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Metal Speciation and Toxicity in Sewage Sludge

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Abstract

Sewage sludge contains metals which can have negative effects on microorganisms, plants and animals when applied to soil. A review is presented which considers the toxicological effects of metals. Improved industrial wastewater management has lead to a decrease in industrial discharge to sewage treatment plants which has reduced the concentrations of metals such as cadmium, chromium, mercury and nickel in the sludge. Urban runoff remains as a major source of metals, especially lead and zinc, although lead concentrations are expected to decrease with the increased use of unleaded petrol. Apart from corrosion products from copper building materials in urban runoff, drinking water pipe corrosion is a major source of copper and this problem is most extensive in hard water areas.

The assessment of bioavailable metal in sludge is discussed. Methods to fractionate the sludge, dissolve the fractions and analyse the metal are reviewed. Extraction of different fractions are difficult to compare, both because of the poor selectivity and specificity of the extractants and the lack of complete information about the exact extraction procedures used. Nevertheless, fractionation of metals in sludge generally reveals that cadmium and zinc are comparably easy to extract which implies that they are mobile in sludge-amended soil and might be expected to be bioavailable, whereas lead and copper are more tightly bound in relatively insoluble fractions. Acidification of sludge releases cadmium, lead, nickel and zinc, but not copper which probably remains as a bound organic form. Cadmium fractionation is difficult due to the low concentrations and subsequent risk for contamination. Complexation by organic material and soil pH are important regulators of metal availability.

Most sludges produced in Sweden have copper and zinc concentrations well below the standard concentrations allowed for spreading sludge on farmland. The concentrations of lead and cadmium often exceed set criteria, especially at larger sewage works. Future exceedance of the sludge criteria is anticipated for cadmium, mercury and lead, while copper continues to be a problem. However, the criteria can be questioned, both because of the limited knowledge about plant uptake mechanisms and long term effects and because of uncertainties in the analysis, particularly in the case of cadmium. Also, only the total concentrations of the metals are limited and metal speciation analysis is not taken into consideration. To gain a better understanding of metals in sewage sludge, more research relating metal speciation to plant uptake is required. There is also a need for improved cadmium analysis and further investigation of the results of chronic exposure to low metal concentrations.

Finally, results for the analysis of metal fractionation and toxicity are presented in a published article. The acute toxicity of sewage sludge to *Photobacterium phosphoreum* is measured and compared to the metal content before and after chemical extraction. The results show that copper is the source of most toxicity in sludge, followed by lead and zinc. A good estimation of sludge metal availability is EDTA (ethylenediaminetetraacetic acid) extraction. EDTA extracts the toxic fraction in all the sludge samples, except in the case of limed sludge, where ammonium acetate was a more adequate extractant.

Contents

Appendix

"Metal fractionation and toxicity of sewage sludge" accepted for publication in Environmental Technology.

List of tables **Page No.**

List of figures Page No.

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1. Presentation of the thesis

This thesis contains a comprehensive review of the literature on metals in sewage sludge and the toxicological and ecological effects of sludge amendment on soil, followed by a research article "Metal fractionation and toxicity of sewage sludge" which has been accepted for publication in Environmental Technology.

The aims and objectives of this thesis are:

(a) Assess and interpret measurements of total metal concentrations in sewage sludge and relate these values to typical regional values and quality standard exceedance.

(b) Review the relevance and selectivity of fractionation methods for sewage sludge.

(c) Connect and link speciation/fractionation results to the effects of sludge amendment on soil ecology.

(d) Consider the connection between total/fractionation results and plant uptake which may ultimately affect human health.

(e) Analyse the toxicity of sewage sludge using a standardised bioassay and compare to metal fractionation procedures to provide a toxic fraction.

(f) Study synergistic/antagonistic effects on this bioassay of combinations of metals.

2. A general consideration of metals in sewage sludge

At the time when most people lived in small villages in rural areas, household by-products and wastewater were naturally returned to farmland in a small, closed system. Today, when people are concentrated in urban areas, the natural cycle of water and nutrients is interrupted and the disposal of sludge has become a problem. To close the system and recirculate the nutrients in sewage sludge, land application of sludge would be preferable. Sludge is rich in nitrogen, phosphorus and micronutrients (1) and application of sludge, with its high content of organic material, to sandy, light textured soils can considerably improve the soil quality (2).

However, the complexity of modern society makes it difficult to keep unwanted substances out of sewage sludge since in many cases all kinds of wastewater enter the same sewer system. Household wastewater is mixed with industrial discharge, increasing traffic pollutes urban runoff and the use of a range of many different chemical substances in the household makes even domestic wastewater a potential problem (3).

Special attention has been paid to the presence of heavy metals in the sludge, partly because of their toxicity and partly because of their persistence in the environment (4,5). A significant proportion of metals entering the sewage works concentrate in sewage sludge (6,7) and therefore sludge-amendment generally increases the total metal content of soils (1,2,8,9,10). This causes some concern about the potential for increased metal concentrations in crops grown in sludge-amended soil. However, many workers have shown that no simple relation exists between total metal content in soil and the concentrations taken up by plants (8,11,12) and it seems difficult to create a model which predicts the bioavailability of metals as a function of total metal content (13-15). Indeed, studies have shown that, under certain conditions, sludge application actually decreases the available metal content of soil by increasing the organic binding sites for metals (11,16-18). Only a fraction of the metals in sludge-amended soils are in a chemical form that can be taken up by plants and animals; undoubtedly, the extent of fractionation between available and non-available metal depends on many factors, such as soil type, pH and other physico-chemical properties of the soil (19-21). The uptake also varies between different plant species. The single most important regulator of plant uptake is, however, the chemical speciation of the metal (22).

It would be preferable if an analysis of "biologically available" metals could be used to supplement measurements of total metal content. Determination of the different biologically relevant species of a metal can be achieved by a comparison of metal speciation in sludge with the results from biological experiments (2,9,15,23,24).

The aim of this review is to discuss different ways of determining total metal content and speciation and to compare these methods with biological experiments. The purpose of this is to find a method which can simply and rapidly predict the suitability of sewage sludge for agricultural application. This study is therefore important in moving towards an assessment and understanding of the ecotoxicological implications of metals in sewage sludge and their application to agricultural soils.

However, in this discussion it must be remembered that sewage sludge is not the only, or even the most important, source of heavy metals in soil. Metals can also enter the soil from atmospheric deposition, fertilisers and agrochemicals. Sewage sludge becomes a major source of metals for the soil to which it is applied, but on a global scale, only Hg, Ni and Zn exceed 2 % of total soil input (1).

3. Metal concentrations in sewage sludge

The metal content in sewage sludge originates from domestic wastewater, urban runoff and industrial discharges. Since some metals are naturally present in the environment, and thus in food, sewage sludge has always had a certain content of metals which have entered the sewer system with domestic wastewater. Metals such as Fe, Mn, Cu and Zn are naturally present in sewage sludge. However, human activities can increase the concentrations above the desired level, mostly by increased contributions from industry and urban runoff. This can lead to harmful effects if the sludge is spread on farmland. Some soils are deficient in certain trace elements, such as Cu and Zn which are necessary for plants and here sludge could be a useful soil improver. Some other metals, such as Cd and Pb, have no known beneficial effect in any biological system and are therefore undesirable in sewage sludge. In the ideal situation, sewage sludge would contain available essential elements in proportions that are beneficial to the soil to which the sludge is applied and to the crops growing on the sludge-amended soil.

Table 3.1 Total metal concentrations in sewage sludge

Metal	Concentration range, $\mu g g^{-1}$ (mean)		References
	Raw sludge	Digested sludge	
Cd	<0.1-95 (22)	5.5-1830 (27)	6,8,9,13,14,19,22,26-39
Cr	40-1970 (488)	219-2240 (467)	6,8,9,13,14,26,32,34, 38,39
Cu	166-1650 (540)	172-4710 (760)	6,8,13,14,19,26-34,36-39
Fe	-	10340-103000 (16100)	29,33,37
Mg	-	3236-12190 (6635)	33,37
Mn	470-1190 (667)	276-490 (371)	14,33,35,37,39
Ni	26-410 (92)	65-4071 (280)	8,9,13,14,26,27,29,30, 32-34,37-40
Pb	18-1940 (620)	319-1138 (731)	8,13,14,19,26,27,30, 32-34,36-40
Zn	153-6100 (1930)	250-7600 (2083)	2,8,9,13,14,19,26-34,36-40

3.1 Total metal concentrations

Measurement of total metal concentration in sewage sludge is interesting for many purposes. An analysis of the total metal concentration can help to locate pollution sources, whether from household, industry or stormwater. In addition, the total metal content is usually reported to provide an estimation of the total metal loading to the soil. However, to assess the potential biological uptake or toxicity after application of sewage sludge, speciation analysis is necessary (25).

Metal concentrations in raw and digested sewage sludge exhibit a wide range of values in the literature, particularly for Cd, Cr, Ni and Pb (table 3.1).

The extent to which industrial discharge and stormwater enter the domestic wastewater system is critical for metal concentrations, particularly for metals such as Cr and Ni which mostly come from industrial sources. Another possible source of metals, particularly for Cr and Ni, is the $FeCl_3$ used as coagulant in some wastewater treatment plants, which can be of different quality depending on the origin of the $FeCl_3$. $FeCl_3$ produced from steel industry by-products contains very high levels of metals, especially Ni (26). Since the major source of Fe and Al is the use of their salts as coagulants, the concentration of these metals is high in sewage sludge and is not always included in an analysis of the metal content of the sludge.

3.1.1 Cadmium

One source of Cd is phosphate, which is used in fertiliser and washing powder. Production and use of batteries, paints and plastics is another important source of Cd (41,42). The industrial discharge of Cd is decreasing due to an increasing awareness of the environmental hazard of Cd. In many countries the use of Cd in paints and plastics is forbidden. Still, the Cd containing products of yesterday can cause problems with water leaching from waste disposal sites and, in the case of incineration, atmospheric fallout.

