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Evaluation of heavy metal availability prior to an *in situ* soil phytoremediation program

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Abstract

Metal mobility and the fractionation of elements and thus the biological uptake of Zn, Pb and Cd by plants were investigated using a simplified analytical procedure for soluble and bioavailable metals using a four-step sequential extraction procedure. Results showed that there was a low proportion of immediately soluble metals, as well as a high proportion of metals that could be released and would so be available for plant uptake. In the sequential extraction procedure, considering the total partition, there was a large proportion of Pb, Cd and Zn extracted in a readily mobile form. In acidic soils the content of metals in ready mobile form (exchangeable-bound to carbonates as well as to Fe and Mn oxides) and bound to organic matter constitutes an important source of potentially available elements. The same pattern was observed in alkaline soils, where almost 80% of the metals could be remobilized and be potentially available to plants. Knowing the metal partitioning and mobility of heavy metals it is very important for evaluating the phytoremediation efficiency.

Introduction

The enrichment of the soils and sediments of the mining district of Cartagena-La Unión in heavy metals constitutes a high risk of pollution in subsidiary areas. The health and environmental hazard of heavy metals depends on the partitioning and fractionation of trace elements in contaminated soils and sediments. The uptake and toxicity of many metals show marked dependence on speciation and these responses are often correlated with the activity of free metal ion (Knight & McGrath 1995; Laxen & Harrison 1981; Parker & Pedler 1997).

Sediments and polluted soils enriched in heavy metals are subjected to erosion, which increase the risks of pollution in the surrounding areas. Hazardous metals like Pb, Zn and Cd take part in the biogeochemical cycles and their mobility depends strongly on their specific chemical forms or different binding forms rather than total element concentration (Galán et al. 2003). In terms of the ecotoxicological aspects, it is necessary to differentiate between, for example, 500 ppm of total lead and 500 ppm of bioavailable lead.

Heavy metals are included in soil minerals, as well as bound to the different phases of soil particles by a variety of mechanisms; mainly absorption, ion exchange, co-precipitation and complexation (Navas & Lindhorfer 2003). To know of the binding of metals with the soil phases and components, is of major interest to assess the connections with other biotic and abiotic elements of the environment (Hirner 1992). The process of identifying and quantifying these different species of metals in a sample is referred to as speciation. Among the procedures to determine element speciation, those based on sequential extraction are the most widely used (Usero et al. 1998). The sequential extraction schemes are considered an essential tool in establishing element fractionation in soils (Gleyzes et al. 2002). Thus, the determination of the different ways of binding gives more information on trace metal mobility, as well as on their availability or toxicity, in comparison with the total element content (Tüzen 2003). The identification of main binding sites and phase associations of trace elements in soils helps in understanding geochemical processes in order to evaluate the remobilization potential and the induced risks. Speciation of the metals can help to assess how strongly they are retained in soil and how easily they may be released into soil solution (Kaasalainen & Yli-Halla 2003), and finally how they can affect environmental and human health.

The use of plants for the remediation of polluted soils may provide a good solution for metal stabilization and for minimizing erosion and associated risks. Phytoremediation has shown great potential as an alternative treatment for the remediation of heavy-metal-contaminated soils and groundwater (Chen & Cutright 2001). For phytoremediation purposes, it is essential to quantitatively determine the trace metals associated with each phase (Gómez Ariza et al. 2000a), to be able to assess the bioavailable fraction. More research regarding metal partitioning for evaluating the phytoremediation efficiency is needed.

This study considers the selectivity of a four-step sequential extraction procedure, based on the capacity of some extracting reagents to remove heavy metals retained from the geochemical phases, for metals mobility assessment. The main objective of this work was to establish the reactivity of metals in polluted soils in relation to the fractions of metals that are immobilized in the minerals or in another soil fraction. This process is needed to be understood prior to the phytoremediation program evaluation. It is essential because of the risks caused by these soils for the near populations and the agricultural and fishing zones that surround this mining area.

