading is reasonable large or the soil pH differs substantially. Roca & Pomares (1991) found significant correlations with DTPA ($r=0{,}78^{*}$) and AB-DTPA ($r=0{,}81^{*}$) extractable Ni in calcareous soils with the Ni content of the edible leaves of lettuce but not in the case of EDTA(pH 4,65) ($r=0{,}73^{ns}$) extractable Ni, although the r value is reasonably high.

4.5 Cobalt

4.5.1 Co in plants

There are significant differences in the plant Co concentration (mg/kg) and uptake (μg/plant) of the different plant types (Tables 16 and 17; Appendix 3: Tables 3 and 4). The concentration of Co in lettuce tends to be higher than in the wheat and bean pods, but not for all treatments.

Data concerning Co toxicity in plants is scarce. However, some work has been done on Co deficiencies in plants (Kabata-Pendias & Pendias, 1992). There are no reports of Co toxicity to animals attributed to the consumption of natural feedstuffs. However, in certain areas, and under the influence of man-induced pollution, the excess of Co in plants may be a health risk (Kabata-Pendias & Pendias, 1992). Levels of Co are normally very low in foods (Reilly, 1991) because the uptake of Co from contaminated soils is restricted (Purves, 1985).

The most striking observation is the pronounced effect of soil pH on Co concentration of foliage or bean pods. According to the data available for Co levels in plants (Table 16), the concentrations found at low pH are high and in the case of lettuce may even cause the yield to decrease. The yield is exponentially well correlated to the Co concentration of lettuce at low pH ($R^2=0{,}54$). On the other hand, the lettuce yield may simply regulate the Co uptake. The yield differences at the 2 t/ha $CaCO_3$ application rate therefore may be the result of a pH induced toxicity of elements such as Co, Fe or Mn. Narwal *et al*, (1983) also found a decrease in Co concentration of fodder rape with increased soil pH.

A small amount of Co was added to the soil by the OSAs: 0,13 mg/kg being the maximum (OSA 10 at 20 t/ha). The differences in Co uptake could therefore be due to soil conditions being altered by the different treatments. There are no significant differences between OSA types but there are significant differences due to the application rate of the OSAs (Appendix 3: Table 3). It is difficult to determine what the specific influence of the different OSA application rates are because the plant types are influenced in different ways (Table 17). Narwal *et al*, (1983) found significant decreases in Co concentration in fodder rape with the application of sewage sludge. The sewage sludge however contained only 8,1 mg/kg Co.

Table 16. Average and critical concentrations of Co in plants as reported by different authors.

Source of data	Lettuce	Wheat	Bean pods	Vegetables	Cereals	Barley and cabbage	Plants in general
	Co (mg/kg dry matter)						
Range for this experiment at low soil pH	7,62-23,66	4,54-11,27	3,47-13,98				
Range for this experiment at high soil pH	1,31-3,34	1,04-1,88	1,08-2,19				
Kabata-Pendias & Pendias (1992): Average plant concentration	0,046-0,21	0,015-0,38					
Kabata-Pendias & Pendias (1992): Sufficient or normal							0,02-1
Kabata-Pendias & Pendias (1992): Excessive or toxic							15-50
Reilly (1991): Average plant concentration				0,025	0,046		
MacNicol & Beckett (1985): Upper critical levels			4-40 (leaves)			6-25	
Wallace, Alexander & Chaudhry (1977): Toxic symptoms			43-142 (leaves)				

Table 17. Mean Co concentration (mg/kg dry matter) and total Co uptake (μg/plant) of plants as influenced by $CaCO_3$ application rate, OSA type and OSA application rate.

Plant	$CaCO_3$ rate (t/ha)	Control 0 t/ha OSA	OSA type and application rate (t/ha)					
			OSA 10			OSA 20		
			5	10	20	5	10	20
		Co concentration (mg/kg dry matter)[2]						
Lettuce	2	23,66 b[1]	13,89 a	9,87 a	9,58 a	11,54 a	10,76 a	7,62 a
	11	3,34 a	2,11 ab	1,31 b	1,93 ab	2,91 ab	2,60 ab	2,27 ab
Wheat	2	5,08 a	5,38 a	4,64 a	4,54 a	6,44 a	9,51 a	11,27 a
	11	1,31 a	1,06 a	1,25 a	1,88 a	1,25 a	1,09 a	1,04 a
Beans	2	8,82 a	8,72 a	13,98 a	8,88 a	2,97 b	4,11 b	3,47 b
	11	1,53 a	2,19 a	1,97 a	1,45 a	1,36 a	1,39 a	1,08 a
		Co uptake (μg/plant)						
Lettuce	2	34,36 a	27,07 a	25,24 a	40,48 a	29,88 a	29,58 a	30,95 a
	11	0,48 a	1,27 a	0,51 a	0,72 a	0,46 a	0,72 a	0,73 a
Wheat	2	12,49 ab	14,93 a	12,90 ab	12,22 ab	14,87 ab	24,59 b	32,22 b
	11	3,76 a	2,78 a	3,06 a	4,43 a	3,08 a	2,74 a	2,56 a
Beans	2	25,18 a	27,30 a	30,73 a	25,27 a	12,87 a	24,87 a	18,73 a
	11	0,78 ab	1,70 ab	0,99 ab	1,07 ab	0,39 a	1,13 ab	2,75 b

ND = not detected

[1] Values in the row followed by the same symbol do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 4 replicates.

4.5.2 Co in soil

The EPA method 3050 once again shows no significant differences in soil Co between treatments due to the large variation in results but also possibly due to the small amounts of Co added by the OSAs. NH_4-EDTA, DTPA and AB-DTPA show significant differences in extractable soil Co with plant type and $CaCO_3$ application rate, this effect being common for all three the extractants (Appendix 3: Tables 5, 7 and 8). The influence of plant type on the extractability of soil Co can be explained in the same way as for Ni and will not be discussed further.

The mean Co concentration in the soil for EPA method 3050 is between 14,13 and 17,58 mg/kg soil, which is below the limit of 20 mg/kg (Department of National Health and Population Development, 1991) (Table 18). The mean NH_4-EDTA extractable Co is between 9,16 and 11,55 mg/kg soil which is once again just below and above the limit of 10 mg/kg given by Bruemmer & van der Merwe (1989). However, the soil pH(H_2O) at the 2 t/ha $CaCO_3$ is below 5,0 and the above limit is only applicable if the soil pH($CaCl_2$) is above 5,5. It therefore seems as if the extractable Co concentration in the soil is very high especially at the low soil pH and this apparently results in the high plant Co concentrations at the low soil pH.

The OSA type and application rate do not cause any significant differences in Co extractability using Duncan's multiple range test (Table 18) although there are significant differences using the ANOVA analysis of variance (Appendix 3). It therefore seems as if the OSA applications did not have any influence on Co extractability in the soil.

The increased $CaCO_3$ application rate resulted in significant decreases in Co extractability for all three extractants, which is a reflection of the decreased availability of Co at high pH. NH_4-EDTA extractable Co however, decreased only slightly with an increase in pH.

Table 18. Soil extractable Co found with the various extractants as influenced by $CaCO_3$ application rate, OSA type and OSA application rate.

Extractant	$CaCO_3$ rate (t/ha)	Control 0 t/ha OSA	OSA type and application rate (t/ha)					
			OSA 10			OSA 20		
			5	10	20	5	10	20
		Co in soil (mg/kg soil)						
NH_4-EDTA[2]	2	11,55 a[1]	10,57 a	11,00 a	10,79 a	10,94 a	10,34 a	11,16 a
	11	10,52 a	10,14 a	10,32 a	9,16 a	9,96 a	10,29 a	10,39 a
EPA method 3050[3]	2	14,82 a	NA	16, 33 a	17,58 a	NA	17,16 a	15,62 a
	11	15,99 a	NA	16,83 a	16,79 a	NA	15,89 a	14,13 a
DTPA[3]	2	5,22 a	NA	5,04 a	5,06 a	NA	4,59 a	4,29 a
	11	1,07 a	NA	1,22 a	1,23 a	NA	1,19 a	1,27 a
AB-DTPA[3]	2	5,89 a	NA	5,82 a	5,53 a	NA	5,47 a	5,11 a
	11	1,66 a	NA	1,70 a	1,79 a	NA	1,57 a	1,68 a

NA = Not analyzed

[1] Values in the row followed by the same symbol do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 12 replicates.

[3] Mean of 6 replicates.

4.5.3 Relationship between soil Co and plant Co

NH_4-EDTA, DTPA and AB-DTPA extractable soil Co are well correlated with plant uptake, except in the case of NH_4-EDTA extractable soil Co and the bean pods (Table 19), if both $CaCO_3$ rates are included in the regressions. AB-DTPA extractable soil Co is best correlated with plant Co for all three the plant types (Table 19).

Table 19. The significant ($P<0,05$) multiple linear regression equations for Co concentration (mg/kg dry matter) and uptake (μg/plant) in the plants as related to the extractable Co in the soil as determined by the different soil extractants.

Extractant	Plant type	CaCO$_3$ rate (t/ha)	Plant concentration (mg/kg) or uptake (μg/plant)	Regression equation[1]	R^2 value
NH$_4$-EDTA	Lettuce	2	Concentration	ComgL2=49,80-3,69NH$_4$Co	0,34**
		2 & 11	Concentration	ComgL=56,30-19,96NH$_4$Co-5,25pH	0,70***
			Uptake	CoμgL=-36,50+5,69NH$_4$Co	0,11*
	Wheat	2	Concentration	ComgW2=18,00-1,07NH$_4$Co	0,28**
			Uptake	CoμgW2=44,52-2,53NH$_4$Co	0,23**
		11	Concentration	ComgW11=3,05-0,17NH$_4$Co	0,16*
		2 & 11	Concentration	ComgW=26,08-0,80NH$_4$Co-2,37pH	0,66***
			Uptake	CoμgW=66,60-1,86NH$_4$Co-6,30pH	0,63***
	Bean pods	11	Uptake	CoμgB11=-2,36+0,36NH$_4$Co	0,24*
DTPA	Lettuce	2	Concentration	ComgL2=-9,99+3,96DTPACo	0,55*
		2 & 11	Concentration	ComgL=-0,96+2,24DTPACo	0,78***
			Uptake	CoμgL=-7,90+7,42DTPACo	0,79***
	Wheat	2 & 11	Concentration	ComgW=-0,43+1,55DTPACo	0,76***
			Uptake	CoμgW=-1,72+4,40DTPACo	0,76***
	Bean pods	2	Concentration	ComgB2=-9,06+3,84DTPACo	0,63**
		2 & 11	Concentration	ComgB=-1,55+2,30DTPACo	0,72***
			Uptake	CoμgB=-2,64+5,69DTPACo	0,72***
AB-DTPA	Lettuce	2	Concentration	ComgL2=-14,36+3,94ABCo	0,46*
			Uptake	CoμgL2=455,92-11,59ABCo-77,13pH	0,70*
		2 & 11	Concentration	ComgL=-1,53+1,92ABCo	0,76***
			Uptake	CoμgL=-10,78+6,61ABCo	0,82***
	Wheat	2	Concentration	ComgW2=-7,45+2,70ABCo	0,40*
		2 & 11	Concentration	ComgW=-1,52+1,62ABCo	0,79***
			Uptake	CoμgW=-4,95+4,63ABCo	0,80***
	Bean pods	2	Concentration	ComgB2=-16,09+4,95ABCo	0,72**
		11	Concentration	ComgB11=2,56-0,92ABCo	0,45*
		2 & 11	Concentration	ComgB=-33,45+4,42ABCo+4,19pH	0,82***
			Uptake	CoμgB=-5,77+5,96ABCo	0,76***

* Significant at $P<0,05$
** Significant at $P<0,01$
*** Significant at $P<0,001$

[1] ComgL/ComgW/ComgB = Co concentration (mg/kg dry matter) in lettuce, wheat and bean pods; CoμgL/CoμgW/CoμgB = Total Co uptake (μg/plant) by lettuce, wheat and bean pods; NH$_4$Co = Co in soil (mg/kg) determined by using the NH$_4$-EDTA extraction method; DTPACo = Co in the soil (mg/kg) determined by using the DTPA extraction method; ABCo = Co in the soil (mg/kg) determined by using the AB-DTPA extraction method; pH = Soil pH after the experiment.

However, if the two $CaCO_3$ rates are studied separately the correlations are far poorer and in some cases there are no significant correlations.

As for Ni, the poor correlations at the two $CaCO_3$ application rates individually can be ascribed to the small application of Co to the soil. There are however still very good correlations with DTPA and AB-DTPA as extractants, especially at low soil pH, which supports the theory that plant yield at low soil pH may have been influenced by excess Co.

4.6 Cadmium

4.6.1 Cd in plants

There are significant differences in the Cd concentration of the different plant types (Appendix 3: Table 3). Lettuce has the highest concentration followed by wheat and then the bean pods (Table 20). Sauerbeck (1991) also found that lettuce accumulated Cd most, followed by wheat and then bean pods (Table 21).

In human and animal nutrition, Cd is a cumulative poison. Therefore, its content in food and feed plants has been widely studied. The threshold concentration in animal feed plants may be a bit higher than those established for human food plants and may differ for each kind of animal. However, in view of variable and secondary effects of Cd in the food chain, it is desirable to minimize its concentrations in crops (Kabata-Pendias & Pendias, 1992). Table 21 gives average and phytotoxic levels of Cd in plants, while Table 22 provides a summary of some countries' legislation for Cd in foodstuffs. Some of the countries not mentioned in table 22, such as the USA, Canada, Sweden and the United kingdom had no limits for Cd in foodstuffs when surveyed in 1986, all for a number of different reasons (Simpson & Curnow, 1988). According to the data in Table 21 the Cd concentrations in this experiment are average and not phytotoxic. However, according to legislation in some countries the Cd levels exceed the permissible concentration (Table 22). There is therefore some discrepancy in the legislation as the maximum permissible concentration in some countries is lower than the average concentration in plants. Some research will therefore have to be conducted to verify the validity of the legislation.

The wheat and bean pods do not show any differences in Co concentration with different OSA application rates and OSA types (Table 20). However, the lettuce plants are influenced significantly by the different OSA application rates and OSA types (Table 20). OSA 10 causes a decrease in Cd plant concentration (mg/kg) with increased application rates while OSA 20 tends to increase Cd plant concentration. The decrease in Cd plant concentration (mg/kg) is probably due to a dilution effect because of an increase in plant yield. This decrease is eliminated if the plant yield is taken into account when using plant uptake (μg/plant) as the basis for comparison (Table 20).