Cadmium is a metal of concern due to its toxicity and relatively high mobility in sludge-amended soil (11). Many of the reported raw sludges have Cd concentrations between 10 and 30 μgg^{-1}. Two higher concentrations were found in the literature, 88 μgg^{-1} (14) and 95 μgg^{-1} (8), both published before 1983. On the other hand, some sludges analysed more recently contain less than 2 μgg^{-1} (26). The mean cadmium concentration in digested sludge is only about 20 % higher than in undigested sludge (table 3.1). This could be expected because of the approximately two-fold concentration of metals following digestion due to loss of organic material. However, the difference between the highest and the lowest value is so great (5.5-1830 μgg^{-1}) that it is difficult to draw any conclusions in this direction. Most digested sludges contain Cd concentrations between 5.5 and 55 μgg^{-1}, but there are some sludges that contain 178-287 μgg^{-1}, (13,33,37) and two of these values are Chicago sludge before 1982 (13,37). One extremely high Cd concentration, 1830 μgg^{-1}, is found in a sludge from a sewage treatment plant which receives wastewater from a factory producing batteries (39).

Some workers have found a decrease of Cd levels with time (43). This might be a result

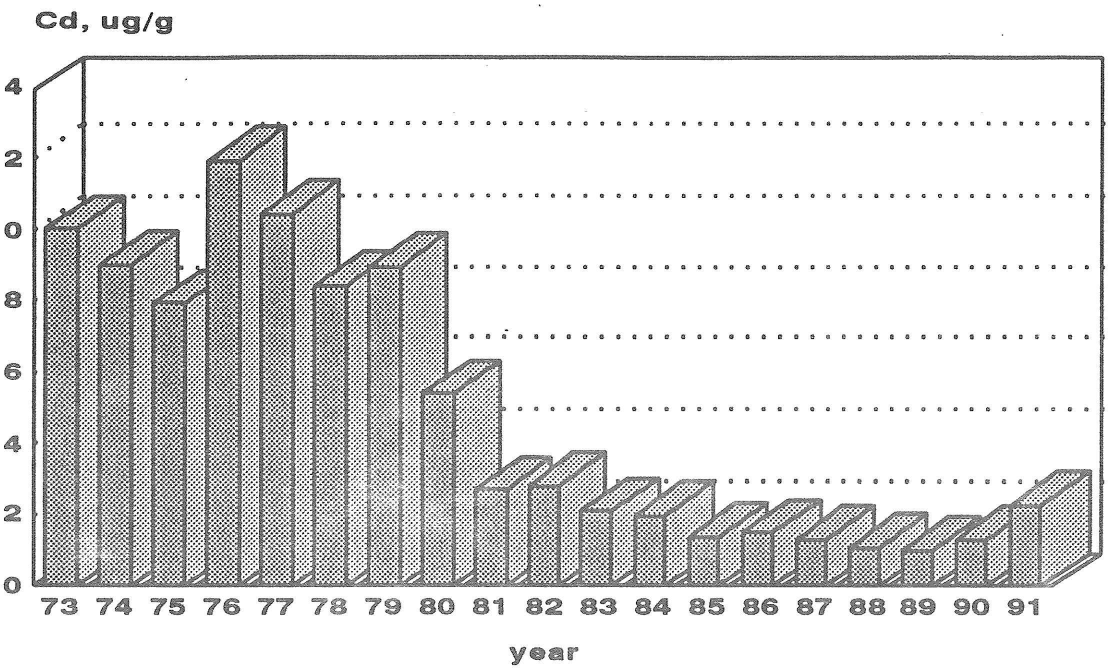


Figure 3.1 Cadmium concentrations in sewage sludge from Ryaverket sewage treatment plant in Göteborg, Sweden (43). The Cd concentration for 1991 is measured in digested sludge rather than raw sludge which explains the elevated value.

of a decrease of the industrial discharge entering the sewage works. The lower reported levels might also be a consequence of improved analytical methods pushing down the detection limits and decreasing the risk for blank contamination. Nowadays, Cd concentrations less than 2 $\mu g g^{-1}$ are frequently reported (26,43) and the analytical errors are probably significant at these low levels (42).

3.1.2 Chromium

The main source of Cr is industry (41), mainly leather tanneries (1), and where no industrial discharge containing Cr reaches the sewage works the concentration of Cr is in the range 50-500 $\mu g g^{-1}$. The highest value is found in a digested sludge from Chicago (13), but that value is a mean for the years 1972-1987 and Cr content is decreasing with time. The highest value for undigested sludge is from a Polish sludge especially rich in Cr and Zn (9). One of the lowest Cr concentrations was found in Whitlingham, UK (6) where the average concentration on Sunday was much lower than on the other days of the week, suggesting an industrial source with a 6 day working week.

3.1.3 Copper

The Cu content in sludge originates from a variety of sources which are difficult to control. Some of the Cu comes from industry (41), but with increasing control of industrial effluents the importance of the contribution of urban runoff to the sewage system has been revealed. Car brake linings release Cu and corroding roofs and drain pipes contribute to the Cu content of urban runoff (44). Another important source of Cu is drinking water pipes. Many houses have Cu pipes for household connections which, under favourable conditions for corrosion, provide a significant contribution to the sewage Cu content. This corrosion is dependent on water pH and hardness/alkalinity (45), which means that it can be controlled to some extent by adjusting to the correct pH, hardness and alkalinity at the waterworks. Also, the combination of materials in the water system is important (46). Corrosion of household connections is enhanced by the comparably long residence time of the water. Since Cu pipes are so common, replacement of the Cu is not realistic at the moment and therefore more emphasis should be placed on minimising pipe corrosion by water quality adjustment before leaving the waterworks.

Copper is a metal of concern in sewage sludge since it is both toxic and present in high concentrations. Due to the fact that a considerable proportion of Cu originates from water pipes and urban runoff, very low concentration levels are hard to find. Most sludges have concentrations within the range 160-1200 μgg^{-1}, and it seems difficult to find a sludge with a concentration below that. The sludge from Anderson, Ind. (33) was exceptionally contaminated with Cu and contained 4710 μgg^{-1}, twice as much as any other sludge. This sludge also contained the highest levels of Zn, and very high concentrations of Cd, Ni and Pb.

3.1.4 Nickel

The dominant source for Ni is industry, mostly dry cell battery manufacturers (1) and the metallurgical industry (41). The highest concentration of Ni, 4071 μgg^{-1}, is found in the same sludge as the highest Cd concentration; a sludge contaminated with metals from a factory that produces batteries (39). The other high value, 2280 μgg^{-1}, comes from the Anderson sludge (33) which was previously mentioned for its high Cu content. All other sludges contained less than 721 μgg^{-1}, typically 44-290 μgg^{-1} for raw sludge, and 65-390 μgg^{-1} for digested sludge.

3.1.5 Lead

An important source of lead is urban runoff which contains particles from car exhausts. However, the introduction of catalytic converters on automobiles has created an increasing demand for unleaded petrol. This has resulted in a decrease of lead concentration in the fine fractions of road dust (47), but since it can take some years before the larger size fractions of road dust are washed down into the sewer system, this decrease of lead concentration might not become evident in the sludge concentration in the immediate future. The other important source is car battery manufacturers (1,41). Before the introduction of DDT, a considerable contribution of lead to the environment was a direct result of the use of lead-containing pesticides (48). All sludge concentrations above 1050 μgg^{-1} are from 1982 or before, which reflects the decreasing industrial use of lead.

3.1.6 Zinc

Zinc is used in galvanized steel and is released into the environment by weathering and corrosion (41). As a consequence of the widespread use of galvanized steel, Zn is ubiquitous and enters the sewer system through urban runoff, household wastewater and industrial effluents. Thus, the concentration varies between different sludges and no values found here are exceptionally high or low. Zinc is usually present in high concentrations in sludge, but since the toxicity of Zn is relatively low it has not been regarded as a major problem for land application.

3.2 Less frequently reported metals

In table 3.2 some of the less frequently reported metals are listed. Most of these metals emanate from one or a few sources which makes the concentrations vary within a wide range depending on the presence or absence of a point source. Examples of this are: Ag, which is used in photographic processing (1,41); As which is a mining by-product (49,50) and is used as impregnating agent for wood and in pesticides (41,48,50); Hg, from dry cell battery manufacturers (1); Mo, which is used as an an additive to stainless steel and is discharged from the shale oil industry (51). Other metals are mostly a result of atmospheric fallout; Vanadium from combustion and refining of oil (52) and Cs from nuclear reactions (atomic bombs and nuclear power plants) (53,54). The concentrations of Cs have increased in some parts of Europe and west Asia after the Chernobyl nuclear reactor accident.

Some metals of interest are missing in table 3.2, for example Pt, Tl and Ti. Platinum is used in catalytic converters on automobiles and it has been reported that the increase in their use might result in an increasing concentration of this toxic metal in the environment; concentrations of 2-10 ngg^{-1} have been reported for road dust (47). Thallium is also very toxic. It is a by-product in mining (55) and is used in rat poisons. Titanium is the tenth most commom element in the earths crust (56). Titanium oxide is the white pigment used for painting traffic markings on asphalt and a considerable quantity of Ti wears off every year. Concentrations of 800-3000 μgg^{-1} have been reported for road dust (57) which will enter the sewer system with urban runoff.

3.3 Methods for digestion and analysis

Total metal content can be analysed in many different ways depending on the chemistry of the sample matrix. What exactly total metal content means is sometimes not clear since many authors fail to describe their methods. The concentrations of metal reported will depend on digestion method and analysis. It is certainly preferable to analyse a standard sludge (*eg* an EC standard sludge), assert the accuracy of the method and report a standard deviation.

