CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

FACULTY OF AGROBIOLOGY, FOOD AND NATURAL RESOURCES

DEPARTMENT OF SOIL SCIENCE AND SOIL PROTECTION

The behaviour and fate of potentially toxic elements of anthropogenic origin in the environment, as influenced by soil properties and various leaching solvents

Ph.D. thesis

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Prague 2016

Acknowledgement

PREFACE

The selected publications presented in this thesis were compiled as part of continuous research activities between 2011 and 2016. The studies are all connected by common subject matter; namely, the investigation of toxic metal/metalloid pollution of the environment (particularly soils), their behaviour and interactions with other compounds in soils or soil solution, such as organic acids or immobilizing amendments, and ultimately their fate, with respect to expressing toxicity toward either plant or animal.

All research was conducted within the Department of Soil Science and Soil Protection at Czech University of Life Sciences in Prague, as part of various grant obligations, as independent research or as a part of the thesis work. Grant providers and co-authors are acknowledged within the respective publications. During the course of the doctoral study, a central team evolved, members of which were responsible for major contributions to the initiation, consultation and development of the research, as well as the practical aspects such as sampling and analysis. Consistent primary contributors to the listed research papers are Doc. Ing. Ondřej Drábek, Ph.D., and RNDr. Václav Tejnecký, Ph.D., with project overview and thesis supervision by Prof. Dr. Ing. Luboš Borůvka.

Industrialisation, and its impacts on the environment both past and present, has manifesed itself globally. Consequently, there is global evidence of elevated concentrations of potentially toxic elements (PTE) in the environment, particularly in soils and sediments, which possess reactive surfaces and therefore create a sink (and potential source) of PTE. The significance of the problem calls for greater insight and understanding of PTE in the environment, and solutions that are novel, green, and cost effective. The presented research considers a range of sources of PTE, such as recreational sources like shooting ranges (Ash *et al.* 2013[b]), PTE contamination in fruit production (Ash *et al.* 2012), and those occuring from industrial sources such as mining and smelting (Ash *et al.* 2013[a]; 2014; 2015, Kváčová *et al.* 2014). A theme that runs central to much of the work is the application of low-molecular-mass organic acids (LMMOA) in experimental research. The objective of studying LMMOA is to elucidate their role in the mobilization and redistribution of potentially toxic elements in the soil environment, particularly where PTE concentrations exceed natural background levels (Ash *et al.* 2016[b]). Furthermore, our research included the identification and testing of materials for the capture of PTE following soil washing, such as recycled shredded card, which has a high anionic charge density and alkaline properties (Ash *et al.* 2016[a]).

The approach for research initiation and development considers the three main phases of toxic element contamination: source > pathway > target. Thus, by focusing on these three dynamic phases, it allowed for the development of a compilation of holistic research during the course of the Ph.D. study.

Ash, C., Borůvka, L., Tejnecký, V., Šebek, O., Nikodem, A., Drábek, O. 2013[a]: Temporal dissolution of potentially toxic elements from silver smelting slag by synthetic environmental solutions. *Journal of Environmental Management*. 129, 157-163

Ash, C., Borůvka, L., Tejnecký, V., Šebek, O., Nikodem, A., Drábek, O. 2014: Potentially toxic element distribution in soils from the Ag-smelting slag of Kutná Hora: Descriptive and prediction analyses. *Journal of Geochemical Exploration*. 144, 328-336

Ash, C., Drábek, O., Tejnecký, V., Jehlička, J., Michon, N., Borůvka, L. 2016[a]: Sustainable soil washing: Shredded card filtration of potentially toxic elements after leaching from soil using organic acid solutions. *Plos One*. 11(2)

Ash, C., Tejnecký, V., Borůvka, L., Drábek, O. 2016[b]: Different low-molecular-mass organic acids specifically control leaching of arsenic and lead from contaminated soil. *Journal of Contaminant Hydrology*. 187, 18-30

Ash, C., Tejnecký, V., Šebek, O., Chala, A.T., Drahota, P., Houška, J., Drábek, O. 2015: Redistribution of cadmium and lead fractions in contaminated soil samples due to experimental leaching. *Geoderma*. 241-242, 126-135

Ash, C., Tejnecký, V., Šebek, O., Němeček, K., Zahourová, L., Bakardjieva, P., Drahota, P., Drábek, O. 2013[b]: Fractionation and distribution of risk elements in soil profiles at a Czech shooting range. *Plant, Soil and Environment*. 59, 121-129

Ash, C., Vacek, O., Jakšik, O., Tejnecký, V., Drábek, O. 2012: Elevated soil copper contents in a Bohemian vineyard as a result of fungicide application. *Soil and Water Research*. 4, 151-158

Kváčová, M., Ash, C., Borůvka, L., Pavlů, L., Nikodem, A., Němeček, K., Tejnecký, V., Drábek, O. 2014: Contents of potentially toxic elements in forest soils of the Jizera Mountains region. *Environmental Modeling and Assessment*. 20 (3), 183-195

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LIST OF ABBREVIATIONS

ANOVA – Analysis of variance

BCR SEP – Community Bureau of Reference Sequential Extraction Procedure

DEFRA – Department of Energy, Food, and Rural Affairs

DI_{H2O} – Deionised water

DOC – Dissolved organic carbon

EPA – (United States) Environmental Protection Agency

FA – Fulvic acid

HA – Humic acid

HPLC – High performance liquid chromatography

KH – Kutná Hora

KOLEP – Project on forest soil pollution (KOntaminace LEsních Půd)

LMMOA – Low-molecular-mass organic acid

PTE – Potentially toxic elements

RSS – Rainwater Simulation Solution

SC – Shredded card

SOM – Soil organic matter

LITERATURE REVIEW

1.1 Pollution of soil with potentially toxic elements (PTE)

1.1.1 PTE sources and concentration guidelines

The term potentially toxic elements (PTE hereinafter) refers to any metal or metalloid that has potential to cause harm to plant or animal when presented to the environment in excessive concentration or dangerous form. Concentrations that are deemed excessive are determined according to regulatory guidelines that are proposed by governing bodies or agencies, based on in vivo experiments, or collated background concentrations of trace elements in natural soils and sediments. PTE is a widely accepted, and generally preferred acronym, and is favoured by most authorities e.g., DEFRA, EPA; as opposed to terms such as "risk elements", as the word "risk" is quite broad and can cause ambiguity, especially when followed by the term "element", e.g., 'there is an element of risk'.

Table 1 lists common PTE, their suggested background concentrations for Czech agricultural soils according to Ministry of the Environment of the Czech Republic (1994), and the origin of their natural occurrence. At the time of writing, a nationwide study "KOLEP" involving numerous institutes and universities was in process, with the aim of establishing background concentrations of PTE specifically for Czech forest soils (e.g., Borůvka *et al.* 2015).

Table 1 Background concentrations for PTE extractable with aqua regia and with 2M HNO_3 in Czech agricultural soils (Ministry of the Environment of the Czech Republic 1994)

Contaminant	Aqua regia		2M HNO_3		Natural occurrence
	Sandy soils	Other soils	Sandy soils	Other soils	
As	10	20	2.0	3.0	Cenozoic lake beds, felsic volcanic rock, areas of gold and uranium deposits (Welch *et al.* 1988)
Be	1.5	3.0	0.5	0.7	Bertrandite, beryl, chrysoberyl and phenakite
Cd	0.3	0.4	0.3	0.4	Soil derived from sedimentary rock ~ 0.3 – 11 ppm (Adriano 2001)
Co	16	25	7.0	13	In association with other metals: Cu, Ni, Mn, As and in coal (MEPI 2001)
Cr	85	130	12	20	As chromite ore of igneous origin in mafic and ultramafic rock (Jacobs and Testa 2004)
Cu	45	70	21	35	Cuprite, malachite, azurite and most abundantly chalcopyrite (Adriano 2001)
Hg	0.3	0.4	-	-	In cinnabar as elemental Hg (Environment Agency 2009a)
Mo	0.8	0.8	0.7	0.7	Molybdenite, wulfenite, powellite and combined with sulphur (Adriano 2001)
Ni	40	60	10	15	Basic igneous rocks and mineralized areas (Environment Agency 2009b)
Pb	60	80	30	40	Galena and in silicate minerals: potassium feldspars and pegmatites (Adriano 2001)
V	80	120	10	20	In coal and with uranium (Irwin 1997)
Zn	90	150	40	60	Metamorphic and igneous rock, largely in iron rich minerals (Adriano 2001)

Values are in mg/kg

Pollution of the environment with PTE is a persistent problem, since, unlike organic pollutants, PTE remain indefinitely without degradation, only altering in their species and location. Numerous sources of PTE pollution can be identified, many of which date back to previous centuries.

Soils in all urban areas are generally contaminated with Pb, Zn, Cd, Sb and Cu from traffic, paint and many other non-specific urban sources (Alloway 2013). In rural settings, sewage sludge application and use of fertilizers and pesticides are reasons for enrichment of soil with

PTE, e.g., lead arsenate ($PbHAsO_4$), copper acetoarsenate ($C_4H_6As_6Cu_4O_{16}$), 'Bordeaux Mixture' ($CuSO_4 \cdot 5H_2O + Ca(OH)_2$), copper oxychloride ($3Cu(OH)_2 \cdot CuCl_2$) and phenyl mercuric chloride (C_6H_5ClHg).

Kabata-Pendias (2000) states that energy and mineral consumption by man is the main cause of trace element pollution in the biosphere. It was suggested by Bowen (1979), that when the rate of mining of a given element exceeds the natural rate of cycling by a factor of ten or more, the element must be considered a potential pollutant. Accordingly, the following metals are listed as the potentially most hazardous in the biosphere: Ag, Au, Cd, Cr, Hg, Mn, Pb, Sb, Sn, Te, W, and Zn.

In central Europe, the region termed 'the Black Triangle' corresponds to an area of concentrated burning of fossil fuels during the period between early 1950s up to the mid 1980s. Although major polluting activities (fossil fuel combustion and pyrometallurgic industry) have ceased, effects of atmospheric deposition are still evident (e.g., Markert *et al.* 1996, Knom *et al.* 2009, Sucharova *et al.* 2011, Kváčová *et al.* 2014), and have caused an observable decline in the health and quality of large areas of forest (Borůvka *et al.* 2007).

Smelting of metal rich ores in Central Europe can be dated back to the early Bronze Age, approximately 3000 BC. Evidence suggests that high purity copper was utilized first, as these native copper or oxidic Cu ores were the most accessible. Later, smelting techniques evolved as deeper sulphidic ores and mixed As/Cu ores became obtainable, and often involved a multi-stage roasting prior to smelting (Fokkens and Harding 2013). In many areas of Europe, these primitive on-site smelting practices continued into the 18th Century. Consequently, atmospheric depositions of PTE from smelting, as well as surface depositions of metal rich slags are reasons for extensive contamination in regions with a history of mining. The extensive use of lead throughout human history has resulted in substantial pollution of surface soils on the local scale, with mining and smelting among the main contributors (Alloway 2013). Although more modern smelting techniques may have included some attempt at containment of the pollutants (e.g., stack filters), samples of soil adjacent to lead smelting sites have revealed lead concentrations in excess of 50,000 mg/kg (Vidic *et al.* 2006, Safar *et al.* 2014).

Lithogenic sources of PTE in the soil parent material can vary considerably, depending on the geological character of the area. Large-scale soil contamination can be the result of naturally accelerated bedrock concentration or dust deposition (Meuser 2013). However, such element

concentrations in the parent material should be regarded as a background content when trying to identify pollutants; therefore, when studying PTE in soils, consideration is usually given to their geogenic occurrences, which are unique on a site-by-site basis.

1.1.2 Mining and smelting as a source of PTE in Kutná Hora (KH)

The town of Kutná Hora was chosen as a locality that is representative of an area heavily polluted with PTE due to smelting and mining activities. According to Pauliš and Mikuš (1998), the earliest significant exploitation of silver deposits in the Czech Republic date back to the second half of the 13th century, and by the beginning of the 14th century KH had grown into one of the largest mining towns in Europe. In the first half of the 14th century, annual Ag production stood at 5 – 6 tonnes (with 50 – 100 tonnes of Cu obtained), 90% of which came from mining in KH. Mining activities had started to see a gradual decline by the 15th century, and despite a revival in Ag mining in the 16th century, remaining loads were of lower ore content with annual Ag production reduced to 2 – 4 tonnes, leading to an eventual decline of all mines in KH at the turn of the 17th century.

The geological sub-surface of KH consists of metamorphic rocks, mainly different types of gneiss, and also mica-schists, quartzitic erlan/calc-silicate rocks, and migmatites. Upper layers are made of platform sediments, sandstones and organodetrital limestones/coquina of the Kolín region of the Bohemian Cretaceous Basin, originating from the Cretaceous age. The large-scale ore exploitation, which begun in the early 15th century was due to the development of new smelting technology. This technology used some sulphides as amendments for silver ore smelting. These sulphides contained small or trace amount of arsenic pyrite (As - FeS_2), sphalerite (ZnS) and galenite (PbS). Moreover, with these sulphides some As minerals (alacranite, allargentum and arsenopyrite) also enter the smelting process. Thus, the smelting activities became a massive source of contamination for the entire surrounding environment. The large number of dumps of waste rock and slag contain not only primary minerals of As, but also secondary minerals. Secondary As minerals such as bukovskyite ($Fe^{3+}_2(AsO_4)(SO_4)(OH)$), pitticite ($Fe^{3+}_{20}(AsO_4,PO_4,SO_4)_{13}(OH)_{24}\cdot9H_2O$), and scorodite ($Fe^{3+}(AsO_4)\cdot2H_2O$) were created by weathering of arsenopyrite, and also zykaite ($Fe^{3+}_4(AsO_4)_3(SO_4)(OH)\cdot15H_2O$), kankite ($Fe^{3+}(AsO_4)\cdot3.5H_2O$), and parascorodite ($Fe^{3+}(AsO_4)\cdot2H_2O$) (Bernard and Rost 1992, Fleischer and Mandarino 1991, Pauliš 1999;

2003). A HNO_3 extraction of crushed slag fragments from a KH surface deposit revealed mean PTE concentrations of 10.9 mg/kg Cd, 742 mg/kg Mn, 848 mg/kg Pb, and 24389 mg/kg Zn (Ash *et al.* 2013[a]).

Today, substantial evidence of the towns' industrial and mining past remains. The surrounding landscape of KH is testament to an intensive mining history as evidenced by a network of mineshafts, excavated overburden and spoil heaps, unconfined smelter slag deposits, and large sinkholes caused by tunnel collapses. Slag fragments from the smelting industry litter the region, as it was either dumped to form unconfined heaps, or used in road construction, and even applied to fields as a primitive fertilizer (source of Ca and Mg). In a study by Manasse and Mellini (2002), an in depth analysis of smelter slag produced from base metal smelting (including Ag) identified CaO as one of the major components (> 10% on average), with significant amounts of MgO and K_2O also present.

Unconfined deposits of slag and mining wastes threaten the wider surrounding environment due to eolian deposition of small particles that are heavily loaded with labile metal/metalloids. Therefore, such areas are of interest for study by soil scientists. Králová *et al.* (2010) studied soil in the vicinity of mining wastes at KH, where they observed excessive concentrations of As, Cd, and Zn. Ash *et al.* (2014) conducted a detailed study of the PTE concentrations in soils on and around a major slag deposit in Kutná Hora. Despite determining very high loadings of the studied PTE in soil samples, plant available contents were generally low, which concurs with the findings of Száková *et al.* (2009). Horák and Hejcman (2015) used available data on toxic element distributions in the KH region to group the present PTE based on their likely sources; those uninfluenced by mining activities (Be, Co, Cr, Hg, and V), those originating from smelting processes (Cu, Pb, Zn), and those originating from mining (As, Cd).

1.1.3 Speciation of PTE in the soil environment

Once PTE have entered the soil, by atmospheric deposition, by surface deposition with contaminated floodwaters, or by dissolution of metal bearing wastes and slags, the physicochemical conditions will quickly determine the fate of the PTE. Many forms of an element can occur in soil simultaneously to make up the total concentration. Ions bound in the crystal structure of primary and secondary minerals would generally be considered to be

of geogenic occurrence, unless present as part of an anthropogenic intrusion, such as smelting wastes. Other PTE of anthropogenic origin may become adsorbed on the surfaces of secondary minerals such as clays, oxides and carbonates, bound in solid-state organic matter, or can exist as free ions and soluble organic and inorganic complexes in soil solution (Alloway 2013). The latter soluble species account for the toxic portion of the PTE, as they are readily available for plant uptake or consumption by soil organisms, and can be potentially leached into nearby water sources. Availability is affected by many factors including pH, redox conditions, the presence of competitive ions, types of colloid surface, available water content, and climatic conditions such as temperature and vegetation (Adriano 2001, Alloway 2013).

Manipulation of the chemical conditions in soil in order to detoxify PTE may result in contradictory reactions. For example, arsenite (As^{3+}) can be oxidized to form As^{5+}; this is the preferred ionic species, as arsenates (e.g., AsO_4^{3-}) are known to be readily fixed by soil components such as clays, humus, calcium, and most substantially by hydrous oxides of Fe and Al (Kabata-Pendias 2001). However, in the case of chromium, the reverse is true. Hexavalent Cr^{+6} is the more toxic and mobile species, therefore, objectives for the detoxification of Cr in soil typically include a change of conditions that favour the reduction of Cr^{6+} to Cr^{3+} (e.g., Patterson and Fendorf 1997, Xu *et al.* 2004).

Chemical equilibrium models can be used for the calculation of PTE speciation in solution, e.g., Visual MINTEQ and PHREEQC; These models can provide an estimate of the binding of ions to solid phase (hydr)oxide surfaces and organic matter. Alternatively, a multi-stage sequential extraction procedure (SEP) can be performed on soil samples in order to determine the speciation of an element according to its operationally defined fractions. Many SEPs have been described, but typically, they are modified versions of the original method by Tessier *et al.* (1979). The studies presented in this thesis work utilize a modified Commission of the European Communities Bureau of Reference (BCR) SEP according to a method described by Rauret *et al.* (1999): Target metals are extracted from soil samples in the following successive stages: weakly bound metals (exchangeable) are released into 0.11 mol/L acetic acid. Metals bound to Fe/Mn oxides (reducible) are extracted using 0.1 mol/L hydroxylamine hydrochloride. Metals bound to organic matter and sulphides (oxidizable) are liberated by oxidizing the sample in 8.8 mol/L hydrogen peroxide, followed by dissolution into pH adjusted 1.0 mol/L ammonium acetate. Metals bound in residual soil (residual) are dissolved in a heated mixture of 12.0 mol/L HCl and 14.4 mol/L HNO_3.

1.2 Features of temperate forest soil layers

Forest soils are well defined, and are easily distinguished from other soil groups, due largely to the high turnover and accumulation of soil organic matter in the upper soil layers. Soil organic matter (SOM) occurs in solid, colloidal, and soluble forms in distinct O horizons at the forest soil surface, and intimately mixed with mineral horizons. SOM comprises the majority of the forest floor O horizon, and constitutes between 1 – 15% of upper mineral soil horizons. The accumulation of SOM, and eventually stable humic substances, not only influences the development of soil properties such as soil structure, hydraulic conductivity, and bulk density, but also contributes the majority of the ion exchange complex in forest soil (Binkley and Fisher 2013).

The degree of mixing at the transition between the organic O (L, F and H layers) and mineral A/B horizons depends on factors such as the activity of macrofauna, which are capable of physically disturbing the soil. Accordingly, the organic layer of forest soil is sub-classified; Mor types have a distinct clear boundary between organic and mineral soil, Mull types show a high degree of blending with smoother transitions, and Moder types show signs of animal mixing (Ponge *et al.* 2010). Because the SOM represents such a significant source of cation exchange in forest soils, the degree of mixing is important for the depth of retention of anthropogenic inputs of PTE.

The O horizon is further divided into three structural layers, with less processed fresh litter at the surface, moving down to more fragmented humic material with increasing depth, with highest content of humus at the bottom of the O horizon (Oh layer), or in the transition from the O to A layer (Delft *et al.* 2006). Stable humus is characterized by more polymerized substances that are of higher pH, and have a greater humic to fulvic acid ratio when compared to younger SOM. However, with even further profile depth, a greater fulvic to humic acid ratio can be observed, due to higher solubility of fulvic acids (Brady and Weil 2004). This potentially brings PTE into deeper layers, where they are essentially stored in the soil.

In deeper, subsurface soil horizons, the presence of clays and oxides of Al, Fe and Mn become more abundant, reflecting the areas underlying geology and/or other local parent materials. In these layers, the contribution of secondary minerals toward the exchange

complex may be of greater significance than that of the functional groups of organic compounds. An important consequence of this is that hydrous oxides of Fe/Al are effective in the immobilization of anions such as particular forms of As and P, and are also known for their strong sorption of low-molecular-mass organic acids (LMMOA) (Ali *et al.* 2011, Texas A&M 2015).

1.3 Humic substances and simple organic acids

1.3.1 Humic and fulvic acids

Humic acids (HA) are defined as the fraction of humic substances that is not soluble in solution at pH < 2, whereas fulvic acids (FA) are soluble in water at all pH values. HA can also be differentiated from FA due to certain chemical and physical properties; when compared to FA, HA show a higher degree of polymerization, greater molecular weight and increased carbon content, but lower oxygen content (Stevenson 1994). Humic substances constitute 60 to 80% of the whole soil organic matter. They are suggested to be comprised of huge molecules with variable, complex structures characterized by many aromatic rings (Brady and Weil 2004). However, Piccolo (2001) offers an alternative characterization of the conformational nature of humic substances; in a critical review, it is proposed that, rather than existing as polymers in soil, humic subsatnces are supramolecular associations of self-assembling heterogeneous and relatively small molecules, derived from the degradation and decomposition of dead biological material. Unlike the simple LMMOA, humic acids and fulvic acids are more complex and stronger in their acidity, due to the multiple carboxylic and phenolic groups to which metal ions are firmly bound. Because HA and FA are highly heterogeneous in their composition with variable structure, it is not possible to describe these compounds using a single structural formula (Karpukhin and Bushuev 2007). However, it is suggested that humic materials consist of a skeleton of alkyl/aromatic units cross-linked mainly by oxygen and nitrogen groups with the major functional groups being carboxylic acid, phenolic and alcoholic hydroxyls, ketone and quinone groups (Dube *et al.* 2001).

Differences in solubility are one of the most important features regarding PTE transport in soils. HA are strongly sorbed to clay minerals and, due to their greater molecular mass, are

not so mobile in soil compared to FA (Dube *et al.* 2001). Because of their solubility in water, FA form more soluble metal-complexes. Humic acids on the other hand tend to produce more insoluble inner-sphere metal complexes (Stevenson 1994, Dube *et al.* 2001). For this reason, HA have been applied to contaminated soils with the aim of binding and immobilizing PTE. In a study by Chaturvedi *et al.* (2006), HA were shown to be a particularly effective soil amendment for the sorption of lead. In a study by Borůvka and Drábek (2004), the fulvic acid fraction was shown to account for 98.4, 82.0, and 95.7% of total organically bound Cd, Pb, and Zn respectively in fluvisol samples that were contaminated due to atmospheric deposition of smelter emissions followed by flooding. Potential inorganic ligands in sub-surface aqueous solution are nitrates, sulphate, phosphate, chloride and (bi)carbonate. Even though their concentration is relatively high (millimolar), they are weak ligands with minimal effect on metal complexation, whereas organic compounds of biological origin present in the surface aqueous solution comprise both weak and strong metal complexing ligands; humic and fulvic acids are described as the main natural ligands acting in the subsurface aqueous solution (Berkowitz *et al.* 2008). Because of their solubility and chelating behaviour, FA and HA (mainly as a peat extract) have been marketed as a nutritional supplement in human diet (e.g., Fulvolite™), and in agricultural production, particularly in hydroponics. In both cases, the purpose is to increase the solubility of minerals to enhance their uptake into living cells.

