Abstract

In order to decrease the bioavailability of Pb in soil, phosphate can be added *in situ* to the contaminated land and an almost insoluble mineral, pyromorphite is formed. Due to P-deficiencies in soil, many plants have developed systems to gain access to P in minerals, where one possible action is to exude compounds from their roots. These actions could affect the stability of pyromorphite as well.

In this report the current research on the method is reviewed. Moreover, the effect of rhizospheric processes on the stability of pyromorphite was studied in the laboratory and in the greenhouse. A dissolution study in the laboratory was performed where the impact of different organic anions impact were tested. The organic anions; malate, citrate and oxalate are involved in many rhizospheric processes and were tested at field concentrations. Malate and citrate was shown to have the strongest effect on the dissolution of pyromorphite. The results also concluded that a higher concentration enhanced the dissolution compared to a lower concentration. XRD and SEM studies suggested that dissolution rather than a surface exchange reaction had occurred.

In the greenhouse, Smooth brome grass *(bromus inermis)* grew on a mixture of pyromorphite and soil. The exchangeable fraction of Pb in the rhizospheric fraction was followed, although no conclusions could be drawn from the results. However, the literature review suggests that the remediation method is effective in reducing bioavailable Pb as well as more cost effective and less disruptive than many conventional methods. Before it can be considered as an alternative, particularly in residential areas more long-term field studies should be performed.

CONTENTS

APPENDIX

1 INTRODUCTION

Human activities have caused elevated levels of the heavy metal lead (chemical symbol Pb) in soils all around the world (Chen et al., 2003). Pb interacts strongly with soil particles and is very persistent in the environment. Exposure affects the human health negatively and can lead to irreversible damages of the nervous system etc. (Traina and Laperche, 1999). Due to this health risk, most governments have set up regulations for Pb-contaminated soil and to meet up with these standards, land need to be remediated. One possibility is to stabilize the contaminant at the site. In the case of Pb contaminated soils, a phosphate source is added and very stable Pb-phosphates are precipitated. Laboratory experiments suggest that the bioavailability and mobility of Pb-phosphates are very low (Hettiarachchi and Pierzynski, 2004). Due to the fact that plants have an ability of modifying soil in order to gain access to nutrients, it is possible that in a field situation, these rhizospheric processes could affect the stability of the reaction product. In a worst-case scenario, the stabilized soil could turn toxic again.

2 OBJECTIVES

This degree project aims to study the stability of Pb-phosphates during rhizospheric processes. Focus is set on organic acids, compounds suspected to have a large impact on the stability of Pb-phosphates. The project also includes a literature review of the state-of-the-art in the field of soil remediation by addition of phosphate.

3 MATERIALS AND METHODS

3.1 Literature study

The current literature on the subject remediation of Pb-contaminated soils by addition of phosphate containing materials has been reviewed by reading scientific papers mainly found with the database Web of Science including several journals.

3.2 Laboratory studies

3.2.1 Formation of pyromorphite

Various types of the Pb-phosphate pyromorphite ($Pb_5(PO_4)_3X$, where X=Cl, OH, Br or F) were precipitated according to procedure from Scheckel and Ryan (2002). 0.15M H_3PO_4 was mixed with 0.25M $PbCl_2$ (Fischer Scientific) and 0.25M $PbNO_3$ (Fischer Scientific) respectively in a 1L Nalgene bottle. The solution was purged with N_2 to provide a CO_2 free atmosphere to inhibit formation of $PbCO_3$. The pH of the suspension was adjusted to 7.0 with NaOH and the solution equilibrated for 3 days. The experiments were conducted at room temperature. Another attempt of forming pyromorphite was done with a natural phosphate rock determined by X-ray diffractometry (XRD) to almost pure hydroxyapatite with low amounts of quartz and $PbCl_2$ at a weight ratio 10:1 at various pHs. The reaction products were washed and centrifuged to remove excess material. Thereafter the crystals were freeze dried in order to stop the aging processes (Scheckel and Ryan, 2002).

3.2.1.1 Analysis of reaction product

Scanning electron microscopy (SEM) images were obtained using a Philips XL scanning electron microscope. The samples were mounted on aluminum stubs and coated with gold in order to avoid electron accumulation on the surface. The SEM was run at 1.96mA and 20kV. The XRD analysis was performed with a Bruker D8 discover. XRD patterns give information about the mineralogy of the sample. The XRD used a cobalt source. The instrument was run at 40kV and 20mA.

3.2.2 Dissolution of pyromorphite by organic acids.

The impact of three different organic acids on the dissolution of pyromorphite was tested. Citric acid (BDH, 99.5% purity), oxalic acid (Sigma, 99.6% purity) and malic acid (EM Science, 98.0% purity) were tested at three different concentrations: 0, 20 and 40μM. The study was carried out as a batch experiment. 500ml solutions were prepared from MilliQ-water with the ionic strength kept by 0.01M $NaNO_3$ (Fischer Scientific). The solutions were added to 500ml Erlenmeyer flasks containing 0.5 g pyromorphite. Each treatment was prepared in two different flasks. The flasks were stirred approximately once a day.

The two first sampling days the pH was measured. The experiment was conducted at room temperature.

3.2.2.1 Sampling and analysis

At times ranging from 0.5 to 91days, three 2ml fractions were collected with a glass pipette from each flask. Each fraction was filtered through a 0.45µm filter, which was necessary due to the small size of the pyromorphite crystals. The samples were diluted with MilliQ-water and acidified to 2% with nitric acid before Pb-analysis. Samples were analyzed using an Inductively Charged Plasma Spectrometer (ICP), using the 220.353nm line in argon plasma at 1000W. The sampling time was 0.1s and three measurements were recorded for each sample. By the end of the experiment, remaining pyromorphite in the flasks was washed and freeze-dried. The crystals were studied with XRD and SEM to detect any chemical or morphological changes due to organic acid exposure.

3.2.3 Green house study

The experiment aimed to study how the rhizospheric processes of a grass affected the stability of pyromorphite. It was conducted in the Enhanced Forest Laboratory at the University of Northern British Columbia, Prince George, BC, Canada.

3.2.3.1 Soil source

The soil was sampled in Vanderhoof, BC. The Ministry of Forests tested the soil with respect to nutrients and several minerals, (see appendix 1). pH measurements were performed in the lab (Kalra and Maynard, 1991). Before planting, the soil was mixed thoroughly with ammonium nitrate (50g N/kg soil). To each rhizotron compartment, 1 kg soil was added. A rhizotron is a ca 5*20*40 cm pot with an open top, made of rigid plastic, with a plexi glass window in the front attached with screws, each containing two compartments were used as pots (see figure 1).

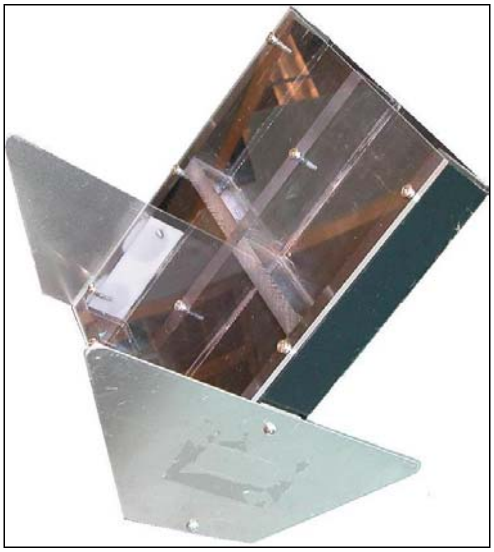

Figure 1. Rhizotron used for the greenhouse experiment.

3.2.3.2 Plant material

Seeds of smooth brome grass (*Bromus inermis*) were germinated directly in the soil. The grass is renowned for its hardiness and drought tolerance. It has a highly developed root system, which is to prefer in a rhizosphere study. The seeding depth was ca 1 cm. Grass was grown in half of the rhizotrons, whereas the soil in the other half of the rhizotrons was left undisturbed.