3.3.1 Digestion methods

To analyse the metal content in a sample it is necessary to dissolve the sample and oxidise the organic matter. It is also desirable to keep the metals in solution which means that the digesting agent should not form insoluble complexes with the metals. The digesting agent is usually a strong acid or combinations of acids, but other substances are sometimes used for oxidation, *eg* hydrogen peroxide (27,58,59). All digestion methods involve some form of heating, either at atmospheric pressure or in a closed vessel where both a high pressure and temperature can be maintained. Many digestion methods have originally been developed for sediments with a significantly lower organic content than sewage sludge. Still, most of them are applicable to sludge samples with

Table 3.2 Concentrations of less frequently reported heavy metals in sewage sludge

Element	Concentration range, $\mu g g^{-1}$	
	Alloway & Jackson, (1)	Lester, Sterritt & Kirk, (44)
Ag	<930	ND-960
As	3-30	10-50
Au	0.25-7	-
B	16-680	200-1430
Ba	9-1004	ND-3000
Co	1-260	ND-2490
Cs	0.45-2.9	-
Hg	0.1-55	0.1-90
La	6.4-380	-
Mo	1-40	0.102-214
Sb	3-44	-
Se	1-10	10-180
Sn	40-700	2.64-329
Sr	-	ND-2230
U	0.8-3.3	-
V	20-400	ND-2100
W	0.9-99.6	-
Zr	4.8-319	-

ND = Not Detected

only minor adjustments. Some methods used for sludge are listed below:

(a) Nitric acid and perchloric acid. Nitric acid has a boiling point of only 120°C which leads to incomplete oxidation when used alone. To increase oxidation efficiency, perchloric acid is added. However, perchloric acid is explosive and should be handled with care (2,59).

(b) Hydrofluoric acid. This acid dissolves silicates, which means that it cannot be used in glass vessels but has to be used, and stored, in teflon (33). Hydrofluoric acid is very corrosive and causes severe damage to human tissues, which means that most workers try to avoid routine use if possible. This acid is not a very effective oxidant and is therefore often combined with other acids when used for samples with high organic content.

(c) Hydrofluoric acid and perchloric acid, 5:1. This is one of the most common procedures, described by, amongst others, Tessier *et al* (60). The combination of these two acids completely dissolves both organic material and silicates. Other workers have tried other proportions such as 10:3 (9).

(d) Tri-acid mixture. Nitric, hydrofluoric and perchloric acids (4:6:1) are heated to 140°C for 3.5 h and boric acid is added (26). A mixture of nitric, sulphuric and perchloric acid (7:2:1) is also used. This mixture is heated in stages to 300°C and boiled in an open vessel for 4 h (29). The higher temperature is made possible by the addition of sulphuric acid which has a boiling point of 339°C. However, some metals such as lead form insoluble salts with sulphate and this leads to low recoveries of these metals (61).

(e) *Aqua Regia* (nitric acid and hydrochloric acid, 1:3). This combination of two effective oxidising agents is even more effective than the acids used singly, due to the production of nitrosyl chloride (58,62,63). *Aqua Regia* digestion is necessary to obtain satisfactory results for elements such as Cd and Sb. This is particularly important in sludge because sludge usually contains silicates which provide binding sites for these metals. Digestion with nitric acid only, leaves the silicates unaffected and metal ions from other fractions of the sludge which are dissolved by the acid can readsorb onto the silicates (64,65).

(f) Microwave digestion. This is a rapid digestion technique which takes advantage of the fact that in a closed vessel where the pressure can be increased, temperatures above the boiling point (at atmospheric pressure) of the acid can be achieved (58). Many workers have experimented with different microwave power, digestion times and vessel material, and multi-step digestion. The use of microwave digestion minimises the loss of volatile elements and shortens the digestion time considerably. Also, this method allows digestion of samples without the use of hydrofluoric acid and perchloric acid which increases the safety of the digestion procedure. The digestion reagents most commonly used are nitric acid and *aqua regia*. Nitric acid is efficient enough for most purposes and can be obtained in very high purity. At a temperature above 170°C the oxidation is very quick and efficient (58). However, in some cases nitric acid is not sufficient and then *Aqua Regia* can be used (62-65). An addition of hydrofluoric acid makes this method suitable for a wider range of elements (65). Sulphuric acid should be used with care because of its high boiling point. At only slightly increased pressure, the sulphuric acid can become hot enough to melt teflon vessels (58). Perchloric acid should be avoided in microwave digestion because of the explosion hazard (58), although some workers have used it for marine samples (66) and peat (67).

3.3.2 Analysis methods

The digested sample is then analysed for metal concentration and there are a number of methods available for this.

(a) Atomic absorption. This method can be used for most metals, but since the metals must be analysed one after the other, it requires a certain amount of sample and can be relatively time-consuming if many metals are analysed in only a few samples. With graphite furnace this method is very sensitive, but problems with residual inorganic and organic matrix interferences can occur especially with sludge samples. Volatile elements such as Cd can be difficult to analyse in complicated media, since the removal of matrix constituents demands temperatures at which the analyte is evaporated (68).

(b) ICP (inductively coupled plasma) is a multi-element analysis which means that many elements can be analysed in a small amount of sample and in a very short time. However, the sensitivity is poorer than with AAS, 1-10 $\mu g l^{-1}$ for Cd, Cu and Zn, up to 100 $\mu g l^{-1}$ for Pb (69).

(c) ASV (anodic stripping voltammetry) is a very sensitive method which can be used for many elements simultaneously. Not only total metals can be measured; by coating the electrode with different materials such as cellulose or Nafion, ASV can be used for direct separation of some metal species. One advantage is that the opportunity for contamination is small (25).

An alternative method for determination of total metal is X-ray fluorescence spectroscopy (30). This is a method which does not require digestion. A very small amount of sample is used, which can cause trouble with the representativity of the sample. This is particularly important in sludge analysis, due to the heterogenity of sewage sludge.

3.4 Comparison of metal concentrations with sewage sludge standards in Sweden

Table 3.3 presents the highest, lowest and median concentrations of some heavy metals in sewage sludge from small, medium and large sewage works in Sweden.

The most obvious trend is that the median metal concentrations increase with increasing number of persons connected. The table shows two exceptions; Cu and Zn decrease a little from the small to the medium size sewage works. Chromium and Pb shows the most dramatic increase for sewage works with more than 20 000 connected persons. The increase in Pb concentration, which is shown in figure 3.2, can probably be traced to urban runoff. Larger communities have a greater percentage of impermeable surfaces with heavy traffic. This leads to both more Pb from car exhausts and, because of the asphalt, that a larger percentage of the road dust enters the sewer system (47). There are some metal concentrations that are exceptionally high. The highest concentrations for Cd, Cu and Zn (36, 3380 and 18930 $\mu g g^{-1}$) are from mining wastewater and the highest Cr value (6019 $\mu g g^{-1}$) is explained by the presence of leather and fur manufacturers. These concentrations are one order of magnitude higher than the normal concentrations. Table 3.4 gives the maximum concentrations permitted for sewage sludge to be used for sludge-amendment of agricultural soils in Sweden (71). Actions to lower the metal concentrations of sewage sludge should be taken at most sewage works in Sweden before 1st January 1995. The limit for Cu can be exceeded by up to double the limit value if it can be shown that the soil is deficient in Cu. To avoid spreading too great a heavy metal loading on a single field, fields that have not received sewage sludge are

Table 3.3 Median concentrations of heavy metals in sludge from Swedish sewage treatment plants (70)

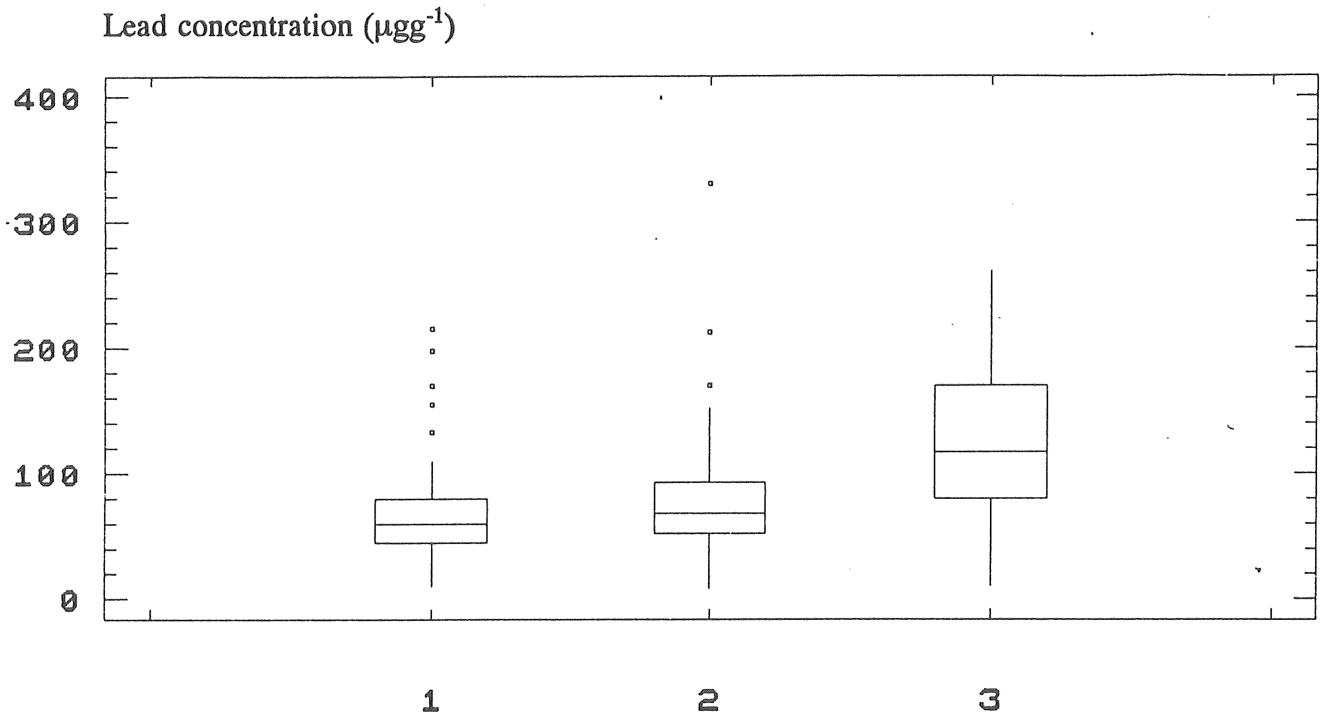
Metal	Range of metal concentrations $\mu g g^{-1}$ (median)		
	No. of persons connected		
	< 5 000	5 000 - 20 000	>20 000
Cd	0.5-6 (2)	<0.1-17 (2)	<1-36 (3)
Co	<1-18 (4)	<1-29 (4)	2-20 (6)
Cr	17-920 (60)	10-429 (67)	22-6019 (103,5)
Cu	48-922 (279)	55-938 (250)	93-3380 (323.5)
Hg	0.4-9 (2)	0.3-14 (2)	<1-6 (4)
Ni	3-64 (13)	4-285 (13)	<1-295 (20.5)
Pb	10-215 (60)	8-330 (68)	10-261 (113.5)
Zn	175-1480 (620)	165-6656 (545)	205-18930 (890)

recommended for sludge-amendment before another dose is spread on the sludge-amended soil. These limits are intended to gradually decrease until a balance is reached between applied metal and the amount of metal removed by the crops.

