In situ immobilization of cadmium and zinc in contaminated soils

Fiction or fixation?

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Leonard Osté

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Abstract

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It is generally assumed that a decrease in metal concentration in the soil solution reduces metal leaching, and metal uptake by and toxicity to plants and soil organisms. *In situ* immobilization is a soil remediation technique that aims at reducing the metal concentration in the soil solution by adding a binding material to the soil. Application of this technique requires understanding of underlying mechanisms and potential side effects.

Both laboratory experiments and model calculations have been performed to gain insight in immobilizing processes. It is essential to quantify metal binding to natural organic matter. The NICA-Donnan model was designed to calculate metal binding by organic materials, but specific Zn parameters were not available due to a lack of analytical data. The Wageningen Donnan Membrane Technique (WDMT) was therefore further developed to measure free Zn concentrations in humic acid solutions.

Many immobilizing materials increase the soil pH. This results in an increased negative charge of soil particles, and hence in a decreased metal mobility. In some cases, the addition of alkaline materials simultaneously increases the dissolved organic matter (DOM) concentration in the soil solution, resulting in increased leaching of metal-DOM complexes. We showed that alkaline soil amendments need to contain enough Ca to suppress the dispersion of organic matter as induced by a pH increase. We also quantified the dispersion of organic matter. It appeared that the Donnan potential of the organic matter, as calculated by the NICA-Donnan model, correlated very well with the DOM concentration.

The addition of alkaline materials strongly decreased the metal concentration in Swiss chard (*Beta vulgaris* L. var. *cicla*). In contrast, the uptake of Cd and Zn by earthworms (*Lumbricus rubellus* and *Eisenia veneta*) was hardly influenced by the addition of alkaline materials. Another experiment showed that the addition of MnO_2, which did not affect soil pH, resulted in a decreased Cd concentration in the earthworm tissue. Apparently, next to dermal uptake, pH independent Cd uptake via the intestine was an important uptake route. Cd uptake by earthworms could be estimated by a soil extraction with 0.1 M triethanolamine and 0.01 M $CaCl_2$ buffered at pH 7.2.

Keywords: beringite, cadmium, DOC, DOM, earthworms, immobilization, leaching, lime, manganese oxides, metal binding, metal uptake, organic matter partitioning, pH, soil contamination, remediation, sorption, Swiss chard, zeolites, zinc.

Abbreviations and Acronyms

CA	Cyclonic ashes (also called: Beringite)
CEC	Cation Exchange Capacity
CLIN	Clinoptilolite
DGT	Diffuse Gradients in Thin-films
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
ECOSAT	Equilibrium Calculation program On Speciation And Transport
FAAS	Flame Atomic Absorption Spectrometry
FAU	Faujasite-type zeolite
FIAM	Free Ion Activity Model
FSHA	Forest Soil Humic Acid
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
ICP MS	Inductively Coupled Plasma with Mass Spectrometry
ICP OES	Inductively Coupled Plasma with Optical Emission Spectrometry
ISE	Ion Specific Electrode
$[Me^{2+}]_{pw}$	Free metal concentration in pore water
MOR	Mordenite-type zeolite
NICA	Non Ideal Competitive Adsorption
NICA-Donnan	NICA combined with an electrostatic Donnan model
NOM	Natural Organic Matter
OM_{mobile}	Potentially mobile fraction of the soil organic matter
PZC	Point of Zero Charge
R^2	Coefficient of determination
RMSE	Root Mean Square Error
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TEA	Triethanolamine
TOC	Total Organic Carbon
WDMT	Wageningen Donnan Membrane Technique
WHC	Water Holding Capacity
WTB	Water Treatment Biosolids

Stellingen

1. Het immobiliseren van cadmium en zink in (matig) zure gronden is praktisch onmogelijk zonder de pH van de bodem te verhogen.

   Dit proefschrift

2. De grote bijdrage van de darmopname resulteert in een klein of verwaarloosbaar effect van immobiliserende stoffen op de accumulatie van cadmium in regenwormen.

   Dit proefschrift

3. De kolomproef zoals beschreven in NEN 7343 is ondeugdelijk om de uitloging van metalen uit verontreinigde grond onder praktijkcondities te schatten.

   Nederlands Normalisatie Instituut. **1995**. *NEN 7343: Uitlogingskarakteristieken van vaste grondachtige en steenachtige bouw- en afvalstoffen. Uitloogtesten. Bepaling van de uitloging van anorganische componenten uit granulaire materialen met een kolomproef.* 1e ed. NNI, Delft.

4. Iemand die is aangewezen op water dat verontreinigd is met koper en/of lood, kan dit het beste innemen in de vorm van koffie.

   Impellitteri, C.A., H.E. Allen, G. Lagos, and M.J. McLaughlin. **2000**. Removal of soluble Cu and Pb by the automatic drip coffee brewing process: Application to risk assessment. *Human and ecological risk assessment* 6:313-322.

5. Bij het huidige budget voor waterbodemsanering is het milieu meer gebaat bij het storten van veel dan het reinigen van weinig baggerspecie.

   Advies en Kenniscentrum Waterbodems (AKWA). 2000. *Verwerking van baggerspecie. Basisdocument voor besluitvorming.* Rijkswaterstaat, Utrecht.

6. Er zijn veel overeenkomsten tussen het gevoerde overheidsbeleid ten aanzien van de veestapel en het autopark, maar een heilige koe blijkt moeilijker te ruimen.

7. Een hoog percentage medewerkers dat dichtbij het werk woont, is positief voor de werksfeer.

8. Topprestaties – bijvoorbeeld in de sport, maar ook in de wetenschap – kenmerken zich primair door schoonheid en niet door maatschappelijk nut.

Stellingen behorende bij het proefschrift "*In situ* immobilization of cadmium and zinc in contaminated soils: fiction or fixation?"

Leonard Osté
Wageningen, augustus 2001.

WTR	Water Treatment Residues
zeo A(Cf)	zeolite type A produced by Crosfield
zeo A(PQ)	zeolite type A produced by the PQ Corporation
zeo P	zeolite type P
zeo X	zeolite type X

Contents

Chapter 1

Introduction

Soil contamination and remediation

Soil remediation policy in the Netherlands

Soil remediation policy has been developed since the contaminated site in Lekkerkerk was found in 1980. From that moment, legislation was urgently needed, because Lekkerkerk appeared to be only one of many contaminated sites in the Netherlands. To speed up legislation, an Interim Soil Remediation Act was developed which came into operation in 1983. The idea that remediation would be a temporary activity was a second reason to develop a temporary act. Within a number of years all contaminated sites would have been cleaned up. The latter assumption appeared incorrect and after eleven years the Interim Soil Remediation Act was incorporated in the Soil Protection Act.

Until 1997 both soil protection and remediation were based on the principle of multifunctionality. In practice, a multifunctional soil should not exceed the target values for contaminants. If a soil has to be remediated, the total content of a contaminant should be reduced until the target value is reached. This policy encountered serious problems: social-economic and planning processes stagnated due to the cost of remediation of contaminated soils. As a result, the public basis for soil remediation decreased (Deelen and Roels, 1997) and the Dutch parliament decided to change the remediation policy in 1997. The change had three main objectives: 1) to realize cheaper soil remediation technologies, 2) to stimulate private initiatives and cofinancing, and 3) to improve legislation and speed up procedures. The new soil remediation policy is increasingly based on site-specific circumstances. Functional remediation, active soil management, and integration of soil remediation in other social-economic developments are the keywords of the new policy. Although the current standards are still based on the total concentration of a contaminant in the soil, the new approach provides better possibilities for so-called 'mild remediation techniques' that are focused on risk reduction, even if the total contaminant concentration still exceeds the target value.

The need for less rigorous remediation techniques is most evident in large (moderately) contaminated areas. Common techniques like excavation or extraction are physically and economically impossible. Moreover, these areas are often used for agriculture and forestry or designated as nature reserves. A thorough soil clean up would have adverse effects on the ecosystem and soil fertility. Mild remediation techniques aim to reduce the negative effects of contaminants, without damaging the essential properties of the soil. The Center for Agriculture and Environment developed a strategy for farmers to prevent soil contamination and to restore soil quality (Weckseler et al., 1995). They checked all available techniques on a number of criteria: environmental effect, cost, suitability for different contaminants, time needed for remediation, possibilities for combination with other techniques, adaptability in agricultural management, and suitability for different soil types. Considering these criteria, only a few techniques appeared to be useful. Bio-remediation is a promising technique for organic micro-pollutants, but metals are non-degradable. At the moment there are three methods that can potentially reduce the toxic effects of heavy metals in contaminated soil: 1) deep ploughing, 2) phytoremediation, and 3) chemical immobilization.

Deep ploughing can give a positive effect if only the upper layer of the soil is contaminated, but there are a few prerequisites. The underlying soil, which is to be mixed with the topsoil, must have a considerable fertility and binding capacity for heavy metals. Furthermore, the 'dilution factor' must be high enough to eliminate harmful effects, and the contaminated soil should not be mixed with soil below the ground water level.

Much attention has been paid to phyto-extraction. The technique is simple and elegant. (Hyper) accumulating plants are grown and the plants, containing a high metal concentration, are removed. The processing of the plants is essential to include in this approach. Ideally, the plants can be used for energy or fiber production. The largest problem of phyto-extraction is the duration of the treatment. (Brooks, 1998). Many hyperaccumulating plants are small plants. Even if the metal concentration is high, the total uptake of metals per hectare is limited (Ernst, 1996). Genetically modified plants and optimization of environmental conditions may improve the efficiency.

In situ immobilization decreases the metal concentration in the soil solution by adding a substance which strongly increases the binding capacity of the system. The total concentration in the soil remains the same, but toxicity and uptake of metals are often related to the soil solution concentration. We consider immobilization the most promising technique for treatment of large areas that have been moderately contaminated with heavy metals.

Heavy metals

Heavy metals are metals with a density over 6000 kg/m^3 (Alloway, 1995). With exception of iron, they belong to the group of trace elements, which means that the average concentration in the earth's crust does not exceed 1 g/kg. They become incorporated into igneous rocks by isomorphous substitution. During natural weathering processes the concentration of trace elements can change. However, contaminated soils are not naturally formed. Human activities introduce heavy metals into the environment. The primary sources are mining activities, including the mining of metal ores, but also fossil fuels and phosphate ores (Lexmond and De Haan, 1984). These activities result in redistribution of heavy metals. Metals are sometimes added to the soil with a certain goal, e.g. pesticides and fertilizers, but often soils are contaminated by solid waste, aerial deposition or contaminated water.

Metals occur in different species in the soil. Metal uptake by and toxicity to plants is weakly related to the metal content in the soil. The availability is related to the free ionic concentration at the biotic surface and the metal flux towards the surface to replenish the ions that have been taken up (Van Leeuwen, 2000). The metal flux is determined by the medium: water content, soil properties, and the lability of metal complexes in solution. The incorporation of all (kinetic) processes is very complicated. Often a good correlation is found between the free ionic concentration in the soil solution and uptake or toxicity. This is known as the Free Ion Activity Model or FIAM (Morel, 1983; Parker et al., 1995).

Cadmium

Two metals are studied in this thesis: cadmium and zinc. Cd is a relatively rare metal (in average soils ca. 0.5 mg/kg), and no biological function of Cd has been found. Main sources in soils are phosphate fertilizer, sewage sludge, and atmospheric deposition from industries. Concentrations in contaminated soils rarely cause acute toxicity to plants; long-term accumulation in mammals is the most important effect. Cd can cause kidney dysfunction and skeletal deformations. A notorious example of Cd toxicity is the 'itai-itai'-disease in Japan just after the second world war. Cd contaminated rice and drinking water combined with deficiencies of Ca, vitamin D, and protein, led to the death of 65 elderly mothers (Alloway, 1995).

Vegetable food is obviously the main route of Cd intake. Therefore, the Dutch Cd standards concerning food products are very low (Warenwet, 2000). Crops can exceed the food standards below the Dutch intervention values for soils.

Zinc

Zn is an essential trace element for humans, animals and higher plants. Zn acts as a catalytic or structural component in numerous enzymes involved in energy metabolism and in transcription and translation (Alloway, 1995). The natural concentration in soils is approximately 100 times higher (ca. 50 mg/kg) than the Cd concentration. Zn, as well as Cd, is relatively mobile in soils. Zn is introduced in soils by sewage sludge addition, atmospheric deposition, manure, and agrochemicals. Plants are relatively sensitive to both Zn deficiency and toxicity. In contrast to Cd, it is unlikely that crop consumption will produce Zn toxicity to mammals. Hoskam et al. (1982) nevertheless described one example of Zn toxicity to foals in the Kempen, but the problem ceased to exist from the moment that the aerial deposition of the Zn factories decreased because of a change in the industrial process. Only the combination of contaminated soil and aerial deposition can produce toxic Zn levels in vegetables.

Heavy metal contaminated soils

Widespread contamination is mostly caused by long-term input of relatively low amounts. There are different potential sources: aerial deposition, flooding by contaminated (river)water, and the long-term spreading of waste, sludge, fertilizers and other products containing heavy metals (Table 1.1).

Table 1.1: Surface area and sources of heavy-metal contaminated land in the Netherlands (modified from Landbouwadviescommissie milieukritische stoffen, 1991).

Area	Source	Surface area (km^2)
The Kempen	Aerial fallout from Zn factories	350
Loess soils Limburg	Aerial fallout from Zn factories	200
Flood plains	Contaminated river water and sediments	250
Harbor sludge soils	Contaminated river water and sediments	5
Toemaakdekken	Town refuse	180
Other agricultural soils	Various (e.g. fertilizer)	50
Total:		1,035

Later estimates of the contaminated area in the Kempen were smaller: 165 km^2 (Nieuwkoop, 1993) and 150 km^2 (Technical Committee on Soil Protection, 1997).

Nevertheless, it is a large area contaminated with Cd and Zn, due to the aerial fallout and waste of Zn factories on both sides of the border between Belgium and the Netherlands.

The zinc industry in the Kempen

The zinc industry has its origin in the part of Belgium where zinc ores could be mined: east of Liège. After the Belgian ore became exhausted, the industry switched to imported ore from Scandinavia. The transport from Antwerp to the Kempen was easier and, moreover, the negative effects of the Zn production (at that moment only emission of sulfur dioxide was known to be harmful) would not raise many problems in a poor and sparsely populated area like the Kempen. Only one factory was built in the Netherlands. Lucien and Emile Dor established the 'Société Anonyme des Zincs de la Campine' near Budel. The village that was built near the factory was called after the directors: 'Budel-Dorplein' (Nieuwkoop, 1993). From 1890 till 1974 Zn was extracted via a thermal process. Three main steps could be distinguished: first roasting to convert Zn sulfide into Zn oxide. This happened at a temperature of 600°C using airflow. Sulfur dioxide and metal gasses were produced during this process. The second step was sintering. Air was blown through a mixture of raw and roasted blend and already sintered material at 1200°C. The unwanted metals (e.g. Cd) and remaining sulfur dioxide were removed and the product was converted from powder to grains: the sinter. In the third step, distillation, the temperature was heated to 1300-1400°C and coke was used to reduce Zn oxide to Zn. The Zn volatilized and had to be condensed without oxidizing again. This was carried out in retorts. The Zn and the remainder of the ore, the slag, were separated in the retort. The Zn was purified afterwards (by melting and distillation) and the slag was dumped as a waste material. Part of it has been used to pave roads with a total length of 832 km. From 1926 the sulfur dioxide was used for the production of sulfuric acid. Later, also Cd was produced (Nieuwkoop, 1993).

The Belgian Zn smelters followed the same procedure. The factory of Overpelt produced 250 tons of Cd waste in 1950 and half the amount was emitted to the air (340 kg of Cd/day) (Staessen et al., 1995). Between 1969 and 1971 all factories changed to an electrolytic process. The Cd production of the Balen and Overpelt smelters in 1992 was 1000 tons, but only 15 kg (0.04 kg/day) was emitted to the air (Staessen et al., 1995). The problem left in the electrolytic process is the waste material. A fluid waste, jarosite, is produced. It is collected in basins on the company site.

The heritage of the Zn smelters in the Dutch part of the Kempen is a large area with increased heavy metal concentrations in the soil, predominantly Cd and Zn. The Technical

Committee on Soil Protection (1997) published an 'Advice on the approach of soil contamination in the Kempen' and classified the situation in the Kempen as 'urgent', which officially means that within four years remediation has to start. Demonstrated effects are (Technical Committee on Soil Protection, 1997):

- leaching of metals to the groundwater;
- transport of metals by the groundwater;
- transport of contaminated sediment and sedimentation of contaminated materials;
- genetic adaptation of plants and invertebrates;
- accumulation of litter in the neighborhood of the Zn smelter;
- kidney dysfunction in humans.

Also farmers experienced the problems of contaminated soil. Some vegetables were difficult to grow without exceeding the food standards. One official court case in which a market gardener was sentenced is known. In 1985 lettuce was declared unfit for consumption because the Cd concentration was 0.37 mg Cd/ kg fresh weight (Warenwet, 1985).

A project-team, called: Active soil management de Kempen, was established to co-ordinate the whole process, comprising aspects such as: limitations in land use, monitoring, remediation, research, and effects of land use change. Immobilization as a soil remediation technique is only one aspect of the whole project, but not unimportant. Recently, Dutch and Belgian scientists and policymakers at a workshop 'Alternative remediation techniques in the Kempen' concluded that immobilization is a suitable and operational technique in areas that will be changed from agriculture to nature (Active soil management de Kempen, 2000).

Immobilization

General aspects

Immobilization covers a large series of techniques to make contaminants less mobile in soil or waste (Vangronsveld and Cunningham, 1998). We defined '*in situ* immobilization of heavy metals in contaminated soil' as the reduction of the metal concentration in the soil solution by adding a metal binding material to the soil without damaging the porous structure. Hence, solidification (producing a solid material in which the contaminant is physically built in) is left out of consideration.

The application of immobilizing materials to large areas does not only require a careful study of the beneficial effects, but also of the negative consequences. Once added to the soil, the treatment chemical can not be removed anymore. A careful study should include three aspects:

1) leaching of metals to deeper soil layers and groundwater,
2) toxicity to and uptake by plants,
3) toxicity to and uptake by soil organisms.

From these three aspects, we can derive a list of criteria for immobilizing agents:

- The material must have a high metal binding capacity at common soil pH (ca. 4-8);
- The additive needs to be durable under the environmental conditions that can occur in the soil;
- The binding agent does not produce undesired side effects with respect to soil structure, fertility and ecosystem;
- The treatment chemical should be cost effective.

Immobilization can be based on binding on the available particle surface (sorption) or on the formation of new particles, incorporating the metals in the structure (precipitation/coprecipitation). Precipitation of metals in an aerobic soil (moderate pH) occurs only at very high concentrations, except for lead. Addition of phosphate (and if necessary chloride) forms lead-phosphate precipitates, particularly (Chloro)pyromorfite ($Pb_5(PO_4)_3Cl$) (Ma et al., 1993; Boisson et al., 1999; Koopmans et al., 1999). Coprecipitation is sometimes mentioned as a long-term effect (for instance during oxidation or weathering). Most attention is paid to sorption processes. Immobilizing materials can enhance sorption in two ways: 1) by adsorption to the surface of the immobilizing material itself, and 2) by reducing the competition of other cations, particularly protons (increase in pH). The latter group (pH increasing materials) is officially beyond our definition, because they hardly bind any metals themselves. They nevertheless decrease the metal concentration in the soil solution, and are therefore included in this study. At first sight, the discrimination between a pH increase and the addition of new sites seems theoretical, but it may have practical implications. Next section focuses on the effects of an increase in pH.

Immobilization by increasing the soil pH

The effect of soil pH on metal uptake by plants has been studied extensively (e.g. Hooda and Alloway, 1996; Oliver et al., 1996; Singh and Myhr, 1998; Krebs et al., 1998), but the results are not always consistent. Generally a decrease in plant uptake was observed, but some studies even found increased crop metal concentrations (Andersson and Siman, 1991;

Sparrow et al., 1993). This might be caused by the fact that not only metal binding by the soil but also uptake via the root surface is pH dependent (Lexmond and Van der Vorm, 1981; Smolders et al., 1999). Plette et al. (1999) quantified both sorption to the soil and sorption to biological surfaces, implicitly assuming that the metal concentration bound by the root surface determines uptake and toxicity. The free metal concentration in pore water is in equilibrium with metals bound to the soil and with metals bound to the root surface. The pH dependency of the sorption processes at both surfaces determines the net effect of a shift in soil pH.

Whereas the mobility of cations generally decreases at a higher pH, the mobility of anions, such as molybdate and arsenate will increase if the pH is increased. In soils that also contain potentially toxic levels of these anions, alkaline additives may produce harmful effects.

An increase in soil pH may change the solid-solution partitioning of the organic matter, resulting in an increased dissolved organic matter (DOM) concentration in the soil solution (e.g. Tipping and Woof, 1991). The metals bound to DOM will leach to deeper soil layers or groundwater. This phenomenon is particularly important for metals that strongly bind to DOM, such as copper (McCarthy and Zachara, 1989; Temminghoff et al., 1998).

Furthermore, the pH influences the ecosystem with respect to vegetation (Roem and Berendse, 2000) and soil organisms (Korthals et al., 1996; Edwards and Bohlen, 1996). This does not need to be a problem in many cases, but one should be aware of it, if alkaline materials are to be used.

Summarizing, an increase in pH can stimulate immobilization of cations, but can also increase the mobility of anions. A pH increase might affect the behavior of organic matter and the composition of the (soil)ecosystem. Increasing the soil pH is a general mechanism, independent of the binding capacity of the immobilizing material. The adsorption capacity gives the material a unique character. In the next section, materials that have been studied are listed and provided with comments. To distinguish the effect of pH and the binding capacity of the material, Yousfi and Bermond (2000) proposed to adjust the pH of the untreated soil to the same pH as the treated soil. The only disadvantage of this approach is that the effects of different materials are compared at different pH values.

Immobilizing materials

The products used in other immobilization studies can be divided into four categories (Table 1.2). Several products could be classified in more groups, because they consist of different components. Calplus, for example, consists of clay and aluminum hydroxide.

Table 1.2: Materials used in immobilization studies.

Group	Materials
1) Lime	$CaCO_3$
	$(Ca,Mg)CO_3$
	$Ca(OH)_2$
2) Alumino silicates	Clay
	Natural and synthetic zeolites
	Incinerator ashes (fly-ashes, cyclonic ashes (formerly: beringite))
	Gravel sludge
3) Al-/Fe-/Mn-(hydr)oxides	Fe bearing (waste)materials (steel shots)
	Hydrous Ferric Oxide (HFO)
	(δ-)Manganese oxide
	Al_{13}-polymer
	Clay-aluminium hydroxide (Calplus, Al-montmorilloniet)
	Water treatment residues/biosolids
4) Materials rich in organic matter	Compost
	Peat
	Sewage sludge

1) Lime

Lime is a well-known soil amendment in agriculture. It is used both to increase the availability of essential nutrients and to decrease the availability of elements harmful to plant, animal and humans. Below pH 7, lime dissolves and neutralizes protons during this process. The dissolution effect of $CaCO_3$ does not work in calcareous soils. In that case it is possible that metals can be bound by $CaCO_3$, but its capacity is not very large.

Also Ca is released during dissolution of lime. It has been postulated that Ca^{2+} added in lime may desorb some of the soil-bound metal ions into soil solution, rendering them available to plants. Calcium is known to be an inhibitor of Cd^{2+} sorption to soil surfaces

(Boekhold et al., 1993), but this mechanism can only have a significant effect, if the pH-induced increase in sorption of Cd^{2+} with liming is of the same order of magnitude as the Ca-induced desorption of Cd^{2+}.

2) Oxides of manganese, iron and aluminum

Manganese

Manganese is a common element in the soil. It is an essential element for plants and animals. The pH and the redox potential of the soil determine the availability. The large number of oxides and hydroxides, in which substitution of Mn^{2+}, Mn^{3+} and Mn^{4+} occurs, complicates the mineralogy (McKenzie, 1989). Manganese oxides are also important for the sorption of other trace elements. The reactivity of the different oxides varies widely, due to differences in structure and surface groups. The most reactive form is synthetic birnessite (sometimes called: δ-MnO_2). The product has a layered structure, but details are not well known. There are different recipes to synthesize birnessite. McKenzie (1981) studied the surface charge of different birnessites and concluded that the most negatively charged oxide is obtained by adding HCl to boiling $KMnO_4$. This product also showed the highest adsorption of different metals (McKenzie, 1980). Different authors confirmed high sorption rates of Cd and Zn (Zasoski and Burau, 1988; Loganathan and Burau, 1973). The high affinity of birnessite can be explained by the low point of zero charge (PZC), which ranges from 1.5 to 2, the relatively large surface area, and the high dielectric constant (Murray, 1975). The immobilizing capacity of birnessite was studied by Mench et al. (1994). Compared to other additives (hydrous ferric oxide, Thomas basic slag, cyclonic ashes, and lime) birnessite exhibited the best potential in reducing the uptake of Cd and Pb by plants. Apart from the price, the critical aspect of birnessite is its stability. Under reducing (anaerobic) conditions the oxide will reduce to Mn^{2+}, which can be toxic to organisms. Reduction might also take place at low pH in aerobic soils.

Iron

Iron oxides are considered important compounds in the soil for heavy metal binding, although the PZC is not as low as the PZC of birnessite. Many iron-bearing materials have been studied and it is frequently suggested that long-term processes further increase and strengthen metal binding. Ainsworth et al. (1994) showed that Cd and cobalt, in contrast to lead, added to a hydrous ferric oxide suspension, were incorporated in the structure during the aging process. Lookman and Vanden Boer (1997) also observed a long-term process. They investigated a shipyard contaminated with blasting waste. Blasting waste contains much iron. They proved that the mobility of Zn, Cu, Ni, and Cd, as determined by a column experiment according to NEN7320, was significantly lower if old weathered blasting waste was used compared to recently used blasting waste. It was explained by the formation of iron oxides. It seems that the addition of not-yet-oxidized iron is more successful in binding heavy metals on a longer term. The addition of $FeSO_4$ (Czupyrna et al., 1989) and of steel shots (metallic iron) (Mench et al., 1994) have been evaluated. A gradual oxidation of Fe^{2+} allows the metal ions to coprecipitate within the newly formed ferric (hydr)oxide. The metal uptake by plants grown on soils treated with steel shots was significantly reduced, both in pot experiments (Mench et al., 1994) as well as in a field experiment one year after treatment (Boisson et al., 1998).

Other iron bearing products that have been studied are Fe-rich, a waste product released during the production of TiO_2 (Chlopecka and Adriano, 1996 and 1997) and recycled iron adsorbent, collected from an impact millroom wheelbrater at a cast-iron facility (Smith, 1996 and 1998). Fe-rich showed a strong decrease in available Zn, but this effect could be explained by the increase in pH. More critical is the fact that Fe-rich contained 20.4 mg Cd k/kg and 1272 mg Cr/kg (Chlopecka and Adriano, 1996). Recycled iron adsorbent was only tested in aqueous systems. Adsorption values were comparable to hydrous ferric oxide (Dzombak and Morel, 1990)

Elevated Cd levels were found in crops in Australia due to Cd contaminated phosphate fertilizer and high chloride concentrations (Smolders and McLaughlin, 1996). McLaughlin et al. (1998b) studied the use of a series of binding additives. Different water treatment biosolids (WTB's) were among them. WTB's were derived form Fe and Al-based chemical flocculation processes for drinking water treatment. If the pumped groundwater is not contaminated the biosolids predominantly contain Fe, Al, Ca and Mn, depending on the groundwater composition. Several WTB's also contain a considerable amount of carbon (up to 20%). Cd uptake by potato tubers was reduced in soils treated with ca. 0.25% WTB's. WTB's containing high carbon contents reduced the uptake most.

Aluminum

Aluminum hydroxides are positively charged under normal soil conditions ($pH<10$). Lothenbach et al. (1997) and Badora et al. (1998) used a soluble polynuclear aluminum complex (Al_{13}). In alkaline solution the Al_{13} units slowly convert to amorphous $Al(OH)_3$. Heavy metals can be taken up into the solid matrix during this process. Significant reductions in $NaNO_3$-extractable Zn were obtained for very small additions of Al_{13} compared to untreated soils with the same pH (Badora et al., 1998).

Aluminum hydroxides are often combined with clay (Keizer and Bruggenwert, 1991; Janssen et al., 1994; Lothenbach et al., 1997). The amount of heavy metals bound on clay-aluminum complexes is higher than the binding on clay or aluminum hydroxide separately. A few reasons can explain this phenomenon. The positive charge of the aluminum hydroxide (specific adsorption) is partly compensated by the negative charge of the clay (non-specific adsorption), so the decrease in the CEC is compensated by an increase in high affinity sites for heavy metals. Furthermore, the aluminum precipitates are spread over the clay in stead of forming a ball, so the surface area increases. The sorption sites are more easily accessible. Finally, small ions (Cu, Zn) just fit in the hydroxide layers between the clay platelets (Keizer and Bruggenwert, 1991). Clay aluminum complexes have a lower affinity for larger cations like Cd and Pb. The uptake of Zn by Swiss chard is significantly reduced after adding clay aluminum complexes to the soil, but the uptake of Cd is not affected (Lexmond and Bruggenwert, 1997).

Red mud

Red mud is an alkaline residue of the alumina industry. Summers et al. (1996) studied the effect on pasture growth on poor sandy soils and evaluated heavy metal uptake by plants. They concluded that the heavy metal uptake decreased after red mud application. Other studies (Lopez et al., 1998; Phillips, 1998) showed that red mud could immobilize heavy metals, but the pH appeared to be the determining factor. McLaughlin et al. (1998a) observed slightly reduced uptake by potatoes after red mud addition. Red mud contains very high amounts of Na and, depending on the origin, it can be contaminated.

3) Alumino silicates

Clays

Montmorillonite type clays were used because of their large CEC (80-120 $mmol_c/100g$), though competition of calcium is very strong (Van Bladel et al., 1993). Sims and Boswell (1978) added bentonite (a commercial montmorillonite clay) to a contaminated sludge treated soil. They observed a decrease in the metal uptake by wheat, but they could not separate the effect of the increase in the CEC from the increase in pH (also a result of the bentonite addition). Lothenbach et al. (1997) studied the effect of Na-montmorillonite. They found that the clay had a relatively low capacity to bind heavy metals.