Figure 2 Smooth brome grass (bromus inermis) growing in forward-tilted rhizotrons.

3.2.3.3 Growth conditions

The grass was grown in a light- and temperature controlled green house. The temperature regime was 21/18ºC and photoperiod 16h. The plants were irrigated daily with MilliQ-water, without fertilization in order to obtain a P-deficient environment. Ammonium nitrate was added three times during the growing period with a total of 167mg N /kg soil and 50mg P/kg soil (potassium phosphate) was added once. These additions took place before the pyromorphite was introduced. During the growing period, the rhizotrons were "flipped" forward so that the roots grew towards the plexi glass window (see figure 2). The rhizotrons were treated equally in terms of fertilization and irrigation.

3.2.3.4 Inserting pyromorphite

After two months of growing, the grass had established a well-developed root system. The plexi glass window was opened and 16 small dialysis membrane-bags (Spectra/Por 1. WVCO 6000 to 8000, product # 132645) filled with 0.5g of pyromorphite and soil, on a 1:10 weight basis, was inserted, (see figure 2). Using tweezers, roots were carefully placed into the membrane-bag together with the soil mixture. The number of replicates is seen in table 1.

Table 1. Treatments in the green house experiment including number of replicates (n). All membranes contained a 1:10 pyromorphite/soil mixture.

Vegetation in the rhizotron	**Treatment**	**n**
Grass	Pyromorphite + soil + roots	8
No grass	Pyromorphite + soil (no roots)	5

3.2.3.5. Sampling and analysis

Almost two months later, the rhizotrons were again opened and the membranes taken out. For each of the membranes, an inner portion and an outer portion were sampled, (see figure 3). Control bulk soil and roots were also collected. The exchangeable fraction of lead was extracted by adding 1M $MgCl_2$ to air-dried sampled soil (sieved <2mm), 10ml/g soil. The solutions were mixed for 1 hour without heat and thereafter centrifuged at 3670 RPM. Roots were air dried and thereafter sonicated in order to obtain the soil root interface (SRI) fraction. Pb-concentration was determined for the exchangeable fraction with an inductively coupled plasma analyzer (ICP). The sampled soil was studied with XRD and SEM to analyze if any changes had occurred. SEM images of roots were taken.

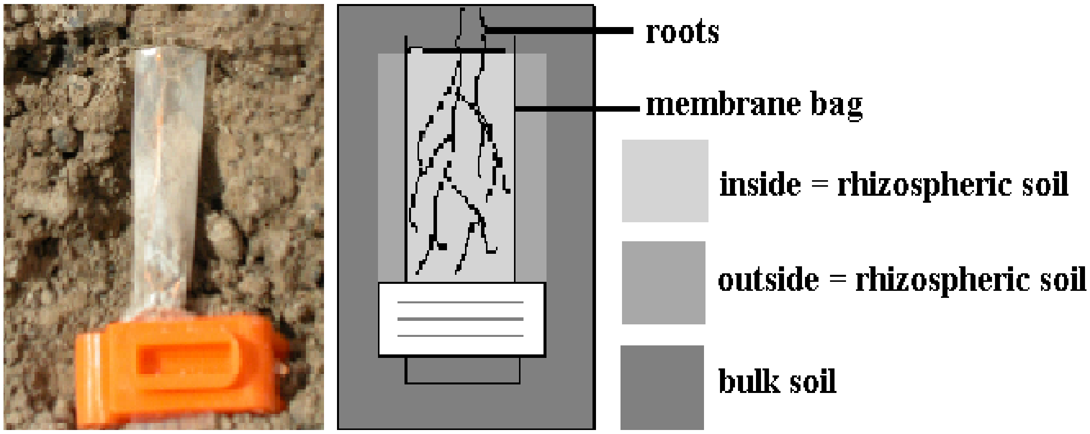

Figure 3. Dialysis membrane filled with a pyromorphite/soil mixture and roots from Smooth brome grass (left). A schematic picture of the dialysis membrane and its surrounding soil portions (right).

4. RESULTS AND DISCUSSION

4.1 Literature study

4.1.1 Lead contamination

4.1.1.1 Background, Sources and Fate in the Environment

Pb is naturally present in the environment and depending on the geophysical characteristics of the site, total Pb concentration in soils ranges from 10 to 200 ppm (Chlopecka and Adriano, 1997). However, elevated levels of Pb are also found in soils due to anthropogenic influences. Two major historical events that influenced the amount of anthropogenic Pb in the environment was the introduction of leaded gasoline 1920's and the usage paints containing Pb. Although most countries have phased out leaded-gasoline and Pb-based paint, there are still large amounts of Pb released to the environment. Today, Pb is used for batteries (63%), pigments (12%), gasoline additives (2.2%) etc. (Schroder et al., 2004). Pb is introduced in the environment from non-point sources such as above-mentioned products as well as from point sources such as smelters and mining activities (Chen et al., 2003). Very high contamination of Pb can be expected in areas with Pb-related activities such as smelters or roads (Markus and McBratney, 2001). There is no gaseous Pb phase at low temperatures, so Pb released to the atmosphere will migrate on airborne particles until they eventually settle to the ground. The metal will be partitioned between the atmosphere, soil particles, soil water, and organisms, depending on its state and the conditions. The persistence of Pb in the environment is very long because metals such as Pb do not degrade further than their elemental state.

Pb interacts strongly with the soil. In soils, Pb is distributed between different phases. It can be found in minerals such as galena (PbS), anglesite ($PbSO_4$), cerussite ($PbCO_3$), and as oxides (e.g., litharge, PbO), adsorbed to Fe and Mn (hydr)oxides, in solution, associated with organic matter or in less soluble fractions, such as Pb-phosphates. Pb oxides, sulfates, carbonates and organic fractions are all relatively soluble in water, and can pose a threat to both the environment and human health (Traina and Laperche, 1999). These soluble Pb compounds are also called non-residual. Adsorbed Pb-compounds such as on Fe and Mn-oxides and Pb-phosphates are less soluble and are referred to as the residual fraction (Cao et al., 2002). The solubility of these residual fractions decreases according to their solubility product constant (K_{sp}) and follows the trend PbO > $PbSO_4$ > $PbCO_3$ > $Pb_5(PO_4)_3X$ (Traina and Laperche, 1999). Most movement of soil-Pb occurs in the topsoil, and downward leakage is not very likely to take place (Chlopecka and Adriano, 1997). The chemical form of any metal governs the bioavailability of metals to organisms and plants (Markus and McBratney, 2001). Pb uptake and transport from soil to plants normally occurs through the solution phase. This makes soils with a large fraction of extractable Pb more environmentally hazardous than soils containing large part of residual Pb fractions (Chen et al., 2003). Therefore, the total metal content of a soil is a not a good measurement of its toxicity.

4.1.1.2 Toxicity, human health aspects and exposure.

Pb is a non-essential element with no known function in the body. The United States Environmental Protection Agency (US-EPA) considers Pb as the second most important hazardous substance (Chen et al., 2003). Chronic exposure of Pb damages the central nervous system, which can cause behavioral changes and impair intelligence. Other effects of Pb exposure are interrupted heme production and impaired hearing. Exposure to Pb depends on several factors such as occupation, age, behavior, diet and proximity to Pb sources. People living close to Pb "hot-spots" are at a higher risk. Children are prone to exposure due to their so-called "hand-to-mouth"-activities (eating soil) and a high intake of food in proportion to their body weight. Around the world, surveys have been conducted to determine human exposure to Pb (Markus and McBratney, 2001) and in the US 12 million children are considered to be in the risk zone (Mielke et al., 1998). Blood Pb-level is the most commonly used biomarker for Pb exposure, and there is for example a strong correlation between blood Pb and exposure to Pb-gasoline (Markus and McBratney, 2001).

