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Original Research

Metal Bioavailability in Long-Term Contaminated Tarnowskie Gory Soils

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Abstract

Metal bioavailability is a key factor in risk assessment procedures for contaminated sites. The goal of our study was to evaluate metal bioavailability and toxicity in long-term contaminated soils in the Tarnowskie Gory area of the Silesia region of Poland. Forty diverse soils were collected throughout the study area. Metal availability was measured using neutral salt extractions, a sequential extraction and an *in vitro* test for Pb bioaccessibility. Effects of soil contamination on microbial activity and wheat growth were examined in a pot study. The study demonstrated relatively low availability of metals in long-term contaminated soils. In the sequential extractions zinc and lead were mainly present as poorly available fractions defined as associated with iron and manganese oxides or the residual fraction. Cadmium was mainly present in the exchangeable fraction but also occurred in the immobile fractions. Extractable metals and their contents in wheat were mostly dependent on soil pH and were not correlated to their total soil concentrations. Percentage Pb bioaccessibility was the lowest in the most contaminated soils. Long-term contamination of the soils by metals did not reduce microbial activity.

Keywords: bioavailability, zinc, cadmium, lead, microbial activity

Introduction

The occurrence of metal toxicity to plants and soil microorganisms or excessive transfer to food chain are related to metal bioavailability. The bioavailable fraction of a metal is its pool that might be absorbed by plant or soil organisms. Trace metals are present in soil in various forms. Total metal content consists of fractions of different solubility and availability. Metal bioavailability depends on its chemical behavior, soil properties and the individual characteristics of the receptor (organism or plant). Among soil properties pH, cation exchange capacity and redox potential play the most important role. Generally, Zn, Pb and Cd availabilities decrease as soil pH increases in the range of pH typical for soils. This reduction of metal availability is an effect of their greater adsorption and precipitation in neutral and alkaline environments [1, 2].

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Chaney and Oliver [3] distinguish four groups of trace elements according to their phyto-availability and risk to food chain:

(I) weakly soluble in soil, absorbed by plants in trace amounts (Cr, Ag),
(II) elements relatively easily absorbed by roots but weakly transported to shoots (Hg, Pb),
(III) elements easily absorbed and transported to shoots, but their phytotoxicity reduces the risk to food chain. Elements are toxic to plants at contents that are not toxic for animals and people (Zn, Cu, Ni),
(IV) elements posing a risk to the food chain. Under certain conditions might be toxic to animals and people at contents non-toxic to plants (Co, Cd).

Various analytical methods are used to describe metal availability. Soil extractions with complexing agents such as DTPA or neutral salt solutions such as calcium chloride or nitrate are the most common procedures for analysis of metal phytoavailability [4, 5, 6]. In certain cases sequential extractions are useful measures for quantification of metal fractions of different solubility and availability [7].

Modern methods of bioavailability measurements are based on using membranes of different porosity and permeability. However, these methods are more often applied to study metal bioavailability in water environments.

Another approach to assessment of metal bioavailability is represented by laboratory or pot biotests in which toxic effects of metals on plant growth or microbial activity are measured [8].

Bioavailability of metals plays a key role in risk assessment for metal contaminated sites such as highly industrialized areas under former and present pressure of metal-ore mining and smelting industry. It is well accepted that analysis of negative effects of soil contamination cannot be based only on total metal contents in soil. We can assume that metal availability depends on metal source and the form of the metal that enters the soil as well as the time of its presence in soil.

Effects of soil contamination have often been overestimated since in numerous studies metals were applied to soil as solutions or salts. Metal salt effects do not reflect real conditions of even highly contaminated soil [9]. In long-term contaminated soils under industrial pressure metals are the object of aging reactions. Furthermore, metals in such soils often originate from different sources including smelter dusts, ore outcrops, metal-rich parent rock material and other point sources. This makes assessment of contamination effects based on total metal contents very inaccurate.