The difference in $CaCO_3$ application rate causes significant differences in the Cd plant concentration and uptake. These differences seem to be most pronounced in lettuce, though still not very large, while the wheat plants seem to show no differences. Korcak & Fanning (1978) found that the soil pH did not influence the Cd uptake of maize to the same extent as for Cu, Ni and Zn. Hornburg & Bruemmer (1993) found that the proportion of mobile Cd only starts decreasing above pH($CaCl_2$) of 6,5. Giordano, Mays & Behel (1979), however, did find lower Cd concentrations in lettuce with liming although they ascribe some of the decrease to improved growth of the plants at the higher soil pH. Sauerbeck (1991) also found differences in Cd content of wheat grains with differences in soil pH. Although liming generally is expected to decrease Cd absorption by raising the soil pH, it is not effective for all soils and plants (Kabata-Pendias & Pendias, 1992). The influence of pH on the Cd uptake by plant therefore probably depends on the plant type, Cd levels of the soil and soil conditions.

Table 20. Mean Cd concentration (mg/kg dry matter) and total Cd uptake (μg/plant) of plants as influenced by $CaCO_3$ application rate, OSA type and OSA application rate.

Plant	$CaCO_3$ rate (t/ha)	Control 0 t/ha OSA	OSA type and application rate (t/ha)					
			OSA 10			OSA 20		
			5	10	20	5	10	20
		Cd concentration (mg/kg dry matter)[2]						
Lettuce	2	0,581 a[1]	0,378 b	0,288 b	0,282 b	0,530 a	0,648 a	0,618 a
	11	0,324 ac	0,201 b	0,179 b	0,240 ab	0,324 abc	0,409 c	0,457 c
Wheat	2	0,111 a	0,214 a	0,188 a	0,072 a	0,113 a	0,269 a	0,201 a
	11	0,110 a	0,126 a	0,163 a	0,164 a	0,118 a	0,145 a	0,130 a
Beans	2	0,086 a	0,010 a	0,205 a	0,022 a	0,010 a	0,050 a	0,041 a
	11	0,032 a	0,011 a	0,012 a	0,010 a	0,029 a	ND a	0,009 a
		Cd uptake (μg/plant)						
Lettuce	2	0,855 a	0,742 a	0,753 a	1,194 ab	1,378 ab	1,800 bc	2,531 c
	11	0,046 a	0,090 a	0,072 a	0,091 a	0,090 a	0,099 a	0,139 a
Wheat	2	0,273 a	0,601 a	0,540 a	0,188 a	0,287 a	0,616 a	0,587 a
	11	0,312 a	0,330 a	0,525 a	0,390 a	0,289 a	0,359 a	0,322 a
Beans	2	0,225 a	0,025 a	0,360 a	0,060 a	0,040 a	0,327 a	0,262 a
	11	0,010 a	0,009 a	0,003 a	0,009 a	0,009 a	ND a	0,016 a

ND = not detected

[1] Values in the row followed by the same symbol do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 4 replicates.

Table 21. Average and critical concentrations of Cd in plants as reported by different authors.

Source of data	Lettuce	Wheat	Bean pods	Food in general
	———mg/kg dry matter———			
Range of the mean concentration at low soil pH in this experiment	0,28-0,65	0,07-0,27	0,01-0,09	
Range of the meant concentration at high soil pH in this experiment	0,18-0,46	0,11-0,22	0-0,03	
Kabata-Pendias & Pendias (1992): Average plant concentration	0,12-0,66		0,29	
Kabata-Pendias & Pendias (1992): Plants grown in contaminated sites	0,5-70	19-47		
MacNicol & Beckett (1985): Upper critical phytotoxic levels	10-95	4-43	5-30 (leaves)	
Reilly (1991): Average concentration				0,095-0,987
Fergusson (1990): Average plant concentration	0,017-3,3	0,012-0,036 (grain)		
Boon & Soltanpour (1992): Plants grown in contaminated garden soils	0,1-34,5			
Garcia, Blessin, Inglett, Kwolek, Carlisle, Hughes & Meister (1981): Cd accumulation in diverse soil media amended with sewage sludge	0,106-3,462	0,312-2,096		
Keefer *et al*, (1986): Cd content of vegetables with a maximum application of 180 t/ha sewage sludge containing 20 mg/kg Cd			<0,10	
Roca & Pomares (1991): Calcareous soil with a maximum application of 1,27 kg/ha Cd	0,20-0,94			
Sauerbeck (1991): Average concentration	ca 4	ca 0,8	ca 0,1	
Bingham *et al*, (1975): Plant tissue Cd levels associated with a 25 % yield decrement	70,0	33,0	1,7	
Schauer *et al*, (1980): Maximum loading of 1,2 kg/ha Cd	1,4-3,9			
Risser & Baker (1990): Suggested tolerance levels of Cd in plants				1
Mitchell *et al*, (1978): Phytotoxic levels	118,147	36,43		

Table 22. Maximum levels of Cd in selected foodstuffs for different countries (Simpson & Curnow, 1988; Verheul, undated).

Country	All food	Cereal products; wheat germ, grain and grain products	Vegetables	Other foodstuffs[1]
	mg/kg dry matter			
Australia		0,2		0,05
Bangladesh	6			
Brazil				1
Chile	0,05			
Colombia	5			
Denmark		0,1	0,1	0,02
Hungary		0,1	0,3	
Malaysia	1			
Netherlands		0,15	Ranging from 0,03 to 0,1 For lettuce - 0,2	
Pakistan	6			
South Africa		0,1	0,1	0,1
Uruguay	5			

[1] Foodstuffs (many not mentioned in this table) not specified in the particular countries' legislation.

4.6.2 Cd in soil

The plant type caused significant differences in the DTPA extractable levels in the soil but will not be discussed further as mentioned before.

The EPA method 3050 could not detect Cd in the soil, probably due to the large dilution by the extract. The other extractants were able to determine Cd levels in the soil and therefore the lack of detection is not due to the absence of Cd in the soil.

The soil Cd content is low and should not cause any environmental danger if compared with findings of other authors (Table 23).

There are significant differences in soil Cd levels with OSA application rate and OSA type for NH_4-EDTA and AB-DTPA extractable Cd (Table 24; Appendix 3: Table 5 and 8). The DTPA extraction does not predict differences in application rate which may have something to do with its buffer capacity (Table 24; Appendix 3: Table 7). Although a maximum of only 0,0255 mg/kg Cd was applied to the soil the NH_4-EDTA and AB-DTPA was able to detect increases in the Cd content of the soil. These two methods therefore seem to be quite suitable to detect Cd loading of the soil.

The different $CaCO_3$ application rates did not influence the Cd extractability by NH_4-EDTA, DTPA and AB-DTPA. Korcak & Fanning (1978) also found that liming did not reduce plant tissue Cd levels or extractable Cd as much as it reduced similar parameters with Zn, Cu, and Ni.

Table 23. Average and maximum permissible Cd concentration (mg/kg) in the soil as extracted by different extractants and reported by different authors.

Source of data	NH_4-EDTA	EPA 3050	DTPA	AB-DTPA
	——mg/kg soil——			
Range for the mean concentration at low soil pH in this experiment	0,012-0,025		0,011-0,031	0,010-0,028
Range for the mean concentration at high soil pH in this experiment	0,012-0,027		0,014-0,022	0,007-0,026
Kabata-Pendias & Pendias (1992): Mean content of surface soils		0,01-1,80		
Kabata-Pendias & Pendias (1992): Cd contamination in surface soils		0,02-1781		
Maximum permissible concentration in soil for:				
South Africa		2		
Germany		1,5		
United Kingdom		3		
Risser & Baker (1990): Suggested maximum cumulative soil levels dependant on soil texture.		2,5-5,0		
Bruemmer & van der Merwe (1989): Maximum permissible concentration with soil pH($CaCl_2$) above 6,0-6,5	1			
Soltanpour (1991): Potentially toxic range				>1,5
Bingham *et al,* (1975): Soil Cd levels associated with a 25% yield decrease for:				
Lettuce			7,8	
Wheat			30,0	
Field beans			24,0	
Boon & Soltanpour (1992): Total contamination of garden soils	0,03-3,1			0,2-14,2
Roca & Pomares (1991): Range with maximum application of 1,60 kg/ha Cd over 3 years	0,12-0,82		0,06-0,19	0,08-0,38
Korcak & Fanning (1978): Sludge treated soils with a maximum of 8 mg/kg Cd applied	0,0-5,6			
Barbarick & Workman (1987): Maximum application of 80,4 tons/ha of sludge containing an average of 13 mg/kg Cd			0,07-0,40	0,07-0,27

Table 24. Soil extractable Cd found with the various extractants as influenced by CaCO$_3$ application rate, OSA type and OSA application rate.

Extractant	CaCO$_3$ rate (t/ha)	Control 0 t/ha OSA	OSA type and application rate (t/ha)					
			OSA 10			OSA 20		
			5	10	20	5	10	20
		Cd in soil (mg/kg soil)						
NH$_4$-EDTA[2]	2	0,012 a	0,012 a	0,012 a	0,012 a	0,015 b	0,018 c	0,025 d
	11	0,012 a	0,012 a	0,012 a	0,012 a	0,014 a	0,019 b	0,027 c
DTPA[3]	2	0,018 ab	NA	0,016 ab	0,011 a	NA	0,031 b	0,031 ab
	11	0,020 a	NA	0,015 a	0,014 a	NA	0,021 a	0,022 a
AB-DTPA[3]	2	0,014 ab	NA	0,011 ab	0,010 a	NA	0,024 bc	0,028 c
	11	0,007 ab	NA	0,008 a	0,013 ab	NA	0,020 b	0,026 b

NA = Not analyzed

[1] Values in the row followed by the same symbol do not differ significantly at P<0,05 according to Duncan's multiple range test.

[2] Mean of 12 replicates.

[3] Mean of 6 replicates.

4.6.2 Relationship between soil Cd and plant Cd

The only significant regression equations for Cd in the soil extracts and Cd in the plants are with lettuce, except one significant correlation for bean pod Cd uptake as related to AB-DTPA extractable Cd at 11 t/ha CaCO$_3$ applied (Table 25). Wheat and bean pod Cd are therefore regulated in some way and not influenced by the soil Cd concentration in the Cd ranges experienced in this experiment. Roca & Pomares (1991) did not find any significant correlations between DTPA, EDTA (pH 4,65) and AB-DTPA extractable metals as related to concentration in the edible leaves of lettuce in calcareous soils. Mitchell *et al,* (1978), however, did find significant correlations for Cd concentrations in lettuce ($r=0,89^{***}$) and wheat leaves ($r=0,56^{*}$) as related to DTPA extractable Cd in the soil. Boon & Soltanpour (1992) found significant correlations for AB-DTPA extractable Cd concentration in the soil as related to Cd concentrations in lettuce leaves.

The soil pH influences the regression of Cd in lettuce as related to Cd in soil for the different extractants (Table 25). Barbarick & Workman (1987) also found that pH influenced the regression equations significantly for Cd concentration in leaves of swiss chard as related to AB-DTPA extractable Cd in the soil. Korcak & Fanning (1978) found different slopes for the regression equations of corn Cd levels as related to DTPA Cd concentrations in the soil for one harvest but not for the second harvest. The influence of soil pH will therefore depend on the specific conditions of the experiment.

Table 25. The significant ($P < 0,05$) multiple linear regression equations for Cd concentration (mg/kg dry matter) and uptake (μg/plant) in the plants as related to the extractable Cd in the soil when determined by the different soil extractants.

Extractant	Plant type	$CaCO_3$ rate (t/ha)	Plant concentration (mg/kg) or uptake (μg/plant)	Regression equation[1]	R^2 value
NH_4-EDTA	Lettuce	2	Concentration	$CdmgL2=0,21+17,28NH_4Cd$	$0,23^{**}$
			Uptake	$Cd\mu gL2=-0,41+112,13NH_4Cd$	$0,52^{***}$
		11	Concentration	$CdmgL11=0,11+13,57NH_4Cd$	$0,41^{**}$
			Uptake	$Cd\mu gL11=0,03+3,03NH_4Cd$	$0,30^{*}$
		2 & 11	Concentration	$CdmgL=0,56+15,62NH_4Cd-0,07pH$	$0,44^{***}$
			Uptake	$Cd\mu gL=3,15+41,65NH_4Cd-0,55pH$	$0,64^{***}$
DTPA	Lettuce	2	Concentration	$CdmgL2=4,05+10,16DTPACd-0,84pH$	$0,86^{**}$
			Uptake	$Cd\mu gL2=0,81+26,60DTPACd$	$0,57^{*}$
		2 & 11	Concentration	$CdmgL=0,24+6,85DTPACd$	$0,43^{**}$
			Uptake	$Cd\mu gL=3,60+21,17DTPACd-0,58pH$	$0,82^{***}$
AB-DTPA	Lettuce	2	Uptake	$Cd\mu gL2=0,50+52,42ABCd$	$0,50^{*}$
		2 & 11	Uptake	$Cd\mu gL=3,81+29,23ABCd-0,63pH$	$0,77^{***}$
	Bean pods	11	Uptake	$Cd\mu gB11=-0,01+1,59ABCd$	$0,50^{*}$

* Significant at $P<0,05$

** Significant at $P<0,01$

*** Significant at $P<0,001$

[1] CdmgL = Cd concentration (mg/kg dry matter) in lettuce; CdμgL/CdμgB = Total Cd uptake (μg/plant) by lettuce or bean pods; NH_4Cd = Cd in soil (mg/kg) determined by using the NH_4-EDTA extraction method; DTPACd = Cd in the soil (mg/kg) determined by using the DTPA extraction method; ABCd = Cd in the soil (mg/kg) determined by using the AB-DTPA extraction method; pH = Soil pH after the experiment.

4.7 Copper

4.7.1 Cu in plants

There are once again significant differences in Cu content of the plant types (Appendix 3: Tables 3 and 4). For Cu, though, wheat generally has the highest concentration followed by lettuce and then bean pods (Table 26).

The appropriate content of Cu in plants is essential both for the health of the plant and for the nutrient supply to man and animals. In several species growing under widely ranging natural conditions, Cu contents of whole plant shoots do not often exceed 20 mg/kg (dry weight), and this value is most often considered to indicate the threshold of excessive contents (Kabata-Pendias & Pendias, 1992). From the data given by other authors the Cu concentrations of the plants in this experiment are high and are tending towards toxic levels for lettuce and wheat (Table 27). The Cu content of the plants are, however, below maximum permissible Cu contents for human consumption and should therefore not pose any toxicity problems to humans (Table 27).

The ANOVA analysis of variance indicates that there are significant differences in the Cu concentration (mg/kg) and uptake (μg/plant) of the plants as a result of the OSA application rates but not the OSA type (Appendix 3: Tables 3 and 4). However, no significant differences in plant Cu concentration (mg/kg) are found if Duncan's Multiple Range Test is used. If the plant yield is taken into account there are, however, significant differences in Cu uptake (μg/plant) by lettuce at 2 t/ha $CaCO_3$ applied and bean pods at 11 t/ha $CaCO_3$ applied (Table 26). Even though there are large increases in Cu content of the soil (9,85 mg/kg Cu at 20 t/ha of OSA 20) this is not reflected in the plant content. The difference in Cu applied to the soil by the two OSA types (9,85 mg/kg Cu at 20 t/ha OSA 20 and 0,17 mg/kg Cu at 20 t/ha OSA 10) is also not reflected in the plant content. It, therefore, seems as if plants do not necessarily increase in Cu content with increased Cu application to the soil, as has also been found by other authors (see Table 27).