Zeolites

Zeolites are naturally occurring crystalline alumino silicate minerals, but they can also be produced synthetically. The framework consists of $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ tetrahedra that are linked at all corners. The framework is open and contains channels and cavities in which cations and water molecules are located (Dyer, 1988). The channel structure of zeolites is responsible for their function as a molecular sieve, but is also important for 'selective' cation exchange. Clinoptilolite is the most often used natural zeolite to remove heavy metals from the soil solution or wastewater. However, the adsorption capacity of natural zeolites is rather low. In batch experiments, the addition of natural zeolite to soil had hardly any effect (Tsadilas et al., 1997; Singh et al., 2000). Weber et al. (1984) did not observe a reduction in heavy metal uptake in sorghum after addition of approximately 6.5% clinoptilolite to an agricultural soil.

Synthetic zeolites have provided better results. Czupyrna (1989) selected a large number of treatments and the zeolite type A appeared to immobilize heavy metals very strongly in batch and column experiments, particularly in combination with $FeSO_4$. Only the pH of the column effluent is mentioned for a treatment with zeolite and $FeSO_4$. It did not differ much from the control soil. Singh et al. (2000) conducted a batch experiment in a 0.01 M $NaClO_4$ solution at $pH = 6$ and concluded that large amounts of Cd were bound by zeolites A, P and Y, compared to natural zeolite, bentonite and cyclonic ashes. Synthetic zeolites were also able to reduce metal uptake by plants (Gworek, 1992; Rebedea and Lepp, 1994). The reduction was partly caused by a pH increase, as a side effect of zeolite addition (Edwards et al., 1999).

Recently, zeolites were synthesized from fly ash (Steenbruggen and Hollman, 1998; Lin et al., 1998). Zeolitization is realized by treating fly ash with NaOH at ca. 90°C. Lin et al. (1998) concluded that both an increase in pH and the binding of Cd on the zeolite-like material were responsible for a strong decrease in extractable Cd (extracted with 0.01 M $CaCl_2$). They also observed a strong reduction of the Cd concentration in the leachate of the zeolite treated soil columns.

Fly and bottom ashes

Fly and bottom ashes are waste products from coal-fired power plants. Depending on the parent coal and the operating conditions of the furnace, the ashes vary in their physical, chemical and mineralogical composition (Bache and Lisk, 1990). Shende et al. (1994) observed a decrease in heavy metal uptake by maize in a calcareous soil ($pH = 7.9$) as long as the fly ash addition was less than 5%. The effect of fly ash was not very clear in an acid soil. In most studies is concluded that the effect of fly ash is comparable to the effect of lime (Bache and Lisk, 1990; Marschner et al., 1995). As many waste products, ashes are often contaminated with heavy metals and toxic anions (e.g. arsenate and molybdate).

Cyclonic ashes (also called: beringite)

Cyclonic ashes are modified alumino silicates originating from the fluidized-bed burning of coal mining refuse. By heating to 800°C the material is partly broken down and recrystallized. The following minerals are present in cyclonic ashes: quartz, illite, calcite ($CaCO_3$), dolomite ($[Ca,Mg]CO_3$), anhydrite ($CaSO_4$), siderite ($FeCO_3$), and ettringite ($6CaO.Al_2O_3.3SO_4.31H_2O$). $pH(H_2O) = 12$, CEC = 20 $mmol_c$/100g (determined in a buffered NH_4OAc solution of pH 7), and the specific surface are is ca. 20 m^2/g (De Boodt, 1991). Cyclonic ashes contain Cd and Zn concentrations close to the Dutch intervention values. The binding mechanism of cyclonic ashes is not quite clear, but part of the effect is attributed to a strong increase in pH. It was used to revegetate the Maatheide, a bare area in the North of Belgium. An addition of 2.5% (w/w) on the Maatheide raised the pH by approximately 2 units (Vangronsveld et al., 1996). Several studies have proven beneficial effects to plants (Mench et al., 1994; Boisson et al., 1998) and reduction of metal leaching to deeper soil layers (Wessolek and Fahrenhorst, 1994).

Gravel sludge

Gravel sludge is an uncontaminated waste product of the gravel industry consisting of different minerals (main constituents are 29% illite, 30% calcite, and 18% quartz) (Lothenbach et al., 1998). Batch experiments show that Zn and Cd uptake by red clover as well as the $NaNO_3$-extractable concentration were significantly decreased in a soil treated with gravel sludge. However, the pH increase can explain most of the reduction in $NaNO_3$-extractable Zn. Krebs et al. (1999) conducted a field plot study growing lettuce and ryegrass on soils treated with gravel sludge. They also concluded that the pH increase contributed to the immobilization effect, but they could not quantify this contribution.

4) Organic matter products

Organic matter is an important component for adsorption of trace elements in many soils. Metals can adsorb on negatively charged phenolic and carboxylic sites of the humics. Addition of organic matter would therefore increase the binding capacity of the soil. Berti and

Cunningham (1997) evaluated the effect of natural humus (composted leaves) on Pb speciation in three different soils. The Pb-fraction extracted by 0.1 M $Ca(NO_3)_2$ and 0.05 M $AgNO_3$ decreased by adding 4 or 10% humus. Also the leachable Pb was reduced. Kireicheva and Glazunova (1994) introduced a mixture of organic matter (65%), zeolite (25%) and alumina (10%) and observed uptake reductions of heavy metals by oats. Ni, Cu, Zn and Pb uptake was 50% at an addition rate of 5% (w/w) compared to the untreated soil. Marschner et al. (1995) applied 0.3% tree bark mulch (no pH shift), but extraction with water did not show a reduction of water extractable metal concentration. The effects of agricultural residues are generally small.

The instability of organic matter is a disadvantage. Solid organic matter can be transformed into dissolved organic matter, resulting in leaching of metals bound to dissolved organic matter. Alternatively, microorganisms can degrade organic matter, resulting in a loss of binding sites.

This thesis

Objectives

The aim of this thesis is to understand the processes that take place after addition of selected immobilizing agents. The metal binding capacity under normal soil conditions is the most important characteristic of an immobilizing agent. Effects of an increase in pH will be distinguished from the addition of new binding sites.

An increase in pH can also increase the dissolved organic matter concentration in the soil solution. This study investigates the underlying mechanisms that determine the partitioning of soil organic matter, and how several alkaline additives affect the dissolved organic matter concentration.

The effects of immobilization on plants have been studied quite frequently, but not much is known about the effects of immobilizing materials on soil organisms. The effects of soil amendments on the leafy vegetable Swiss chard (*Beta vulgaris* L. var. *cicla*) and on the earthworms *Eisenia [Dendrobaena] veneta* Rosa and *Lumbricus rubellus* Hoffmeister are studied. Earthworms were chosen because they are important organisms in the food web and can represent a high fraction of the biomass in the soil.

It is hard to find an immobilizing agent that meets all requirements as listed on page 7, but we decided to include the following materials in our research project: lime, manganese oxide (birnessite), natural and synthetic zeolites, cyclonic ashes, and several iron bearing materials.

Outline

The introduction (Chapter 1) is followed by two supporting chapters. In our study it appeared to be essential to quantify metal binding to natural organic matter. Data concerning Zn binding to natural organic matter are rare. Chapter 2 presents analytical data of Zn binding to humic acid. The free Zn concentration in humic acid solutions was measured by a newly developed technique: the Wageningen Donnan Membrane Technique (WDMT). Our data form an essential part of the database compiled to determine model parameters for the NICA-Donnan model. This model can calculate metal binding to dissolved organic matter, which is important for the speciation in the soil solution.

Chapter 3 focuses on the partitioning of soil organic matter. The ionic composition of the soil solution strongly influences sorption and coagulation of organic matter. A sudden change in the ionic composition, that might take place when an immobilizing chemical is added to the soil, might also change organic matter partitioning. Chapter 3 studies the effect of changes in Ca and pH on dissolved organic matter in soils. The fundamental processes studied in Chapter 3 are used in Chapter 4. Chapter 4 evaluates whether the addition of zeolites or cyclonic ashes indeed influences the partitioning of soil organic matter.

The effects of alkaline immobilizing agents on metal uptake by plants and earthworms are studied in Chapter 5. The alkaline cyclonic ashes are compared to lime in a plant experiment to quantify the additional binding capacity of cyclonic ashes. The effect of cyclonic ashes was also evaluated in an earthworm experiment. Chapter 6 presents the effects of lime (alkaline) and MnO_2 (not influencing pH) on Cd uptake by earthworms. The typical differences in uptake between lime and MnO_2 provided information about metal uptake routes

The results from these studies are integrated and interpreted to more practical recommendations in Chapter 7.

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Chapter 2

Measuring and modeling zinc and cadmium binding by humic acid

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Abstract

Free metal ions in aqueous and terrestrial systems strongly influence bioavailability and toxicity. Most analytical techniques determine the total metal concentration, including the metals bound by dissolved organic matter. The activity can be measured with ion specific electrodes (ISEs) for some metals but an electrode for Zn is not available. As a result, very few data are available on Zn binding by natural organic matter. The aim of this study is to determine free Zn concentrations in purified humic acid solutions using the recently developed Wageningen Donnan Membrane Technique (WDMT). However, several analytical aspects of the WDMT had to be clarified, before reliable data could be composed. Cd was chosen for validation. This study shows that free Cd concentrations as measured by WDMT agreed well with Cd-ISE measurements. It is also shown that the WDMT could be used at high pH and that the presence of divalent ions in the background electrolyte shortened the equilibration time for metals. The WDMT provided consistent results in a range of $p[Cd^{2+}] = 3\text{-}9$ and $p[Zn^{2+}] = 3\text{-}8$ at pH 4, 6 and 8. Metal speciation in humic acid solutions was also calculated by the consistent NICA-Donnan model using generic parameters. The model could excellently describe the experimental data without adjusting any of the parameters ($R^2_{Cd} = 0.994$, $R^2_{Zn} = 0.988$).

Introduction

Bioavailability of trace metals in the environment is influenced by both environmental and physiological factors. The environmental conditions (e.g. pH, total metal content in the soil or sediment, number of binding sites, competing ions, etc.) strongly influence the speciation in solution. The uptake by plants and soft-bodied organisms is often well-correlated to the free ion activity (Bingham et al., 1984; Morel, 1983; Spurgeon and Hopkin, 1996; Hare and Tessier, 1996), though labile complexes (e.g. cadmium chloride and cadmium sulfate complexes) may also influence metal uptake (Smolders and McLaughlin, 1996; Van Leeuwen, 2000) However, it is complicated to determine the free metal concentration in solutions containing natural organic matter (NOM). Hence, speciation models have been used extensively to calculate free metal concentrations in solution based on the total dissolved metal concentration, which could be easily measured. Different models have been developed to describe metal binding by NOM. Dobbs et al. (1989) developed a continuous multiple site ligand model which was incorporated in MINTEQA2. Tipping (1998) derived Model VI, a discrete site/electrostatic model, which accounts for chemical heterogeneity, competition, ionic strength effects and proton-metal exchange. The same aspects were accounted for in a model used by Kinniburgh et al. (1999) This is the consistent Non Ideal Competitive Adsorption equation combined with an electrostatic Donnan model to correct for the variable charge nature, which is referred as: consistent NICA-Donnan model. In stead of discrete sites, as in Model VI, the consistent NICA-Donnan model uses a continuous affinity distribution. All models have one thing in common: they need reliable data to obtain the model parameters. Parameters for Zn binding by humic acid in Model VI are based on 17 data points extracted from two papers written in 1965 and 1971 covering a very limited concentration range (Tipping, 1998).

As stated, direct measurement of free metal ions is difficult. Common analytical equipment (e.g. FAAS, ICP-OES, ICP-MS) measures total concentrations in a sample. Polarographic techniques do not only determine the free metal concentration, but also labile metal complexes. Ion Specific Electrodes (ISEs) have been used frequently to measure free Cd, Ca, or Cu activities (Bresnahan et al., 1978; Milne et al., 1995) but an electrode for Zn is not available. This is probably the main reason for the very limited number of data in literature on Zn binding by humic substances.

Recently, new methods to determine free metal concentrations in solution have been developed. Holm et al. (1995) applied a method using a cation exchange resin. The exchange with the resin highly depends on the ionic strength, the cation composition and the pH of the sample, which is taken into account by a reference experiment. The reference solution should contain exactly the same electrolyte solution, but no complexing ligands. The method appeared to be very sensitive to [Ca^{2+}] in the range from 0.001 M to 0.005 M. Particularly in that range, the free Ca concentration in the sample and reference solution should be equal; otherwise a correction has to be made based on an empirical standard curve (Holm et al., 1995).

Davison and Zhang (1994) introduced the DGT technique (Diffusion Gradients in Thin-films) based on metal fluxes from the aqueous phase through a highly porous hydrogel to an exchange resin, which functions as a sink. The exchange resin functions well between pH 5 and 9. The diffusion rate of metals through the gel depends on the thickness of the gel layer, the metal concentration in the bulk solution, and the diffusion coefficient (Davison et al., 2000). The diffusion coefficient depends on the properties of the gel, the ionic strength of the solution, and the type of ligand. Not only free ions, but also metal complexes can diffuse through the gel, depending on the size of the ligand and the type of gel (Davison et al., 2000). By using at least two DGT devices with different gel compositions, the speciation in solution can be estimated (Zhang and Davison, 2000). The method is suitable for *in situ* measurement in natural waters (Davison and Zhang, 1994) but is complicated for soils. Pore water is not very well mixed, which might result in a concentration gradient towards the membrane filter (Davison et al., 2000), and the interpretation can confuse the diffusion process at moisture contents lower than field capacity (Hooda et al., 1999).

Fitch and Helmke (1989) separated the free and complexed metals via a Donnan equilibrium across an ion exchange membrane. We initially tried their approach, but we could not obtain reliable results. Especially the small, stagnant acceptor solution caused difficulties. Therefore, Temminghoff et al. (2000) and Weng et al. (accepted) further developed the technique, resulting in the Wageningen Donnan Membrane Technique (WDMT). The WDMT cell consists of a donor side flushed with aqueous solution containing free metal ions and metal complexes (e.g. metal-NOM), and an acceptor side flushed with electrolyte solution with approximately the same salt level as the donor solution. The two sides are separated by a negatively charged semi-permeable cation exchange membrane. Cations can easily pass the membrane, whereas the transport rate of negatively charged compounds in solution, e.g. humic acids and Cl^-, is practically zero. When equilibrium is reached, the free metal ion concentration in the acceptor and the donor will become identical if ionic strength, and ionic composition of both solutions are equal. A simple correction can be made, if the donor and acceptor solution are different (Weng et al., accepted). Temminghoff et al (2000) showed that the WDMT could accurately determine free cadmium and copper concentrations in the presence of various (in)organic complexing agents (chloride, EDTA, purified forest soil humic acid). They performed experiments in a background electrolyte ranging from 1 to 10 mM $Ca(NO_3)_2$ whereas pH ranged from 4 to 6.

The objective of this paper comprises the reliable determination of the free Zn concentrations in solutions containing purified Forest Soil Humic Acid (FSHA). Data will be collected over a large Zn range and at different pH values. Furthermore, we model the obtained data with the consistent NICA-Donnan model using a generic set of parameters as determined by Milne (2000) and Milne et al. (2001). However, the experimental conditions to obtain a high-quality data set require more information about the behavior of the WDMT. Cd is chosen to further validate the WDMT, because it can be measured with an ISE and the binding of Cd by humic acid has been studied extensively. Validation of the WDMT comprises 3 aspects. First, metal binding data are ideally measured without strongly competing ions in the system. Therefore, $NaNO_3$ is preferred, but only $Ca(NO_3)_2$ has been used before. We evaluate the possibilities of $NaNO_3$ as a background electrolyte. Second, Temminghoff et al. (2000) compared WDMT measurement to speciation calculations, but we compare WDMT to ISE measurement. Third, the WDMT had been validated for metal complexes with synthetic complexing ligands at pH 5. We evaluate the behavior of the system at pH values up to 8, when CO_2 can cause significant amounts of metal carbonate complexes in the system. After the results for Cd are satisfactory, we will show the WDMT results obtained for Zn.

Theory

Modeling

The consistent NICA-Donnan model is based on the NICA equation, an analytical isotherm for multi-component adsorption to heterogeneous surfaces (Koopal et al., 1994). The NICA model was extended by incorporating non-specific electrostatic binding (Kinniburgh et al., 1996) and by achieving thermodynamic consistency. The consistent NICA-Donnan equation was published by Kinniburgh et al. (1999). Equation 2.1 shows the bimodel form of this equation since it is well known from heterogeneity analysis, that there are two broad distributions, related to carboxylic and phenolic sites (Nederlof et al., 1992).

$$Q=\frac{n_{i,1}}{n_{H,1}}Q_{\max,1}\frac{(\widetilde{K}_{i,1}c_{D,i})^{n_{i,1}}}{\sum_j(\widetilde{K}_{j,1}c_{D,j})^{n_{j,1}}}\times\frac{\left[\sum_j(\widetilde{K}_{j,1}c_{D,j})^{n_{j,1}}\right]^{p_1}}{1+\left[\sum_j(\widetilde{K}_{j,1}c_{D,j})^{n_{j,1}}\right]^{p_1}}$$

$$+\frac{n_{i,2}}{n_{H,2}}Q_{\max,2}\frac{(\widetilde{K}_{i,2}c_{D,i})^{n_{i,2}}}{\sum_j(\widetilde{K}_{j,2}c_{D,j})^{n_{j,1}}}\times\frac{\left[\sum_j(\widetilde{K}_{j,2}c_{D,j})^{n_{j,2}}\right]^{p_2}}{1+\left[\sum_j(\widetilde{K}_{j,2}c_{D,j})^{n_{j,2}}\right]^{p_2}} \qquad [2.1]$$

with

Q_i	adsorbed quantity of component *i* (in mol kg^{-1} organic matter);
$Q_{\max}$	maximum adsorption capacity of the organic matter for protons (in mol kg^{-1} organic matter);
$\widetilde{K}_j$	median of the affinity constants, K_j, of component *j*, where *j* includes component *i*;
n_j	the non-ideality parameter of component *j*;
p	the intrinsic heterogeneity parameter of the organic matter;
Subscript 1	'carboxylic' like type of groups;
subscript 2	'phenolic' like type of groups;
$c_{D,j}$	concentration of the component *j* in the Donnan phase (mol l^{-1}) which is related to the concentration in the bulk solution, c_j.

$$c_{D,j}=e^{-z_j\Psi_D/kT}c_j \qquad [2.2]$$

with

z	the valence of the ion;
Ψ_D	the Donnan potential;
k	the Boltzmann's constant;
T	the temperature (in K).

It is assumed in the Donnan model that there is a uniform distribution of potential within the Donnan volume or 'phase'. The Donnan model does not require any particular assumption about the geometry of the humic particles. This contrasts with the diffuse double layer model in which the potential varies in a systematic way with distance from the interface, and therefore depends on a particular particle geometry. The Donnan volume (V_D) is related to the ionic strength according the following empirical relation (Benedetti et al., 1996):

$$\log V_D = b(1 - \log I) - 1 \tag{2.3}$$

in which I is the ionic strength, and the coefficient b varies with the type of humic substance. There is a close relation between the Donnan volume and the Donnan potential: for a given net charge on the humic particle, a smaller Donnan volume implies a greater concentration of counter ions in the Donnan phase, a greater Boltzmann factor and therefore a greater absolute value of the Donnan potential.

Material and methods

Equipment

A personal-computer-based automatic titrator, developed by Kinniburgh et al. (1995), was used to conduct the ISE experiments. We used four motorized burettes (Metrohm 665 Dosimat) to add metal solutions to the reaction vessel (10^{-3} M and 10^{-1} M metal nitrate solutions), and 0.1 M HNO_3 and 0.1 M NaOH to adjust the pH. All experiments were conducted in a temperature-controlled room at 20°C, and the solution was kept under a nitrogen atmosphere.

The ISE measurements have been done according to Milne et al. (1995). The pH electrodes used were standard glass half-cells measured against a double junction saturated KCl reference electrode. The outer junction of the reference electrode was filled with a mixture of 0.125 M $NaNO_3$ and 0.875 M KNO_3. The pH electrodes were calibrated against two pH buffers of 4 and 7. The Cd electrode (Metrohm 6.0502.110) was calibrated by titrating a cadmium solution in 0.03 M $NaNO_3$ with ethylenediamine (Milne et al., 1995). ISE readings produced calibration curves which were linear for p[Cd^{2+}] between 11 and 3 (slope = -29.94 ± 0.12 mV/p[Cd^{2+}] unit; intercept = -60.66 ± 1.31; R^2 = 0.99992 ± 0.00008; n = 5).

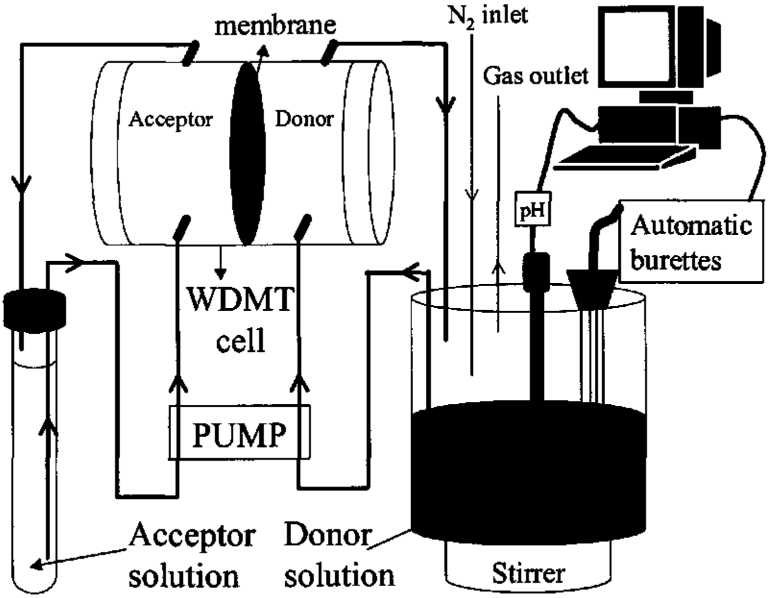

Figure 2.1: Overview of the WDMT equipment.

WDMT experiments were conducted in a system according to Figure 2.1. The WDMT cell and the negatively charged cation exchange membrane (BDH, No.55165 2U) were washed in 0.1 M HNO_3 and rinsed with ultra pure water. The membranes were then saturated with Ca and finally washed with the acceptor solution several times. The pretreatment procedure prevents that cation exchange in the membranes strongly influences the solution composition. The donor side of the cell was connected to the reaction vessel and the acceptor side was connected to a 10 ml test tube (total volume of the acceptor solution was 17 ml). After 24 hours a sample of 0.5 ml was taken from the donor solution and the 10 ml test tube containing acceptor solution was changed. Dissolved organic carbon (DOC) was measured in the donor and acceptor solution (DOC concentration in the acceptor solution was always below the detection limit of 0.5 mg/l). The pH was analyzed in all samples (combined pH electrode, pHC2005-7, Radiometer), as well as Na, Ca, and Cd or Zn, which were determined by inductively coupled plasma combined with optical emission spectrometry (ICP-OES) (Spectro Analytical Instruments). If concentrations were below the ICP-OES detection limit, samples were measured again by ICP with mass spectrometry (ICP-MS) (Perkin Elmer, Elan 6000). Free metal concentrations and adsorbed amounts of metals were calculated according to the scheme in Figure 2.2. The total concentrations (Na, Ca, and Cd or Zn) in the acceptor solution were converted into free ion activities using the ECOSAT computer code to account for inorganic complexes (Keizer and Van Riemsdijk, 1999). The pH was also used as an input variable, next to the total concentration of cations present in the acceptor. Differences in salt levels over the membrane were corrected using the distribution ratio of Na in the donor and acceptor solution. This resulted in the free ion activity in the donor solution. Finally, the inorganic species in the donor solution were calculated. The amount of metals bound to NOM can be calculated based on total metal concentrations, free metal concentrations and the inorganic complexes.

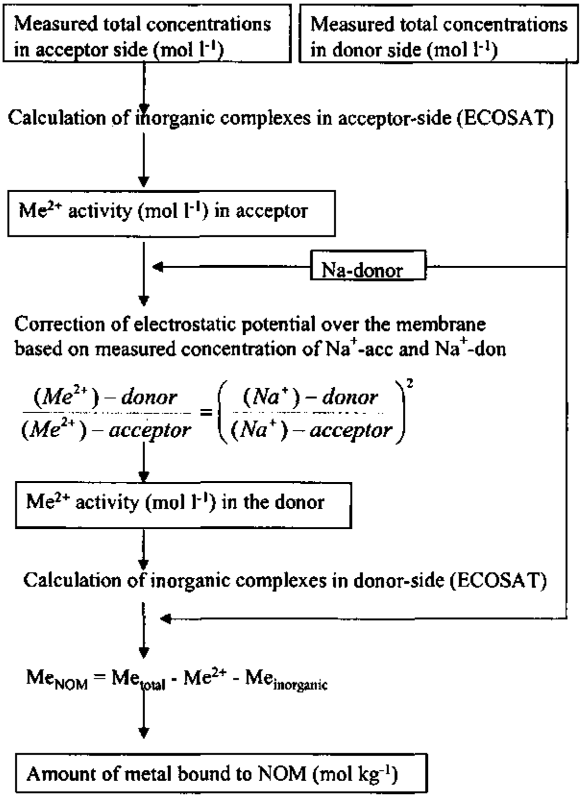

Figure 2.2: Scheme to calculate the free and complexed metal (Me) concentrations in a WDMT cell using measured concentrations in the donor and acceptor side, and a speciation program (e.g. ECOSAT) to calculate the contribution of inorganic complexes.

Effect of the background electrolyte in WDMT cells.

This preliminary experiment had a very simple design without using NOM. Half a liter of a solution, containing 1.78 μM $Cd(NO_3)_2$ in a background electrolyte of 3 mM $NaNO_3$ or 1 mM $Ca(NO_3)_2$, was connected to the donor side. The acceptor solution (3.3% of the total volume) contained the same concentration of background electrolyte but no Cd. Donor and acceptor were sampled every 24 hours during 7 days.

Titration experiments.

All titration experiments had an initial donor solution of 150 ml of FSHA (1.67 g l^{-1}), resulting in an acceptor volume of 10.2% of the total volume. FSHA was extracted from forest floor material. The extraction and purification are described elsewhere (Temminghoff et al., 1997). The solution is adjusted to a specified pH and statted at this pH. The Cd or Zn concentration was increased stepwise after each measurement.

First, a Cd titration ($p[Cd^{2+}]$ = 3-9) was conducted in 0.03 M $NaNO_3$ at pH 4, and 8. This experiment was measured with a Cd-ISE. Second, a similar experiment was performed at pH 4, 6 and 8, but the background electrolyte contained a mixture of 0.03 M $NaNO_3$ and 0.001 M $Ca(NO_3)_2$.

Third, the second experiment was repeated, but measured by WDMT (Figure 2.1) in stead of ISE. The WDMT requires 24 hours per measurement. For this reason, the number of data points of the WDMT is less than the data obtained by ISE. Fourth, Zn binding to FSHA was determined by WDMT since an ISE is not available for Zn ($p[Zn^{2+}]$ = 3-8). The experimental conditions were similar to the Cd experiment, except the $NaNO_3$ concentration at pH 4 and 6, which was 0.003 M.

Modeling.

The parameter determination of complex multi-component models such as the consistent NICA-Donnan model requires a number of decisions to be made. Milne (2000) derived a set of generic parameters for humic acids based on a large number of literature data (Table 2.1). Apart from our data, which were also included in this database, 7 data sets (494 data points) were used for Cd, whereas only one other set (15 data points) was used for Zn. We predicted the Cd or Zn bound to FSHA using the NICA-Donnan model with generic parameters. The free ionic concentrations of Na, Ca, H, Cd, Zn, and the FSHA concentration were used as input parameters. The concentrations bound by FSHA, as predicted by the model, could be compared with the measured concentrations.

Table 2.1: Generic NICA-Donnan parameters for purified humic acids (Milne, 2000).

Parameters	site 1	site 2
b (Eq.3)	0.49	
Q_{max}	3.15	2.55
P	0.62	0.41
log K_H	2.93	8.00
n_H	0.81	0.63
log K_{Ca}	-1.37	-0.43
n_{Ca}	0.78	0.75
log K_{Cd}	-0.20	2.37
n_{Cd}	0.73	0.54
log K_{Zn}	0.11	2.39
n_{Zn}	0.67	0.27

Results and discussion

Further validation of the WDMT comprised three aspects: 1. effect of the background electrolyte, 2. comparison with ISE measurement, and 3. measurement at high pH. Subsequently, we will pay attention to modeling aspects of Cd binding by humic acid. Finally, we will present experimental data and modeling results of Zn binding to FSHA.

Analytical aspects of the WDMT: 1. background electrolyte.

Metal binding data should ideally be measured in absence of strongly competing ions, but the competition of protons is inevitable. In nature, also other (di- or trivalent) ions will play a role. We tested 0.003 M $NaNO_3$, and 0.001 M $Ca(NO_3)_2$ as background electrolytes. The results are presented in Figure 2.3. If no Ca is present in the system, it is clearly shown that Cd does not pass the membrane during the time span of the experiment. This can be explained by the binding capacity of the cation exchange membrane. The negatively charged membrane can bind positively charged ions and prefers divalent to monovalent ions (Weng et al., accepted). In 0.003 M $NaNO_3$, and for the chosen donor volume and cadmium concentration, the membrane binds all Cd that is initially present in the donor solution. When the background electrolyte contains Ca, this divalent ion acts as a competitor reducing Cd binding to the membrane. The Cd concentration in the donor solution in presence of Ca nevertheless decreased somewhat during the first day (Figure 2.3), due to binding of some Cd in the membrane, and due to a small loss of Cd to the acceptor solution. A loss of Cd to the membrane and acceptor solution results in a concentration drop in both donor and acceptor. If both solutions are measured, the equilibrium between free metal and bound metal is well determined. The decrease in Cd concentration in the donor is much smaller when the free metal concentration is buffered by NOM, because a large fraction of Cd will be bound by NOM.