The goal of our study was to evaluate the status of, and indicate factors governing, metal bioavailability in long-term contaminated soils of the Tarnowskie Gory area. The study was performed on a representative group of soils with a wide range of metal content. Various parameters for the characterization of metal bioavailability were used, including extractions with diluted salt solutions, sequential extraction, in vitro test and plant/microbial pot test.

Experimental Procedures

Samples Collection

Forty samples were collected from the top 0–20 cm soil layer of arable lands in the Tarnowskie Gory area. Sampling sites were located in 10 districts of Tarnowskie Gory: Repty (7 samples), Bobrowniki (5), Tarnowskie Góry (12), Rybna (5), Stare Tarnowice (2), Strzybnica (1), Żyglin (2), Miasteczko Śląskie (2), Sowice (2) and Pniowiec (2). Soils were collected throughout the region to ensure a wide range of soil properties such as organic matter or clay content as well as metal content. Tarnowskie Gory is a metal mining area located in the northwestern part of Upper Silesia, the most industrialized region of Poland. Tarnowskie Gory is one of the most contaminated parts of Upper Silesia. Soils of the studied area were under strong pressure of industrial emissions, especially in the second half of the 20th century. However, contamination in the Tarnowskie Gory area is of a complex character since soils of the area are partly developed from shallow and metal-rich trias limestones (Bobrowniki or Repty) and due to occurrence of ore outcrops mixed with topsoil (Tarnowskie Gory town).

Soil Analyses

Soil samples were air dried, crushed and sieved through a 1 mm mesh prior to analysis. Analysis of basic soil properties included pH, particle size distribution, amorphous iron oxides and total organic matter. Soil pH was measured in a slurry with 1:2 v/v soil:water ratio. Particle size distribution was determined by hydrometer method [10]. Organic matter was measured by the Tiurin method after hot digestion of sample with potassium dichromate.

Total metal contents were analyzed by atomic absorption spectrometry after hot aqua regia sample digestion protocol (digestion in mixture of concentrated nitric and hydrochloric acids, followed by refluxing in 3 M hydrochloric acid) [11].

The following forms and fractions of metals were analyzed to characterize metals availability and solubility:

a) Tessier sequential extraction [12],
b) 0.01 M $CaCl_2$ extraction (5 g:50 ml soil:solution ratio, shaken for 2 h in room temperature)
c) 0.01 M $Sr(NO_3)_2$ extraction (10 g:20 ml soil:solution ratio, shaken for 2 h in room temperature,
d) bioaccessible Pb was measured using the simplified Ruby et al. method [13] under simulated gastric conditions (at pH 2.2, 37^{o}C) shaken at 30 rpm for 1 hour with 1g:100 ml soil:solution ratio.

Pot Study

A pot study with the same forty soils was conducted to study the toxicological effect of soil contamination on soil microbial activity and wheat growth. The study was conducted in 3.0 liter plastic pots. Soils were put into pots while still moist after thorough mixing. Each pot contained 2.2 kg of oven-dried soil. After adjustment of water content to 70% of the content at field water capacity, soils were incubated for 2 weeks at 20-22^{o}C. Then microbial parameters were determined. The CO_2 released by each soil sample in a 10-day period was measured to quantify soil respiration. The nitrification potential was expressed as the rate of nitrate production after substrate (ammonia sulfate) application to sample. $N\text{-}NO_3^-$ concentration was measured 0, 14 and 48 h after substrate addition. Dehydrogenase and urease activities were measured according to standard protocols [14, 15].

The pot study with spring wheat (*Triticum aestivum*, L.) was conducted directly after microbial activity measurements. Plants were harvested at full maturity. Dried straw and grain samples were analyzed for macro- and microelements. Plant material was prepared for analyses by ashing in a muffle oven at 480°C for 16 hours, followed by digestion in concentrated HNO_3 and refluxing with 3 M HCl. Element concentrations in filtrates were measured by AAS.