A comparison of tables 3.3 and 3.4 shows that the median concentration for Cr, Cu, Ni and Zn in Sweden is well below the allowed concentrations and, at the small and medium size sewage works, median concentrations of Cd, Hg and Pb are also below the limits. This means that most of the sewage works produce sludge that can be spread on farmland. However, sludge from many of the larger sewage works are close to, or above, the limit for Cd, Hg and Pb. Sewage works situated in hardwater areas often exceed the limit concentration for Cu due to drinking water pipe corrosion.

3.5 Discussion

Metals in the environment can be divided into several groups according to the present and potential hazards that are connected with them and if they are biologically essential. The extent of environmental damage is dependent on concentration, speciation and



1. < 5 000 persons connected
2. 5 000-20 000 persons connected
3. > 20 000 persons connected

Figure 3.2 Lead concentrations in sludge from sewage treatment plants in Sweden, μgg^{-1}. The box covers the middle 50 % of the data values, the "whiskers" extend out to the extremes if they are within 1.5 times the box size. Values further away from the bulk of the data are plotted as separate points. The central line is at the median.

Table 3.4 Swedish maximum allowable values for total metal concentrations in sewage sludge (71)

Metal	Limit value (μgg^{-1})
Cd	2
Cr	150
Cu	600
Hg	2.5
Ni	100
Pb	100
Zn	1500

toxicity of the available species. For essential elements, both too much and too little can cause adverse effects on plants and animals.

Some metals are very abundant, usually insoluble and have low toxicity. Examples are Ti, Fe and Mn. These metals already exist naturally in soil in very high concentrations and do not exert any toxic effects under normal circumstances. The application of sludge with a very high content of Fe on soil is not likely to lead to any significant increase in total concentration.

Some metals are necessary in very small amounts, yet toxic in higher concentrations, for example, Cu, Co, Se and Zn. There are soils that are deficient in these metals and which could be considerably improved by sludge amendment (72). On the other hand, high concentrations of these metals are toxic and sewage works accepting industrial effluents can receive concentrations high enough to render the sewage sludge unsuitable for use on farmland.

One of the metals which most often exceeds the concentration limits set up by the authorities for spreading on farmland is Cu. Since Cu usually emanates from very diffuse sources such as water pipes and urban runoff, the concentrations are difficult to control. Water hardness and pH affect the Cu concentrations from pipe corrosion. Perhaps a solution to the problem with Cu in sewage sludge could be to earmark high Cu content sludge for use on Cu-deficient soils only (72). This illustrates the importance of checking the total level of a metal both before and after sludge amendment, since the same sludge could be beneficial to a Cu deficient soil and very unsuitable on a soil already high in available Cu. This group of metals is sometimes overlooked in the discussions about environmental hazards. Instead, public interest centres on highly toxic metals.

Cd, Hg and Pb are not naturally present in the environment (except in very limited areas) and have no known biological function. The ideal concentration of these metals is zero. However, these metals have been known and used for a long time and anthropogenic activities have spread these metals in the environment for hundreds of years (42). This means that the background is higher than zero, even in "unpolluted" areas. An immediate remedy for these metals is to stop using them, but even then it will take a long time before their environmental concentrations decrease considerably, because of the large amounts diffusely spread all over the world. Cadmium, Hg and Pb have been very much discussed and public interest has focused on these three metals which are often considered some of the most dangerous threats to the environment. Their industrial use has decreased and many countries have banned their use for the many purposes where a substitute can be found. Still, there is a lot left to do with these metals and probably their concentration is unlikely to reach zero.

There are also metals which are not present in high concentrations but which are finding increased use. Examples are Pt and other metals used in catalytic converters. Pt is very toxic and behaves chemically very much like Hg, it can for instance undergo methylation (47). This faciliates the incorporation of Pt in the biosphere which makes it a potential danger to plants and animals. Since the use of these metals have recently increased from a very low level, we have an opportunity to follow the pathway of the metal from anthropogenic source to environment, for example the Pt concentrations through car exhausts, road dusts, urban sewers, sewage sludge, soil and into the food chain. This might result in valuable information about the spreading of metals in the environment.

An important limitation in the use of sludge metal criteria is that only the total concentration of the different metals are considered. No account is taken of the speciation or fractionation of the metals. The potential effects of metals added to soil by sludge-amendment can be realted to the chemical form of the metal and in which fraction of the sludge it is found. Soil characteristics are also important to consider. Factors such as pH are important for the behaviour of the metals. There are soils which are deficient in some metals, for example Cu, and a relatively high content of this metal in the added sludge could be beneficial to the soil. These examples show that the sludge criteria for maximum metal content do not provide enough information to determine the suitability of a certain sludge for application to agricultural soil.

4. Metal speciation

Measuring total metal content is the first step in estimating the effect of metals in sludge-amended soil, but it is not sufficient for the determination of biological effects (8,11,25). The uptake of metals by microbes, plants and animals is closely related to the chemical species of the metals. One way of investigating which metal species are present in sewage sludge and sludge-amended soil is by extraction with different substances supposed to dissolve a specific fraction of the sample and thus render the metals in that fraction available for analysis. In sequential extraction, extractants are applied to the same sample, one following after the other with the weakest reagent first. In single extraction, the sample is divided into sub-samples and each of them is treated with one extractant. In simultaneous extraction the extractants are applied in combinations and more than one fraction can be extracted simultaneously (31). Many workers have developed their own extraction scheme (60) and a list of some of the most commonly used schemes is made by Lake *et al* (10). Some of the extractants are listed in table 4.1.

The use of the extraction procedures summarised in table 4.1 is based on the assumption that the analysed material, *eg* sewage sludge, consists of a few well-defined fractions, usually termed exchangeable, bound to carbonates, bound to Fe/Mn oxides, bound to organic matter and residual. These fractions are operationally defined, but they are often assumed to have a physico-chemical meaning.

The exchangeable metals are very loosely bound and are extracted by the addition of another positive ion in excess, *eg* Mg^{2+}, Ba^{2+} or NH_4^+. The carbonate fraction is dissolved by changing the pH to 5 or adding chemicals which dissolve carbonates, *eg* ammonium chloride or ammonium nitrate. To dissolve the Fe/Mn oxides and extract the metals co-precipitated with these, the oxides have to be reduced under acidic conditions. The organic matter is oxidised at a pH around 2. Very often H_2O_2 is used in this oxidation. The fraction termed residual contains the metals that are not extracted by the other extractants. The determination of the metal content in the residual phase is made by the same methods as the total metal content described above.

The assumption that physico-chemical fractions are selectively isolated is a simplified, and sometimes even incorrect, description of a sludge sample. This has caused some workers to criticise extraction methods for lack of scientific meaning (73,74). In reality, sewage sludge consists of many different fractions which are not very well defined, and sometimes not even completely known with respect to structure and proportions (7,27). The term organic matter, for instance, covers a very wide range of compounds which have different complexation capacity for metals, different molecular structure and are oxidised in varying degrees (30). The metals in this fraction may be adsorbed onto an organic particle or incorporated into a cell protein. Naturally, there is no extractant which completely extracts all organically bound metals and nothing else (8,60,74,75).

The main problems occurring in extraction analysis are lack of selectivity, incomplete extraction and readsorption (8,30,73,74,76,77). An extractant with good selectivity should extract only the desired fraction and leave the other fractions unaffected. To completely dissolve one, perhaps not very clearly defined, fraction, without affecting the

Table 4.1 Extractants used for metal speciation in sludge (and sediments)

Extractant	Experimental conditions	Extracted fraction	Reference
$MgCl_2$, 1 M	pH 7.0, 1 h, agitation	Exchangeable	8,26,60
NaOAc, 1 M	pH 8.2, 1 h, agitation	Exchangeable	60
NaOAc, 1 M	pH 7.0, 1 h, agitation	Exchangeable	8
$BaCl_2$, 1 M	pH 8.1, 1 h	Exchangeable	8
NH_4CH_3COO, 1 M	pH 7.0, 1 h	Exchangeable	8
KNO_3	pH 6.9, 16 h	Exchangeable	27,30,31
KF, 0.5 M	pH 5.8, 16 h	Adsorbed	27,30,31
NH_4NO_3, 1 M	pH 7.0, 1 h	Carbonate	8
NH_4Cl, 1 M	pH 7.0, 1 h	Carbonate	8
NH_4CLO_4, 1 M	pH 7.0, 1 h	Carbonate	8
NaOAc, 1 M	pH 5.0, 5 h, agitation	Carbonate	8,26,60
EDTA	pH 7.1, 16 h	Carbonate	27,30,31
$NH_2OH{\cdot}HCl$, 0.1 M in 0.01 M HNO_3	pH 2.0, 12 h	Fe/Mn-oxides	8
$(COONH_4)_2$, 0.2 M + $(COOH)_2$, 0.2 M	pH 3.0, 2 h	Fe/Mn-oxides	8,26
$NH_2OH{\cdot}HCl$, 0.04 M in 25 % HAc	96°C, 6 h, occasional agitation	Fe/Mn-oxides	8,60
NaOH, 0.5 M		Organic	30
NaOCl, 25 %	pH 9.5	Organic	30
$Na_4P_2O_7$,0.1 M	pH 8.8, 16 or 24 h	Organic	26,27,30,31
HNO_3, H_2O_2 and NH_4OAc	85°C, pH 2, 2+3 h, intermittent agitation	Organic	60
HNO_3, 6.0 M	pH 3.8, 16 h	Sulphide	30,31

others, is of course extremely difficult, if at all possible (74,78). As an example, all of the exchangeable metal ions would be extracted by sodium acetate, pH 5 (carbonate fraction) and therefore exchangeable metal has to be removed first. As the oxides and carbonates often have an important structural function, the dissolution of these fractions would expose parts of the particle, which were hidden in the interior of the particle, to the surface, thus revealing more exchangeable metal ions (74). This also makes it difficult to dissolve one fraction completely. To do this, all of this fraction has to be available for contact with the extractant. However, there are examples of organic particles completely covered by Fe or Mn hydrous oxides, which have to be removed first (74).