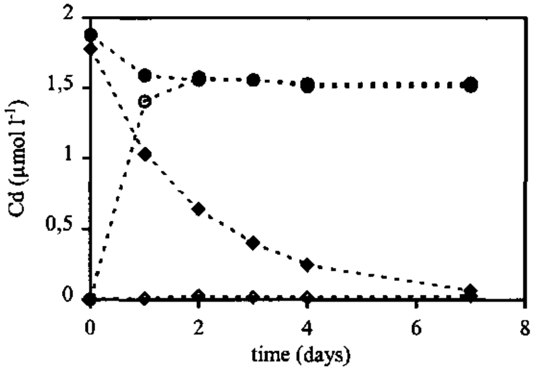

Figure 2.3: The Cd concentration in the donor and acceptor side as a function of the background electrolyte during 7 days. Closed symbols represent measurement in the donor solution whereas open symbols represent measurement in the acceptor solution. Circles: in presence of 0.001 M $Ca(NO_3)_2$, diamonds: in presence of 0.003 M $NaNO_3$.

Based on this experiment, we decided to use a mixture of 0.03 M $NaNO_3$ and 0.001 M $Ca(NO_3)_2$ as a background solution in WDMT experiments. $NaNO_3$ defines the ionic strength and $Ca(NO_3)_2$ ensures a sufficient metal transport rate across the membrane in the other experiments.

Analytical aspects of the WDMT: 2. comparison with ISE.

Figure 2.4 shows the Cd data measured by ISE and WDMT. The comparison between the two analytical techniques is extremely good. Slight differences can be observed only at high Cd concentrations at pH 4. As a remark, we would like to report that three Cd selective electrodes failed during use. One of them was newly purchased. The fourth electrode worked properly during all experiments. Milne et al. (1995) also reported that two of their electrodes failed. Apparently, a Cd-ISE is a very sensitive instrument.

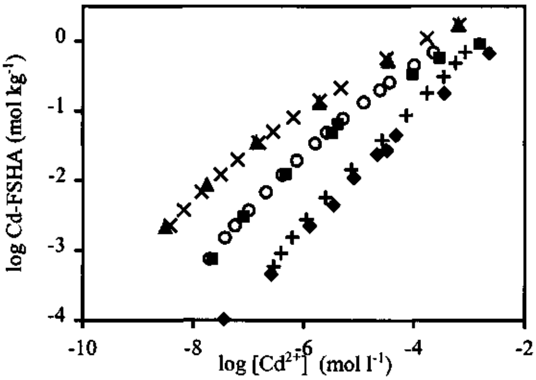

Figure 2.4: Free Cd versus bound Cd (log/log) as measured by WDMT and ISE. ◆ pH 4-WDMT, ■ pH 6-WDMT, ▲ pH 8-WDMT, + pH 4-ISE, ○ pH 6-ISE, × pH 8-ISE.

Analytical aspects of the WDMT: 3. high pH.

The donor side of the cell is kept under nitrogen to prevent carbonate complexes in the solution at higher pH values. To obtain a CO_2-free acceptor solution at pH 8 requires extra experimental efforts. If the acceptor solution is not kept under nitrogen atmosphere, the acceptor solution will be acidified by CO_2 diffusion. At the same time, the donor and acceptor strive towards equilibrium in proton activity by transporting protons from the acceptor to the donor solution. These two processes, an increase in protons by acidification and a decrease in protons by transport to the donor side, result in a non-equilibrium situation for protons. The net result created a dynamic steady state, in which the pH in the acceptor solution was approximately 6.5 at $pH_{donor} = 8$. Concentration differences between donor and acceptor might lead to an electrostatic potential over the membrane, but the very low proton concentrations at near neutral pH do not significantly contribute to the ionic strength. Although there is not necessarily Donnan equilibrium for protons over the membrane, there will be Donnan equilibrium for the metal ions. The advantage of the rather low pH in the acceptor is that metal carbonate complexes are insignificant.

A difference in pH between donor and acceptor requires pH measurement in both donor and acceptor. If the pH in the donor is assumed to be equal to the pH in the acceptor, this will lead to an erroneous calculation of free metal ions, because of the correction for carbonate and hydrolysis complexes in the acceptor which are not present in reality. The higher volume of the acceptor, compared to original design of Fitch and Helmke (1989) is an advantage of the

WDMT cells. Measurement of pH, but also multi-element analysis are easier if more sample solution is available.

Modeling Cd binding by FSHA.

Determination of Cd binding to humic substances in the presence of only monovalent ions, can not be easily realized when the WDMT is used. Ca is needed to facilitate the metal transport across the membrane. The competition between Ca and Cd complicates the determination of ion specific model parameters. To assess the effect of Ca competition, we compared the results of the Cd titration in a Ca/Na background with the Cd titration in a Na background. The data obtained at pH 4 and 8 are presented in Figure 2.5.

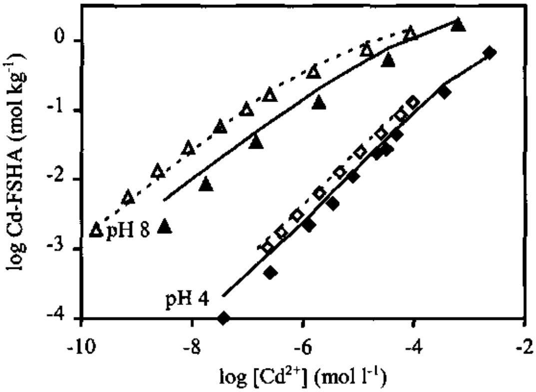

Figure 2.5: Cd bound to humic acid versus free Cd (log/log) with and without Ca in the background electrolyte. Closed symbols and solid lines represent data and predictions in 0.03 M $NaNO_3$ and 0.001 M $Ca(NO_3)_2$; open symbols and dotted lines are data and predictions in 0.03 M $NaNO_3$.

Clear differences in Cd adsorption can be observed when Ca is present or absent, particularly at pH 8. A slightly larger Ca effect at higher pH values was also observed by Kinniburgh et al. (1999). The experimental data in Figure 2.5 were calculated by the consistent NICA-Donnan model using the generic model constants (Table 2.1) for humic acid, which have been determined recently (Milne, 2000; Milne et al., 2001). The measured free Ca, Na, H, and Cd concentrations as well as FSHA concentrations were used as the input variables to calculate the amounts of Cd bound to FSHA. The average effect of Ca as predicted by the model using generic parameters is quite reasonable. The modeling results (lines in Figure 2.5) also show a larger effect of Ca at pH 8. Generally, the effect of Ca is slightly underestimated, especially at low Cd concentrations. The predictions could be improved if specific parameters for this humic acid would have been used.

Not only the Cd, but also the Ca concentrations were measured. Similar to Figure 2.5, we can compare the measured [Ca^{2+}] with the Ca bound to FSHA. The Ca bound to FSHA is slightly overestimated by the model (data not shown), but the trends were predicted well. The predicted log Ca_{bound} is, on average, 0.3 log units higher compared to the measured value.

Both the influence of Ca competition on Cd binding and the comparison of measurements and predictions of Ca bound by FSHA indicate that the effect of Ca is reasonably taken into account. The lines in Figure 2.6a represent the model predictions as calculated by the consistent NICA-Donnan model using the generic parameters presented in Table 2.1. The model is very well capable of describing the Cd data measured in competition with Ca without adjusting any parameter (RMSE = 0.226; R^2 = 0.994). The Cd bound to FSHA was slightly overestimated particularly at low Cd concentrations.

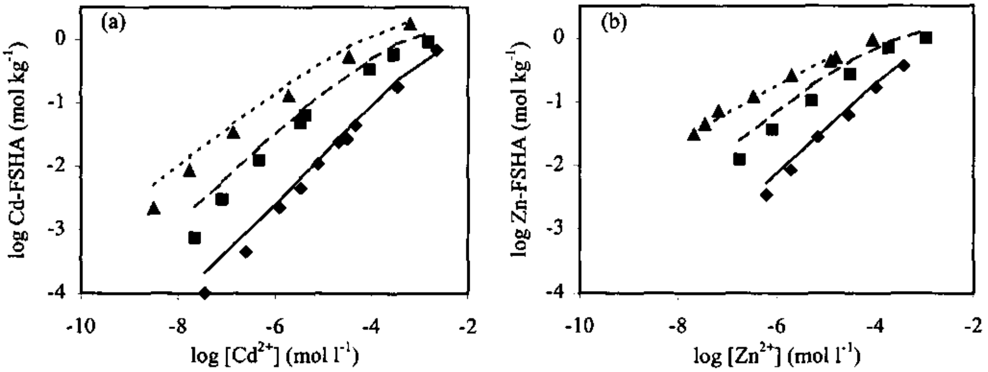

Figure 2.6: Cd (a) and Zn (b) bound to humic acid versus free Cd (a) and Zn (b) (log/log) at 3 different pH values in a mixed background electrolyte containing 0.03 M $NaNO_3$ and 0.001 M $Ca(NO_3)_2$. ◆ = pH 4, ■ = pH 6, ▲ = pH 8. Lines represent model calculations: solid line = pH 4, dashed line = pH 6, dotted line = pH 8.

Zn binding by FSHA measured by WDMT and modeled with NICA-Donnan.

Zn binding by FSHA is presented in Figure 2.6b. The trends are similar to Cd; lower binding at lower pH values. Zn is bound slightly stronger by FSHA compared to Cd (Figure 2.6a). The detection limit for Zn is higher than for Cd, because it is mainly determined by contamination in the laboratory.

The calculation of the Zn bound by FSHA is represented by the lines in Figure 2.6b. The model predicts the Zn data even better than Cd (RMSE = 0.099; $R^2 = 0.988$), but this is not surprising since our data formed the major part of the Zn database used by Milne (2000), which is the basis for the generic parameters.

Conclusions

The WDMT is an excellent method to determine free metal ion concentrations in complex solutions, without any interference of labile complexes. Sufficient time for equilibration is required and an abundant divalent ion, such as Ca, is needed as a background electrolyte in case divalent metals are to be measured at low concentrations. The method provides reliable results over a large metal and pH range and is suitable for multi-element analysis.

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Chapter 3

The solid-solution partitioning of organic matter in soils as influenced by an increase in pH or Ca concentration

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Abstract

Organic matter is an important component of the soil with regard to the binding of contaminants. Hence, the partitioning of organic matter influences the partitioning of soil contaminants. The partitioning of organic matter is complicated but, in any case, influenced by the ionic composition of the soil solution. This study focuses on the behavior of organic matter after a change in the ionic composition of the soil solution, particularly in Ca concentration and pH. Different amounts of $Ca(NO_3)_2$ and NaOH were added to soil suspensions. The dissolved organic carbon (DOC) concentration increased with increasing pH, but Ca addition had the opposite effect. A stronger increase in DOC was observed if a single dose of NaOH was added, compared to a gradual addition of the same amount of NaOH. Cation binding by organic matter in the supernatant was calculated using the consistent NICA-Donnan model. The log DOC concentration appeared to be correlated to the Donnan potential, calculated under the simplifying assumption that all DOM equals humic acid. This correlation was found for all eight neutral to acidic soils used in this study, though the slope and the elevation of the regression lines varied. The slope varied by a factor of 2 and the elevation appeared to be strongly influenced by the DOC concentration in the untreated soils, which was related to the total organic matter in the soil. Finally, we predicted the DOC concentration as influenced by $Ca(NO_3)_2$ and NaOH on the basis of an extraction of untreated soil with 0.03 M $NaNO_3$. The predicted potential of the organic matter appeared a useful parameter to predict the DOC concentration in soil suspensions after strong changes in ionic composition.

Introduction

Organic matter is an important soil component. It has a nutritional function by serving as a source of N, P, and S. Organic matter also promotes a good soil structure and has a high binding capacity for cations and organic contaminants. The bulk of the organic matter is present in the solid phase; a very small part is present as dissolved organic matter (DOM). Organic matter is retained in the solid phase in different states: a) macromolecular insoluble complexes, b) complexes bound together by protons, di- and trivalent ions, c) molecules adsorbed to clay or oxide surfaces, or d) organic matter in the interlayers of expanding-type clay minerals (Stevenson, 1994). The ionic composition of the soil solution influences all states, but particularly b and c: coagulation/dissolution and ad-/desorption.

Ong and Bisque (1968) evaluated coagulation of humic substances in electrolyte solutions (160 mg/l). They varied concentrations of mono-, di-, and trivalent ions at pH 7. Critical concentrations, at which the humic started to coagulate, were found at 600, 7.2, and 1.1 mmol/l for Na, Ca, and Al respectively. Similar results were found by Temminghoff et al. (1998), who studied coagulation of purified forest soil humic acid (370 mg/l) at pH 4 and 6. At pH 4, 100 mmol Na/l was not enough to induce any coagulation, whereas Ca and Al induced coagulation at 3 and 0.16 mmol/l respectively. Tipping and Ohnstad (1984) studied the effect of both Ca and pH on the coagulation of aquatic humic substances (61 mg/l). They did not observe any coagulation at a $CaCl_2$ concentration less than 10^{-3} M independent of pH. At higher $CaCl_2$ concentrations coagulation occurred in all pH treatments (3.1, 6.6, 10.7).

Sorption of organic matter has often been studied using pure mineral surfaces as a sorbent (Gu et al., 1994; Spark et al., 1997; Avena and Koopal, 1998; Filius et al., 2000). They all found a pH-dependent high affinity adsorption isotherm, characterized by an initially steep slope and a semi-plateau at a certain amount of sorbed organic matter. The pH dependency was mainly expressed in the level of the plateau. An increase in pH decreased the maximum sorption capacity of the mineral phases. This was particularly the case for the pH dependent sorption sites on the Al and Fe oxides. They showed the highest binding capacity for organic matter, followed by clay minerals; SiO_2 does not play a role (Spark et al., 1997; Shen, 1999b).

The role of both coagulation and sorption mechanisms makes it very complex to quantify DOM in field soils. It is even more difficult if the parent material of DOM varies. The origin of DOM in field soils is mainly determined by the leaching from fresh litter and relatively stable humus, but the microbial biomass and root exudates may also contribute to the DOM (Kalbitz et al., 2000), as would manure application. This implies that mainly long-term processes play a role in the DOM concentration in stable soils. It is therefore not surprising that a simple relationship between a soil chemical parameter like pH and the DOM concentration is not found (Dolfing and Scheltens, 1999). Apparently, the organic matter is adapted to the actual environmental conditions.

However, if the environmental conditions change, soil chemistry might change. The stable solid-solution partitioning of organic matter is disturbed and this can lead to a (temporary) dispersion of organic matter until a new stable situation is reached (Avena and Koopal, 1998; Balesdent et al., 1998). A decrease in the cation concentration will result in both dissolution and desorption of organic matter. Particularly H^+ and Ca^{2+} are important in soils with a moderate pH. An increased pH increases the negative charge of the organic matter molecules and other solid particles with pH dependent sites, and a decreased Ca^{2+} concentration predominantly creates a more negative electrostatic potential. Both mechanisms lead to an increased repulsion between the organic matter molecules.

The cationic composition of the soil solution might change after addition of chemicals, such as: fertilizers, waste materials, and lime. Other examples of soil additives are immobilizing materials, aimed at transforming soil contaminants, especially heavy metals, into less mobile and less toxic species. Many additives that have been evaluated, are alkaline materials (e.g. Vangronsveld et al., 1996; Lothenbach et al., 1998; Edwards et al., 1999). However, a sudden increase in pH does not only increase metal binding to the soil, but might also increase the concentration of DOM in the soil solution (Dolfing and Scheltens, 1999; You et al., 1999). Next to the addition of chemicals, the change of land use (e.g. transformation from agriculture to forestry) can change the chemical composition (Balesdent et al., 1998; Römkens and Salomons, 1998). It is important to understand the solid-solution partitioning of organic matter as influenced by these changes in ionic composition in the soil solution, because the leaching of DOM can strongly contribute to the leaching of metals and other contaminants to the subsoil or the groundwater (McCarthy and Zachara, 1989; Zhu and Alva, 1993; Temminghoff et al., 1997; Römkens and Dolfing, 1998).

The effect of changes in pH (realized by additions of NaOH) has been studied frequently (Dolfing and Scheltens, 1999; You et al., 1999; Tipping and Woof, 1991). Shen (1999a) studied the effect of NaOH addition to a soil suspension on DOM. He found a strong increase if the soil was suspended in 0.01 M NaCl, but found hardly any increase in DOM in 0.003 M $CaCl_2$. Römkens and Dolfing (1998) added Ca to a soil suspension and observed a decrease in DOM. No soil experiments have been reported as far as we know in which both pH and Ca were varied independently. Besides the limited number of data, the quantitative modeling of organic matter partitioning is rare. Tipping and Woof (1991) divided the soil organic matter (SOM) into a potentially mobile fraction and an permanently immobile fraction. Basically, they related the DOM concentration to the amount of potentially mobile organic matter (OM_{mobile}) and its net electrical charge (Z) according to Equation 3.1:

$$DOM \sim OM_{mobile} * e^{cZ} \qquad [3.1]$$

The net electrical charge of the OM_{mobile} was calculated by the speciation model (Model IV) developed by Tipping and Hurley (1988). The parameter c is constant for all soils. To apply this approach to different soils, Tipping and Woof (1991) assumed a distribution of OM_{mobile} over 10 fractions, each with its own partitioning behavior. This approach was applied to predict DOM in 16 acid organic soils to which acid and base and applied. Conceptually, this approach is good, but the disadvantage is the introduction of two fitting parameters: both the total amount of OM_{mobile} and its distribution over the fractions have to be optimized for each individual soil.

The objective of this study was to study organic matter partitioning in acidic to neutral soils as influenced by changes in pH and Ca levels. A simple model to describe the DOM concentrations was developed from an experiment with one soil. The approach was then applied to 8 other soils to test its general validity. Finally, we predicted the DOM concentration as influenced by the additions of NaOH and $Ca(NO_3)_2$, using our model and analytical results of a single batch experiment with untreated soil.

Experimental Section

Soils

Nine agricultural soils were used in this study. Three of them (K1, K2, and K3) originated from the Kempen, an area in the south of the Netherlands contaminated with Cd and Zn by aerial deposition. The E-numbered soils were obtained from different uncontaminated sites in the Netherlands. Numbers in this paper correspond to the numbers used by Van Erp et al. (2001), who characterized the soils. Additional analyses have been done with respect to Fe and Al (hydr)oxides. Characteristics of the soils are presented in Table 3.1. The percentage of soil organic carbon was determined after wet oxidation by sulfochromic acid (Walinga et al., 1992). Free Al, Mn, and Fe compounds were extracted in a buffer of citrate and dithionite (Holmgren, 1967). The detailed procedure was described by Buurman et al (1996). Clay content was determined by sieve and pipette; unbuffered 0.01 M $BaCl_2$ was used for the cation exchange capacity (CEC) and the pH was measured in 0.01 M $CaCl_2$ (Houba et al., 1995). In the remainder of this paper, we will use dissolved organic carbon (DOC) and solid organic carbon (SOC), because these quantities were measured. DOM and SOM were used in speciation calculations by assuming a carbon content of 50% (w/w).

Experiment 1: DOC in soil K1 as influenced by addition of $Ca(NO_3)_2$ and/or NaOH

Three grams of soil K1 (Table 3.1) were suspended in 30 ml of a mixture of 0.03 M $NaNO_3$, 0.01 M $Ca(NO_3)_2$, and 0.03 M NaOH. The mixing ratio varied, depending on the desired Ca level (11 levels ranging from 0 to 0.025 mol/kg of dry soil) and pH (4 levels varying from pH 6 to 8.7). Extra NaOH additions were needed several times to obtain a constant pH for each Ca series. The tubes were shaken end-over-end for 96 hours. The pH was measured in a settling suspension. After centrifuging for 15 minutes at 1,800 *g*, the supernatant was analyzed for DOC (SK^{12} TOC/DOC analyzer, Skalar), Na, K, Mg, Ca, Al, Fe, Mn, Cd, Cu, and Zn (ICP-OES, Thermo Flame, Spectro Analytical Instruments). DOC samples were carefully acidified to below pH 6 to prevent interference from inorganic carbon. It was checked that the DOC had not flocculated before analysis. ICP-OES samples were analyzed after acidification with concentrated HNO_3 (1% v/v).

Table 3.1 Characteristics of the soils

Properties	K1	K2	K3	E1	E7	E23	E25	E29	E31
%C	2.2	2.0	2.1	2.7	3.1	8.3	14	0.6	2.3
% clay	7.0	2	3	13	4	25	28	3	26
% $CaCO_3$	0	0	0	0	0	0	0	0.71	1.86
$Fe_{citr/dith}$ (g/kg)	2.5	0.48	3.0	5.8	0.79	18.7	9.1	0.80	8.2
CEC (cmol+/kg)	7.8	ND	ND	6.9	5.8	25.3	33.4	3.2	24.9
pH (0.01 M $CaCl_2$)	5.2	4.9	5.3	4.2	4.9	4.9	5.5	6.8	6.9

ND = not determined.

Experiment 2: DOC in different soils as influenced by addition of $Ca(NO_3)_2$ and/or NaOH.

A similar experiment was conducted using 9 soils (Table 3.1). The crosses in Table 3.2 indicate the treatments that were included for each soil. Single doses of NaOH/$Ca(NO_3)_2$ were added; no additional pH corrections were made. The soils without NaOH and $Ca(NO_3)_2$ (just an extraction with 0.03 M $NaNO_3$ in a 1:10 solid:solution ratio) will further be referred to as the untreated soils. The experimental conditions and analytical procedures were similar to the previous experiment.

Table 3.2: soil treatments of experiment 2.

$Ca(NO_3)_2$	NaOH (mmol/kg)			
mmol/kg	0	30	60	90
0	x	x	x	x
16.7	x	x		
33.3			x	x

Modeling

The pH-dependent charge of the organic molecules as well as the ionic composition of the solution appeared crucial factors determining the solubility of organic matter. It was therefore important to know the speciation of the ions present in the system. The total metal concentration in the solution may comprise free ions, inorganic complexes (e.g. carbonates or hydroxides), and metal complexes with organic matter. We measured the total concentrations in solution and used the computer program ECOSAT to calculate the speciation (Keizer and Van Riemsdijk, 1999). Cation binding to dissolved organic matter was described with the consistent Non-Ideal Competitive Adsorption model combined with a Donnan model to account for non-specific cation exchange. The model is referred to as the consistent NICA-Donnan model (Kinniburgh et al., 1999). It accounts for chemical heterogeneity of the organic matter, non-ideal binding behavior and competition between different ions for the same binding sites. The organic matter is electrostatically considered as a gel having a Donnan potential. In this study we used a generic parameter set for humic acids without any adjustments (Milne, 2000). The generic parameter set was based on the description of a wide variety of literature data for metal ion binding to humic acids.

Results and Discussion

Description of DOC in soil K1

Figure 3.1a shows log DOC as a function of total Ca in the system. The total Ca concentration in the system is the amount initially present (= 0.035 mol/kg) plus the amount added as $Ca(NO_3)_2$. The pH (± 0.1) of each series is presented in the legend. Addition of Ca decreases the DOC concentration. The effect is larger at higher pH values. An obvious pH effect can be observed in Figure 3.1a, particularly at low Ca levels. The pH effect almost disappears if log DOC is presented as a function of the dissolved Ca concentration in the supernatant (Figure 3.1b). Apparently, pH plays a minor role compared to the dissolved Ca concentration (including free and complexed Ca). This was also found by Temminghoff et al. (1998), who observed humic acid coagulation at certain Cu or Ca concentrations, but found hardly any differences between pH 4 and 6.

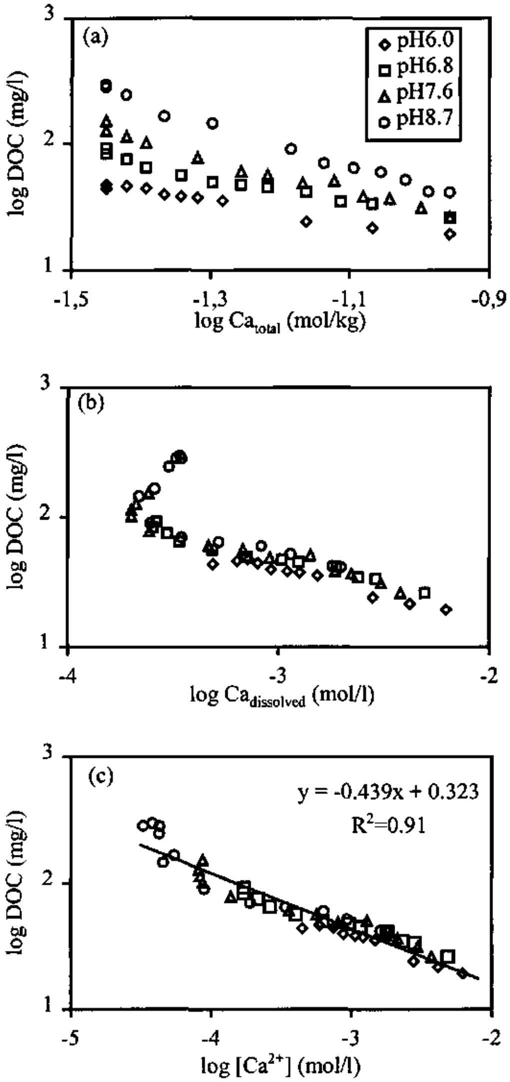

Figure 3.1: Log DOC versus (a) log total Ca, (b) log dissolved Ca, and (c) the calculated log [Ca^{2+}] in a suspension of soil K1 at four different pH values.

Figure 3.1b shows that the dissolved Ca concentration increases at high DOC concentrations, which can be explained by the formation of Ca-DOC complexes in the supernatant. It might be better to use the free ionic concentrations in the soil as a predictive variable for DOC. Hence, we calculated the speciation in the supernatant. Measured total solution concentrations of Al, Ca, Fe, K, Mg, Na, Zn, and the pH were used as input variables. The generic parameters for purified humic acid were used to run the consistent NICA-Donnan model, assuming that the organic matter in the supernatant consists of humic acid. Figure 3.1c shows a good correlation between log DOC and the calculated log $[Ca^{2+}]$.

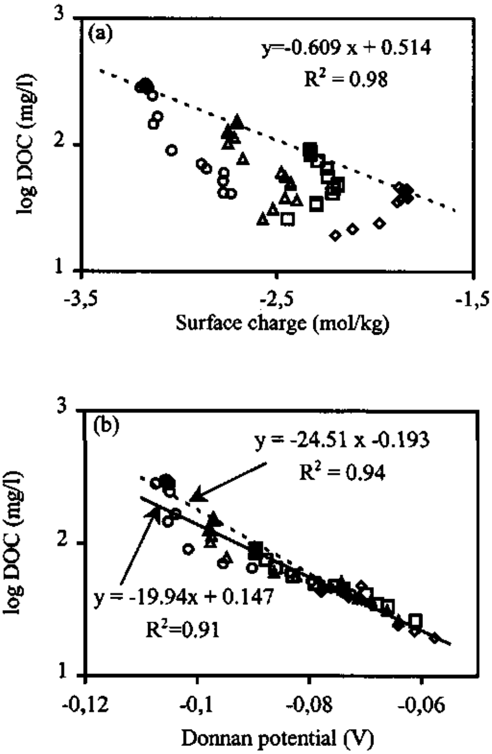

Figure 3.2: Log DOC versus (a) the calculated net electrical charge, and (b) the Donnan potential in a suspension of soil K1 at four different pH values. ◇ = pH 6.0; □ = pH 6.8; Δ = pH 7.6; O = pH 8.7. Closed symbols refer to treatments without $Ca(NO_3)_2$ addition (only adjusted with NaOH). Open symbols refer to treatments with $Ca(NO_3)_2$ and subsequently adjusted to the required pH.

From a theoretical point of view, we preferred a more general approach in stead of using a correlation with a single ion. Tipping and Woof (1991) used the net electrical charge as a basis for their predictions. We calculated the net electrical charge using the consistent NICA-

Donnan model. Figure 3.2a shows the relationship between log DOC and the charge. If only NaOH is used to vary the pH, the calculated net electrical charge correlates nicely with the DOC concentration (black data points and dashed line in Figure 3.2a). However, if Ca is added, the net electrical charge no longer correlates with the DOC concentration. Figure 3.2b shows log DOC versus the calculated potential in the Donnan gel (ψ_D). Here too, a difference, albeit slightly, is observed between the treatments with (open data points; solid line) and without Ca (black data points; dashed line), but the correlation is still strong even if Ca treatments are included. The data only show some scattering at very negative potentials (high pH; low Ca concentration). This might be partly attributed to experimental complications concerning CO_2 at high pH values. Calculations of carbonate complexes were based on atmospheric CO_2-pressure, which might not have been reached completely. Furthermore, the speciation calculations may have provided some uncertainty, particularly at higher pH values. Despite these uncertainties, the single parameter ψ_D can be used to quantify the effects caused by changes in solution conditions on the dispersion of organic matter in soil K1.

DOC in different soils

An experiment using 9 soils (including K1) was conducted to validate the relationship as shown in Figure 3.2b. Figure 3.3 shows ψ_D versus log DOC for all soils, except E29. This soil was left out, because the lowest NaOH-level (30 mmol/kg) increased the pH already to 11, which was outside the range of a natural soil. Also the data with pH > 9 in soil E31 have been excluded for that reason. The black dots in Figure 3.3 represent the untreated soils. The addition of hydroxide generates a more negative potential, whereas the addition of only calcium would result in a less negative potential. The shift in ψ_D produced by the addition of a fixed dose of NaOH or $Ca(NO_3)_2$ varies for each soil, depending on the buffer capacity of the soil.