Basic statistics and analysis of relationships between variables were performed using SAS software [16]. Pearson correlation coefficients were calculated to analyze relationships between various soil, microbial and plant parameters.

Results and Discussion

Properties of Soils

Tarnowskie Gory soils had a wide range of such properties as pH (from very acidic to alkaline), organic matter content (1.1 – 11.1%) and particle size distribution (from sandy soils to silty clay loams) (Figure 1).

A wide range of total metal concentrations was also found (Figure 2). Zinc, lead and cadmium concentrations ranged from background to levels recognized as high contamination [17]. Copper and nickel contents were not as high as Zn, Pb and Cd in soils of Tarnowskie Gory area according to pollution criteria [17]. No significant sources of contamination by these metals were present in the region. These two metals are most likely from pedogenic sources and the contents measured were at a level usually found in uncontaminated soils of agricultural regions [18]. Since their contents are low and of natural character they are not widely discussed in this paper.

Dust emissions from intensive smelting activity were the major source of soil contamination with Zn, Pb and Cd in the region. This supposition is confirmed by the decreasing content of metals with depth in the soil profiles [19]. However, these metals partly originate from natural sources – metal rich parent rock material and metal ore outcrops mixed with the top level of soil when cultivated [20].

Total Zn, Pb and Cd contents were positively correlated with clay content and slightly less strongly correlated with organic matter and soil pH (Table 1). Greater metal contents in finer textured soils are related to their greater sorption capacity and better ability to retain metals from dustfall in topsoil.

Sequential Extraction

A sequential extraction procedure [12] was used to determine the solid-phase association of metals. The soil sample is digested in succeeding extracting solutions to mobilize metal fractions with decreasing mobility in the following sequence: exchangeable, linked to carbonates, Fe/Mn oxides, organic matter and residual form.

The extraction showed high variability between soils. However, to generalize both Zn and Pb were mostly present as adsorbed or occluded by Fe/Mn oxides (on average 28.6 and 55.1 % of total Zn and Pb, respectively). This is a relatively stable and weakly available fraction. A significant contribution (on average 43.5%) to total Zn content

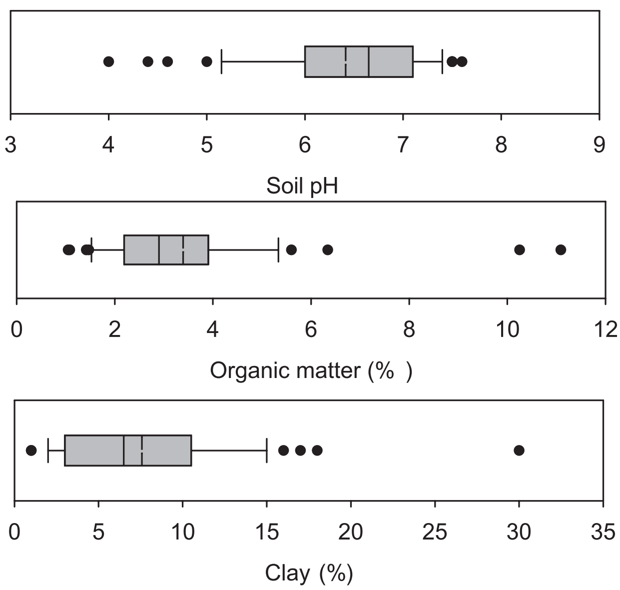

Fig. 1. Summary statistics for basic properties of soils collected in the Tarnowskie Gory area. Error bars are presented with median (solid line), mean (dash line), 10th and 90th percentiles (whiskers) and all samples with values outside10th and 90th percentiles (dots).

