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Heavy metal bioavailability in a soil affected by mineral sulphides contamination following the mine spillage at Aznalcóllar (Spain)

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Abstract

A field experiment, lasting 14 months, was carried out in order to assess the effect of organic amendment and lime addition on the bioavailability of heavy metals in contaminated soils. The experiment took place in a soil affected by acid, highly toxic pyritic waste from the Aznalcóllar mine (Seville, Spain) in April 1998. The following treatments were applied (3 plots per treatment): cow manure, a mature compost, lime (to plots having pH < 4), and control without amendment. During the study two crops of *Brassica juncea* were grown, with two additions of each organic amendment. Throughout the study, the evolution of soil pH, total and available (DTPA-extractable) heavy metals content (Zn, Cu, Mn, Fe, Pb and Cd), electrical conductivity (EC), soluble sulphates and plant growth and heavy metal uptake were followed. The study indicates that: (1) soil acidification, due to the oxidation of metallic sulphides in the soil, increased heavy metal bioavailability; (2) liming succeeded in controlling the soil acidification; and (3) the organic materials generally promoted fixation of heavy metals in non-available soil fractions, with Cu bioavailability being particularly affected by the organic treatments.

Abbreviations: DTPA – diethylenetriamine-pentaacetic acid; EC – electrical conductivity; OM – organic matter

Introduction

In April 1998 a dam wall enclosing pyritic mine sludge at Aznalcóllar (Seville, Spain) was broken and about 5 million m^3 of a highly toxic pyrite waste spread along the Guadiamar river, covering 45 km^2 of the surrounding arable land. The solid phase (9×10^5 m^3 of toxic tailings) spread 37 km downstream. The mineral phases consisted of different sulphides, such as pyrite (83.1%), sphalerite (5.4%), galena (2.1%), chalcopyrite (1.4%) and arsenopyrite (0.9%) and numerous trace metals (Almodovar et al. 1998). Although most of the sludge, together with the surface soil, was removed, heavy metal levels (especially Zn, Cd and Cu) of soils in some areas are still present in concentrations that are phytotoxic. The main source of these elements was the solution phase of the spill, which penetrated into the soil and the solid phase for the other elements (Simón et al. 2001).

The sulphides from the sludge remaining in the soil can, under adequate moisture and aeration conditions, be oxidised to sulphates with the formation of sulphuric acid, such that pH falls markedly (Förstner & Wittmann 1983). Soil pH is one of the main factors controlling the solubility and bioavailability of heavy metals, together with redox potential, soil texture, CEC, and organic matter (OM) content (Ross 1994).

The concept of soil metal bioavailability refers to metal fractions in chemical forms which can be taken up by different soil organisms and by plants (Mench et al. 2000). The most widely used approach to predict the bioavailability of toxic metals in soils is to choose a chemical extractant, or a series of extractants, thought to remove particular chemical phases of metals in the soil, which correlate well with amounts of metals taken up by plants grown in the soil (Turner 1994; Bañuelos 2000). Of the weak chelating agents, DTPA has been used successfully as an extractant for characterising bioavailability of soil metals (Lindsay & Norvell 1969; Gough et al. 1980; Lee et al. 1983; Ross 1994).

Addition of humified OM such as compost, together with lime to raise soil pH (Kuo et al. 1985), is a common practice for immobilisation of heavy metals and improvement of soil conditions, to facilitate re-vegetation of contaminated soils (Williamson & Johnson 1981). Conversely, soluble OM present in fresh manure can increase the short-term solubility of heavy metals (Almås et al. 1999), through the formation of soluble metal chelates. However, Walker et al. (2003) indicated that the effect of OM on heavy metal bioavailability in calcareous soils was often not related to the OM composition or degree of humification.

A remediation programme has been carried out in the area affected by the Aznalcóllar spill to effect metal immobilisation in these strongly contaminated soils, to reduce phytotoxicity and thus to re-establish a vegetation cover. The aim of this field experiment was to assess the effect of soil amendments (OM and lime) on the bioavailability of heavy metals in a zone close to Aznalcóllar. Special interest has been paid to the evolution of soil pH and sulphate formation, as these parameters can strongly affect heavy metal bioavailability and therefore the bioremediation programme.