Table 26. Mean Cu concentration (mg/kg dry matter) and total Cu uptake (μg/plant) of plants as influenced by $CaCO_3$ application rate, OSA type and OSA application rate.

Plant	$CaCO_3$ rate (t/ha)	Control 0 t/ha OSA	OSA type and application rate (t/ha)					
			OSA 10			OSA 20		
			5	10	20	5	10	20
		Cu concentration (mg/kg dry matter)[2]						
Lettuce	2	9,90 a	7,77 a	8,66 a	13,27 a	8,51 a	10,64 a	11,00 a
	11	3,67 a	4,78 a	5,84 a	4,48 a	3,42 a	4,79 a	5,12 a
Wheat	2	14,81 a	15,28 a	15,98 a	12,62 a	13,63 a	17,99 a	17,58 a
	11	14,48 a	17,04 a	13,37 a	19,26 a	18,42 a	14,63 a	16,89 a
Beans	2	4,51 a	4,44 a	5,29 a	4,07 a	3,62 a	6,21 a	5,62 a
	11	2,14 a	3,74 a	2,80 a	2,22 a	2,07 a	3,04 a	3,23 a
		Cu uptake (μg/plant)						
Lettuce	2	14,48 a	15,33 a	22,83 ab	57,08 b	22,26 ab	30,57 ab	45,96 b
	11	0,53 a	2,71 a	3,22 a	1,67 a	0,56 a	1,33 a	1,79 a
Wheat	2	36,48 a	42,74 a	45,07 a	33,89 a	32,36 a	48,20 a	51,50 a
	11	41,67 a	44,25 a	33,24 a	45,43 a	45,44 a	36,81 a	42,72 a
Beans	2	13,04 a	14,58 a	19,38 a	12,66 a	15,72 a	37,98 a	30,20 a
	11	1,11 ab	2,86 a	1,64 a	1,69 a	0,61 a	2,71 a	8,41 b

[1] Values in the row followed by the same symbol do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 4 replicates.

Table 27. Average and critical concentrations of Cu in plants as reported by different authors.

Source of data	Lettuce	Wheat	Bean pods	Plants in general
	mg/kg dry matter			
Range of the mean concentration at low soil pH in this experiment	7,77-13,27	12,62-17,99	3,62-6,21	
Range of the mean concentration at high soil pH in this experiment	3,42-5,84	13,37-19,26	2,07-3,74	
Kabata-Pendias & Pendias (1992): Average plant concentration	6-8,1	0,6-10,3 (grain)	5,1-8	5-30
Kabata-Pendias & Pendias (1992): Plants grown in contaminated sites	64	21 (14-70 grass tops)		
Kabata-Pendias & Pendias (1992): Excessive or toxic levels				20-100
Kabata-Pendias & Pendias (1992): Tolerable in agronomic crops				50
MacNicol & Beckett (1985): Upper critical phytotoxic levels	8-23	11-18	15-30 (leaves)	5-64
Reilly (1991): Average concentration				ca. 2 or less
Barbarick & Workman (1987): Maximum application of 80,4 tons/ha sludge containing an average of 1018,5 mg/kg Cu		1,0-2,9 (straw) 4,2-6,3 (grain)		
Reuter & Robinson (1986): Adequate levels	7-80	>1,0 - >2,2	5-15 (leaves)	
Reuter & Robinson (1986): Toxic levels	>21	>18		
Keefer *et al,* (1986): Cu content of vegetables with a maximum application of 180 t/ha sewage sludge containing 6500 mg/kg Cu			9,5-11,3	
Schauer *et al,* (1980): Maximum loading of 150 kg/ha Cu	11-17			
Roca & Pomares (1991): Calcareous soil with a maximum application of 115,10 kg/ha Cu	12-15			
Sauerbeck (1991): Average concentration	ca 8	ca 5	ca 7	
Mitchell *et al,* (1978): Phytotoxic levels	8,2-10,7	11,8-15,4		
Verheul (undated): Legislation for different countries				20-50

The soil pH influences the Cu content of the plants significantly (Appendix 3: Table 3 and 4). However, the decreases in plant Cu at high soil pH appear only in the lettuce and the bean pods (Table 26). Narwal *et al,*

(1983) found a decrease in Cu concentration in fodder rape with increased soil pH, except at a high sludge application rate were the opposite was found. It is therefore clear that generalisations about the influence of soil pH on plant content of Cu cannot be made and that every soil-plant system should be studied independently.

4.7.2 Cu in soil

The NH_4-EDTA and DTPA extractable soil Cu is once again significantly influenced by the plant type and will not be discussed further as mentioned before (Appendix 3: Tables 5 to 8).

If the Cu concentration of the soil is compared with data found by other authors it is clear that there is sufficient Cu in the soil to sustain normal growth. Further, the soil Cu content is also not excessive (Table 28).

The OSA application rates and OSA types resulted in significant differences in the amounts of Cu extracted by the different extractants (Table 29; Appendix 3: Tables 5 to 8). The Cu loading of the soil by OSA 10 is 0,17 mg/kg at 20 t/ha and for OSA 20 it is 9,85 mg/kg at 20 t/ha. These large differences in Cu loading is quite clearly reflected in the results obtained by the different soil extractants (Table 29). The EPA method is able to extract almost all of the added Cu and at the current rate it should take only 3-4 more applications of OSA 20 at 20 t/ha to reach the German limit of 60 mg/kg in the soil. It will, however, according to predictions take more than 13 years, at the current application rate, to reach the NH_4-EDTA limit of 60 mg/kg.

The soil pH caused significant differences in the Cu extracted, even by EPA method 3050 (Appendix 3: Table 5 to 8). The Cu extracted generally decreased with increased soil pH, with the exception of NH_4-EDTA were it increased. This increase is probably due to the buffer capacity of the NH_4-EDTA being exceeded, causing the % EDTA complexed with Cu to increase as predicted by Norvell & Lindsay (1969). The influence of soil pH on the EPA method 3050 is slightly puzzling and is possibly due to precipitation with Ca or reasonably strong fixation by the soil at the higher soil pH. Korcak & Fanning (1978) also found a decrease in DTPA Cu extractability with increased soil pH.

Table 28. Average and maximum permissible Cu concentration (mg/kg) in the soil as extracted by different extractants and reported by different authors.

Source of data	NH_4-EDTA	EPA 3050	DTPA	AB-DTPA
	——————mg/kg soil——————			
Range for the mean concentration at low soil pH in this experiment	4,55-8,26	23,22-31,06	3,05-5,45	6,29-10,72
Range for the mean concentration at high soil pH in this experiment	5,74-9,82	22,13-30,09	2,37-4,52	6,07-9,45
Bruemmer & van der Merwe (1989): Maximum permissible concentration with soil pH($CaCl_2$) above 4,5	60			
Kabata-Pendias & Pendias (1992): Mean content of surface soils		1-323		
Kabata-Pendias & Pendias (1992): Cu contamination in surface soils		3-3700		
Maximum permissible concentration in soil for: South Africa Germany United Kingdom: pH 6,0-7,0 pH 5,0<5,5		 100 60 135 60		
Soltanpour (1991): Low Medium High				0-0,2 0,3-0,5 >0,5
Lindsay & Norvell (1978): Critically low			<0,2	
Roca & Pomares (1991): Range with maximum application of 147,90 kg/ha Cu over 3 years	11,7-22,6		4,3-9,8	9,1-21,5
Korcak & Fanning (1978): Sludge treated soils with a maximum of 314 mg/kg Cu applied			0,2-116	
Sims *et al,* (1991): Sludged sites with a total loading rate of 140 kg/ha Cu		1,5-14,0	0,2-4,6	
Barbarick & Workman (1987): Maximum application of 80,4 tons/ha of sludge containing an average of 170 mg/kg Cu			1,2-10,1	2,2-12,2

Table 29. Soil extractable Cu found with the various extractants as influenced by $CaCO_3$ application rate, OSA type and OSA application rate.

Extractant	$CaCO_3$ rate (t/ha)	Control 0 t/ha OSA	OSA type and application rate (t/ha)					
			OSA 10			OSA 20		
			5	10	20	5	10	20
		Cu in soil (mg/kg soil)						
NH_4-EDTA[2]	2	4,96 a	4,60a	5,11 a	4,55 a	5, 74 b	6,28 c	8,26 d
	11	5,82 a	5,76 a	6,21 a	5,74 a	7,16 b	8,12 b	9,82 c
EPA method 3050[3]	2	23,22 a	NA	24,07 a	25,61 a	NA	26,57 a	31,06 b
	11	22,13 a	NA	23,00 a	23,19 a	NA	24,68 a	30,09 b
DTPA[3]	2	3,10 a	NA	3,05 a	2,83 a	NA	4,16 b	5,45 c
	11	2,41 a	NA	2,47 a	2,37 a	NA	3,53 b	4,52 c
AB-DTPA[3]	2	6,55 a	NA	6,45 a	6,29 a	NA	8,60 b	10,72 c
	11	6,12 a	NA	6,07 a	6,09 a	NA	7,79 b	9,45 c

NA = Not analyzed

[1] Values in the row followed by the same symbol do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 12 replicates.

[3] Mean of 6 replicates.

4.7.3 Relationship between soil Cu and plant Cu

The Cu content of the soil is only correlated with Cu content of the bean pods. The only exception is a significant correlation for Cu uptake in lettuce if both pH levels are included as related to EPA method 3050 extractable Cu (Table 30). Further, there are only good correlations if plant yield is considered (Cu uptake, μg/plant). The plant in general does therefore not reflect the increased Cu content of the soil except for bean

Table 30. The significant ($P<0,05$) multiple linear regression equations for Cu concentration (mg/kg dry matter) and uptake (μg/plant) in the plants as related to the extractable Cu in the soil as determined by the different soil extractants.

Extractant	Plant type	$CaCO_3$ rate (t/ha)	Plant concentration (mg/kg) or uptake (μg/plant)	Regression equation[1]	R^2 value
NH_4-EDTA	Bean pods	2	Uptake	$Cu\mu gB2=-6,81+4,70NH_4Cu$	$0,26^{**}$
		11	Uptake	$Cu\mu gB11=-4,04+0,98NH_4Cu$	$0,49^{***}$
		2 & 11	Uptake	$Cu\mu gB=52,40+1,90NH_4Cu-9,20pH$	$0,49^{***}$
DTPA	Bean pods	11	Uptake	$Cu\mu gB11=-2,27+1,84DTPACu$	$0,59^{**}$
		2 & 11	Uptake	$Cu\mu gB=-12,53+7,50DTPACu$	$0,34^{**}$
AB-DTPA	Bean pods	11	Uptake	$Cu\mu gB11=-6,22+1,32ABCu$	$0,74^{**}$
EPA method 3050	Lettuce	2 & 11	Uptake	$Cu\mu gL=56,68+0,85EPACu-15,55pH$	$0,70^{***}$
	Bean pods	2	Uptake	$Cu\mu gB2=-76,33+3,71EPACu$	$0,67^{**}$
		11	Uptake	$Cu\mu gB11=-9,22+0,50EPACu$	$0,65^{**}$
		2 & 11	Uptake	$Cu\mu gB=8,13+1,76EPACu-7,05pH$	$0,66^{***}$

* Significant at $P<0,05$

** Significant at $P<0,01$

*** Significant at $P<0,001$

[1] CuμgL/CuμgB = Total Cu uptake (μg/plant) by lettuce and bean pods; NH_4Cu = Cu in soil (mg/kg) determined by using the NH_4-EDTA extraction method; DTPACu = Cu in the soil (mg/kg) determined by using the DTPA extraction method; ABCu = Cu in the soil (mg/kg) determined by using the AB-DTPA extraction method; EPACu = Cu in the soil (mg/kg) determined by using the EPA method 3050; pH = soil pH after the experiment.

pods were the influence of OSA application rate and type had a similar influence on plant yield and Cu content of the soil. The increased plant yield therefore resulted in an increase in total Cu uptake (μg/plant).

Barbarick & Workman (1987) found significant correlations with swiss chard as related to AB-DTPA Cu levels in the soil but commented that AB-DTPA may not provide a good correlation with plant levels unless relatively large quantities of Cu are added to the soil. In contrast, they did not find significant correlations between Cu content of swiss card and DTPA extractable soil Cu. In a field trial they also found no significant correlations for AB-DTPA and DTPA Cu levels in the soil as related to straw or grain Cu levels in wheat, indicating that Cu added in the sludge was not readily available to the plant. Roca & Pomares (1991) also did not find significant correlations with DTPA, EDTA (pH 4,65) and AB-DTPA extractable Cu with lettuce in calcareous soils. Mitchell *et al,* (1978) and Korcak & Fanning (1978), however, did find significant correlations for Cu in lettuce, wheat and corn as related to DTPA Cu in soil, with very large quantities of Cu added to the soil. It therefore appears as if significant correlations are only obtained if very large Cu applications are made to the soil. The plants are able to regulate Cu uptake to a certain extent if the Cu applications are not excessive.

4.8 Zinc

4.8.1 Zn in plants

The plant types have significantly different Zn concentrations (Appendix 3: Tables 3 and 4). The lettuce, in general, has the highest Zn concentrations (mg/kg) followed by the bean pods and then the wheat (Table 31).

Zn is probably one of the most thoroughly investigated of all the nutritionally important trace elements. Plants and animals normally contain large amounts of Zn which is essential for normal growth (Reilly, 1991). The Zn concentration of the plants in this experiment are high yet probably not phytotoxic, but are very close to phytotoxic levels if compared to the results of other authors (Table 32). Most of the Zn concentrations of the plants are higher than the permissible levels for human consumption with the exception of wheat with 11 t/ha $CaCO_3$ applied. As in the case of Cd the Zn legislation for human consumption seems strict and should be studied further to determine the suitability of these levels.

The OSA application rate causes significant differences in the Zn uptake by the plants, according to the ANOVA analysis of variance (Appendix 3: Tables 3 and 4). However, these differences are not consistent for the different plant types (Table 31). For Zn concentration (mg/kg) in lettuce the 20 t/ha OSA 10 at 2 t/ha $CaCO_3$ was significantly lower than the other treatments. If yield is taken into account (μg/plant) the Zn in the control is lower than for the other treatments and higher in the OSA 20 treatment. For wheat there are only significant differences for Zn concentration (mg/kg) at 11 t/ha $CaCO_3$ applied. The control is lower than all the treatments excluding the 5 t/ha OSA 10 treatments. The 5 t/ha OSA 10 at 11 t/ha $CaCO_3$ also causes significantly lower Zn concentrations than 20 t/ha OSA 20 at 11 t/ha $CaCO_3$ applied. In the bean pods there are significant differences only at the 11 t/ha $CaCO_3$ application. The Zn concentration (mg/kg) of the bean pods for the 20 t/ha OSA 20 is lower than the other treatments with the exception of the control and 5 t/ha OSA 20. For the Zn uptake (μg/plant) though, the 5 t/ha OSA 20 is lower than the other treatments with the exception of the control. It is therefore clear that there is no consistent pattern in the Zn content of the plants.