The bioavailability of solid-Pb in the human body will depend on its stability. The stability of a compound *in vivo* will be different to the stability in a natural environment. If the compound dissolves in the body it will enter the bloodstream and either accumulate in tissues or be removed from the body by different detoxification systems (Traina and

Laperche, 1999). In the human body, organic acids work as chelating agents. Therefore chelators such as EDTA are used in medical treatment of Pb-poisoning cases (Scheckel and Ryan 2002b).

4.1.1.3 Regulations and remediation

Proper remediation procedures of Pb-contaminated soils are important because of the health risk associated with Pb contamination. Policies and regulations regarding Pb-contaminated soils vary between countries. Often, the acceptable levels of Pb depend on the land-use. For example, lower levels are accepted in residential areas. US-EPA has 400 mg/kg soil for residential soil and 1000 mg/kg soil for industrial sites as remediation goal, whereas Australia has 300mg/kg soil and 1500mg/kg respectively (Markus and McBratney, 2001). These are values of total amount Pb. This measure is not always the best for estimation of the environmental risk. Therefore, many countries have also regulatory limit for extractable Pb. In the US, the limit is 5 mg/l (Chen et al., 2003). There are two major soil remediation techniques; decontamination and stabilization. Both have in-situ as well as ex-situ applications. An example of an ex-situ method is excavation followed by chemical or physical treatment. Containment and soil flushing are examples of in-situ methods. Most of the existing remediation methods are very costly (see table 2) and/or have a large impact on the landscape (Cotter-Howells, 1996). Around the world, there is a great need of remediation of contaminated soils. Unfortunately, often there is not enough money and/or technology to ensure that the public health is not threatened. Therefore, there is a great need for less costly (and more ecologically friendly) methods. Currently, several new in situ remediation techniques are being investigated such as phytoremediation and chemical immobilization. Phytoremediaton is done by growing plants known for accumulating the contaminant in their tissue, on contaminated soil. The plants are harvested after the growing period. An example of chemical immobilization is to immobilize Pb by adding phosphorous. The phosphate is added with the goal to decrease the solubility of the contaminant. Chemical immobilization of Pb by phosphorous will be reviewed in this paper. A focus will be put on how rhizospheric processes affect the stability of the reaction products.

Table 2. Estimated cost for various remediation alternatives for 1 hectare Pb-contaminated soil (a contamination grade of 0.2 % w/w Pb is used for all alternatives except for phytoremediation with 0.14% w/w pb). (source: Hettiarachchi and Pierzynski, 2004

Method		Cost (USD)
Site decontamination		
	Off site	1,600,000
	Soil washing	790,000
	Phytoextraction	279,000
Site stabilization		
	Asphalt capping	160,000
	Soil capping	130,000
	In situ stabilization	60,000

4.1.2 Pb immobilization by phosphate

4.1.2.1 The Remediation Method

The objective of chemical immobilizing of metal contaminated soil is to transform soluble phases to less soluble phases. By adding phosphate to a Pb-contaminated soil, soluble Pb-phases will precipitate to more stable Pb-phosphates such as pyromorphite. This reduces both the availability and mobility of Pb. These compounds are almost insoluble and geochemical stable over a wide pH range (Hettiarachchi and Pierzynski, 2004). Extended research has been performed on the formation as well as the mobility and bioavailability of Pb-phosphates (Arnich et al., 2002, Bautier et al., 2001, Brown et al., 2004, Cao et al., 2003, Chen et al., 2003, Chlopecka and Adriano, 1997, Cotter-Howells, 1996a, Cotter-Howells, 1996b, Eusden et al., 2002, Laperache, 1996, Laperache, 1997, Ma, 1994, Melamed et al., 2003, Sayer, 1999, Scheckel and Ryan, 2002a, Scheckel and Ryan, 2002b, Yang et al., 2001, Zhu et al., 2003).

4.1.2.2 Formation of Pyromorphite

Pyromorphite is a Pb-phosphate with the molecular formula $Pb_5(PO_4)_3X$ where X = F, Cl, Br or OH. It is part of the apatite family and is coordinated in a hexagonal crystal system. The crystals are small and round, about 1µm in diameter, see figure 4. The main formation mechanism for pyromorphite from hydroxyapatite (HA) is by dissolution followed by precipitation (Melamed et al., 2003);

$Ca_{10}(PO_4)_6(OH)_2 + 14H^+ \rightarrow 10Ca^{2+} + 6H_2PO_4^- + 2H_2O$ (1)

$10Pb^{2+} + 6H_2PO_4- + 2H_2O \rightarrow Pb_{10}(PO_4)_6(OH)_2 + 14H^+$ (2)

Another possible formation route is by surface exchange, where Pb would absorb to the surface of the apatite particle and a cat ion-exchange occur (Arnich et al., 2002):

$Ca_{10}(PO_4)_6(OH)_2 + x\ Pb^{2+} \rightarrow Ca_{10-x}Pb_x(PO_4)_6\ x\ (OH)_2 + x\ Ca^{2+}$ (3)

These two reactions occur concurrently. Thermodynamically, it is expected that Pb contaminated soil will eventually form pyromorphite if sufficient P is available. In fact, naturally formed Pb-phosphates have been identified in mine waste (Eusden et al 2002), at industrial sites (Bautier et al., 2001), as well in urban and roadside soils (Cotter-Howells, 1996). However, under most environmental conditions, the solubility of Pb-solids is low and P-sources are scarce. Therefore the kinetics are slow and the reaction far from equilibrium. Natural formation of pyromorphite is enhanced by an acidic pH and a high organic carbon (OC) content in soil (Bautier et al., 2001) which both increase the dissolution rate of P- and Pb-sources in soil. The impact on already formed pyromorphite is less, due to the lower solubility compared to other Pb-sources. According to field sampling less than 2% of the total Pb in contaminated soil is found as Pb-phosphates (Cotter-Howells, 1996b). Since the formation of pyromorphite is dependent on sufficient amounts of P, P is added to contaminated sites to induce the conversion. A number of different P-sources have been tested for this purpose, where hydroxyapatite (HA) ($Ca_5(PO_4)_3OH$) , phosphoric acid (H_3PO_4) and natural phosphate rock (PR) are the most commonly used. H_3PO_4, is a relatively weak acid with $pK_{a1} = 2.12$, $pK_{a2} = 7.21$ and $pK_{a3} = 12.67$ (Lide, 1993). HA can be found in nature in phosphate rock and in bones and it is also industrially manufactured. Its solubility in water is low. The dissolution of HA increases with decreasing pH:

$Ca_5(PO_4)_3OH + 7H_3O^+ \leftrightarrow 3H_2PO_4^- + 5Ca^{2+} + 8H_2O$ (4)

(PR) is a general term for rock that is rich in phosphate. Most PR is made of apatite-like minerals (Hinsinger, 2001).