Materials and methods

Experimental procedure

The experimental site was located on the right margin of the Guadiamar river (longitude W 06°13′00″, latitude N 37°26′21″), 10 km downstream from the Aznalcóllar mine. The site (20 × 30 m) was divided into 12 plots (8 × 4 m each, with a margin of 1 m between plots). The soil was a non-calcareous loam with 19.7% clay, 34.3% silt and 46% sand, classified as Typic Xerofluvent (American Soil Taxonomy), with about 1.1% OM. The following treatments were applied (3 plots per organic treatment): cow manure, which provided soluble and easily mineralisable OM (OM 188, water soluble-C 33.4, NaOH extractable-C 30.1, total-N 6.8, all g kg^{-1}); a mature compost with highly humified OM (OM 875, water soluble-C 26.4, NaOH extractable-C 125, total-N 26.6, all g kg^{-1}); control, without organic amendment (repeated 6 times because of the great differences of soil pH and heavy metal content between plots); lime, applied to highly acidic plots. The organic amendments were added at annual rates of 13.6 t ha^{-1} of compost, and 36 t ha^{-1} of manure (on a dry matter basis) in order to increase the soil OM content to about 1.6%. The initial liming procedure was carried out using 25 kg per plot of a by-product from the industrial extraction of sugar from sugar beet, which contained 85% $CaCO_3$ (equivalent to 21 kg $CaCO_3$ per plot).

During the study, two crops of *Brassica juncea* (L.) Czern (cv. Z1) were grown, with two organic amendment additions (one month before each sowing). All plots were fertilised with an 8 : 15 : 15 N : P : K inorganic fertiliser at a rate equivalent to 750 kg ha^{-1} (2.4 kg per plot). After the first crop, the plots were divided into two or three subplots due to the great variation of contamination and pH within plots, and plots showing excessive soil acidification were limed at an appropriate rate to increase pH values to about 6.0. Soil samples were taken on four different dates: March, May and December 2000, and April 2001, corresponding to the first sowing and harvest and the second sowing and harvest, respectively. All samples were taken at 0–20 cm in depth, and were air dried and sieved to <2 mm for analysis. The evolution of soil pH, total and plant-available heavy metals content (Zn, Cu, Mn, Fe, Pb and Cd), sulphate concentration and electrical conductivity (EC) were followed throughout the study. Sulphate concentration and EC were determined at the four samplings in 9 of the 12 plots, corresponding to three plots per treatment (control, manure and compost). Plant growth (fresh and dry weight) and heavy metal concentrations were also determined.

Analytical methods

Total metal concentrations in plant material and soil were determined following HNO_3/$HClO_4$ digestion. Plant-available metals in soil were analysed after extraction with DTPA-$CaCl_2$-triethanolamine (Lindsay & Norvell 1969). Extracted metals were analysed by atomic absorption spectrometry. Soil pH was measured in a saturated soil paste. EC was determined in a 1:5 aqueous soil extract and sulphate content was determined by turbidimetry with $BaCl_2$ (Abrisqueta et al. 1962). All analyses were done at least in duplicate.

Statistical analysis

Using SPSS version 10.0 software (SPSS Inc.), the data were subjected to one way ANOVA and differences between means were determined using the least significant difference test (at $P < 0.05$). Regression equations were calculated using SigmaPlot version 5.0 software, by the least-square procedure where correlation coefficient (r) was calculated, and the non-linear least-square procedure (Marquardt-Levenberg algorithm), where residual mean square (RMS) and F-value of the curve fit were calculated.

Results and discussion

The soil at the experimental site was characterised by a wide variation in total metal concentrations between and within plots, as the analysis of variance showed (Table 1), Zn, Pb and Cu being the principal pollutants. Simón et al. (1999) had identified these same elements as the main pollutants of the soil before removal of the mining sludge from the surface. The removal of a few cm of the topsoil together with the sludge in the cleaning procedure was not equally effective in all areas and patches of sludge still remained in the soil. In addition, acidic water, accumulated in surface puddles, could have contributed to the heterogeneity of pH and heavy metal distribution. The pH of the soil was very variable in the different plots at the beginning of the experiment (Figure 1). Acidification was indicated by the decrease of pH values (median: from 5.85 in March 2000 to 2.60 in May 2000), still in a very wide range, at the first harvest of *B. juncea* (May 2000). The oxidation of metal sulphide, in pyrite ore, to sulphate is an acidification process (Nordstrom 1982). Simón et al. (2001) found that the oxidation of tailings from the Aznalcóllar spill was very rapid when exposed to air at the soil surface. Therefore, the sulphides of sludge remaining in the soil of the current studies could have been oxidised rapidly, and the adequate moisture content of the soil during the growth period could have favoured the process, with the consequent decrease of pH values. The general liming procedure initiated after harvesting the first crop succeeded in controlling the soil pH, increasing the pH to values adequate for plant growth, while the dry conditions in summer also may have inhibited the oxidation process. The low OM and $CaCO_3$ contents of this soil gave it a low buffer capacity, allowing these drastic changes in soil pH.