Table 31. Mean Zn concentration (mg/kg dry matter) and total Zn uptake (μg/plant) of plants as influenced by $CaCO_3$ application rate, OSA type and OSA application rate.

Plant	**$CaCO_3$ rate (t/ha)**	**Control 0 t/ha OSA**	**OSA type and application rate (t/ha)**					
			OSA 10			**OSA 20**		
			5	**10**	**20**	**5**	**10**	**20**
		Zn concentration (mg/kg dry matter)[2]						
Lettuce	2	148,96 a	162,09 a	135,12 a	95,78 b	131,30 a	156,10 a	143,73 a
	11	120,88 a	111,34 a	127,68 a	99,77 a	117,90 a	105,42 a	148,33 a
Wheat	2	74,49 a	63,33 a	65,85 a	55,89 a	70,72 a	67,34 a	59,05 a
	11	34,88 a	41,19 ab	51,32 bc	49,09 bc	47,39 bc	55,92 bc	62,18 c
Beans	2	99,84 a	83,61 a	132,32 a	83,20 a	50,21 a	36,80 a	46,70 a
	11	78,28 ab	112,27 a	122,35 a	120,40 a	84,45 ab	73,50 a	37,03 b
		Zn uptake (μg/plant)						
Lettuce	2	219,94 a	317,41 b	349,09 b	405,53 b	340,82 b	433,16 b	574,59 c
	11	17,60 a	56,41 a	57,34 a	36,39 a	16,43 a	23,92 a	44,27 a
Wheat	2	183,79 a	178,25 a	185,45 a	149,93 a	160,74 a	181,78 a	172,30 a
	11	100,09 a	107,42 a	137,09 a	115,55 a	116,62 a	142,05 a	160,34 a
Beans	2	261,28 a	256,71 a	213,38 a	224,16 a	215,03 a	219,32 a	262,40 a
	11	37,97 ab	82,45 a	64,46 a	79,97 a	24,48 b	51,68 a	93,52 a

[1] Values in the row followed by the same symbol do not differ significantly at P<0,05 according to Duncan's multiple range test.

[2] Mean of 4 replicates.

Table 32. Average and critical concentrations of Zn in plants as reported by different authors.

Source of data	**Lettuce**	**Wheat**	**Bean pods**	**Plants in general**
	——mg/kg dry matter——			
Range of the mean concentration at low soil pH in this experiment	95,78-162,09	55,89-74,49	36,80-132,32	
Range of the mean concentration at high soil pH in this experiment	99,77-148,33	34,88-62,18	37,03-122,35	
Kabata-Pendias & Pendias (1992): Average plant concentration	44-73	5-67 (grain)	32-38	27-150
Kabata-Pendias & Pendias (1992): Plants grown in contaminated sites	55-530	65-350 (grass)		21-4510
Kabata-Pendias & Pendias (1992): Excessive or toxic levels				100-400
Kabata-Pendias & Pendias (1992): Tolerable in agronomic crops				300
MacNicol & Beckett (1985): Upper critical phytotoxic levels	150-530	108-500	60-250 (leaves)	60-900
Reilly (1991): Average concentration				0,2-19,0
Barbarick & Workman (1987): Maximum application of 80,4 tons/ha sludge containing an average of 1920,0 mg/kg Zn		2,8-13,2 (straw) 23-44 (grain)		
Reuter & Robinson (1986): Adequate levels	25-150	15-390	30-100 (leaves)	
Reuter & Robinson (1986): Toxic levels	96-665	>66-560		
Keefer *et al,* (1986): Zn content of vegetables with a maximum application of 180 t/ha sewage sludge containing 1300 mg/kg Zn			35-47	
Schauer *et al,* (1980): Maximum loading of 99 kg/ha Zn	46-133			
Roca & Pomares (1991): Calcareous soil with a maximum application of 443,0 kg/ha Zn	52-63			
Sauerbeck (1991): Average concentration	ca 170	ca 180	ca 160	
Mitchell *et al,* (1978): Phytotoxic levels	380-1058	189-655		
Verheul (undated): Legislation for different countries				40-50

Barbarick & Workman (1987) found that increasing sludge rates, in a field experiment, consistently produced significant increases in Zn levels of grain. Schauer *et al* (1980) also observed significant increases in the concentration of Zn in the edible tissue for increased levels of sludge application.

Although the ANOVA analysis of variance indicates significant differences in plant concentration (mg/kg) due to the different $CaCO_3$ application rates (Appendix 3: Tables 3 and 4) these differences are not that clear if the data is studied (Table 31). It is only the Zn concentration of wheat that tends to decrease clearly due to increased $CaCO_3$ application rates. Other authors also found significant decreases in Zn concentrations in plants due to liming (John & van Laerhoven, 1976; Narwal *et al*, 1983).

4.8.2 Zn in soil

The soil Zn extractions again show significant differences due to the plant type grown (Appendix 3: Tables 5 to 8), and will once again not be discussed further.

Contamination of Zn from some source occurred for all 4 replicates of the 10 t/ha OSA 10 at 2 t/ha $CaCO_3$ for the lettuce pots. The Zn must have been mixed in during the preparation because all the replicates have about the same soil concentration, using all 4 extraction methods. The plant concentration for this treatment is also slightly higher which may indicate that the contamination may have taken place during mixing. The 4 replicates were included in the data in Table 33 and for the statistical analysis. When studying the data this must, therefore, be kept in mind.

The soil Zn concentration should be sufficient for normal plant growth but should by no means be phytotoxic or detrimental to the environment, if compared with data from other authors (Table 34).

Once again due to large variation in results the EPA method 3050 was unable to detect significant differences between treatments except for a few interactions which may be a result of the contaminated treatment being significantly higher (Table 33; Appendix 3: Table 6). Even though few significant differences were determined the EPA method 3050 showed the same trend as the other extractants.

Table 33. Soil extractable Zn found with the various extractants as influenced by $CaCO_3$ application rate, OSA type and OSA application rate.

Extractant	$CaCO_3$ rate (t/ha)	Control 0 t/ha OSA	OSA type and application rate (t/ha)					
			OSA 10			OSA 20		
			5	10	20	5	10	20
		Zn in soil (mg/kg soil)						
NH_4-EDTA[2]	2	2,14 a	2,64 b	2,90 b	3,04 b	2,89 b	4,05 c	5,61 d
	11	1,94 a	3,04 ab	5,74 b	3,05 bc	2,61 ac	3,84 bc	5,77 d
EPA method 3050[3]	2	13,72 a	NA	14,62 a	14,59 a	NA	15,28 a	17,35 a
	11	15, 07 a	NA	18,49 a	13,45 a	NA	17,55 a	16,81 a
DTPA[3]	2	1,77 a	NA	2,38 b	2,74 bc	NA	3,06 c	4,40 d
	11	1,16 a	NA	3,53 a	1,57 a	NA	2,11 a	2,84 a
AB-DTPA[3]	2	4,40 a	NA	4,82 a	5,62 a	NA	7,52 b	9,52 b
	11	2,46 a	NA	6,96 ab	4,42 b	NA	4,95 b	7,12 b

NA = Not analyzed

[1] Values in the row followed by the same symbol do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 12 replicates.

[3] Mean of 6 replicates.

OSA application rate and OSA type resulted in significant differences in Zn extracted by NH_4-EDTA, DTPA and AB-DTPA (Table 33; Appendix 3: Tables 5, 7 and 8). With the application of 20 t/ha of OSA 20, 7,18 mg/kg Zn is applied to the soil while with 20 t/ha of OSA 10, 1,52 mg/kg Zn is applied. These increases are well detected by NH_4-EDTA, DTPA and AB-DTPA except for DTPA at 11 t/ha $CaCO_3$ applied, were the increases are very small and not statistically significant (Table 33). At 20 t/ha/year it should take more than 15 years to reach the >70 mg/kg Zn proposed by Soltanpour (1991) for AB-DTPA extractable Zn and longer for the other limits (Table 34).

Table 34. Average and maximum permissible Zn concentration (mg/kg) in the soil as extracted by different extractants and reported by different authors.

Source of data	NH_4-EDTA	EPA 3050	DTPA	AB-DTPA
	———mg/kg soil———			
Range for the mean concentration at low soil pH in this experiment	2,14-5,61	13,72-17,35	1,77-4,40	4,40-9,52
Range for the mean concentration at high soil pH in this experiment	1,94-5,77	13,45-18,49	1,16-3,53	2,46-7,12
Bruemmer & van der Merwe (1989): Maximum permissible concentration with soil pH($CaCl_2$) above 5,5	100			
Kabata-Pendias & Pendias (1992): Mean content of surface soils		3-770		
Kabata-Pendias & Pendias (1992): Zn contamination in surface soils		15-180 000		
Maximum permissible concentration in soil for:				
South Africa		185		
Germany		200		
United Kingdom: pH 6,0-7,0		300		
pH 5,0<5,5		200		
Soltanpour (1991): Low				0-0,9
Medium				1,0-1,5
High				>1,5
Potentially toxic range				>70
Lindsay & Norvell (1978): Critically low			0,6-0,8	
Roca & Pomares (1991): Range with maximum application of 556,00 kg/ha Zn over 3 years	16,1-55,1		4,6-20,3	6,4-27,8
Korcak & Fanning (1978): Sludge treated soils with a maximum of 827 mg/kg Zn applied			0,6-406	
Sims *et al,* (1991): Sludged sites with a total loading rate of 560 kg/ha Zn		7,5-47,0	0,3-10,8	
Barbarick & Workman (1987): Maximum application of 80,4 tons/ha of sludge containing an average of 1920 mg/kg Zn			0,7-13,9	0,8-16,8

The soil pH caused significant differences in the DTPA and AB-DTPA extractable Zn but not in the NH_4-EDTA extractable Zn. As was the case for Cu the relatively high extraction by NH_4-EDTA at high soil pH could be as a result of the buffer capacity of the extractant being exceeded and so doing increasing the % EDTA complexed with Zn as predicted by Norvell & Lindsay (1969) and Lindsay & Norvell (1969). Korcak & Fanning (1978) also found that the DTPA extractable Zn decreased with liming of the soil.

4.8.3 Relationship between soil Zn and plant Zn

There are significant correlations for Zn in the soil as related to the plant Zn content (Table 35). Good correlations are obtained for lettuce uptake using NH_4-EDTA and DTPA extractable Zn but not with AB-DTPA extractable Zn. Lettuce uptake of Zn is also well correlated with NH_4-EDTA Zn if both soil pH levels are included. Further, wheat Zn concentration (mg/kg) at 11 t/ha $CaCO_3$ pH is well correlated with NH_4-EDTA and AB-DTPA Zn but not with DTPA Zn. The reason for the good correlation with lettuce uptake (μg/plant) could be because plant yield influenced the total Zn uptake by lettuce significantly.

Barbarick & Workman (1987) found significant correlations for Zn in wheat grains with DTPA and AB-DTPA extractable Zn in the soil, in a field trial. However, nonsignificant correlations with Zn in the straw of wheat was found. Roca & Pomares (1991) also found nonsignificant correlations with the edible leaves of lettuce in calcareous soils for DTPA, EDTA (pH 4,65) and AB-DTPA extractable soil Zn. They, however, did find significant correlations for these extractions with the wrapper leaves of lettuce. For very large applications of Zn to the soil, Mitchell *et al*, (1978) found significant correlations for Zn concentrations in lettuce and wheat leaves in relation to DTPA extractable Zn in the soil. It therefore seems as if correlations for plant content of Zn with soil Zn content, determined by some extractant, depends not only on the plant type but also the plant part used as well as the soil Zn levels.

Table 35. The significant ($P<0,05$) multiple linear regression equations for Zn concentration (mg/kg dry matter) and uptake (μg/plant) in the plants as related to the extractable Zn in the soil as determined by the different soil extractants.

Extractant	Plant type	$CaCO_3$ rate (t/ha)	Plant concentration (mg/kg) or uptake (μg/plant)	Regression equation[1]	R^2 value
NH_4-EDTA	Lettuce	2	Uptake	$Zn\mu gL2=34,60+83,87NH_4Zn$	$0,56^{***}$
		2 & 11	Concentration	$ZnmgL=159,86+4,72NH_4Zn-8,62pH$	$0,15^{*}$
			Uptake	$Zn\mu gL=1042,43+11,82NH_4Zn-157,31pH$	$0,76^{***}$
	Wheat	11	Concentration	$ZnmgW11=30,05+6,24NH_4Zn$	$0,54^{***}$
			Uptake	$Zn\mu gW11=76,27+16,07NH_4Zn$	$0,33^{**}$
	Bean pods	11	Concentration	$ZnmgB11=137,30-15,26NH_4Zn$	$0,22^{*}$
DTPA	Lettuce	2	Uptake	$Zn\mu gL2=86,85+103,69DTPAZn$	$0,72^{**}$
	Bean pods	11	Uptake	$Zn\mu gB11=32,72+18,79DTPAZn$	$0,40^{*}$
		2 & 11	Uptake	$Zn\mu gB=12,68+0,56DTPAZn$	$0,31^{*}$
AB-DTPA	Wheat	11	Concentration	$ZnmgW11=35,80+2,94ABZn$	$0,63^{*}$

* Significant at $P<0,05$
** Significant at $P<0,01$
*** Significant at $P<0,001$

[1] ZnmgL/ZnmgW/ZnmgB = Zn concentration (mg/kg dry matter) in lettuce, wheat and bean pods; ZnμgL/ZnμgW/ZnμgB = Total Zn uptake (μg/plant) by lettuce, wheat and bean pods; NH_4Zn = Zn in soil (mg/kg) determined by using the NH_4-EDTA extraction method; DTPAZn = Zn in the soil (mg/kg) determined by using the DTPA extraction method; ABZn = Zn in the soil (mg/kg) determined by using the AB-DTPA extraction method; pH = Soil pH after the experiment.

4.9 Comparison of metal extractabilities by the various extractants

When comparing the extractants with each other one aspect that should be noted is the influence of soil pH on the buffer capacity of the extractants. DTPA and AB-DTPA was developed for high pH soils while NH_4-EDTA was developed for low pH soils.

According to the results of Haynes & Swift (1983) and Adams & Kissel (1989) the buffer capacity of the NH_4-EDTA and DTPA could have been exceeded in this experiment, while the AB-DTPA extractant buffers the soil effectively. The results of this experiment confirms it, as discussed previously.