1.3.2 Low-molecular-mass organic acids

Low-molecular-mass organic acids (LMMOA) are simple carboxylic acids of low molecular mass. Their occurrence in the environment and in organisms is extensive; in soils, the conditions of release are largely provided by the slow decomposition of abundant soil organic matter and are usually confined to the upper portion of weathering profiles (Gardner 1982). LMMOA in soil have a number of other sources, including microbial organisms, plant roots, and fungi. Table 2 lists LMMOA present in the soil environment according to Strobel (2001).

Table 2 LMMOA of mono-, di-, and tri-carboxylic structure, commonly found in soil solutions, adapted from Strobel (2001)

Organic acid	Molecular weight	Dissociation (pKa)
Formic	46.03	3.75
Acetic	60.05	4.76
Propionic	74.08	1.84
Butyric	88.11	4.82
Valeric	102.14	4.84
Lactic	90.08	3.86
Pyruvic	88.06	2.39
Oxalic	90.04	1.25; 4.19
Malonic	104.06	2.85; 5.70
Succinic	118.09	4.21; 5.64
Malic	134.09	3.40; 5.20
Tartaric	150.09	2.89; 4.40
Fumaric	116.08	3.03; 4.44
Maleic	116.08	1.92; 6.07
Citric	192.43	3.13; 4.76; 6.40
Aconitic	174.11	2.80; 4.46 (trans isomer)

Rhizosphere soil differs considerably from bulk soil, as its chemical composition is altered significantly either directly by the activity of plants roots, or by the enhanced population of microflora, with LMMOA being among the most important root exudates (Walker *et al.* 2003). Due to the weak acidic nature of LMMOA, these substances are important in many chemical reactions and processes in soils and soil solutions (Strobel 2001), such as the mobilisation of plant nutrients (e.g. Fe, P) and in the protection of plants roots when exposed to rhizotoxic concentrations of Al^{3+} in acid soils (Van Hees *et al.* 2002). LMMOA in soil function as ligands, increasing the total amount of dissolved cations in soil solutions by chelation/complexation (Hue *et al.* 1986, Fox 1995), therefore complexes of LMMOA with metals are readily transported in soil pores through the soil profile (Strobel 2001). It has been

suggested that organic acids may also promote desorption of metals by dissolving the minerals that adsorb the metal (Collins 2004, Tao *et al.* 2006, Ash *et al.* 2016). The extractability of PTE in rhizosphere soil is influenced by the ionic species and contents, which are dependent on the pH and chemical compositions of root exudates, such as LMMOA (Chiang *et al.* 2006).

LMMOA in soil occur as products of many microbial and biological functions, but the most significant and concentrated are those that are present in rhizosphere soil as exudates of mycorrhiza and plant roots. The importance of organic acid exudates in the transport of metals is particularly evident in hyper-accumulating plant species, which have been shown to contain high endogenous levels of organic acids (Boominathan and Doran 2003). An assessment of the Cd-hyper-accumulator *Solanum nigrum* revealed a lower soil solution pH, increased contents of LMMOA in the root exudates and greater Cd translocation and accumulation than a control plant, thus emphasizing the significance of LMMOA on metal mobility (Bao *et al.* 2011). The major sources and sinks of LMMOA, and pathways for their mineralization are shown in Figure 1. The figure shows the loss of LMMOA from the soil system as CO_2 upon mineralization. In addition to being subject to microbial decomposition, LMMOA can also dissociate by means of photolysis reactions when exposed to UV light.
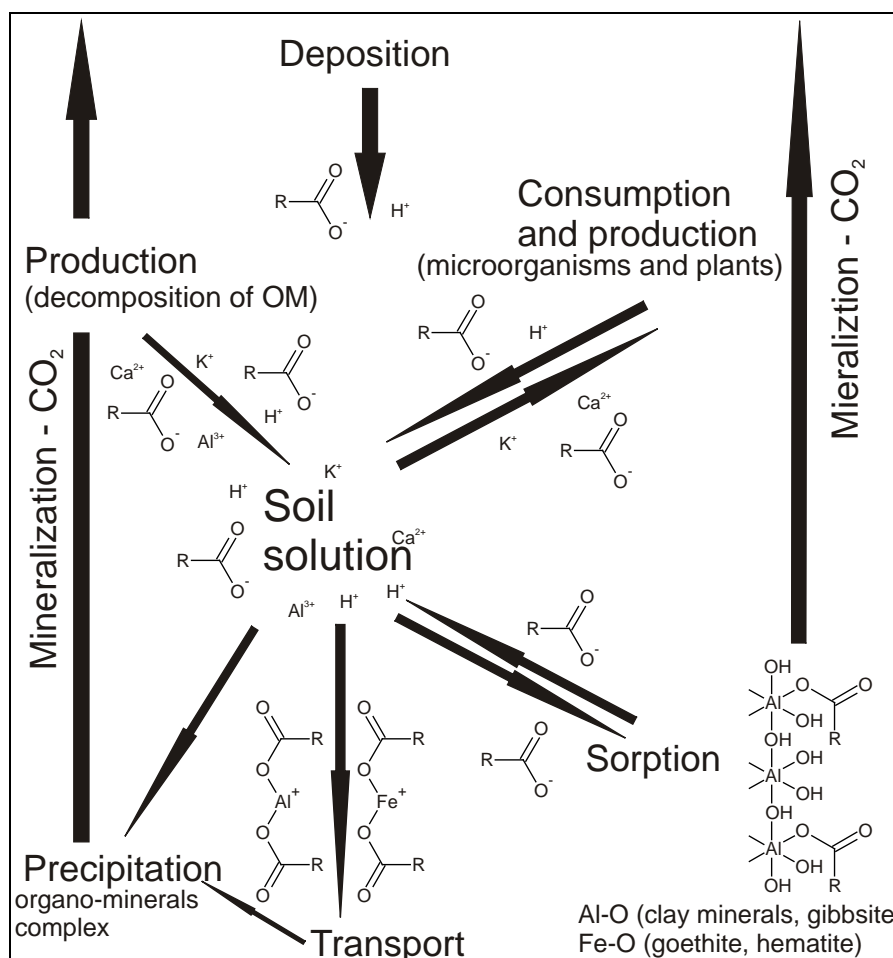
Figure 1 Sources and sinks of LMMOA in soil, and possible pathways for their transformation, according to Tejnecký *et al.* (2014)

In the soil environment, concentrations of LMMOA are generally dependent on variables such as vegetation, season and mineral affinity. Levels reported for different acids vary between nM to mM, and soil solution concentrations of the dominant organic acids can range from 1 to 100 mM (Van Hees *et al.* 2002; 2005). However, in common soil solutions, the concentrations of LMMOA are suggested to be usually up to 1 mM (Ettler *et al.* 2004). Collins (2004) also states in a review of LMMOA separation techniques, that soil solution concentrations of mono-carboxylic acids commonly occur up to 1 mM. Di- and tri-carboxylic acids on the other hand are usually found at much lower concentrations of <50 µM, except for in the rhizosphere, where concentrations can be higher. Tejnecký *et al.* (2016) studied the temporal differences of LMMOA (lactate, acetate, formate, oxalate) concentrations in organic

and organo-mineral horizons of beech and spruce forest. The month of sampling was shown to be a significant factor on the amount of measured LMMOA, with highest variability observed for oxalate (maximum in Apr/May, minimum in Jun/Jul). Strongest seasonal differences of LMMOA content were found in the organic O horizon as compared to the deeper A/B horizon.

The list of LMMOA in soils is somewhat extensive (Table 2), and so the selection and combination of organic acids chosen for study is seldom uniform. Almost every plant root on the planet is expected to have a chemically, physically and biologically unique rhizosphere (Jones and Hinsinger 2008). In an assessment of temperate rainforest soils, Chiang *et al.* (2006) found that the contents of LMMOA in soil samples showed the following trend: oxalic acid > succinic acid > malonic acid > fumaric acid, with the content of oxalic acid accounting for more than 60% of the total LMMOA. The most commonly identified LMMOA in coniferous forest soils are oxalic, citric, acetic, and formic acids (Van Hees *et al.* 2005). A more recent study, focusing on the extraction techniques of LMMOA from a podzol soil O and E horizon, determined that citrate and isocitrate were the most abundant acids in the aforementioned horizons respectively (Ali *et al.* 2011). Citric acid was also the most common acid to be found underneath six North American tree species, often occurring with lactic acid (Dijkstra 2001). The low-molecular-mass aliphatic organic acids extracted in water and present in soil solution from O, A, Bh and Bt horizons from a group of forested Ultisols, Entisols and Spodosols were identified by high performance liquid chromatography (Fox and Comerford 1990). Oxalic acid was found in all samples and was present generally in the highest concentrations. Oxalic acid concentrations in soil solution ranged from 25 to 1000 µM*,* and were greater in the Bh and Bt horizon soils than in the A horizon. High concentrations of formic acid were also identified in most soils, ranging from 5 to 174 µM in soil solution. Trace amounts of citric, acetic, malic, lactic, aconitic, and succinic acids were detected in some samples.

1.3.3 Low-molecular-mass organic acid metal complexes

LMMOA that occur as exudates from roots and fungi, decomposing organic matter residues and microbial secretions can influence metal solubility and uptake through their indirect effects on microbial activity, and direct effects through acidification, chelation, precipitation,

and oxidation-reduction reactions (Uren and Reisenauer 1988). The complexes formed between metal ions and inorganic ligands are much weaker than the complexes formed with organic ligands (Stumm and Morgan 1981). The affinity for metals exhibited by LMMOA is due to their carboxylic functionality (Berkowitz *et al.* 2008). However, increased metal adsorption onto soil in the presence of LMMOA has also been described (Schwab *et al.* 2005, Li *et al.* 2006). Furthermore, if a free metal cation is the only biologically active form of an element, then metal-organic complexation reduces free metal cation concentrations, resulting in lower bioavailability of the element. If the metal becomes toxic in high concentrations, organisms may produce organic acids to enhance the formation of metal-organic complexes to lower toxicity (Prapaipong *et al.*1999). Despite this, a complexed metal in the subsurface behaves differently than the original species, in terms of its solubility, retention, persistence and transport. In general, the status quo is that a complexed metal is more soluble in water, less likely to be held on the solid phase, and more easily transported through a porous medium (Berkowitz *et al.* 2008).

The effect that LMMOA have specifically on Al mobility in soils has been studied (Jones *et al.* 1996, Van Hees *et al.* 2000, Li *et al.* 2006). Results showed that certain acids, particularly citric, oxalic, and malonic acids, have strong Al-complexation capacity and are effective in Al mobilization, whereas weaker organic acids promoted the retention of mobilized Al by the soil exchangeable sites (Li *et al.* 2006). Malic acid in particular was shown to be an inducible root mechanism for the detoxification of rhizospheric Al^{3+} in acidic soil, with malate predominantly complexing Al in solution in favour of other metal cations with a high degree of stability (Jones *et al.* 1996).

LMMOA are degraded relatively quickly in soil, and have half-lives ranging from 0.5 to 12 hours (Jones *et al.* 2001). Among other factors, rate of mineralization is dependent upon microbial biomass and community structure (Jones 1998), and sorption processes have been shown to significantly control the decomposition rates of organic acids by reducing mobility and bioavailability (Van Hees *et al.* 2003). Degradation of LMMOA complexed with heavy metals has been described by Renella *et al.* (2004); their results suggest that the trend of degradability depends on the metal with which the organic acid is complexed. Biodegradability ranked in the following order Mg > Zn ≈ Pb ≈ Cu > Cd with metal complexes with citrate being generally more degradable than oxalate-metal complexes.

1.3.4 Application of organic acids in soil washing, treatment, and leaching experiments

The potential for LMMOA use in remediation of soils contaminated with PTE by leaching them out of the soil has been recognized. Schwab *et al.* (2008) investigated the mobility of Cd, Pb, and Zn by various LMMOA at 10 mM concentrations by means of thin layer chromatography. Citric acid proved to be the most effective, showing highest frontal distance (R_f) values for all three elements, followed by malic, fumaric and tartaric acids for Zn, Cd and Pb respectively. For most organic acids studied, R_f values followed the trend Zn > Cd > Pb (Schwab *et al.* 2008). Wasay *et al.* (2001) used salts of weak organic acids (citrate, tartarate or oxalate-citrate) in a column leaching experiment. Their results showed that citrate and tartarate effectively removed heavy metals, particularly Pb and Cd, from three polluted soils while leaching little macro-nutrients and improving soil structure. Not only do LMMOA influence speciation, mobility and extractability of metals, but also influence transformations of Al and Fe in soil, thus affecting the structural stability and texture of soil, as can be observed in Podzol type soils (Brady and Weil 2004, Chiang *et al.* 2006). Results from leaching tests by Ash *et al.* (2016) also confirm the dissolution of mineral oxides in the presence of organic acids, as evidenced by correlation of Fe/Al with extraction of As by LMMOA solutions at varying concentration. The efficiency of As release was determined not by the number of carboxyl groups on the LMMOA, but rather by their acidity (pKa dissociation constant). Overall, the impact of rhizospheric processes on the speciation and extractability of metals is not well understood, and the impacts of rhizosphere chemistry on the forest ecosystem require further study (Chiang *et al.* 2006).

Due to certain properties of organic acids, there is a demand for their isolation and application in some non-toxicity related soil remediation situations, for example, in the treatment of alkaline soils, particularly in dry semi-arid or arid regions. By incorporating organic acids into irrigation water, mobile Ca^{2+} and Mg^{2+} displace Na^+, improving soil structure. Irrigation water then allows the organic acids to combine with or leach salts into deeper horizons where they cannot affect plant growth (SRT 2012). Among many of the fertility issues relating to the availability of phosphorus for plant uptake, organic acids can interfere with the pathways of P availability in the following ways. Firstly, organic acids produced by plant roots and microbial decay, which serve as organic anions, are attracted to positive charges and hydroxyls on the surface of clays and hydrous oxides, therefore competing with P ions for fixation sites. Secondly, organic acids can entrap reactive Al and

Fe in stable chelates, rendering them unavailable for reaction with P ions in solution (Brady and Weil 2004). Conversely, because phosphate is known to be strongly adsorbed to mineral soils (Ali *et al.* 2011), phosphate compounds have been used as an agent for the extraction of LMMOA from soil samples (Chen *et al.* 2001, Van Hees *et al.* 2003, Ali *et al.* 2011). Other poorly soluble nutrient elements that organic acids have been suggested to mobilize include Mn, Cu, Zn and Fe (Jones *et al.* 2003).

One of the criticisms identified in the analysis of LMMOA is that quantification of these acids in soil solution may be vastly underestimated as they do not reveal the large spatial heterogeneity that may exist in their concentration, e.g., around roots or microbes. Moreover, there is a knowledge gap concerning the utilization of organic acids by micro-organisms and the forms that they are capable of degrading, e.g., metal-complexed organic acids (Jones *et al.* 2003). Other discrepancies in the analysis of LMMOA have been observed; for example, Drábek *et al.* (2015) showed that the identification of LMMOA in solution by means of HPLC becomes distorted due to co-elution of organic acids that have formed complexes with soluble Al.

Due to the potential of LMMOA to form soluble complexes with various metals, a number of researchers have focused on their application in soil leaching experiments. The effects of selected LMMOA on desorption of Cd and Pb have been studied by Qin *et al.* (2004), but with variable initial solution pH at constant LMMOA concentration (0.01 mol/L). The order of desorption of metals followed the descending order citric acid > malic acid > acetic acid, which corresponds to both the number of organic acid functional groups and pKa values (3.13, 3.40, and 4.76 respectively). Furthermore, citric acid was capable of releasing metals effectively from soil at a higher pH range (maximum desorption at pH 6) than malic and acetic acids (maximum desorption at pH 3). Studies by Ettler *et al.* (2003) were conducted to simulate the presence of vegetation on metal contaminated mine wastes and the effect that root exudates have on the mobility of metals. Preliminary leaching tests were performed using citric solutions (20 and 8 mM) in order to determine the kinetics of release of Pb, Cd, Zn and As. At the lower (8 mM) concentration, despite initial extraction of Pb and Zn, after 200 hours no metals were detectable in the citric acid solution, possibly due to decomposition of citrate, resulting in re-adsorption or precipitation of metals. Ash *et al.* (2013[a]) experienced similar results, whereby at low concentration (1 mM), citric acid solution had shown high decomposition rates and poor extraction of PTE (Cd, Mn, Pb, Zn) compared to a pH adjusted water solution. In a later leaching study by Ettler *et al.* (2009), acetic and oxalic acids were

included in addition to the citric acid. It was then identified that acetic and oxalic acids released the higher amounts of metal, this time at much lower (500 µM) concentrations, contradictory to findings in other studies that have stated citric acid as being the most effective of the LMMOA in metal mobilization (Qin *et al.* 2004, Li *et al.* 2006). Reasons were attributed to complex formations being dependent on pH. However, it was concluded that at 500 µM concentrations, all of the investigated LMMOA were shown to influence the mobility of metals released into soil solutions (Ettler *et al.* 2009). Schwab *et al.* (2008) found citric acid to have the highest frontal distance (R_f) values from a number of studied LMMOA, but when applied to a column leaching study, the citric acid had minimal impact on the movement of Pb. It is in contrast to findings by Ash *et al.* (2016), whereby citric acid was a far superior reagent for leaching of Pb in both organic and mineral soil layers, as compared to oxalic and acetic acids. The enhanced leaching of Pb by citric acid was attributed to formation of stable Pb complexes, which only citrate is capable of. Fedje *et al.* (2013) established that the final pH in the leachate strongly influences the metal leachability. Their results showed that a pH < 2 is needed to achieve a high leaching yield, while <50 w% of most metals were leached when the pH was higher than 2 or below 10. In a batch soil washing experiment by Li *et al.* (2012), the removal of Cr specifically from individual operationally defined fractions (4 step BCR SEP) by citric acid/sodium citrate was identified. Their results showed that Cr was leached primarily from oxidizable and residual fractions, and that Cr was redistributed to the acid extractable fraction. This result indicated that soil washing by citric acid may be useful for the transfer of Cr fixed in strongly bound fractions to more easily extracted fractions, possibly as a precursor to secondary washing with less destructive solvents. Moon *et al.* (2012) found that tartaric and oxalic acids of 1 M concentration were effective at removing significant amounts of Zn from contaminated soil, but increasing concentration to 2 M did not yield any added increase in Zn dissolution for any of these acids. Despite the promising results of batch soil washing using 1 M tartaric and oxalic acids, which removed 83.9% and 73.9% of total Zn from soil, the residual Zn content in soil after washing did not meet the regulatory limit of the country where the study was conducted (Republic of Korea). Only the studied inorganic acids ($HCl > HNO_3 > H_2SO_4 > H_3PO_4$) were able to remove Zn to a level of acceptance. Fedje *et al.* (2013) studied a range of solvents, which included lactic acid, for a 2 h soil leaching experiment. Although the 1 M lactic acid solution showed a significant removal of Cu from samples, one of the key findings presented was the importance of pre-treatment of samples prior to washing. For example, the

incineration of samples to oxidize organic matter ,thus assumingly liberating metals that can then be more easily leached, only served to encapsulate the Cu, rendering it less soluble.

1.4 Column and batch leaching experiments

Column leaching experiments are a commonly used tool for the simulation of a given solution percolating through a porous medium, as the mobilization mechanisms are suggested to be close to those found in the field (Jean-Soro *et al*. 2012). To a certain extent, they allow the user to determine the level of binding and mobility of trace metals through applied leaching of the solution of interest; metals dissolved in the extract solution represent an environmental hazard and metals left in the soil following soil washing/leaching are likely to be present in chemically-stable mineral forms and bound to non-labile soil fractions (Leštan *et al.* 2008). It is therefore possible to make predictions about these interactions in the environment based on the outcome of the experiments. The leaching process can be defined by the five stages shown in Table 3.

Table 3 Liquid-solid leaching stages (Van Der Sloot 1997)

| Stage	Description
1	The solvent is transferred from the bulk solution to the solid surface
2	The solvent penetrates or diffuses into the solid (intra-particle diffusion)
3	The solute dissolves from the solid into the solvent
4	The solute diffuses through the mixture to the surface of the solid (intra-particle diffusion)
5	The solute is transferred to the bulk solution

Transfer of solute into the bulk solution (stages 3 – 5) depends on the carboxyl groups of LMMOA to participate in ligand exchange between a H^+, and the metal at the solid surface. Thus, for a favorable release of metals with time, unreacted solvent (ligands of organic acids) are needed to be constantly replenished at the solid/liquid interface. A dynamic column flow type leaching experiment is advantageous over a batch experiment, as the solvent is continually replacing solvent that has already reacted with metals, thus increasing the rate of metal leaching. Leaching experiments have frequently proven to be an effective tool for the simulation of solutions (rainfall, or a chelant such as surface produced organic acids) percolating through a porous medium in the environment e.g., leaching through a soil profile. This can be implemented in a hydro-dynamic way, such as in column leaching experiments (Schwab *et al.* 2008, Jean-Soro *et al.* 2012, Zou *et al.* 2015, Ash *et al.* 2016) or by creating a series (batch) of hydrostatic vessels, which are analysed at predetermined times (Ettler *et al.* 2003; 2009, Ash *et al.* 2013[a]); both have advantages and disadvantages. Column experiments represent a mobile scenario with solvent passing the soil sample, and the possibility to collect a number of samples as frequently as required, without the need of relying heavily on interpolation to plot results. On the other hand, due to the continuous consumption of solvent and the demand for the continual presence of an operator, column experiments are not practical for performing over long periods of time. In contrast, with respect to the time to equilibrium, batch experiments can be designed with an indefinite time limit. However, because the reaction is hydrostatic and confined, it has to be assumed that variables such as redox reactions, pH, decomposition of organic acids etc. will be different to how they may be in a hydrodynamic situation. Generally, batch experiments are carried out to attain some state of equilibrium or quasi-equilibrium. Column experiments usually simulate field conditions such as fluid flow, mass transfer and dissolution mechanisms in a more realistic manner (Van Der Sloot *et al.* 1997). As the use of column experiments has progressed, adjustments have been made to improve the accuracy of results and reduce chemical interferences. Researchers have employed a number of different methods for sample introduction when making column leaching experiment, including the use of peristaltic pumps (Jean-Soro *et al.* 2012), dosing or proportioning pumps (Schwab *et al.* 2008), and gravity fed leaching (Hodson *et al.* 2001).

Prior to introducing experimental solvents of EDTA or citric acid into a column of Ni and Cr contaminated sample, Jean-Soro *et al.* (2012) pre-conditioned the sample by passing deionised water through the solid sample until pH, conductivity and absorbance had reached a state of equilibrium. This equilibrium was achieved in <200 V/Vp (sample pore volumes).

The same experiment also featured variables such as alternating the flow with chelant/deionised water in sequences, and applying deionised water continuously after applications of the test solvents.