In nature, most metals are present in very low concentrations compared to the number of binding sites available. This means that the surfaces of the particles are not saturated with metals. If one fraction is dissolved, free metal ions enter the solution. These metal ions can then readsorb onto the other fractions (76). The readsorbed metals are not detected in the analysis of the solution which leads to a considerable analytical error. The pretreatment of the sample can also cause errors. It has been shown that drying the sample can affect metal distribution (27,30,78,79). For example, anaerobically digested sludge can contain sulphides which are easily oxidised if the samples are air dried (80).

Of course, analytical problems such as reproducibility and contamination have to be taken into consideration (25). Experiments with spiked sludges or model sediments have gone some way in helping to solve these problems (28,30,76). No completely satisfactory method has been found for the speciation of metals in sewage sludge. Still, at the present time there is no better method than extraction for evaluating the metal distribution and thus the potential bioavailability of metals.

4.1 Extraction results

A significant proportion of the literature on the single and sequential extraction of metal fractions has been devoted to sediments. However, there are a few that have performed extractions on sewage sludge. Table 4.2 shows results for a digested sewage sludge obtained using a sequential extraction scheme (14).

It is interesting to note that the sludge shows a different fractionation pattern before and after drying. The liquid samples displayed more variation between replicates than the corresponding dried samples (27). The dried samples gave more reproducible results, but it can be discussed whether it is really desirable to obtain results which, although reproducible, might not reflect the true fractionation of the sewage sludge.

A discussion of speciation results for different metals follows.

4.1.1 Cadmium

Most workers have found the largest part of the Cd in the carbonate fraction, extracted by EDTA (27,30) or NaOAc (26), or in the organic fraction, extracted by $Na_4P_2O_7$ (26,31).

Some Cd can also be found in the exchangeable (extracted by KNO_3) and adsorbed (extracted by KF) fractions (31). However, some workers have reported results that disagree with these findings. In one study, Cd was found to 60 % in the organic and sulphide fraction (81) which agreed with earlier results by the same authors (7). Another study showed that 73 % of the Cd was found in the Fe/Mn oxide fraction (9). Cadmium

Table 4.2 Distribution of metal forms (in % of total metal) in digested sludge by sequential extraction (14)

Metal	Extractant*				
	KNO_3	KF	$Na_2P_2O_7$	EDTA	HNO_3
Cd	0	0	14.8	48.8	17.5
Cu	6.4	10.4	10.4	22.5	35.1
Ni	13.9	8.3	14.2	32.2	6.8
Pb	0	8.8	29.1	61.4	4.4
Zn	0.3	0.4	50.3	18.2	9.3

* KNO_3 - exchangeable, KF - adsorbed, $Na_2P_2O_7$ - organically bound, EDTA - carbonate, HNO_3 - sulphide.

is usually not found in the residual fraction, which reflects the low natural concentration of Cd.

A separation of fresh wet sludge into a range of particle sizes showed that Cd was mainly associated with the low molecular weight fraction (82). After centrifugation, 3-25 % of the Cd was found in the supernatant (81).

Spiking the sewage sludge with Cd results in higher concentrations of Cd in the carbonate, organic and sulphide fractions, thus indicating that the additional Cd may be in a more tightly bound, and thus less bioavailable, form (31).

The overall conclusion is that Cd is generally weakly bound. Cadmium is also the only metal which is present in significant amounts in the exchangeable or water soluble phases. This indicates that Cd could be mobile in soil water and probably also comparably available for uptake by organisms.

4.1.2 Chromium

Studies have shown that 64 % of the Cr is found in the oxidisable phase, extracted by H_2O_2 (7,81). This is confirmed by other workers who found 71 % of the Cr in the organic fraction extracted by H_2O_2 (9). Another study found 38% in the Fe/Mn oxide fraction and 49 % in the residual fraction (26). No workers have found more than a few percent of the Cr in the adsorbed or exchangeable fraction (7,9,26,81). Only a few workers have interested themselves in Cr concentrations in sewage sludge which makes it difficult to draw any general conclusions. However, it seems that Cr would be relatively strongly bound in sludge applied to soils and thus it would be unlikely to leak out into soil water or be available for plant uptake .

4.1.3 Copper

Copper has a different distribution pattern than Cd and requires stronger reagents to be extracted. Around 75 % of the Cu was found in the organic, sulphide and carbonate fractions in one study (28) and other studies found more than 90 % of the Cu in the oxidisable phase extracted by H_2O_2 (7,81). These trends are confirmed by other workers (31). Other workers found 32 % of the Cu in the Fe/Mn oxide and 55 % in the residual phase (26). One study has shown that 70 % of the Cu is found in the sulphide phase (27). However, another study could not comfirm this but demonstrated instead that most of the Cu was extractable with $MgCl_2$ (30) which can be due to formation of a chloro complex. However, most workers have found Cu to be strongly bound to the sludge, mostly in the organic phase. This means that Cu is not likely to be bioavailable, unless the organic phase is degraded.

4.1.4 Nickel

According to some workers, Ni is mostly found in the EDTA-extractable carbonate fraction which contains 50 % of the Ni, while the rest of the Ni is quite evenly distributed in the other fractions (27). This was confirmed in a study on model compounds (30). However, other workers have found results which totally disagree. In one study, 55% of the Ni was found in the residual fraction, 32 % in the Fe/Mn oxide fraction and only 12 % in the carbonate ("acid soluble") fraction (26). This may be a result of addition of residual Ni with the coagulant which has been proved to contain significant concentrations of Ni. Another study shows that 43 % of the Ni is found in the Fe/Mn oxide fraction and 20 % in the carbonate, organic and residual fractions, respectively (9). However, too few results are yet available to draw any reliable conclusions concerning which fraction Ni would really be expected to be found in.

4.1.5 Lead

The oxidisable fraction has been shown to contain 85 % of the Pb and the rest is found in the residual fraction (7,81). Another study points out the residual fraction as the most important fraction for Pb, containing 72 % (26). Others have found 53 % of the Pb in the carbonate (EDTA-extractable) fraction and 36 % in the organic fraction, extracted by $Na_4P_2O_7$ (27). The adsorbed or exchangeable fraction contained less than 1 % (7,27) or around 4 % (26). A study on model compounds showed that 70 % of the Pb in the liquid sample and 86 % of the Pb in the dried sample was extracted by H_2O_2 when discrete extraction was used. The percentage for NaOAc was 76% and 47% respectively and for $Na_4P_2O_7$, 22% and 47% (30). For a sequential extraction procedure the percentage was 70% and 72% for NaoAc and less than 15% for all other extractions, which shows the difficulty of comparing sequential extractions to single extractions or even to other sequential extractions when applying the extractants in a different order (30). The overall conclusion must be that most of the Pb is found in the fractions that are not easily extracted, which means that Pb would be expected to be comparably unavailable to organisms and to have low mobility in sludge-amended soils.

4.1.6 Zinc

Zinc was found to be readily extracted from sewage sludge. An extraction with Tessier's scheme found 72 % of the Zn in the Fe/Mn oxide fraction, 15 and 17 % in the carbonate and organic fractions, 3 % in the exchangeable fraction and only 2 % in the residual fraction (9). Another study confirmed the Fe/Mn oxides as an important fraction containing 32 % of the Zn, but on the other hand this study found as much as 42 % of the Zn in the residual phase (26). A discrete extraction procedure applied to a model compound showed yet another result (30). H_2O_2 extracted 95 % of the Zn in liquid sludge and 100 % in the dried sludge, NaOAc extracted 83 and 58 % respectively and NaOH, supposed to extract the organic fraction, extracted 73 and 45 %. This is confirmed in other studies where some workers found 85 % of the Zn in the organic fraction of digested sewage sludge (7,81) and other workers found 50% of the Zn in the organic fraction and the rest in the carbonate and sulphide fractions (27). However, the almost complete extraction of Zn by H_2O_2 does not necessarily mean that all the Zn is to be found in the organic fraction but rather is an illustration of the lack of selectivity of some extractants (30). A comparison to a sequential extraction shows that $MgCl_2$, which is assumed to extract the exchangeable fraction, is the most effective extractant for Zn and extracts 37 % of the Zn in the liquid sludge and 59 % in the dried sludge. This can be compared to the 44 and 30 % extracted by NaOAc which is supposed to extract the organic fraction (30). This study clearly indicates that it is almost impossible to determine the distribution of a metal in a sewage sludge without a very thorough description of the method and that it is necessary to repeat the same extractants in the same order if a comparison between two experiments is to be performed. In this study, the impact of drying is also clearly shown; $Na_4P_2O_7$ extracted 8 % of the Zn in the liquid sludge, but as much as 60 % in the same sludge after drying (30).

A study of metal speciation of spiked sludge showed that 50 % of the Zn is organically bound in both primary, secondary and digested sludges and that the additional Zn in the spiked sludge redistributes itself in more or less the same fashion (28). The same authors have also investigated the effects of a thousandfold increase of Zn and found minor changes in the fractionation pattern, mostly an increase in the EDTA-extractable fraction (83). However, all studies do not show the same results. One study confirmed that most of the Zn, 66 %, is found in the organic fraction, but the additional Zn was found almost entirely in the carbonate and the sulphide fractions, whereas the Zn content in the organic fraction did not significantly increase (31).

The results of Zn speciation and fractionation show that Zn is most likely to be found in the organic phase. However, the results are not unequivocal.

4.2 Interpretation of speciation results

A comparison of different studies of metal distribution in sewage sludge by extraction is difficult. Sewage sludge from different sewage works give different results. Also, the different metals have different distribution patterns. For example, Cd is the only metal that is found to any extent in the exchangeable fraction and very little Cd is found in the residual fraction, while extraction of Pb requires dissolution of the organic and Fe/Mn oxides, or in some sludges even the residual fraction.