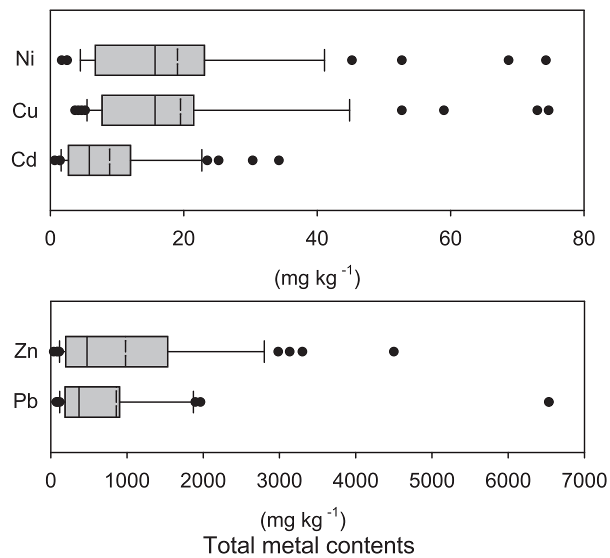

Fig. 2. Total metal contents in soils collected in the Tarnowskie Gory area. Error bars are presented with median (solid line), mean (dash line), 10th and 90th percentiles (whiskers) and all samples with values outside10th and 90th percentiles (dots).

Table 1. Correlation coefficients between soil properties and total metal contents.

	Clay	Organic matter	pH_{water}
Zn	0.82 ***	0.49 **	0.43 **
Pb	0.69 ***	0.52 ***	0.24 [n]
Cd	0.76 ***	0.60 ***	0.51 ***

*** significant at 0.001 probability level
** significant at 0.01 probability level
[n] not significant

Table 2. Percent contribution of different metal fractions to total content of metal in soil.

Metal fraction	Zn	Pb	Cd
	percent of total content		
Exchangeable	7.0[1] (0.05-40.0)[2]	2.7 (0.01-22.7)	30.0 (10.4-48.2)
Carbonates	8.1 (0.8-18.4)	7,9 (0.7-18.8)	16.9 (7.7-26.2)
Fe/Mn oxides	28.6 (2.7-57.1)	55.1 (12.4-76.3)	25.6 (7.1-59.6)
Organic	12.8 (0.8-27.9)	15.1 (3.0-21.7)	4.8 (0.5-10.7)
Residual	43.5 (11.6-95.1)	19.2 (1.2-82.6)	22.7 (2.7-60.9)

[1] mean value
[2] range in parenthesis

was also defined as the non-available residual form related to crystalline structures of minerals (Table 2).

Cadmium was present in more available fractions. The exchangeable fraction (extracted with 1 M $MgCl_2$) occupied 10-48% of total Cd with a mean contribution equal to 30%, but its substantial fraction was in immobile forms (Table 2).

Neutral Salt Extractions

Despite numerous studies of contamination levels done for different parts of Silesia, little is known about the solubility of metals in soils of the region. Extractions with diluted neutral salts as calcium chloride or strontium nitrate are procedures giving good predictions of metal availability to plants. The extraction solution causes only a slight pH shift so the extraction is performed under a pH equal to the pH of the soil [21]. A calcium nitrate extraction was proposed as an alternative to the more widely used Ca-chloride extraction because chlorides complex many metals (especially Cd), increasing their solubility [22]. Using strontium nitrate instead of Ca-chloride allows the measurement of available calcium. Sr-nitrate extractions have been successful in the prediction of Zn, Cd and Ni phytoavailability [6, 23].

Wide ranges of extractable Zn and Cd were found for the analyzed soils. Generally, Ca-chloride solution extracted more metals than Sr-nitrate (Figure 3). Lead concentrations in filtrates were below detection in most samples.

Ca-chloride and Sr-nitrate extractable Zn and Cd were not correlated to total metal contents in Tarnowskie Gory soils. The main factor controlling metal extractability was soil pH - correlations were significant at 0.001 probability level (Table 3). There was no relationship between organic matter content and metal solubility. Clay content had a weak effect on Ca-chloride extractable Zn and Cd. The results prove that the level of soil contamination with metals does not directly reflect metal availability.