AREAS OF STUDY

The research presented in this thesis was conducted on five different localities. The first location shown in Figure 2 is a vineyard in northern Prague, which was used for collection of a number of samples as part of earlier research dating back to 2010 (Ash *et al.* 2012).
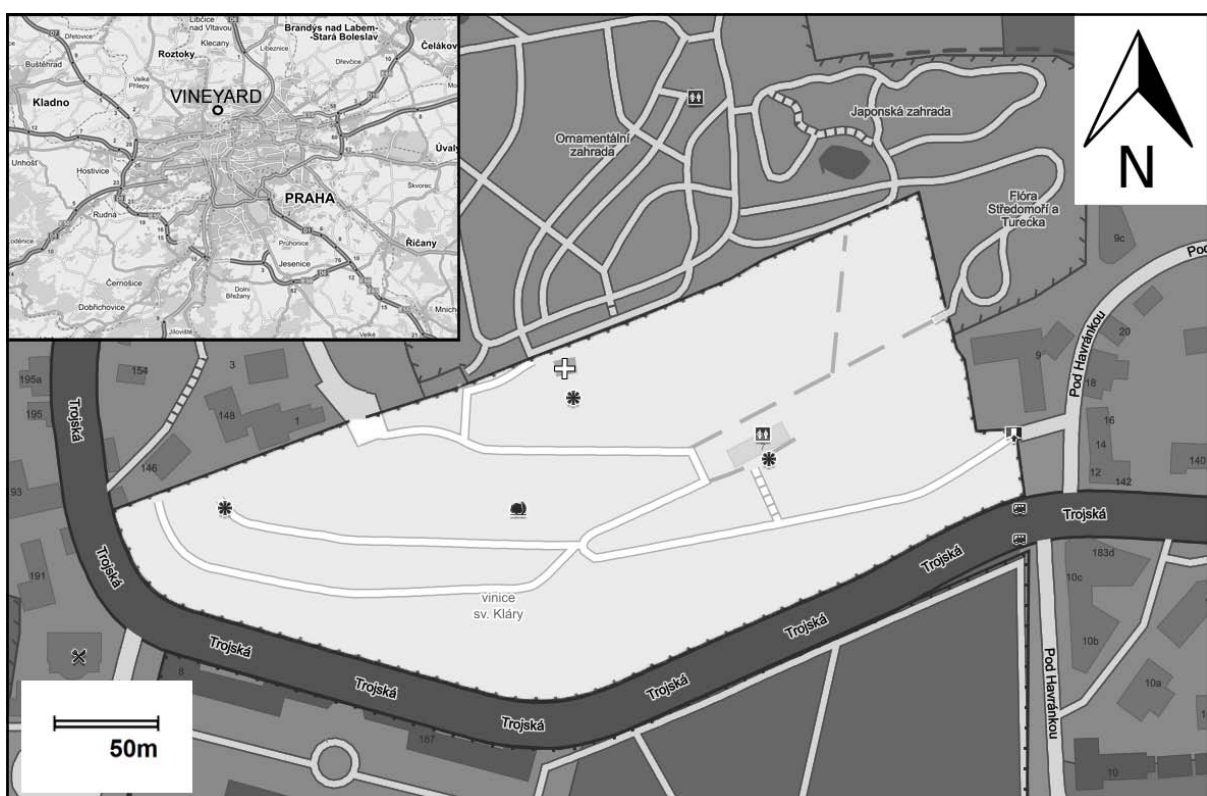
Figure 2 Location of Vineyard sv. Klára, in an urbanised area to the north of the Vltava River in Prague

In 2009, samples were collected from different soil depths at various points within a recreational shooting range. Results of the study are published in Ash *et al.* (2013[b]); the area is representative of a locality characterised by alkaline soil in the Moravia region (Figure 3)
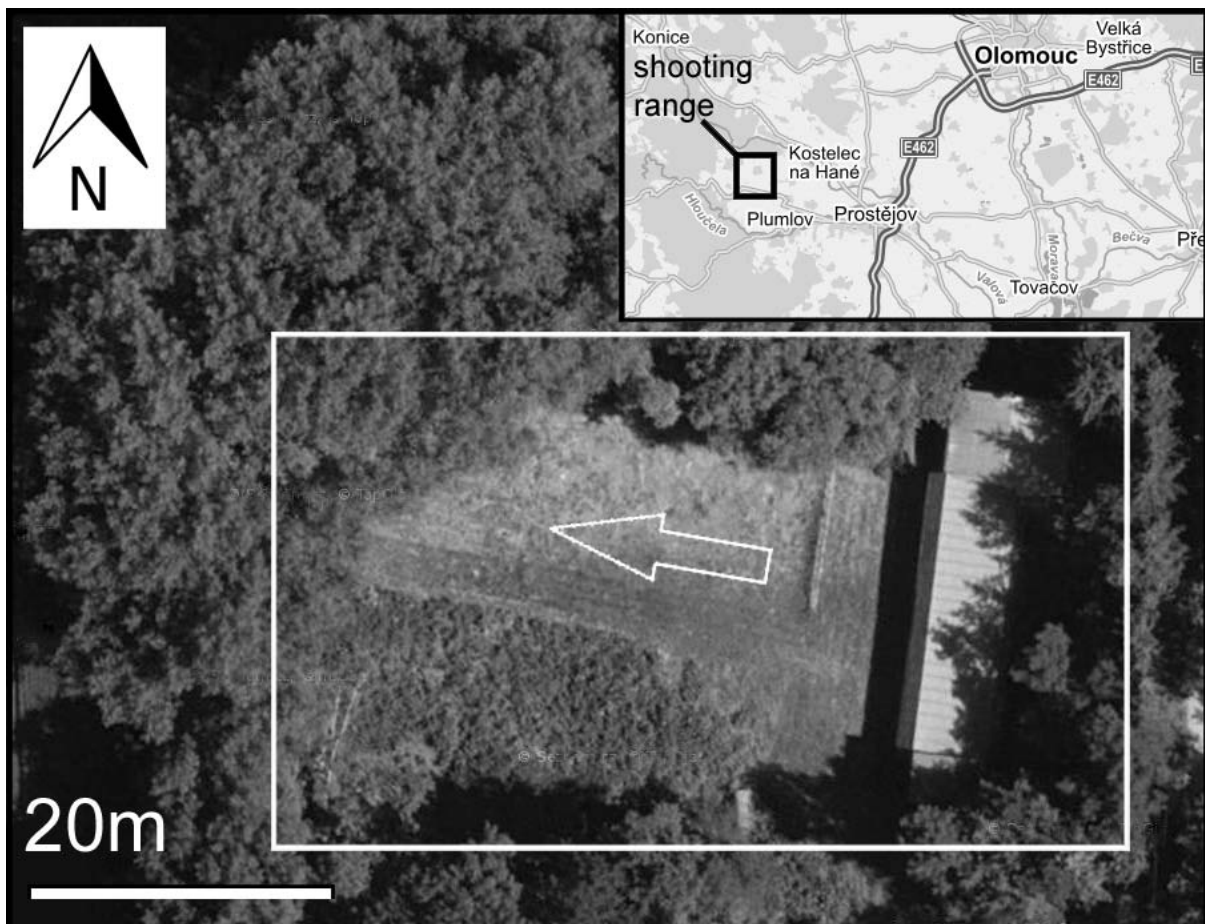
Figure 3 Location of a recreational shooting range in Moravia, Czech Republic that was used for sample collection. The firing line is positioned under the constructed canopy and the direction of fire is from east to west

The Jizera Mountains were sampled at a number of different locations as a part of thesis work obligations of former student Ing. Michaela Kváčová. Statistical analyses were performed to relate the sampled soil layers to soil properties and stand factors (Kváčová *et al.* 2014); specific locations of the samples are shown in the map in Figure 4.
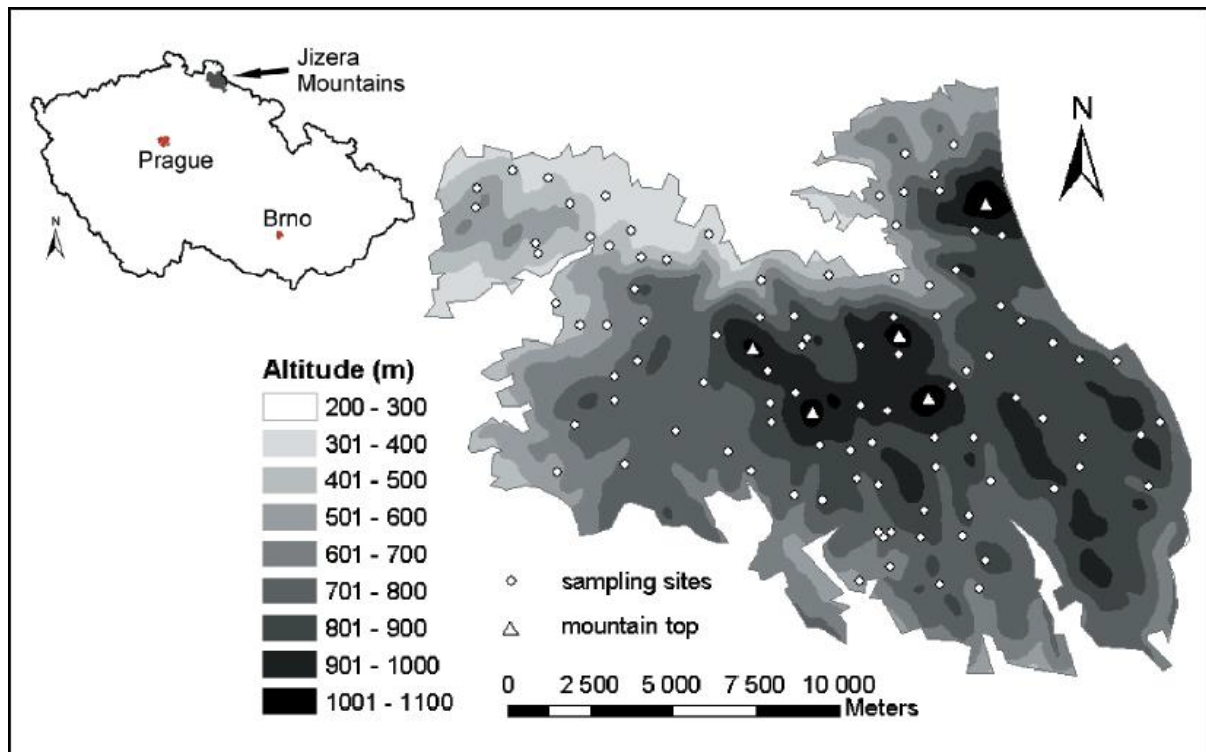
Figure 4 Map of the Jizera Mountains region in northern Czech Republic where samples were collected from multiple locations with various altitudes and stand factors

The location selected for sampling in the studies of Ash *et al.* (2013[a]; 2014) represents an area contaminated with PTE due to historic smelting activities and subsequent deposition of the associated wastes. The site consists of a significant slag deposit, which is situated alongside the River Vrchlice, to the south-west of Kutná Hora, as shown in Figure 5.
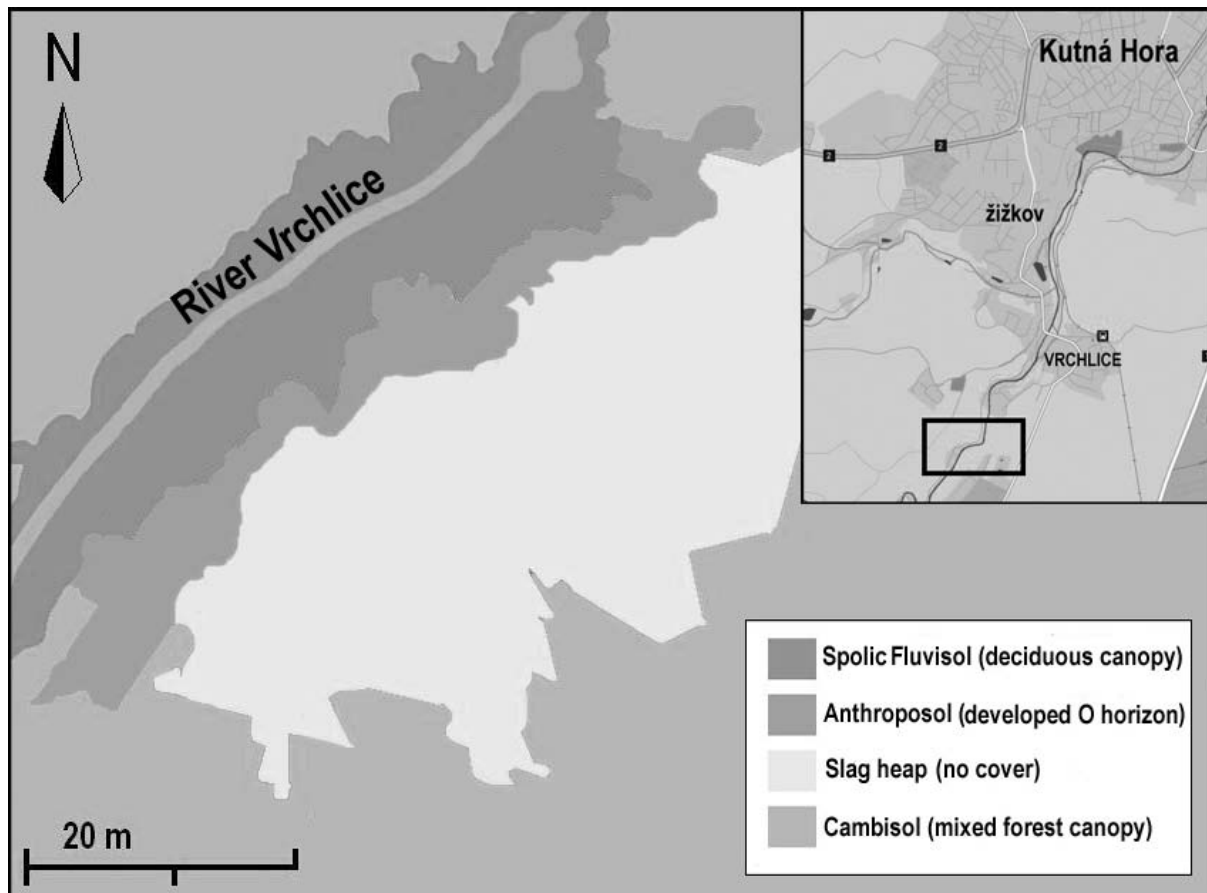
Figure 5 Map showing the location of the studied slag heap in the historic mining region of Kutná Hora, the Czech Republic

A small, forested area to the north-west of Přibram was used for sample collection as part of two studies (Ash *et al.* 2016[a]; 2016[b]); the site is representative of a wooded area that has been affected by long-term deposition of atmospheric pollutants. In this case, the pollution source is from a secondary lead smelter, which is positioned not more than 500 m upwind of the study area (Figure 6).
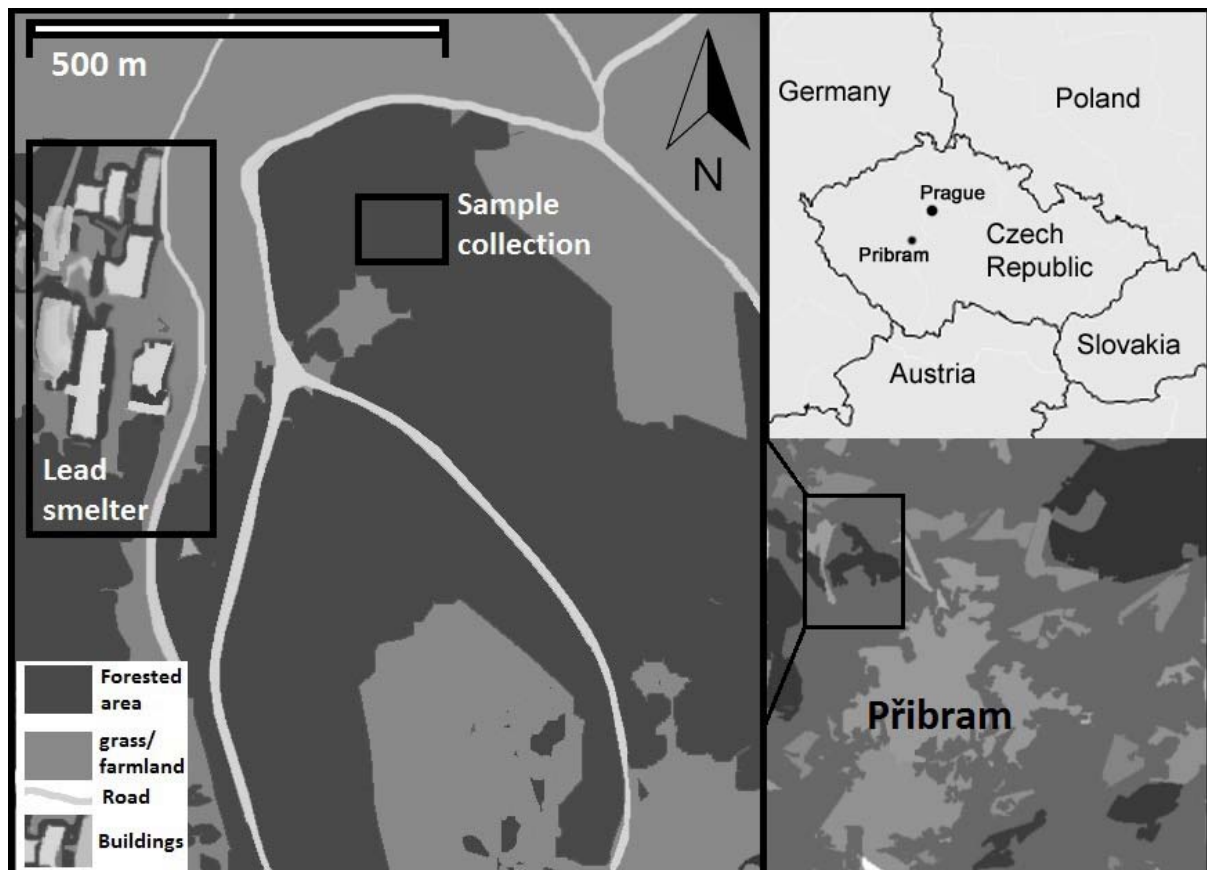
Figure 6 Map showing the location of the studied soils in the district of Přibram, the Czech Republic

HYPOTHESES AND AIMS

3.1 Elevated soil copper contents in a Bohemian vineyard as a result of fungicide application

Hypotheses: The distribution of copper at the soil surface within the vineyard will reflect the slope position and the organic matter content i.e. there will be an accumulation of copper in lower parts of the slope and in samples with a greater content of organic matter.

The general purpose of the study was to characterise a vineyard of the Bohemian region in terms of potentially toxic element distribution (mainly Cu), by means of distribution maps that can be used to identify associations between elements and soil properties.

3.2 Fractionation and distribution of risk elements in soil profiles at a Czech shooting range

Hypotheses: 1) In areas of concentrated shooting, dissolution of ammunition and migration of potentially toxic elements will be observed. 2) Mobility and bonding of PTE can be effectively monitored by a combination of single extraction methods and sequential extraction procedures.

The aim of the study was to clarify the mobility of selected PTE (Cu, Ni, Pb, Sb, Zn) being released from bullets at various soil depths, and to assess the effectiveness of sequential extraction analyses for tracking the mobility and binding of individual elements in a contaminated alkaline soil.

3.3 Temporal dissolution of potentially toxic elements from silver smelting slag by synthetic environmental solutions

Hypotheses: It is anticipated, that the highest dissolution of studied elements will occur in the 1 mM citric acid solution, followed by the rainwater simulation solution (deionised water acidified using inorganic acid), followed by the control (deionised water).

The aim of this experiment was to study the temporal solubility of PTE (Pb, Zn, Cd, Mn) from slag into solutions that are representative of solvents, which occur in the environment. The study was designed to get an indication of the concentrations of contaminants being mobilised during chemical weathering, and thus can help determine the important pathways for migration of PTE into the environment.

3.4 Potentially toxic element distribution in soils from the Ag-smelting slag of Kutná Hora: Descriptive and prediction analyses

Hypotheses: Element specific enrichment is expected in soils that have higher pH, greater soil organic matter content, or higher clay content. Furthermore, all studied soil and sediment samples will have a PTE content that exceeds the background levels for the typical respective soil types, and will exceed regulation guideline values for each of the studied PTE.

The purpose of this study was to elucidate the distribution of PTE in soils with varying properties around a large historical smelter slag deposit, and to find correlations of soil properties with PTE concentrations that were obtainable by extractants corresponding to releasable and plant available contents. The overall study aim was to contribute to the database of knowledge concerning smelter slag and the potential for pollutants that are weathering from slag to migrate into surrounding environmental systems.

3.5 Contents of potentially toxic elements in forest soils of the Jizera Mountains region

Hypotheses: It is anticipated that, due to past atmospheric depositions of PTE, the level of contamination will generally exceed guideline values set for Czech agricultural soils. Highest contents of PTE are expected in soils that are covered with coniferous canopy as opposed to open areas or those covered with deciduous trees.

The main aim of this study was to determine the level of PTE (Cd, Cu, Mn, Pb, Zn) pollution in samples from the organic and mineral horizons of forest soils in the Jizera Mountains region. The objective was to assess the distribution of PTE and their relationship to stand factors (vegetation cover type and altitude), and to use surface mapping for the visual projection of PTE distribution.

3.6 Redistribution of cadmium and lead fractions in contaminated soil samples due to experimental leaching

Hypotheses: 1) After 720 hrs of saturation, a contaminated organic surface soil sample will develop a similar Cd and Pb fractionation (according to BCR sequential extraction procedure) to that of a naturally developed fluvisol sample. 2) Addition of a $CaCO_3$ amendment to contaminated soil will reduce overall leaching of both Cd and Pb, and the decreased release will be due primarily to increased adsorption onto the easily exchangeable fraction, as compared to a non-amended sample.

The main aim of the study was to provide a description of temporal changes of Cd and Pb binding phases in contaminated soil after leaching, in hydro-static and hydro-dynamic scenarios. The study also seeks to determine the effects of a $CaCO_3$ amendment on Cd and Pb binding dynamics in the studied soils. Results from this study offer insight to the behaviour of Cd and Pb in contaminated soils that are subjected to periodic and prolonged saturation.

3.7 Sustainable soil washing: Shredded card filtration of potentially toxic elements after leaching from soil using organic acid solutions

Hypotheses: The solution concentration of PTE (As, Cd, Pb) after leaching through contaminated soil sample will be significantly decreased when a filter layer composed of shredded card is used. Greatest sorption (and retention) is expected for elements that are occurring mainly in cation form, due to the anionic nature of the shredded card surfaces.

The study aim was to analyze different leaching solutions (oxalic acid, formic acid, $CaCl_2$, deionized water) for content of As, Cd, and Pb after passing through columns of contaminated soil with/without a shredded cardboard (SC) filter. We further aim to assess SC for its effectiveness at retaining PTE after leaching with acidified deionized water. The study attempts to determine whether SC could be applied as an effective filter to remove PTE from the extract solution following soil washing.

3.8 Different low-molecular-mass organic acids specifically control leaching of arsenic and lead from contaminated soil

Hypotheses: 1) Cumulative amounts of As and Pb released from soil samples by LMMOA of differing molar concentration will not be linear but will plateau in the upper concentration range (up to 25 mM). 2) Differences in the extent of As and Pb leaching will reflect the carboxylic structure of LMMOA; an organic acid with a greater number of carboxyl groups is a more effective extracting agent than an acid of simpler carboxylic structure (citric > oxalic > acetic). 3) The major leaching mechanism for As and Pb from soil is similar for each of the studied LMMOA.

The aim of this study was to describe trends of As and Pb release from soil by continuous leaching using different LMMOA (acetic, oxalic, and citric acid), and to identify the most effective LMMOA (type and concentration) for the extraction of As and Pb in organic and mineral soil samples. The resulting data bring new insight into the roles that are played by LMMOA in polluted soils.

SUMMARY

During the course of the thesis work, research aims evolved to accommodate new findings and insights gained from preceding projects. Initial and somewhat arbitrary studies included the characterisation of Cu contamination at a Bohemian vineyard, the assessment of different extraction methodologies for determining the risk of ammunition dissolution at a shooting range, and compiling results from unpublished research that aims to map the distribution of PTE in forest soils of the Jizera Mountains. Congruent to the realisation of these independent research projects, a vision was starting to form as to what the focus of the PhD. research should be concerning. Following a study that was performed as part of earlier MSc. thesis preparations, a broad research aim began to evolve, which was to present work that contributes to a greater understanding of the behaviour and fate of PTE in the soil environment, with a particular emphasis on chemical leaching. Thanks to the development of a network of peers and colleagues, combined research efforts were manifested in a number of project outputs in the form of published results. The following text summarises those results and the research process.