Materials and methods

Site description

Cartagena-La Union mountain, located on the east side of Murcia province in SE of the Iberian Peninsula, is a Mediterranean area with more than 2500 years of mining tradition; nevertheless this activity has been abandoned over the last few decades. Piles of mine waste from ancient times, as well as from more recent intensive ones from large-scale exploitations, are rich in various metals such as lead (Pb), iron (Fe) and zinc (Zn).

Samples were collected from an old mining area (110–0 m a.s.l.; 37°37′20″ N, 0°50′55″ W – 37°40′03″ N, 0°48′12″ W). This region typified as termomediterranean area shows temperature averages 18 °C throughout the year and an annual precipitation that ranges from 200 mm to 300 mm. Several anthropogenic soils profiles, strongly affected by heavy metals in mining ponds were studied. A total of 16 samples were extracted till an average depth of 30 cm.

The methodology used in this work to study the metal partitioning on mining soils and sediments has been specially designed for mining areas enriched in iron oxides and in heavy metals, and is thus ideal for this study. Another part of this work has been to evaluate the environmental risks associated to the above-mentioned polluted soils and sediments. For this purpose, a specialized bibliography has been consulted, and a new innovative approach has been developed to find the specific objectives of this work. It must be taken into consideration that metals are mainly associated with organic matter, carbonates, oxides and hydroxides, so these fractions must be evaluated. Electrical conductivity and pH are also parameters of interest.

Analytical procedures

Soil samples were air-dried and crushed to pass through a 2 mm sieve, homogenized and stored in plastic bags prior to laboratory analysis. Representative aliquots were ground in an electric agate mortar. Soil electrical conductivity (E.C., dS m^{-1}) and real pH values were determined using glass electrodes in a 1:1 soil:water paste. Potential pH values were also measured with potassium chloride solution (KCl 1M) (National Soil Survey Center 1996).

A simplified analytical procedure for sequential extraction that identifies those forms of heavy metals that are dangerous for the environment must distinguish between two fractions:

1. Soluble: immediately soluble metals in the form of free cations or soluble complexes are extractable with deionised water.
2. Bioavailable: 'Exchangeable' metals can be determined using a 1 M solution of nitric acid (HNO_3). Metals extracted include those fractions complexed by organic matter and adsorbed by or co-precipitated with hydrous oxides, carbonates, and sulphides (National Soil Survey Center 1996).

Metals adsorbed and/or complexed, in other words: metallic oxides, hydroxides, amorphous aluminium silicates and humic substances, possess reactive surfaces on which heavy metals tend to be selectively adsorbed. To quantify the concentration of heavy metals in this form it is necessary to use an extractant able to form particularly stable bonds with these elements. A solution of diethylen triamino pentaacetic acid (DTPA), in the presence of calcium chloride and triethanol amine (pH 8.1) and a solution of 1% ethylenediaminetetraacetic acid (EDTA: pH 4.5) were used as extractants (Norvell 1984). The complexing capacities of DTPA and EDTA are able to simulate the effect of root secretions with respect to heavy metals in nutritional processes (Barbafieri et al. 1996).

In each step a soil-extractant ratio of 1:5 (4 g/20 ml in centrifuge propylene tubes of 50 ml) was used. The samples were agitated for 3 h and then centrifuged at 12,199 rpm (18,000 rcf) for 15 min (Avanti TM Centrifuge J-25, Beckman Coulter TM). The extraction solutions were filtered with an Albert N° 242 filter paper. Total concentrations of metals were determined by flame atomic absorption spectroscopy (UNICAM 969 AA spectrometer).

To determine the partitioning of edaphic metals, the sequential extraction scheme proposed by Tessier et al. (1979), and modified by Gomez Ariza et al. (2000a) for metal polluted and iron oxide rich sediments is a good option. This procedure had the following operational fractions:

F1 – Exchangeable and bound to carbonates (acid): contains metals adsorbed onto clay minerals that are sensitive to changes of pH.