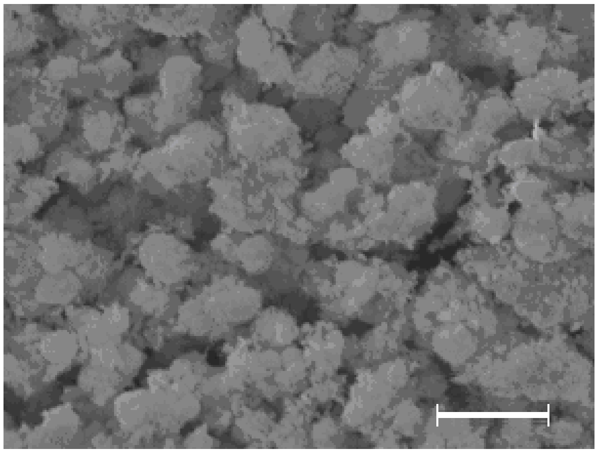

Figure 4. SEM micrographs of pyromorphite crystals. The scale bar represents ca 5 µm. (Source. my own experiments)

4.1.2.3 Dissolution of Pyromorphite

Pyromorphite dissolves according to the following reaction:

$$Pb_5(PO_4)_3Cl + 6H^+ \leftrightarrow 5Pb^{2+} + 3H_2PO_4^- + Cl^- \qquad (5)$$

The reaction is driven to the right when the pH is low and/or when PO_4^- or Pb^{2+} is removed from the solution (Laperche et al., 1997). Several different solubility products (K_{sp}) can be found in literature. For example, Scheckel and Ryan (2002a) reported a $K_{sp}= 10^{-25.05}$, for pH 2.12-7.21 which includes most environmental conditions. K_{sp} within the pyromorphite-group vary according the following scheme (Traina and Laperche, 1999);

$Pb_5(PO_4)_3F > Pb_5(PO_4)_3OH > Pb_5(PO_4)_3Br > Pb_5(PO_4)_3Cl$

4.1.2.4 Experiments conducted on pyromorphite formation and dissolution.

Formation, identification and stability of Pb-phosphates have been studied quantitatively and qualitatively. A decreased (or increased) amount of Pb in the aqueous solution phase has been used as a measure of efficiency of an amendment. The effectiveness in terms of change from non-residual to residual Pb-fractions in soil has been extensively studied with sequential extraction, SE (Cao et al., 2002, Chen et al., 2003, Zhu et al., 2003, Chlopecka and Adriano, 1997, Melamed et al., 2003). The method separate five or six soil fractions; (water soluble), exchangeable, bound to carbonates, bound to Fe and Mn oxides, bound to organic matter and residual (Melamed et al., 2003). A third approach to estimate the efficiency of decreasing solubility of Pb in soil is by a Toxicity

Characteristic Leaching Procedure (TCLP) that simulates the leaching process a waste will undergo in a landfill. Yet, a reduction is of Pb in a sample is not equivalent with pyromorphite formation and thus has to be verified by other methods. Pyromorphite can be identified by studying the reaction product with scanning electron microscopy (SEM) (shows surface morphology), energy dispersive x-ray spectrometer (EDX) (specifies what elements are present) and/or X-ray diffractometry (XRD) (determines what minerals are present in a sample).

In the lab, pyromorphite have been formed by reaction of aqueous Pb and different P-sources. P-sources tested are; H_3PO_4 (Scheckel and Ryan, 2002a), H_3PO_4 containing soft-drinks (Scheckel and Ryan, 2002b) and HA (Ma, 1996, Arnich et al., 2002). All experiments resulted in reductions of aqueous Pb up to 99% (Arnich et al., 2002). Presence of different ions; N, K, Ca and calcite (Ma, 1996) and salts; NaCl and $NaClO_4$ (Arnich et al., 2002) has been shown to not affect the immobilization. However, EDTA has been shown to significantly reduce the formation of Pb-phosphates (Ma, 1996). A higher ratio P/Pb enhances the reduction. The thermostability of pyromorphite increases with increased aging time up to about 24h, increasing time thereafter is not significant. However, during aging there is no difference in the chemistry or crystallinity can be seen(Scheckel and Ryan, 2002a). In the field, most Pb is present in solid-phases (Laperche et al., 1996). Thus, reactions between P and different solid-phases of Pb have been conducted in the lab as well in the field. Pyromorphite has been formed from the lead-minerals PbO and $PbCO_3$ and HA (Laperche et al., 1996), Pb-based paint and soft-drink derived H_3PO_4 (Scheckel and Ryan, 2002b). Contaminated soil with various origin has been treated with HA (Laperche et al., 1996, Chen et al., 2003, Ma, 1996, Zhu et al., 2003, Chlopecka and Adriano, 1997), H_3PO_4 (Yang et al., 2001, Eusden et al., 2002, Chen et al., 2003, Melamed et al., 2003, Cao et al., 2002), PR (Zhu et al., 2003, Chen et al 2003, Melamed et al., 2003, Cao et al., 2002), NaH_2PO_4 (Cotter-Howell, 1996a), and $Ca(H_2PO_4)_2$ (Chen et al., 2003, Melamed et al., 2003, Cao et al., 2002). The formation of pyromorphite has been reported to be pH dependent (Laperche, 1996) where pH 5 is to prefer rather than pH 6 or 7. The residual fraction of Pb increased and the exchangeable fraction decreased after a P amendment in all studies (Zhu et al., 2003, Chen et al., 2003, Melamed et al., 2003, Cao et al., 2002, Chlopecka and Adriano, 1997). Three of the studies were long-term field-studies (Chen et al., 2003, Melamed et al., 2003, Cao et al., 2002). These were conducted under similar conditions. The soil was first acidified with H_3PO_4 and then H_3PO_4 (T1) $Ca(H_2PO_4)_2$ (T2), and PR (T3) was added. Pyromorphite was identified for all treatment after 330 days. Cao (2002) found the greatest amount pyromorphite for T3, possibly due to long-term supply of P form PR. In two of the experiments PR was the most efficient treatment in converting non-residual fractions to the residual fraction (Chen et al., 2003, Cao et al., 2002), whereas $Ca(H_2PO_4)_2$ was most effective in the third (Melamed et al., 2003). It was mainly carbonate bound Pb that converted into residual Pb. The effect decreased further down in the soil profile. TCLP studies showed that the elevated levels in the topsoil decreased to below the EPA-value for all treatments (Chen et al 2003, Melamed et al 2003). The highest TCLP-Pb was found 10-20 cm down in the soil profile. All treatments reduced the contamination grade throughout the soil profile, but only $Ca(H_2PO_4)_2$ in Melamed et al. (2003)'s experiment managed to reduce the whole soil profile to below the EPA critical value (5mg/l). Although, these results indicate that the remediation method has a potential to work on a long-term basis. Zhu et al. (2003) compared the effect of PR, HA+SSP (single super phosphate, a common fertilizer) and HA on contaminated soil in field. HA was most efficient followed by PR and HA+SSP.

The use of P-amendments on Pb-contaminated soil evidently boosts the formation of Pb-phosphates, which is shown by the increase pf Pb in the residual fractions. Although different experiments suggest different types of P-amendments, the results definitely show that chemical immobilization of Pb by P is a promising remediation alternative. However, it is important to remember that the type of contamination, P-amendment, soil, experimental design etc. affect the results of the remediation.

4.1.3.0. Availability of pyromorphite

The availability of a metal is dependent on the organism. Therefore it is necessary to use methods specific to the type of target-group in question. In this literature study, availability of Pb has been divided into the subgroups; bioaccessibility, bioavailability and phytoavailablity.

4.1.3.1. Bioaccessibility

The bioaccessibility refers to human uptake. It is the soluble fraction of an element in the gut. Bioaccessibility of pyromorphite has been studied with physiologically based extraction tests (PBET) (Yang et al., 2001) and in vitro gastrointestinal (IVG) method (Schroder et al., 2004). These *in vitro* tests aim to mimic the human body by simulating the stomach and the small intestine of a young child, (the main target organism for lead poisoning). Besides from using the correct pH, temperature, residence time and chemistry, it includes mixing. An IVG study also includes food. Simple types of PBET, measuring the extractable Pb at a pH simulating the stomach and the human GI-system respectively, have also been conducted (Scheckel and Ryan 2002b, Brown et al., 2004). PBET studies have shown that H_3PO_4 reduced the bioaccessible Pb significantly in an aqueous solution (Scheckel and Ryan 2002) and for contaminated soil (Brown et al., 2004, Yang et al., 2001). Increased amounts of acid added, as well as increased aging time, reduced the amount of bioaccessible Pb (Yang et al., 2001). The IVG method performed by Brown et.al (2004) showed that contaminated rock treated with PR resulted in higher concentration of bioaccessible Pb compared to H_3PO_4.