An initial study was conducted with a focus on characterizing the PTE content in soils on and around a significant smelter slag deposit in the Kutná Hora region. Respective of background values, highly elevated concentrations of PTE were identified in soils that had developed on the slag heap and in the adjacent river bank, with a generally increased enrichment below the surface. Particularly extreme values were observed for Cu and Zn. Despite determining high overall contents, generally low availability of PTE was observed. Cadmium and Mn were the most easily available elements. Statistical analysis showed low availability of Cu in soils formed under tree cover, whereas Mn availability was significantly increased under a deciduous tree canopy. Likewise, when correlated with Corg, available Mn (and As) showed positive correlation, while Cu (and Pb) had negative correlation. With the exception of Ag, all elements showed negative correlation with pH, while Pb and Cu availability showed negative correlation with clay content. By determining the PTE in the soil as a proportion of its source (PTE content in slag) it was possible to predict points at which PTE accumulate according to a given soil parameter. We achieved statistically significant trends for Pb, Cd, Cu and Zn when plotted in this way with pH as the independent variable, and it was possible to interpolate the threshold pH value at which PTE accumulate in soil (~ pH 4.5, 5.0, 5.0,

>6.0 respectively). Analysis of the adjacent Vrchlice river sediment revealed PTE values that exceed natural background concentrations. It is therefore evident that PTE are being mobilised from the slag and settling in surrounding surface waters. However, analysis of the river water did not reveal the presence of PTE, only in a riverbank depression where water flow is restricted, we did detect Mn and Zn in water samples; intrusion of slag fragments and deposition of PTE in stagnant wells may result in chronic persistence of PTE in the water.

Concurrent to the investigation of soil and sediments surrounding the KH slag heap, samples of slag were also collected. Slag deposits are considered a source of high levels of PTE. However, some contention in the literature suggests that low availability due to encapsulation of PTE in slag limits the threat of their migration into the environment. Extracting agents were used to replicate potential leaching scenarios that are analogous to natural chemical weathering. Slag was submersed in a rainwater simulation solution (RSS), weak citric acid solution, and a control (deionised water) for a one-month period, with solution analyses made at intervals of 1, 24, 168 and 720 hrs. The mineralogical character of slag was responsible for buffering of solvents to within a narrow range (around pH 5.5), which may moderate the transport of some PTE (especially Pb); smelter slag often consists of high portions of basic oxides due to lime fluxes commonly used in medieval smelting. Decomposition of citric acid with time may have had implications on metal solubility, however, the consequent changes in metals solubility are indirect, likely to be more related to pH. A common observation among metals in citric acid was the short time to equilibrium, which is in contrast to RSS whereby a continued release of Cd, Mn, Zn occurred, suggesting that water in the environment e.g., flooding or precipitation events, is significant for the mobilization of these elements. Overall levels of PTE in solution after 1 month were greatest in RSS, with the exception of Pb, which had generally low solubility in all solvents. Removal of Pb from solution was probably due to pH instability at low concentration. Statistical analysis showed that, in the case of Cd and Zn, the heterogeneity of slag is strongly reflected in dissolution of these elements as significant concentration differences were observed between slag samples. It is evident that chemical weathering by environmental solvents is responsible for the transport of notable quantities of certain PTE, which will inevitably settle in surrounding soils and surface waters. However, the large amount of basic compounds in smelter slag can immobilize some contaminants; thus, physical weathering and aeolian transport should also be considered as likely major pathways for the distribution of PTE into soil and water near slag deposits. An impermeable

cover would therefore offer effective mitigation of the movement of PTE due to the effects of both chemical and physical weathering.

Following the previously described research, consideration was given to the layer of organic matter that was developing at the slag heap toe-slope and the thick organic horizon that comprises much of the adjacent fluvisol; both are developed from the same vegetation litter, but water regime is vastly different. A primary hypothesis was formed that: after artificial prolonged saturation of soil organic matter from the slag heap, a soil fraction distribution similar to that of a naturally formed fluvisol sample will occur. The main aim was to provide a description of temporal changes of Cd and Pb binding phases in contaminated soil after leaching, in both hydrostatic and hydrodynamic wetting scenarios. The study also aimed at determining the effects of a $CaCO_3$ amendment on Cd and Pb binding dynamics in the studied soils. During one month of saturation, the removal of Cd and Pb from contaminated O and B horizon soil samples occurred primarily from the residual fraction, with a significant redistribution onto the Fe- oxides (identified mainly as magnetite and hematite) and partially onto the organic matter. After 720 h, the distribution pattern of Cd and Pb in contaminated O horizon soil closely matched the distribution pattern of that in a well-established spolic fluvisol sample. In batch experiments, $CaCO_3$ amendment resulted in decreased leaching of Cd and Pb, although the fraction distribution patterns were practically indistinguishable from untreated samples. In a hydrodynamic leaching test, Fe-oxides and organic matter were shown to be important phases of Cd retention. Pb was leached mainly from the reducible fraction whereas the oxidizable and residual fractions were of greater importance for Pb retention. Based on findings in this study, further research could be focused on the climatic and spatial environmental parameters that control PTE soil speciation dynamics with time, with a particular emphasis on prediction of PTE fractions in contaminated soils based on water regime.

After some adjustments were made to the concept column leaching method following its use in the abovementioned experiment, the improved apparatus was implemented further in a sorption study designed to assess shredded card (SC) for its ability to immobilize PTE from solution (oxalic acid, formic acid, $CaCl_2$, and water) flowing through a column. A peristaltic pump was used to leach PTE from contaminated soil before passing through the SC sorbent.

The general aim was to determine the feasibility of using recycled card as a sustainable and effective filter of PTE from solution following soil washing. Increases in solution pH (as

much as pH +4.49) were observed after leaching through SC. Sorption of PTE onto SC varied between the studied PTE, and according to type of leaching solution. In oxalic acid, all of the studied PTE showed considerable sorption. In formic acid, Pb sorption was very efficient, As and Cd were bound to a lesser extent. In DI_{H2O}, only Pb showed a high rate of sorption. A generally greater degree of sorption was achieved for PTE that had been leached from a mineral soil sample. Further attention is needed to clarify the role of DOC, and the influence that the degree of humification of source soil has on PTE sorption. Desorption experiments showed that subsequent release of PTE from SC varies according to the source of the PTE (organic vs. mineral soil), and the type of leaching solution from which PTE were initially removed. The soil type from which PTE are leached not only dictates the amount of sorption (respective of total contents), but also has an effect on the subsequent release of the PTE; a greater adsorbed proportion of PTE generally results in lower release of that element. Arsenic was the PTE most readily leached in all desorption experiments. A high rate of Cd sorption from the organic acid solutions was followed by strong retention during desorption experiments. For sorption of Cd onto SC from DI_{H2O}, the desorption experiment indicated a good level of retention. For SC samples with initial Pb sorption from LMMOA and $CaCl_2$, desorption in acidified water was relatively low $\leqslant 10\%$ after 5 hrs of leaching). SC was able to retain Pb after adsorption in DI_{H2O}, with losses of no more than 8.5% of total lead. Shredded card showed good potential as a sorbent of toxic elements in various solutions. We suggest using recycled card in trial experiments, as a filter of washing effluent from soil contaminated with inorganic pollutants; once PTE are sorbed onto SC, it can be reduced to an ash by burning, significantly reducing the amount of contaminated material that needs to be disposed of.

In the final phase of the thesis work, column leaching experiments were again utilised. Leaching was applied to soil samples polluted with As and Pb (a Cambisol polluted by way of atmospheric deposition from a lead processing plant) in order to determine the specificity of LMMOA related release for individual potentially toxic elements, at varying organic acid concentrations (0.5, 1, 2.5, 5, 10 and 25 mM). Leaching of As into LMMOA solutions followed the order oxalic acid > citric acid > acetic acid in both organic and mineral soils. The observed differences in extraction of As into the studied acids were validated by ANOVA and multiple range tests. The mechanism of ligand-enhanced dissolution of mineral oxides followed by As release into solutions of LMMOA has been recognised, and is supported by findings in this study. Highly significant correlation was observed between As

in oxalic and citric acids and the content of Al and Fe in leaching solutions, which infers that hydrous oxides of Al and Fe are major sources of soluble As in the presence of LMMOA. BCR sequential extraction confirmed a significant decrease of metal oxide-bound As after leaching for both soils. A greater portion of As was leached from the mineral soil sample in a much shorter duration than the surface organic soil by both oxalic and citric acids, suggesting that subsurface mineral soil layers are more vulnerable to As toxicity. Significant releases of Pb into the studied LMMOA were observed, with extractability from both soils following the general order: citric > oxalic > acetic. Soil AB, which represents a mineral soil layer, was shown to be more susceptible to leaching of Pb than surface soil samples that were characterised by a high content of organic matter and a greater ability to buffer against acidic solution pH changes. High affinity to humic matter is known to play a key role in the behaviour of Pb, and the movement of Pb to deeper horizons is likely to depend on transport of dissolved Pb-organic complexes. Superior leaching efficiency of citric acid was attributed to stable complex formation structures with Pb ions, which are not demonstrated by the other acids. The hypothesis that carboxylic structure is the key variable for leaching of metal/metalloids did not hold true for As. It is apparent that acid dissociation is the key controlling factor, releasing As by way of mineral dissolution. In the case of Pb, the carboxylic structure of the studied LMMOA reflected the amount of Pb released into, and held in solution (tri-carboxyl citric > di-carboxyl oxalic > mono-carboxyl acetic). Results obtained in the study are evidence that the extent of As and Pb leaching in contaminated surface and subsurface soil depends significantly on the types of carboxylic acid involved, and that the mechanisms of As and Pb release differ between the studied LMMOA. Implications of the type of acid and the specific metal that can be mobilised most effectively become increasingly significant where LMMOA concentrations are highest, such as in rhizosphere soil.

CONCLUDING COMMENTS

The aim of the thesis was to contribute work that enhances scientific understanding of the behaviour and fate of PTE in soil, particularly with respect to chemical leaching. Initial research activities focused on the descriptive characterization of a medieval smelter slag deposit in the Kutná Hora region. We determined significant concentrations of numerous PTE (Pb, Cd, Mn, Cu, Ag, Zn, As) in soils and sediments near the slag heap, but the same elements were largely undetected in water samples. Despite observing extremely high loadings of PTE in the area, their availability was limited due to the input of organic matter along much of the base of the slag. To better elucidate the potential pathways for transfer of PTE from slag into the environment, batch leaching tests were performed, using crushed slag and experimental solutions of citric acid and simulated rainwater. Citric acid (1 mM) was able to leach small amounts of Pb, but was characterized by a short time to decomposition during the experiment, regarding Pb release, citric acid still outperformed rainwater simulation and a water control. On the other hand, favourable dissolution of other PTE (Cd, Mn, Zn) occurred in solutions of deionised water. The experiment proved that chemical leaching is a possible means for transport of PTE into adjacant soils, however, aeolian transport and other physical erosion mechanisms should be considered as likely major pathways for toxic element transfer. At the same study area, samples of organic matter taken from the slag toe-slope were saturated, and then compared with a natural fluvisol sample for PTE fractionation. We found that during prolonged wetting, the chemical speciation of PTE changed; removal of Cd and Pb from soil occurred mainly from the residual fraction, with a significant redistribution onto the Fe/Mn oxides and partially onto organic matter. Furthermore, the fractionation of Cd and Pb closely matched the distribution pattern of a spolic fluvisol sample. The significance of the finding is that with further research, perhaps by way of modelling it could be possible to anticipate PTE fractionation of contaminated soils, based on the water regime. Moving into the remediation aspect of contaminated soils research, a sorption study was designed to assess shredded card (SC) for its ability to immobilize PTE from different solutions flowing through a column. The general aim was to determine the feasibility of using recycled card as a sustainable and effective filter of PTE from solution following soil washing. The material performed well for the capture and retention of PTE, particularly Cd and Pb. We propose that SC can be applied as a promising experimental material for the filtration of PTE from leaching effluent following soil washing.

Furthermore, concerning the actual soil washing, low-molecular-mass organic acids offer a less destructive, biodegradable alternative to strong inorganic acids, which are detrimental to a number of soil characteristics that dictate soil quality, such as microbial health, retention of DOC, and soil structure and texture. The thesis research culminated in a study that aimed to test different LMMOA for their ability to leach PTE from contaminated soil, and to identify the associated mechanism of PTE release. A process of ligand-enhanced dissolution of mineral oxides with congruent As release was recognised; our data strongly support the idea that hydrous oxides of Al and Fe are major sources of soluble As. Leaching data also suggests that subsurface soil layers are more vulnerable to As toxicity. Likewise, subsurface soil was also more susceptible to leaching of Pb than soil of upper horizons that are typically characterised by a high content of organic matter. Transfer of Pb to deeper horizons is likely to depend on vertical leaching of dissolved Pb-organic complexes. Results from the study provide sufficient evidence that leaching of As and Pb from soil by LMMOA depends significantly on the type of carboxylic acid. Moreover, the mechanisms of As and Pb release differ according to type of LMMOA. Accordingly, we can consider soils in which a large production of LMMOA is occurring, and in particular rhizosphere soil, to be far more vulnerable to PTE toxicity due to enhanced solubility, than soils that are absent of LMMOA. Furthermore, the extent of PTE leaching in rhizosphere soil depends upon the LMMOA composition, which varies according to the plant species.

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FEATURED PUBLICATIONS

1. Elevated soil copper contents in a Bohemian vineyard as a result of fungicide application

2. Fractionation and distribution of risk elements in soil profiles at a Czech shooting range

3. Temporal dissolution of potentially toxic elements from silver smelting slag by synthetic environmental solutions
   - Conference poster - EuroLeague for Life Sciences (ELLS) student conference 'Future Cities, Future Life', Alnarp, Sweden, November 2012

4. Potentially toxic element distribution in soils from the Ag-smelting slag of Kutná Hora: Descriptive and prediction analyses

5. Contents of potentially toxic elements in forest soils of the Jizera Mountains region

6. Redistribution of cadmium and lead fractions in contaminated soil samples due to experimental leaching

7. Sustainable soil washing: Shredded card filtration of potentially toxic elements after leaching from soil using organic acid solutions

8. Different low-molecular-mass organic acids specifically control leaching of arsenic and lead from contaminated soil
   - Conference poster – International conference of the Society for Environmental Geochemistry and Health, Arlington, Texas, March 2015

Elevated Soil Copper Content in a Bohemian Vineyard as a Result of Fungicide Application

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Abstract: A set of fifty samples were taken from soil surface layers of an anonymous vineyard in the Bohemia region of the Czech Republic. Samples were analysed for basic soil parameters including pH and humus content and quality and for potentially toxic elements Cu, Pb, Zn, Cd and Mn. When compared to soil guideline values, mean Cu exceeded the limit by 280%. Although other elements did not exceed the guideline value, Zn and Cd surpassed background concentrations by 108 and 187%, respectively. Mn did not occur in concentrations that are considered excessive with respect to common natural soil levels. A statistically significant correlation was observed for Cu and C_{ox}, but no significant correlation could be made between Cu and humus quality, suggesting that in this case, Cu retention in surface soil is more dependent on quantity of humic substances rather than humus quality. The unnaturally high accumulation of Cu in the surface horizon is certainly of anthropogenic origin; most likely due to long term application of copper-based fungicides. High observed values of Cd are probably a result of the soils parent material and application of fertilizers which may have contained cadmium compounds. Distribution maps illustrated a common trend of potentially toxic element accumulation in the western section of the study area plot. However, many variables which were unmeasured in this study could account for the distribution and therefore more detailed study is required before conclusions can be made regarding this occurrence. An analysis of wine produced at the vineyard showed that although the end product beverage contained insignificant amounts of Cu in both red and white wines, the white wine contained 0.026 mg/l of Cd; a value which could contribute considerably to exceeding the dietary allowable intake of cadmium. The investigation should be extended to analyse a wider range of wines produced from the vineyard with a focus on soil-plant-fruit-wine transfer of cadmium and/or production processes.

Keywords: Bordeaux mixture; copper toxicity; fungicide; potentially toxic elements; vineyard soil

The application of liquid copper based treatments is a well established practice for combating a range of fungal plant diseases, and their use in vineyards are extensive. The Bordeaux mixture ($CuSO_4$ and $Ca(OH)_2$) is a common and long-used solution for such treatments worldwide. A well known property of Cu is its potential to accumulate in soil surface horizons, and due to the fact that many European vineyards are often centuries old, repeated use of copper based fungicides has resulted in the intensification of soil Cu concentrations beyond regulation toxicity limits. Komárek *et al.* (2008) proved that this is evident in various vineyards of the Czech Republic whereby in most of their studied vineyard soils, soil Cu levels did not meet the regulatory limits for agricultural soils set by the Czech Ministry of the Environment, and total Cd exceeded the limits in all cases. In the same study, from 5 vineyards of differing regions, the average Cu concentration in the upper 10 cm was

shown to be 1.8 times greater than the underlying 10 cm layer, highlighting the persistence of copper complexes which form with soil organic matter (SOM) at the surface. In addition to its affinity for organic matter, Cu forms and mobility are strongly controlled by pH, with most bioavailable Cu being bound to Fe and Mn hydroxides or converted to residual form at pH greater than ~ 6 (Komárek *et al.* 2010; Fan *et al.* 2011). However, mobility can increase at pH values above ~ 7.5 due to the solubilization of SOM and formation of Cu-SOM complexes (Komárek *et al.* 2010). Ion activity measurements on soil samples by Karlsson *et al.* (2006) showed that less than 0.2% of total Cu was in the free form Cu^{2+} at pH 4.8–6.3.

When present in toxic concentrations, Cu is known to inhibit plant growth and to interfere with important cellular processes such as photosynthesis and respiration, therefore plants grown in the presence of excessive levels of Cu normally show reduced biomass and symptoms of chlorosis (Yruela 2005). Besides the direct toxicity toward plants from potentially toxic elements (PTE), fungicides are thought to reduce the biochemical functions in soil making them more susceptible to contamination. Wang *et al.* (2009) made a comparison of soil microbial properties between apple orchards of differing ages and a reference soil to determine the relationship between Cu accumulation and microbial activity. A significant correlation was observed between $CaCl_2$-extractable Cu and soil microbial biomass carbon, suggesting that elevated soil Cu concentrations were responsible for significant reductions in soil microbial health and activity. Enzyme activity assays also showed a similar trend, and declined with the increasing levels of Cu in orchard soils. Conversely, in a study by Norgrove (2007), little to no negative consequences were observed within 3 years of high application rates of copper fungicide with respect to earthworm activity and there was no evidence of any negative effect on litter decomposition or soil bulk density.

MATERIAL AND METHODS

This study focuses on an anonymous small wine producer of the Bohemia region in the Czech Republic. The studied vineyard is situated between 220–265 m a.s.l. and covers an area of 3.5 ha. The area is part of the Czech Cretaceous Basin, consist-ing largely of Proterozoic rocks (shales, greywackes, lydites, spilite) covered with Quaternary sediments of variable thickness, made up of mostly sandy gravels and sands, occaisioning the presence of eolian sediments – loess or calcareous drift sands. Samples were taken using an irregular sampling network from fifty points within the vineyard (Figure. 1). Surface layer of soil was collected to a depth of 20 cm. Samples were air dried, sieved to < 2 mm and the following basic soil characteristics were assessed: C_{ox}, quality of humic substances, pH_{H_2O} and pH_{KCl}. 2M HNO_3 extractable concentrations of selected PTE (Cu, Pb, Zn, Cd) were identified and mapped using ArcGIS software. PTE levels were compared to Czech soil guideline values for agricultural soils (SGV) and background concentrations in order to establish the extent of the contamination.

C_{ox} was measured by a modified Tyurin's oxidimetric titration; potassium dichromate in sulphuric acid titrated with Mohr's salt (Pospíšil 1964). Quality of humic substances was calculated from spectrophometric analysis, taken as the ratio of a pyrophosphate soil extract absorbance at wavelengths 400 and 600 nm (A400/600) according to Podlešáková *et al.* (1992). For PTE analysis, 5 g of soil sample (< 2 mm) was shaken with 50 ml of 2M HNO_3 for 6 h. The sample was then centrifuged and the supernatant analysed for risk elements by FAAS spectrometry; Varian 280 FS with SIPS 20 using Analytika standards (Analytika Ltd., Prague, Czech Republic). pH_{H_2O} and pH_{KCl} were measured with a WTW inolab pH level 1 pH probe. Wine which had been produced from grapes grown at the study area was purchased; one white (Riesling Kabinett 2011) and one red (Pinot Noir 2010), and analysed for PTEs. The method was reproduced from Sperková and Suchánek (2005). 50 ml of
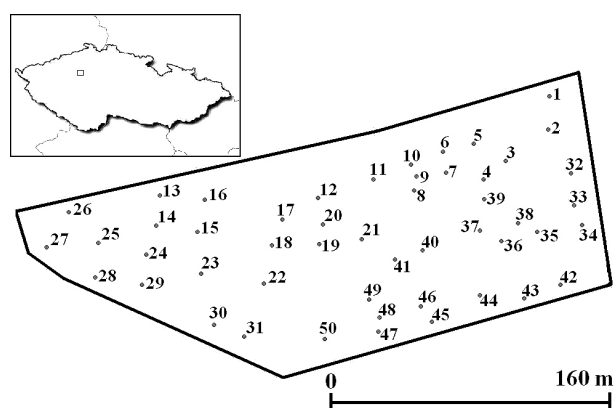

Figure 1. Vineyard outline map and sampling points

Table 1. Mean and standard deviation of potentially toxic elements (PTE) from fifty samples (mg/kg)

	Cu	Pb	Zn	Cd	Mn
Mean	139.564	40.81	65.298	0.75	657.128
SD	66.84	22.37	60.32	82.07	246.29

SD – standard deviation

wine was heated at 190°C for approximately 4 h to volatilize the ethanol and the residual wine dissolved in 25 ml of 0.1M HNO_3. Samples were prepared in triplicate and the solution was analysed for elements by FAAS. Detection limits for elements were as follows: Cu 0.035, Mn 0.020, Cd 0.018, Pb 0.09, Zn 0.013 mg/l.

Correlation statistics were prepared in MS Excel and Chi-square analysis calculated according to a method written by Taylor (2007). Distribution maps were created using ESRI ArcGIS 10.0 (Esri, Redlands, CA, USA).

RESULTS AND DISCUSSION

Potentially toxic elements. To assess the degree of enrichment of PTE in the upper 20 cm of vineyard soil, concentrations were related to SGV and background concentrations for the Czech Republic according to Beneš (1993). Average soil PTE contents are expressed as a percentage of the reference levels (Table 2.).

Copper was the only element to occur higher on average than the Czech SGV (2.8 times higher), although in some soil samples Zn and Cd also exceed the SGV. Reasons for an excess of soil Cd may be attributable to the nature of the parent material of the vineyard, which was derived primarily from sandstones, known for its high natural content of Cd (Adriano 2001), and is also likely to have arisen as a direct result of traditional fungicide applications which may have contained Cd compounds or from phosphorus-fertilizers, manures

Table 2. Mean soil potentially toxic elements (PTE) values as a percentage of soil guideline values (SGV) and background concentration

	Cu	Pb	Zn	Cd
SGV (%)	280	57	65	75
Background (%)	400	100	108	187

or to a lesser extent liming (FMAF 2000). Cu is by far the most significant pollutant as almost every sample is contaminated as indicated by the standard deviation in Figure 2. In this study the mean soil Cu concentration is one of the highest seen in a Czech vineyard. However, in a study by Pietrzak and McPhail (2004) at a vineyard in Victoria, Australia, total mean Cu reached as high as 228.9 mg/kg.