The same sludge can give different results depending on the treatment of the sludge both at the sewage works and in the laboratory. Dewatering, digestion, composting and liming alter the metal speciation and drying of the sludge prior to the analysis can also disturb the metal distribution in the sludge.

Although the sludge fractions have the same names in different studies, they are not in the physical meaning distinguishable but are defined as the part of the sludge which is extracted by one or more extractants. For example, table 4.1 shows that the carbonate fraction can be extracted by five different extractants, each of them giving a slightly different result. Some studies have also clearly shown that sequential, simultaneous or single extractions give different results. In the case of sequential extraction, the order of application is critical important.

The conclusion must be that it is of utmost importance to report all parameters including the exact extraction procedure when reporting results from fractionation experiments. Satisfactory conclusions can only be obtained if sufficient attention is paid to clearly defining the conditions for extraction. Also, all metals have to be studied individually, since different metals behave differently in the sludge, both because of their different chemical properties and because of their different origin.

5. Sludge-amended soils

The behaviour of heavy metals in sludge and sludge-amended soil depends on many parameters, such as the complexing capacity and the pH of the sludge and the soil. These parameters can change with time which means that to determine the bioavailability of metals some years after sludge application, a new study of the soil parameters and metal speciation is needed.

5.1 Complexing agents and stability constants in sewage sludge.

Sewage sludge contains a variety of complexing agents, mostly organic. Some enter the sludge with the sewage, while some originate in the process, for example, chemicals added in the waterworks and bacterial extracellular polymers (6,40). The presence of these complexing agents is one of the most important factors affecting the distribution of metal between the different fractions (17). However, the effect of complex formation varies between metals. It has been shown that most Cu in sludge-amended soil solution is in the form of organic complexes, whereas complexes of Cd account for only a small proportion of the total metal in the soil solution (17). However, sludge leachate with high organic content can bind, and thus transport, enough Cd to pose a risk to groundwater quality (84). When all the fractions of a sludge-amended soil are taken into account, a considerable part of Cd, Ni and Zn is complex bound to organic matter. Humic substances appear to be the most important complexant for Cd (42). Sludge addition seems to increase the complexation capacity of soil, but at the same time, complex stability decreases (17). However, the overall effect is that sludge application restricts heavy metal mobility, and thus toxicity, in soil (1,11,17,85). A considerable portion of soil organic matter is humic acids which have a large binding capacity for metals. These binding sites are usually far from saturated and sludge-amendment can change the soil conditions in a way that favours complexation of the added metals. This provides one explanation to the fact that sludge-amendment can result in lower levels of mobile metals, even though total metal concentrations may be increased (16). There are also results that show increased mobility of metals after sludge-amendment (23).

There are different kinds of binding sites, but it has been shown that most of them are carboxyl, phenol and hydroxyl functional groups. There are also amide and thiol groups (14). Different metals bind to different groups. The binding capacity for Cd, Cu and Zn is decreased if the carboxyl and phenol groups are methylated, but Ni is affected very little (14). The structure of these binding sites is changed during anaerobic digestion at the sewage treatment plant which means that the binding capacity for metals is different in raw and in digested sludge (7). Also, the binding capacity and the strength of the complex depends on factors such as pH and the presence of competing metal ions, for example Ca in limed sludge. Composting of sewage sludge changes the speciation significantly. Most metal concentrations increase, but Cu and Ni concentrations decrease (32). This has the important implication that a sludge that did meet the requirements for spreading on soil before composting may not meet these requirements after composting. This is especially true for Cd which can show an increase of up to 58% (32).

5.2 Metal uptake and soil characteristics

The interest in bioavailability has focused mostly on plants, since measurement of plant metal concentrations are important to assess the human exposure of metals. Plant uptake depends very much on soil parameters. As mentioned earlier, sludge amendment can decrease the bioavailability of metals because of the additional binding sites that are provided in the sludge (1,16,17). Liming is a very important factor which decreases metal uptake (86). Sludge-bound metals are more easily taken up by wheat in sandy soil than plants growing on silty clay loam soils (2).

One of the most important factors is soil pH (20). A low pH enhances metal uptake. An experiment with progressive acidification of dried and liquid sludge revealed that significant quantities of Zn were mobilised at pH 4. Cadmium and Pb were not mobilised until pH 2, but Ni was easily mobilised at all pH values from 7 and downwards. All metals showed increasing mobility with decreasing pH, except Cu, which was hardly mobile at all above pH 0.5 (27).

Nitrogen-containing fertilisers can enhance Cd uptake (87). This can be explained in terms of ion exchange processes. The added base cations not only displace part of the exchangeable Cd, but also lower the soil pH by displacing H^+ ions. This shows the importance of taking into account not only the properties of the sewage sludge before applying it to soil, but also the characteristics of the soil itself. Also, the combination of sludge-amendment and other fertilisers or lime can affect metal uptake. Other important factors are CEC (Cation Exchange Capacity) and clay content (23).

5.3 Persistence of metals in sludge-amended soil

Metals in sludge-amended soil can leave the soil in two ways: they can be taken up by plants and animals or they can migrate to a lower layer where they can leak out into adjacent waterways or groundwater. A study of migration of metals in soil amended with sewage sludge containing large amounts of Cd and Ni showed that Cd is the metal which migrates most readily in soil, 60-80 cm in 7 years. Cd was found mostly in the exchangeable phase. Ni, which was primarily associated with the oxidisable phase, migrated 40-60 cm, Pb, found in the acid-soluble and reducible phases, migrated 20-40 cm and Cr, which was mainly in the residual phase, remained in the upper layer (39). The actual depth of metal penetration is dependent on the soil type. In this case (39) the soil had a very coarse texture which would faciliate the movement of the metals. However, these results indicate a connection between speciation and migration. In other types of soils, it has been shown that the metals associated with the sewage remained in the topsoil for many years, even though the area was regularly ploughed (88).

Other workers have investigated the migration of metals in soil and found that most metals remain in the upper 5 cm, but movements to a depth of 10 cm are observed for all seven metals, Cd, Cr, Cu, Mo, Ni Pb and Zn (89). The results are shown in figure 5.3.

It must also be remembered that the structure of the binding sites, and thus the speciation, can change significantly with time after application to soil. This may lead to

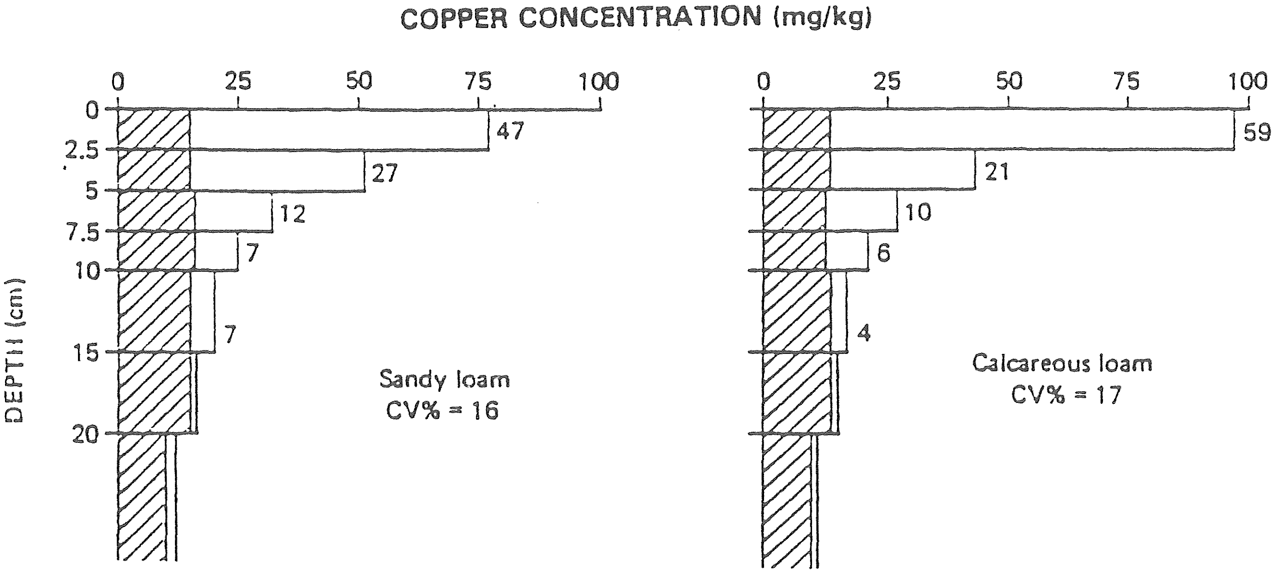


Figure 5.3 Distribution of Cu through soil profiles, from Davis *et al* (89)

remobilisation of metals, thus rendering formerly immobile metals bioavailable (14). However, it has been shown that this process can be very slow (4). This means that the metals persist in soils for many years and less than 0.05 % of the sludge metals added annually are removed in the crop (1,4).

6. The biological relevance of metal speciation in sewage sludge and sludge amended soils

All metal speciation schemes suffer from severe problems with specificity, selectivity and readsorption. Even if all these problems are solved, there is still the question of what the different fractionation results really mean in terms of bioavailability (76). A good analytical reproducibility does not necessarily imply meaningful results. The only way to determine the usefulness of these extractions is by comparison with biological experiments. Many workers have investigated the impact of elevated metal concentrations on living organisms, and some of them have compared different extractants to uptake by plants and concentrations in animal tissue.

6.1 Uptake and effects of metals on different organisms

Heavy metals can enter the metabolism and thus have toxic effects on most life-forms. However, since metabolism differs between microbes, plants and animals, these groups will be considered separately.