All soils show the same trend; a nice correlation is shown between DOC and the calculated ψ_D. An unexpected observation was that the slope of soil K1 is –28.01 (Figure 3.3) in stead of –19.94 (Figure 3.2b). There is one difference in the experimental set up between experiments 1 and 2. NaOH was added several times in Figure 3.2b till the required pH was reached, whereas a single dose was added in Figure 3.3. The initial shock of the NaOH addition led to extreme pH conditions and a (partly) irreversible dispersion of organic matter. Careful consideration of both experiments revealed that the final pH and $[Ca^{2+}]$ after 96 hours was not influenced by the way of NaOH addition. As a result, the Donnan potential (strongly

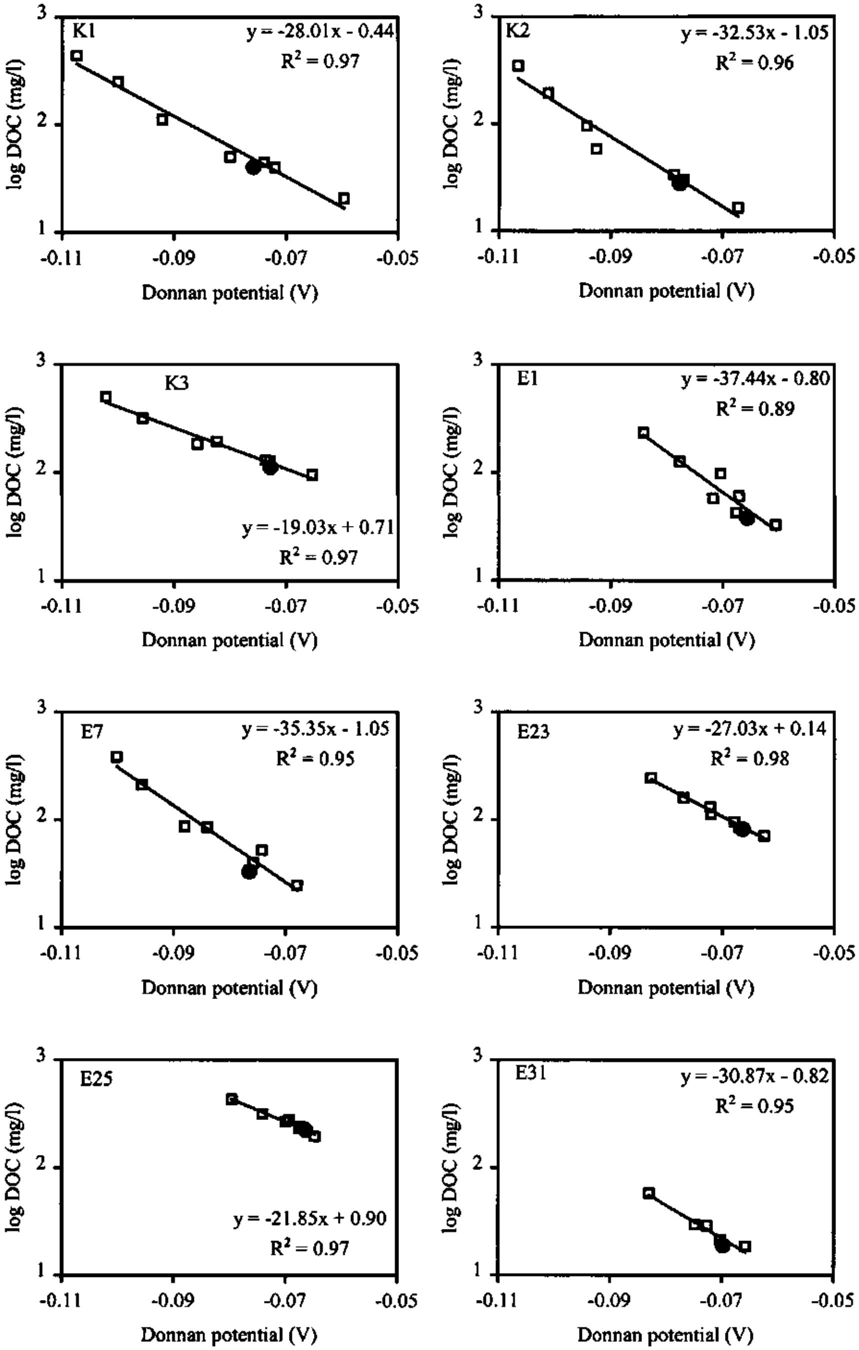

Figure 3.3: Relationship between log DOC and Donnan potential as influence by Ca and pH in 8 different soils. The line represents the regression as calculated from all data. The equation is written in each graph. ●: untreated soils; □: soils treated with NaOH or $Ca(NO_3)_2$.

influenced by Ca) showed a different correlation with DOC in both experiments. This phenomenon might have practical implications: a slow change in ionic composition, which happens after changing land use, might have less influence on the organic matter partitioning compared to a sudden change after the addition of chemicals.

Both the slope and the elevation of the lines in Figure 3.3 show considerable differences between the soils. The slopes range from -19 to -38 (factor of 2) with a median of -29.4. A few reasons could be responsible for the varying slopes. The adsorption capacity of mineral phases for organic matter molecules might influence the dispersion rate. Filius et al. (2000) showed that iron oxides influence the electrostatics of adsorbed organic matter molecules. Both Spark et al. (1997) and Shen (1999b) found that Al and Fe oxides showed the highest binding capacity and clay contributed to a lesser extent. An alternative explanation might be that the original proton or calcium activity in the untreated soils influenced the state of the solid organic matter which might influence the slope. However, neither the clay %, CEC or extractable Fe content nor pH or free Ca concentration of the untreated soil provided a strong correlation with the slopes in Figure 3.3. Extractable Al compounds were negligible in these soils.

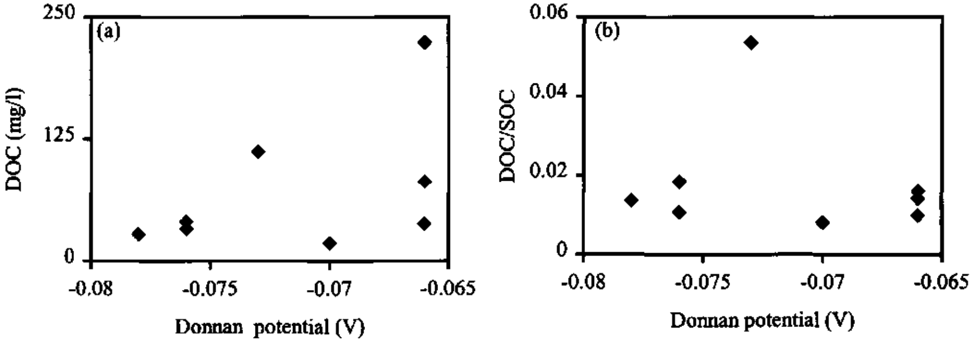

Figure 3.4: DOC (a) and DOC/SOC (b) as a function of the Donnan potential for 8 untreated soils.

The differences in elevation of the lines are most obvious if E25 and E31 are compared (Figure 3.3). The elevation is strongly influenced by the DOC concentration in the untreated soil, which appeared to be correlated to the SOC content of the soil. These phenomena are illustrated by Figure 3.4. Figure 3.4a shows the DOC concentrations for each soil as a function of the calculated Donnan potential. The potential of the untreated soils lies between –66 and –78 mV. This is a relatively small range for soils that varied widely in soil characteristics (Table 3.1), but within that range the DOC concentrations varied by a factor of 12. If the DOC (g/kg) was normalized to the SOC (g/kg), the variation was reduced to a factor of 6.6, due to soil K3 (Figure 3.4b). All other untreated soils had a DOC/SOC ratio, which varied roughly between 0.01 and 0.02. The differences in the elevation of the lines in Figure 3.3 would have been smaller after incorporation of the SOC contents in our equation.

Noteworthy is the fact that the DOC/SOC ratios found in this experiment were about 60 times higher than average values found in pore water of Dutch field soils (Peijnenburg, personal communication). The experimental conditions (e.g. dried soil versus moist field soil, temperature, pore water versus $NaNO_3$ background electrolyte, sampling methods, and different solid:solution ratios) could be responsible for this difference in DOC/SOC (Kaiser et al., 2001).

Prediction of DOC as influenced by cations

Until now, we needed to know the speciation in solution (including DOC) in each treatment before we could calculate ψ_D. Thus, we could only describe the DOC concentration. Ideally, one would be able to predict the DOC concentration for a range of conditions based on one simple experiment with an untreated soil. We used the following procedure to predict DOC. First, the analytical results of the supernatant of the untreated soils (without any Ca or OH added) were used to calculate free ion concentrations (see section Modeling). The free ion concentrations, in turn, were used to calculate the total amount of metals in the organic phase, which was less than the total metal amount in the system. For these calculations, the solid and dissolved organic matter were considered one pool, having the same binding properties as humic acid. Tipping and Woof (1991) made the same assumption for the OM_{mobile}. The calculated metal amounts in the organic phase plus the additions of NaOH and $Ca(NO_3)_2$ were used as input values for the prediction of the pH, free metal concentrations, and ψ_D in the treatments shown in Table 3.2. ψ_D was used to predict the DOC concentration according to Equation 3.2.

$$\text{Log DOC} = -29.4\ \psi_D + C \qquad [3.2]$$

The value of –29.4 is the median of the slopes in Figure 3.3. The intercept C determines the elevation of the line and is obtained by filling in the calculated ψ_D and the DOC concentration measured in the experiment with untreated soil in Equation 3.2. Two underlying simplifying assumptions were included in this procedure. We assumed that changes in proton and cation concentrations in the suspensions were fully due to changes in ion binding by the organic matter in the soil, whereas effects due to the clay content, and metal (hydr)oxides were ignored. Furthermore, organic matter was considered one pool with the binding properties of humic acid.

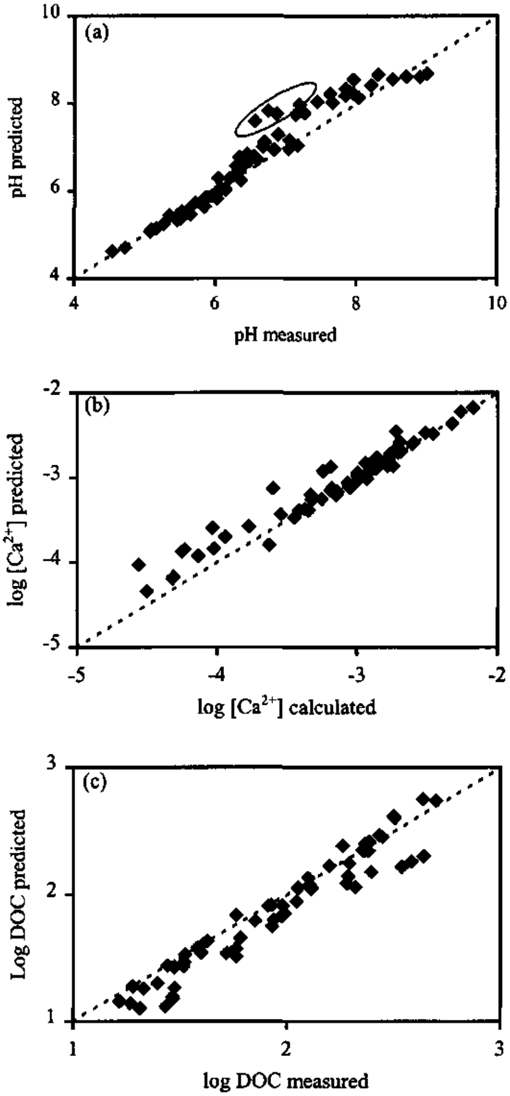

Figure 3.5: (a) Predicted pH versus measured pH in all treatments and all soils; the oval closure marks the highest NaOH additions in soil E1 and E7 (see text). (b) Predicted log [Ca^{2+}] versus calculated log [Ca^{2+}] in all treatments and all soils. (c) Predicted log DOC according to Equation 3.2 versus measured log DOC. Dashed lines are 1:1 lines.

The pH, free metal concentration and ψ_D of each treatment can now be predicted by speciation calculations according to the above-mentioned procedure. The pH has also been measured, so we could verify the reliability of our predictions. Figure 3.5a shows the predicted versus the measured pH (Figure 3.5a). It is remarkable that the prediction is reasonable, considering the simplifying assumptions and the large pH range (4 units). The largest differences between measured and predicted value were obtained for the highest NaOH doses (with and without Ca) in soil E1 and E7 (oval enclosure in Figure 3.5a). The Ca concentration strongly affects the calculated ψ_D. Therefore, we also compared the predicted free Ca concentration, following the above-described procedure, to the value calculated in the supernatant, based on measured total concentrations in solution. Figure 3.5b shows that also in this case the correspondence is quite good. Only at low Ca levels the predicted values are systematically too high.

The quality of the predicted data in 3.5a and 3.5b suggests that also the Donnan potential is at least reasonably predicted. The predicted ψ_D is used to obtain the predicted DOC concentration using Equation 3.2. Figure 3.5c presents the relationship between the measured and predicted DOC concentration. The predicted DOC concentrations are generally a bit lower than the measured values, but the absolute deviation is the largest at high DOC concentrations. The predictions can be considerably improved, if a soil specific value for the slope (Equation 3.2) is used, but this requires more experimental work. However, the prediction procedure is very simple and without fitting parameters: the analytical data of one single batch experiment, model calculation using a generic parameter set, and a median value for the slope of Equation 3.2 were used. This method can be used as a quick procedure to estimate whether soil treatment with alkaline (waste) materials or other materials that strongly influence the ionic composition will affect the DOC concentrations, and, as a result, the leaching of contaminants bound by DOC.

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Chapter 4

Immobilization of heavy metals in soils using alkaline additives especially synthetic zeolites

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Abstract

In situ immobilization of heavy metals in contaminated soils is a technique to improve soil quality. Synthetic zeolites are potentially useful additives to bind heavy metals. This study selected the most effective zeolite in cadmium and zinc binding out of six synthetic zeolites (mordenite-type, faujasite-type, zeolite X, zeolite P, and zeolite A) and one natural zeolite (clinoptilolite). Zeolite A appeared to have the highest binding capacity between pH 5 and 6.5 and was stable above pH 5.5. The second objective of this study was to investigate the effects of zeolite addition on the dissolved organic matter (DOM) concentration. Since zeolites increase the pH and bind Ca, their application might lead to dispersion of organic matter. In a batch experiment, the DOM concentration increased by a factor of 5 when the pH increased from 6 to 8 as a result of zeolite A addition. A strong increase in DOM was also found in the leachate of soil columns, particularly in the beginning of the experiment. This resulted in a higher metal leaching caused by metal-DOM complexes. In contrast, the free ionic concentration of Cd and Zn was strongly decreased after the addition of zeolites, which might explain the reduction in metal uptake observed in plant experiments. Pretreatment of zeolites with acid (to prevent a pH increase) or Ca (to coagulate organic matter) could suppress the increase in DOM, but did also decrease the binding capacity of the zeolites due to competition of protons or Ca.

Introduction

Common soil remediation technologies are suitable for relatively small and heavily contaminated sites, but are financially and physically inefficient in large moderately contaminated areas. New techniques are being developed to treat these areas, such as immobilization and phytoremediation (Vangronsveld and Cunningham, 1998). *In situ* immobilization reduces negative effects of contaminants by adding an immobilizing chemical to the soil. The additives must obviously possess a high binding capacity, but they should not impair soil structure, soil fertility or the ecosystem. Hence, evaluation of potential immobilizing agents should not only include the metal binding capacity, but also possible side effects.

A lot of additives have been screened for their potential to immobilize heavy metals in soils. Many of them are alkaline materials. Examples of alkaline additives are: lime (Hooda and Alloway, 1996; Singh and Myhr, 1998), zeolites (Czupyrna et al., 1989; Gworek, 1992; Edwards et al., 1999), many incinerator ashes (Shende et al., 1994; Vangronsveld et al., 1996), Fe rich, which is an iron oxide released during the industrial production of TiO_2 pigment (Chlopecka and Adriano, 1997), gravel sludge (Lothenbach et al., 1998), hydroxyapatite (Boisson et al., 1999), and Thomas basic slag (Mench et al., 1994). Next to the binding sites on the surface of the immobilizing material, also an increase in soil pH contributes to the immobilization of heavy metals in soil. An increase in pH does not provide new binding sites, but makes existing sites in the soil more reactive towards metal binding due to a decreased proton competition. An increase in pH and the addition of new sites do not always produce similar effects. Oste et al. (in press) showed that the addition of lime (pH increase), and manganese oxides (addition of new sites) could decrease the free Cd concentration in soil pore water to the same extent, but that the Cd uptake by earthworms was significantly more reduced by MnO_2 compared to lime. Furthermore, an increase in pH may increase the dissolved organic matter concentration and microbial activity (Haynes and Naidu, 1998) and might change the ecosystem with respect to vegetation (Roem and Berendse, 2000) and soil organisms (Korthals et al., 1996; Edwards and Bohlen, 1996). Nevertheless, a pH increase is often effective in reducing metal leaching and plant uptake. Alkaline additives can thus improve the quality of many contaminated soils.

This study is mainly focused on the use of zeolites as immobilizing agents. Zeolites are crystalline alumino silicate minerals. They are generally formed in nature when water of high pH and high salt content interacts with volcanic ash causing a rapid crystal formation. The framework consists of $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ tetrahedra linked at all corners. The framework is open and contains channels and cavities in which cations and water molecules are located (Dyer, 1988). The channel structure of zeolites is responsible for their function as a molecular sieve, but is also important for 'selective' cation exchange. The selectivity for different ions is determined by several factors (Dyer, 1988):

- size and state of solvation of the ions;
- the charge (Si:Al ratio) and geometry of the framework;
- the number of cation sites available for occupation inside the framework;
- temperature.

Natural zeolites have been used for a long time in Japan to improve soil quality. Farmers add zeolites to the soil to control soil pH and to improve ammonium retention (Dwyer and Dyer, 1984). Natural zeolites are used in wastewater treatment to remove ammonium ions and heavy metals (Zamzow et al., 1990). Weber et al. (1984) investigated the effect of a natural zeolite (clinoptilolite) on heavy metal uptake by plants from an agricultural field. They did not observe a reduction in heavy-metal uptake in sorghum even at an addition rate of approximately 6.5%. Other studies concerning the addition of natural zeolites to soil also show little or no effect on the availability of metals (Mineyev et al., 1989; Chlopecka and Adriano, 1996 and 1997; Baydina, 1996).

Synthetic zeolites have yielded better results. Czupyrna et al. (1989) selected a large number of additives and the zeolite type A appeared to immobilize heavy metals very strongly in batch and column experiments, certainly when the application was combined with addition of $FeSO_4$. Singh et al. (2000) measured high adsorption of Cd to the synthetic zeolites A, P and Y, compared to natural zeolite. The batch experiment was carried out in 0.01 M $NaClO_4$ solution at pH 6-7. Gworek (1992) added 1% synthetic zeolites A and X to a Cd-contaminated loamy soil ($pH_{KCl} = 7.8$) and grew lettuce and ryegrass. The Cd uptake in both plants was significantly reduced in the treated soils. Zeolite X performed slightly better compared to zeolite A. Zinc uptake could also be reduced after adding zeolite A to a loamy sandy soil ($pH_{KCl} = 6.4$) (Gworek, 1994). The effect of zeolites on soil pH was not reported, but particularly in the Cd-contaminated soil (pH = 7.8), it is unlikely that an increase in pH will have strongly contributed to the reduction in plant uptake. Other work on synthetic zeolites has been done by Lepp and co-workers (Rebedea and Lepp, 1994; Rebedea et al., 1997; Edwards et al., 1999). Rebedea and Lepp (1994) used zeolites A and P to evaluate metal uptake by plants in pots and in the field, and leaching from columns. The pot experiment showed that zeolites significantly reduced metal uptake, but the reduction was partly caused by a pH increase, a side effect of zeolite addition. Edwards et al. (1999) compared zeolites and lime to quantify the contribution of ion exchange and alkalization. Two soils were treated with three levels of zeolites A, P, and X and watered to field capacity. After 3 months, soil solution samples were taken using a displacement technique. Unfortunately, only one liming level was applied, but a rough estimation indicates that the reduction in the soil solution independent of the alkalization effect is up to a factor of 2 for Cd and Zn.

Although the binding capacity of synthetic zeolites can be very high, an increase in soil pH without a simultaneous addition of Ca, can raise the DOM concentration in the soil (Chapter 3). An increase in DOM might result in increased leaching of metals bound to DOM (e.g. McCarthy and Zachara, 1989; Temminghoff et al., 1997).

Therefore, this study has two main objectives. The first one is to evaluate the Cd and Zn binding capacity and stability of synthetic zeolites. The second objective is to assess the effect of zeolites on leaching of DOM and metals bound to DOM, and the possibilities to limit leaching, without losing the metal binding capacity of zeolites.

Table 4.1: Characteristics of soils and additives.

Properties	Soil K1	Zeo A(PQ)	CA	Soil K4
% C	2.2	-	-	2.3
% clay (< 2 μm)	7	-	-	3
% 2-50 μm	4	-	-	12
CEC (cmol+/kg)	7.8	560*	-	-
pH (0.01 M $CaCl_2$)	5.2	10.1	9.5	5.8
Na_{total} (mmol/kg)	2.22	5,522	71.3	1.0
Ca_{total} (mmol/kg)	35.4	5.24	1,191	64.8
Al_{total} (mmol/kg)	109	5,889	2,185	175
Cd_{total} (mmol/kg)	0.030	<0.001	0.018	0.055
Zn_{total} (mmol/kg)	3.05	0.26	2.91	8.23

*Value supplied by The PQ Corporation

Materials and methods

Soils

The soils used in this study originated from the Kempen, an area in the South of the Netherlands contaminated with air-borne Cd and Zn emitted by different Zn smelters in the region. The molar ratio of Cd to Zn in these soils was approximately 1:100 (Boekhold et al., 1992a; Wilkens and Loch, 1997). Soil characteristics are presented in Table 4.1. The percentage of organic carbon was determined after wet oxidation by sulfochromic acid (Walinga et al., 1992). Clay content was determined by sieve and pipet; unbuffered 0.01 M $BaCl_2$ was used for the cation exchange capacity (CEC) and the pH was measured in 0.01 M $CaCl_2$ (Houba et al., 1995).

Binding of Cd and Zn to zeolites in a batch system

We tested 1 natural, and 6 synthetic zeolites in a batch experiment. A mordenite-type (MOR), a faujasite-type (FAU), zeolite A (zeo A(PQ)) and zeolite X (zeo X) were obtained from The PQ Corporation (Kansas City, KS, USA). Zeolite P (zeo P) and another zeolite A (zeo A(Cf)) were provided by Crosfield (Eijsden, the Netherlands). All synthetic zeolites were supplied in the Na-form. Natural zeolite, clinoptilolite (CLIN), was provided by Minerais de la Mediterranée (Balaruc-les-Bains, France). The binding capacity was determined at three concentrations of Cd and Zn by shaking 0.2 g of zeolite in 180 ml of an artificial soil solution adjusted to pH 5 or 6.5 with 0.1 M HNO_3 at 20°C. The pH was measured with a pH-electrode (Radiometer, Copenhagen, DK). The artificial soil solution contained 0.5 mM K, 0.5 mM Na, 0.3 mM Mg, and 1 mM Ca all added as chloride salts, resulting in an ionic strength of 5 mM. Cd and Zn were added to this solution in a molar ratio of 1:100. Added Zn levels were 1.5, 5, and 10 µM, whereas Cd levels equaled 0.015, 0.05, and 0.1 µM. After a shaking period of approximately two weeks, the suspensions were centrifuged at 1800 *g* for 15 minutes. After acidification, the supernatant was analyzed for Ca, K, Mg, and Na by inductively coupled plasma with optical emission spectrometry (ICP-OES, Spectro Analytical Instruments, Kleve, Germany). Zn was analyzed by flame atomic absorption spectrometery (FAAS) with Smith Hiefje background correction (Instrumental Laboratory-S11, Thermo Jarrell Ash, Breda, the Netherlands), and Cd by graphite furnace atomic absorption spectrometry (GFAAS) with Zeeman background correction (Varian SpectrAA 300-Zeeman, Sunnyvale, CA, USA).

Zeolite A(PQ) was chosen to determine more detailed adsorption characteristics for Cd and Zn in a 1:100 ratio. Again, 0.2 g of zeolite was added to 180 ml 0.005 M salt solution, containing 0, 0.156, 0.313, 0.625, 1.25, or 5 μM $ZnCl_2$ and adjusted to 0.005 M with $CaCl_2$. The pH was 5, 5.7, and 6.5. After shaking for two weeks, centrifuging, and acidifying, the samples were analyzed for Zn by FAAS, and for Cd by GFAAS.

Stability of zeolites as influenced by pH

Zeolite P, zeolites A(Cf) and A(PQ) were used in this experiment. Five grams of zeolite were suspended in 50 ml of demineralized water and pH adjusted to lower values with 2.5 M HNO_3. The pH ranged from 9 to 4. The experimental procedure was similar to the previous experiment, except that in this experiment Al, Ca, K, Mg, Na were measured by ICP-OES.

Effects of zeolites and other alkaline materials on DOC in soil suspensions

A batch experiment was designed to study the solid-solution partitioning of organic matter after zeolite A addition to a soil. Cyclonic ashes, formerly called: beringite (provided by Dr. J. Vangronsveld, LUC, Diepenbeek, Belgium), were also included in this experiment, because they contain much Ca. Characteristics of this material are presented in Table 4.1. Three grams of soil K1 (Table 4.1) were suspended in 30 ml of a 0.03 M $NaNO_3$-solution in a 40 ml centrifuge tube. NaOH was added as a solution (0.03 M); $Ca(OH)_2$, zeolite A(PQ) and cyclonic ashes were added as a suspension (strongly stirred). The highest additions increased the pH to 9. The tubes were shaken for 96 hours, centrifuged and the supernatant was analyzed for DOC (SK^{12} TOC/DOC analyzer, Skalar, Breda, the Netherlands). Acidified samples were analyzed for Al, Ca, K, Na, and Zn by ICP-OES.

Effects on the leaching of DOC from zeolite-treated soil

We conducted an experiment using four columns: untreated soil, and soils treated with 0.5% zeolite A(PQ), 2.5% zeolite A(PQ), or 0.3% lime ($Ca(OH)_2$). Soil K4 (Table 4.1) was mixed with additives and moistened to a water content of 0.18 l/kg. The soil was left at room temperature in the dark for four weeks and then dried. The column experiments were carried out according to the Dutch protocol for leaching tests (Nederlands Normalisatie Instituut, 1995). The columns (internal diameter: 5 cm, length: 22 cm) were completely filled with approximately 650 g of dry soil and leached with ultra-pure water (acidified to pH 4 with HNO_3) from the bottom to the top at a speed of 15 ml/hour. Total liquid-solid ratio (volume-weight) over the whole experiment is 10, corresponding to approximately 30 pore volumes. A glass fiber pre-filter (Schleicher & Schuell, Dassel, Germany) to prevent blockage, and a 0.45 μm membrane filter (Schleicher & Schuell, Dassel, Germany), to separate the solid and dissolved phase, were fixed at the top of the column. The protocol prescribes collection of the leachate in 7 fractions, but we collected approximately 100 fractions with an increasing volume. All fractions were analyzed for pH, and DOC, Al, Ca, Na by ICP-OES, Zn and Cd were measured by ICP-MS (Elan 6000, Perkin Elmer, Norwalk, CT, USA).

Effects on the leaching of DOC from soil mixed with pretreated zeolites

The experimental set up is similar to the first column experiment. Next to an untreated soil column, and a 0.1% $CaOH_2$ treatment, there were three columns containing soil with 0.5% pretreated zeolite. The pretreatments comprised: (1) addition of acid to soil pH; (2) addition of Ca; (3) addition of acid and Ca. The acid treatments were done in a zeolite suspension with concentrated HNO_3 to pH 5.8. Ca treatment comprised the addition of 1 mol Ca/kg zeolite and stirring the suspension overnight. Subsequently, the zeolites were washed three times with 0.001 M $Ca(NO_3)_2$. The combined Ca/H treatments got the same treatments as the Ca-treated zeolite, but also concentrated HNO_3 was added in each step (also in the first step).

Modeling

The approach developed in Chapter 3 described a relationship between DOC concentrations in batch experiments and the electrical Donnan potential of humic acid (ψ). The average ψ was determined by speciation calculations using ECOSAT, a computer code for speciation and transport in soil-water systems (Keizer and Van Riemsdijk, 1999). Both organic and inorganic metal complexes were included. Metal-DOM complexes were calculated using the consistent Non Ideal Competitive Adsorption model combined with an electrostatic model that considers the organic matter as a gel with a Donnan potential. In the remainder of the paper, we will refer to the model as the consistent NICA-Donnan model (Kinniburgh et al., 1999). Na, Ca, Zn, DOC, and pH as determined in the supernatant were used as input variables in the model. Generic NICA-Donnan parameters for humic acid were obtained from Milne (2000). The relationship between ψ (V) and DOC (mg/l) obtained for the Kempen soil (K1) in Chapter 3 is:

$$DOC = 0.363 * 10^{-28.01*\psi} \qquad [4.1]$$

We used this equation to describe the DOC in soil suspensions treated with NaOH, $Ca(OH)_2$, zeolite A, and cyclonic ashes.

Results and discussion

Selection of zeolites

The batch experiments without soil were designed to select the most effective zeolites with respect to metal binding at moderate pH. Table 4.2 shows the percentage of added metal that is bound to the zeolites.

Table 4.2: Percentages of Cd, Zn, and Ca bound by different zeolites at pH 5 and 6.5.

	pH	Cd (%)	Zn (%)	Ca (%)
MOR	5	3.6	3.9	57.1
	6.5	16.5	13.5	57.5
FAU	5	84.8	72.8	80.3
	6.5	90.9	78.9	84.0
Zeo A(PQ)	5	94.4	87.3	82.4
	6.5	99.9	99.1	98.7
Zeo X	5	95.8	37.0	46.1
	6.5	99.3	97.4	97.3
Zeo P	5	91.2	82.1	77.1
	6.5	99.8	98.2	98.1
Zeo A(Cf)	5	73.0	94.4	76.7
	6.5	100	97.5	99.4
CLIN	5	0	8.4	0
	6.5	11.3	18.9	0

It took approximately two weeks to get the zeolite suspensions at the required pH values. We added HNO_3 in rather small steps to prevent destruction of the zeolite framework caused by an overdose of acid. However, if the required pH was reached at a certain day, the pH had increased again by the following day. This phenomenon was also observed by Singh et al. (2000). We continued adjusting the pH till the change over 24 hours was less than 0.1 pH unit. Although we started with an artificial soil solution, the large CEC of the zeolites and soluble salts in the material changed the initial solution completely, resulting in a strongly increased Na concentration. The adsorption values in Table 4.2 are averages of three Cd/Zn levels. The binding rates vary widely between different zeolites. The amount of metals bound to the natural zeolite (CLIN) is almost negligible, but also the mordenite-type and faujasite-type do not show a high metal binding capacity. Zeolites A, P and X have many adsorption values exceeding 90%. Zeolite X shows a strong decrease in Zn binding at pH 5, whereas zeolite P shows a strong decrease in Cd binding at pH 5. Zeolite A(PQ) seems to have a slightly higher binding capacity than zeolite A(Cf), but the differences were not significant (t-test, $p > 0.05$). Based on these results, the stability experiment was conducted with both zeolites A, because of their high binding capacity, and with zeolite X, because of its preference for Cd.