Following the scheme proposed by Gomez Ariza et al. (2000a) for polluted and Fe-rich sediments, metals that are exchangeable and bound to carbonate were evaluated together using NH_4OAc at pH 5. This extractant was selected because reagents normally used for the evaluation of the exchangeable phase ($MgCl_2$ and NH_4Oac:pH 7) cannot distinguish between this and the bound to carbonate phases. It has been observed that NH_4OAc does not produce interferences in the instrumental measurements using FAAS (Flame Atomic Absorption Spectrometry) and GFAAS (Graphite Furnace Atomic Absorption Spectrometry). This extractant present a high complexing power due to the acetate ion, which may prevent readsorption problems and precipitation of released ions (except for Hg).

F2 – Bound to oxides of Fe and Mn (reductant): accounts for metals bonded to the reducible soil components, such as Mn hydrated oxides and particularly amorphous or poorly crystalline oxyhydroxides.

The hydroxylamine is the reducing reagent most often used, due to its ability to dissolve different metallic oxides (Gleyzes et al. 2002; Tessier et al. 1979). The highest levels of metal bound to the reducible phase can be found using the highest concentration of hydroxylamine (Gómez Ariza et al. 2000b). Considering the most unfavourable case, several repetitive extractions (up to a total of eight) might be needed to completely release the metal bound to iron oxides (Gómez Ariza et al. 2000a). Galán et al. (2003) used only one extraction for a case similar to the present study. Other authors have indicated that a portion of the oxides are coated or occluded by the organic matter in the sediments. Due to the low content of organic matter (OM) in mining soils and sediments, it is unnecessary to use an additional hydroxylamine extraction of the sediments treated with hydrogen peroxide (H_2O_2) and NH_4OAc (Gómez Ariza et al. 2000a).

F3 – Bound to organic matter (oxidant): accounts for metals bonded to sulphides and organic materials. Here it has however been shown that primary sulphides cannot be totally dissolved in this step (Förstner 1985).

Hydrogen peroxide in an acidic medium is generally used to oxidize organic matter in soil and sediments analysis, even though the oxidation of all forms of organic matter is not complete. There are more efficient extractants but they may however attack the silicate lattices thus disturbing later analysis (Tessier et al. 1979).

F4 – Residual: it accounts for metals bonded to the crystalline structure of the lithogenic materials and sulphides not dissolved in step 3. The determination of the structural metals was not the purpose of this research, so strong acids like fluorhidric acid (HF) were not used.

The residue from fraction 3 can be extracted for 2 h at 140 °C with 10 ml of concentrated HNO_3. After the centrifugation the supernatant was transferred to a 25 ml volumetric flask and diluted with water (Gauthreaux et al. 2000).

The results were expressed as a percentage of the total of samples analyzed. In fractionation studies the metal behaviour between acidic and alkaline soils was differentiated, because soil pH is a variable that influences heavy metals adsorption, retention and movement (de Matos et al. 2001). The pattern of partition of the heavy metals was considered without contemplating the pH of the soils ('total partition').

Results and discussion

Characterisation of soils

Soil pH values ranges from 2.5 to 7.5 and electric conductivity varies between 6 and 20 mS m^{-1}. Soils contain low amounts of organic matter, with 54–71% sand, 24–36% silt and 5–13% clay. Clays are bonded to Fe oxides and a great percentage of soils surface are covered by hardpans. Total metal content is elevated and range between 100–570 mg kg^{-1} of Cu, 1800–9000 mg kg^{-1} of Pb and 2000–12,000 mg kg $^{-1}$ of Zn. These conditions make an unfavourable environment for plant survival, but some species like *Piptatherum miliaceum*, *Lygeum spartum* and *Helichrysum decumbens* can be found in these sites.

Soluble and bioavailable metals

The percentages of metals extracted by EDTA were higher than the other extractants (Figure 1). Thus, these percentages of extractable metals indicate that plants could mobilize a high proportion of metals (more than 50%).