Both the solubility of micronutrients in the soil and the stability of micronutrient metal-chelate complexes can be highly pH-dependent (Haynes & Swift, 1983). Therefore, the extraction should be buffered successfully to eliminate variation as a result of the pH influence on the metal-chelate complexes. However, because of the importance of soil pH in determining micronutrient solubility the soil pH should be taken into account when predicting bioavailability. Salt extractions that function at the natural soil pH may, therefore, be more suitable for taking soil pH into account.

Significant regression equations between trace element extraction by the different soil extractants are given in Table 36 and 37 at the different pH levels.

Table 36. The best regression equations for NH_4-EDTA, DTPA, AB-DTPA and EPA method 3050 as related to each other for Ni, Co, Cd, Cu, Zn for both $CaCO_3$ application rates.

Extractants	Element	Regression equation[1]	R^2 value
EPA 3050 vs. NH_4-EDTA	Cu	EPACu=24,71+1,22NH_4Cu-1,26pH	$0,38^{***}$
	Zn	EPAZn=11,42+1,13NH_4Zn	$0,40^{***}$
EPA 3050 vs. DTPA	Cu	EPACu=17,02+2,45DTPACu	$0,44^{***}$
	Zn	EPAZn=5,18+1,71DTPAZn+1,06pH	$0,37^{***}$
EPA 3050 vs. AB-DTPA	Cu	EPACu=13,13+1,64ABCu	$0,48^{***}$
	Zn	EPAZn=4,91+0,82ABZn+1,05pH	$0,34^{***}$
DTPA vs. NH_4-EDTA	Ni	DTPANi=2,75+0,35NH_4Ni-0,38pH	$0,82^{***}$
	Cu	DTPACu=3,73+0,43NH_4Cu-0,54pH	$0,70^{***}$
	ZN	DTPAZn=2,92+0,58NH_4Zn-0,44pH	$0,88^{***}$
AB-DTPA vs. NH_4-EDTA	Ni	ABNi=5,36+0,96NH_4Ni-0,73pH	$0,69^{***}$
	Cd	ABCd=0,001+0,96NH_4Cd	$0,34^{***}$
	Cu	ABCu=6,68+0,73NH_4Cu-0,69pH	$0,75^{***}$
	Zn	ABZn=6,67+1,13NH_4Zn-0,89pH	$0,83^{***}$
DTPA vs. AB-DTPA	Ni	DTPANi=0,24+0,33ABNi	$0,57^{***}$
	Co	DTPACo=-0,37+0,94ABCo	$0,96^{***}$
	Cd	DTPACd=0,01+0,50ABCd	$0,19^{***}$
	Cu	DTPACu=-0,21+0,59ABCu-0,13pH	$0,91^{***}$
	Zn	DTPAZn=0,04+0,44ABZn	$0,76^{***}$

*** Significant at P<0,001

[1] EPACu/EPAZn = Cu and Zn in soil (mg/kg) determined by using EPA method 3050; NH_4Ni/NH_4Cd/NH_4Cu/NH_4Zn = Ni, Cd, Cu, and Zn in soil (mg/kg) determined by using the NH_4-EDTA extraction method; DTPANi/DTPACo/DTPACd/DTPACu/DTPAZn = Ni, Co, Cd, Cu, and Zn in soil (mg/kg) determined by using the DTPA extraction method; ABNi/ABCo/ABCd/ABCu/ABZn = Ni, Co, Cd, Cu, and Zn in soil (mg/kg) determined by using the AB-DTPA extraction method; pH = Soil pH after experiment.

Table 37. The best regression equations for NH$_4$-EDTA, DTPA, AB-DTPA and EPA method 3050 as related to each other for Ni, Co, Cd, Cu, ZN at the two application rates of CaCO$_3$ (2 and 11 t/ha).

Extractants	Element	CaCO$_3$ rate (t/ha)	Regression equation[1]	R^2 value
EPA 3050 vs. NH$_4$-EDTA	Cu	2	EPACu2=17,10+1,52NH$_4$Cu2	0,48***
		11	EPACu11=17,35+1,02NH$_4$Cu11	0,29**
	Zn	11	EPAZn11=11,34+1,20NH$_4$Zn11	0,56***
EPA 3050 vs. DTPA	Cu	2	EPACu2=-22,19+2,33DTPACu2+8,50pH	0,54***
		11	EPACu11=16,86+2,53DTPACu11	0,39***
	Zn	11	EPAZn11=11,94+1,93DTPAZn11	0,52***
EPA 3050 vs. AB-DTPA	Cu	2	EPACu2=-25,34+1,35ABCu2+8,80pH	0,53***
		11	EPACu11=10,77+1,95ABCu11	0,52***
	Zn	2	EPAZn2=11,82+0,52ABZn2	0,14*
		11	EPAZn11=11,40+0,94ABZn11	0,43***
DTPA vs. NH$_4$-EDTA	Ni	2	DTPANi2=1,02+0,35NH$_4$Ni2	0,17*
		11	DTPANi11=0,24+0,30NH$_4$Ni11	0,38***
	Cd	2	DTPACd2=0,01+0,92NH$_4$Cd2	0,15*
	Cu	2	DTPACu2=0,60+0,53NH$_4$Cu2	0,78***
		11	DTPACu11=10,17+0,33NH$_4$Cu11-1,37pH	0,62***
	Zn	2	DTPAZn2=-8,02+0,67NH$_4$Zn2+1,85pH	0,86***
		11	DTPAZn11+-0,12+0,58NH$_4$Zn11	0,91***
AB-DTPA vs. NH$_4$-EDTA	Ni	2	ABNi2=1,24+1,30NH$_4$Ni2	0,26**
	Co	11	ABCo11=2,65-0,10NH$_4$Co11	0,14*
	Cd	2	ABCd2=0,00+1,13NH$_4$Cd2	0,37***
		11	ABCd11=0,00+0,88NH$_4$Cd11	0,38***
	Cu	2	ABCu2=2,19+0,94NH$_4$Cu2	0,85***
		11	ABCu11=3,04+0,57NH$_4$Cu11	0,66***
	Zn	2	ABZn2=1,13+1,53NH$_4$Zn2	0,74***
		11	ABZn11=0,82+1,06NH$_4$Zn11	0,90***
DTPA vs. AB-DTPA	Co	2	DTPACo2=-7,90+0,96ABCo2+1,59pH	0,79***
		11	DTPACo11=-0,05+0,74ABCo11	0,71***
	Cd	2	DTPACd2=0,01+0,55ABCd2	0,19*
		11	DTPACd11=0,01+0,41ABCd11	0,16*
	Cu	2	DTPACu2=-0,65+0,56ABCu2	0,92***
		11	DTPACu11=-1,42+0,63ABCu11	0,88***
	Zn	2	DTPAZn2=-8,54+0,33ABZn2+2,00pH	0,67***
		11	DTPAZn11=-0,29+0,49ABZn11	0,82***

* Significant at $P<0,05$

** Significant at $P<0,01$

*** Significant at $P<0,001$

[1] EPACu2/EPAZn2/EPACu11/EPAZn11 = Cu and Zn in soil (mg/kg) determined by using EPA method 3050 for the two different CaCO$_3$ application rates; NH$_4$Ni2/NH$_4$Cd2/NH$_4$Cu2/NH$_4$Zn2/NH$_4$Ni11/NH$_4$Co11/NH$_4$Cd11/NH$_4$Cu11/ NH$_4$Zn11 = Ni, Co, Cd, Cu, and Zn in soil (mg/kg) determined by using the NH$_4$-EDTA extraction method for the two CaCO$_3$ application rate; DTPANi2/DTPACo2/DTPACd2/DTPACu2/DTPAZn2/DTPANi11/DTPACo11/DTPACd11/ DTPACu11/DTPAZn11 = Ni, Co, Cd, Cu, and Zn in soil (mg/kg) determined by using the DTPA extraction method for the two CaCO$_3$ application rates; ABNi2/ABCo2/ABCd2/ABCu2/ABZn2/ABNi11/ABCo11/ABCd11/ABCu11/ABZn11= Ni, Co, Cd, Cu, and Zn in soil (mg/kg) determined by using the AB-DTPA extraction method for the two CaCO$_3$ application rates; pH = Soil pH after the experiment.

For Ni the regression equations between the extractants are good, excluding EPA method 3050, with the inclusion of both soil pH levels. At the two soil pH levels individually they are however not significantly or poorly correlated. This tendency is very much the same as for the regression equations between the extractable soil Ni and total plant Ni uptake and the reason for this should be the same, as discussed previously.

DTPA and AB-DTPA extractable soil Co are basically the only two that are well related to each other. The Co form extracted by EDTA from the soil is, therefore, different from that of DTPA.

There are no really good correlations between extractable Cd as extracted by the different extractants. AB-DTPA-Cd and NH_4-EDTA-Cd are, however, correlated the best. The Cd concentration of the soil is very low and it is therefore difficult to obtain good correlations.

Extractable Cu and Zn are well correlated between most of the extractants. The Cu and Zn form extracted by the different extractants from the soil is therefore probably the same.

Soil pH significantly improves the regression equation for metals extractable with NH_4-EDTA as related to DTPA and AB-DTPA if both pH levels are included, with the exception of Cd. This once again indicates that the buffer capacity of the NH_4-EDTA was exceeded causing significant differences in trace element extraction at the different soil pH levels.

Hornburg & Bruemmer (1993) found good correlations between Na_2EDTA (pH 4,6) and DTPA extractable Cd, Cu, and Zn in soils with varying composition. In this experiment the NH_4-EDTA and DTPA Cd was, however, not as well correlated. Barbarick & Workman (1989) also obtained good correlations between DTPA and AB-DTPA extractable Cd, Cu, Ni, and Zn in a pot trial at high (8,0) and low (5,3) soil pH as well as in a field trial at a more uniform soil pH (6,5-7,8). Roca & Pomares (1991) also found good correlations for EDTA (pH 4,65) and AB-DTPA as related to DTPA extractable Ni, Cd, Cu, and Zn in calcareous soils. These correlations are far better than those obtained in this experiment.

Sims *et al,* (1991) found very similar results as in this experiment for DTPA extractable Ni, Cd, Cu, and Zn as related to EPA method 3050. They found that there are nonsignificant correlations in the cases of Cd and Ni but significant correlations with Cu and Zn although that of Zn is not that good.

4.10 Growth cabinet vs. glasshouse

From the results of the experiment conducted in a growth cabinet it appears as if there is no contamination of Ni, Cd, Co, Cu, or Zn in the glasshouse from the atmosphere. There was, however, the possible contamination during the soil preparation of one treatment and its replicates, as discussed previously. The differences that did occur between the growth cabinet and glasshouse is probably as a result of the differences in growth conditions between the two environments (Tables 38 to 41).

The yield is better in the growth cabinet if compared with that in the glasshouse. Some of the differences in plant concentration of trace elements can, therefore, be as a result of the difference in yield.

With the NH_4-EDTA extraction it is only Cu and Zn that showed differences between the places where the plants were grown. For Cu there is an increase in Cu extracted from the soil in the glasshouse pots over that of the growth cabinet for both the 2 t/ha $CaCO_3$ treatments. For Zn, however, the 11 t/ha $CaCO_3$ application is lower in the glasshouse than in the growth cabinet. These differences could be due to the higher temperatures in the glasshouse as compared to the growth cabinet. The higher temperatures cause the organic material to decompose quicker and more completely, allowing the Cu to be more available for uptake and extraction. At the 11 t/ha $CaCO_3$ application higher pH renders the Zn more unavailable.

Table 38. Mean trace element (Ni, Co, Cd, Cu, Zn) concentration (mg/kg dry matter) of wheat grown in a glasshouse and growth cabinet for three different treatments.

Place	Treatment	Element[2]				
		Ni	Co	Cd	Cu	Zn
In growth cabinet	1	4,53 ab[1]	8,14 ac	0,19 a	13,57 a	70,94 a
	2	3,67 ab	6,24 ac	0,22 a	14,09 a	81,40 a
	3	1,63 a	0,72 b	0,23 a	14,95 a	60,72 a
In glasshouse	1	4,17 ab	4,78 c	0,08 a	12,96 a	64,09 a
	2	6,14 b	13,40 a	0,24 a	20,45 a	65,39 a
	3	1,12 a	1,04 b	0,13 a	16,89 a	62,18 a

Treatment 1 = 0 t/ha OSA 20 + 2 t/ha $CaCO_3$
Treatment 2 = 20 t/ha OSA 20 + 2 t/ha $CaCO_3$
Treatment 3 = 20 t/ha OSA 20 + 11 t/ha $CaCO_3$

[1] Value followed by the same symbol in the column do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 4 replicates.

Table 39. Mean total trace element (Ni, Co, Cd, Cu, Zn) uptake per plant (μg/plant) by wheat in a glasshouse and growth cabinet for three different treatments.

Place	Treatment	Element[2]				
		Ni	Co	Cd	Cu	Zn
In growth cabinet	1	18,23 a[1]	32,63 a	0,76 ac	54,92 a	387,26 a
	2	13,64 ab	23,07 a	0,81 a	52,05 a	298,32 a
	3	4,48 b	1,97 b	0,62 ac	40,71 a	165,87 b
In glasshouse	1	10,23 ab	11,86 c	0,20 bc	32,25 a	159,94 b
	2	17,49 ab	37,62 a	0,68 ac	58,78 a	185,78 b
	3	3,01 b	2,56 b	0,32 ac	42,72 a	160,34 b

Treatment 1 = 0 t/ha OSA 20 + 2 t/ha $CaCO_3$
Treatment 2 = 20 t/ha OSA 20 + 2 t/ha $CaCO_3$
Treatment 3 = 20 t/ha OSA 20 + 11 t/ha $CaCO_3$

[1] Value followed by the same symbol in the column do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 4 replicates.

Table 40. Mean trace element (Ni, Co, Cd, Cu, Zn) concentration (mg/kg soil) in soil extracted with NH_4-EDTA, for wheat grown in a glasshouse and growth cabinet with three different treatments.

Place	Treatment	Element[2]				
		Ni	Co	Cd	Cu	Zn
In growth cabinet	1	1,92 a[1]	11,52 a	0,01 b	3,71 a	1,90 a
	2	2,27 ab	10,60 ab	0,02 a	6,73 b	5,14 b
	3	1,87 a	8,94 b	0,02 a	8,48 c	5,66 b
In glasshouse	1	2,08 ab	11,76 a	0,01 b	5,09 d	2,03 a
	2	2,39 b	10,14 ab	0,02 a	8,51 c	5,39 b
	3	1,82 a	9,98 ab	0,02 a	9,11 c	4,60 c

Treatment 1 = 0 t/ha OSA 20 + 2 t/ha $CaCO_3$
Treatment 2 = 20 t/ha OSA 20 + 2 t/ha $CaCO_3$
Treatment 3 = 20 t/ha OSA 20 + 11 t/ha $CaCO_3$

[1] Value followed by the same symbol in the column do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 4 replicates.