Stability

The stability experiment showed that the Al concentration increased at low pH values (Figure 4.1). The measured concentrations indicated a supersaturated solution, possibly because of alumino silicate colloids. The maximum dissolved Al concentrations in Figure 4.1 accounted for only 5% of the total Al present in the system, but damage to the surface layer might strongly affect the binding behavior. Singh et al. (2000) found that the Cd sorption to zeolite A increased from less than 10% at pH 4 to more than 95% at pH 5.8. X-ray diffraction patterns confirmed the destruction of the mineral structure under acidic conditions. Although zeolite X appeared to be more stable, we considered also zeolite A stable enough for use. Moreover, zeolite A(PQ) had a slightly higher binding capacity, particularly for Zn (Table 4.2), and was cheaper (personal communication A. Dube, PQ Corporation).

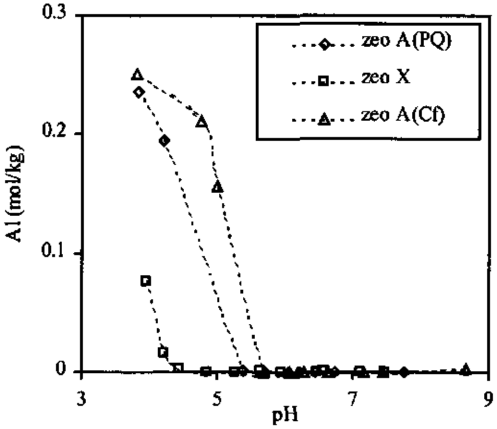

Figure 4.1: Aluminum released by adding HNO_3 to a zeolite suspension.

Cd and Zn binding by zeolite A at pH 5, 5.7, and 6.5

More detailed adsorption experiments were therefore performed using zeolite A(PQ). Figure 4.2 shows the Zn and Cd adsorption to zeolite A(PQ). A clear pH dependency can be observed, though the differences between pH 5 and pH 5.7 are remarkably small, whereas the metal binding at pH 6.5 is much higher. It might be possible that the dissolution behavior of the zeolite between pH 5 and 6 (Figure 4.1) is responsible for this difference. The analytical detection limit made it impossible to measure lower Cd concentrations (Figure 4.2b).

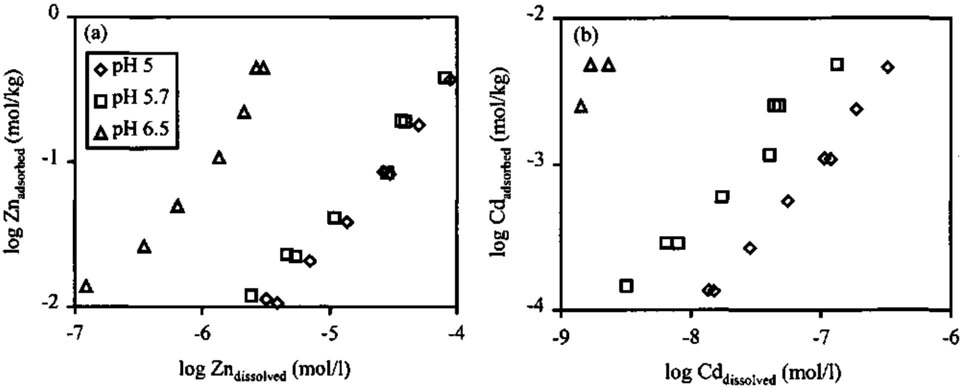

Figure 4.2: Adsorption of Zn (a) and Cd (b) on zeolite A(PQ) at three pH levels.

We compared the Cd sorption characteristics of this zeolite with a Kempen soil using a pH-dependent Freundlich equation (Boekhold et al., 1992b). If the Cd loading on the solid phase was similar, the Cd concentration in solution was at least 100 times lower in a zeolite suspension compared to a soil suspension at pH 5, and 10^{-4} mol Cd/kg. The difference between soil and zeolite could rise to a factor of 2000 at pH 6.5, and 10^{-2} mol Cd/kg.

Effects of zeolites on DOM in soils

Figure 4.3a presents the DOC concentrations influenced by an increase in pH as realized by different materials. Addition of NaOH increases the DOC concentration by a factor of 5 when the pH shifts from 6 to 8, whereas no increase in DOC was observed if the pH was increased with $Ca(OH)_2$. Figure 4.3a also shows that zeolite A acts like NaOH and that the Ca-rich cyclonic ashes (CA) behave similarly to lime. The various treatments affect the calcium concentration in solution differently (Figure 4.3b). The addition of CA leads to a rise in calcium concentration, whereas addition of zeolites or NaOH leads to a decrease in soluble calcium. The effects on the soluble Zn concentration are shown in Figure 4.3c. Initially, Zn decreases in all treatments (Figure 4.3c), but only the CA treatment shows a continuous decrease to almost zero. Addition of $Ca(OH)_2$ can not decrease the Zn concentration to less than 5 mM. The differences between CA and $Ca(OH)_2$ can partly be attributed to the decrease in organic matter, but probably also to the metal binding capacity of the CA at high addition rates (10, 20 and 25% in case of the three highest data points). Treatment with NaOH or zeolites induces an increased Zn concentration in solution above pH 6.5 and 7 respectively. The increase in DOM can explain the increased Zn concentrations at higher pH values.

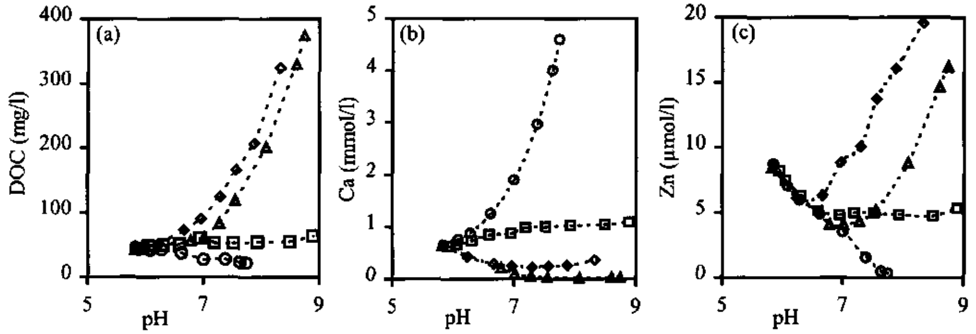

Figure 4.3: DOC (a), Ca (b) and Zn (c) measured in suspensions containing soil K1 and different additives. ◇ NaOH; □, $Ca(OH)_2$; O, cyclonic ashes; △, zeolite A.

Each measured DOC value, as shown in Figure 4.3a, can also be calculated with Equation 4.1. The lines in Figure 4.4 represent the calculated data points that are connected to each other. The predictions are in perfect agreement with the data in case of the CA and lime treatments, whereas the calculations of the NaOH treatment are reasonable. The line in Figure 4.4 representing the zeolite treatment shows a higher calculated DOC concentration between pH6 and 8 than the NaOH treatment. This is not surprising, since zeolites decrease the Ca concentration, leading to a more negative Donnan potential, and thus a higher DOC concentration (Equation 4.1). In contrast to the calculations, the measured data show a slightly lower DOC concentration in the zeolite treatment compared to the NaOH treatment. Apparently, the zeolite addition produces other effects on the DOC than only the changes in pH and the concentration of cations in solution which is accounted for in our calculations. However, general trends are well predicted, also for the zeolite treatments.

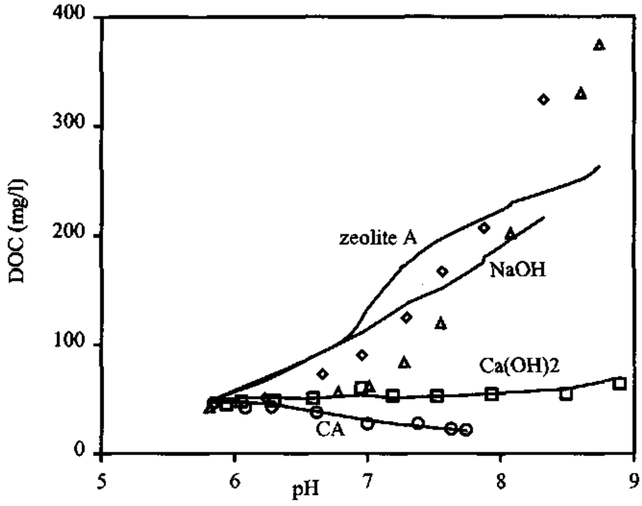

Figure 4.4: Measured (symbols) and calculated (lines) DOC concentrations in suspensions containing soil K1 and different additives. ◇ NaOH; □, Ca(OH)₂; O, cyclonic ashes; △, zeolite A.

Leaching from zeolite-treated soil columns

Figure 4.5a presents the pH in the leachate of the first column experiment. The zeolite-treated columns show an elevated pH during the whole experiment compared to the untreated soil. The lime-treated column started above pH 8, but at the end the pH had decreased to 7.6. Figure 4.5b to 4.5f show cumulative amounts of leached elements, because the differences in concentration between the first few pore volumes and those at the end of the experiment are large and difficult to show in one graph.

The results of the batch experiments regarding DOC (Figure 4.3a) are confirmed by the results of our first column experiment. Figure 4.5b shows that addition of zeolite A (Na-form) increased DOC leaching. The DOC concentrations in the first tube were up to 14 g/l compared to 0.4 g/l in the control soil. After 3 pore volumes, the amount of DOC leached from the 2.5%-zeolite-treated column was still a factor of 6 higher than from the untreated column (Figure 4.5b). In the later stages of the experiment, the differences in DOC concentrations between the 2.5%-zeolite treatment and the control were less than a factor of 2. Apparently, a change in ionic composition of the solution generated a fast increase of DOC, and subsequently the situation became more or less stable again. The lime treatment did not show an increased DOC leaching, although the pH was 8 in the first few pore volumes. The Ca in the lime prevented the dispersion of organic matter (Figure 4.5d).

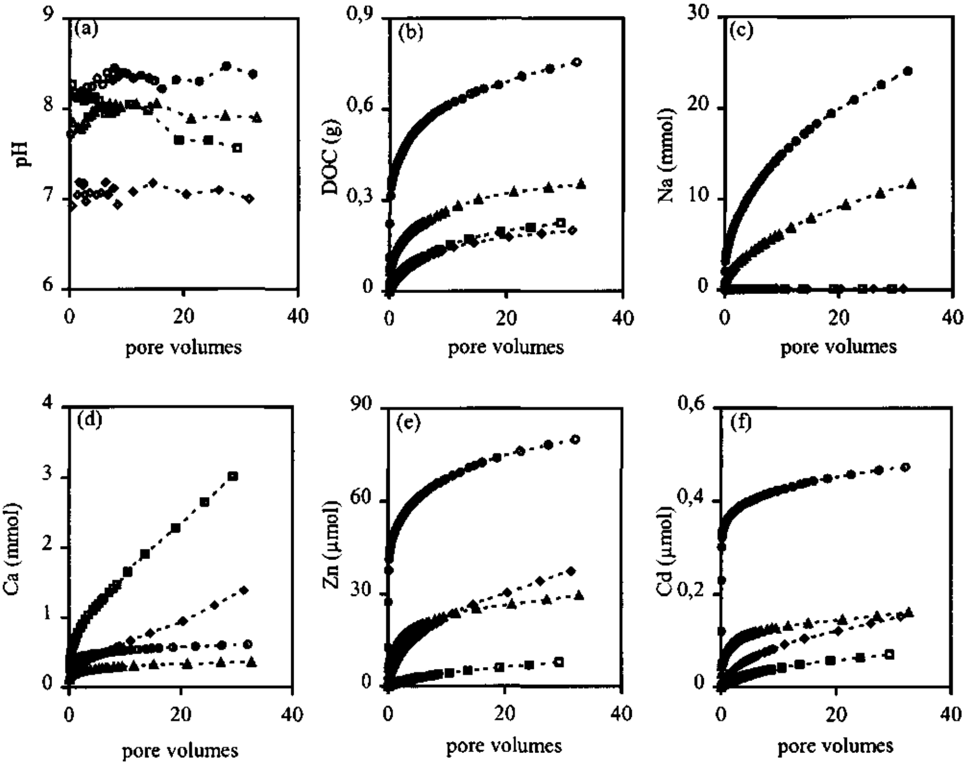

Figure 4.5: (a) pH measured in the leachate of the first column experiment; (b-f) cumulative amounts of Na, Ca, Zn, and Cd leached from the columns. ◇ no additives; □, 0.3% $Ca(OH)_2$; △, 0.5% zeolite A; ○, 2.5% zeolite A.

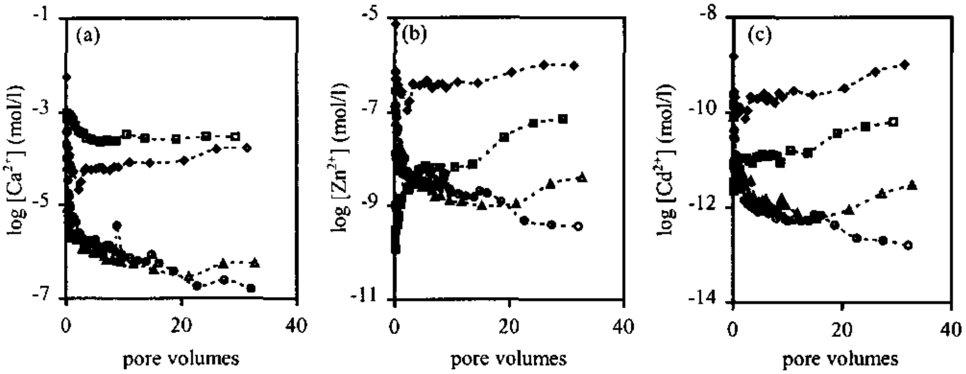

Figure 4.6: Free ionic concentrations of (a) Ca, (b) Zn, and (c) Cd based upon speciation calculations in the column leachates. ◇ no additives; □, 0.3% $Ca(OH)_2$; △, 0.5% zeolite A; ○, 2.5% zeolite A.

Increased Zn and Cd leaching was observed in the soil with 2.5% zeolite A as a result of the DOC leaching (Figure 4.5e and 4.5f). This was particularly the case in the first part of the experiment. In the second part, the cumulative amounts of Cd and Zn leached from the untreated column showed a slightly steeper slope, meaning a higher concentration in the leachate in the untreated soil than in the treated soil. The lower metal leaching from the zeolite-treated columns at the end of the experiment might have two reasons. First, the high leaching in the beginning removed approximately 1.25% of the total Cd and 1% of the total Zn. This is the most mobile fraction and it might be possible that the remaining fraction is more difficult to extract. Another explanation may be the effect of the binding capacity of the zeolite. The zeolite addition decreases the free metal concentration, due to its binding capacity and alkalinity. When the DOC concentration is back to more normal levels, it will also lead to a decreased Zn leaching in the later stage of the experiment.

The leaching of calcium (Figure 4.5d) was always less in 0.5%-zeolite treatment compared to the control, although the DOC leaching was much higher. At 2.5% zeolite, the Ca leaching in the beginning was higher than in the control, due to the very high DOC levels, but the total leaching at the end of the experiment was clearly below the control, due to the Ca binding capacity of the zeolites. The high Ca binding capacity was not surprising, because these zeolites had been developed to soften laundry washwater.

The total leaching of Cd and Zn for the 2.5% zeolite treatment was much greater than for the control, indicating that the effect of the increased leaching to DOC won over the effect of binding of the metals to the zeolite. The effect of the metal binding can be explicated if the free metal ion concentration in the leachates is calculated, assuming that the DOC behaves similarly to 'generic' humic acid. The results (Figure 4.6) show that the concentrations of free Cd, Zn, and Ca in the zeolite-treated soils are much lower than in the control over the whole range of the experiment. The low free metal concentrations may explain the reduced uptake by plants found in other studies (Rebedea et al. 1997, Gworek, 1992 and 1994).

Leaching from soil mixed with pretreated zeolites

A low Ca concentration and an increase in pH favor the dispersion of the soil organic matter. The dispersion of organic matter can be prevented if the pH of the system would not change or if the Ca level is high enough (as in case of lime and cyclonic ashes). To realize these conditions, we pretreated zeolite A by titrating it with acid and/or Ca. The results of the column experiment with pretreated zeolites are presented in Figure 4.7. The pH differences between the various treatments (Figure 4.7a) are smaller than in Figure 4.5a. The pH in the

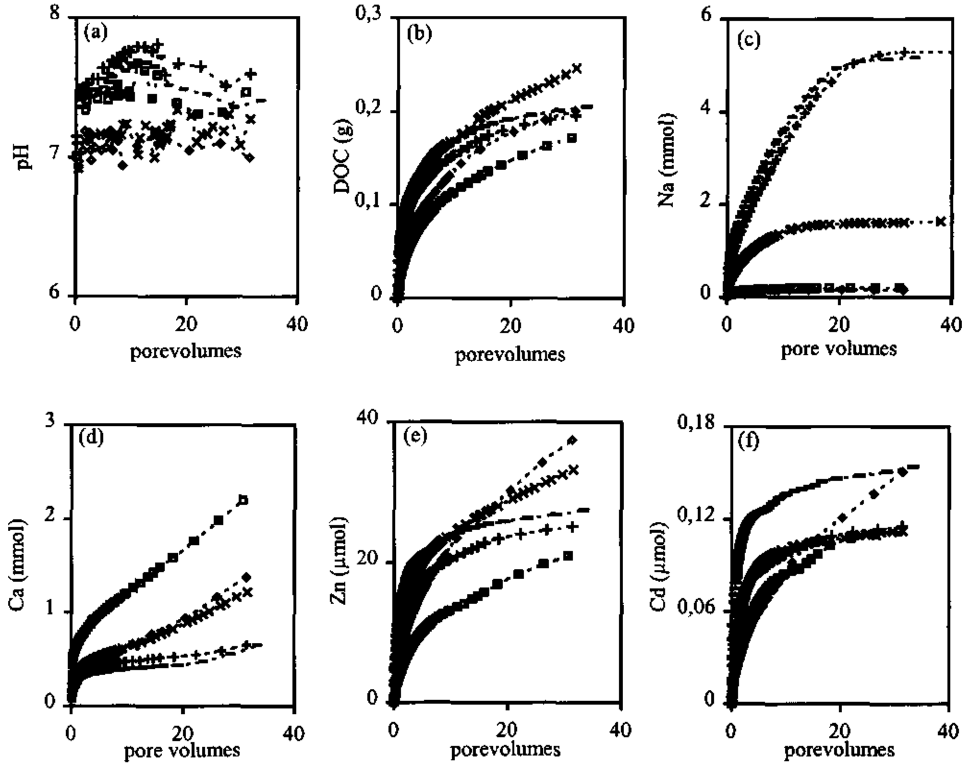

Figure 4.7: (a) pH measured in the leachate of the second column experiment; (b-f) cumulative amounts of Na, Ca, Zn, and Cd leached from the columns. ◇ no additives; □, 0.1% $Ca(OH)_2$; +, 0.5% Ca-treated zeolite A; ×, 0.5% H-treated zeolite A; –, 0.5% Ca/H-treated zeolite A.

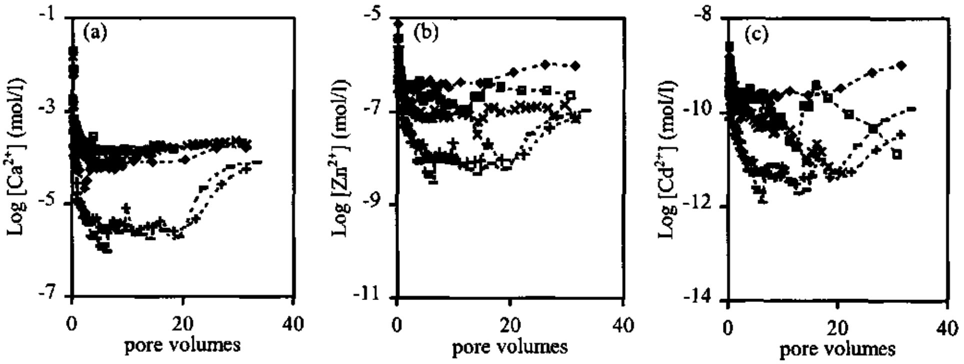

Figure 4.8: Free ionic concentrations of (a) Ca, (b) Zn, and (c) Cd based upon speciation calculations in the column leachates. ◇ no additives; □, 0.1% $Ca(OH)_2$; +, 0.5% Ca-treated zeolite A; ×, 0.5% H-treated zeolite A; –, 0.5% Ca/H-treated zeolite A.

columns containing H-treated zeolites is almost equal to the pH in the untreated column, but Ca and Ca/H treated zeolites still increase the pH by approximately 0.5 unit. The amount of DOC leached from the columns is close to the control soil (Figure 4.7b). In the first experiment, 0.3% lime did not affect DOC leaching, but here 0.1% lime slightly decreased the DOC leaching. The behavior of the soil mixed with H-treated zeolite A is unexpected. The pH of this column deviates from the Ca- and Ca/H-treated zeolites, but also the leaching of DOC, Na, and Ca is different (Figure 4.7b, 4.7c, and 4.7d). This must be attributed to the pretreatment procedure. The number of acid additions and the amounts per addition probably affected the behavior of the pretreated zeolite. Figure 4.7e and 4.7f show that the metal leaching from the control soil is higher after ca. 10 pore volumes (steeper slope), suggesting that treatment of a soil with zeolite will reduce the leaching on a longer term. However, the liming treatment was the most successful treatment in reducing Zn and did also perform well in case of Cd. The competition with calcium and protons that were present in the pretreated zeolites made the zeolites less effective with respect to metal binding. This becomes even more clear if the free ionic concentrations are calculated (Figure 4.8). The overall differences between treated and untreated soils are much smaller in Figure 4.8 compared to Figure 4.6.

Conclusions

Synthetic zeolite A has a high capacity to bind heavy metals, and the alkaline character of the material increases metal sorption to the soil. Na-saturated zeolite A also binds high amounts of Ca, decreasing the competition of Ca in the soil. This study did not reveal whether the effect of a decreased proton and calcium concentration will sustain over a long period. If the pH decreases again, the binding to the zeolites will decrease as well, because metal binding appeared to be very pH dependent between pH 5 and 6.5.

Moreover, the synthetic zeolites used in this study simultaneously induce dispersion of soil organic matter, leading to higher in stead of lower metal leaching from soil columns. In field or laboratory experiments without leachate collection, the organic matter leaching will not be noticed. Although the total metal leaching increases, zeolites can decrease the free metal concentration in the soil solution, leading to a decreased metal uptake by plants.

Pretreatment of zeolites with H or Ca can prevent high DOM leaching. The reverse side is that pretreatment limits the binding of Cd and Zn. If a decrease in leaching to deeper soil layers is required and particularly in poorly buffered soils, zeolites are not suitable. However, a small amount of zeolites may work well in soils with a high acid buffering capacity and a pH > 6, particularly to reduce metal uptake by plants.

In general, alkaline additives have to be evaluated whether they contain enough Ca to prevent increased DOM leaching from soils as a result of their alkalinity. A high amount of Ca, as in the cyclonic ashes, reduces DOM leaching, resulting in a decreased metal leaching.

Acknowledgements

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Chapter 5

The effect of beringite on Cd and Zn uptake by plants and earthworms: More than a liming effect?

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Abstract

Metal contaminated soils are potentially harmful to plants, animals and humans. Harmful effects are often related to the free metal concentration in the soil solution. Immobilization is a potentially useful method to improve the quality of metal contaminated soils by transforming free metal ions into species that are less mobile and less toxic. The effect of many immobilizing products can be attributed to sorption on the surface of the material. Alkaline materials also enhance adsorption to soil particles by decreasing proton competition. Immobilization should preferably be evaluated independently of soil pH so as to discriminate between these processes. In this study, the immobilizing effect of beringite, an alkaline alumino silicate, was compared with that of lime. Plants were grown on a soil contaminated with cadmium and zinc and treated with graded amounts of beringite or lime. Metal availability, as determined by a 0.01 M $CaCl_2$-extraction, and metal uptake by plants strongly decreased in all treated soils. Beringite did not reduce metal availability more than liming when the obtained pH levels were similar. The effect of beringite can, therefore, be explained as a liming effect, at least for the duration of our experiment (ten weeks). The effect of beringite and lime on metal accumulation by earthworms (*Eisenia veneta* and *Lumbricus rubellus*) was small or not significant, although the $CaCl_2$-extractable metal concentration in treated soils decreased by more than 90%. We conclude that immobilizing agents based on a liming effect can decrease metal uptake by plants, but they will hardly affect metal uptake by earthworms. Hence, these materials are able to reduce negative ecological effects of metal contamination on plants and herbivores, but not on earthworm predators.

Introduction

Heavy metal contaminated soils form a widespread problem. They can bring about direct toxic effects to plants and animals or cause secondary poisoning and groundwater contamination. Soil remediation is necessary if harmful effects have to be prevented or reduced. Nevertheless, soil remediation is a drastic intervention, particularly when dealing with extensive areas. Common techniques like excavation or extraction are then physically and economically not feasible. Moreover, such areas are often used for agriculture and forestry or are designated as nature reserves. The techniques mentioned above would have adverse effects on the ecosystem and on soil fertility and productivity. Thus, so-called mild remediation techniques are clearly needed to reduce the harmful effects of soil contaminants, without damaging essential properties of the soil. Two of these are mentioned frequently: phytoremediation, and immobilization (Krebs and Gupta, 1994). We will focus on in situ immobilization.

We define in situ immobilization of heavy metals in contaminated soil as reduction of the metal concentration in the soil solution by adding a binding agent to the soil, leaving the porous structure intact. The metals are not removed from the soil, but are transformed into a less mobile form. Mobility can be interpreted as the physicochemical mobility in the soil, but also includes the transport rate from the soil into organisms. Hence, three criteria are important to assess the success of a treatment: effect on metal leaching to subsoil and groundwater, uptake by and toxic effects on plants and uptake by and toxic effects on soil organisms.

Immobilization techniques are based on the formation of new particles (precipitation or coprecipitation) or on the binding to the surface of existing solid particles (sorption). Two materials will be used in this study: lime, and beringite. Lime enhances sorption of heavy metal cations by reducing the H^+ concentration in the soil. Hence, proton binding to negatively charged sites decreases, and metal adsorption increases. Liming should not be considered as an addition of new binding sites but as a method to make the existing sites in the soil available for metal binding. Beringite is a modified alumino silicate that originates from the fluidized-bed burning of coal mining refuse. The binding mechanism of beringite is not quite clear, but adsorption and (co)precipitation may play roles (De Boodt, 1991). The increase in pH, caused by beringite treatment, might also be an important contributing factor to metal immobilization. Application of 2.5% (w/w) beringite to the soil of the Maatheide, a Cd and Zn contaminated area in the northern part of Belgium, raised the pH by approximately

2 units (Vangronsveld et al., 1996). Several studies have shown beneficial effects on plants (Vangronsveld et al., 1996; Mench et al., 1994a; Boisson et al., 1998) and a reduction of metal leaching (Wessolek and Fahrenhorst, 1994), but the process responsible for the effect is still unclear. If (co)precipitation or sorption to the surface of beringite plays an important role, the immobilizing effect of beringite will exceed the effect of liming to the same soil pH.

We evaluated the effect of lime and beringite on metal uptake by plants and earthworms from soils contaminated with Cd and Zn. Metal availability to plants is mainly determined by the free ion in the soil solution (Parker et al., 1995). We studied the uptake by a leafy vegetable, since leafy vegetables accumulate relatively high quantities of heavy metals (Wiersma et al., 1986). Zinc levels in vegetables are generally not toxic to humans, but plants can accumulate Cd to levels which can produce toxic effects in animals and humans.

Metal uptake by earthworms was studied, because earthworms are central organisms in the food web of terrestrial ecosystems (Spurgeon and Hopkin, 1996a). Birds, wild mammals, and some snakes may have a diet consisting for more than 50% of earthworms (MacDonald, 1983). Contaminants may enter the earthworm body through the intestine from ingested food, and through the skin (Lee, 1985). The validity of the equilibrium partitioning theory, assuming a direct relationship between the contaminant concentration in pore water and the content in the worm, has been demonstrated for organic chemicals (Ma et al, 1998). With regard to metals, Kiewiet and Ma (1991) showed that Cd and Pb concentrations in earthworms were related to concentrations in solution in experiments with earthworms in reconstituted ground water. This relationship seems less clear in soil. It is questionable whether the equilibrium partitioning theory holds for metals, due to their complicated chemical speciation. The conditions in the gut are obviously important for intestinal uptake of metals with pH being an important parameter. The pH in the gut is within one unit of neutrality (Wallwork, 1983). Whether a decrease in metal concentration in the soil solution, as a result of adding an immobilizing agent, can decrease metal uptake by earthworms is not yet known.

The objectives of this study are to compare the immobilizing effects of beringite and lime, and to investigate the effects of beringite on earthworms.

Materials and methods

Experiments

Four experiments were conducted. All contaminated soils (G553, AB and G&K) were sampled in the Dutch or Belgian Kempen, an area contaminated with Cd and Zn due to former emissions of five zinc smelters. Reference soils (WK and KOBG) were uncontaminated soils from the Netherlands. General characteristics are presented in Table 5.1. Total metal concentrations were determined by aqua regia extraction: 2 g of soil and 16 ml of a concentrated HCl/HNO_3 mixture, in a ratio of 3:1 by volume, were boiled under reflux for 2 hours.