6.1.1 Microbial effects

If the sludge is spread on soil, metals may have a direct toxic effect on soil microorganisms. Some workers have studied the influence of various heavy metals on soil microorganisms (24). Fungi and actinomycetes were the most resistant soil microorganisms and *Azotobacter* and nitrifiers appeared to be the most sensitive. This study also showed that different metal species have different toxicity. Zinc and Cu were shown to suppress enzyme activity and respiration. Small doses of these metals had a beneficial effect on microbial growth which perhaps could be explained if the soil was deficient in these metals. However, small doses of As and Pb gave a similar beneficial effect, although these metals have no known biological function. All examined compounds of Hg were very harmful to soil bacteria and stimulated actinomycetes. This means that adding toxic metals to soil does not kill all forms of life to the same extent, but rather changes the population structure of the microbial community.

Metals are often involved in the metabolism of the microorganism. One way to metabolise metals is methylation. This is a process which makes the metals more hydrophobic and therefore more easily incorporated in the biosphere. Biomethylation has been reported for Pb (40) and for Pt (47).

However, investigation of these problems is difficult because of the complexity of both the sludge matrix and the microbial population. The short generation time of the organisms makes it necessary to take into account phenomena such as adaption of bacteria and changes in the population composition (90). For example, bacteria of the *Flavobacteria* species are naturally Cd resistant and *Pseudomonas* bacteria can develop tolerance in sludge-amended soils (1). However, in assessing concentration limits, it cannot be assumed that organisms will evolve an increased tolerance (90).

6.1.2 Metal concentrations in plants

All plants have means to take up essential metals from soil. These mechanisms are designed to provide the plants with the metals necessary for plant growth and function. Different plants accumulate different amounts of metals. Most dicotyledoneous plants absorb heavy metals to a greater extent than monocotyledoneous (23). Cereals and legumes accumulate less metals than leafy plants like lettuce and spinach (11). Carrots and tomatoes accumulate several metals to a significant degree (14). The distribution of the metal in the plant is important in determining whether the metals may enter the food chain. Fortunately, it seems that peas and beans have lower levels of metals in the edible parts of the plant compared to the leaves, while cereals generally do not accumulate metals in the grain (14,23).

The uptake mechanism functions in different ways for different metals. Some plants seem to have the ability to discriminate against heavy metals by either excluding them or retaining them in the roots (23). One study shows that Cd, Cu, Ni and Zn can be retained in the roots, whereas root concentrations of Cr and Pb were even lower than the soil concentrations. The authors state that this is not the result of some barrier function, but rather depends on the particularly strong binding of Cr and Pb in the soil (23).

Other workers have found that Zn and Cd are taken up by roots and transported to the aerial parts of the plant, Cu and Pb are mostly retained in the roots and Ni seems to be evenly distributed in the plants (14).

The uptake of different metals for 12 food crops was investigated (1). The species that accumulated most Cd were, tobacco > lettuce > spinach > celery > cabbage; most Pb were, kale > ryegrass > celery; most Cu were, sugar beet > some varieties of barley; most Ni were, sugar beet > ryegrass > mangold > turnip and most Zn were, sugar beet > mangold > turnip. The Cd, Cu, Pb and Zn contents were generally highest in the roots. Cadmium was accumulated by spinach and lettuce which had relatively low Ni content, whereas ryegrass which accumulates Ni had low Cd concentrations (23).

Tobacco has been recognised as a high accumulator of heavy metals such as Cd. Considering that smoking of tobacco products has been identified as a significant contributor to the human Cd load, it has been suggested that tobacco should not be grown on sludge-amended soil (91). Experiments have shown that tobacco plant concentrations of Cd, Cu, Mn, Ni and Zn increase with increased sludge application rates, Fe concentration decreases and Pb concentration is unaffected.

Metals taken up by plants have different effects. An increase in wheat plant concentration of Cr, Cu, Mn and Zn, caused both by irrigation with sewage and by sludge amendment was observed to give decreased activity of the enzymes; aspartate amino transferase, alanine amino transferase and peroxidase (92).

6.1.3 Effects on animals

Lead uptake by earthworms has been studied (93,94). Calcium in the soil seems to affect Pb uptake; Ca in the diet suppresses lead accumulation by earthworms and earthworm species that have active calcium secreting glands have been found to contain lower Pb concentrations than species with less active Ca excretion inhabiting the same soil. A measurement of Cd, Cu, Pb and Zn in earthworms revealed that Cd concentrations were generally higher in the earthworms than in the soil, whereas Cu and Pb concentrations were lower (95). The results suggest that Cu and Zn accumulation may be physiologically regulated in earthworms. Other workers confirm these results and suggest that the earthworm *Lumbricus rubellus* can be used as an indicator species for investigating the rates of heavy metal uptake within terrestrial ecosystems (96).

Other workers have investigated the effect of heavy metals on development, reproductive capacity and viability of the grasshopper *Aiolopus thalassinus* (97). Cadmium, Hg and Pb had adverse effects on both lifespan and weight of the adult grasshoppers and the hatchability of the nymphs was also affected.

However, the most interesting question is of course how the metals affect animals which are used for food. Pharmacokinetic analyses have been conducted on published experimental data to estimate biotransfer factors for bovine milk (98). Some metals, especially Pb and As, can be transferred to milk when cows are fed with food containing metal salts, but metals from sewage sludge were not found to produce significant elevations of metals in the milk. These findings suggest that metals in sludge may not be as bioavailable as metal salts.

6.2 Prediction models

Since heavy metals have adverse effects on organisms and thus are a potential threat to crops grown on sludge-amended soils, methods to predict metal uptake, for example in plants, have become interesting.

6.2.1 Computer modeling

The United States Environmental Protection Agency (USEPA) has developed a model for predicting heavy metal uptake by plants. This model is meant to be used for deciding whether a sludge can be permitted to use for land application. However, it has been shown that it does not work as it was meant (13). The USEPA model predicts that plant tissue metal concentration will be linearly related to metal loading rates in the soil, independent of soil structure, pH and other soil parameters. A comparison with field experiments shows that the model underpredicts Cd concentrations in leaves from soils with $pH < 7$ and overestimates leaf Cu for all fields and corn grain Cd and Zn concentrations. At one site, almost all the plant tissue metal concentrations were overestimated with this model. These results show the difficulty of making general predictions of metal uptake without thorough analysis of the local soil characteristics. For example, studies comparing limed soil to unlimed soil have shown that soil pH is crucial for metal uptake by the plants (86).

GEOCHEM is a multi-purpose computer program for calculating the equilibrium speciation of the chemical elements in a soil solution. This program has been partially successful in the correlation of plant uptake to simple parameters (11,15). Corn uptake of Cd was strongly correlated with the concentration of $CdCl^+$ in soil, but not to the

Cd^{2+} concentration. One drawback of this model is that an extensive amount of input data is needed which demands a thorough investigation of the soil and sludge characteristics.

A simpler appoach is to calculate transfer coefficients (TC) for different metals. This coefficient is often defined as metal concentration in plant / metal concentration in soil (23). A TC above 1 means that the metal is accumulated in the plants and a TC below 1 means that the plant takes up less than the average soil concentration. Cd and Zn have TC, 1-10; Ni and Cu have TC, 0.1-1 and Pb, Cr and Hg have TC, 0.01-0.1 (23).

6.2.2 Correlations between metal uptake and extractions

The aim of all extraction methods is to find a correlation between biological uptake and an extraction method, preferably a single extractant. As previously mentioned, this is extremely difficult due to the great differences between different sludges and different species.

The uptake of Cd by four vegetables grown on soils contaminated from a variety of sources was investigated in a study in which 23 different soil parameters were compared with Cd concentration in the vegetables (11). Out of these 23 variables, only 8 were significantly related to Cd accumulation. The most frequently occurring parameter was total Cd, which was inversely related to plant Cd. This illustrates that just because a sewage sludge has a high metal content it is not necessarily unsuitable for agricultural application. Of course, the opposite is also very true; a low metal concentration is no guarantee for "safe" plant concentrations. Some workers concluded that plant availability of heavy metals is not always predictable by extraction with DTPA (33).

However, there are some workers who have managed to find a significant correlation between a soil test and plant heavy metal concentrations. The results in table 6.1 identify DTPA and $CaCl_2$ as the most suitable extractants to mimic plant uptake, at least for metals such as Cd, Cu and Zn (1). Investigations of transfer of Cd to food crops also showed that DTPA extraction predicts Cd uptake for potatoes, lettuce and cabbage better than $CaCl_2$, EDTA and NH_4NO_3 (86). Other workers confirm the suitability of $CaCl_2$ for predicting metal concentrations in clover, barley and red beet (99).

Addition of a chelating agent such as EDTA or DNP (2,4-Dinitrophenol) to soil can result in lower uptake of heavy metals in plants. Tomatoes showed a reduced accumulation of Cd from soil to which EDTA and DNP was added (100). This may be caused by a decrease in the concentrations of free Cd ions. On the other hand, EDTA and DNP addition enhanced the transport of Cd from the roots into the above-ground plant parts. These results suggest that Cd-complexes are formed in the roots, which indicates that EDTA can be taken up by the roots, perhaps as a Cd-EDTA complex. This means that if the interest is mainly focused on the metal concentrations in the edible parts of the plant, addition of EDTA (and perhaps also DNP) can have both beneficial and adverse effects.