Table 41. Mean yield and soil pH for wheat grown in a glasshouse and growth cabinet for three different treatments.

Place	Treatment	pH[2]	yield[2]
In growth cabinet	1	4,80 a[1]	4,05 a
	2	5,11 b	3,65 a
	3	6,84 c	2,74 b
In glasshouse	1	4,60 d	2,53 b
	2	4,64 e	2,84 b
	3	6,85 c	2,54 b

Treatment 1 = 0 t/ha OSA 20 + 2 t/ha $CaCO_3$
Treatment 2 = 20 t/ha OSA 20 + 2 t/ha $CaCO_3$
Treatment 3 = 20 t/ha OSA 20 + 11 t/ha $CaCO_3$

[1] Value followed by the same symbol in the column do not differ significantly at $P<0,05$ according to Duncan's multiple range test.

[2] Mean of 4 replicates.

CHAPTER 5

CONCLUSIONS

The application of OSAs in this experiment did not cause detrimental effects to the environment for the five trace elements studied. In many cases there were even a decrease in plant concentration of heavy metals. It must, however, be kept in mind that only one application of each OSA was made at moderate rates of application. Therefore it can safely be said that the OSAs used will not cause environmental problems, due to trace element pollution, if used in moderation. However, there may be problems due to the build-up of trace elements in the soil caused by the repeated application of the OSAs. This can, however, be regulated so that the benefits of OSA application can be reaped without causing detrimental effects on the environment.

Simplified assumptions concerning which plants or plant parts absorb the most trace elements cannot be made. It rather depends on the species of plant and on the metal concerned. In this experiment the lettuce plants accumulated the most Co, Cd, and Zn while the wheat accumulated the most Cu and the bean pods the most Ni.

The soil pH plays a very important role in the plant bioavailability and soil extractability of trace elements. An increase in soil pH mostly caused the plant concentration (mg/kg) of trace elements to decrease. There are, however, some exceptions. The Cd and Cu concentration of wheat were not influenced by $CaCO_3$ application, while for Zn the lettuce and bean pods concentrations were not influenced by $CaCO_3$ application rates. Generalizations, concerning the plant concentration as influenced by soil pH should therefore be made with caution, taking into account the complete plant-soil-metal system.

The influence of $CaCO_3$ application rate on the soil extractability of trace elements could be due to one of three factors or a combination of factors: (1) The increased Ca^{2+} content of the soil results in competition for the complexing sites of the extractant and consequently less Ni is extracted; (2) the buffer capacity of the extractant could be exceeded and the pH of the extracting solution may be increased or decreased causing changes in the stability constants of Ni complexes; (3) the Ni may be precipitated or may be sorbed more by the soil components at the higher soil pH (Harter, 1983; Bruemmer *et al*, 1988). The buffer capacity of the NH_4-EDTA and DTPA was probably exceeded and this caused the metal-chelate complexes to be influenced. The Cu and Zn extractability by NH_4-EDTA did not decrease with increased $CaCO_3$ application rate and this is probably due to the buffer capacity of the NH_4-EDTA that was exceeded. The Cd extractability by all the extractants was not influenced by $CaCO_3$ application rate probably because the Cd is very soluble at the pH levels of this experiment (Hornburg & Bruemmer, 1993). However, in general the trace element extractability decreased with increased $CaCO_3$ application rate for NH_4-EDTA, DTPA and AB-DTPA. The most probable reason for the decrease in metal extractability with increased $CaCO_3$ application rate is that the metal solubilities decrease due to precipitation and sorption at the high soil pH. Competition with the trace elements for the complexing sites of the extractants by Ca^{2+} probably does not play an important role in decreasing the extractability with increased $CaCO_3$ application. AB-DTPA was developed to prevent the competition with Ca^{2+} (Soltanpour, 1985) and therefore the decrease should be as a result of the decreased solubility of the metals due to the rise in soil pH.

EPA method 3050 could not predict bioavailability of trace elements to plants well nor the loading of the soil by trace elements. The reason for this is that there was poor duplication of results. This variation could be as a result of poor sample homogeneity. The samples should have been pulverized before analysis by this method. The method is further very laborious to conduct and not suitable for routine analysis. The other extractants predict soil metal loading very well even with very small increases in metal content of the soil. Good predictions of plant bioavailability was also obtained for certain extractant, metal and plant combinations but not in as many cases as expected. Of the metals the best correlations were obtained between Co plant content and soil extractability. Ni correlations were influenced substantially by soil pH while for Cd, Cu, and Zn poor correlations were obtained.

Legislation for the maximum permissible trace element content in environmental samples is not always in agreement with metal levels normally found for environmental samples. For Ni the maximum permissible total Ni concentration in the soil for South Africa is lower than the average total Ni concentration of the soil given by many authors (Kabata-Pendias & Pendias, 1992). Therefore, the question is raised whether the maximum permissible levels are not too strict. The same situation arises for Cd where the average concentration in plants are higher than the maximum permissible Cd concentration in foodstuffs for many countries. Some research is therefore needed to set safe, but not unnecessarily strict, legislation. To be able to do this, knowledge of South Africa's background levels for trace elements is necessary, as a basis to work from.

Due to the limitations of pot studies it is important to conduct long-term field studies to determine the actual situation in practice. It is further important to study interactions between trace elements and how they influence the pollution danger of a specific trace element. A holistic approach to trace element pollution is therefore needed.

CHAPTER 6

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APPENDIX 1

LEGISLATION

1.1 South Africa

In South Africa there is no single regulation for the control of all the aspects of the disposal of sludge and organic wastes in general. It is, however, very clear that all the necessary provisions are available to make regulations for its control (Oberholster, 1983). The government prefers to initiate control by means of guidelines or norms, which can easily be reviewed and amended as experience is gained and also provision can be made for *ad hoc* decisions where necessary, without being hampered by rigid statutory provisions. These guidelines are then slowly developed to become more and more comprehensive, to finally be promulgated as regulations (Oberholster, 1983).

Although there is no single regulation that controls all the aspects of the disposal of sewage sludge there are currently five sets of legislation which impact on sewage sludge application on agricultural land.

-Act 36 of 1947: Fertilizers Registration Act - Dept. of Agriculture

-Act 43 of 1983: Conservation of Agricultural Resources Act - Dept. of Agriculture

-Act 73 of 1989: Environment Conservation Act - Dept. of Water Affairs and Forestry

-Act 54 of 1956: Water Act - Dept. of Water Affairs and Forestry

-Act 63 of 1977: Health Act with its associated sludge guidelines (A11/2/5/4, 2nd Draft), Dec. 1991 - Dept. of National Health and Population Development.

At present the only guidelines for the use of sewage sludge in South Africa is given by the Dept. of National Health and Population Development and will be discussed further. However, the legislation is being co-ordinated to supply the users and suppliers with one document for the safe use and disposal of sewage sludge. The guidelines given here will therefore be changed to some extent in the not to distant future.

Sewage sludge is basically classified into 3 types, namely A, B and C, in decreasing order of its potential to cause odour nuisances and fly-breeding as well as to transmit pathogenic organisms to man and his environment.

A fourth type of sludge, a type D sludge, is also classified for unrestricted use on land at a maximum application rate of 8 dry t/ha/yr. A type D sludge is a sludge that is certified to comply with the following quality requirements:

(i) Stabilised - should not cause odour nuisances or fly-breeding

(ii) Contains no viable Ascaris ova per 10 g dry sludge

(iii) Maximum of 0 Salmonella organisms per 10 g dry sludge

(iv) Maximum of 1000 Faecal Coliforms per 10 g dry sludge, immediately after treatment (disinfection/sterilisation).

Maximum metal and inorganic content in mg/kg dry sludge must further be less than that given in Table 1. The user must also be informed about the moisture and N P K content and warned that no more than 8 t/ha/yr dry sludge may be applied to the soil and that the pH of the soil should preferably be higher than 6.

The maximum permissible metal and inorganic content in soil is also limited by these guidelines. There are also limits set for the maximum amount of metal and inorganic contaminants that can be applied to soil in a year and in 25 years (Table 2).

1.2 **USA (EPA)** (Environmental Protection Agency, 1993)

In USA the EPA (Environmental Protection Agency) set limits (or equations for calculating these limits) for 10 pollutant elements when sewage sludge is used or disposed of by one or more methods. The EPA developed these numerical limits by using exposure assessment models designed to protect individuals, plants, animals or other organisms potentially at greater risk from pollutants in sewage sludge. The numerical limits derived from the exposure assessment models are designed to protect public health or the environment from reasonably anticipated adverse effects.

In determining the limits for the pollutants in sewage sludge certain assumptions were made. The American estimated national median background concentrations for inorganic pollutants in soil was used in the models. For organic pollutants the agency decided to use a zero background concentration level in the models. Further

Table 1. Maximum metal and inorganic content in mg/kg dry sludge for a sludge to be classified as a Type D sludge in South Africa.

Element	**Maximum metal and inorganic content in sewage sludge**
	— mg/kg dry sludge —
Cd	20
Co	100
Cr	1750
Cu	750
Hg	10
Mo	25
Ni	200
Pb	400
Zn	2750
As	15
Se	15
B	80
F	400

Table 2. Maximum total soil metal and inorganic content and maximum permissible application amounts thereof in South Africa.

Element	Maximum permissible metal and inorganic content in soil	Maximum amount of metal and inorganic contaminants that can be applied to soil	
	—mg/kg—	kg/ha/25 yr	g/ha/yr—
Cd	2	4	160
Co	20	20	800
Cr	80	350	14000
Cu	100	150	6000
Hg	0,5	2	80
Mo	2,3	5	200
Ni	15	40	1600
Pb	56	80	3200
Zn	185	550	22000
As	2	3	120
Se	2	3	120
B	10	16	640
F	50	80	3200

it was considered that the sewage sludge is only incorporated into the top 15 cm of the soil and 1330 kg/m^3 soil bulk density was assumed.

Site-specific parameters are not included in the legislation. For example no limit for soil pH is set. They argue that data from low pH studies were also used in the exposure assessment models. The EPA therefore believes that the numerical limits protect a majority of U.S. soil conditions without requiring pH control for all agricultural land practices.

The final standards set by the EPA consists of general requirements, pollutant limits, management practices, operational standards, and requirements that address frequency of monitoring, record keeping, and reporting. The standards can very shortly be summarized as follows (more specifically for agricultural soil and heavy metals):

(i) No sewage sludge shall be applied to land if the concentration of any pollutant in the sewage sludge exceeds the ceiling concentration for the pollutants, given in Table 3.

(ii) For the use on agricultural land more specifically the following applies:

(a) The cumulative loading rates for each pollutant shall not exceed the limits given in Table 4.

(b) The concentration of each pollutant in the sewage sludge shall not exceed the concentration given in Table 5.

(c) The product of the concentration of each pollutant in the sewage sludge and the annual whole sludge application rate for the sewage sludge shall not cause the rates given in Table 6 to be exceeded.

(iii) Sewage sludge may be applied to agricultural land without limitations if certain standards are met with regards to the sludge. The pollutant concentrations in Table 5 must be met. Either the density of Fecal Coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of *Salmonella sp.* bacteria in the sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed. Further, the temperature and/or pH of the sewage sludge shall be maintained at a specific value for a period of time or the density of enteric viruses in the sewage sludge prior to pathogen treatment is less than one Plaque-forming Unit per four grams of total solids (dry weight basis) and the density of viable helminth ova in the sewage sludge prior to pathogen treatment should be less than one per four grams of total solids (dry weight basis). Further, certain vector attraction reduction requirements must be met. These are requirements that reduce the characteristics of sewage sludge that reduces the attraction of rodents, flies, mosquitoes, or other organisms capable of transporting infectious agents by the sewage sludge. If these standards are not met certain general requirements and management practices apply for the use of sewage sludge on agricultural soil.

Table 3. Ceiling concentration for pollutants in sewage sludge in the USA (Environmental Protection Agency, 1993).

Element	Ceiling concentration
	— mg/kg dry sludge —
As	75
Cd	85
Cr	3000
Cu	4300
Pb	840
Hg	57
Mo	75
Ni	420
Se	100
Zn	7500

Table 4. Cumulative pollutant loading rates for the addition of sewage sludge to agricultural land in the USA (Environmental Protection Agency, 1993) .

Element	Cumulative pollutant loading rates
	——kg/ha——
As	41
Cd	39
Cr	3000
Cu	1500
Pb	300
Hg	17
Mo	18
Ni	420
Se	100
Zn	2800

Table 5. Maximum permissible pollutant concentration in sewage sludge for agricultural use (Environmental Protection Agency, 1993).

Element	Average concentration
	–mg/kg dry sludge–
As	41
Cd	39
Cr	1200
Cu	1500
Pb	300
Hg	17
Mo	18
Ni	420
Se	36
Zn	2800

Table 6. Maximum annual application rate of pollutants by the application of sewage sludge on agricultural soil (Environmental Protection Agency, 1993).

Element	Annual pollutant application rate
	kg/ha/365 day period
As	2,0
Cd	1,9
Cr	150
Cu	75
Pb	15
Hg	0,85
Mo	0,9
Ni	21
Se	5,0
Zn	140

1.3 **United Kingdom** (Omex Environmental Ltd, 1990)

A short summary of the main points in the code of practice for the use of sewage sludge in agriculture in the United Kingdom follows.

(i) In the United Kingdom the soil should be analyzed (for pH, Zn, Cu, Ni, Cd, Pb, Hg, Cr, Mo, Se, As, F) before the first application of sewage sludge on agricultural land and at least every twentieth year while sludge is being used.

(ii) Except for dedicated sites the potentially toxic element (PTE) concentrations in arable soils must not exceed the limits given in Table 7, and that for surface applications to grasslands the limits given in Table 8, and no sludge should be applied to any site where the soil concentration of any of the elements is at or above these values. Exceptions can be made for soils containing naturally high Mo values but then only in accordance with expert advice.

(iii) The sewage sludge must also be analyzed (for dry matter %, organic matter %, pH, total N, NH_4-N, Total P, Zn, Cu, Ni, Cd, Pb, Hg, Cr, Mo, Se, As, F) at least every 6 months and every time significant changes occur in the quality of the sewage treated.

(iv) The 10 year average annual rates of application of these elements in sludge must not exceed those set out in Table 7. This value is determined by averaging over the 10 year period ending with the year of calculation.

(v) Except for applications to grassland no limits have been set for PTE concentrations in sludge used in agriculture. Sludge to be surface applied to grassland should not contain lead or fluoride in excess of 1200 or 1000 mg/kg dry solids, respectively.

(vi) Sludge must not be applied to land which is managed with a pH of less than 5,0. For soils where the pH is less than 5,2 specialist agricultural advice should be sought before making sludge applications.