4.1.3.2 Bioavailability

Bioavailability is to what extent a contaminant will be assimilated within the body. Since, *in vitro* studies can't completely predict the bioavailability in human, *in vivo* studies (e.g. animal testing), though expensive and controversial, are considered necessary.
Rats fed with H_3PO_4 treated contaminated soil (Brown et al., 2004) confirmed that the presence of phosphoric acid reduced the overall uptake with 26%. Tissue absorption was used as a bioassay. HA's potential to reduce the absorption of Pb from $PbCl_2$ was tested by two different experiments. In the first rats were fed with a pyromorphite solution and the uptake was measured. In the second experiment a pyromorphite solution was pumped through rat tissue (Arnich et al., 2002). The first experiment showed that the treatment reduced the uptake with 63%. HA neither affected absorption nor the concentration in the blood for the rats being pumped with solution.

4.1.3.3 Phytoavailability

The fraction of a contaminant available for plant uptake is referred to as the phytoavailable fraction. This is generally measured by the uptake by plants grown on contaminated soils. Several research groups have studied the uptake of Pb in plants after treated with P in the greenhouse (Chlopecka and Adriano, 1997, Laperche et al., 1997, Zhu et al., 2003) or in field studies (Brown et al., 2004, Cao et al., 2002). All studies concluded that the Pb concentrations in shoot decrease with a P-amendment. However, the results are inconsistent whether the concentration in roots increases or decreases after a P addition. In plants, Pb accumulates in roots (Chlopecka and Adriano, 1997) and Pb-phosphates have been identified on the root-surfaces. This Pb-phosphate formation is thought to further inhibit a translocation from root to shoot. Apatite was more effective in reducing the uptake than a natural phosphate rock (PR). The amount apatite added and the decrease in Pb in the above ground tissue was correlated linearly (Laperche et al., 1997). Ground PR was two times as effective in reducing Pb uptake compared to ungrounded PR, which is consistent with that dissolution of a mineral is a function of surface area exposed to the solution (Brandt et al., 2003). In another experiment, H_3PO_4 and $Ca(H_2(PO_4)_2H_2O$ reduced Pb in shoot tissue, whereas plants grown with PR as P-amendment had the same plant Pb/soil Pb ratio as plants grown on control soil (Brown et al., 2004). The exchangeable fraction of a soil can be considered as an equivalent to the available fraction for plants. Although, Brown et al. (2004) found that the correlation between plant Pb and extractable soil Pb was only $r = 0.18$. This underlines that precautions has to be taken when comparing different dataset with each other.

4.1.4. The impact of rhizospheric processes on the stability of pyromorphite.

4.1.4.1 P-deficiency in soil

Phosphorous (P) is, after nitrogen, the most important element for plant growth. It is used to synthesize essential cell components such as DNA/RNA, phospholipids and ATP Plants mainly take up P as phosphate ions (PO_4^{3-}) but also to some extent as organic-P. In soil, most P is found in minerals such as apatite, a form that is unavailable to plants. Therefore, many plants modify the soil to enhance the dissolution of the unavailable soil-P. These processes occur in the fraction of the soil surrounding the root that is called the rhizosphere (Gobran and Stephen, 1996). The rhizosphere soil shows, due to the large influence of root activities, a significantly different environment compared to the bulk soil. This buffer zone helps the ecosystem to withstand stresses, such as nutrient deficiencies. Plants under P-stress may change the chemical equilibrium of the dissolution reaction by either take up PO_4^{3-} or decrease the pH in the soil. Both these reactions will drive the dissolution of P-minerals to the right, (see equation 4, p15). Another possible action is to facilitate biological weathering by providing better living conditions for mycorrhiza whose hypae can extend to “unreachable”-soil (Jones, 1998). Finally the plants can exude different types of root exudates that will enhance the dissolution.

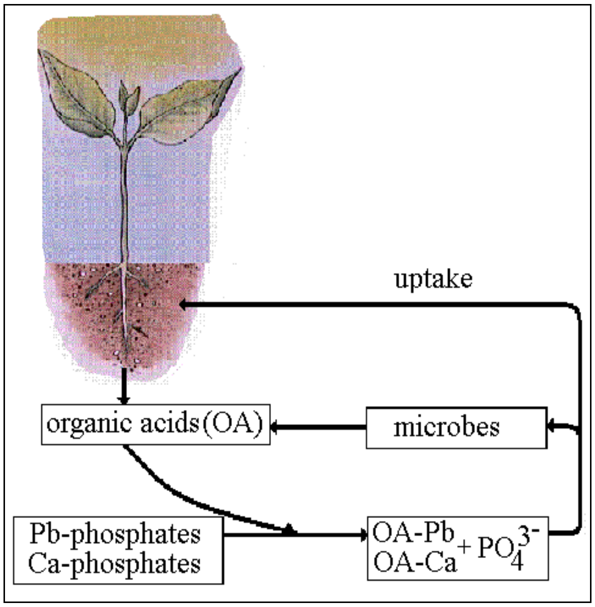

Figure 5 How organic acids work as complex binders and thereby effect the phosphate availability in soil. (Source: Dakora and Donalds, 2002)

Several plants have been reported to release organic acids under P-stress. Due to the pH in the cytosol and in the soil environment, it is organic anions and not acids that are released from the roots, see pKa values (see table 3) (Ryan et.al. 2001). The anions travel across the cell membrane to the soil where the reactions occur. The reactions that occur in the soil are not well understood. The main mechanisms for dissolution of P-minerals by organic anions are by replacing P in the mineral structure and forming strong complexes with the metal ions (see figure 5). These two reactions occur concurrently, but the second mechanism is dominant (Dakora and Donald, 2002). Citrate, malate and oxalate are examples of anions experimentally found in soil. The concentrations are in the µM range. In soil, their persistence varies from 0.5 to 6 hours.

It has been shown that root exudation varies along the length of the root as well as with plant species, age and soil type where plants grown on calcareous soils tend to exude more organic anions than plants growing on soils with a lower pH.

Table 3 Data of common organic anions exudated from plants under P-deficiency. pKa-values, the dominant form at pH 7 and Field concentrations in µM (source van Hees et.al., 2003).

Type of acid	**Formula**	**pKa**	**Dominant form**	**Field conc.**
Citric acid	$C_6H_8O_7$	3.06 / 4.74 / 5.4	Citrate^{3-}	9.3
Malic acid	$C_4H_6O_5$	3.4 / 5.05	Malate^{2-}	0.3
Oxalic acid	$C_2H_4O_2$	1.19 / 4.21	Oxalate^{2-}	6.9

Table 4. Formation constants between organic anions and Ca^{2+} (source Ryan et.al., 2001).

Metal	**Citrate^{3-}**	**Citrate^{2-}**	**Oxalate^{2-}**	**Malate^{2-}**
Ca^{2+}	4.68	-	3.0	2.66

4.1.4.2 Organic acid complexes with soil metals.

Organic anions form strong complexes with Ca^{2+}, see table 4. The formation constant describes the affinity of the acid for Ca^{2+}. Organic anions do not only form strong complexes with Ca^{2+} and other common soil cations, also heavy metals can be chelated. This put forward a concern about the plants ability to dissolve Pb-phosphates. The tricarboxylate citrate^{3-} chelates more strongly than the dicarboxylates malate^{2-} and oxalate^{2-}. The acids can form several different complexes with lead. The dissolution is pH dependent as well, and is governed by the pK_a values of the acid (see table 2). This intricate system of complexes makes it difficult to compare log K values of the organic acids directly. The following log K values for complexes with oxalate and citrate are available in the literature (Puigdomenech, 2001; Stumm and Morgan, 1996);

$$Pb^{2+} + Ox^{2-} \leftrightarrow PbOx \qquad \log K = 5.42 \tag{6}$$

$$Pb^{2+} + 2Ox^{2-} \leftrightarrow Pb(Ox)_2^{\ 2-} \qquad \log K = 7.55 \tag{7}$$

$$Pb^{2+} + Cit^{3-} \leftrightarrow PbCit^{-} \qquad \log K = 5.4 \tag{8}$$

$$Pb^{2+} + 2Cit^{3-} \leftrightarrow Pb(Cit)_2^{\ 4-} \qquad \log K = 8.1 \tag{9}$$

Although, it is likely that mM-concentrations are required to effectively solubilize P (Ryan et. al., 2001).