Pb Bioaccessibility

The Ruby in-vitro method [13] was developed for assessment of lead bioaccessibility, a parameter predicting Pb absorption by humans in the case of soil ingestion. Incidental soil ingestion might be one of the primary human exposure pathways in the contaminated residential sites, especially for children. The method was calibrated in feeding studies with rats giving good predictions of Pb absorption. *In vitro* bioaccessibility of Pb measured in Silesian soils ranged from 7.2 to 268 mg kg^{-1} of soil and was not strongly related to total Pb. Relatively high r value for correlation (r=0.79) between these two variables was caused by two extreme samples with total Pb over 6000 mg kg^{-1} (Figure 4A). Under 2000 mg kg^{-1} of

Table 3. Correlation coefficients between soil properties and Ca-chloride and Sr-nitrate-extractable metals.

Metal	Total metal content	Clay	Organic matter	pH_{water}
Ca-chloride extraction				
Zn	-0.33 *	-0.36 *	-0.01 ^{n}	-0.75 ***
Cd	-0.31 ^{n}	-0,35 *	-0.16 ^{n}	-0.67 ***
Sr-nitrate extraction				
Zn	-0.23 ^{n}	-0,28 ^{n}	-0.08 ^{n}	-0.55 ***
Cd	-0.17 ^{n}	-0,17 ^{n}	-0.12 ^{n}	-0.49 **

*** significant at 0.001 probability level
** significant at 0.01 probability level
* significant at 0.05 probability level
^{n} not significant

Table 4. Variability of parameters characterizing microbial activity.

Activity parameter	Unit	Mean	Range	RSD[1]
Urease	mg $N\text{-}NH_4$ kg^{-1}	68.2	35.0 – 129.8	35.8
Dehydrogenase	µg TPF g^{-1}	114.7	40.6 – 233.7	36.1
Nitrification	regression coefficient	0.25	0.02 – 0.70	63.7
Respiration	µg $C\text{-}CO_2$ g^{-1} $24h^{-1}$	9.0	3.6 – 20.6	46.1

[1] percentage relative standard deviation (RSD=SD/mean)

total Pb there was no apparent relationship. Bioaccessible Pb expressed as a percent of total concentration was relatively low [1.3-30.6%]; for most contaminated soils (total Pb>1000) even lower: [1.3-13.6%] (Figure 4B) and was correlated with clay (r= -0,66), total P (r= -0.67) (Figures 4C,D), total Al (r=-0.81), pH (r= -0.72) and was not correlated with organic matter. The relationship with total P is likely an effect of precipitation of insoluble Pb phosphates in P-rich soils [24, 25]. Lead bioaccessibility in Tarnowskie Gory soils expressed as a percent of total content was distinctly lower than the percentage measured for contaminated soils of a military shooting facility [26].

Microbial Activity

Four microbial activity parameters were measured in soils of interest, including two specific processes of nitrogen transformation (nitrification activity and urea hydrolysis by urease) and two parameters characterizing basal microbial activity (dehydrogenase activity and soil respiration). The greatest variability was found for nitrification (Table 4). Correlation coefficients between total metal contents and microbial parameters are shown in Table 5. All four activities were positively correlated to total metal contents, which means that the greater metal content, the greater activity of microbial processes. This may be explained by the fact that other soil properties influencing biological activity such as clay content and organic matter content were positively correlated to metal concentrations in soils.

The most significant soil variables affecting biological activity were: total nitrogen (positively), organic matter content and soil particle size distribution (sand content– negatively, clay content– positively) (Table 5).