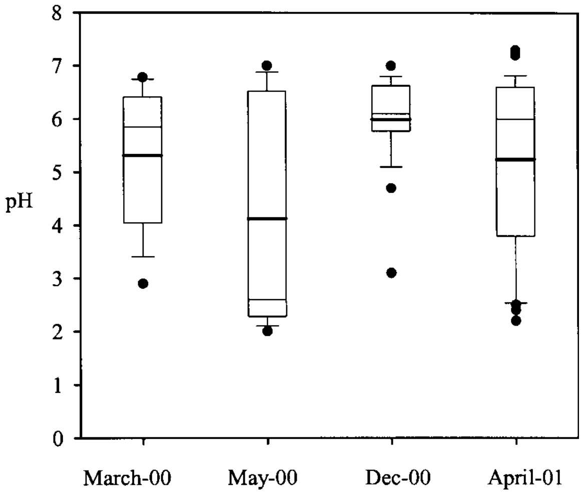

Figure 1. Distribution of the pH values of the soil during the experiment. The box indicates the upper and lower quartiles, the thick line within the box indicates the mean and the thin line indicates the median; the vertical lines indicate the 90–10 percentiles and the dots are outliers.

The sulphate concentrations ranged from 1.0 to 17.8 g kg^{-1} in the samplings, and showed a close relationship with EC, which ranged from 0.70 to 4.29 dS m^{-1} (Table 2). Both data fitted a linear regression equation at a high significant level ($P < 0.001$, Figure 2b). Sulphate concentrations had a clear effect also on pH values of the soil (Figure 2a), and their values fitted to a sigmoidal logistic equation ($P < 0.001$). The data indicate that the decrease of soil pH was a consequence of sulphate formation due to sulphide oxidation. Plots with pH values close to 7 had the lowest concentrations of sulphates. Liming decreased sulphate concentration in soil solution as a consequence of pH increase and precipitation of part of these soluble sulphates as $CaSO_4$. Also, with time, the concentration of oxidisable sulphides decreased, which contributed to pH stabilisation. As can be seen in Table 2, at the end of the experiment sulphate concentrations were the lowest of all samplings. Redox conditions can also influence sulphate formation. It has been shown in a pot experiment with this soil (Walker et al., pers. comm.), that fresh manure stopped acidification and raised soil pH, mainly because the added OM was easily oxidisable, leading to less-oxidising conditions in the soil and so diminishing sulphide oxidation.

Plant survival and biomass production of *B. juncea* depended greatly upon soil pH, with both being very low, or zero, in the strongly acidic plots or subplots (pH < 3.0) for both crops. However, with respect to the organic amendments, the biomass production (fresh weight) in the first harvest was 2869 ± 507 kg ha^{-1} in plots with cow manure, 977 ± 195 kg ha^{-1} with

Table 1. Total concentration (mg kg^{-1}) of heavy metals in the experimental plots and subplots (l = left, c = centre, r = right). Mean values of three samplings (May 2000, December 2000 and April 2001), and standard error