4.1.4.3 Current research on how plants affect Pb-phosphates.

Researchers have been concerned whether plants can affect the long-term stability of pyromorphite in the field. This problem has been studied both directly and indirectly. Ma (1996) simulated the P-sink of a plant by placing an anion-resin in a dissolution batch. The results showed that the dissolution of pyromorphite slightly increased when P was removed from the solution, though not to levels where pyromorphite could be considered unstable. Microorganisms have been reported to produce exudates that can precipitate metals (Gadd, 2000). Sayer et al. (1999) showed that the fungus *Aspergillus niger* could dissolve pyromorphite when grown on agar with pyromorphite as only P-source. The fungus utilized the phosphate and formed Pb-oxalate-dihydrate and Pb-carbonates, from the excess Pb. However, in a natural environment, *A.niger* would most likely use more soluble P-sources if they were available. Grass grown on sand, containing no P, mixed with pyromorphite had up to 30 times higher concentration of Pb in their shoot tissue compared to the control (Sayer et al., 1999, Laperche et al., 1997). Laperche demonstrated that if apatite was added to the mixture, the shoot-concentration reduced significantly. These results suggest that a P-deficiency can promote dissolution of pyromorphite, but if other more soluble P-sources such as apatite are available, the stability of pyromorphite remains. Finally, it has been showed that the biochemical actions of the roots can not only dissolve pyromorphite, but also induce pyromorphite formation (Cotter-Howells, 1996a). Grass was grown in a contaminated soil mixed with peat. XRD and SEM analyses verified that Pb-phosphates were present in the rhizosphere soil whereas they were absent in the control soil.

Thus, all data suggest that plants and microorganisms have the capability to induce pyromorphite dissolution under P-deficiency. To what extent this occur in a natural environment has yet to be shown. It seems likely that if sufficient amount of other more soluble P-sources are present, plant will not use pyromorphite as a P source. However, these findings suggest that phosphate-remediated soil must be monitored to ensure that enough P is present in the soil.

4.1.5 Advantages and disadvantages of different types of P-sources

In terms of immobilizing Pb from soil, phosphate has a positive impact on the environment. However, the addition can be detrimental in other aspects. The main hazard is that P is lost by runoff or leaching, which can cause eutrophication. The retention and P distribution in the soil-profile has been demonstrated by Chen et al. (2003), Melamed et al. (2003) and Cao et al. (2002) who compared PR, H_3PO_4 and $Ca(H_2PO_4)$ in soil. The amount leached was 6-14%. PR leached the least amount of P while H_3PO_4 leached the most. Soil pH (Cao et al., 2002) was reduced for all treatments. The greatest pH decrease was for H_3PO_4 and the least for PR. Another experiment showed that H_3PO_4 added to a soil caused a pH decrease of 3 units (Yang et al., 2001). A decrease in soil-pH can dissolve other heavy metals that don't bind as strongly to phosphate and may also increase the mobility of P. These adverse effects can lead to groundwater contamination and more toxic soils. In many experiments, phosphoric acid has been the most efficient amendment in converting soil-Pb to pyromorphite. However, it has also been shown to cause the largest pH reductions and P losses. PR affects the environment least in terms of pH reduction and P retention capacity. From long-term studies, the results with PR have been very positive. This is due to the slow but constant release of P.

4.2 Laboratory studies

4.2.1 Formation of pyromorphite

When H_3PO_4 and $PbCl_2$ as well as $PbNO_3$ and H_3PO_4 were mixed, a white powder precipitated immediately. The same phenomenon occurred when a Pb-source reacted with a natural phosphate rock (hydroxyapatite). The white powder mixed with the rock. All precipitates were characterized by XRD and SEM. Figure 6 shows XRD patterns and SEM images for the reaction products of $PbCl_2$ reacted with H_3PO_4, and the natural hydroxyapatite respectively. Figure 6a and b indicated a large difference in XRD pattern. Figure 6c and d showed two significantly different crystals, which support that two different products were formed. Analysis of the XRD spectra suggested that chloropyromorphite was formed by the reaction between $PbCl_2$ and H_3PO_4, whereas hydroxypyromorphite was suggested when $PbNO_3$ was used as Pb-source. The reaction product from phosphate rock and $PbCl_2$ at pH 7 was determined to be a mixture of laurionite (PbOHCl) and phosgenite ($Pb_2Cl_2CO_3$). When the phosphate rock was dissolved in H_3PO_4 or the reaction occurred at a low pH (1 and 2), the reaction product

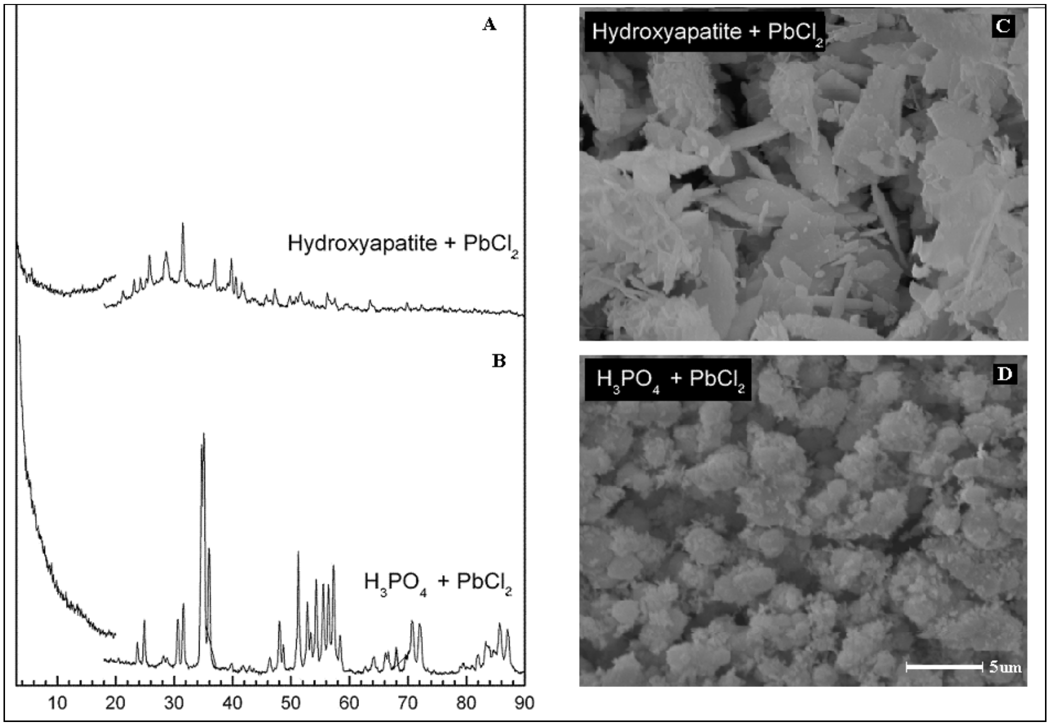

Figure 6. XRD spectra (A and B), and SEM images (C and D) for the reaction products between hydroxyapatite and $PbCl_2$ (A and C) and H_3PO_4 and $PbCl_2$ (B and D).

was identified as hydroxypyromorphite. Figure 6d showed that chloropyromorphite crystals were round and small, about 1µm in diameter.