Table 5.1: Soil characteristics.

Properties	-----------Contaminated soils----------			------Reference soils------	
	G553	AB	G&K	KOBG	WK
$\%C_{org}$	2.2	1.7	2.1	2.9	1.6
% clay	7.0	6.5	7.0	2	3.0
WHC (l/kg)	0.29	ND	0.29	0.30	0.27
pH (0.01 M $CaCl_2$)	5.2	4.4	5.3	5.4	4.4
Total Cd (mg/kg)	3.4	1.6	9.6	0.12	0.2
Total Zn (mg/kg)	199	85	437	22	31

WHC = Water Holding Capacity; ND = not determined.

In experiment 1, we compared metal uptake by plants grown on beringite treated soils to plants on lime treated soils in a greenhouse study with Swiss chard (*Beta vulgaris* L. var. *cicla*). Four weeks before sowing, portions of 2.5 kg of soil G553 (Table 5.1) had been mixed with 0, 1.25, 2.5, 5, and 10% of beringite or with 0, 0.05, 0.1, 0.2, and 0.4 % of lime ($Ca(OH)_2$). All percentages in this sections are presented as mass ratios. The lime treatments covered the same pH range as the beringite treatments. Fertilizers applied (per kg of soil) were: 8 mmol of $NaNO_3$, 4 mmol of K_2HPO_4, 1 mmol of $MgSO_4$, 1 mmol of $MgCl_2$, 0.06 mmol of H_3BO_3, 0.04 mmol of $CuSO_4$, 0.12 mmol of $MnSO_4$, and 0.01 mmol of Mo as $(NH_4)_6Mo_7O_{24}$. The soil was wetted with deionized water to a moisture content of 0.17 L/kg (60% of the water holding capacity). Each treatment was replicated three times. The soil was stored in the dark in polyethylene bags at room temperature. After four weeks, each portion of 2.5 kg of soil was put in pots and sown to Swiss chard. Plants were watered frequently with de-ionized water to keep the moisture content constant. Another fertilizer application of 8 mmol of $Ca(NO_3)_2$ (per kg of soil) was added to the pots two weeks after sowing. Plants were harvested after six weeks. At the same time soil samples were taken from each pot. Plants were digested and soils were extracted with 0.01 M $CaCl_2$. Cadmium and Zn were determined in all samples.

The effects of beringite on earthworms were studied in experiments 2, 3, and 4. These experiments were all conducted in pots in a climate chamber. Earthworms had been kept in a reference soil (KOBG in experiment 2 and WK in experiments 3 and 4) for at least two weeks before the experiment started. Earthworms were sampled at the start of the experiments. At the end of each experiment, soil samples were taken for $CaCl_2$-extraction, and the earthworms were collected to measure the metal content in the body tissues.

In experiment 2 we used soil AB (Table 5.1), either untreated, treated with 0.2% lime ($Ca(OH)_2$) or treated with 3% beringite. Each treatment was replicated four times including reference soil KOBG. Additives were mixed with the soil and deionized water was added to a moisture content of 0.18 L/kg. The soil was put in plastic bags in the dark at room temperature for a period of four weeks. Subsequently, 1-L pots were filled with 650 g of soil and five adult earthworms (*Lumbricus rubellus* Hoffmeister) collected from the field were added. The worms were exposed to the soil for four weeks under light conditions at 15°C, and additional food was supplied. In addition to metal uptake, growth and cocoon production were measured in this experiment.

Experiment 3 was set up to study the effect of different amounts of beringite on earthworms. Portions of 1.5 kg soil G&K (Table 5.1) were treated with 0, 2.5, 5, and 7.5% in four-fold replication. Moisture content was adjusted to 0.19 L/kg with de-ionized water (= 70% of the water holding capacity). The soil was transferred to pots after an incubation period of four weeks. The temperature was kept at 18°C and a photoperiod of 16L:8D was maintained. Twelve adult earthworms (*Eisenia [Dendrobaena] veneta* Rosa), obtained from a local vermiculturist, were added to each pot and kept in the soil for three weeks. Water and food were supplied twice a week.

Experiment 4 was designed to study the influence of time on metal accumulation in the earthworm tissue and the effect of beringite on the level and establishment of a steady state. The experimental conditions of this experiment were similar to those of experiment 3. Three kilograms of soil G553 was treated with 0 or 7.5% beringite in four replicates. We added 24 earthworms per pot and sampled six earthworms after one, two, four, and eight weeks.

Soil analyses

Soil samples were sieved to less than 2 mm and extracted with $CaCl_2$ (Houba et al., 2000). Three grams of air-dried soil and 30 ml of 0.01 M $CaCl_2$ were shaken for 24 hours. The pH was determined in suspension. Suspensions were centrifuged at 1,800 *g* for 10 minutes. The Zn concentration in the supernatant was determined by flame atomic absorption spectrometry with Smith-Hieftje background correction (IL-S11, Thermo Jarrell Ash, Breda, The Netherlands). Cadmium was measured by graphite furnace atomic absorption spectrometry with Zeeman background correction (VarianSpectrAA 300/400,Varian, Sunnyvale, CA, USA).

Plant analyses

Leaves and petioles of Swiss chard plants were dried at 70°C and ground. A sequential digestion procedure using HF, H_2O_2, and HNO_3 in a closed-system microwave was used (Novozamsky et al., 1996). Eight milliliters of 20% HF were added to 0.5 g of dry plant material and evaporated to dryness. Five milliliters of 65% HNO_3 and 3 ml of 30% H_2O_2 were used for the microwave digestion. Zinc was determined by inductively coupled plasma optical emission spectrometry (Spectro Analytical Instruments, Kleve, Germany) and Cd was determined by flame atomic absorption spectrometry.

Earthworm metal analyses

Sampled earthworms were rinsed and kept in petri dishes on moist filter paper for 3 days to empty their gut. The filter papers were changed every day to reduce coprophagy. One or two earthworms were put in a vial, frozen, and subsequently freeze-dried. The dry matter was transferred into a digestion tube. The worms were digested in concentrated HNO_3 (Marinussen et al., 1997a), and Cd and Zn were determined by inductively coupled plasma optical emission spectrometry.

Statistical analysis

The significance of all treatment effects was determined by analysis of variance. When differences were found, Tukey's multiple comparison test was used to determine differences between individual treatments. Log-transformed data were used for metal analyses in soils, plants, and earthworms. A normal distribution was assumed for survival and cocoon production. The software package SAS® was used for the calculations (release 6.12 for Windows, SAS; Cary, NC, USA).

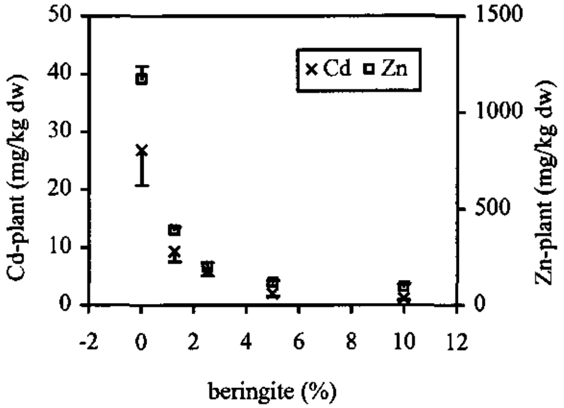

Figure 5.1: Effect of beringite on the concentrations of Cd (left axis) and Zn (right axis) in Swiss chard. dw = dry weight, error bars = standard deviation.

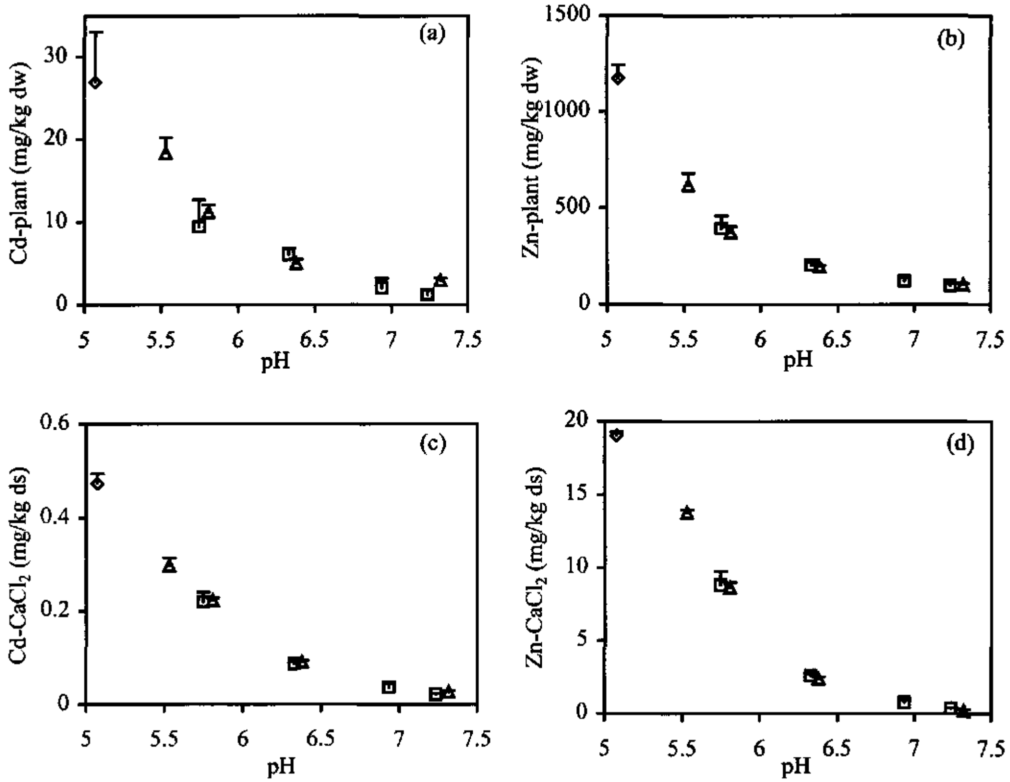

Figure 5.2: Cd and Zn concentrations in Swiss chard (a,b) and $CaCl_2$-extractable Cd and Zn concentrations from soil G553 (c,d) as a function of soil pH. ◇ = untreated; □ = beringite treated, Δ = lime treated, dw = dry weight, ds = dry soil, error bars = standard deviation.

Results

Effects on Swiss chard

Figure 5.1 presents the effect of beringite addition on Cd and Zn uptake by Swiss chard. A decrease in metal uptake is clear even at the lowest beringite rate. If we present metal uptake as a function of soil pH (Figure 5.2a and 5.2b), no difference could be found between soils treated with beringite and soils treated with lime at the same obtained soil pH. Similar trends were found for the $CaCl_2$-extractable metal concentrations (Figure 5.2c and 5.2d). The similarity of the curves shown in Figure 5.2 indicates a strong correlation between the $CaCl_2$-extractable metal concentration and the metal uptake by Swiss chard. Calculated coefficients of determination are $r^2_{Zn} = 0.91$ and $r^2_{Cd} = 0.90$. This confirms the predictive power of an extraction with 0.01 M $CaCl_2$ to estimate plant uptake of Cd and Zn (Novozamsky et al., 1993).

Effects on earthworms

Table 5.2 shows that lime and beringite in experiment 2 raised the pH from 4.4 to 6.1 and 5.4 respectively. As a result, the $CaCl_2$-extractable Cd and Zn concentrations decreased significantly, but the decrease in available metal concentrations did not significantly affect survival, cocoon production, growth and Zn tissue concentration ($p > 0.05$). A clear difference could be observed between the uncontaminated and contaminated soils with respect to Cd accumulation. The Cd tissue concentration in soil KOBG did not differ from the initial Cd content (3.3 mg/kg), but increased to 11.9 mg/kg in the untreated contaminated soil. A small,

Table 5.2: Results of experiment 2 (with standard deviations within parentheses).

Parameters	Untreated	3% beringite	0.2% lime	KOBG
pH-$CaCl_2$	4.4 (0.1)[a]	5.4 (0.0)	6.1 (0.1)	ND[b]
Cd-$CaCl_2$ (mg/kg)	0.51 (0.00)	0.21 (0.01)	0.07 (0.02)	ND
Zn-$CaCl_2$ (mg/kg)	44.3 (0.1)	18.6 (0.7)	5.2 (1.5)	ND
Survival (n=5 per pot)	4 (0.8)	4.3 (1.5)	2.8 (2.6)	5.0 (0.0)
Cocoons per pot	21 (5.4)	21.8 (5.0)	25.5 (16.2)	18 (3.6)
Growth per worm (mg)	243 (112)	323 (203)	223 (210)	384 (122)
Cd-worm (mg/kg)*	13.3 (1.4)	11.9 (0.6)	14.8 (1.3)	4.0 (0.72)
Zn-worm (mg/kg)**	1027 (204)	983 (200)	960 (116)	854 (121)

ND = not determined; * initial Cd-worm = 3.3 (0.9) mg/kg; ** initial Zn-worm = 1024 (156) mg/kg.

but significant, difference ($p < 0.05$) could be determined between the lime and beringite treatment, though both treatments did not differ from the untreated contaminated soil.

The results of experiment 3 also show that beringite does not significantly decrease the metal content in the earthworm tissue (Figure 5.3a and 5.3b), even though it clearly reduces the $CaCl_2$-extractable soil metal concentrations (Figure 5.3c and 5.3d). A relationship between uptake and $CaCl_2$-extractable amount is therefore absent for both Cd and Zn. A contrast between Cd and Zn uptake by earthworms is observed when comparing reference soil WK and the untreated soil. Cadmium uptake was low in the reference soil and high in the contaminated soil, while the Zn tissue concentration is the same in both soils. This must be attributed to ability of earthworms to regulate Zn in their body (Morgan and Morgan, 1988).

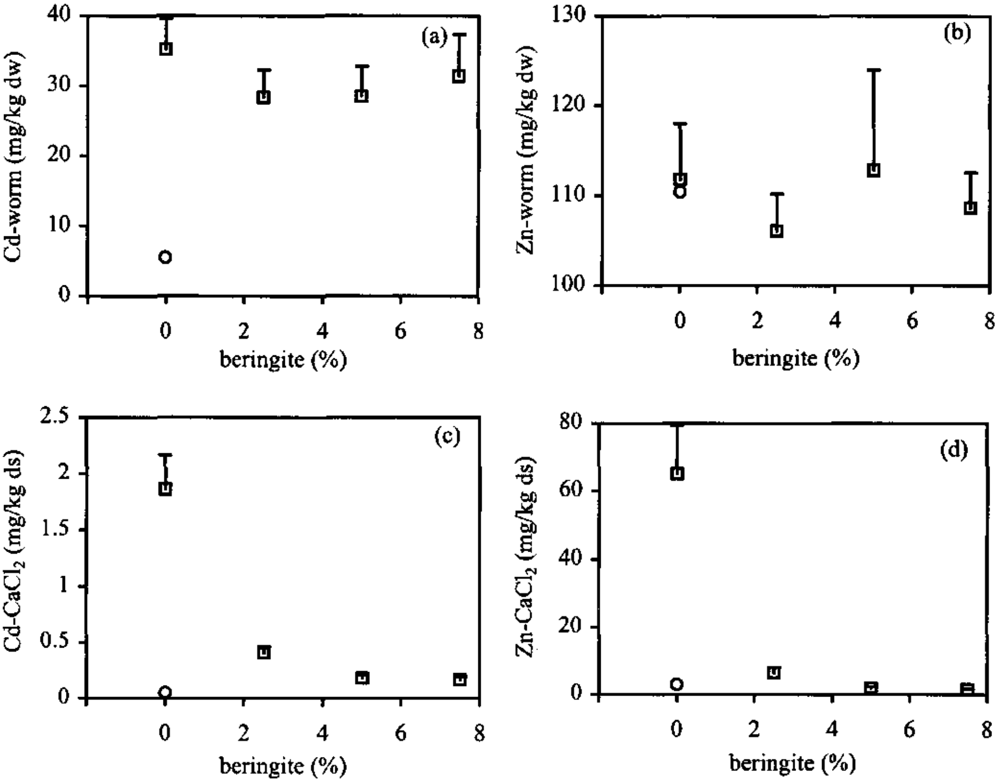

Figure 5.3: Metal contents in earthworm tissue (a,b) and $CaCl_2$-extractable metal concentrations (c,d) in soil G&K vs. different amounts of beringite. Note that the vertical axis of Figure 5.3b does not start at 0. □ = contaminated soil, O = reference soil WK at the start of the experiment, dw = dry weight, ds = dry soil, error bars = standard deviation.

Figure 5.4a shows the rate of Cd accumulation by *Eisenia veneta*. Worms in beringite-treated soils show a small, but significant, decrease in Cd accumulation ($p < 0.005$). No significant interaction between time and treatment was found ($p > 0.05$), which means that the dashed lines in Figure 5.4a did not diverge during the eight-week period. Both lines in Figure 5.4a increased during the eight-week period, though the slope tended to decrease between four and eight weeks. It took longer than eight weeks to reach a steady state for Cd, but Zn behaved differently. The Zn concentration in the earthworm tissue decreased in both soils (Figure 5.4b). $CaCl_2$-extractable Cd contents, measured at the end of the experiment, decreased from 0.45 mg/kg in the untreated soil to 0.03 mg/kg in the beringite treatment. Zn concentrations decreased from 23.6 to 0.29 mg/kg. In this experiment we again observed a strong effect of beringite on the chemical availability of Cd and Zn, while the effect on the accumulation by earthworms remained small.

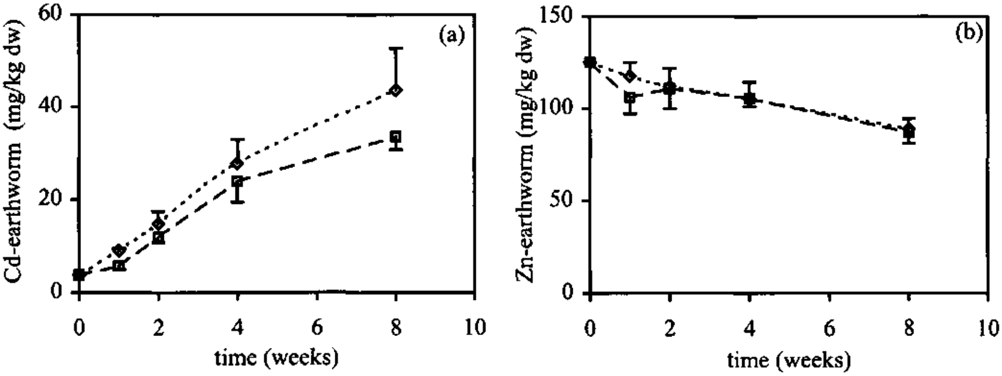

Figure 5.4: Cd (a) and Zn (b) content in earthworms in soil G553 during an eight-week period. ◇ = untreated; □ = beringite treated, dw = dry weight, error bars = standard deviation.

Discussion

Effects of lime and beringite on plant uptake

Experiment 1 shows that beringite and lime can reduce metal uptake by plants very effectively in a sandy soil, confirming earlier immobilization studies (Vangronsveld et al., 1996; Mench et al., 1994a; Boisson et al., 1998). We could not observe any difference between the effect of beringite and lime. As stated earlier, liming should be considered as a method to make existing binding sites available for metal binding. A binding agent containing many reactive sites would reduce the metal concentration in the pore water more than lime added to obtain the same soil pH. As beringite fails to show such an additional effect, it may be considered to act as a liming material. In contrast to lime, beringite raises the pH for a long period (Vangronsveld et al., 1996).

The effect of liming has been studied extensively. Most studies report decreasing metal uptake in plants as a result of liming (Lexmond, 1980; Hooda and Alloway, 1996; Krebs et al., 1998) though not all studies find pronounced effects (Maier et al., 1997; Singh and Myhr, 1998). Different reasons can explain these varying results: plant species (uptake mechanism, root exudates, etc.), type of experiment (greenhouse, field), or increased calcium competition (Maier et al., 1997). A more fundamental explanation of the variable effects of liming is given by Plette et al. (1999), who suggest that liming leads to an increased metal sorption to soil, but simultaneously to an increase in metal binding at the root surface. If beringite is comparable to lime, it might have the same limitations. It would therefore be interesting to study the effects of beringite under circumstances that the effects of lime are small or absent.

Effect of beringite on metal uptake by earthworms

Considering the earthworm experiments of this study, the uptake of Zn and Cd by earthworms is hardly influenced by beringite. From the plant experiment, we conclude that a rise in pH is the most important immobilization mechanism of beringite. Although little is known about the effect of beringite on earthworms, we can compare our results with studies examining the influence of lime on metal uptake by earthworms. Most studies show that uptake of Zn (and also Cu) by earthworms is not strongly influenced by a varying pH (Spurgeon and Hopkin, 1996b; Marinussen et al., 1997b). This is explained by physiological regulation of these metals in the earthworm body (Ireland, 1975; Morgan and Morgan, 1988). Spurgeon and Hopkin (1999) found that Cu and Zn were primarily detoxified by excretion. Our Zn data confirm these studies: Zn tissue concentrations neither correlated with the total metal contents nor with the $CaCl_2$-extractable Zn concentrations.

Earthworm studies regarding the effect of lime on Cd uptake show ambiguous findings varying from distinct effects (Perämäki et al., 1992; Benninger-Truax and Taylor, 1993), weak effects (Beyer et al., 1982 and 1987) to insignificant effects (Perämäki et al., 1992; Bengtsson et al., 1986). The variation in experimental conditions might be responsible for the above-mentioned differences. The experimental conditions differed in soil type, pH range, Cd level, time span, earthworm species, and whether it was a field or laboratory experiment. Different effects were also observed within individual studies. Perämäki et al. (1992) found a clear pH effect in a short-term experiment (35 days), whereas they did not observe such an effect in an experiment over 230 days. Bengtsson et al. (1986) did not find any time effects, but observed a stronger effect of pH at 14 compared to 94 mg Cd per kg soil. However, a comparison of the cited studies could not identify any experimental factor that clearly influenced pH dependency.

In the studies that observed a significant pH effect, the Cd levels in earthworm tissues were lower at a higher pH. However, the influence of pH on Cd uptake is much smaller than the influence of pH on Cd in the soil solution. The same phenomenon is shown in this study with respect to beringite addition. Two reasons can be responsible for the limited effect of a change in pH on earthworm tissue concentrations: pH-independent uptake in the gut and pH-dependent uptake through the skin. As stated in the introduction, the pH of the earthworm gut is near neutral. Ingested particles will be brought to this pH irrespective of the pH in the soil. Next to intestinal uptake, dermal uptake is considered an important pathway. Plette et al. (1999) suggested that metal uptake through the body wall is determined by sorption processes at the biological surface. The sorption processes are affected by competition of other cations, such as H and Ca. Although an increase in pH decreases Cd in the soil solution, it also increases Cd adsorption to the worm surface, due to a decreased proton competition. The net effect of a decreased concentration in solution and an increased sorption rate is as yet unpredictable and could be close to zero.

Influence of time on metal uptake by earthworms

Experiment 4 suggests a continuous accumulation of Cd in *E. veneta*, although the slopes tended to decrease after four weeks. Other studies also reported a long-term increase in the Cd content in earthworms. Corp and Morgan (1991) sampled a contaminated field soil and kept native and introduced *L. rubellus* in the laboratory for 31 days. In a soil containing 3.4 mg Cd/kg, similar to soil G553, the Cd content in native worms was almost twice as high as in introduced earthworms. Rietra and Ma (1997) studied accumulation of Cd by *L. rubellus* and measured increasing tissue concentrations during 100 days. Perämäki et al. (1992) measured an increasing Cd content in *Aporrectodea caliginosa* over a period of more than 200 days.

The decrease in Zn in the earthworm tissue in experiment 4 is noteworthy, because the Zn level in soil G553 is much higher than in reference soil WK (Table 5.1). Competition of Zn and Cd might be a reason. The decrease in Zn content is of the same order of magnitude as the increase in Cd. Marinussen et al. (1997c) found a replacement of Cu by Cd in the tissue of *E. veneta* when the Cd concentration in a Cu contaminated soil was increased. Kiewiet and Ma (1991) reported competition of Cd and Ca for *L. rubellus* in water. They did not find an effect of Ca on Pb uptake. Weltje (1998) reviewed mixture toxicity of heavy metals to earthworms and mainly found antagonistic relations between different metals.

Accumulation and toxicity

If beringite does not decrease the accumulation of metals by earthworms, it will not reduce the risk of secondary poisoning of earthworm predators, but can beringite prevent toxicity to earthworms? Accumulation and toxicity can be independent parameters. The contaminant concentrations in our study were less than the levels required to decrease cocoon production or survival, but other studies have reported a distinct influence of pH on metal toxicity to earthworms. Van Gestel and Van Dis (1988) observed a significantly reduced Cd toxicity in a soil limed to pH 7 compared to the original soil (pH 4.1). Ma (1984) observed increased Cu toxicity at lower soil pH. Spurgeon and Hopkin (1996b) found such a relationship for Zn, while they did not observe an effect on accumulation. Because beringite increases soil pH, these studies suggest that beringite might be able to reduce metal toxicity to earthworms.

Conclusions

Beringite reduces the $CaCl_2$-extractable and plant available amount of Cd and Zn to the same extent as lime does. Additional sorption on the beringite surface, independent of a pH increase, could not be determined within the time span of this experiment. Whereas Cd and Zn uptake is strongly decreased in plants, beringite hardly influences Cd and Zn accumulation by earthworms. Hence, alkaline soil additives do not reduce the risk of secondary poisoning of earthworm predators.

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Chapter 6

Cd uptake by earthworms as related to the availability in the soil and the intestine

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Abstract

The free metal concentration in the soil solution is often considered a key parameter for metal uptake by and toxicity to soft-bodied soil organisms. The equilibrium partitioning theory, which assumes a relationship between the contaminant concentration in pore water and the contaminant concentration in the body tissue, can be used to describe uptake by earthworms. This theory has proved useful for organic chemicals, but its applicability is less clear for metals. In this study the Cd concentration in soil pore water (pw) was varied by increasing the soil pH by the addition of lime ($Ca(OH)_2$), and by adding manganese oxide (MnO_2), which has a high metal binding capacity. Both lime (0.135% w/w) and MnO_2 (1% w/w) decreased $[Cd^{2+}]_{pw}$ by a factor of 25, while Cd_{worm} was only reduced by a factor of 1.3 in lime-treated soils and 2.5 in MnO_2-treated soils. Cadmium uptake was weakly related to the free metal concentration ($R^2_{adj} = 0.66$). Adding pH as an explanatory variable increased R^2_{adj} to 0.89, indicating that Cd uptake from pore water is pH dependent, which might be attributed to competition of protons and Cd at the surface of the earthworm body. However, previous earthworm experiments in reconstituted groundwater showed a conspicuously smaller pH dependent Cd uptake. The differences in metal uptake between earthworms in lime- and MnO_2-treated soils are therefore more likely to reflect the predominance of pH independency of intestinal uptake of Cd. Equilibrating the soil with a solution of 0.01 M $CaCl_2$ and 0.1 M of triethanolamine (buffered at pH 7.2), simulating the conditions prevailing in the worm intestine, yielded free Cd concentrations that were closely ($R^2_{adj} = 0.83$) and linearly related to the Cd concentration in the earthworm tissue.

Introduction

Soil quality assessment should take account of toxic effects of contaminants on plants, animals, and human beings. The free metal concentration in the soil solution is often considered to be a key factor in the toxicity of heavy metals to terrestrial plants (Parker et al., 1995), microorganisms (Plette et al., 1999), and soft-bodied invertebrates (Spurgeon and Hopkin, 1996; Campbell, 1995). Hence, the quality of a metal contaminated soil can not be improved only by removing the metal from the soil but also by reducing the free metal concentration in the soil solution. This can be achieved by applying soil amendments that enhance metal binding to the solid phase. Immobilization is a nondestructive *in situ* technique suitable for large areas (Vangronsveld and Cunningham. 1998). Different criteria have been used to evaluate the effect of immobilization, such as effects on leaching (Lin et al., 1998; Wessolek and Fahrenhorst, 1994), plants (Mench et al., 1994; Rebedea and Lepp, 1994), and soil organisms, for example bacteria (Vangronsveld et al., 2000) and earthworms (Pearson et al., 2000; Oste et al., 2001). Earthworms are an important food source for a variety of other animals (Edwards and Bohlen, 1996), which implies that accumulation of metals in earthworms can produce harmful effects on the ecosystem as a result of secondary poisoning. Previous experiments have shown that Cd and Zn accumulation in earthworms were weakly or not at all affected by soil amendments (Oste et al., 1998; Oste et al., 2001). For Zn this can be explained by physiological regulation, but Cd is a non-essential element that is hardly eliminated and therefore accumulates for a long period in worms (Spurgeon and Hopkin, 1999). Apparently, metal uptake by earthworms is either not directly related to the concentration in the soil solution or also dependent on other variables (notably pH) that were affected by the amendments (Oste et al., 2001). A good relationship however was found for organic micro-contaminants (Van Gestel and Ma, 1998; Belfroid et al., 1995). Earthworms differ from plants and microorganisms, because two pathways have to be considered: dermal and intestinal uptake.

The objective of this paper is to evaluate the applicability of the equilibrium partitioning theory for metal uptake by earthworms. We designed an experiment using one total Cd concentration in the soil (9.9 mg/kg). The Cd concentration in soil pore water was varied by adding lime and/or manganese oxide to the soil. Lime decreases the Cd concentration by increasing pH, resulting in a higher availability of the binding sites in the soil. Manganese oxide can also decrease Cd in pore water, but the binding capacity of the soil system is increased without changing the pH. The effects of the additions were evaluated by measuring the Cd tissue concentration in the earthworm *Lumbricus rubellus* Hoffmeister.

Theory

Metal uptake routes

To assess the contribution of both pathways, it is necessary to know the conditions in the soil (dermal uptake) and the digestive tract (intestinal uptake). Figure 6.1 presents an overview of both uptake routes. The large shaded box represents the solid phase. Inside the box is the pore water to which the body wall of the earthworm is exposed. Both solid particles and solution are ingested and egested by the earthworm.