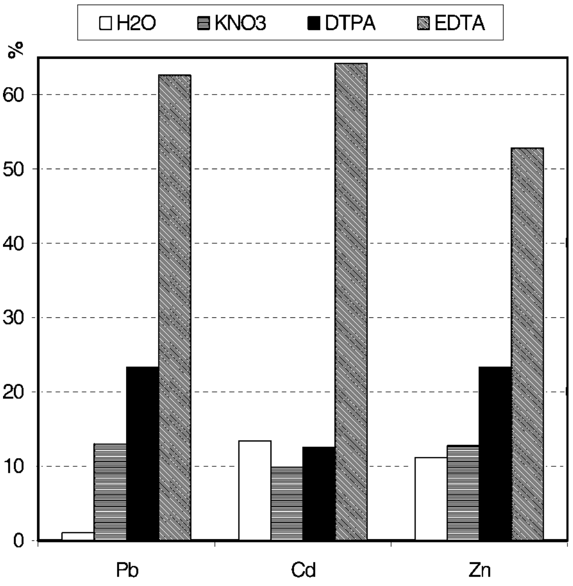

Figure 1. Percentage of soluble and bioavailable metals.

There were a low proportion of metals that could be immediately solubilized (Cd 13%, Zn 11% and Pb 1%). These results indicate that Pb is practically insoluble in water. In general, Pb mobility was low because of its low solubility (Lindsay 1979); which may be further reduced as a result of its interactions with the soil's solid phase *via* sorption and ion exchange (Dong et al. 2000). The proportion of metals released by KNO_3 was also low, except for Pb that showed to be more soluble than in water. Otherwise Cd was less soluble in KNO_3 than in water, what can be related to co-precipitation processes. Prokop et al. (2003) mention that binding to the soil components reduced the bioavailable fraction of Cd.

Fractionation of metals

Acidic soils

In acidic soils heavy metals exhibit a distinctive partitioning pattern (Figure 2a–c). Thus, the percentage of Pb in the fractions was found as 49.6% bound to residual fraction, 34.3% accumulated in the reducible fraction, 14.7% exchangeable-bound to carbonates and 0.1% bound to organic matter. The proportion of Pb that could be in the solution phase (F1–2) was 49%. Fe (oxy) hydroxides are good sorbents for aqueous Pb and their dissolution or precipitation may release or sorb Pb (Dong et al. 2000).

The distribution of Cd in the samples was found as bound to residual 49.6% > bound to organic matter 31.4% > exchangeable-bound to carbonates 10.6% > Fe/Mn oxide 8.5%. The proportion of Cd that was biologically available was 19.1%. The high amount of Cd in the oxidizing phase is attributed to the fact that cadmium

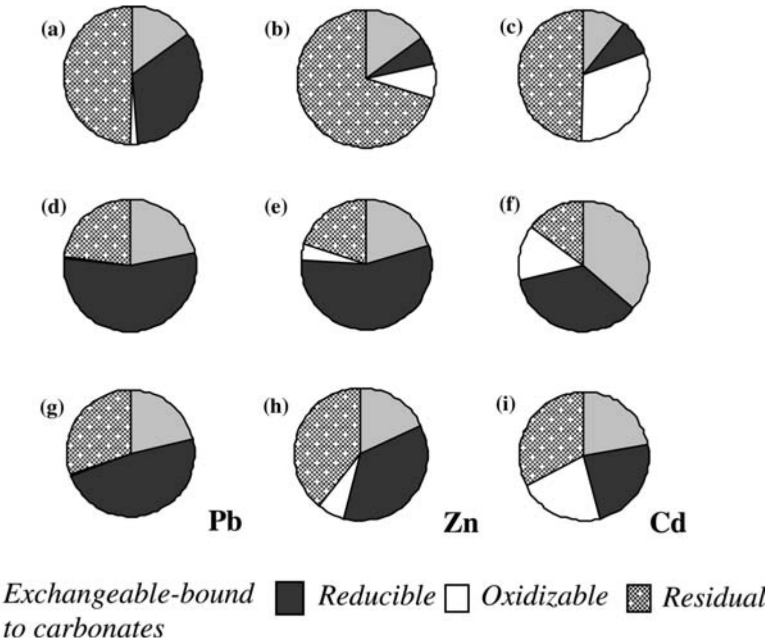

Figure 2. Fractionation of metals: Pb, Zn and Cd in acidic soils (a–c), in alkaline soils (d–f), and the total partition (g–i).

sorbs weakly on organic matter, as well as on silicate clays and oxides, unless the pH is higher than 6 (McBride & Martínez 1994).