Laperche et al. (1996) detected a pyromorphite peak when hydroxyapatite was reacted with enriched Pb-soil samples at pH 5, but not at pH 7.7. He could not exclude a possible co-precipitation of other metals. The crystals formed were formed as small needles. They measured ca 0.2µm · 2µm that were quite different from the crystals formed in our experiment. Arnich et al. (2003) formed almost pure pyromorphite from synthetic hydroxyapatite and $PbCl_2$ at a weight ratio of 10:1 at pH 5.3-6.5. At a weight ratio 5:1, a mixture of calcium and Pb-phosphate formed. Their SEM image for the reaction product (10:1) was very similar to our pyromorphite formed from H_3PO_4. The reason for our failure in forming pyromorphite from hydroxyapatite at higher pH, was most likely due to the low solubility of the mineral. This resulted in too low concentrations in available phosphate to satisfy the need for pyromorphite formation. Since the rate of dissolution is linearly correlated to the surface area, the large crystals of our hydroxyapatite mineral contributed to the low solubility (Brandt et al., 2003).

4.2.2 Pyromorphite dissolution by organic acids.

Previously experiments on weathering by organic acids have often been performed at unnaturally high levels. The concentrations of organic acids used in my experiment belonged to a range that can be expected in rhizospheric soil (Dakora and Donald, 2002, Jones, 1998). This experimental set-up was considered to mimic the natural environment the most. As expected, the pH was lowest for the highest concentration of acid used in the flasks (see table 5). The pH increased about 0.5 units in a day. Because of problems with the pH-meter, the pH was only measured the first two days.

Table 5. Average pH for various concentrations of organic acids in the dissolution flasks day 1 and day 2

Acid concentration	Day 1	Day 2
0 µM	4.8	5.3
20 µM	4.4	4.9
40 µM	4.0	4.6

In my experiment, the dissolution of pyromorphite was measured as the release of Pb-ions from pyromorphite. The concentration Pb in the sampled fractions was determined by ICP. The dissolution of pyromorphite in the presence of citric acid up to day 20 is shown in figure 7. The dissolution reached equilibrium for each concentration within a day. The data supported the theory that solubility increases with increased concentration. However, the dissolution of pyromorphite rises and falls slightly throughout the experiment. The same pattern could be seen for all tested acids.

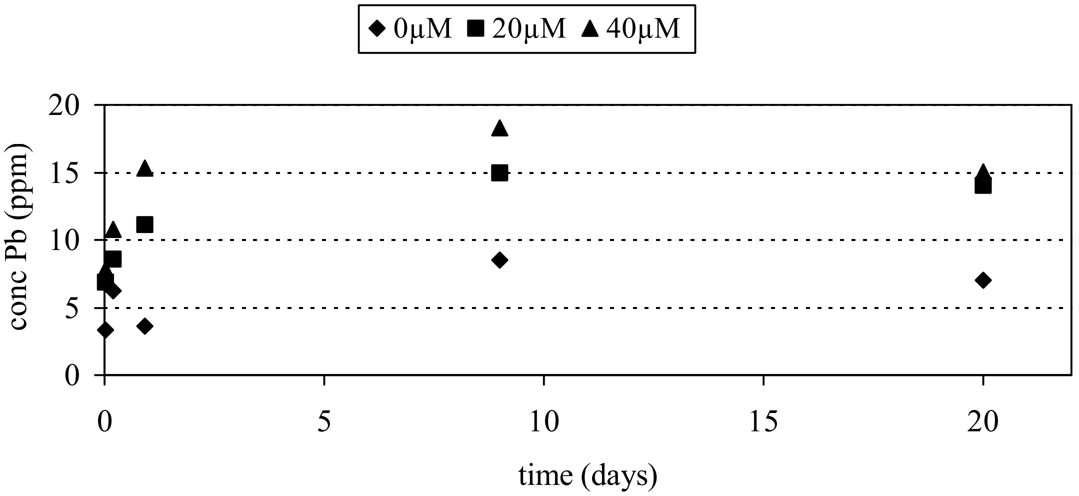

Figure 7. Concentration of Pb in solutions of pyromorphite dissolved in 0, 20 and 40 µM citric acid at different times.

The solubility of pyromorphite in presence of various organic acids at concentrations of 20 µM and 40 µM is shown in figure 8 and 9. The amount of dissolved Pb was dependent on the type of acid used. Up to day 20, malic acid and citric acid had the strongest effect on pyromorphite dissolution whereas oxalic acid had a lower effect. All acids were more effective in dissolving pyromorphite compared to the control. After 20 days the concentration of Pb in the solution was 14 ppm when the pyromorphite was treated with 20µM citric acid or malic acid compared to 8 ppm for the control and almost 10 ppm for oxalic acid. At 40 µM, the concentration of Pb in the solution varied from 12 ppm (oxalic acid) to 20 ppm for malic acid. After 90 days, the trend was changing, and the concentration of Pb decreased. At 40 µM, only malic acid differed from water.

Jones (1998) reported citric acid as the most efficient organic acid in releasing P, and thereafter oxalic and malic acid. Log K values for complexes between citrate and oxalate see equation 6-9 (Stumm and Morgan, 1996 and Puigdomenech, 2001) suggested that citrate form somewhat stronger complexes with Pb than oxalate. Oxalic acid was supposed to have the lowest effect on the dissolution in terms of H^+ affinity, see pKa values (table 3). These types of comparisons are difficult to make due to the complicated system of complexes between Pb and organic anions as well as with H^+. However, from the results in our experiment we could conclude that oxalic acid had a somewhat lower influence on pyromorphite dissolution compared to citric- and malic acid. After 90 days neither of the concentrations of oxalic acid was significantly more effective in dissolving pyromorphite than the control.

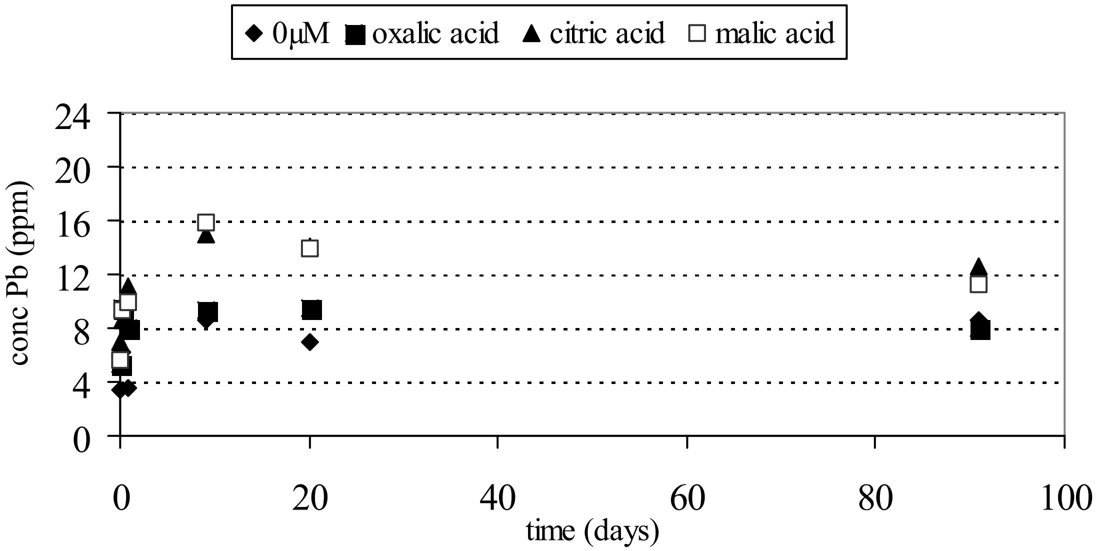

Figure 8. Concentration of Pb in solutions of pyromorphite dissolved in 0 µM and 20 µM citric-, malic and oxalic acid.