(vii) To minimise the risk, sludge applications must be co-ordinated in time with planting, grazing or harvesting operations. The acceptable use of sewage sludge is also controlled. Other environmental protection measures such as odour control and water pollution control are also given in the code of practice.

Table 7. Maximum permissible concentrations of potentially toxic elements in soil after application of sewage sludge and maximum annual rates of addition (Omex Environmental Ltd, 1990).

POTENTIALLY TOXIC ELEMENTS (PTE)	MAXIMUM PERMISSIBLE CONCENTRATION OF PTE IN SOIL (mg/kg dry solids)				Maximum permissible average annual rate of PTE addition over a 10 year period (kg/ha)
	pH 5,0<5,5	pH 5,5<6,0	pH 6,0-7,0	pH >7,0	
Zn	200	250	300	450	15
Cu	80	100	135	200	7,5
Ni	50	60	75	110	3
	For pH 5,0 and above				
Cd	3				0,15
Pb	300				15
Hg	1				0,1
Cr	400(Provisional)				15(Provisional)
Mo	4				0,2
Se	3				0,15
As	50				0,7
F	500				20

Table 8. Maximum permissible concentrations of potentially toxic elements in soil under grass after application of sewage sludge when samples taken to a depth of 7,5cm (Omex Environmental Ltd, 1990).

Potentially toxic elements (PTE)	**Maximum permissible concentration of PTE in soil (mg/kg dry solids)**			
	pH 5,0<5,5	**pH 5,5<6,0**	**pH 6,0-7,0**	**pH >7,0**
Zn	330	420	500	750
Cu	130	170	225	330
Ni	80	100	125	180
	For pH 5,0 and above			
Cd	3			
Pb	300			
Hg	1,5			
Cr	600(Provisional)			
Mo	4			
Se	5			
As	50			
F	500			

1.4 **Germany** (Töpfer, 1992)

The German legislation is very strict and contrary to the American legislation takes into account soil science aspects. Some of the directives in the German law are:

(i) The heavy metal content (total Cd, Pb, Hg, Cr, Ni, Cu, Zn) of the sewage sludge must be analyzed. This analysis must be done every 6 months or sooner as determined by the responsible authorities.

(ii) The heavy metal content may not exceed the statutory values given in Table 9. Furthermore, if the application is to be done on a light textured soil with less than 5% clay or a soil $pH(CaCl_2)$ between 5 and 6, then the Cd content must be below 5 mg/kg and the Zn content below 2000 mg/kg.

(iii) The soil heavy metal content (total Cd, Pb, Hg, Cr, Ni, Cu, Zn) must be determined before the first addition of sewage sludge takes place and then at least every 10 years or less as determined by the responsible authorities.

Table 9. Maximum permissible concentrations of heavy metals in sewage sludge (Töpfer, 1992).

Element	Maximum permissible concentration
	–mg/kg dry sludge–
Pb	900
Cd	10
Cr	900
Cu	800
Ni	200
Hg	8
Zn	2500

Table 10. Maximum permissible concentrations of heavy metals in soil for the application of sewage sludge (Töpfer, 1992).

Element	Maximum permissible concentration mg/kg dry soil
	—mg/kg dry soil—
Pb	100
Cd	1,5
Cr	100
Cu	60
Ni	50
Hg	1
Zn	200

(iv) The statutory values in Table 10 for soil may not be exceeded. If the application is to take place on a soil with less than 5 % clay or a soil pH ($CaCl_2$) between 5 and 6 then the Cd in the soil must be less than 1 mg/kg and the Zn less than 200 mg/kg.

(v) The sewage sludge must also be analyzed for total N and NH_4-N, P, K, Mg, dry residue, organic carbon, total content of bases and pH.

(vi) No more than 5 tons dry mass per ha in 3 years (1,67 t.dr.m./ha/year) of sewage sludge may be added to the soil. If the toxic element content of the sewage sludge is less than half of that permitted in Table 9, then 10 tons dry material per ha in 3 years may be added to the soil.

(vii) The soil pH and plant available P, K and Mg in the sewage sludge must be determined. Addition of sewage sludge is not permitted if the soil pH($CaCl_2$) is below 5,0.

(viii) The amount, method and time of addition must be adjusted according to the plant requirements, soil type and general environmental conditions of every specific case.

(ix) Raw sewage sludge may not be added to soil.

(x) Fruit and vegetables may not receive sewage sludge additions. The last addition of sewage sludge must have been made at least 2 years before planting.

(xi) Forage crops may only receive sewage sludge before planting.

(xii) No sewage sludge may be added to grasslands, forests, or protected areas.

1.5 Summary of legislations and guidelines

Legislation and guidelines pertaining to trace elements and the addition of sewage sludge to agricultural soil can be summarized into two tables, one for sewage sludge content (Table 11) and another for the soil content (Table 12).

Table 11. Maximum permissible contaminant concentrations (mg/kg, dry basis) in sewage sludges for application to agricultural land given by different countries (Department of National Health and Population Development, 1991; Töpfer, 1992; Smith & Vasiloudis, 1991).

COUNTRY	Cd	Cr	Cu	Pb	Ni	Zn	Hg	As	Se	Co	F	Mo
	mg/kg, dry bais											
SOUTH AFRICA	20	1750	750	400	200	2750	10	15	15	100	400	25
USA	39	1200	1500	300	420	2800	17	41	36	-	-	18
BELGIUM	10	500	500	300	100	2000	10	10	25	-	-	-
DENMARK	8	-	-	400	30	-	6	-	-	-	-	-
FINLAND	30	1000	3000	1200	500	5000	25	-	-	-	-	-
FRANCE	20	1000	1000	800	200	3000	10	-	100	-	-	-
GERMANY	10	900	800	900	200	2500	8	-	-	-	-	-
NETHERLANDS	10	500	600	500	100	2000	10	10	-	-	-	-
NORWAY	10	200	1500	300	100	3000	7	-	-	-	-	-
SWEDEN	15	1000	3000	300	500	10000	8	-	-	-	-	-
SWITZERLAND	30	1000	1000	1000	200	3000	10	-	-	-	-	20
CANADA*	20	-	-	500	180	1850	5	75	14	-	-	20
EEC-RECOMMENDED	20	750	1000	750	300	2500	16	-	-	-	-	-
EEC-MANDATORY	40	-	1500	1000	400	3000	-	-	-	-	-	-
LOWEST RECOMMENDED VALUE	8	200	500	300	30	1850	5	10	14	100	400	20
HIGHEST RECOMMENDED VALUE	40	1200	3000	1200	500	10000	25	75	100	100	400	20
MEAN OF RECOMMENDED VALUES	19	891	1332	588	233	3367	11	30	38	100	400	20
MEDIAN RECOMMENDED VALUE	20	1000	1000	500	200	2775	10	15	25	100	400	20

*CANADA: Values apply to sludges and sludge-based commercial products containing < 5% nitrogen.

Table 12. Maximum permissible soil concentrations in South Africa, Germany, and the U.K.

Element	South Africa[1]	Germany[2]	United Kingdom[3]
	———mg/kg dry soil———		
Cd	2	1,5	3
Co	20	-	-
Cr	80	100	400 (Provisional)
Cu	100	60	135 (pH 6,0-7,0)
Hg	0,5	1	1
Mo	2,3	-	4
Ni	15	50	75 (pH 6,0-7,0)
Pb	56	100	300
Zn	185	200	300 (pH 6,0-7,0)
As	2	20	50
Se	2	5	3
B	10	25	-
F	50	200	500
Tl	-	1	-
U	-	5	-
CN^-	-	5	-

[1] Department of National Health and Population Development (1991)

[2] Schachtschabel, Blume, Bruemmer, Hartge & Schwertmann (1989) and Töpfer (1992).

[3] Omex Environmental Ltd (1990)

1.6 Extractable vs. total elemental content

All the above-mentioned legislation or guidelines were set using the total content of trace elements in the soil determined by using acid digestion methods. Bruemmer and Van der Merwe (1989) suggest the use of NH_4-EDTA in legislation to determine the maximum permissible trace element concentration in the soil.

According to Bruemmer and van der Merwe (1989) the amount of NH_4-EDTA-extractable heavy metals gives satisfactory estimates of the total pool of potentially available heavy metals. This pool comprises those fractions which can deliver metals from solid phases to the soil solution, i.e. the non-silicate bound fractions. They therefore suggest that the NH_4-EDTA extractable fraction of potentially available metals in soils is the best basis for preliminary threshold values for heavy metals. Table 13 gives these threshold values.

Table 13. The suggested preliminary threshold values for NH_4-EDTA (pH 4,5) extractable heavy metals for the soils of South Africa.

Element	NH_4-EDTA extractable
	–mg/kg soil
Cd	1
Co	10
Cr	50
Cu	60
Hg	1
Ni	20
Pb	100
Zn	100

Further investigation should take place to improve these values and to incorporate other elements.

The mobility and availability of the heavy metals in soils depends to a high degree on soil pH. In Table 14 pH values are listed for different elements, below which an increased mobility and availability of these elements can be expected. Therefore, the suggested threshold values for the different heavy metals in soils (Table 13) can only be used as limits for soil pollution if the pH values of the soils are not below the values listed in Table 14 (Bruemmer & Van der Merwe, 1989).

Table 14. Mobility of different metals in soils in relation to soil pH.

Metal	Increasing mobility below pH ($CaCl_2$)
Cd	6,0-6,5
Mn	5,5
Ni	5,5
Co	5,5
Zn	5,5
Al	4,5
Cu	4,5
Cr(III)	4,0-4,5
Pb	4,0
Hg	4,0
Fe(III)	3,5

The NH_4-EDTA extraction, to some extent, takes into account the influence soil type has on the availability of trace elements to plants. Therefore, threshold values based on NH_4-EDTA extractable heavy metals, to some extent, takes soil variability into account.

APPENDIX 2

EXTRACTION AND DIGESTION METHODS

1 Digestion of plant material (7:3 HNO_3:$HClO_4$ digestion)[1]

1. Weigh 1 g (accurate to 0,1 mg) prepared plant material into a digestion tube (280 mm x 20 mm).
2. Add 7 cm^3 concentrated HNO_3 and 3 cm^3 $HClO_4$ (70%).
3. Mix thoroughly until all the material is damp.
4. Leave overnight in a fume cupboard.
5. Digest in a Technicon digestion block. Begin at 50 °C for 15 minutes; 100 °C for 15 minutes; 150 °C for 15 minutes; 200 °C for 15 minutes. Brown fumes disappear and heavy, dens, white fumes appear.
6. Add slowly 30 % H_2O_2 until fumes are white.
7. Heat for a further 20 minutes till the white fumes of H_2O_2 disappear.
8. Leave to cool.
9. While still warm add 5 cm^3 HNO_3 (1:1).
10. Wash over into a 100 cm^3 volumetric flask.
11. Fill to the mark with distilled water.

2. NH_4-EDTA extraction (The Non-Affiliated Soil Analysis Work Committee, 1990)

1. Place 10 g air-dry soil in an extraction bottle.
2. Add 30 cm^3 0,02 mol.dm^{-3} $(NH_4)_2EDTA$ solution to the soil.
3. Shake horizontally for 60 minutes at 180 cycles per minute at a constant temperature of 20 ± 2 °C.
4. Filter immediately through Whatman No. 40 paper into suitable containers.

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[1] Loock, A. 1992. Personal communication)

3. **EPA Method 3050 acid digestion of sediments, sludges, and soils** (EPA, 1986)

1. Mix the sample thoroughly to achieve homogeneity. For each digestion procedure, weigh to the nearest 0,01 g and transfer to a conical beaker 1,00-2,00 g of sample.
2. Add 10 ml of 1:1 HNO_3, mix the slurry, and cover with a watch glass. Heat the sample to 95 °C and reflux for 10 to 15 minutes without boiling.
3. Allow the sample to cool, add 5 ml of concentrated HNO_3, replace the watch glass, and reflux for 30 minutes.
4. Repeat this last step to ensure complete oxidation.
5. Using a ribbed watch glass, allow the solution to evaporate to 5 ml without boiling, while maintaining a covering of solution over the bottom of the beaker.
6. After the sample has been cooled, add 2 ml of water and 3 ml of 30% H_2O_2.
7. Cover the beaker with a watch glass and return the covered beaker to the hot plate for warming and to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence.
8. Heat until effervescence subsides and cool the beaker.
9. Continue to add 30% H_2O_2 in 1 ml aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged. Do not add more than a total of 10 ml 30 % H_2O_2.
10. Cover the sample with a ribbed watch glass and continue heating the acid-peroxide digestate until the volume has been reduced to approximately 5 ml.
11. After cooling, dilute to 100 ml with water.
12. Filter through Whatman No. 41 filter paper and dilute to 100 ml with water.

4. DTPA extraction (Adapted from Lindsay & Norvell, 1978)

Preparation of DTPA extraction solution

The DTPA extracting solution contains 0,005 M DTPA, 0,01 M $CaCl_2$, 0,1 M TEA adjusted to pH 7,30.

1. To prepare 10 liters DTPA extraction solution dissolve 149,2 g of reagent grade $(HOCH_2CH_2)_3N$ (TEA), 19,67 g of diethylenetriaminepentaacetic acid (DTPA), and 14,7g of $CaCl_2.2H_2O$ in approximately 200 ml of distilled water.
2. Allow to dissolve and dilute to approximately 9 liters.
3. Adjust the pH to 7,30 ± 0,05 with 1:1 HCL while stirring and dilute to 10 liters.
4. The solution is stable for several months.

Extracting procedure

1. Place 10 g air-dry soil in an extraction bottle.
2. Add 20 ml DTPA extraction solution to the soil.
3. Shake horizontally for 2 hours at 180 cycles per minute at a constant temperature of 20 ± 2 °C.
4. Filter immediately through Whatman NO. 42 into a suitable containers.

5. AB-DTPA extraction (Soltanpour, 1991)

Preparation of NH_4HCO_3-DTPA extraction solution

1. Dissolve 1,97 g of DTPA in 800 ml of pure water.
2. Add about 2 ml of 1:1 NH_4OH to facilitate dissolution.
3. After DTPA is in solution, add 79,06 g of NH_4HCO_3, and stir gently until dissolved.

4. Adjust pH to 7,6 using either NH_4OH or HCl.
5. Bring to a final volume of 1 liter.
6. The extracting solution is unstable with regard to pH and should be made immediately before using of kept under mineral oil.

Extracting procedure

1. Place 10 g of soil in 250 ml Erlenmeyer flask.
2. Add 20 ml of extracting solution and shake on a reciprocating shaker at 180 cycles per minute for 15 minutes. The flasks are kept open.
3. Filter through a medium-speed filter paper.
4. Place 1 ml of concentrated HNO_3 in a 10 ml beaker.
5. Carefully add 10 ml of AB-DTPA extract or standard solution to the beaker and mix on a shaker for 15 minutes to eliminate carbonate species.