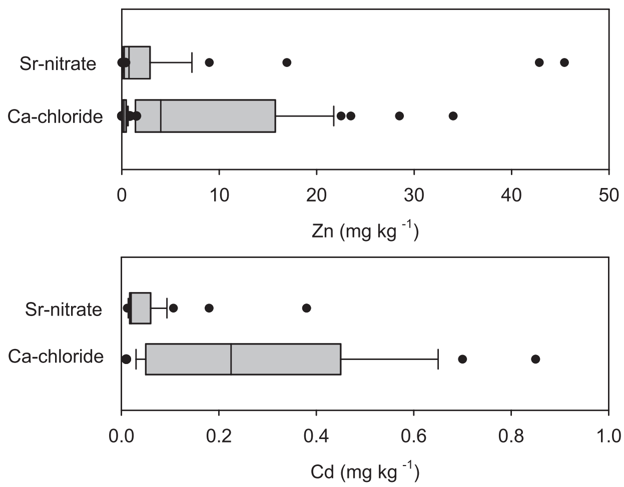

Fig. 3. The contents of zinc and cadmium extractable with Ca-chloride and Sr-nitrate. Error bars are presented with median (solid line), 10th and 90th percentiles (whiskers) and all samples with values outside10th and 90th percentiles (dots).

Table 5. Correlation coefficients between total metal concentrations and microbial activity parameters.

Metal	Urease	Dehydrogenase	Nitrification	Respiration
Zn total	0.52 ***	0.40 *	0.54 ***	0.41 **
Pb total	0.45 **	0.27 [n]	0.35 *	0.32 *
Cd total	0.55 ***	0.49 **	0.59 ***	0.45 **
N total	0.70 ***	0.78 ***	0.36 *	0.65 ***
Organic matter	0.64 ***	0.62 ***	0.36 *	0.58 ***
pH	0.25 [n]	0.33 *	0.56 ***	0.10 [n]
Clay	0.56 ***	0.58 ***	0.61 ***	0.36 *

*** significant at 0.001 probability level
** significant at 0.01 probability level
* significant at 0.05 probability level
[n] not significant

Table 6. Correlation coefficients between total or extractable metal contents and their concentrations in plants.

Metal		Soil metal fraction		
		Total	Extractable	
			$CaCl_2$	$Sr(NO_3)_2$
Zn	grain	-0.19 [n]	0.56 ***	0.73 ***
	straw	-0.17 [n]	0.65 ***	0.75 ***
Pb	grain	0.28 [n]	X[1]	X
	straw	0.10 [n]	X	X
Cd	grain	-0.08 [n]	0.34 *	0.23 n
	straw	-0.22 [n]	0.85 ***	0.65 ***

*** significant at 0.001 probability level
** significant at 0.01 probability level
* significant at 0.05 probability level
[n] not significant
[1] Pb extractable with Ca-chloride and Sr-nitrate below detection in most soils

Our results are in agreement with the statement of Angle et al. [27] that total content of metal in soil is not a sufficient measure of its effect on microorganisms.

No significant relationships were found between Zn and Cd extractability and microbial parameters. Lack of toxicity in soils defined as heavily contaminated can be associated with the fact that metals in these soils are present in forms and structures of limited bioavailability. Soluble metals, at the level commonly found in the polluted soils from Silesia, seem not to be toxic to microorganisms. Angle et al. [27] demonstrated that even more sensitive bacteria isolated from contaminated soil are resistant to metal levels higher than those extracted from the contaminated soils. Microorganisms are tolerant to metal-soil contamination and are usually more resistant in more contaminated soil. It is also possible that microorganisms in metal-contaminated soils have developed some adaptation mechanisms. Their resistance may result both from their adaptation ability and the fact that the most sensitive microorganisms die, causing a flush of activity of more resistant species due to lower competition. According to Palmborg and Nordgren [28], changes in population structure in metal-contaminated soils are obvious, but they do not reduce overall soil microorganism activity. However, the lack of toxicity to such specific processes as nitrification (claimed as the sensitive test [29, 30]) in our study suggests low availability of metals in Tarnowskie Gory soils.

Wheat Yield and Composition

The yield of wheat grain ranged from 1.7 to 9.1 g per pot while shoot biomass was also diversified between soils (1.3-12.9 g per pot). Soil pH was the only soil parameter significantly affecting grain yield (correlation coefficient r=0.41, significant at 0.01 probability level). No significant relationships were found for straw yield, including extractable fractions of metals.