Cu, Zn and Cd all exceed the Czech background concentration on average, whereas Pb is on the upper limit (Table 2). Pb appears to be the least problematic PTE at the study area as even the upper error bar does not surpass the SGV (Figure 2). These findings are consistent with the data obtained by Komárek *et al.* (2008) for the same study area whereby total contents of soil Cu, Pb and Cd as a percentage of SGV were 228, 77 and 76%, respectively, closely matching the results of our study. Other vineyards across the Czech Republic showed slightly different trends. Pb was far lower in all cases and Cd was greater than the SGV at one Moravian vineyard (Komárek *et al.* 2008). However, each individual vineyard should be treated on a case by case basis as there are many variables to consider. Mn was measured in all samples but as there is no SGV or background level published for this element in the Czech Republic it has been omitted from the tables. The average Mn concentration from samples was 657.1 mg/kg, a level at which Mn is not likely to exhibit toxic effects given that normal soils contain an average Mn concentration of 850 mg/kg (range of 100 to
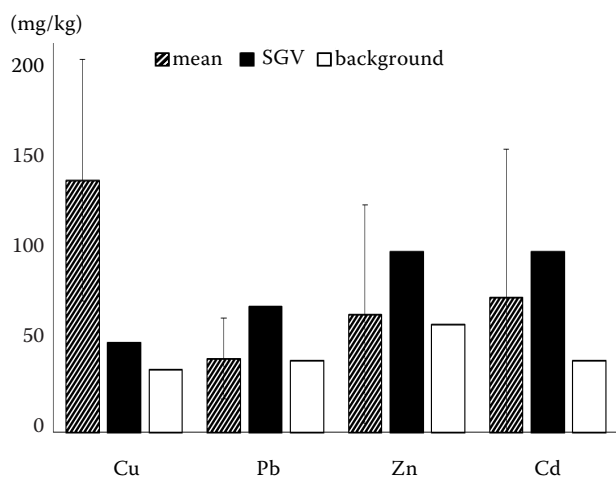

Figure 2. Mean soil potentially toxic elements (PTE) compared to soil guideline values (SGV) and background concentrations of the Czech Republic
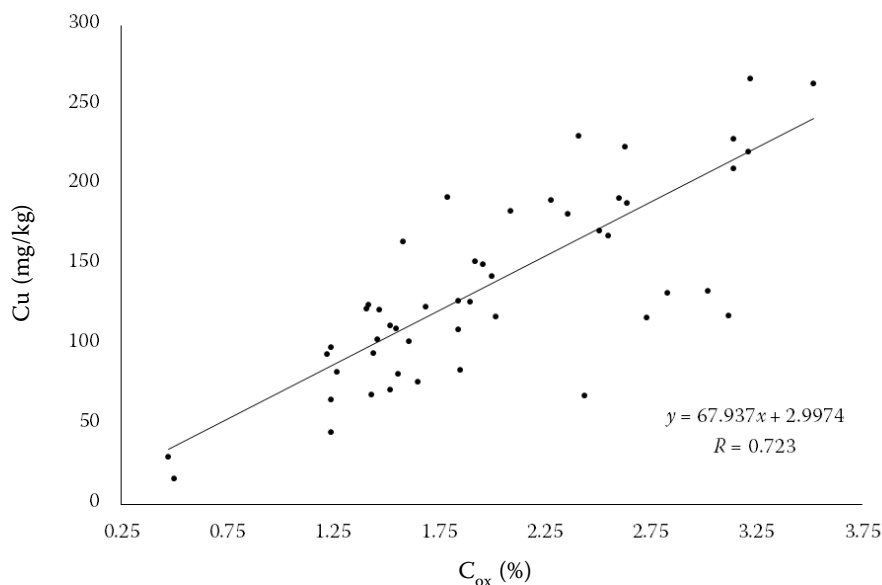
Figure 3. Dependence of Cu retained in the upper 20 cm of soil on oxidizable carbon percentage

4000 mg/kg) (ADRIANO 2001). Mn toxicity is dependent on soil pH and redox and is most dangerous for plants in the exchangeable Mn^{2+} form occurring mainly under acidic conditions, whereas other species of manganese which predominate at higher pH (Mn^{3+}, Mn^{4+}) are not available for plant uptake (MILLALEO *et al*. 2010).

Organic matter. An assessment was made to relate the distribution of Cu to known parameters which affect its mobility. Figure 3 confirms the dependence between surface horizon Cu retention and organic matter, in this case nitric acid extractable Cu correlated with percentage of oxidizable carbon. The *R* statistic indicates a strong positive correlation which is highly significant due to exceeding the critical value of 0.304 for Pearson's correlation. The relationship was further confirmed by Chi-square analysis, whereby a statistically strong dependence was achieved between variables C_{ox} and Cu concentration (test statistic 34.6 > critical *P* = 9.488). However, Figure 4 indicates that there is no statistical significance for the correlation of humus quality with the occurrence of Cu in the upper 20 cm of soil (critical *P* = 0.304
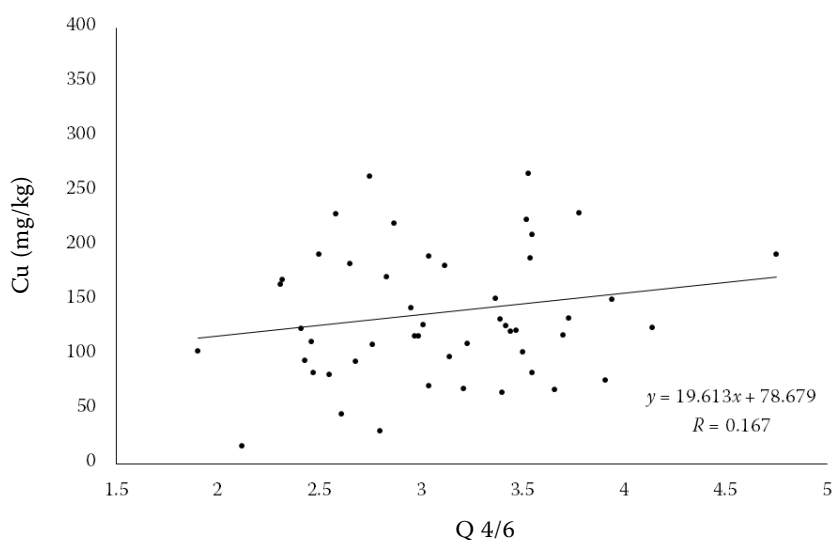

Figure 4. Dependence of Cu retained in the upper 20 cm of soil on humus quality

Table 3. pH $_{H_2O}$ and pH $_{KCl}$

	pH	
	H$_2$O	KCl
Mean	6.17	5.68
SD	0.97	1.17
Variance	0.95	1.36

SD – standard deviation

> R = 0.167) suggesting that Cu retention in the vineyard surface layers is more strongly determined by humus quantity rather than humus quality. For further study, multiple horizon sampling to allow for a leaching depth analysis of PTEs would serve as an expedient tool in better understanding Cu mobility. In other studies it was determined that Cu concentrations are always greatest at the surface due to retention on the soil sorption complex and their concentrations decrease with increasing depth, confirming the anthropogenic origin of Cu in vineyard soils (Pietrzak & McPhail 2004; Komárek *et al.* 2008).

When C$_{ox}$ is mapped with Cu concentration among samples, a pattern becomes clear that the areas with the greatest accumulation of copper correspond to those with the higher contents of oxidizable carbon at the north western corner of the vineyard. Further investigation is required to determine reasons for the higher portion of or-

ganic matter in this part of the vineyard, possibly considering factors such as wind direction, slope, plant species and mass of plants grown at differing sample points etc. Also, the spraying regime by employees is not known and may well have a significant impact on the distribution of fungicide throughout the vineyard. Additionally, knowing hydrological and soil water parameters such as soil permeability, infiltration and runoff rates, water retention etc. and K$_{ow}$ value of fungicides would assist in the assessment of fungicide movement and hence PTE transport within the vineyard.

Soil pH. No apparent relationship between soil pH and distribution of PTE was observed in this case.

Distribution maps. Figure 6 illustrates the distribution of elements throughout the studied vineyard. There appears to be a common trend of accumulation in the western part of the site for all elements. There are some apparent hot-spots of Cd contamination where values are considerably higher than the surrounding values, the reason for which is undetermined. Pb shows a particularly strong trend of surface soil accumulation in the western corner of the vineyard which is very similar to the pattern for Zn. The map of Cu distribution illustrates a trend of overall contamination although the highest values also occur in the far western corner of the study area.

Wine analysis. Two bottles of wine produced at the studied vineyard were purchased; one bottle of white (Riesling Kabinett 2011) and one red
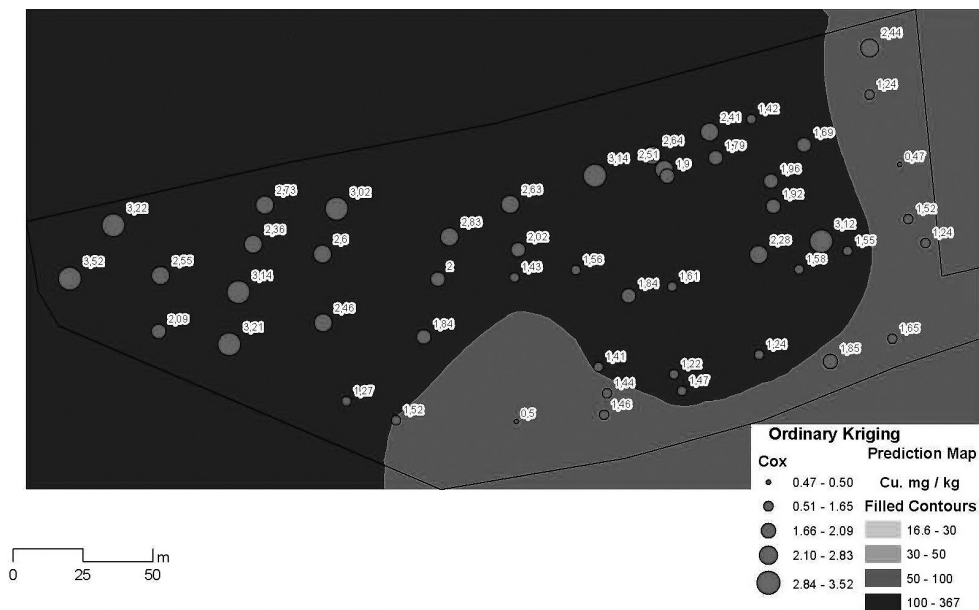

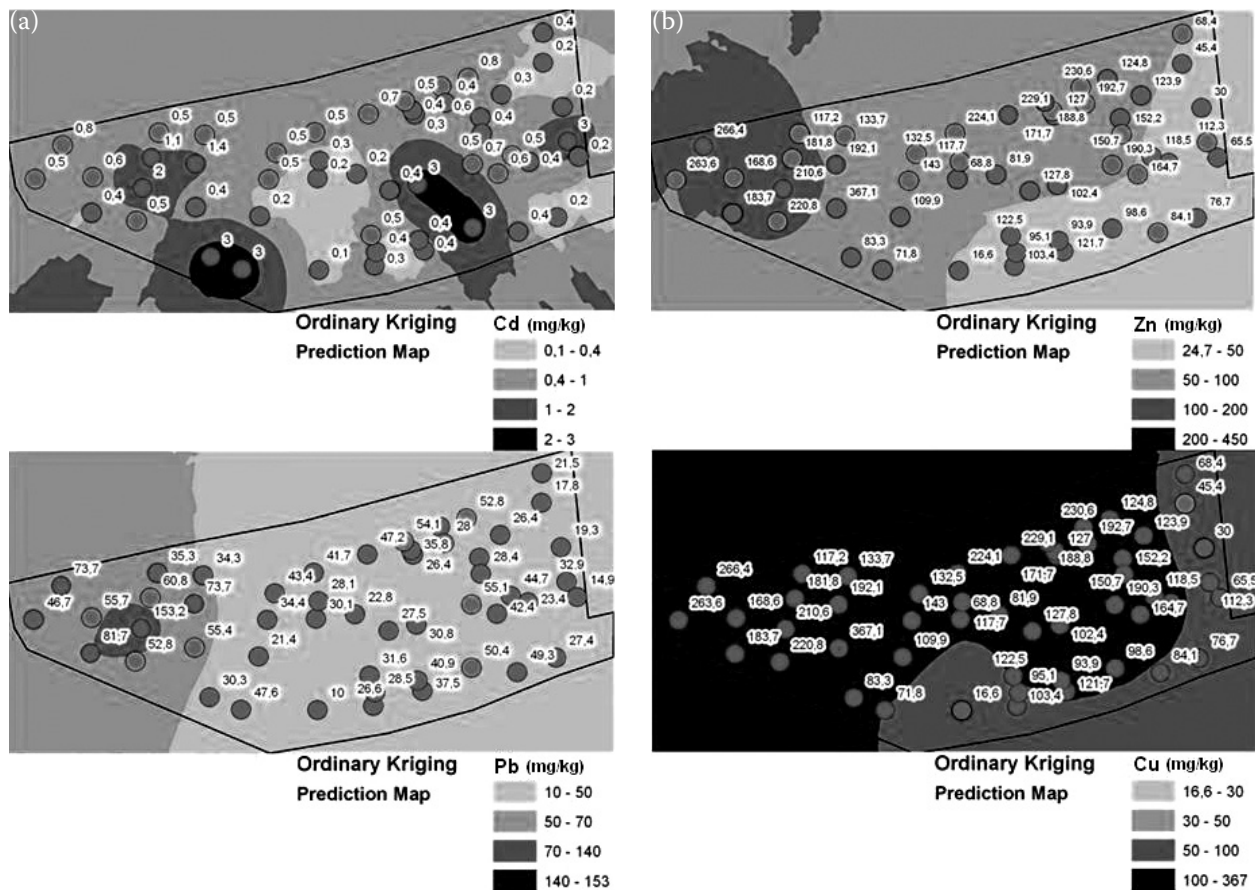Figure 5. Overlay of oxidizable carbon and Cu occurrence in samples

Figure 6. Distribution maps: (a) potentially toxic elements (PTE) Cd and Pb, (b) potentially toxic elements (PTE) Zn and Cu

(Pinot Noir 2010). The wine was analysed for its PTE contents to determine whether or not there is a significant threat of soil contaminants reaching the end product beverage. Mean results from 3 replicates are given in Table 4 with two supermarket wines for reference.

A guideline was set by IPCS (1998) delineating a rough estimate of no more than approximately 3 mg/day oral intake of Cu, and EPA (2004) publish guidelines for human exposure to Cu which includes a drinking water standard of 1.3 mg/l.

With regards to copper, which is the main element of concern, both of our study vineyard wines show insignificant amounts which would have a minimal contribution to reaching the daily allowable intake of Cu. The white wine values were all below detection limits suggesting that red wine production results in greater Cu accumulation, although this should be the subject of further and more thorough investigation. The supermarket wines had a greater content of Cu than for the studied vineyard wine, but the value still represents a fraction of the guideline al-

Table 4. Mean values (standard deviation) of analysed wine samples (in mg/l)

	Cu	Mn	Cd	Pb	Zn
Study area red	0.032 (0.012)	2.393 (0.208)	b.d.l.	b.d.l.	0.296 (0.039)
Study area white	b.d.l.	1.206 (0.053)	0.026 (0.001)	b.d.l.	0.163 (0.008)
Modry portugal	0.086 (0.021)	1.072 (0.040)	b.d.l.	b.d.l.	0.368 (0.011)
Poezie cervena	0.083 (0.023)	1.100 (0.055)	b.d.l.	b.d.l.	0.920 (0.036)

b.d.l. – below the determination limit

lowances. Manganese in the studied vineyard red wine was almost twice the value for the white and more than twice the supermarket wines. The only cadmium which was in a large enough concentration to be detected by the analysis equipment was in the studied vineyard white wine. Unpolluted water usually contains below 0.001 mg/l (Friberg *et al.* 1986) and the dietary daily intake of Cd for most areas is estimated to be in the range of 0.01–0.040 mg (IPCS 1992). Therefore, the white wine produced from grapes at the studied vineyard contains an amount of cadmium which in conjunction with normal dietary consumption, could increase Cd intake to well above average. All Pb analysis gave results below the determination limit, and concentrations of zinc were highest in the two supermarket wines.

CONCLUSIONS

Excessive concentrations of Cu (respective of background concentrations and guideline values for agricultural soils) were consistently evident in the upper 20 cm of the vineyard soil. It is possible to say with confidence that the copper is of anthropogenic origin, most likely as a result of repeated fungicide application. It is apparent from statistical analysis that in this case the retention of copper in surface soil is more dependent on humus quantity rather than the quality of humic substances (although the range of obtained values for Q 4/6 is small). On average, Cd and Zn were found to exceed background concentrations only. Grape vines are very deep rooting plants, therefore suggestions for further study include sampling at depth from all horizons to gain more data concerning PTE leaching, retention and potential uptake.

By studying the distribution maps, a common trend of PTE accumulation was observed in the western corner of the map. Further analysis and more specific data are needed to determine the reason for the distribution.

The analysis of wine samples showed that Cu, although highly concentrated in the surface soil layer, is not being transferred to the end product beverage at a level that is considered dangerous. However, the study area white wine contained an unacceptable level of cadmium, which may have occurred as soil-plant-fruit-wine transfer or as a result of production processes. It is therefore recommended that the study be extended to sample a wide range of wines produced at the studied vineyard, with an analytical focus on all potential transfer stages within the grape vine plant.

Although the observed high soil copper content does not result in any apparant detrimental effect with respect to the consumers beverage, it is not practicable to claim that the elevated levels of soil Cu do not effect the health of the plant and its grape production and/or the health of soil micro and macro fauna which maintain soil quality.

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Fractionation and distribution of risk elements in soil profiles at a Czech shooting range

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ABSTRACT

The fractionation and vertical distribution of risk elements in soil profiles at a shooting range characterized by alkaline soil were studied. High loading rates of lead (Pb) and antimony (Sb) were observed in the upper 30 cm of soil. Copper (Cu) contents are elevated compared to control soil but do not exceed guideline limits for agricultural soil. Zinc (Zn) and nickel (Ni) did not occur in excessive levels; however, easily mobilizable Zn fraction was higher in surface samples, whereas Ni appears to be relatively immobile. Pb and Cu loading in surface soils can be identified as fraction distributions are easily distinguished from those of non-polluted soils. At the firing line Cu, Sb and Zn are most abundant whereas Ni and Pb are concentrated at the target zone. Sequential extraction procedures proved effective for the monitoring of shooting range soils. However, data should be backed by other scenario dependent analyses to confirm the environmental fate of shooting range contaminants.

Keywords: lead; inorganic contaminants; BCR sequential extraction; soil probe; alkaline soil

Shooting ranges (SR) are the second largest source of Pb contamination, with annual depositions ranging from 10 to 60 000 tons in different countries (Ahmad et al. 2012). In addition to lead, SR soils are burdened with a range of risk elements (RE) present in excessive concentrations. Bullet lead exists as an alloy consisting of 90% Pb or more (Sorvari et al. 2006), which can contain variable quantities of Cu, Ni, Sb, Sn, As, Bi, Ag (Dufosse and Touron 1998, Randich et al. 2002). Jackets and casings contain Cu and Zn in varying proportions (Ackermann et al. 2009).

Bound RE can be observed in the environment by using simple extraction methods (Rauret 1998, Ettler et al. 2007) or by applying sequential extraction (SE) methods (Ettler et al. 2005, Bacon and Davidson 2008) which can provide information about the mode of occurrence (natural vs. anthropogenic), potential availability, mobilization and transport of trace metals (Filgueiras et al. 2002).

The aim of the study is to clarify the mobility of selected RE (Cu, Ni, Pb, Sb, Zn) being released from bullets at various soil depths. We assess the effectiveness of SE analyses for tracking the

Table 1. Basic soil properties in target area profiles

Profile layer (cm)	CEC (mmol/100 mL)	pH_{H_2O}	pH_{KCl}	TOC (%)	TIC (%)
SP 1 (0–12)	12.50	8.48	7.65	4.89	1.48
SP 1 (12–31)	9.13	8.27	7.59	2.57	3.20
SP 1 (31–x)	5.75	6.56	5.91	1.24	0.00
SP 2 (0–14)	11.75	7.95	7.17	2.70	0.40
SP 2 (14–29)	9.63	7.96	7.26	1.32	6.15
SP 2 (29–60)	7.25	7.78	7.25	0.60	9.85
SP 2 (60–90)	6.25	7.85	7.39	0.26	9.80
SP 3 (6–12)	11.00	7.80	7.03	2.55	0.43
SP 3 (12–37)	9.75	7.87	7.02	2.23	0.58
SP 3 (37–x)	13.13	7.78	7.20	0.97	3.20
Control (0–5)	8.67	7.79	7.05	1.80	0.94

SP – soil pit; CEC – cation exchange capacity; TOC – total organic carbon; TIC – total inorganic carbon

mobility and binding of individual elements in a contaminated alkaline soil.

Scientific hypothesis. (i) In areas of concentrated shooting, a dissolution of ammunition and migration of RE in soil will be observed. (ii) Mobility and bonding of RE can be effectively monitored by a combination of single extraction methods and SE procedures.

MATERIAL AND METHODS

Sampling. Samples were taken from an outdoor SR in eastern Czech Republic which is characterized by alkaline soil (Table 1). The range is principally an unregulated open grass strip, with no barriers between it and surrounding fields. At the target zone there is a banked soil backstop, leading up to a thicket of trees and a field used for growing crops. The SR has been in use for over 40 years with irregular operating intensity.

Figure 1 shows the sampling plan. Pits were dug to expose horizons at the target area (soil pits 1, 2, 3), and samples were taken from each layer. Surface samples were collected from topsoil 0–5 cm at the firing line, half way and in front of the target area. Control soil was taken from the top 5 cm of a nearby field (100 m from the study area, not influenced by activities of the SR).