Treatment	Plot	Subplots	Zn	Cu	Pb	Cd
Control	1[3]	l	517 ± 34.7	132 ± 12.6	172 ± 21.7	2.1 ± 1.51
		c	483 ± 38.5	178 ± 9.3	387 ± 73.0	2.2 ± 2.20
		r	553 ± 76.6	143 ± 30.2	222 ± 49.9	1.6 ± 0.54
Compost	2	lc	957 ± 202.5	223 ± 20.8	408 ± 126.9	2.2 ± 1.29
		r	719 ± 304.8	174 ± 66.0	509 ± 158.8	0.6 ± 0.43
Control	3	l	1367 ± 83.7	284 ± 11.1	623 ± 184.5	2.6 ± 1.72
		cr	1126 ± 59.6	292 ± 28.2	757 ± 319.0	2.9 ± 1.11
Manure	4	l	953 ± 92.6	205 ± 7.2	503 ± 59.1	1.4 ± 1.02
		cr	1265 ± 333.9	299 ± 53.7	783 ± 274.8	2.3 ± 1.16
Compost	5	l	1147 ± 129.0	226 ± 36.1	534 ± 135.6	2.4 ± 0.90
		cr	1010 ± 328.8	217 ± 34.7	467 ± 166.5	1.6 ± 1.25
Control	6	l	985 ± 77.3	202 ± 18.9	437 ± 78.0	1.8 ± 1.25
		cr	833 ± 318.0	240 ± 48.0	604 ± 335.6	1.6 ± 0.95
Control	7	l	989 ± 140.0	244 ± 16.4	696 ± 138.2	2.0 ± 1.49
		r	668 ± 248.3	231 ± 32.1	678 ± 223.4	1.0 ± 1.12
Compost	8	l	874 ± 356.4	219 ± 40.7	428 ± 165.5	1.8 ± 0.42
		r	808 ± 260.9	213 ± 27.7	425 ± 45.6	1.6 ± 1.19
Control	9	l	703 ± 220.9	188 ± 29.1	387 ± 50.1	1.1 ± 0.75
		r	668 ± 222.7	179 ± 22.1	334 ± 37.0	1.6 ± 0.42
Manure	10	l	917 ± 6.8	195 ± 26.0	412 ± 124.9	2.2 ± 1.63
		cr	532 ± 35.1	154 ± 11.8	249 ± 20.4	1.2 ± 0.82
Control	11	l	727 ± 62.7	126 ± 1.0	208 ± 4.5	2.0 ± 1.11
		cr	457 ± 67.6	176 ± 14.3	503 ± 71.9	0.6 ± 0.48
Manure	12	lc	529 ± 40.3	129 ± 6.5	227 ± 21.3	0.9 ± 0.68
		r	465 ± 9.4	168 ± 11.9	319 ± 106.4	0.9 ± 0.63
ANOVA						
F plots[1]			6.330***	8.182***	4.142***	0.729 n.s.
lsd			275	45.4	215	1.53
F plots × subplot[2]			3.746***	5.351***	2.572***	0.605 n.s.
lsd			381	59.8	301	2.28

[1] n=6

[2] n=3.

[3] n=9 in plot 1, lsd for comparison with plot 251 for Zn, 41.5 for Cu 196 for Pb, and 1.40 for Cd.

*** Significant at $P < 0.001$; n.s. not significant.

Table 2. Range, mean and median of pH, sulphate concentration and EC for subplots at each sampling

Samplings	pH			[SO$_4^{2-}$] (g kg^{-1})			EC (dS m^{-1})		
	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median
March 2000	2.9–6.8	5.3	5.9	8.3–12.5	10.7	10.8	2.29–3.01	2.71	2.75
May 2000	2.0–7.0	4.1	2.6	3.1–17.8	11.1	10.8	1.21–4.29	2.83	2.72
December 2000	3.1–7.0	6.0	6.1	7.4–13.3	10.3	10.3	2.02–3.22	2.70	2.74
April 2001	2.2–7.3	5.2	6.0	1.0–10.7	7.9	9.4	0.70–2.69	2.17	2.46