Sewage sludge has proved to be an excellent medium for growing the alga *Chlorella*

Reagent	Element(s)	Crop	Ref.
0.005 *M* DTPA	Zn, Cd, Cu, Mn	Tobacco	Adamu et al. (1989)
0.005 *M* DTPA	Ni	Lettuce	Browne et al. (1984)
0.005 *M* DTPA	Cu	Soya beans	Adams and Kissel (1989)
0.005 *M* DTPA	Cu, Cd		Morel et al. (1988)
0.005 *M* DTPA	Cd	Wheat	Juwarka and Shende (1986)
0.005 *M* DTPA	Zn, Cu, Mn, Ni, Cd	Tobacco	Mulchi et al. (1987)
0.005 *M* DTPA	Cd	Potato	Jackson (1990)
Ammonium bicarbonate DTPA	Cd, Cu, Ni, Pb, Zn	Swiss chard	Barbarick and Workman (1987)
Ammonium bicarbonate DTPA	Mo		Pierzynski and Jacobs (1986)
DTPA, EDTA, $CaCl_2$	Zn, Cu, Ni	Ryegrass	Sanders et al. (1987)
EDTA	Cd, Zn		Fujii and Corey (1986)
0.43 *M* acetic acid	Ni	Ryegrass, clover	Burridge and Berrow (1984)
0.01 *M* $CaCl_2$	Zn, Cu, Ni		Sanders et al. (1987)
0.01 *M* $CaCl_2$	Cd, Zn		Sauerbeck and Styperek (1984)
0.01 *M* $CaCl_2$	Cd	Lettuce, radish, carrot, cabbage	Alloway et al. (1985)
0.1 *M* $NaNO_3$	Cd		Hani and Gupta (1985)
1 *M* NH_4NO_3	Cd	Lettuce, radish, carrot, cabbage	Morgan and Alloway (1985)

Table 6.1 Soil tests significantly correlated with plant heavy metal concentration, from Alloway and Jackson (1).

pyrenoidosa, but the growth is inhibited by heavy metals. Addition of EDTA favours the growth of algal cells in activated sludge, thus implying that the toxicity of the various heavy metals is removed by this action. However, excessive EDTA addition might lead to nutrient deficiency due to chelation of essential elements (101). Generally, current research points to EDTA as a mediator of metal toxicity in sewage sludge and therefore it would appear that an extraction with EDTA may be a suitable indication of metal toxicity or bioavailability (table 6.1). Figure 6.1 shows the correlation between the HNO_3 and $NaNO_3$ soluble Cu and the Cu content of rye grass (102).

The concentration of Cd found in earthworms (*Lumbricus rubellus* and *Dendrodilus rubidus*) shows a relation to the fraction extracted by 0.5 % acetic acid that approaches direct proportionality (95).

6.3 Are metals in sewage sludge a threat to human health?

Many cases of metal poisoning has been reported in history. Very often, reported health hazards are discovered in industrial workers who have been exposed to high levels over a long period. Less is known of the long term effects of trace concentrations.

Acute Cd poisoning was described as early as 1867, the symptoms being giddiness, vomiting, syncope, respiration difficulties, loss of consciousness and cramps (42). It took almost 100 years to recognise the health hazards resulting from long term exposure. Kidney and lung damage, anosmia (loss of the sense of smell), anaemia, hypertension and severe osteoporosis are symptoms of Cd poisoning. The "Itai-Itai" incident in Japan showed that Cd poisoning could be a danger to the general public and not only to workers in industries using Cd. The source of Cd in that case was rice which had been irrigated with water contaminated with mining effluents. Cadmium is also believed to be carcinogenic (42).

Lead poisoning is considered a problem for workers in battery factories, but also for children exposed to elevated soil Pb levels (18). The main hazard in the case of children

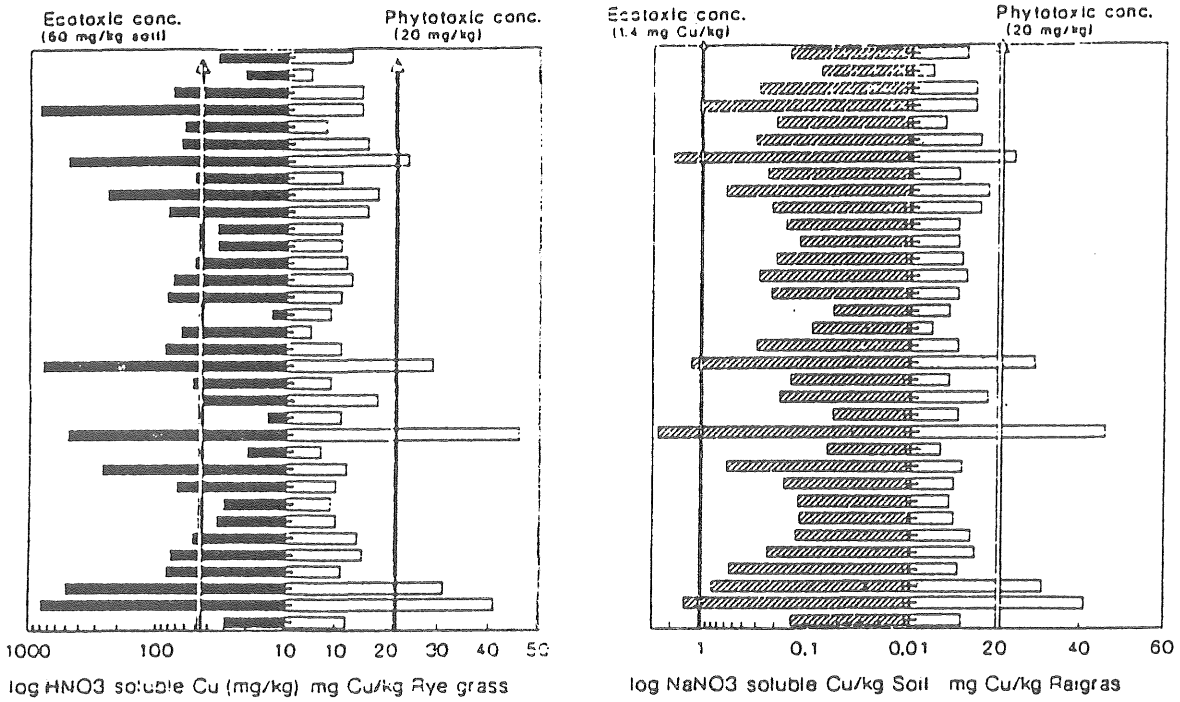


Figure 6.1 Effect of HNO_3 and $NaNO_3$ soluble Cu concentration in different soils and the Cu contents of rye grass (102)

seems to be lead-based paint and lead dust from traffic. Iron and Ca deficiency causes increased uptake of Pb. Lead poisoning causes neurological damage and disturbs the function of the red blood cells (103).

Consumption of fish and seafoods is the primary exposure route for Hg. For As, which is suspected to cause skin diseases, including cancer, contaminated drinking water is the most important environmental route (103).

Lately, some attention have been paid to the problem with metal levels that are too low to give easily recognisable acute effects. Many heavy metals are believed to exert synergistic effects in combination with other metals, and some believe that several million people suffer from subclinical metal poisoning (103). It is difficult to discover the effects of low doses of a combination of many metals, since the symptoms are sometimes very diffuse and general and the correlation to metal poisoning is sometimes not very clear. Some examples of the symptoms of heavy metal poisoning are hypertonia, immune suppression and allergies. Metals like Al, As, Be, Cd, Co, Cr, Hg, Ni and Sb can adversely affect human reproduction, from infertility and genotoxic effects to pregnancy complications, low birth weight and impaired postnatal development. At least 18 trace elements (Al, As, Be, Bi, Cd, Co, Cr, Cu, Fe, La, Mn, Ni, Pb, Sb, Se, Ti, V and Zn) have been shown to initiate or promote cancer in animals (103). The possibility of widespread health effects from low levels of metals points out a field with a great need of more investigation and is also a strong reason to limit the distribution of heavy metals in the biosphere.

At present the heavy metal content in Swedish soils is comparably low. It is doubtful if metals from sewage sludge can cause any acute effects on human health. It is even difficult to show any correlation of metal concentrations with plant uptake. However, the possibility of chronic effects remains. A deeper knowledge of the interactions of metals in sewage sludge with the soil environment is necessary to assess the potential of metal transfer into the food chain. Further investigation of the key parameters affecting uptake is required to understand metal speciation and its consequences for human health.

7. Future research and conclusions

7.1 Conclusions

Some general conclusions can be drawn from this study of the literature:

(a) The most studied metals in sewage sludge are Cd, Cr, Cu, Ni, Pb and Zn. The main sources are industrial discharge (especially for Cr and Ni), urban runoff and domestic wastewater. Corrosion of drinking water pipes is an important source of Cu. The contribution from industry is decreasing, which makes urban runoff more important.

(b) There are a number of methods to fractionate sludge metals with different chemical extractants. The most commonly used methods divide the sludge into the following fractions: exchangeable, carbonate, Fe/Mn oxides, organic and residual. However, the selectivity and specificity of most of these methods is poor and it is difficult to compare the results from different methods.

(c) The fractionation results show that Cd is probably the most mobile metal, followed by Zn. These two metals might be expected to be bioavailable to a significant extent. Copper is mostly bound in the organic fraction and is not likely to be taken up by organisms. The most tightly bound metal is Pb, which means that it would be comparably unavailable to organisms.

(d) The plant uptake of metals from sludge-amended soil depend not only on metal speciation in the sludge, but also on soil properties, such as pH and the presence of complexing agents. Plant uptake also varies between different plants and different metals. Tobacco, for instance, accumulates Cd very efficiently and is therefore not recommended for cultivation on sludge-amended soil. Leafy plants accumulate more metals than cereals and legumes. Fortunately, many plants have low metal concentrations in their edible parts.

(e) DTPA, $CaCl_2$ and EDTA extraction have been found to correlate positively with plant uptake in some experiments. However, the results are not unambiguous. There seems to be no simple relation between plant uptake and total metal level.

7.2 Further work

The use of sewage sludge as a soil improver is in many ways a good idea; it turns a by-product which must be disposed of into a valuable source of plant nutrients. However, before agricultural use, evaluation of sludge must be carried out in order to prevent adverse effects of sludge-bound metals on plants and animals. How this should be done is a matter of discussion and a review of the literature points to the following questions as important for future research:

(a) The question whether a sewage sludge containing metals is suitable for agricultural application should be determined on the basis of the condition of the receiving soil. In determining the limit concentrations not only acute toxicity, but also long term effects have to be considered. The maximum tolerable levels depend on soil factors such as pH, CEC and content of organic complexing substances. Also important is the metal concentration already present in the soil.

(b) To determine the effects of sludge-amendment, more research is needed about how uptake of metals by organisms is affected by soil parameters. Special attention should be paid to the influence of soil pH. It has been shown that a lower pH enhances mobility of metals in the soil, and thus the bioavailability, which indicates that a lower metal concentration can be tolerated on acidified soils. This is especially important in Sweden, where many soils have a poor buffer capacity.

(c) Metal fractionation could be a valuable tool in assessing the bioavailability of sludge metals. However, there is a need for a more clear definition of the meaning of a metal fraction. The results of metal fractionation must also be related to soil ecotoxicology.

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