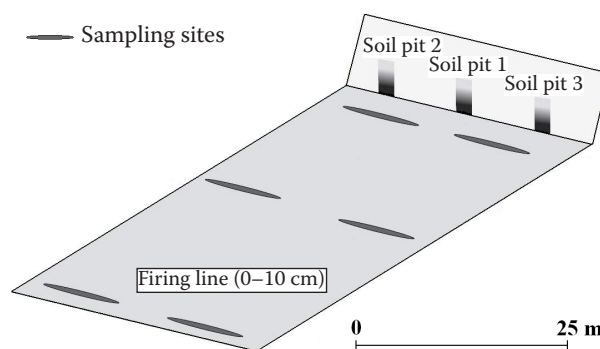**Sample treatment**. Samples were air dried, sieved < 2 mm and bullet fragments were sepe-

Figure 1. Shooting range sampling map

rated. The following basic soil properties were determined: pH (pH_{H_2O}, pH_{KCl}), cation exchange capacity, total organic carbon (TOC) and total inorganic carbon (TIC) content and soil texture classification (Pospíšil 1964, Carter and Gregorich 2008). Simple extractions were made using 2 mol/L HNO_3 (releasable) and total content determined by microwave digestion in a mixture of concentrated acids; $HF/HNO_3/HClO_4$ (Vaněk et al. 2010). The three step BCR SE was applied to detect binding of contaminants in soils. Contents of Pb, Ni and Zn in individual fractions were determined using Varian Spectra AA280 FS (fast sequence, Mulgrave, Australia) FAAS under standard analytical conditions. Calibration was matrix-matched with

Table 2. EDX (energy – dispersive X-ray spectroscopy) analysis of bullet projectile from target area

Localization	Element	Jacketed bullets 1	Non-jacketed bullets 2	Non-jacketed bullets 3
		composition (%)		
Bullet core	Pb	94.5	96.7	95.2
	Al	0.24	3.26	2.02
	Sb	3.78	–	2.78
	Fe	1.44	–	–
Mantel[a]	Fe	66.6	–	–
	O	33.4	–	–
Mantel[b]	Cu	83.2	–	–
	Fe	4.02	–	–
	Pb	4.3	–	–
	Zn	8.32	–	–

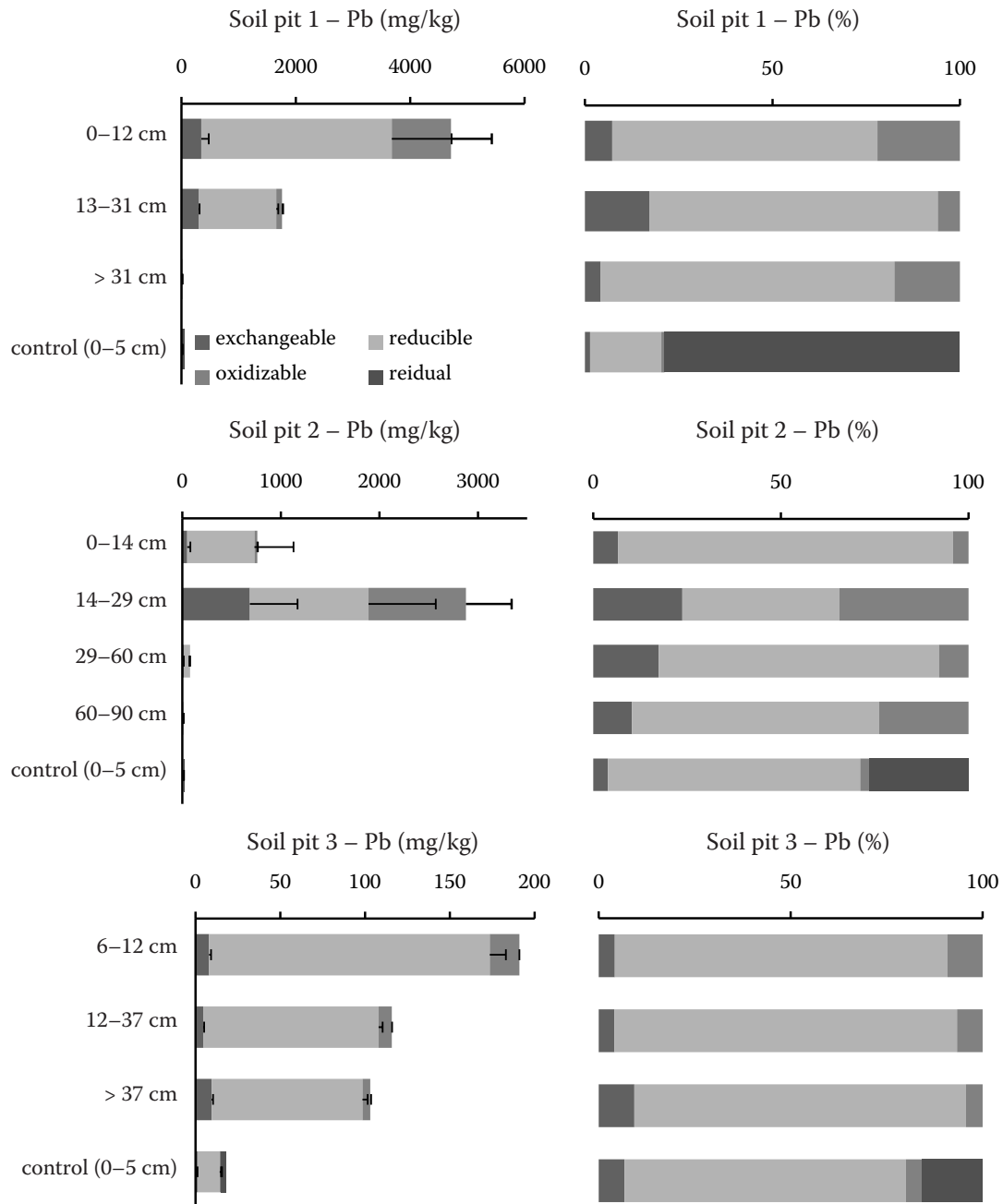[a]bulk mantle; [b]thin layer between mantle and core

Figure 2. Sum of all Pb fractions

standards from Analytika s.r.o. (Prague, Czech Republic) Cu and Sb were determined by ICP-OES (Thermo Scientific iCAP 6500 Radial ICP, Cambridge, UK). A procedural blank was run for each extraction step, samples were analyzed in triplicates. A combination of energy – dispersive X-ray spectroscopy (EDX) and X-ray diffraction (XRD) was used to identify composition and mineralogy of projectile remains in soil samples using SEM PHILIPS XL 30 CP (Amsterdam, the Netherlands) equipped with EDX detector and

PANalytical X'Pert Pro diffractometer (Almelo, the Netherlands), respectively.

RESULTS AND DISCUSSION

Basic soil parameters. The study area is characterized by alkaline soil, texture is classed as silt loam. Mineralogical composition was identified for control samples and soil pit samples from the target area. Quartz was detected as the prevailing mineral, less
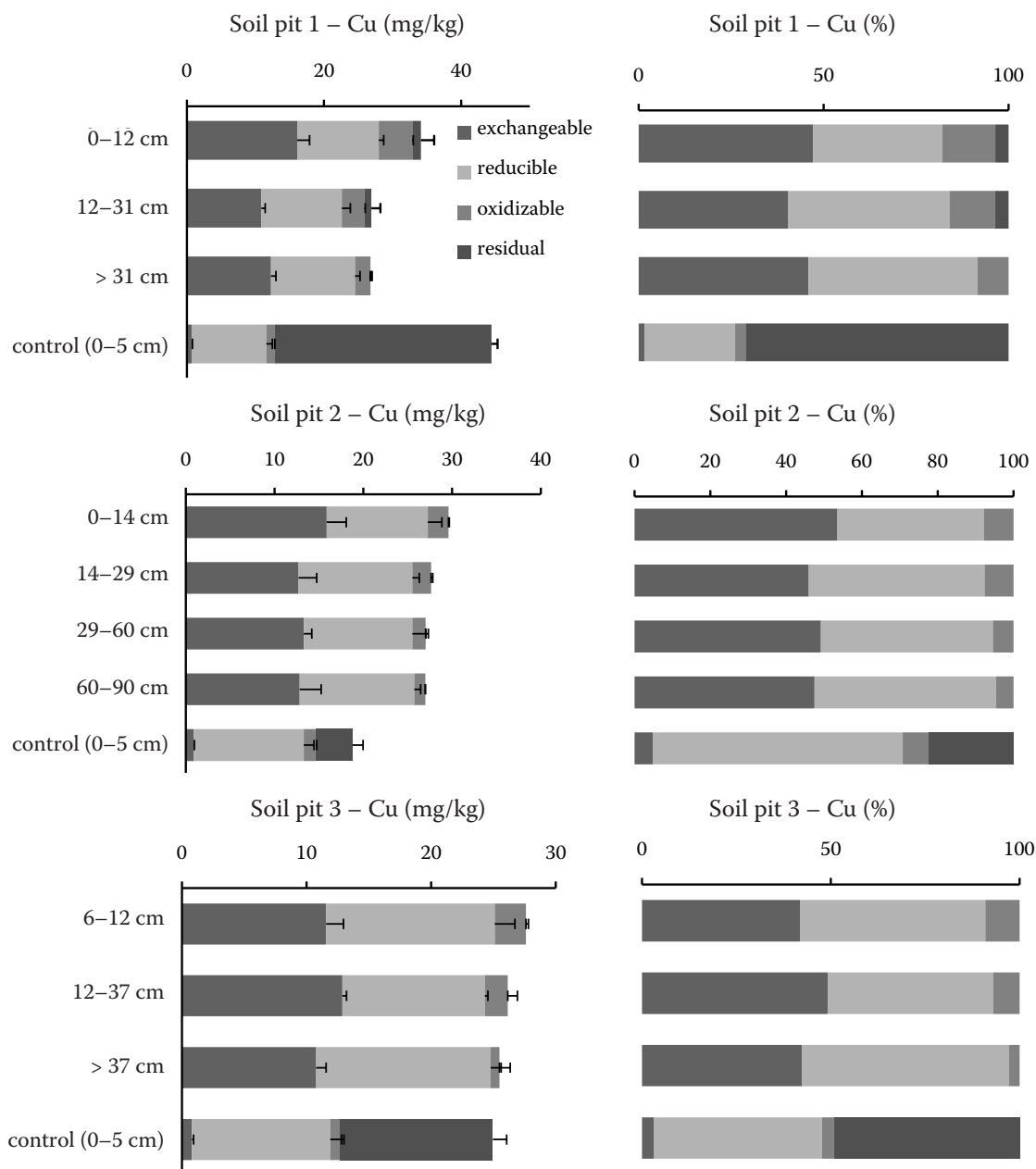
Figure 3. Sum of all Cu fractions

abundant was – albite, calcite and muscovite/illite and occasioning – dolomite, titanite, K-feldspar.

Bullet composition. Approximately 80% of bullets recovered were non-jacketed all lead alloy, most likely fired from pistols. The lesser proportion of recovered projectiles was either full metal jacket bullets most often fired from rifles, or semi-jacket type bullets.

An increased sorption of RE (mainly Pb) was observed at the bullet surface by newly formed minerals in the weathering crust: Lepidocrocite $\gamma\text{-Fe}^{3+}O(OH)$, goethite $\alpha\text{-Fe}^{3+}O(OH)$, akaganeite $\beta\text{-Fe}^{3+}(O.OH.Cl)$ and whewellite. In agreement with other findings (Lin et al. 1995, Cao et al. 2003) Pb secondary minerals present at the projectile surface occurred mainly in the form of cerussite $PbCO_3$ and hydrocerussite $Pb_3(CO_3)_2(OH)_2$. Table 2 lists elemental percentages of the major bullet components.

Target area. In soil pits 1 and 2, compared to control soil a significant enrichment of RE was observed, particularly for Sb and Pb. Surface enrichment of Cu, Ni and Zn was not so pronounced. Compared to guideline limits (GL) for agricultural
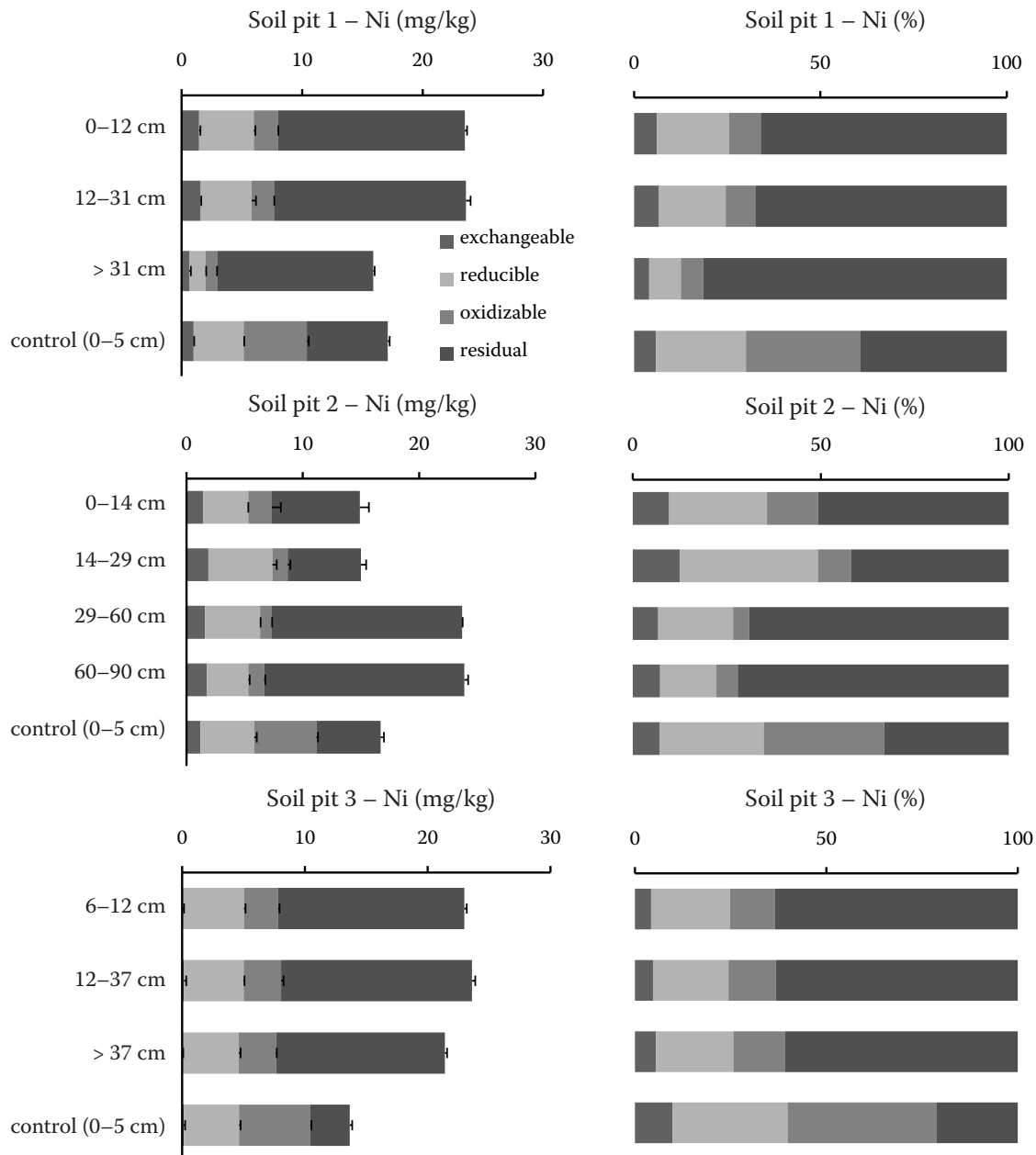
Figure 4. Sum of all Ni fractions

soils (Beneš 1993) the 2 mol/L HNO_3 extraction shows concentrations of Pb to be highly excessive.

Sequential extraction. Figures 2–5 illustrate the RE contents within each sampled horizon and percentage distribution of fractions: exchangeable, reducible, oxidizable and residual.

There is a well defined enrichment of Pb in the upper 30 cm of soil, after which Pb concentrations are within the GL. Speculations can be made as to reason why the underlying horizon in soil pit 2 is more highly enriched in Pb than the other soil pits. Cao et al. (2003) observed a substantial amount

of downward migrating Pb in the subsurface soil which was linked to possible enhanced solubilization of organic complexes at alkaline pH and Knechtenhoffer et al. (2003) determined that it is not uncommon for preferential flow to mobilize significant amounts of Pb to subsurface horizons. The time frame of bullet decomposition in soil was observed by Jorgensen and Willems (1987) whereby 5–17% transformation from metallic Pb pellet to free ionic Pb had taken place within 6–13 years.

The residual Pb content was found to be negligible and fractions follow the order (red. > ox. > exch.
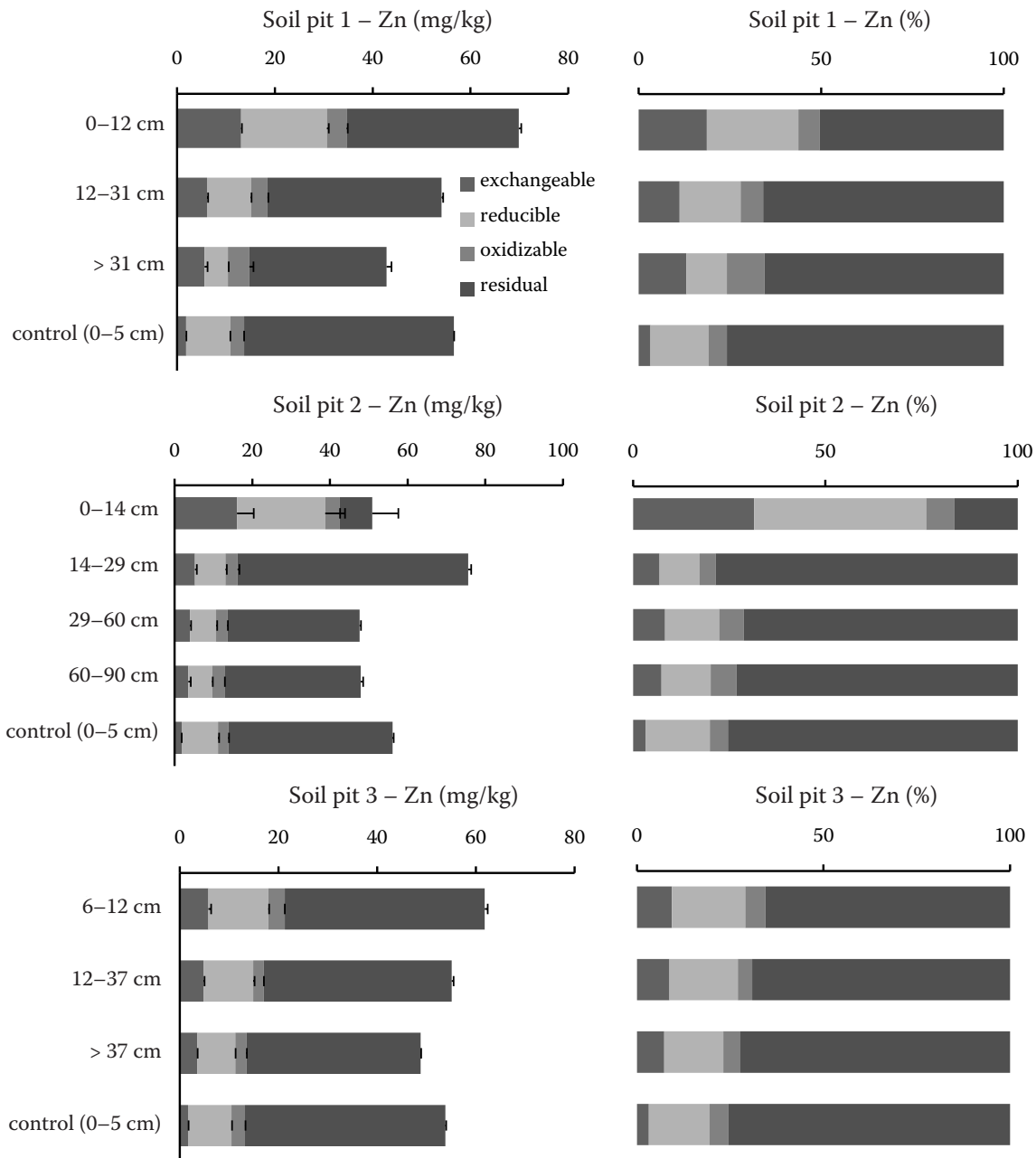
Figure 5. Sum of all Zn fractions

> res.). This fraction order was also observed by Conesa et al. (2010) and is common among many long term polltued soils regardless of the source of Pb as shown by other investigations (Takáč et al. 2009, Chrastný et al. 2010). Exchangeable Pb percentage in surface samples forms a weak negative correlation with pH_{H_2O} and TOC ($R = 0.415$ and 0.475, respectively). Leaching studies and long-term extraction experimentation could be applied to samples to aid prediction of Pb mobility.

An even distribution of Cu throughout the soil profile may be due to downward migration with soluble complexes (EPA 1992). Exchangeable Cu accounts for almost half of the total content in the alkaline soil whereas in the control sample < 5% Cu is easily exchanged, with the majority being bound to Fe/Mn oxides (Figure 3). Organically bound Cu is firmly coupled with the presence of TOC ($R = 0.932$).

Nickel fraction percentages in target area soil are in agreement with those commonly observed

Figure 6. Content of Sb extracted by 2 mol/L HNO$_3$

in normal soils, predominantly in residual and Fe/Mn-bound fractions (Kabata-Pendias and Mukherjee 2007). Results of SE performed on two SR soils by Conesa 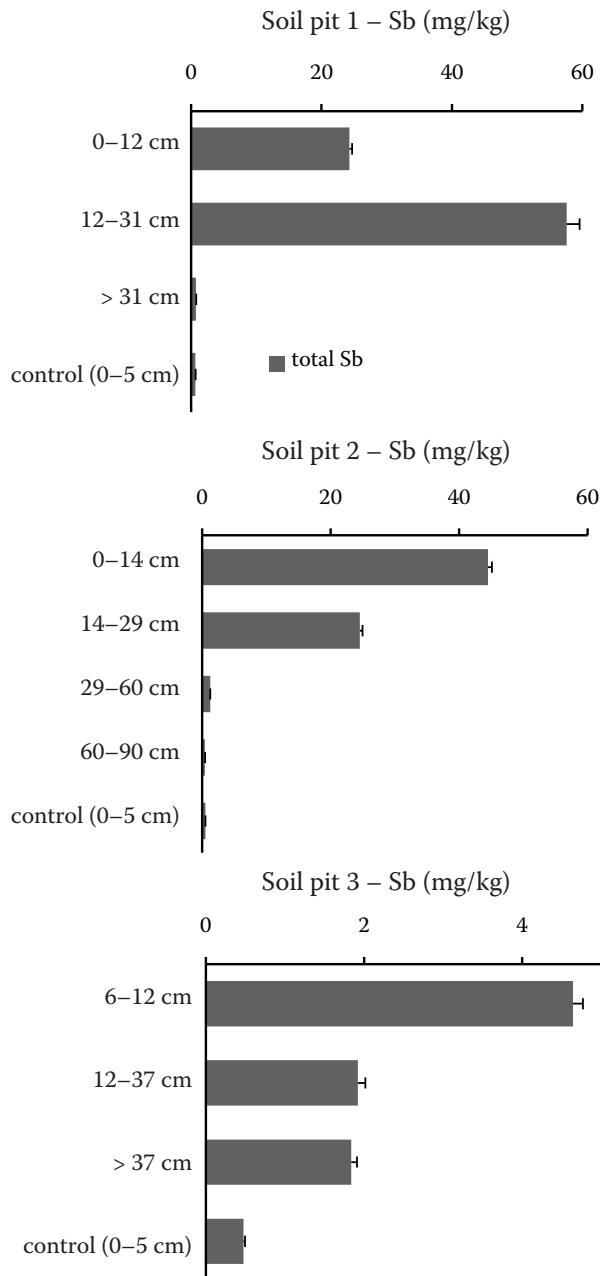et al. (2010) matched the analysis of our SR whereby more than 90% of Ni resides in the last three steps (Figure 4). Nickel does not form insoluble precipitates in unpolluted soils and therefore retention of Ni is exclusively through adsorption mechanisms (EPA 1992) as evident in the control soil.

Zinc concentrations in SR soils typically surpass background levels but are seldom excessive (Knechtenhofer et al. 2003, Migliorini et al. 2004). Total Zn content did not exceed 50% of the GL even at the surface, implying that this is not a highly problematic element. Tessier et al. (1980) found the majority of Zn in polluted soils to be associated with Fe/Mn oxides and Pustišek et al. (2001) observed that the exchangeable Zn portion also increases in samples which are increasingly contaminated. Despite low overall values, in the case of soil pits 1 and 2, a distinction can be made between fraction distributions of control soil and SR surface layers (Figure 5).

Individual fractions of Sb occurred below detection; therefore 2 mol/L HNO$_3$ extraction results are given (Figure 6). With the exception of soil pit 1 surface layer, there is a trend of decreasing Sb with depth, which becomes more pronounced after 30 cm. The results matched those of Vleek et al. (2011), whereby SR soil Sb concentrations are excessive in the upper 30 cm of a soil core after which the content decreases.

Two apparent trends exist with respect to RE distributions along the bullets path (Figure 7). (i) Soil contains elevated concentrations of Cu, Sb and Zn at the firing line and concentrations generally decrease with distance away from this point. (ii) Target area soil contains elevated concentrations of Ni and Pb and concentrations generally decrease with distance from the target mound. The observed distribution is consistent with the amounts that would be expected due to the composition of either bullet or casing.