APPENDIX 3

ANOVA ANALYSIS OF VARIANCE

Table 1. F-values and their level of significance from the analysis of variance of the crop yield for lettuce, wheat and bean pods as influenced by OSA type, OSA application rate and $CaCO_3$ application rate.

Effect	df^1	Yield		
		lettuce	wheat	beans
		F-values and their level of significance		
B	3	29,76***	0,57^{ns}	10,57***
C	1	1,79^{ns}	0,30^{ns}	12,90***
D	1	704,36***	0,20^{ns}	228,30***
BC	3	0,25^{ns}	1,40^{ns}	6,43***
BD	3	7,69***	3,07*	1,94^{ns}
CD	1	6,47*	0,30^{ns}	4,47*
BCD	3	3,01*	0,30^{ns}	2,32^{ns}

B =OSA application rate
C =OSA type
D =$CaCO_3$ application rate
^{1} Degrees of freedom of effect
* Significant at the <0,05 probability level
** Significant at the <0,01 probability level
*** Significant at the <0,001 probability level
^{ns} Not Significant

Table 2. F-values and their level of significance from the analysis of variance of the soil pH after completion of the experiment.

Effect	df[1]	pH F-values and their level of significance
A	2	$73,01^{***}$
B	3	$6,34^{***}$
C	1	$45,45^{***}$
D	1	$33571,92^{***}$
AB	6	$3,12^{**}$
AC	2	$2,98^{ns}$
BC	3	$6,46^{***}$
AD	2	$59,38^{***}$
BD	3	$7,96^{***}$
CD	1	$22,86^{***}$
ABC	6	$0,86^{ns}$
ABD	6	$1,39^{ns}$
ACD	2	$5,62^{**}$
BCD	3	$3,86^{*}$
ABCD	6	$0,87^{ns}$

A =plant type
B =OSA application rate
C =OSA type
D =$CaCO_3$ application rate
[1] Degrees of freedom of effect
* Significant at the $<0,05$ probability level
** Significant at the $<0,01$ probability level
*** Significant at the $<0,001$ probability level
^{ns} Not Significant

Table 3. F-values and their level of significance from the analysis of variance for the trace element (Ni, Co, Cd, Cu, Zn) concentration (mg/kg dry matter) of the plant material.

Effect	df[1]	Element				
		Ni	Co	Cd	Cu	Zn
		F-values and their level of significance				
A	2	1254,20***	74,80***	312,60***	333,34***	184,24***
B	3	0,87^{ns}	6,31***	1,79^{ns}	2,80*	2,68*
C	1	1,30^{ns}	2,71^{ns}	7,40**	0,08^{ns}	12,39***
D	1	3302,40***	988,65***	24,68***	89,66***	14,20***
AB	6	0,82^{ns}	7,11***	3,26**	0,64^{ns}	2,44*
AC	2	1,55^{ns}	17,76***	9,90***	0,39^{ns}	30,53***
BC	3	2,04^{ns}	0,72^{ns}	2,33^{ns}	1,51^{ns}	2,97*
AD	2	1099,80***	1,50^{ns}	5,57**	27,90***	13,68***
BD	3	3,68*	1,66^{ns}	1,12^{ns}	1,34^{ns}	6,36***
CD	1	1,96^{ns}	1,40^{ns}	1,13^{ns}	0,77^{ns}	0,31^{ns}
ABC	6	1,40^{ns}	2,30*	1,86^{ns}	0,80^{ns}	5,55***
ABD	6	0,21^{ns}	1,96^{ns}	1,05^{ns}	1,06^{ns}	3,02**
ACD	2	0,23^{ns}	13,06***	0,24^{ns}	0,10^{ns}	0,28^{ns}
BCD	3	0,37^{ns}	1,68^{ns}	1,93^{ns}	0,21^{ns}	1,25^{ns}
ABCD	6	2,98**	2,47*	0,46^{ns}	1,12^{ns}	2,38*

A =plant type
B =OSA application rate
C =OSA type
D =$CaCO_3$ application rate
[1] Degrees of freedom of effect
* Significant at the <0,05 probability level
** Significant at the <0,01 probability level
*** Significant at the <0,001 probability level
^{ns} Not Significant

Table 4. F-values and their level of significance from the analysis of variance for the total trace element (Ni, Co, Cd, Cu, Zn) uptake (μg/plant) of the plant material.

Effect	df[1]	Element				
		Ni	Co	Cd	Cu	Zn
		F-values and their level of significance				
A	2	826,08***	23,23***	126,48***	364,23***	30,69***
B	3	3,03*	3,20*	4,18**	17,20***	15,27***
C	1	7,44**	0,06^{ns}	3,59^{ns}	0,70^{ns}	1,56^{ns}
D	1	2988,80***	2135,90***	202,59***	599,46***	1202,50***
AB	6	1,86^{ns}	1,25^{ns}	3,24**	3,72**	5,46***
AC	2	0,47^{ns}	2,23^{ns}	4,37*	2,13^{ns}	2,20^{ns}
BC	3	4,59**	2,03^{ns}	3,20*	4,85**	5,10**
AD	2	753,37***	126,19***	78,38***	167,80***	222,50***
BD	3	0,94^{ns}	1,35^{ns}	2,06^{ns}	1,41^{ns}	3,72*
CD	1	1,06^{ns}	0,19^{ns}	7,93**	3,43^{ns}	7,17**
ABC	6	5,06***	3,03**	0,98^{ns}	3,86**	1,58^{ns}
ABD	6	3,24**	2,87*	2,08^{ns}	2,68*	3,33**
ACD	2	1,07^{ns}	4,47*	2,70^{ns}	0,97^{ns}	4,50*
BCD	3	0,08^{ns}	0,02^{ns}	2,32^{ns}	1,22^{ns}	1,50^{ns}
ABCD	6	4,16***	3,64**	1,03^{ns}	2,10^{ns}	1,46^{ns}

A =plant type
B =OSA application rate
C =OSA type
D =$CaCO_3$ application rate
[1] Degrees of freedom of effect
* Significant at the <0,05 probability level
** Significant at the <0,01 probability level
*** Significant at the <0,001 probability level
^{ns} Not Significant

Table 5. F-values and their level of significance from the analysis of variance for the NH$_4$-EDTA extractable trace element (Ni, Co, Cd, Cu, Zn) concentration (mg/kg soil) of the soil.

Effect	df[1]	Element				
		Ni	Co	Cd	Cu	Zn
		F-values and their level of significance				
A	2	14,56***	27,73***	0,99^{ns}	6,45**	201,27***
B	3	33,02***	2,70*	100,26***	36,21***	286,89***
C	1	37,99***	0,73^{ns}	317,78***	227,77***	99,63***
D	1	342,07***	20,12***	0,11^{ns}	136,98***	1,43^{ns}
AB	6	2,40*	4,29***	1,09^{ns}	1,54^{ns}	9,42***
AC	2	0,74^{ns}	1,12^{ns}	1,92^{ns}	4,60*	25,26***
BC	3	12,70***	2,07^{ns}	91,44***	46,73***	63,79***
AD	2	1,75^{ns}	4,40*	1,12^{ns}	0,34^{ns}	2,87^{ns}
BD	3	5,22**	1,17^{ns}	2,38^{ns}	0,79^{ns}	10,39***
CD	1	6,98**	0,55^{ns}	0,09^{ns}	0,00^{ns}	16,80***
ABC	6	1,73^{ns}	1,28^{ns}	0,47^{ns}	1,74^{ns}	10,16***
ABD	6	1,66^{ns}	2,15^{ns}	1,83^{ns}	1,40^{ns}	6,71***
ACD	2	4,95**	0,32^{ns}	0,35^{ns}	2,99^{ns}	13,49***
BCD	3	0,79^{ns}	0,91^{ns}	1,52^{ns}	0,42^{ns}	7,65***
ABCD	6	1,07^{ns}	0,21^{ns}	1,47^{ns}	0,51^{ns}	13,74***

A =plant type
B =OSA application rate
C =OSA type
D =CaCO$_3$ application rate
[1] Degrees of freedom of effect
* Significant at the $<0,05$ probability level
** Significant at the $<0,01$ probability level
*** Significant at the $<0,001$ probability level
^{ns} Not Significant

Table 6. F-values and their level of significance from the analysis of variance for the trace element (Ni, Co, Cd, Cu, Zn) concentration (mg/kg dry matter) in the soil determined by the EPA method 3050.

Effect	df[1]	Element			
		Ni	Co	Cu	Zn
		F-values and their level of significance			
A	2	1,94^{ns}	1,52^{ns}	0,22^{ns}	0,91^{ns}
B	2	0,49^{ns}	2,02^{ns}	21,57***	2,13^{ns}
C	1	0,05^{ns}	0,17^{ns}	20,93***	2,52^{ns}
D	1	0,93^{ns}	0,52^{ns}	5,60*	1,95^{ns}
AB	4	1,42^{ns}	0,11^{ns}	0,25^{ns}	1,58^{ns}
AC	2	1,15^{ns}	1,65^{ns}	0,07^{ns}	1,52^{ns}
BC	2	2,00^{ns}	0,13^{ns}	9,05***	1,78^{ns}
AD	2	0,23^{ns}	0,48^{ns}	0,09^{ns}	3,98*
BD	2	1,30^{ns}	0,59^{ns}	0,08^{ns}	1,84^{ns}
CD	1	0,14^{ns}	2,64^{ns}	0,03^{ns}	0,00^{ns}
ABC	4	1,49^{ns}	0,42^{ns}	0,51^{ns}	1,14^{ns}
ABD	4	0,41^{ns}	1,27^{ns}	2,38^{ns}	3,37*
ACD	2	0,76^{ns}	0,11^{ns}	2,12^{ns}	1,01^{ns}
BCD	2	0,18^{ns}	0,94^{ns}	0,20^{ns}	0,05^{ns}
ABCD	4	1,22^{ns}	0,72^{ns}	1,50^{ns}	1,99^{ns}

A =plant type
B =OSA application rate
C =OSA type
D =$CaCO_3$ application rate
[1] Degrees of freedom of effect
* Significant at the <0,05 probability level
** Significant at the <0,01 probability level
*** Significant at the <0,001 probability level
^{ns} Not Significant

Table 7. F-values and their level of significance from the analysis of variance for the trace element (Ni, Co, Cd, Cu, Zn) concentration (mg/kg dry soil) in the soil as extracted with DTPA.

Effect	df[1]	Element				
		Ni	Co	Cd	Cu	Zn
		F-values and their level of significance				
A	2	$17,66^{***}$	$16,34^{***}$	$3,28^{*}$	$6,00^{**}$	$75,91^{***}$
B	2	$11,02^{***}$	$0,10^{ns}$	$0,14^{ns}$	$63,37^{***}$	$173,03^{***}$
C	1	$2,90^{ns}$	$2,02^{ns}$	$15,30^{***}$	$273,71^{***}$	$27,53^{***}$
D	1	$716,92^{***}$	$1609,60^{***}$	$2,68^{ns}$	$113,53^{***}$	$81,24^{***}$
AB	4	$4,56^{**}$	$2,89^{*}$	$6,46^{***}$	$1,01^{ns}$	$33,26^{***}$
AC	2	$0,51^{ns}$	$3,38^{*}$	$2,08^{ns}$	$1,93^{ns}$	$14,79^{***}$
BC	2	$0,77^{ns}$	$0,50^{ns}$	$3,99^{*}$	$91,62^{***}$	$65,42^{***}$
AD	2	$6,14^{**}$	$1,98^{ns}$	$0,54^{ns}$	$5,31^{**}$	$24,88^{***}$
BD	2	$2,41^{ns}$	$5,04^{*}$	$0,41^{ns}$	$1,20^{ns}$	$37,20^{***}$
CD	1	$0,39^{ns}$	$1,43^{ns}$	$2,90^{ns}$	$0,04^{ns}$	$35,56^{***}$
ABC	4	$1,02^{ns}$	$1,32^{ns}$	$1,71^{ns}$	$0,52^{ns}$	$26,74^{***}$
ABD	4	$3,43^{*}$	$0,82^{ns}$	$0,86^{ns}$	$0,63^{ns}$	$29,09^{***}$
ACD	2	$0,11^{ns}$	$8,23^{**}$	$2,31^{ns}$	$1,92^{ns}$	$24,89^{***}$
BCD	2	$0,42^{ns}$	$1,09^{ns}$	$0,74^{ns}$	$0,12^{ns}$	$21,57^{***}$
ABCD	4	$0,11^{ns}$	$2,25^{ns}$	$0,85^{ns}$	$1,93^{ns}$	$30,76^{***}$

A =plant type
B =OSA application rate
C =OSA type
D =$CaCO_3$ application rate
[1] Degrees of freedom of effect
* Significant at the $<0,05$ probability level
** Significant at the $<0,01$ probability level
*** Significant at the $<0,001$ probability level
^{ns} Not Significant

Table 8. F-values and their level of significance from the analysis of variance for the trace element (Ni, Co, Cd, Cu, Zn) concentration (mg/kg) in the soil as extracted with AB-DTPA.

Effect	df[1]	Element				
		Ni	Co	Cd	Cu	Zn
		F-values and their level of significance				
A	2	4,50*	67,69***	0,66^{ns}	2,89^{ns}	38,00***
B	2	2,60^{ns}	1,07^{ns}	12,10***	277,46***	86,49***
C	1	4,75*	6,19*	35,57***	1074,70***	29,21***
D	1	223,60***	3364,70***	2,97^{ns}	97,81***	61,44***
AB	4	2,05^{ns}	6,67***	1,41^{ns}	0,84^{ns}	4,21**
AC	2	1,43^{ns}	7,02**	0,06^{ns}	0,92^{ns}	3,66*
BC	2	2,36^{ns}	1,55^{ns}	8,88***	335,59***	11,98***
AD	2	0,50^{ns}	6,65**	2,25^{ns}	4,60*	3,74*
BD	2	3,17^{ns}	4,95*	0,69^{ns}	0,23^{ns}	8,96***
CD	1	6,07*	0,04^{ns}	0,54^{ns}	8,24**	5,27*
ABC	4	0,91^{ns}	1,87^{ns}	0,48^{ns}	3,63*	3,08*
ABD	4	0,57^{ns}	1,15^{ns}	2,79*	0,20^{ns}	3,03*
ACD	2	3,53*	12,41***	1,66^{ns}	2,19^{ns}	2,63^{ns}
BCD	2	1,52^{ns}	0,22^{ns}	0,24^{ns}	3,10^{ns}	4,17*
ABCD	4	1,68^{ns}	3,70*	2,45^{ns}	0,58^{ns}	1,97^{ns}

A =plant type
B =OSA application rate
C =OSA type
D =CaCO$_3$ application rate
[1] Degrees of freedom of effect
* Significant at the <0,05 probability level
** Significant at the <0,01 probability level
*** Significant at the <0,001 probability level
^{ns} Not Significant