The distribution of Zn in the samples was found as bound to residual 70.3% > exchangeable-bound to carbonates 14.6% > bound to organic matter 8% > Fe/Mn oxide 7%. The proportion of Zn that could be in the solution phase was 21.6%. These results do not agree with Mulligan et al. (2001), which mentioned that under acidic conditions, zinc is one of the most soluble and mobile of trace metals cations, being hold in exchangeable forms on clays and organic matter. This was however true for the situation found for Zn in alkaline soils.

Thus, considering the fractions with environmental importance the proportion of metals in the readily soluble phases, bounded to carbonates and oxides, was higher than the proportion associated with organic matter (except for Cd). This pattern could be related to the high percentage of Fe oxides and clay minerals and to the low content of organic matter in these soils.

A small proportion of metals in the readily soluble phases were found comparing to the residual phase, mainly for Zn and Cd. Several studies have however documented that the high acidity increases the mobility of heavy metals significantly (Chen & Cutright 2001; Lindsay 1979; Xian & Shokohifard 1989). Nevertheless, in the considered superficial soil layers, this soluble fraction seems to be poor. This may be caused by the scanty but intense rains typical of the Mediterranean climate that provoke an intense erosion of the soils, and also by the particular texture of these anthropogenic soils. In any case, this aspect should need further studies to be clarified.

Alkaline soils

Figure 2 d–f summarises the speciation of the heavy metals Pb, Cd and Zn in alkaline soils. Each trace metal exhibits a distinctive partitioning pattern. The distribution of Pb in the samples was found as to be bound to Fe/Mn oxide 54.6% > residual 23% > exchangeable-bound to carbonates 22.2% > bound to organic matter 0.1%. A considerable proportion of Pb (76.8%) could be in the solution phase (F 1–2). This pattern of partition shown by Pb was expected. Chemisorptions on oxides and silicate clays and precipitation as carbonate, hydroxide or phosphate are the mechanisms responsible for lead immobilisation, all of them being favoured at higher pH, although in alkaline soils its solubility may increase by formation of soluble Pb-organic and Pb-hydroxy complexes (McBride & Martínez 1994).

In the case of Cd, the highest concentrations were bonded to the exchangeable-bound to carbonates (36.1%) and reducible fractions (35.6%). They constitute the proportion of Cd that could be biologically available. The concentration of Cd in the other fractions was found as 13.3% in F3 and 15% in F4. Mulligan et al. (2001) report that above pH 7.5 Cd is not very mobile. Its divalent form is soluble but can also form complexes with organic matter and oxides.

The distribution of Zn was found as to be bound to Fe/Mn oxide 55.7% > exchangeable-bound to carbonates 20.4% > residual 19.9% > bound to organic matter 4%. The concentrations in F1 and F2 were found to be 76.1%, which indicates that a considerable amount of Zn could be in the solution phase. McBride & Martínez (1994) detected that at higher pH however, chemisorptions on oxides and aluminosilicates lowers the solubility of Zn. Otherwise at high pH, Zn is bioavailable due to the solubility of its organic and mineral colloids (Mulligan et al. 2001).

The high amount of metals released constitutes an important source of potentially available trace metals that under certain conditions could be remobilized and taken up by plants. Because of the fact that the high pH condition keeps the metals forms as hydroxyl species, aqueous metal complexes and carbonate-bound forms, preventing metal speciation into free ionic form. So the exchangeable form, which is the highly mobile form, is decreased (Chuan et al. 1996; Gee et al. 1997; Lindsay 1979; Song et al. 1999).