Trends identified by SE and analysis of basic soil properties suggest that Pb retention in upper horizons is enhanced by alkaline soil conditions and sufficient organic carbon contents. Excessive concentrations in subsurface horizons may be due to enhanced formation of soluble organic ligand-Pb complexes at alkaline pH or preferential flow. SR soils can be identified primarily by a greater share of reducible Pb fractions. Despite low total values of Cu, there is a marked difference in fraction distribution between non-polluted soils and those affected by SR activities. Compared to background concentrations, Ni does not appear to be a problematic element. Zinc concentrations are also not excessive but Zn is increasingly bioavailable in surface layers. Antimony is highly enriched in the upper 30 cm of soil. High concentrations observed at the firing line suggest that bullet casings or barrel discharges are the greatest
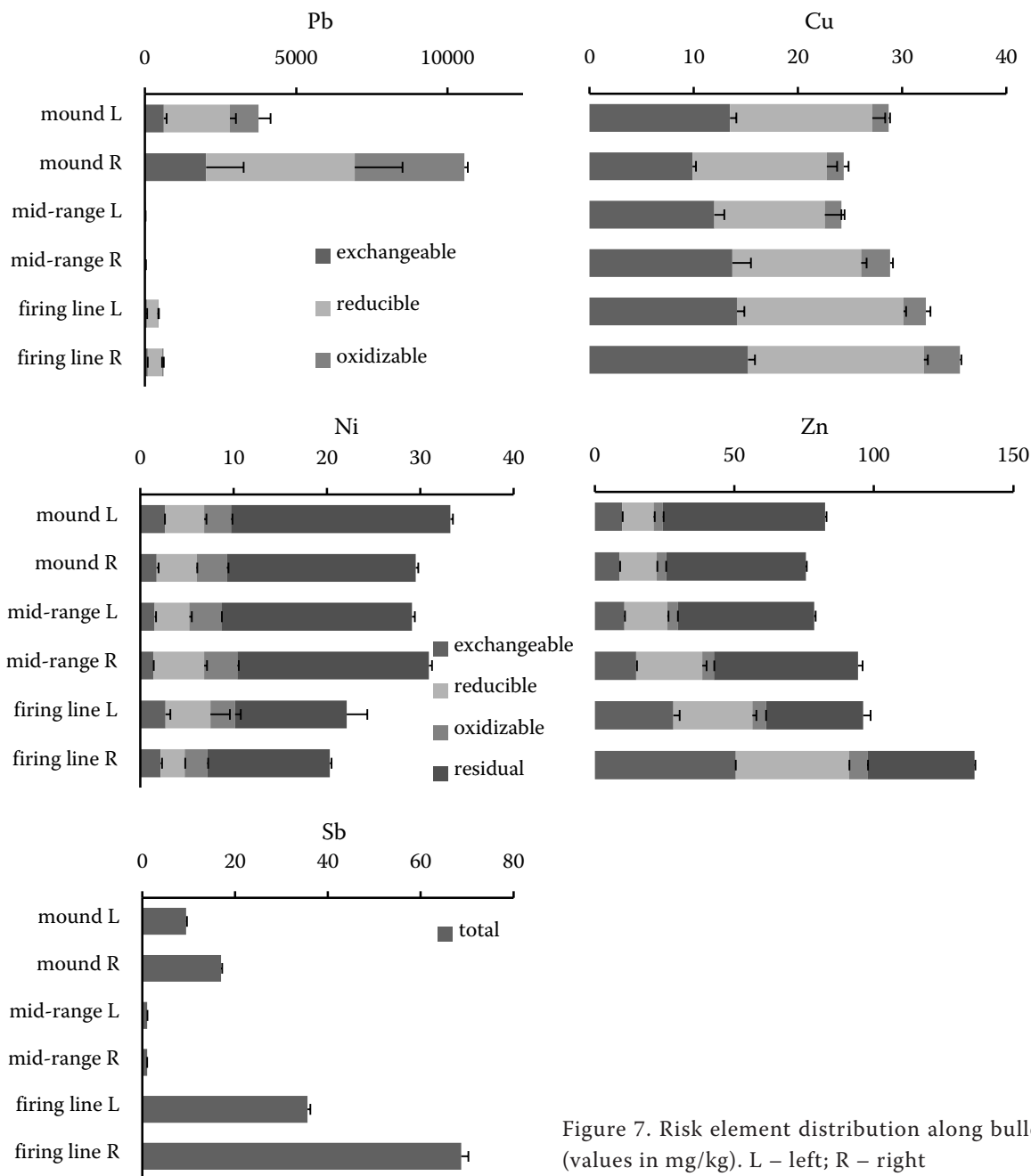
Figure 7. Risk element distribution along bullet path (values in mg/kg). L – left; R – right

source of Sb. In areas of concentrated shooting dissolution of ammunition and vertical migration of risk elements (Pb, Ni, Sb) was observed in the soil profile. Sequential extraction procedures are effective for monitoring shooting range soils. However, SE observations should be supported by other analysis tools specific to the aims of the user in order to confirm the environmental fate of SR contaminants. Anthropogenic inputs to a soil can be identified by the pattern of fraction distribution, irrespective of total concentrations. Single extrac-

tion procedures are suitable only for comparison of REs to guideline values and/or between study sites.

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Temporal dissolution of potentially toxic elements from silver smelting slag by synthetic environmental solutions

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ARTICLE INFO

ABSTRACT

Waste slag which is created during precious metal smelting contains high levels of potentially toxic elements (PTE) which can be mobilised from unconfined deposits into the local environment. This paper examines the extractability of selected PTE (Pb, Zn, Cd, Mn) from slag samples by synthetic solutions designed to replicate those in the environment. Extracting agents were used to replicate potential leaching scenarios which are analogous to natural chemical weathering. Slag was submersed in a rainwater simulation solution (RSS), weak citric acid solution (representing rhizosphere secretions) and control solutions (deionised water) for a one month period with solution analyses made at intervals of 1, 24, 168 and 720 h. In 1 mM citric acid, dissolution of Cd and Zn showed little change with time, although for Zn the initial dissolution was considerable. Lead in citric acid was characterized by overall poor extractability. Mn solubility increased until an equilibrium state occurred within 24 h. The solubility of studied metals in citric acid can be characterized by a short time to equilibrium. RSS proved to be an effective solvent that, unlike citric acid solution, extracted increasing concentrations of Cd, Mn and Zn with time. Solubility of Pb in RSS was again very low. When taken as a proportion of a single 2 M HNO_3 extraction which was applied to slag samples, Cd was the element most readily leached into RSS and control samples. In both studied solvents, slag heterogeneity is prominent in the case of Cd and Zn solubility. Contact time with solvent appears to be an important variable for the release of PTE from slag into solution. The purpose of this study was to provide insight into the environmental chemical dissolution of PTE from slag, which causes their enrichment in surrounding soils and surface waters.

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1. Introduction

Silver mining was a thriving industry in the Czech town of Kutná Hora (KH) which emerged as a result of the exploitation of Ag bearing loads discovered, possibly as early as the 10th century (Pauliš and Mikuš, 1998). A large expansion of exploitation of the minerals in the region reigned from the second half of the 13th century and production continued to be strong up until the turn of the 17th century where it experienced a gradual decline as remaining ores became less economically feasible to extract. To obtain silver, a smelting process is required which results in the formation of large quantities of slag wastes, which typically contain high concentrations of potentially toxic heavy metals and metalloids, plus various minerals due to the ore composition and fluxes added during the smelting process.

Disposal of mine wastes and other materials which can act as sources of mobile potentially toxic elements (PTE) should be done so with consideration of the composition and discharge of their leachates. Slags of historical smelting practices were typically deposited with no control over the fate of weathering products, and thus attention has been given to the behaviour of smelter wastes in a number of recent studies (Ettler et al., 2009a; Kierczak et al., 2013; Lima and Bernardez, 2011). Although the mining history of KH is of high cultural value and today much attention is given to the mines which are an integral part of the town's economy via tourism, there are a limited number of studies which have focused directly on the smelter slag. A study by Králová et al. (2010) examines soils close to the mines, whereby high concentrations of As, Cd and Zn were observed. Excessive concentrations of As, Cd, Cu, Mn, Pb, and Zn extractable by 2 M HNO_3 were

identified in soils directly surrounding the KH slag during the development of a thesis study (Ash, 2012). However, investigations into the forms of PTE originating from the smelter slag of KH have typically identified very low available and easily mobilised forms compared to the (pseudo) total concentrations (Ash, 2012; Száková et al., 2009).

The release of inorganic contaminants bound in slag from solid to liquid phase due to mineral dissolution, desorption and complexation processes can be affected by a range of parameters, such as pH, redox reactions, dissolved organic matter and microbial activities which are in turn dependent on the soil solution. One approach for investigating the mobility of PTE and minerals from slag is to subject these materials to a range of solutions that can occur in the natural environment. Piatak et al. (2004) proposed an effective precipitation experiment using a method based on a field leach test developed by Hagemann and Briggs (2000), whereby crushed and sieved samples were subjected to extraction by deionised (DI) water or a solution that replicates local precipitation (mixture of H_2SO_4 and HNO_3 with DI water to achieve pH 4.2). Alternatively, the focus may be on the application of low molecular mass organic acids (LMMOA) which commonly occur in nature. Ettler et al. (2003, 2004, 2009b) have developed a number of studies dedicated to the use of LMMOA in leaching experimentation. Initial studies were conducted to simulate the presence of vegetation on metal contaminated deposits and the effect that root exudates have on the mobility of metals (Ettler et al., 2003). Preliminary dissolution tests were performed using citric acid solutions (20 and 8 mM) in order to determine the kinetics of release of Pb, Cd, Zn and As. These concentrations are particularly strong compared to those found in naturally occurring soil solutions and were designed to demonstrate maximum reactivity of contaminated material. LMMOA influence a range of soil properties and chemical behaviours, most notably by altering pH and functioning as ligands, increasing the total amount of dissolved cations in soil solutions by chelation/complexation (Fox, 1995). Houben and Sonnet (2012) showed that compared to plant free controls, root activity doubled the rate of Zn release from smithsonite to leachates and that metal concentration in leachates was controlled by DOC originating in organic compounds produced by rhizosphere activity. In some studies, the cultivation of microorganisms to enhance metal chelation due to LMMOA production in non-sulphidic ores has shown mixed results (Seh-Bardon et al., 2012; Deng et al., 2013).

A significant feature of smelter slag is the mineralogy and the influence that dissolution and subsequent formation of mineral compounds has on the solubility of selected analytes (Ahmed et al., 2000). Maskall et al. (1996) studied the mobility of Pb at various slag deposits, where despite finding high surface concentrations, relatively small amounts were found to be in mobile forms. The insolubility of Pb was partly attributed to the weathering of Ca bearing minerals which raised the pH of the slag. In a recent study, Vítková et al. (2011) proved that metal leaching from smelter slag has strong dependence on granulometry and Kierczak et al. (2013) noted differences in extractability of metals between slags of differing cooling rates. In the field, slag varies in size from large rocks to fine dust according to the degree of weathering, therefore the rate of leaching in the environment is also variable.

The aim of this experiment is to study the temporal solubility of selected PTE from slag into solutions which are representative of those which exist in the environment. Testing different solvents on the dissolution of PTE gives an indication of the concentration of contaminants that are being mobilised during chemical weathering and will help determine the significant pathways for migration of PTE into surrounding environmental media.

2. Material and methods

2.1. Study area and sampling

Sample material used in this study was taken from a large deposit along the River Vrchlice, 1.5 km south of Kutná Hora. The slag deposit is approximately 100 m in length, with areas of varying slope, cover and stages of vegetative development. Multiple slag pieces of varying size were collected by hand from the surface of the heap at three places, and labelled simply as samples 1, 2 and 3. The three positions at which slag was collected were loosely randomized, but samples were widely spaced, situated around half way up the heap and from an area free of plants or litter.

2.2. Experimental procedure

The LMMOA chosen for analysis was citrate (Lach-Ner s.r.o) prepared to 1 mM concentration by diluting with DI water. Levels reported for different LMMOA vary between nM to mM, and soil solution concentrations of the dominant organic acids can range from 1 to 100 mM (Van Hees et al., 2002, 2005). However, in common soil solutions, the concentration of LMMOA is usually up to 1 mM (Ettler et al., 2004). The rainwater simulation solution (RSS) for this experiment was inspired by rainwater samples collected from the Jizera Mountains in the Czech Republic as part of an experiment by Tejnecký et al. (2009). The relatively acidic rainwater from this region gives a maximum case scenario. The solution was replicated using DI water with pH adjusted to 4.6 using 65% HNO_3 (Lach-Ner s.r.o). Extractions were performed in reaction vessels (250 mL, 8 oz opaque P.E bottles) into which 200 mL of solvent was added to slag sample. Prior to experimentation, slag pieces were washed to remove soil and debris, ground to <2 mm and mixed to produce a homogeneous sample. Smelter slags are highly heterogeneous in structural material and mineral composition as well as chemically, therefore, in studies which have focused on the analysis of smelter slag (Ettler et al., 2009a; Gee et al., 1997; Manasse and Mellini, 2002), for bulk analysis, sample material is homogenized to give a mean composition. The ratio of solvent to slag differed for reaction vessels depending on which solvent was used. For RSS, samples were prepared in a 20:1 solvent:slag (v:w) ratio, whereas citric acid samples were prepared in a 10:1 solvent:slag ratio, the purpose of this was to increase the concentration of slag in order to amplify reactivity in the anticipation of citrate quickly decomposing. The volume of LMMOA used in this experiment does not necessarily mirror the volumes that are produced in rhizosphere solutions, it simply offers a scenario where contact between slag and citric acid is ensured and sufficient volumes can be analysed. Reaction vessels were mechanically shaken for 5 min using an orbital shaker then left undisturbed at room temperature until the required analysis at 1, 24, 168 and 720 h. Samples for each time period were prepared independently. A control (DI water, pH 5.5), was used for RSS and LMMOA in the respective solvent:slag ratios 20:1 and 10:1. At the end of each required time period, solutions were disturbed by inverting the bottle, then filtered through Filtrak 390 filter paper (Niederschlag, Germany). Single extractions were made to slag samples to determine the releasable content of PTE; by shaking (as before) 5 g of slag with 50 mL of 2 M HNO_3 for 6 h at 21 °C. The sample was centrifuged at 11,000 rpm then filtered (Filtrak 390). Analytical standards were matrix matched and made from certified reference material 'water calibration solution CZ 9001 ÷ CZ 9200' by Analytica s.r.o. Determination of target elements was made using an iCAP 6500 radial ICP emission spectrometer, Thermo Scientific, UK (determination limit: Cd 0.002; Mn 0.005; Pb 0.03; Zn 0.005 mg/L). Standard reference materials NIST 1640 and NIST 1643d were used

to check the quality of element determination in the aqueous extract. Citric acid, RSS and 2 M HNO₃ extractions were made in duplicates, and a procedural blank was run for each extraction step. For the detection of changes in citrate, samples were additionally filtered through a nylon 0.45 μm syringe filter, then analysed using an ion chromatograph ICS 1600 equipped with IonPac AS11-HC. Quality control and assurance of IC analysis is described in a paper by Tejnecký et al. (2013). pH measurements were taken for each solution at the start of the experiment and after 24, 168 and 720 h using a WTW inoLab pH probe. To determine significant differences between slag samples and for changes in PTE content between analysis times, ANOVA and Kruskal Wallis tests were made respectively, using IBM SPSS. Graphs with uncertainty estimates were prepared in Microsoft excel.

3. Results and discussion

3.1. Citric acid solution

Fig. 1 shows that the citric acid solvent is effective in the dissolution of a small quantity of Cd. However, after 1 h little (or no) further extraction occurs. Except for with sample 1, the results suggest that Cd had reached a state of equilibrium. There also exists a statistically confirmed difference in Cd concentration between samples (Table 2). Solubility of Mn is stated as being highly dependent on pH and redox potential (Kabata-Pendias, 2001). Godo

and Reisenauer (1980) plotted a decrease in soluble Mn in 0.01 M citrate solution from pH 4.5–5.5. However, Kabata-Pendias (2001) describe the ability of Mn to form organic ligand complexes even in the alkaline pH range. In the current study, complexation between Mn and citrate may account for the increased soluble Mn content up to 24 h, after which there is little significant change. Equilibrium appears to have been reached within 24 h, a small decrease after 24 h observed in sample 1 is questionable due to wide standard error. Of the extracting agents tested, citric acid was able to extract the greatest amount of Pb, although the overall concentration is relatively low <1.5 mg/kg. There was a significant decrease in Pb solubility between 1 h−24 and 168 h (Table 1), after which an increase was observed for samples 2 and 3, however, Pb content in all samples was small at the end of the experiment. The decrease in Pb in solution over time appears to be inversely related with the change in pH of solution (see Section 3.3). Kourgiantakis et al. (2000) found that the addition of NH₄OH or KOH to a Pb-citrate reaction mixture induced precipitation, and raising pH beyond 4 resulted in the immediate formation of a precipitate containing lead and citrate. Seh-Bardon et al. (2012) experimented with the use of the fungus Aspergillus Fumigatus in mine tailing leaching column experiments. When applied to the column surface, production by the fungus of up to 25 mM citric acid was induced and consequently resulted in the removal of 60% of Pb over 80 days. Deng et al. (2013) found that a mixture of bio-produced organic acids by Penicillium Chrysogenum were more efficient in the
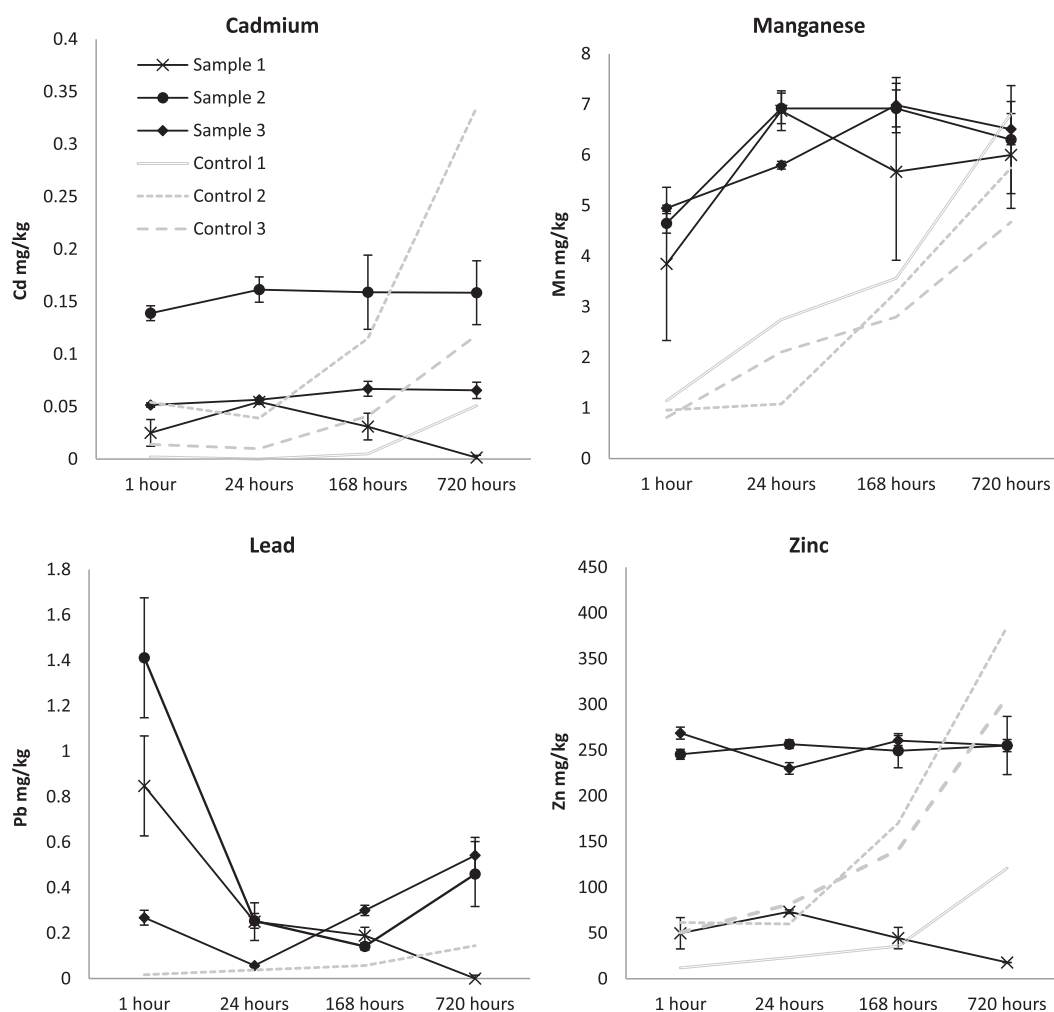

Fig. 1. Change in soluble potentially toxic element concentrations extracted with 1 mM citric acid solution (Pb in control 1 and 3 = below determination limit).

Table 1
Kruskal Wallis test for soluble PTE content change between sampling times in citric acid replicates from all slag samples combined.

1 mM citric acid solution				
	Mean rank			
	Cd	Mn	Pb	Zn
1 h	10.17	4.33	17.17	13.5
24 h	14.08	15.50	6.67	11.67
168 h	13.58	16.17	7.33	13.17
720 h	12.17	14.00	16.50	11.67
P	0.775	0.012*	0.005*	0.952
(1 h–24 h)	0.127	0.004*	0.016*	0.749
(1 h–168 h)	0.575	0.037*	0.016*	0.749
(1 h–720 h)	0.873	0.004*	0.394	0.749
(24 h–168 h)	1.000	0.522	0.749	0.631
(24 h–720 h)	1.000	0.522	0.011*	1.000
(168 h–720 h)	0.631	0.423	0.011*	0.631

*Significant at < 0.05 (Tabulated output from multiple Kruskal–Wallis tests without correction).

Fig. 2. Decomposition of citrate in citric acid solutions over one month of experimentation.

3.2. Rainwater simulation solution

Trends identified for RSS (Fig. 3) are more consistent than for citric acid. Initial concentrations of Cd extractable by RSS are slightly lower than the initial concentration extractable by citric acid. However, the soluble portion of Cd in RSS continues to increase as the experiment progresses, eventually reaching a value of 0.32 mg/kg for sample 3. Despite the common trend, there is quite a wide range between the final concentrations of Cd in RSS for the three samples. The trend of Mn dissolution from slag in RSS is similar to that of Cd with an increasing release over time but in a more linear fashion. The highest detected value for Mn in RSS (5.66 mg/kg sample 1) corresponds to a solution concentration of 0.288 mg/L which can be compared with the highest concentration in the control of 0.4 mg/L, a concentration which is still below the level in soil solution when Mn begins to exhibit toxicity toward most crop plants (Hue et al., 2001). It was apparent for sample 1 that Mn in the control exceeded that of RSS. Although the control solution is less acidic, the result may reflect the low ionic strength of DI water and the potential for cations to be drawn into solution via osmotic forces. Heterogeneity of slag samples should also be considered a possible cause of inconsistent results within samples. The amount of Pb that was extracted by RSS was minimal. A small amount of Pb appears to be detectable in solution initially, but the concentration decreases after just 24 h, followed by a possible small increase after 1 week (Table 3). Overall concentrations of Pb in all solutions are inconsequential and no value for Pb in control samples could be obtained. Kierczak et al. (2013) also found a DI water extract to be a poor extractant of Pb from slag (0.022 mg/L after 24 h), although they did find citric acid to be effective (5.1 mg/L Pb after 24 h). The differing experience with citric acid may be attributed to its stronger concentration (20 mM) and continuous shaking. Similar to the observed trend for Cd and Mn, in RSS the desorption of Zn continues for the duration of the experiment, reaching a maximum value of 426.4 mg/kg Zn in sample 3. As the trend of Zn dissolution was still sharply increasing at the end of the experiment, the possibility that further extraction may be achieved with more time cannot be dismissed. This is in contrast to the findings of Kierczak et al. (2013) whereby DI water and stream water did not remove significant quantities of Zn. The heterogeneity of metal dissolution between samples is most prominent for Cd and Zn, as was the case in citric acid (Table4).