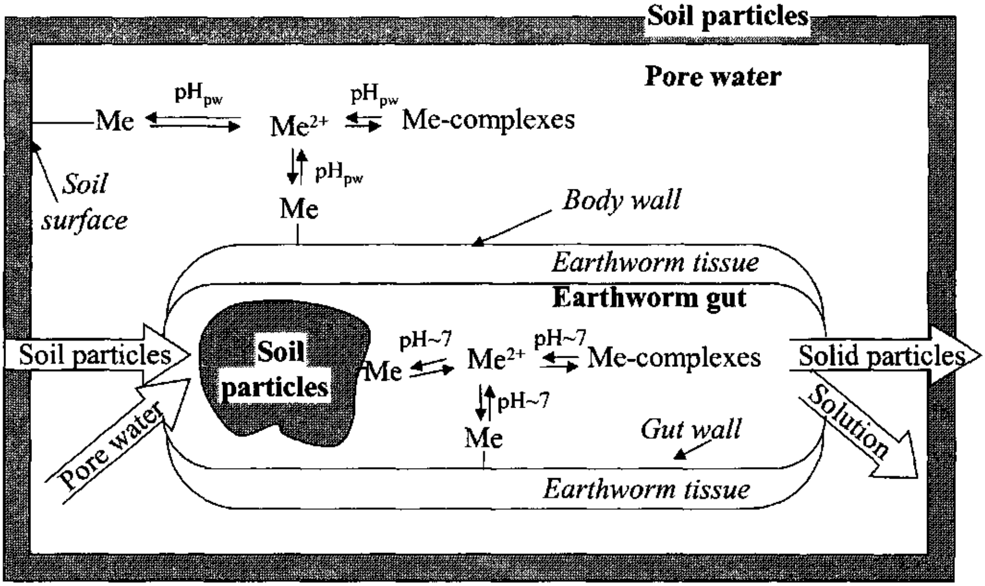

Figure 6.1: Schematic view of earthworm exposure to metal; Me = metal, pw = pore water.

It is often assumed that diffusion of metal ions through the body wall forms the major route of metal uptake by earthworms (Spurgeon and Hopkin, 1996; Reinecke et al., 1997). Dermal uptake can be described by the equilibrium partitioning theory (Morel, 1983; Shea, 1988), presuming a direct relationship between the earthworm tissue concentration and the free metal concentration in pore water. The free metal concentration is strongly influenced by sorption to the solid phase. Sorption to soil particles is determined by soil composition and environmental conditions, such as pH and salt level (Alloway, 1995). However, environmental conditions, like pH, can also influence metal binding to bacterial surfaces (De Lurdes Simoes Gonsalves et al., 1987; Plette et al., 1999). Studies with aquatic organisms (Campbell, 1995; Hare and Tessier, 1996) and plants in nutrient solutions (Lexmond and Van der Vorm, 1981) revealed that metal uptake was also influenced by competition of protons.

All together, the free metal concentration in pore water $[Me^{2+}]_{pw}$ is in dynamic equilibrium with metals bound to the soil and with metals bound to the earthworm body wall, and both reactions are pH dependent. Figure 6.1 only shows competition of protons, but divalent macro-ions, such as Ca, have also been shown to influence these reactions (Kiewiet and Ma, 1991). The pH dependency of sorption processes at both surfaces determines the net effect of a shift in soil pH. It might even be possible that the processes fully compensate each other.

The other pathway shown in Figure 6.1 represents ingested material. Earthworms are known to be selective consumers. Morgan and Morgan (1999) found that the Cd content in earthworms was strongly influenced by the ingested amount, resulting in a higher Cd uptake by *Aporrectodea caliginosa* compared to *Lumbricus rubellus*. The amount of Cd ingested by worms will be practically independent of soil pH because the amount of Cd in pore water is very small compared to the amount of Cd bound to the solids at all pH values. This can be different in aquatic ecosystems. Hare and Tessier (1996) measured Cd uptake by an aquatic insect larva living on plankton. They found a significant influence of pH on Cd uptake and assumed that protons and metal ions competed for the biological uptake site. However, an alternative explanation is that plankton was more contaminated at higher pH values, due to a higher absorption and adsorption at higher pH. As this phenomenon is not relevant to our case it could be excluded from Figure 6.1.

As stated, the ingested amount of metals is important, but intestinal uptake will also be affected by the conditions prevailing in the gut. Once the material is ingested, it is buffered to near neutral pH (Wallwork, 1983). Three processes may be responsible for this remarkably stable pH: secretion of $CaCO_3$ by the calciferous gland (Laverack, 1963), ammonia secretion (Wallwork, 1983), and mucus production (Trigo and Lavelle, 1993). Mixing and grinding of the ingested material take place in the strongly muscular gizzard and subsequently the organic material is partly digested by a number of enzymes and absorbed in the gut (Laverack, 1963). While passing the gut, the organic carbon content decreased by approximately 35% in both *Lumbricus terrestris* and *Aporrectodea longa* (Morgan and Morgan, 1992). The decrease in organic carbon probably decreases the number of metal binding sites, but it is difficult to quantify this effect.

In brief, the constant pH and the digestion of organic matter are typical properties of the earthworms themselves that deviate from the conditions in the soil. Absorption of heavy metals through the gut wall will be influenced by these factors. Hence, the uptake via the gut will be independent of the soil pH, and the digestion of organic matter might result in a higher availability than expected from soil organic matter.

Speciation in pore water

With respect to dermal uptake, the free metal concentration is considered an important factor. The total concentration in pore water consists of free Cd ions, inorganic Cd complexes (e.g., $CdCl^+$ or $CdOH^+$), and Cd complexed to dissolved organic matter (Cd-DOM). The free Cd concentration can be calculated from the total Cd concentration in pore water using computer codes like ECOSAT (Keizer and Van Riemsdijk, 1999). Cadmium complexation to DOM has been described with the Non-Ideal Consistent Competitive Adsorption model combined with an electrostatic Donnan model, to correct for the variable charge of organic matter, which is referred to as the consistent NICA-Donnan model (Kinniburgh et al., 1999). This model includes surface heterogeneity of the organic matter, nonideal adsorption of cations and competition with different ions (e.g., Ca^{2+} and H^+). The organic matter is electrostatically considered as a gel having a Donnan potential.

The model parameters for DOM as used in ECOSAT have been derived for purified humic acid (HA) (Kinniburgh et al., 1999), but pore water also contains fulvic acid (FA). This might produce a small error in the estimation of the Cd complexation to DOM.

Calculation of cadmium uptake from the soil pore water.

Plette et al. (1999) showed that an extended Freundlich equation, a common equation to describe Cd binding to soil organic matter and soil, could also describe Cd binding to biological surfaces (Equation 6.1).

$$Cd_{\text{bound}} = K\,(Cd^{2+})^{n}\,(H^{+})^{m} \qquad [6.1]$$

The non-linearity parameters n and m are organism specific and K is only a constant if other environmental parameters (e.g. $[Ca^{2+}]$, ionic strength, temperature) remain constant. Equation 6.1 will be linearized by a log transformation and Cd concentrations (in mol/l) will be used in stead of Cd activity in the remainder of this paper. Cadmium bound to soil or biota is expressed in mol/kg. Log K, n, and m can be estimated by linear regression of log-transformed data.

Boekhold and Van der Zee (1992) derived Equation 6.2 for Cd sorption to a sandy soil containing 2.5% organic carbon in a background electrolyte of 0.005 M $CaCl_2$.

$$\text{Log } Cd_{soil} = 0.77 * \log [Cd^{2+}] + 0.5 * pH - 2.46 \qquad [6.2]$$

Log $[Cd^{2+}]$ ranges from –5.2 to –4.0, $R^2 = 0.993$, n = 14

The pH coefficient of 0.5 appeared to be applicable to other soils as well. Kiewiet and Ma (1991) kept *L. rubellus* in reconstituted groundwater during 24 hours and varied pH and Ca independently. Application of Equation 6.1 to the Cd uptake at varying pH provides Equation 6.3.

$$\text{Log } Cd_{worm} = 0.68 * \log [Cd^{2+}] + 0.057 * pH - 0.48 \qquad [6.3]$$

Log $[Cd^{2+}]$ ranges from –5.2 to –4.2, $R^2_{adj} = 0.83$, n = 60

A combination of Equation 6.2 and 6.3 provides 6.4:

$$\text{Log } Cd_{worm} = 0.88 * \log Cd_{soil} - 0.38 * pH + 1.94 \qquad [6.4]$$

Obviously, a change of 1 unit in log Cd_{soil}, has a larger effect than a change of 1 unit in pH. Equation 6.4 nevertheless predicts that an increase in soil pH of 1 unit, without altering the total Cd content, will reduce the Cd concentration in earthworms by a factor of nearly 3 if uptake through the skin is the dominant route.

Materials and methods

Experimental design

An uncontaminated sandy soil from the Wildekamp field near Wageningen, The Netherlands, was spiked with cadmium (Table 6.1). Particle size distribution was determined by sieve and pipette, organic carbon (OC) according to Kurmies, and the actual cation exchange capacity (CEC) with 0.01 M $BaCl_2$. 'Total' metal concentrations were determined by aqua regia extraction: 2 g of soil and 16 ml of a concentrated HCl/HNO_3 mixture, in a ratio of 3:1 by volume, were boiled under reflux for 2 hours. All methods are described in Houba et al. (1995).

The factorial experiment consisted of four treatments: two lime ($Ca(OH)_2$) levels (0 and 0.135% (w/w)) and two manganese oxide (MnO_2) levels (0 and 1% (w/w)). Each treatment was replicated six times. Five field-collected earthworms (*Lumbricus rubellus* Hoffmeister) in the mature adult stage were kept in 650 g of soil in 1-L pots at 15 ± 1°C for seven weeks under continuous light conditions in a climate chamber. Additional food was supplied to each pot. A Rhizon soil moisture sampler (Rhizosphere Research Products, Wageningen, The Netherlands) was put horizontally in each pot.

Table 6.1: Soil characteristics.

Particle size		OC	WHC	CEC	Total metal contents			
< 2 µm	> 50 µm				Cd	Zn	Mn	Ca
(%)	(%)	(%)	l/kg	$Cmol_c$/kg	mg/kg	mg/kg	mg/kg	mg/kg
3.0	85.7	2.0	0.27	3.5	9.9	31	143	663

WHC = Water Holding Capacity

Treatment of the soils

Four weeks before the earthworms were put into the soil, a solution containing 71 µmol of $Cd(NO_3)_2$ in distilled water was added to each soil batch of 800 g, resulting in a Cd concentration of 10 mg/kg dry weight. After adding the Cd solution and adjusting the moisture content of the soil to 19% (ca. 70% of the water holding capacity), we added MnO_2 (10 g/kg dry weight) and/or $Ca(OH)_2$ (1.35 g/kg dry weight). MnO_2 was prepared in our laboratory according to Buser et al. (1954) by adding HCl to an excess of $KMnO_4$ at 90°C. This type of manganese oxide, called δ-MnO_2 or birnessite, is known for its high metal binding capacity (Mench et al., 1994; Vangronsveld and Cunningham, 1998). A reference soil was included as a positive control of the cocoon production and survival rate in this experiment. The soils were stored in plastic bags in the dark at room temperature until use.

Sampling and analysis

When the soil was transferred to the experimental jars, samples were taken and extracted with 0.01 M $CaCl_2$ (Houba et al., 2000). The soil was also extracted with a mixture of 0.01 M $CaCl_2$ and 0.1 M triethanolamine (TEA) adjusted to pH 7.2. The procedure was similar as for a single extraction with 0.01 M $CaCl_2$: 3 grams of soil were suspended in 30 ml of the extractant and shaken for 24 hours. After centrifuging at 1,800 *g*, Cd was measured in the supernatant.

During the exposure of the earthworms we sampled pore water in weeks 1, 4, and 7 to measure pH and dissolved organic carbon (DOC). DOC was determined by an SK^{12} TOC/DOC analyzer (Skalar, Breda, The Netherlands). After acidification to 0.1 M HNO_3 samples were analyzed for Al, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, and Zn by ICP-AES (Spectro Analytical Instruments, Kleve, Germany), by flame atomic absorption spectrometry with Smith-Hieftje background correction (Instrumental Laboratory-S11, Thermo Jarrell Ash, Breda, The Netherlands), or by ICP-MS (Elan 6000, Perkin Elmer, Norwalk, CT, USA) depending on the concentration.

At the end of the experiment, the earthworms were collected, counted, rinsed, and weighed. Cocoons were counted by wet-sieving of the soil. The earthworms were kept on wetted filter paper in petri dishes for 48 hours to empty their gut. The filter paper was refreshed after 24 hours to prevent coprophagy. Subsequently, the worms were freeze-dried and digested individually in 5 ml concentrated HNO_3 during 2 hours at 120°C. Then 1 ml of H_2O_2 was added three times to prevent boiling over and the temperature was increased and kept at 150°C for 1 hour. The solution was transferred to 50 ml flasks and Cd was measured by flame atomic absorption spectrometry.

Statistical analysis

The significance of all treatment effects was tested by analysis of variance. Log-transformed data were used, except for survival and cocoon production. The software package SAS® was used for the calculations (release 6.12 for Windows, SAS, Cary, NC, USA).

Results

Pore water analyses

The Cd concentration in pore water strongly decreased in the soils treated with lime and MnO_2. The results, presented in Figure 6.2, represent the average of the six pots. The pore water samples in week 1, 4 and 7 were averaged, because no differences were measured in the course of time. Lime increased pH by approximately 1.6 units (Table 6.2), which equals a decrease in proton concentration by a

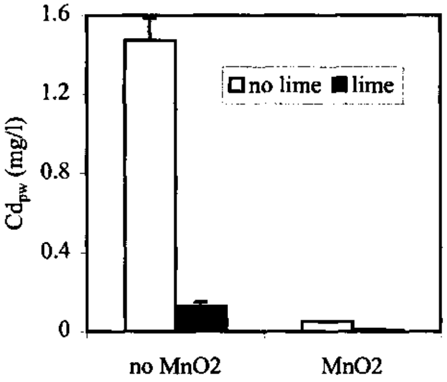

Figure 6.2: Total Cd concentration in pore water in each treatment. Error bars represent the standard deviation.

factor of 40. A decreased proton competition results in a higher binding of Cd to soil particles. MnO_2 decreased $[Cd^{2+}]_{pw}$ because of binding to the MnO_2 surface and without an overall effect on pH (Table 6.3). MnO_2 showed an interaction effect with lime because it slightly decreased the pH in the lime treatments and increased the pH in the unlimed soil (Table 6.2).

Table 6.2: Results of pore water analyses (in mg/l, except for pH) ± standard deviation.

Variables	Without MnO_2		MnO_2	
	Unlimed	Limed	Unlimed	Limed
pH	4.43 ± 0.06	6.21 ± 0.15	4.64 ± 0.04	6.07 ± 0.17
DOC	155 ± 49	222 ± 26	131 ± 8	180 ± 25
Na	13.9 ± 1.9	12.1 ± 1.1	15.1 ± 1.1	12.1 ± 0.5
K	84 ± 12	72 ± 5	525 ± 16	391 ± 11
Ca	225 ± 27	480 ± 64	215 ± 11	406 ± 61
Cd	1.473 ± 0.111	0.131 ± 0.021	0.051 ± 0.002	0.009 ± 0.002
Mn	48.1 ± 4.9	6.6 ± 1.6	3.5 ± 0.4	0.7 ± .0.6

Table 6.3: The influence of lime and MnO_2 on pore water concentrations and earthworm parameters (*** = $p<0.0005$; ** = $p<0.005$; * = $p<0.05$; ns = not significant). Data were log transformed, except survival and cocoon production.

Parameter	pH	DOC	K	Ca	Cd	Mn	survival	cocoon	Cd_{worm}	Mn_{worm}
Lime	***	***	***	***	***	***	ns	ns	**	ns
MnO_2	ns	*	***	ns	***	***	ns	ns	***	***
Lime * MnO_2	**	ns	ns	ns	***	***	ns	ns	ns	ns

The Mn concentration in pore water showed a decrease in the soil treated with lime (Table 6.2). The Mn concentration in pore water also decreased when MnO_2 was added, although the total Mn content in the soil increased from 0.14 to 4.49 g/kg. MnO_2 did not affect the Ca concentration significantly (Table 6.3) but strongly increased the K concentration. This can be explained by the use of $KMnO_4$ in the production of MnO_2, although the material was washed with $CaCl_2$ several times. However, K has only a limited effect on the uptake of Cd by *L.*

rubellus (Rietra and Ma, 1997). Lime increased [Ca^{2+}] (Table 6.2). Addition of 1.3 g of $Ca(OH)_2$ per kg soil tripled the total amount of Ca in the soil (Table 6.1). Other elements were influenced neither by lime nor by MnO_2.

The DOC concentrations increased with increasing pH because of a more negatively charged surface of organic matter. The DOC concentrations slightly decreased in the MnO_2 treatments. The DOC concentrations are generally high but well reproducible both in time and within treatments. No artifacts may be expected from the moisture samplers because they were tested before use and proved to be inert also in other studies (Knight et al., 1998).

Distribution and speciation of Cd

Cadmium in the pots could be present in earthworms or pore water or bound to the solid phase. The latter fraction was the most important; over 95% of total Cd was bound to the soil in all treatments. The fraction in pore water ranged from 0.015 to 3%, whereas the fraction present in the earthworms ranged from 0 to 2% (partly depending on the number of worms left in the pots).

Thermodynamic modeling indicated that over 99% of the Cd in pore water was present either as a free ion or complexed with dissolved organic matter. Cadmium bound to dissolved organic matter varied from 15 to 85%. Liming slightly increased the DOC concentration resulting in a higher percentage of complexed Cd. The model further calculated that approximately 94% of the Ca in pore water existed as a free ion in all treatments. The concentrations of Ca are at a level that most of the Ca is present as a free ion and small changes in the complexed amount do not affect the speciation.

Earthworms

The Cd concentration in *L. rubellus* is presented in Figure 6.3a. Both lime and MnO_2 influenced the Cd content in the worm significantly (Table 6.3), though MnO_2 decreased the Cd content in the worm more than lime. The manganese content in the worms strongly increased with a MnO_2 addition (Figure 6.3b), whereas the Mn in the solution phase decreased (Table 6.2).

The treatments neither affected the survival rate nor the cocoon production significantly (Table 6.3). The average number of cocoons produced per pot was 19.3 ± 12.3 and approximately 60% of the worms survived in the treated soils and the clean reference soil. The survival rate seems low, but this was also observed by Spurgeon et al. (2000), who added

10% of peat to a soil low in organic matter (2.35%) to ensure adequate survival. However, such an addition would have changed the availability of metals.

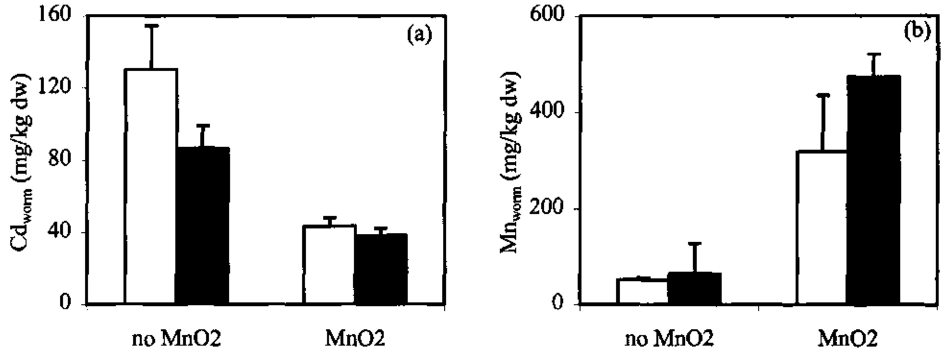

Figure 6.3: Content of Cd (a) and Mn (b) in the earthworm *Lumbricus rubellus* in each treatment. Error bars represent the satndard deviation; dw = dry weight, open bars = no lime, filled bars = lime.

Discussion

Effect of MnO_2

We have shown that addition of MnO_2 results in a lower Cd uptake by earthworms under conditions of constant pH, $[Ca^{2+}]$ and total Cd_{soil}. Both the influence of competing elements in the soil solution and the ingestion of Cd are constant. The addition of MnO_2 reduced Cd_{worm} by a factor of approximately 2.5, whereas $[Cd^{2+}]_{pw}$ was reduced by a factor of circa 25. It is possible to conclude that uptake of Cd by *L. rubellus* is not only determined by the total Cd concentration in the soil, although a previous experiment did not always show a decrease in metal uptake by *Eisenia veneta* in MnO_2 treated soils (Oste et al., 1998).

Another effect of MnO_2 addition is the increase in Mn in the earthworm tissue (Figure 6.3b). A few reasons can be responsible. First, chemical reduction of MnO_2 might occur, particularly at lower pH values, but the Mn concentrations in pore water proved that reduction of MnO_2 into Mn^{2+} did not occur in the soil (Table 6.2). Second, mobilization of Mn in the gut might induce higher uptake. However, the neutral pH in the gut (Wallwork, 1983) does not suggest a large effect. The most plausible explanation is that MnO_2 particles left in the gut showed up in the analysis. The increase in Mn observed in the worms corresponds with ca. 30 mg of soil in a worm with a fresh weight of 2 g. We noted small amounts of minerals after digestion. Cadmium bound to the soil particles did not influence Cd_{worm}, because Cd_{soil} was much lower than Cd_{worm}.

Effect of lime

The Cd uptake is affected by the increase of 1.6 unit in pH, but the effects are small, particularly in the MnO_2-treated soils. A reduction by a factor of 5, as predicted by Equation 6.4, would have resulted in a large overestimation of the pH effect. The results confirm earlier experiments indicating small or insignificant effects of liming on Cd uptake by earthworms (Bengtsson et al., 1986; Oste et al., 2001). Sample et al. (1999) compiled a database and derived uptake models for different metals. They also concluded that Cd uptake was hardly influenced by pH.

Lime treatment results in a decrease in proton competition, but also in a simultaneous increase in Ca, introducing another competing ion. Hence, $\log[Ca^{2+}]$ and pH correlated positively in our experiment ($R^2 = 0.78$). Lime decreased the proton activity by a factor of 40, and increased $[Ca^{2+}]$ by a factor of 2 (Table 6.2), suggesting that the effect of pH dominated. It is nevertheless impossible to distinguish these 2 factors in our experiment. Also in field soils as used by Janssen et al. (1997a), pH and $[Ca^{2+}]$ were correlated ($R^2 = 0.76$).

Table 6.4: Regression equations obtained from different studies (Cd_{worm} in mol/kg dry weight, $[Cd^{2+}]$ in mol/l, 1 mol Cd = 112.4 g Cd).

	Equation	Statistics	Experimental
A	$\log Cd_{worm} = 0.19*\log[Cd^{2+}] - 2.00$	$R^2_{adj} = 0.66$ $n = 19$	Equation A & B: *L. rubellus* in lime/MnO_2 treated soil for 49 days, $p[Cd^{2+}] = 4.9\text{-}7.8$, pH = 4.4-6.2 (this study).
B	$\log Cd_{worm} = 0.30*\log[Cd^{2+}] + 0.18*pH - 2.30$	$R^2_{adj} = 0.89$ $n = 19$	
C	$\log Cd_{worm} = 0.31*\log[Cd^{2+}] + 0.30*pH - 2.68$	$R^2_{adj} = 0.70$ $n = 18$	E. andrei in 18 different soils for 21 days, $p[Cd^{2+}] = 6.7\text{-}12.2$, pH = 3.4-7.9 (Janssen et al., 1997b).
D	$\log Cd_{worm} = 0.41*\log[Cd^{2+}] + 0.38*pH - 2.17$	$R^2_{adj} = 0.78$ $n = 19$	E. andrei in 19 different soils for 63 days, $p[Cd^{2+}] = 6.7\text{-}11.4$, pH = 3.4-7.9 (Peijnenburg et al., 1999).
E	$\log Cd_{worm} = 0.97*\log[Cd^{2+}]_{TEA} + 3.63$	$R^2_{adj} = 0.83$ $n = 18$	TEA-extraction (this study)

Relationship between $[Cd^{2+}]_{pw}$ and Cd_{worm}

According to Figure 6.1, dermal uptake is determined by the free ion concentration. However, Equation A (Table 6.4) shows only a moderate correlation ($R^2_{adj} = 0.66$). Figure

6.4a shows a plot of Cd_{worm} versus $[Cd^{2+}]_{pw}$. Both the addition of MnO_2 and the addition of lime decrease the $[Cd^{2+}]$ from 10^{-5} M to $3*10^{-7}$ M, but Cd uptake by *L. rubellus* is twice as high in the limed soil compared to the MnO_2-treated soil. Equation B (Table 6.4) shows a regression analysis assuming pH dependent uptake from pore water. The description of the data is strongly improved ($R^2_{adj} = 0.89$).

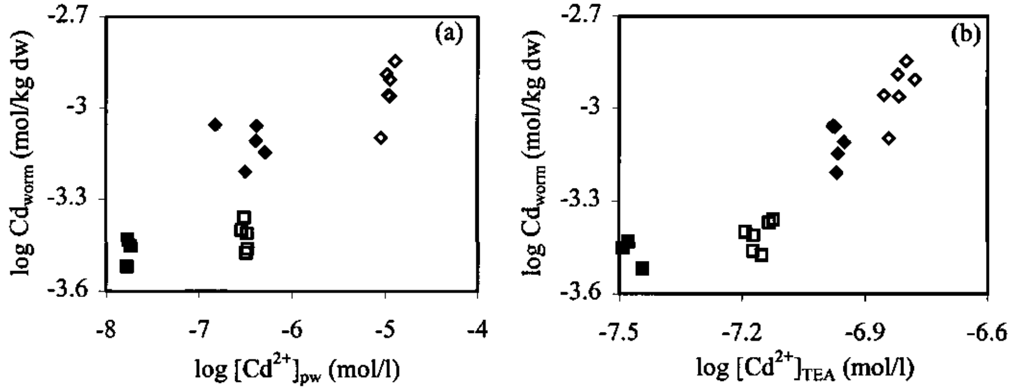

Figure 6.4: Log Cd_{worm} as a function of log $[Cd^{2+}]_{pw}$ (a) and log $[Cd^{2+}]_{TEA}$ (b). Data represent individual pots; dw = dry weight; no treatment (◇), lime (◆), MnO_2 (□), lime and MnO_2 (■).

We also considered two other studies in which Cd in pore water was measured (Janssen et al., 1997b; Peijnenburg et al., 1999). Janssen et al. (1997b) exposed earthworms to 20 Dutch field soils for 3 weeks, sampled pore water and used the MINTEQ model to calculate the free Cd concentration. They found no significant correlation between free Cd concentration in pore water and the Cd content in *Eisenia andrei* ($R^2 = 0.17$). We recalculated the Cd speciation in the soil solution using ECOSAT (including the consistent NICA-Donnan model), but we still did not find a correlation between the free Cd concentration (p$[Cd^{2+}]$ ranged from –12 to –6.7) and the tissue concentration in the worm ($R^2 = 0.05$). If pH is also incorporated as an explanatory variable in our regression analysis, as is shown in equation C (Table 6.4), the correlation is strongly improved ($R^2_{adj} = 0.70$). Peijnenburg et al. (1999) used the same soils and pore water data to conduct another earthworm experiment using *E. andrei*. The $[Cd^{2+}]$ concentration as calculated by ECOSAT, and the pH were correlated to Cd_{worm} after 63 days, resulting in Equation D (Table 6.4). Both studies show that Cd_{worm} can be described reasonably well as the result of a pH dependent uptake process. As in our own experiment (pH coefficient = 0.18; Equation B, Table 6.4), however, the value of the pH coefficients in Equations C and D (0.30 and 0.38) are conspicuously higher than the value obtained from a water experiment (0.057; Equation 6.2). If uptake from pore water would be the most important uptake route, these coefficients should be similar. Apparently, intestinal uptake of soil particles can not be neglected. The high pH coefficients in equations B, C, and D (Table 6.4) compensate for pH dependent sorption to the soil.

We should nevertheless be careful to compare water and soil experiments. The time span of the soil experiments (3 to 9 weeks) differed from the water experiments (24 hours), but the Cd concentrations in the earthworm tissues are in the same range. Although these differences in exposure produce some uncertainty in the comparison of the water and soil experiments, we conclude that the net effect as predicted by Equation 6.4 does not agree with our results. Another difference between the water and soil experiments was that the soil pH was increased by adding $Ca(OH)_2$, whereas NaOH was used to increase the pH in the water experiments. As a result, the pH variable in Equation B (Table 6.4) implicitly accounts for a change in $[Ca^{2+}]$.

This study shows that dermal and intestinal uptake of metals need different approaches and that the equilibrium partitioning theory is not directly applicable to metals. The theory holds for uncharged organic chemicals because their binding behavior and both intestinal and dermal uptake are pH independent. Thus, even if intestinal uptake were the most important route, the concentration in soil pore water and in the worm would still correlate well.

Mild extraction method to predict Cd uptake by earthworm

If intestinal uptake is an important pathway, the free Cd concentration in the gut will determine Cd uptake (Figure 6.1). A neutral pH and degradation of organic matter characterize the conditions in the gut. To estimate the availability in the gut, we evaluated the use of a mild extraction procedure at a constant pH. An extraction with diethylenetriaminepetaaceticacid (DTPA), triethanolamine (TEA), and $CaCl_2$ buffered at pH 7.2 has been used in several studies (Lindsay and Norvell, 1978). Approximately 50% of the total Cd is extractable in the presence of DTPA (Beyer et al., 1982; Abdul Rida and Bouché, 1997), indicating that this mixture is a rather strong extractant. We modified this procedure by omitting the DTPA; furthermore, we changed the solid-solution ratio and shaking time, and refer to it as TEA-extraction. The results showed a reasonable correlation between the TEA-extractable Cd and Cd_{worm} ($R^2 = 0.78$; data not shown). The final pH in the unlimed soils was 7.13 ± 0.015; in the limed soil the pH was 7.23 ± 0.006. This difference may influence the amount of Cd bound to the soil, but also the Cd complexed to TEA, because TEA acts as a complexing agent. The binding of Cd to TEA could be calculated using a similar approach as for inorganic Cd-complexes in the pore water. Constants were obtained from Smith and

Martell (1975). Only 6 to 8.5% of the dissolved Cd was present as a free ion. Figure 6.4b shows the Cd_{worm} versus $[Cd^{2+}]_{TEA}$. Equation E (Table 6.4) shows that the Cd coefficient is almost 1, indicating a linear relationship between Cd_{worm} and $[Cd^{2+}]_{TEA}$. A TEA-extraction might therefore be a useful predictive tool for Cd accumulation in earthworms.