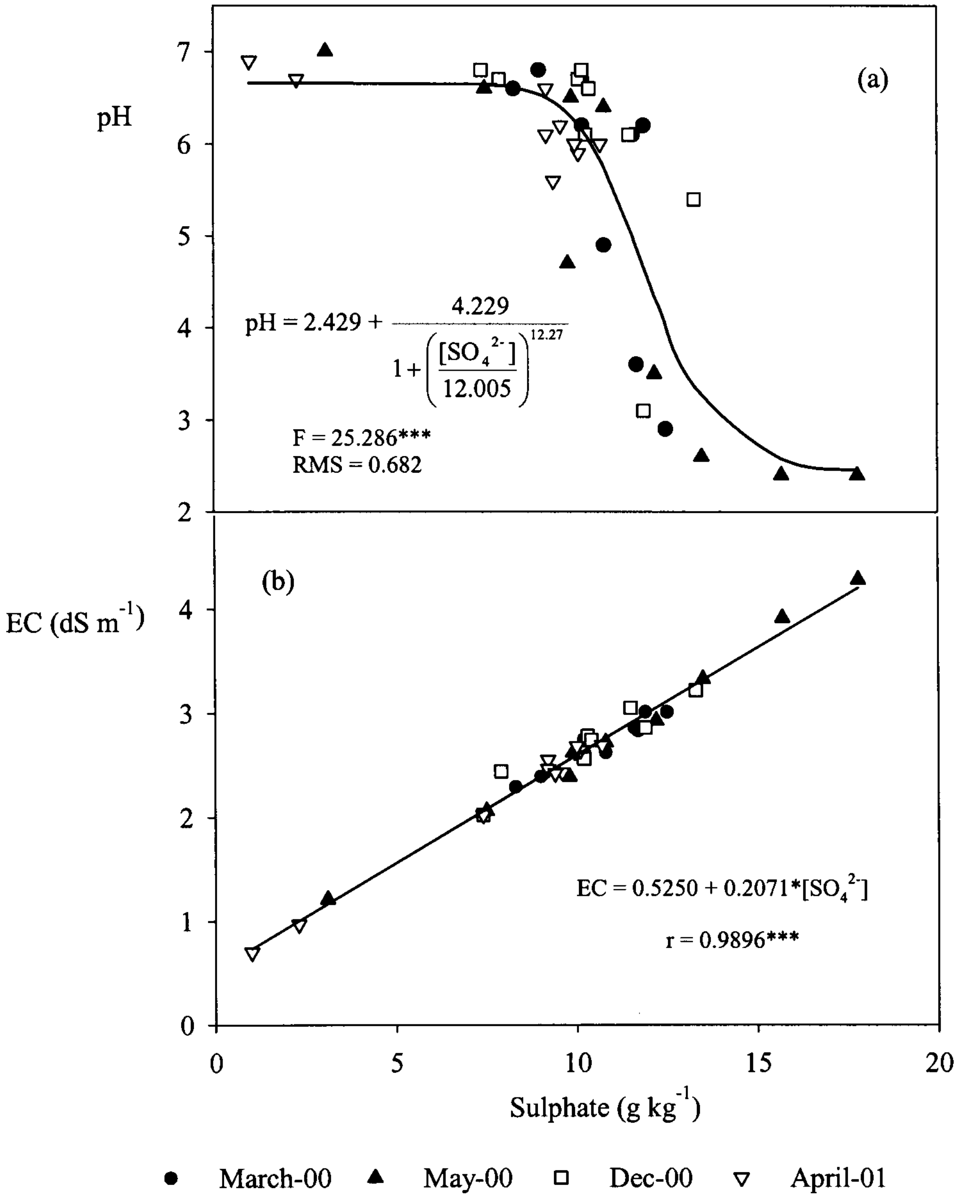

Figure 2. Relationship between sulphate concentration and (a) pH, and (b) electrical conductivity. Symbols are experimental results at different samplings and lines are the fitted regression equations.

compost and 1354 ± 470 kg ha^{-1} in control plots without organic amendments. In the second harvest, the highest production was obtained with compost (2186±563 kg ha^{-1}), followed by control (1631±946 kg ha^{-1}) and manure (518 ± 135 kg ha^{-1}).

The bioavailable (DTPA-extractable) concentrations of Zn, Cu, Fe and Mn in soil were in a very wide range in all samplings (Figures 3–4), due to the differing total metal concentrations in each plot (Table 1). After the first harvest, the range and mean values were (Figure 3) (n = 22): Zn 85.8–965, mean 363 mg kg^{-1}; Cu 10.7–108.8, mean 36.5 mg kg^{-1}; Pb 0.0–11.4, mean 1.94 mg kg^{-1}; Cd 0.6–3.3, mean 1.6 mg kg^{-1}; Fe 65.4–3369, mean 679 mg kg^{-1}; Mn 41.9–808, mean 245 mg kg^{-1}. The highest values were found in zones of very low pH (Figure 3), which gave the highest concentrations of Zn (2029 mg kg^{-1}) and Cu (71 mg kg^{-1}) in plants (data discussed elsewhere; Bernal et al. 2003). After the second crop, soil concentrations of available Zn, Fe and Mn decreased, even in subplots where pH tended to decrease, indicating the immobilisation of metals (Figure 4). The range and mean values were (n = 29): Zn 57.6–319, mean 166.6 mg kg^{-1}; Cu 10.6–43.7, mean 31.9 mg kg^{-1}; Pb 0.0–11.3, mean 1.48 mg kg^{-1}; Cd 0.3–2.4, mean 0.86 mg kg^{-1}; Fe 62.0–1395, mean 303.3 mg kg^{-1}; Mn 46.0-376, mean 120.6 mg kg^{-1}. There was a clear direct relationship between both Zn and Mn concentrations and sulphate concentration in the soils in both samplings (r = 0.720, $P < 0.001$; r = 0.728, $P < 0.001$, respectively), while the correlation was not significant for Fe, as it can form secondary minerals in soils as described here (oxides, hydrous oxides, jarosites, etc.). During the experiment, the mean percentage of total Zn extracted with DTPA, averaged across all the plots, decreased form 44.2% to 26.7% due to pH stabilisation, although the organic amendments may have had a significant effect as occurred in previous work (Narwal & Singh 1998; Walker et al. 2003). The percentage of extracted Pb was rather low (average 0.8%) in all samplings despite the high total concentration levels of this metal and it showed an inverse relationship with sulphate concentration (r = −0.745, $P < 0.001$). This may reflect the fact that Pb can form insoluble compounds with different anions, including SO_4^{2-}, and it can be easily adsorbed at the surfaces of Fe-oxides (Ross 1994). The mean value of DTPA-extractable Cu of all plots did not decrease after the second crop (Figure 4), perhaps due to the OM additions, as Cu concentration did not show a correlation with pH or sulphate concentration of soil. An increased solubility of Cu as a consequence of the formation of stable Cu complexes with soluble organic molecules can be found in the pH range 6-7 (Hornburg & Brümer 1993).