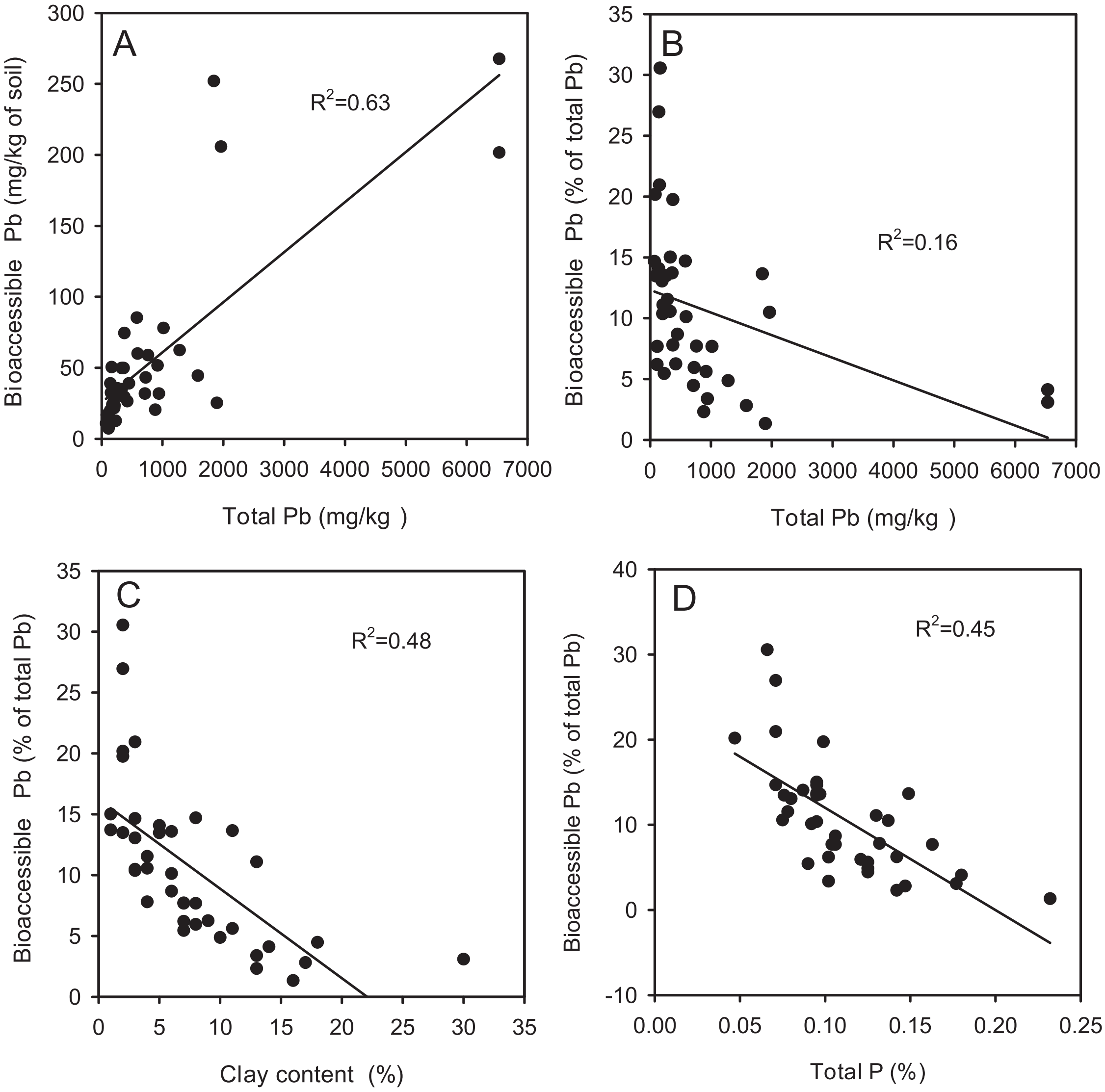

Fig. 4. The relationships between soil properties and Pb in-vitro bioaccessibility.