Total partition

Pb is mostly present in the reducible fraction (47.9%) of the soils (Figure 2 g–i). However, a considerable proportion (30.2%) appears to be accumulated in the residual fraction. The concentration of Pb in the exchangeable-bound to carbonates and bound to oxides of Fe and Mn are 69.20%. A considerable proportion of Pb could be in the solution phase (F 1–2), in agreement with Li & Thornton (2001) which found that the exchangeable-bound to carbonates and Fe/Mn oxide phases are the largest fractions for Pb in soils. The percent of Pb found in the oxidisable fraction is very low (0.7%). These findings confirm that under oxidizing conditions, Fe (oxy) hydroxides tend to immobilize Pb (Gambrel et al. 1980), whereas under reducing conditions they dissolve to release Pb (Gambrel 1994).

In the case of Cd, the distribution pattern among the geochemical fractions is quite heterogeneous, and consequently the binding with different phases varies widely. The highest concentrations are bonded mainly to the residual fraction (32.8%). Therefore, incorporation into the crystalline structures of mineral particles could be regarded as the major transport mechanism for these metals. The concentration in the exchangeable-bound to carbonates and bound to oxides of Fe and Mn, that could be biologically available are 46%.

A large amount of Zn was in the residual fraction (39%). The second most abundant phase was the Fe–Mn oxide fraction (36.3%). The bound to organic matter fraction accounts for 6.71%. The exchangeable-bound to carbonates phase is also important (17.9%). These results agree with the observation of Li & Thornton (2001). Usero et al. (1998) using also the Tessier method found that Zn is preferably bound to iron-manganese oxides. In the other hand, Maskalland & Thornton (1998) indicate that in contaminated soils, Zn is mainly found in the residual fractions.

The big proportion of metals in the readily soluble phases may be related to the high percentage of Fe oxides and clay minerals in these soils. The small proportion of metals bounded to organic matter could be based on the scarcity of this fraction in the soils.

Conclusions

The main goal of the research was to understand metal mobility and the fractionation of elements, as well as the lability and biological uptake of Zn, Pb and Cd by plants. This is the main first step towards implementation of an efficient phytoremediation program.

Related to the bioavailable fraction, there were a low proportion of immediately soluble metals that could be easily liberated into soil solution and taken up by plants. Otherwise the EDTA extraction showed that there was a high proportion of metals that could be released and therefore be available to plants.

Of the total amounts of Pb, Cd and Zn, a large proportion was extracted in a readily mobile form, which may have further implications on environmental risks. There was however a large proportion extracted in the residual phase. But in this phase the metals were contained in the crystal lattices of minerals with strong bindings and consequently they will not be released into the environment (Navas & Lindhorfer 2003). The percentages of heavy metals in the oxidisable phase are much lower, and in these forms metals are less soluble and change in the acidic and redox conditions are needed in order to release the metals into the ecosystem.

In acidic soils the content of metals in a ready mobile form (F1 and F2) and in the oxidisable fraction constitutes an important source of potentially available trace metals that could be remobilized and incorporated to the soil solution (Riba et al. 2002). The same pattern was shown in alkaline soils, where a big proportion of metals (about 80%) could be remobilized and are potentially available to plants.

Knowledge of metal binding phases gives valuable information of how to improve the plant uptake of these metals. So in order to plan and carry out the remediation of the affected soils, quick information about the speciation of heavy metals in soils is an essential tool. It provides helpful data about easily bioavailable heavy metals that can be taken by plants and the amount of each metal that can be remobilized under certain conditions. This knowledge will allow an improved efficiency of the phytoremediation program, with special considerations in the minimization of environmental and health risks. Furthermore, other studies and aspects of this problem, such as the influence of soil properties and soil forming factors on transformation and movement of anthropogenic trace elements (Burt et al. 2003) should be taken into account.

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