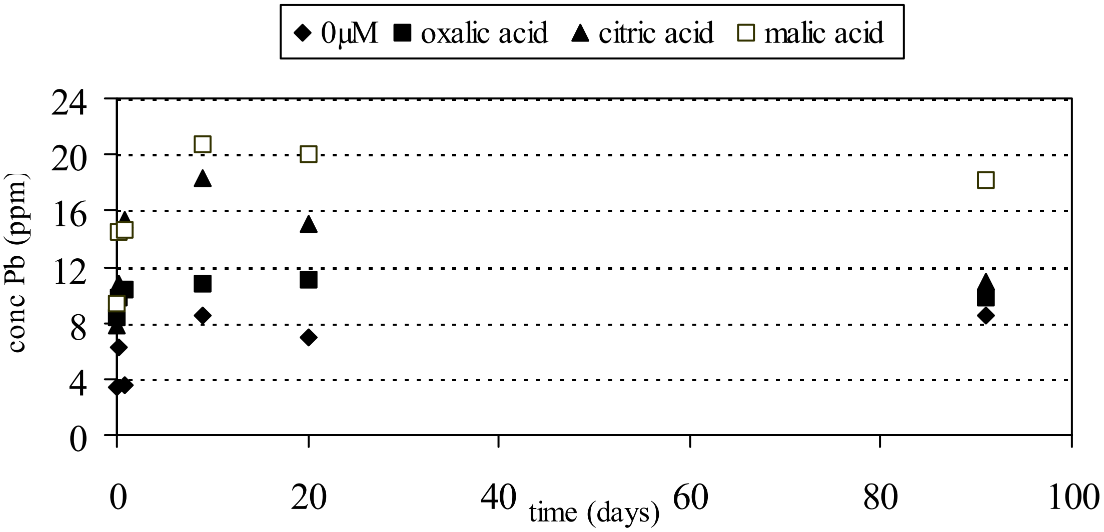

Figure 9. Concentration of Pb in solutions of pyromorphite dissolved in MilliQ-water (0µM acid) and 40 µM citric-, malic and oxalic acid.

Organic acids replace P in the mineral structure and/or forming complexes with the metal ions (Jones, 1998). SEM and XRD studies from the remaining pyromorphite did not show any changes in crystallinity or mineralogy compared with the start product. This implied that dissolution mechanism rather than a surface exchange has occurred. Ryan et al. (2001) suggested that mM concentrations of organic acids were required to increase the solubility of P. This explains why the organic acids in this experiment only have a slight effect on the dissolution of pyromorphite. I cannot explain why the concentration of Pb in solution is less after 20 (and 91) days compared to after 10 days. One possibility was due to aging of the pyromorphite.

4.2.2.1 Sources of error

A few analyzed samples had extreme values and were considered as outliers and excluded. These might be a result of that single pyromorphite crystals had managed to get through the filter. It would have been interesting to follow the pH change throughout the experiment. Due to problems with the pH probe it was only measured the first two days. Between the last and the second sampling occasion, the ICP was repaired. This might have affected the results. The concentration Pb in the last sample from the last sampling occasion for 40 µM citric acid was lower than expected and the only occasion when a higher acid concentration resulted in a lower release of Pb. Therefore it is to suspect that something went wrong during that sampling.

4.2.3 Dissolution of pyromorphite in the green house

Being the first experiment of its kind, the greenhouse experiment suffered quite a few "child-deceases". The grass did initially not grow well and showed thin, discolored stems, which is a sign of a nutrient deficiency. After a few amendments of N and P the grass seemed to recover. Average concentrations of Pb in the exctracted solutions from soil samples were determined (see table 5). Pb could not be detected in extracted solutions from bulk soil samples. The concentration inside the membranes was significantly larger than outside the membrane. This result was expected since the outside originally only consisted of pure soil. Roots or no roots on the inside of the membrane had no significant effect on the Pb concentration in the extracted solution. This was tested with a heteroscedastic, one-sided t-test (confidence level 95%). Outside the membrane, samples with roots in the inside had a significantly higher concentration of Pb. This could be interpreted in two ways. Either action of roots has increased the dissolution of pyromorphite and Pb-ions have traveled across the membrane to the outside and thereby increased the amount of lead on the outside. Anther possibility is that the increased concentration of Pb on the outside is due to experimental deficiencies. Inserting the pyromorphite/soil to membranes without roots resulted in no spill on the outside. When roots were included, on the other hand, the membranes became "bouncier" and more soil-mixture was lost.

Table 5. Average concentration. of Pb (ppm) in extracted solutions from soil samples (± standard error)

Treatment	Sample location	Conc. Pb
Pyromorphite/soil		
	Inside the membrane	117 (14.3)
	Outside the membrane	0.5 (0.1)
Pyromorphite/soil + Roots		
	Inside the membrane	103 (7.7)
	Outside the membrane	1.6 (0.3)

SEM-pictures of the roots showed that pyromorphite had accumulated on the sonicated root-surface, see figure 10. Several researchers, among others Chlopecka and Adriano (1997) have also identified Pb-phosphates on root surfaces for plants growing in Pb-contaminated soil. If rhizospheric processes of roots dissolve pyromorphite it is to expect that new types of Pb-solids form. Reformation of pyromorphite is not very likely, especially in a P deficient environment. Neither studies with SEM or XRD could detect any chemical or morphological changes for pyromorphite in soil. Although XRD and SEM sometimes fail to detect minerals and differences, these results suggest that the impact of roots was low. Previous experiments (Sayer et al., 1999, Laperche et al., 1997) suggest that the rhizospheric processes plants under P deficiency have the capability of dissolving pyromorphite. When there is sufficient P in soil, plants will not use pyromorphite as a P source.

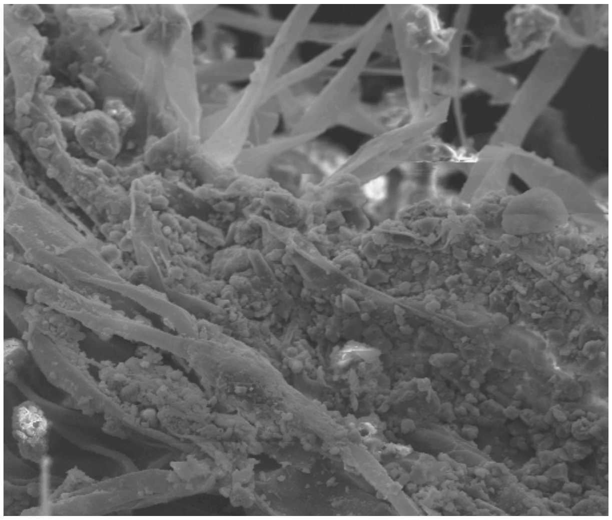

Figure 10. SEM image of pyromorphite accumulated on the root surface.

4.2.3.1 Sources of error

Since P was added to the rhizotron, it is possible that there might have been sufficient P in the soil so that the grass did not use the pyromorphite as a P-source.

Although the membranes were described as very persistent, quite a few did not withstand the processes in the rhizotron, see figure 11. These were of course not included in the results but the number of replicates was much fewer than wished than I hoped for. It was more complicated than expected to insert the pyromorphite/soil mixture to the membrane as well as collecting the soil samples afterwards. This resulted in inconsistencies that might have affected the results. The two months that the experiment lasted is short compared to a real field situation. Future experiments should consider alternative ways of measuring the dissolution of pyromorphite. Using rhizons might be one option.

Figure 11. Broken dialysis membrane.

5 CONCLUSIONS

5.1 Literature study

All living beings will continue to be exposed to Pb. The major health risk underlines the importance of remediation to reduce the exposure.

The in-situ stabilization of Pb by adding P has been proven as a possible remediation technique. There is always a possibility of Pb^{2+} release from residual Pb-fractions, if the environmental conditions change. Therefore, the long-term fate in field should be further investigated, particularly before it can be used for soils in residential areas. Rhizospheric processes can alter the stability of the reaction products, but as long as more soluble P-sources are available this is of less significance.

5.2 Laboratory studies.

The lab experiment showed that organic acids increased the solubility of pyromorphite. A higher concentration caused a higher dissolution. The type of organic acid used affected the solubility. Malic acid had the strongest effect on the solubility of pyromorphite. The greenhouse experiment could not confirm the impact of rhizospheric processes on the dissolution of pyromorphite in soil.

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7 ACKNOWLEDGEMENTS

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