The effect of the organic amendments on the bioavailability of metals was difficult to observe due to the great variability of total metal concentration and pH. In any case, pH seemed to be the controlling factor in metal availability, to a greater extent than the organic treatments. However, the addition of organic amendments improved plant growth (Bernal et al. 2002) which is of great relevance for soil reclamation.

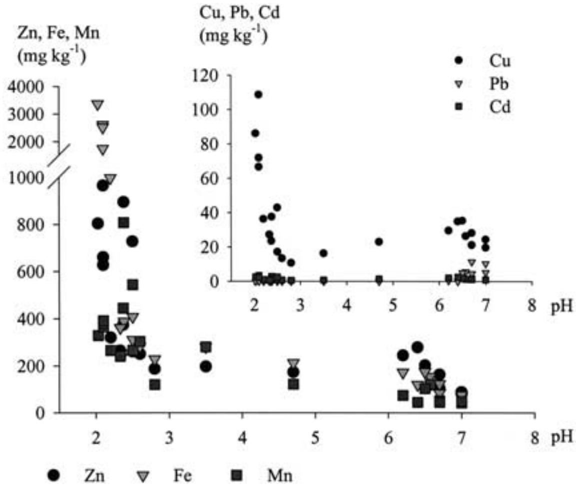

Figure 3. Concentrations of DTPA-extractable heavy metals in soil (May 2000) as a function of soil pH.

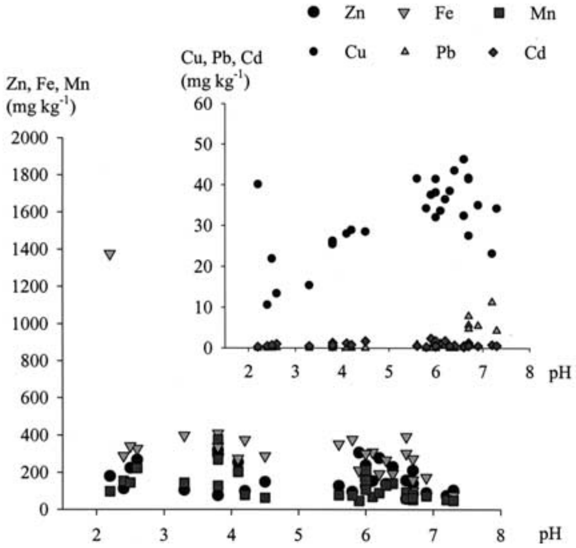

Figure 4. Concentrations of DTPA-extractable heavy metals in soil (April 2001) as a function of soil pH.

Conclusions

The soil studied was highly contaminated by Zn, Cu and Pb, with a wide range of pH. High sulphate concentrations were observed in plots concomitant with low pH, which indicated that soil acidification occurred as a consequence of sulphide oxidation. Plant survival, biomass production and heavy metal contents were conditioned by soil pH. Soil pH was also the main factor affecting metal bioavailability. The production of sulphate by sulphide oxidation increased solubility of Zn and Mn, and therefore their concentrations in plant-available (DTPA-extractable) fractions. However, the bioavailability of Cu did not decrease with either soil pH increase or with time, indicating that the organic treatments might have had a significant effect. Further studies are required to determine the effects of pH and organic amendments on Cu availability to plants. The application of both lime, for controlling the soil pH, and OM, for improving plant growth, were shown to be good strategies for bioremediation of this soil contaminated by heavy metals and liable to acidification.

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