Lead and cadmium contents in straw were obviously non-toxic to plants: 0.4-12.6 and 0.12-3.9 mg kg^{-1} for Pb and Cd, respectively. Zn contents in straw ranged between 23.4 and 788.0 mg kg^{-1}. The Zn contents above phytotoxicity level (usually 400-500 mg kg^{-1}) were observed only on two acidic soils (pH 4.0 and 4.6).

Straw Zn was correlated with soil pH (Figure 5) but was not correlated to total Zn content in soil (Table 6). Similar results were found for Zn content in wheat grain (Table 6). Total contents of Pb and cadmium also did not correspond to the contents of these elements in wheat straw and grain. There was no correlation between exchangeable Cd (the first fraction in sequential analysis extracted with 1M $MgCl_2$) and the content of this metal in wheat.

The contents of Ca-chloride and Sr-nitrate extractable Zn well predicted the Zn content both in grain and straw (Table 6). A slightly better fit between extractable Zn and plant Zn was found for the Sr-nitrate extraction,

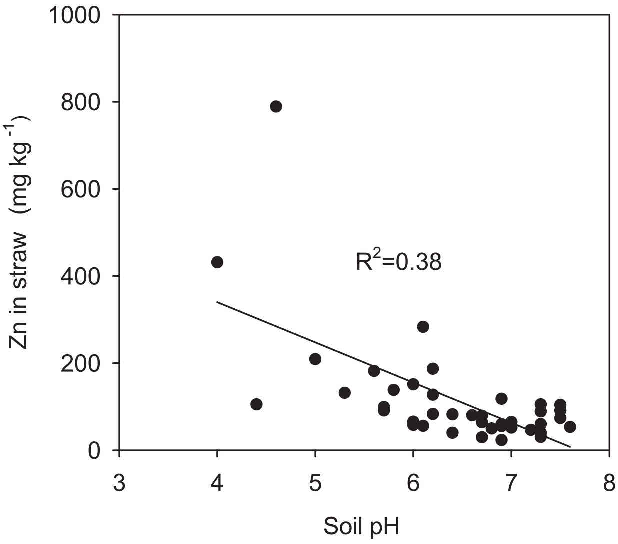

Fig. 5. The relationship betwen soil pH and Zn content in wheat straw.

which confirms the usefulness of this test [6, 23]. High correlation coefficients were found between extractable Cd (higher for Ca-chloride extraction) and Cd content in wheat straw while relationships for Cd in grain were not satisfying (Table 6).

In summary, despite high metal contents in most soils from the studied region, bioavailability of metals is relatively low. Bioavailability of Zn, Pb and Cd as measured using neutral salt extractions, plant metals content or in-vitro Pb test was mostly dependent on parameters other than total metal contents such as pH or clay content. There was no visible relationship between total soil metals and their bioavailability expressed by any measured parameter. The bioavailability of the most contaminated soils of the region is low, which is related to relatively high contents of clay colloids and organic matter in these soils. High sorption capacity in combination with the partly natural character of the metal presence in soils (parent rock material rich with metals) makes the metals less available. Furthermore, metals entering the soil are subject to aging reactions, lowering their mobility over time if soil pH is not decreased [32]. Sequential extraction showed that the greatest fraction of soil Zn and Pb is present in stable forms linked to Fe/Mn oxides or extremely immobile residual forms that may be solubilized using only concentrated mineral acids. In contrast, the most significant fraction of Cd was the exchangeable form. However, this fraction does not strictly reflect the metal availability to plants. Soil contamination with metals did not reduce microbial activity of soils, including nitrification potential which is claimed to be a highly sensitive parameter. The most contaminated soils were the most microbially active, due to their relatively high organic matter and clay contents and neutral pH optimal for bacterial activity.

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