Conclusions

Lime decreases $[Cd^{2+}]_{pw}$ but simultaneously increases pH and $[Ca^{2+}]$. In contrast, MnO_2 decreases $[Cd^{2+}]_{pw}$ without affecting pH or $[Ca^{2+}]$. MnO_2 therefore offers an opportunity to vary the concentration of $[Cd^{2+}]$ in the same soil system at a fixed total Cd content in the soil and fixed pH.

The experiment in which MnO_2 was added to a contaminated soil shows that the total Cd concentration does not solely determine the Cd content in earthworm tissues. On the other hand, our experiment also revealed that $[Cd^{2+}]_{pw}$ did not correlate very well to worm tissue concentrations. Regression equations using pH and log $[Cd^{2+}]_{pw}$ as explanatory variables could describe Cd uptake by *L. rubellus* reasonably well. They may be useful for practical purposes, but they do not reflect the real processes. The pH coefficient clearly deviates from the value obtained from water experiments, indicating that intestinal uptake predominates. A TEA extraction of the soil simulates the Cd availability in the gut (constant pH) and showed a good agreement with the Cd concentration in the earthworms. It might be a promising tool to estimate Cd uptake by earthworms.

With respect to soil remediation, the addition of immobilizing materials in a Cd-contaminated soil will have a limited effect on the Cd accumulation by earthworms. Nevertheless, additives that increase the number of binding sites will perform better than materials that only increase the pH.

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Chapter 7

Potential applications of immobilization for *in situ* soil remediation: Summary and discussion

Summary

Basic concepts

Heavy metals in soils can produce harmful effects on plants, animals and human beings. It has been generally accepted that uptake and toxicity of metals is more related to the soluble metal fraction, and particularly the free metal concentration, than to the metal content in the soil. Hence, reducing the free ionic concentration in the soil solution can reduce harmful effects. A decrease in free metal concentration can be achieved by adding materials to the soil that transform free metal ions into less mobile species.

The measurement of free metal concentrations in solution is complicated. Available analytical techniques are limited in measuring free metal concentrations in solutions containing natural organic matter. Most data sets have been obtained by using an Ion Specific Electrode, but no electrode is available for Zn. The recently developed Wageningen Donnan Membrane Technique can solve this problem. Chapter 2 focused on the measurement of metal binding to dissolved organic matter. First, the WDMT was further developed, particularly with respect to the validation, the ionic composition of the background electrolyte, and the measurement at high pH. Free Cd and Zn concentrations were measured in humic acid solutions over a large concentration range ($p[Cd^{2+}] = 3\text{-}9$; $p[Zn^{2+}] = 3\text{-}8$) and at different pH values (4, 6, and 8). The WDMT required a relatively large sample volume and was labor-intensive. Therefore, the measured data were used to derive specific NICA-Donnan model parameters for Cd and Zn. The model was used in the other chapters to calculate the speciation in soil solutions.

The behavior of organic matter plays a crucial role in the mobility of metals. Not only the binding of metals by (dissolved) organic matter (Chapter 2) is important, but also the solid-solution partitioning of organic matter itself. An important factor influencing the solid-solution partitioning is the ionic composition of the solution which was studied in Chapter 3.

An increase in pH without a simultaneous addition Ca could strongly increase the dissolved organic matter concentration. The mobilization of organic matter also led to a mobilization of metals, due to the metal binding by organic matter molecules. It appeared that the electrostatic Donnan potential as calculated by the NICA-Donnan model could well describe the DOM concentration as affected by the addition of OH^- and Ca^{2+}.

In situ *immobilization*

The potentials of *in situ* immobilization of heavy metals in contaminated soil were investigated in the remainder of the thesis using the basic concepts obtained in Chapters 2 and 3. The first question was which materials could be used as immobilizing agents. The materials that had been used in immobilization studies were divided into 4 groups (Table 1.1): lime, alumino silicates, Fe/Al/Mn-(hydr)oxides, and materials rich in organic matter.

The second question is how selected materials should be evaluated. The introduction mentioned three aspects: 1) leaching to deeper soil layers or groundwater, 2) toxicity to and uptake by plants, and 3) toxicity to and uptake by soil organisms.

Leaching of contaminants is not restricted to the free metal concentration, but to the total concentration in the soil solution. Hence, inorganic metal complexes (e.g. metal chlorides, and hydroxides) and metals bound by DOM also contribute to leaching. In Chapter 4, the leaching from zeolite-treated soils was investigated. Although the binding capacity of some synthetic zeolites was very high, Cd and Zn leaching from soil columns treated with zeolite A was higher compared to an untreated soil column. The high leaching could be explained by the strongly increased leaching of organic matter. The theory to describe the solid-solution partitioning of organic matter, as developed in Chapter 3, could be applied in Chapter 4. Synthetic zeolites increased the pH and decreased the Ca concentration, creating ideal conditions to transform solid organic matter into DOM. Ca-rich materials, also increasing the pH, did not enhance the dispersion of organic matter.

Uptake of Cd and Zn by plants and earthworms was studied in Chapter 5. The available metal concentration in the soil was estimated by extracting the soil with 0.01 M $CaCl_2$. Lime and cyclonic ashes strongly reduced the extractable metal concentrations, but cyclonic ashes did not reduce the metal concentrations more than liming when the obtained pH levels were similar. The uptake by Swiss chard was strongly correlated to the extractable metal concentrations, but metal uptake by earthworms was hardly influenced by the alkaline materials used in this study.

Chapter 6 focused on the effect of lime and MnO_2 on Cd uptake by earthworms. MnO_2 had a very high binding capacity for Cd, without adjusting the pH, whereas lime only increased the pH. MnO_2 reduced Cd uptake by earthworms more than lime at a similar reduction of Cd in the pore water. Apparently, intestinal uptake was an important uptake route. The pH in the intestine is reported as approximately 7, independent of soil pH. The binding capacity of MnO_2 reduced metal uptake also in the intestine at neutral pH, whereas the increase in soil pH did not affect the pH in the intestine.

Discussion

We will discuss the potentials for immobilization as a soil remediation technique, following the aspects mentioned in Table 7.1.

Table 7.1: Properties of a good immobilizing agent

- high metal binding capacity
- selective metal binding
- durable
- no negative side effects
- cheap

Metal binding capacity

The metal binding capacity of sorption materials can be based on two principles: sorption to the surface of the binding material (new sites) or sorption to existing sites in the soil by decreasing proton competition (pH increase).

Figure 7.1 presents 0.01 M $CaCl_2$-extractable Cd as a percentage of the Cd content in the soil after addition of various amendments. The line represents the effect of liming in soil G553. All data points deviating from the line indicate other effects than only an increase in pH and simultaneous increase in Ca content. Changes in dissolved organic matter concentration, and in ionic strength or ionic composition, which may contribute to small deviations from the liming effect, are suppressed by 0.01 M $CaCl_2$. Data points that are clearly below the line (Figure 7.1) are most probably caused by the addition of new binding sites. MnO_2 shows such an effect. However, an increase in pH effectively reduces the metal mobility as evaluated with an extraction with 0.01 M $CaCl_2$. Also plant availability is reduced after an increase in pH (Chapter 6), so why do we bother about the mechanism? This thesis shows that this difference between a pH increase and the addition of new binding sites does not only have a theoretical value, but also practical implications. Chapter 4 shows that alkaline additives poor in Ca can strongly increase the DOM concentration, resulting in an increased metal leaching. Chapter 5 and 6 show that alkaline additives hardly reduce the Cd uptake in earthworms. MnO_2 reduces Cd uptake by the earthworm *Lumbricus rubellus*, though the effect is limited compared to the decrease in $CaCl_2$-extractable Cd.

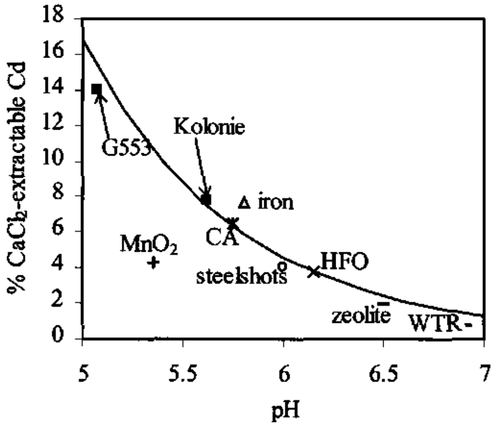

Figure 7.1: $CaCl_2$-extractable Cd concentration as a percentage of the total Cd in the soil versus the obtained pH after addition of immobilizing agents. The line is a regression line of soil G553 after different amounts of lime. Black squares = untreated soils, CA = cyclonic ashes, iron = Fe (Merck, analytical grade), steelshots = waste product of the cast iron industry, zeolite = zeolite A (Na-form), WTR = (drinking) water treatments residue.

Selectivity

This thesis is mainly focused on Cd and Zn. MnO_2 appeared to have a high binding capacity for both metals. However, MnO_2 is less effective in Cu contaminated soils (Oste, unpublished results). On the other hand, Calplus was found to be effective for Zn and Cu, but not for Cd. Extrapolation from one to another metal is dangerous without detailed knowledge of the binding mechanism.

Whereas heavy metals are not always bound sufficiently strong, other elements should preferably not be bound. Materials that can immobilize heavy metals sometimes immobilize elements that are beneficial to the soil. The synthetic zeolites used in Chapter 4 have a high affinity for Ca, decreasing heavy metal binding and increasing leaching of organic matter.

Durability

Once mixed with the soil, immobilizing materials can not be removed again. Hence, it is very important that soil additives are stable over a long period. If long-term degradation or weathering processes take place, this may lead to remobilization, but it should be prevented that the situation gets worse than before soil treatment. The biodegradability of organic matter containing products, resulting in an increased transport of contaminants, is an example of such a risk. Also the addition of MnO_2 to anaerobic and acid soils may cause Mn toxicity.

Side effects

The examples mentioned at the end of the previous paragraph are typically unwanted side effects. The following aspects should be considered to assess potential side effects of an immobilizing agent: toxicity of the material itself (after dissolution or degradation), toxicity of contaminants in the additive, toxicity as a result of reactions induced by the additive, effect on soil structure, effect on DOM.

MnO_2 can be reduced to Mn^{2+} at low redox potential and/or low pH. It can therefore not be used in (temporarily) water-logged soils, and the applicability in aerobic acidic soils is unclear. In one of the plant experiments Mn-uptake was significantly increased, but there were no toxic symptoms found. In the earthworm experiments, Mn in pore water even decreased, due to binding of Mn^{2+} to MnO_2. The behavior of MnO_2 under field conditions is difficult to predict.

The contamination level in the amendment can be a problem in waste products. Incinerator ashes generally contain a series of contaminants, but also other industrial waste products, such as Fe-rich, can contain substantial amounts of contaminants. High contamination levels do not necessarily lead to higher concentrations in the soil solution, but contaminants can be released on the long term and availability via the solid phase (e.g. soil ingestion) is less dependent of solution concentrations.

Sometimes, materials chemically react after addition to the soil. Iron particles or Fe^{2+} ions are oxidized to ferric (hydr)oxide. The reaction may change the pH, which could be compensated by simultaneous addition of an alkaline substance. The oxidation of iron may also reduce MnO_2 present in the soil. The latter could not be proved in our plant experiment in soils treated with steelshots.

Soil additives can both improve and damage the soil structure. The amount of Ca in the material plays an important role as well as the particle size and type. Cyclonic ashes, containing much Ca, improve the water binding capacity and the structure of the soil, whereas synthetic zeolites in the Na-form can damage the soil structure.

Carrier molecules can facilitate transport of contaminants. DOM can act as a metal carrier in the soil solution. Alkaline additives with little or no Ca can increase metal leaching due to the increase in DOM. In contrast, Ca-rich materials can even suppress leaching of DOM, resulting in decreased metal leaching.

Cost

The price of *in situ* immobilization is highly determined by the price of the additive. Treatment of the upper 35 cm of 1 ha with 1% additive requires approximately 50 tons. Only cheap materials can be considered. Industrially produced end products, such as synthetic zeolites or Calplus, will cost € 0.75 to € 1.00 per kg. The MnO_2 used in this study has been synthesized in the laboratory only and is probably much more expensive.

Several products are waste products and hence attractive because of the price. The enormous variety of waste materials makes it very complicated to judge the different products generally. Incinerator ashes, for example, can widely vary in pH and contamination level. The properties of waste materials are determined by the composition of source material and the industrial process. One important prerequisite is that they are not contaminated if the soil is only moderately contaminated. Materials than can be obtained without contamination are drinking water treatment residues, gravel sludge, and steel shots (from a cast iron factory).

Samenvatting

Schadelijke effecten van zware metalen zijn in het algemeen sterker gerelateerd aan de metaalconcentratie in het bodemvocht dan aan het metaalgehalte in de bodem. Gangbare saneringstechnieken zijn gericht op de verwijdering van de metalen uit de bodem, maar schadelijke effecten kunnen ook worden beperkt of weggenomen door de metaalconcentratie in het bodemvocht te verlagen. Dit laatste kan worden gerealiseerd door menging van metaalbindende stoffen door de grond. De mogelijk te gebruiken stoffen zijn, op basis van structuur en samenstelling, ingedeeld in vier groepen. 1) kalk, 2) aluminiumsilicaten, 3) Al-/Fe-/Mn-(hydr)oxides, 4) organischestofrijke materialen. Hoewel deze stoffen de metalen slechts minder mobiel maken, wordt de techniek immobilisatie genoemd.

Immobilisatie heeft een aantal voordelen ten opzichte van gangbare saneringstechnieken. De techniek is doorgaans goedkoper dan gangbare saneringen, hoewel dit in sterke mate wordt bepaald door de prijs van het additief. Bovendien is immobilisatie aantrekkelijk, omdat het een eenmalige kortdurende ingreep betreft, waarna de grond weer gebruikt kan worden zoals daarvoor. Een goede immobilisator laat namelijk de bodemstructuur, de bodemvruchtbaarheid en het bodemleven intact, terwijl veel gangbare grondreinigingsmethoden een eindproduct geven, dat als teelaarde ongeschikt is. Een derde voordeel is dat ook nieuwe verontreiniging, zoals de huidige depositie, wordt geïmmobiliseerd.

Immobilisatie heeft echter ook een aantal beperkingen. Het inbrengen van additieven is lastig in gebieden met bebouwing of begroeiing. Ook in het geval van diepe verontreinigingen treden er fysieke beperkingen op. Verder wordt alleen de blootstellingsroute via het bodemvocht gereduceerd. De opname van metalen als gevolg van grondinname door mensen en hogere diersoorten wordt vooral bepaald door de omstandigheden in het maagdarmkanaal. Tenslotte wordt de verontreiniging niet verwijderd en dat maakt monitoring noodzakelijk om onplezierige verrassingen te voorkomen.

Bovengenoemde voor- en nadelen maken duidelijk dat immobilisatie niet in alle opzichten een succesvolle oplossing biedt. Aan de andere kant geldt dat we tot nu toe in het geval van gecompliceerde verontreinigingen niet verder komen dan nietsdoen. Dit proefschrift heeft als doel meer inzicht te krijgen in de werking van diverse immobiliserende stoffen. Alvorens dat te doen, wordt aandacht besteed aan twee fundamentelere processen. Hoofdstuk 2 gaat in op het meten en modelleren van vrije metaalionen in oplossing. In hoofdstuk 3 wordt het gedrag van organische stof in de bodem bestudeerd, vooral met betrekking tot de verdeling over de opgeloste en de vaste fase.

In het begin van deze samenvatting is opgemerkt dat schadelijke effecten vooral worden veroorzaakt door de metaalconcentratie in het bodemvocht, maar het bodemvocht bevat metalen in verschillende gedaantes (species). Vaak worden schadelijke effecten niet gerelateerd aan de totale metaalconcentratie, maar aan de concentratie van één van de aanwezige species, namelijk de vrije metaalionen. Deze metaalionen zijn analytisch moeilijk te onderscheiden van andere species, zoals anorganische metaalcomplexen (zoals $CdCl^+$) of metaal dat is gebonden aan opgeloste organische stof. Met een ion-selectieve electrode kunnen vrije metaalionen wel gemeten worden, maar voor Zn bestaat geen ion-selectieve electrode. Daarom zijn er voor zink nagenoeg geen betrouwbare data beschikbaar. In hoofdstuk 2 wordt de Wageningen Donnan Membrane Technique verder ontwikkeld en gebruikt om vrije zinkconcentraties te meten in oplossingen waarin ook opgeloste organische stof aanwezig is. Deze data zijn gebruikt om modelparameters te schatten, zodat we in het vervolg de vrije metaalconcentratie kunnen berekenen op basis van de totale metaalconcentratie in de bodemoplossing, die eenvoudig is te meten. Het gebruik van zo'n computermodel bespaart ingewikkeld meetwerk.

In hoofdstuk 2 hebben we de binding van metalen aan opgeloste organische stof bestudeerd. De concentratie opgeloste organische stof in het bodemvocht is van groot belang, omdat daaraan veel metalen kunnen worden gebonden. De metalen kunnen op die manier door de bodem getransporteerd worden en zo uitspoelen naar diepere lagen en grondwater. De concentratie opgeloste organische stof kan echter variëren. Hoofdstuk 3 laat zien dat organische stof in de bodem, onder invloed van een verhoging van de pH zonder gelijktijdige toevoeging van calcium, in oplossing kan gaan.

Aangezien veel stoffen die gebruikt worden voor immobilisatie tegelijkertijd de pH verhogen, wordt de theorie uit hoofdstuk 3 toegepast in hoofdstuk 4. Daaruit blijkt dat toevoeging van industrieel geproduceerde zeolieten zonder calcium kan leiden tot verhoogde uitspoeling van organische stof en daardoor verhoogde uitspoeling van Cd en Zn, ook al bleek in schudproeven dat deze zeolieten veel metaal konden binden. Als de zeolieten voorbehandeld worden met Ca, kan de mobilisatie van organische stof weliswaar worden beperkt, maar de bindingscapaciteit van voorbehandelde zeolieten is sterk verminderd, omdat Ca een concurrent is voor de bindingsplaatsen aan het zeoliet. Andere pH-verhogende materialen, die Ca bevatten, kunnen voorkómen dat er organische stof uitspoelt.

In hoofdstuk 5 en 6 wordt het effect van immobilisatie op planten (de bladgroente snijbiet) en regenwormen bestudeerd. Het blijkt dat de Cd- en Zn-gehaltes in snijbiet gecorreleerd zijn aan de Cd- en Zn-gehaltes in oplossing. Zowel kalk als cycloonassen (ook wel beringiet genoemd) zijn in staat om het metaalgehalte in snijbiet te verlagen. Voor regenwormen ligt dat anders. pH-verhogende additieven blijken het metaalgehalte nauwelijks te beïnvloeden. Voor Zn kan dit worden verklaard, doordat regenwormen hun zinkgehalte kunnen reguleren, maar Cd nemen ze passief op. In hoofdstuk 6 concluderen we dat de Cd-opname in belangrijke mate wordt bepaald door opname in de darm van de regenworm. De pH in de darm is ongeveer neutraal, onafhankelijk van de pH van de bodem. Een verandering van de pH van de grond heeft dus geen effect op de metaalbeschikbaarheid in de darm. De toevoeging van MnO_2 (wat resulteert in een verhoging van de bindingscapaciteit zonder verhoging van de pH) leidt wel tot een verlaging van de Cd-opname, maar zeker niet in dezelfde mate als de verlaging in het bodemvocht. Er is een goede correlatie gevonden tussen het Cd-gehalte in de regenworm en de Cd-concentratie in oplossing na extractie van de grond met 0.01 M $CaCl_2$ gebufferd op pH 7.2 door triethanolamine.

In de hoofdstukken 4, 5 en 6 worden drie aspecten behandeld met betrekking tot de werkzaamheid van immobilisatie: (a) metaaluitspoeling naar diepere bodemlagen en grondwater, (b) opname door en toxiciteit voor planten (snijbiet) en (c) opname door en toxiciteit voor bodemleven (regenwormen). In het ideale geval heeft immobilisatie een langdurig positief effect op deze drie aspecten. Dit betekent dat er een aantal eisen aan een stof worden gesteld. Een goede immobilisator heeft een hoge metaalbindende capaciteit, bindt metalen selectief, is stabiel, vertoont geen negatieve bijeffecten en is goedkoop.

In de praktijk zal voornamelijk geïmmobiliseerd worden met pH-verhogende materialen. Voorwaarde is, dat ze voldoende Ca bevatten om een toename van opgeloste organische stof te onderdrukken, dat ze niet verontreinigd zijn en dat ze een langdurige pH-verhoging bewerkstelligen. Het laatste is niet noodzakelijk als het gaat om bouwland, omdat daar regelmatige bewerking plaatsvindt. De cycloonassen, waarmee in 1990 een perceel op de Maatheide in België is behandeld, hebben bewezen dat ze de pH van de grond langdurig kunnen verhogen. Momenteel wordt in België naar een vervangend product gezocht. Uiteraard is bij het zoeken naar alkalische producten de prijs van groot belang. In dat kader komt een alkalisch afvalproduct als gravelslib in aanmerking, maar ook het door ons gebruikte drinkwaterzuiveringsslib bleek de pH te verhogen.

Als pH-verhoging om welke reden dan ook niet gewenst is, wordt het lastig. MnO_2 is kostentechnisch niet aantrekkelijk en de stabiliteit is onzeker. IJzerhoudende stoffen lijken de beste optie in dat geval. Er zijn aanwijzingen dat verouderingsprocessen de metaalbinding versterken, al moet dit met betrekking tot immobilisatie met ijzerhoudende additieven nog worden vastgesteld in langetermijn-experimenten.

Levensloop

Leonard (Lein Andries) Osté werd geboren op 15 juli 1970 in Terneuzen. In 1988 behaalde hij het VWO-diploma aan het Christelijk Streeklyceum te Ede. In datzelfde jaar begon hij aan een studie Milieuhygiëne aan de Landbouwuniversiteit te Wageningen. In 1994 rondde hij deze studie af met twee afstudeervakken en een stage. Tijdens een afstudeervak bodemscheikunde heeft hij zich beziggehouden met het Basic-Stern model rond bolvormige deeltjes onder leiding van Peter Venema. Een tweede afstudeervak, bij de sectie bodemhygiëne en –verontreiniging, ging over de beschikbaarheid van lage concentraties organische microverontreinigingen (olie, PAK's e.d.) in landfarming-systemen. Dit onderzoek werd uitgevoerd bij het Staring Centrum - DLO bij Joop Harmsen. De studie werd afgerond met een stage bij Professor Colin Ferguson at the Centre for Research into the Built Environment (CRBE), Nottingham Trent University in Nottingham (Groot-Brittanië). Dit betrof een literatuurstudie naar de opname van zware metalen door groenten. Deze informatie is gebruikt bij de ontwikkeling van een risico-evaluatiemodel voor verontreinigde bodems.

Van januari tot juni 1995 is hij werkzaam geweest op het secretariaat van de vakgroep Bodemkunde en Plantevoeding. Vervolgens werkte hij tot en met december 1995 als projectmedewerker bij Ingenieursbureau Grabowsky & Poort. Dit betrof een Bijzonder Inventariserend Onderzoek (BIO) naar slootdempingen in de provincie Zuid-Holland. Het eerste halfjaar van 1996 was hij werkzaam als docent wis-, natuur-, en scheikunde aan de christelijke scholengemeenschap Het Streek te Ede.

In augustus 1996 werd hij aangesteld als Assistent in Opleiding bij de toenmalige vakgroep Bodemkunde en Plantevoeding, die tegenwoordig deel uitmaakt van de sectie Bodemkwaliteit. Het promotie-onderzoek heeft geleid tot dit proefschrift. Van april tot augustus 2000 was hij tevens werkzaam bij de leerstoelgroep Milieusysteemanalyse als docent voor het vak Milieuhygiëne I.

Vanaf 1 mei 2001 heeft hij een baan als medewerker Waterbodems bij de afdeling EMD van het Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwaterbehandeling (RIZA) te Lelystad.

Nawoord

Met enige aarzeling heb ik in juni 1996 een brief geschreven op een AIO-vacature getiteld: 'In situ-immobilisatie van zware metalen in verontreinigde grond'. Ik was al in 1994 afgestudeerd en niet specifiek op zoek naar een onderzoeksbaan, maar mijn tijdelijk werk op het secretariaat in 1995, gaf me een goed gevoel over de sfeer en organisatie bij de toenmalige vakgroep Bodemkunde en Plantevoeding. Gelukkig durfde de vakgroep het ook met mij aan. Nu terugkijkend was de genoemde aarzeling onterecht. Het is een heerlijk gevoel dat 't boekje af is, maar het is met genoegen tot stand gekomen. Het plezier in 'onderzoek doen' is toegenomen.

Dat is allereerst te danken aan mijn begeleiders, te beginnen met Theo Lexmond. Je was verantwoordelijk voor het projectvoorstel, dat buitengewoon bruikbaar bleek tot het einde toe. Verder kon ik altijd van je enorme kennis gebruik maken als dat nodig was en is je interesse voor mijn werk onverminderd gebleven, ook al waren het voor jou woelige tijden bij Wageningen Universiteit. Frans de Haan en Willem van Riemsdijk wil ik bedanken voor hun taak als promotor. Terwijl Frans het werk op afstand heeft gevolgd, heeft Willem zich intensief met de begeleiding beziggehouden. Met je aanstekelijke enthousiasme voor de wetenschap en ons werk in het bijzonder, heb je me enorm gestimuleerd. Vaak kwam je na de bespreking van een concept nog langs met suggesties die je achteraf had bedacht, waaruit grote betrokkenheid bleek. Verder wil ik Erwin Temminghoff bedanken voor adviezen met betrekking tot de analytische aspecten en voor het becommentariëren van diverse conceptartikelen.

De meeste tijd heb ik echter niet doorgebracht met begeleiders, maar met de mensen op de werkvloer en dat heeft de hele sectie geweten. Vooral Gerdine Gaikhorst en Egbert Nab hebben mij de nodige labvaardigheden bijgebracht, maar alle mensen op het lab zijn wel op een of andere manier betrokken geweest bij het werk. Arie van de Berg, Monique Driessen, Rein van Eck, Toos de Wild en Eric Heij, bedankt voor alle adviezen, metingen en gezelligheid.

In de kas hebben Willem Menkveld, Jaap Nelemans, Arie Brader, Peter Pellen en Willeke van Tintelen mij ingewijd in de uitvoering van potproeven, maar ook het verzamelen van Kempengrond was een hele belevenis. Simon Maasland was mijn steun en toeverlaat zodra mijn technisch vernuft het liet afweten (en daar is weinig voor nodig). Zelfs het secretariaat heb ik niet geheel met rust gelaten. Ik wist immers precies welke informatie het secretariaat heeft en daar heb ik dankbaar gebruik van gemaakt. Als tegenprestatie wisten ze mij regelmatig te vinden om af te sluiten.

De plezierige werksfeer werd verder gegarandeerd door het 'volk' in de kelder. De zeilweekenden, het weekend Berlijn, de borrels in Loburgh en de pancakeparty zijn natuurlijk onvergetelijk, maar ook de dagelijkse lunches, opgevrolijkt door letje en Sonja van de Dreijenborch-kantine, zorgden voor de nodige gezelligheid. Twee mensen wil ik in het bijzonder bedanken: mijn kamergenoot Anke Wolthoorn voor de dagelijkse gezelligheid en de altijd aardige antwoorden die je gaf als ik weer 'ns zat te mopperen tegen mijn computer of andere onzin zat uit te kramen. De ander is Jeroen Filius. Het gemak van het dagelijkse contact heeft onze vriendschap alleen maar hechter gemaakt. Ik hoop dat we die band in de toekomst kunnen vasthouden.

Mijn project lag duidelijk binnen het interesseveld van Wageningse studenten. Tien studenten hebben een afstudeervak in het kader van dit project uitgevoerd. In chronologische volgorde: Steven Bakker, Michal Oppenheimer, Stan Cals, Gerlinde Roskam, Daphne Bücker, Jeroen van de Berg, Roger Saanen, Katrien Verkampen, Suzanne van Dijk en Tessa Pancras. Met veel plezier kijk ik terug op de samenwerking (dat drukt de verhoudingen beter uit dan begeleiding). Het maakte mijn werk afwisselender, gezelliger en de resultaten waren vaak zeer bruikbaar. Diverse studenten zullen een 'déjà vu' beleven als ze het boekje doorbladeren.

Ook een aantal mensen buiten de sectie Bodemkwaliteit heeft een bijdrage geleverd. De regenwormproeven in hoofdstuk 5 en 6 zijn uitgevoerd in samenwerking met Wim Ma, Jos Bodt en Jan Dolfing van Alterra, een mooie kruisbestuiving van universitair en toegepast onderzoek, waar ik in ieder geval veel van heb geleerd.

We gaan steeds verder van huis. In de startfase heeft Jaco Vangronsveld (LUC, Diepenbeek, België) ons geholpen met kennis en materialen (cycloonassen en gronden), hetgeen meteen vaart in het onderzoek heeft gebracht. De werkbezoeken na Consoil '98 aan de groepen van Nick Lepp (Liverpool John Moores University), Brian Alloway (Reading University) en Jason Weeks (NERC, Monks Wood) waren zeer inspirerend. Samen met Claus Svendsen, werkzaam bij de laatstgenoemde groep heb ik spontaan wat experimenteel werk uitgevoerd. *Claus, although we did not write an ace paper, I really enjoyed our co-operation.*

Thank you for the valuable discussions and pleasant time when you came over to Ede. Sorry for the kilometres you had to cycle.

Tenslotte wil ik alle mensen buiten het werk bedanken, die de afgelopen jaren met belangstelling mijn werkzaamheden hebben gevolgd: ouders, broers, verder familie en vrienden. Het laatste dankwoord is voor Gery; bedankt voor al het vertrouwen en de grote vrijheid die je me gaf om dit proefschrift op mijn manier te realiseren.

Leonard