With the exception of Pb which did not yield data for the control solution, the selected PTE display a close correlation in the RSS and the control solution. The RSS is representative of strongly acidic rain
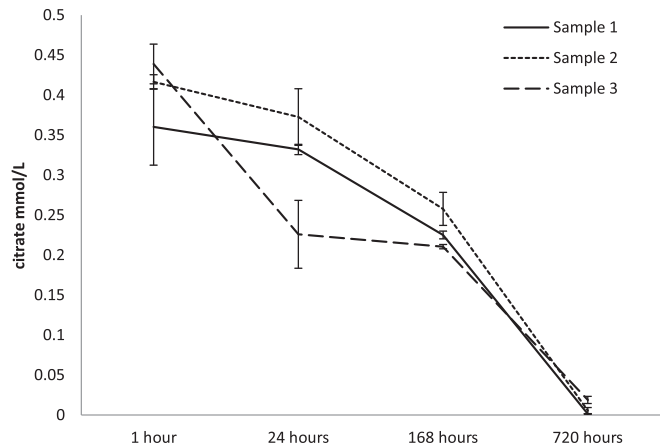
removal of Cd, Mn, Zn from a slag/soil mixture than other individually prepared LMMOA. On the other hand, 0.5% citric acid was able to leach more Pb than the bioleach. Reasons for successful Pb extraction may include the fact that the mixture was kept to a temperature of 30 °C and shaken constantly for 4 days. Although citric acid can be effective in Pb chelation and transport, it is apparent that the stability of this process is dependent on pH and efficiency is greatly enhanced when citric acid is highly concentrated. There was significant variation in the dissolution of Zn between sample 1 and the other two samples (Table 2), highlighting the heterogeneity of the studied material. Although a value of around 250 mg/kg Zn was observed in citric acid for samples 2 and 3, equilibrium occurred sometime within the first hour, thus it is necessary to make analyses within the first hour to determine the causes and trends. The short time to equilibrium that was observed for the studied metals in citric acid is an important characteristic with regards to its implications in the environment, particularly in the case of Zn which has the potential to be scavenged from slag to a relatively high concentration in a short contact time.

The stability and decomposition of citrate was monitored in all three samples (Fig. 2). In each case, trends were more or less parallel. Data presented in Fig. 2 and their relation to trends observed in Fig. 1 suggest that the rate of decomposition of citrate over one month of experimentation does not directly affect the solubility of the studied metals, with the exception of Pb whose availability may be partially related to the dissociation of citrate. Citrate concentrations decreased over the duration of the experiment to infinitesimal levels after one month. Ettler et al. (2004) had experienced similar results in a leaching experiment whereby citric acid solutions made up to 20 mM and 8 mM had decreased after 24 h to 15.5 and 5 mM respectively and after 168 h the 8 mM solution had dropped below detection limit. Hashimoto (2007) observed very high citrate sorption onto the mineral phase, reducing its biodegradation by up to 99% in acidic soil. Rapid sorption of citrate to Al and Fe oxides may also contribute to the low solution concentration observed after just 1 h (half the initial concentration).

Table 2
Analysis of variance between samples 1, 2 and 3 for citric acid extractions − P (F).

	1 h	24 h	168 h	720 h
Cd	0.002** (98.6)	0.001** (150)	0.022* (17.8)	0.007** (37.8)
Mn	0.515 (0.84)	0.049* (9.65)	0.479 (0.95)	0.853 (0.17)
Pb	0.024* (16.5)	0.050 (9.61)	0.020* (18.7)	–
Zn	0.001** (236)	0.000** (888)	0.001** (172.4)	0.002** (106)

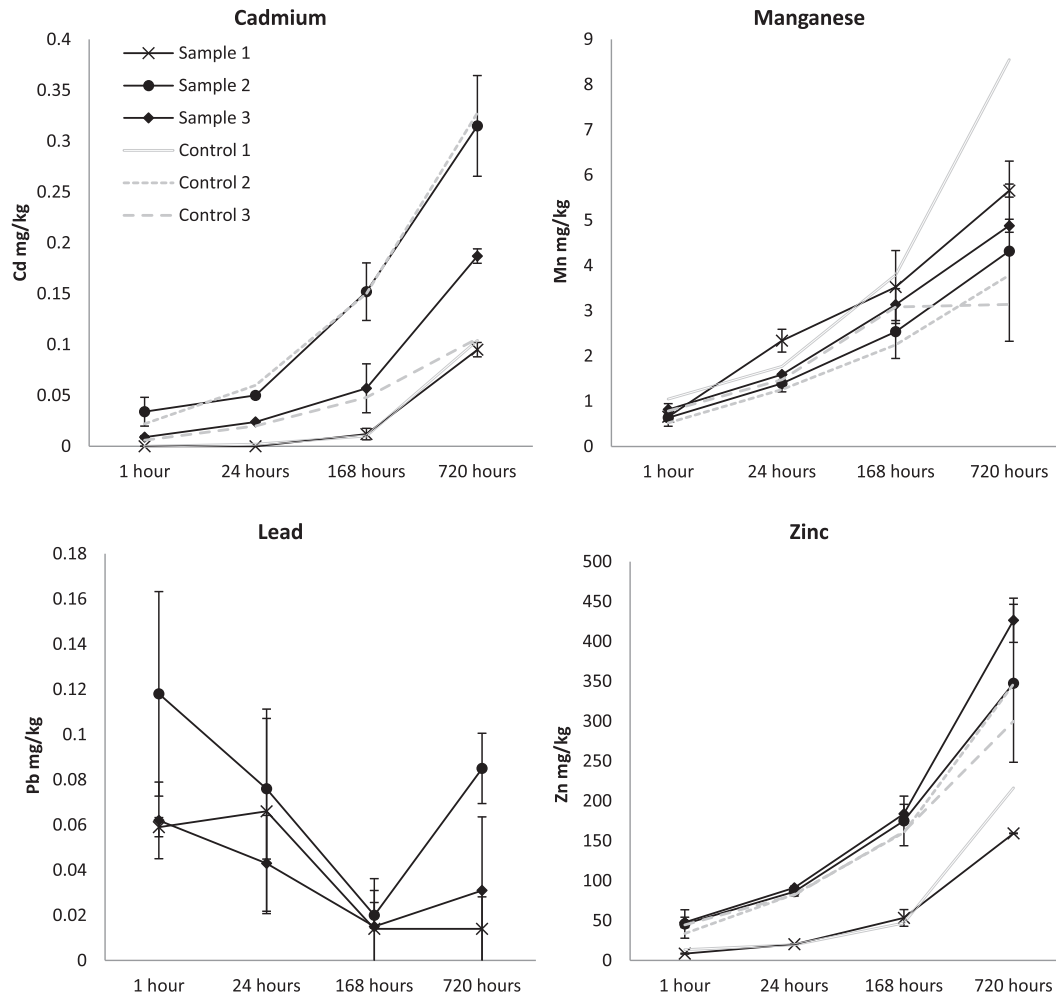*Significant at < 0.05, **Significant at < 0.01, − values below detection.

Fig. 3. Change in soluble potentially toxic element concentrations extracted with rainwater simulation solution (Pb in all control samples = below determination limit).

(pH 4.6), however, the selected metals in both solvents show highly significant correlations (Cd R = 0.967; Mn R = 0.881; Zn R = 0.955, at 5% sig. critical value = 0.532). Thus, in the environment, sustained periods of any rain are likely to mobilise Cd, Mn and Zn from slag into surrounding soils and surface waters.

Table 3
Kruskal Wallis test for soluble PTE content change between sampling times in RSS replicates from all slag samples combined.

Rainwater simulation solution				
	Mean rank			
	Cd	Mn	Pb	Zn
1 h	5.75	3.50	17.75	5.17
24 h	10.42	9.83	15.75	9.50
168 h	13.00	15.83	5.42	14.83
720 h	20.83	20.83	11.08	20.50
P	0.002*	0.000*	0.013*	0.001*
(1 h–24 h)	0.076	0.004*	0.423	0.109
(1 h–168 h)	0.065	0.004*	0.004*	0.010*
(1 h–720 h)	0.004*	0.004*	0.173	0.004*
(24 h–168 h)	0.377	0.010*	0.006*	0.109
(24 h–720 h)	0.004*	0.004*	0.228	0.004*
(168 h–720 h)	0.025*	0.025*	0.229	0.055

*Significant at <0.05 (Tabulated output from multiple Kruskal–Wallis tests without correction).

Table 5 presents concentrations of PTE in solution after one month of extraction as a percentage of the slag PTE concentration that was obtained by a single 2 M HNO_3 extraction. The heterogeneity of the slag being studied is evident (not only between samples but also between replicates, particularly for Pb and Zn). Dissolution tests on slag sample 2 generated generally higher PTE concentrations than the other two samples. Respective to nitric acid extractable contents, Cd and Zn appear to be the most readily dissolved elements, whereas Pb is somewhat insoluble. This is consistent with the general trend in extraction/leaching experimentation when using a mild extractant. Sahuquillo et al. (2003) summarized a series of leaching experiments on contaminated soils, where low extraction yields (<1% of total) were usually observed for all metals with the exception of Zn when using weak extracting solutions. In contaminated soils Cd is typically bound in

Table 4
Analysis of variance between samples 1, 2 and 3 for rainwater simulation solution extractions − P (F).

	1 h	24 h	168 h	720 h
Cd	0.069 (7.38)	0.001** (130)	0.016* (21.7)	0.011* (28.7)
Mn	0.482 (0.94)	0.027* (15.3)	0.388 (1.32)	0.572 (0.68)
Pb	0.205 (2.82)	0.652 (0.49)	0.925 (0.08)	0.990 (5.49)
Zn	0.062 (8.12)	0.000** (274)	0.013* (26.2)	0.043* (10.7)

*Significant at < 0.05, **Significant at 0.01.

Table 5
PTE solution content (after 1 month) as a percentage of content extractable by 2 M HNO$_3$.

		Cd	Mn	Pb	Zn
Sample 1	2 M HNO$_3$ (mg/kg)	10.9 ± 0.93	742 ± 30.3	684 ± 35.9	21,135 ± 724
%	Citric acid	0.01 ± 0.02	0.81 ± 0.14	–	0.08 ± 0.00
	RSS	0.87 ± 0.06	0.76 ± 0.02	0.004 ± 0.00	0.75 ± 0.00
	Water control 1	0.47	0.92	–	0.57
	Water control 2	0.95	1.15	–	1.02
Sample 2	2 M HNO$_3$ (mg/kg)	10.7 ± 0.33	554 ± 11.3	848 ± 477	19,540 ± 291
%	Citric acid	1.49 ± 0.28	1.14 ± 0.19	0.054 ± 0.02	1.31 ± 0.16
	RSS	2.95 ± 0.46	0.78 ± 0.36	0.010 ± 0.00	1.78 ± 0.51
	Water control 1	3.14	1.04	0.017	1.97
	Water control 2	3.08	0.68	–	1.77
Sample 3	2 M HNO$_3$ (mg/kg)	8.97 ± 0.32	897 ± 23.6	320 ± 11.7	24,389 ± 588
%	Citric acid	0.73 ± 0.09	0.73 ± 0.04	0.169 ± 0.03	1.05 ± 0.03
	RSS	2.08 ± 0.08	0.54 ± 0.02	0.002 ± 0.01	1.75 ± 0.11
	Water control 1	1.32	0.52	–	1.26
	Water control 2	1.18	0.35	–	1.23

(± = standard error, $n = 2$).

the exchangeable fraction (Waterlot et al., 2011) and is known for its easy solubility and bioavailability (Rosselli et al., 2003). A column leaching experiment was performed by Navarro et al. (2008) on smelter slag to simulate the effect of percolating rainwater whereby DI water was passed through a slag sample core (ø 15 cm, L 75 cm) at a flow rate of 2.4 L/hr for 5 h. Effluents which were analysed at the end of experimentation showed a significant mobilization of Pb (>2 mg/L). Despite having similar slag composition, this is in contrast to our findings (max. 0.0059 mg/L) and suggests that the dissociation and dissolution of Pb is less dependent on contact time, but more dependent on a continuous supply of replenished solvent. Contact time with solvent is, however, an important variable in controlling the precipitation and re-adsorption of Pb as it is submersed in stagnant solution, particularly when important chemical parameters such as pH and dissolution of primary and secondary phase minerals are highly dynamic. Lima and Bernardez (2011) highlight the importance of the PTE phase in slag relating to its leachability, e.g. metallic Pb particles were readily leached at pH < 2.8 but Zn which occurred as complex phases remained stable even under highly acidic conditions.

3.3. pH changes of solvents

A clear shift in the pH of the test solvents was observed over the course of the experiment, whereby all solvents that are reacting
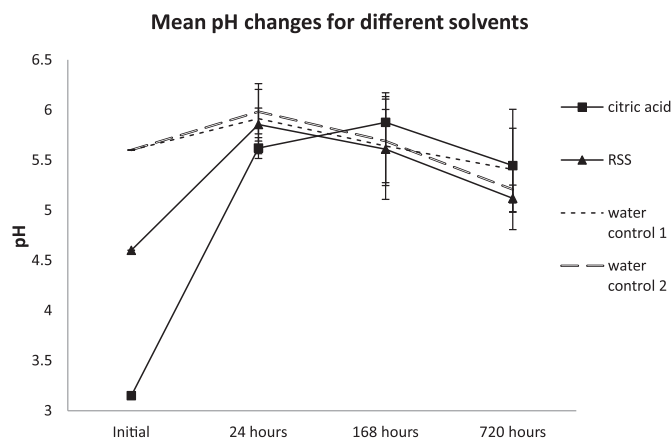
Fig. 4. Mean pH change from samples 1, 2, 3 over one month for leaching experiment solutions.

with the slag appear to be buffered to within a narrow pH range at the end of the experiment (Fig. 4). Initial pH values within a range of 2.3 converge after one month to within a narrow range (0.55) between the pH of approximately 5–5.5. The pH values of our analysed solvents fluctuate slightly after 24 h, although the majority of the pH adjustment had already taken place within the first 24 h, closely matching the results of Ettler et al. (2004) and Kierczak et al. (2013), confirming the inability of citrate to maintain its acidity over a range of concentrations. The observed buffering effect is likely to be induced by mineral compounds reacting during their release from slag due to dissolution by solvents. The chemical composition of smelter slag usually consists of high portions of basic oxides, typically including >10% CaO by weight due to lime fluxes commonly used in medieval smelting (Manasse and Mellini, 2002). Moreover, it was stated by Pauliš and Mikuš (1998) that the slag used in this study was once applied as an early fertilizer to local agricultural soils due to its high content of Ca and Mg.

4. Conclusions

The mineralogical make up of slag was responsible for the buffering of solvents to within a narrow range around pH 5.5 which may moderate the transport of some PTE (especially Pb). The decomposition of citric acid with time may have had small implications on metal solubility, however, the consequent changes in metals solubility are indirect, likely to be more related to pH. A common observation among metals in citric acid was the short time to equilibrium, which is in contrast to RSS whereby a continued increase in soluble Cd, Mn, Zn occurred, suggesting that even in a hydro-static situation, water in the environment is a significant variable concerning the mobilization of these elements. Overall levels of PTE in solution after 1 month were greatest in RSS, with the exception of Pb, which had generally low solubility in all solvents. Removal of Pb from solution was probably due to pH instability at low concentration. Statistical analysis showed that, in the case of Cd and Zn, the heterogeneity of slag is strongly reflected in dissolution of these elements as significant concentration differences were obtained between slag samples. It is evident that chemical weathering by environmental solvents is responsible for the transport of notable quantities of certain PTE which will inevitably settle in surrounding soils and surface waters. However, the large amount of basic compounds in smelter slag can immobilize some contaminants, thus physical weathering and aeolian transport should also be considered as significant pathways for the distribution of PTE into soils and waters in the vicinity of slag deposits. An impermeable cover would therefore be anticipated to

offer effective mitigation of the movement of PTE due to the effects of both chemical and physical weathering.

Acknowledgements

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Potentially toxic element distribution in soils from the Ag-smelting slag of Kutná Hora (Czech Republic): Descriptive and prediction analyses

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ARTICLE INFO

ABSTRACT

The distribution and environmental hazard of potentially toxic elements in the vicinity of a large smelter heap located in the traditional mining town of Kutná Hora, Czech Republic were studied. To understand their occurrence and to predict their mobility and deposition, trends and patterns in PTE distribution were related to soil properties and environmental variables. Slag samples and slag impacted soils were characterized by high 2 M HNO_3 extractable PTE contents which generally increased with depth, but relatively low plant available fractions; Cd and Mn were the most available elements. Manganese availability was significantly higher in soils under vegetation canopy whereas availability of Cu decreased. Organic matter and pH were identified as significant variables controlling availability of Mn, As and Mn, Zn respectively. Availability of Pb and Cu had significant negative correlation with soil clay content. Mean values of Pb, Mn, Cu, Zn and As from sediment samples exceeded the background value for non-polluted alluvial sediment by 1.8, 1.5, 3.4, 9.8 and 1.3 times respectively. Despite the elevated presence of PTE in river sediments, only Mn and Zn were detected in water samples, but at levels which are not considered hazardous. Included in the paper is a method proposal for quickly and easily identifying threshold values of a chosen soil property which control accumulation of PTE in soil respective of their source. The slag heap at Kutná Hora is a proven source of PTE with evidence of their mobility/transport into the surrounding environment. Although the environment is highly heterogeneous, and the mobility of PTE can be dependent upon pedogenic, geologic, ecological, hydrological and climatic variables, overall the study area was characterized by relatively low availability of PTE, with a limited threat to healthy plant development in the immediate surroundings of the smelter slag heap.

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1. Introduction

The Central Bohemian town of Kutná Hora (KH) in the Czech Republic emerged as a consequence of the presence of precious metal bearing veins which form a part of the town's underlying geology. At its peak in the late 14th century, approximately 6–7 tonnes of silver were produced annually from the KH mining industry (Pauliš and Mikuš, 1998). The common method of extraction was to process roasted ores in smelters, whose operation required the use of pyritic sulphides and lead, plus various other fluxes which could have been added. These additives along with the natural metal rich composition of the ores result in a high residual concentration of potentially toxic elements (PTE) bound in the slag. Slag was initially considered to be relatively inert and resistant to weathering but, due to more recent studies, the current understanding is that slag is a reactive and significant source of PTE including, As, Cd, Pb and Zn (Ash et al., 2013; Ettler et al., 2003, 2009; Piatak et al., 2004; Rosado et al., 2008), as evident by secondary minerals and the identification that PTE concentrations are often

highest in the smaller particle fractions, enriched in weathered material (Rosado et al., 2008). Likely sources of high PTE inputs at the study area are apparent from aerial imagery (Fig. 1) whereby a distinct absence of vegetation is visible, which in turn reflects the soils which have developed either on or around the slag (Fig. 2). Sulphide oxidation and surface controlled dissolution of minerals and glass will release metals to pore water solutions where they have the potential to be transported to underlying, adjacent and downstream aquatic systems (Lottermoser, 2002). For slag dumps which develop acid conditions, PTE can be potentially leached into groundwater as demonstrated by Navarro et al. (2008). The KH slag deposit is an interesting site for investigation due to its close proximity to sensitive environmental receptors such as the adjacent Vrchlice River and mixed forest which surrounds the slag. Lead and Zn were identified as being among the most excessive metals associated with silver smelter slag in a number of studies (Ettler et al., 2009; Lottermoser, 2002; Manasse and Mellini, 2002; Navarro et al., 2008), and previous analysis of the KH slag identified excessive 2 M HNO_3 extractable concentrations of PTE (max. mg/kg Cu 1027; Mn 496; Pb 966; Zn 4097; Cd 6.07) (Rubešová, 2011). However, little is known about the migration of these contaminants into the riverbank soils and potential impacts which may result downstream. The primary

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focus of this study is on the distribution of PTE between soils of varying properties and how these properties correlate with PTE concentrations obtainable by extractants of different strengths (2 M HNO_3 and 1 M NH_4NO_3). The Vrchlice River sediment and waters were sampled so as to determine the presence and concentrations of PTE. A method is described for the prediction of soil parameter values at which PTE accumulate in soils, respective of their source concentration. The overall study aim is to contribute to the database of knowledge concerning smelter slag and the potential for pollutants which are weathered from slag to migrate into surrounding environmental systems.

2. Site characterization and description

The town of KH is situated on the Kutná Hora Crystalline Unit, represented by micaschist and orthogneiss zones. An extensive description of the geology of KH can be found in Kachlík (1999). The mining operations in KH followed the path of Ag-enriched quartz veins which formed from metamorphosed igneous material (Pauliš and Mikuš, 1998). These veins also contain high concentrations of a range of PTE due to the occurrence of minerals such as arsenopyrite (As), chalcopyrite and tetrahedrite (Cu), galena (Pb) and sphalerite (Zn). The structure of the slag wastes, as well as the chemical composition, is highly heterogeneous. Slag pieces occur in a variety of sizes and shapes from up to 50 cm diameter fragments to gravelly shards and highly weathered fine particles. The chemistry of the slag wastes reflects not just the mineralogy of the roasted silver ores but is also a result of the addition of fluxes used in the medieval smelting process. To reduce the melting temperature of silver, lead sulphides, charcoal, vitrol (Fe, Cu, Zn sulphates) and calcite were often added to the smelter, resulting in a mixed end product of slag and argentiferous metals bearing Ag and Pb (Helm-Clark, 2001).

The scale of the smelter waste heap can be seen in Fig. 1. The unvegetated predominant mass of slag lines the southern side of River Vrchlice for more than 100 m. Slag pieces are not limited to the main pile, but also occur within the river and can be found within the subsurface of soils not just in the vicinity of the smelter sites, but all over the KH region as past disposal options for the vast amount of material included road construction, burial and mixing into agricultural soils. Around the fringes of the slag heap there can be found pioneer plants such as mosses, lichens, grasses, heath plants and poorly developed shrubs and trees. At the edge of the uncovered slag heap there is a sharp transition into a zone of well established deciduous trees, mainly oak and beech (on the area denoted Spolic fluvisol in Fig. 2). Upland to the slag pile (to the south-east direction of the map) there is a well established mixed forest of oak, birch, beech, spruce and pine on developed cambisol soils which are not influenced by the intrusion of slag. The Kutná Hora region can potentially receive precipitation throughout the year with an average annual rainfall of 450 mm. However, most precipitation occurs in the summer months with July being the wettest month. The winter is comparatively drier with most precipitation coming in the form of snow. All water in the area shown on the map flows into the valley which corresponds to the position of the Vrchlice River in Fig. 2 and the direction of river flow is from sample 1 s > 3 s (SW–NE). The area marked L in Fig. 2 refers to the location of a shallow (~20 cm deep) and widened lagoon type formation with reduced flow where sediment and suspended contaminants are likely to settle.

3. Sampling and analysis methodology

Sampling positions were plotted in the form of three transects (1, 2 and 3) which cover different soil types between the slag heap and the river (see Fig. 2). At the highest point, samples were taken within the banks of the slag heap (positions 'a'); at the bottom of the slag heap is a level area, with sporadic grass and light vegetation (positions 'b'); beyond the level area there is a steep bank adjacent to the river which is characterized by constantly moist and periodically submerged soil with a high input of organic matter (positions 'c'). Approximate sampling positions are shown in Fig. 2 where shallow pits were made at each point. Sampling pits were dug to the depth of around 30 cm, from which samples of soil and slag fragment were taken according to a layer depth (approx. 0–10 and 10–30 cm), these samples were labelled A and B respectively. For making reference to control soils,
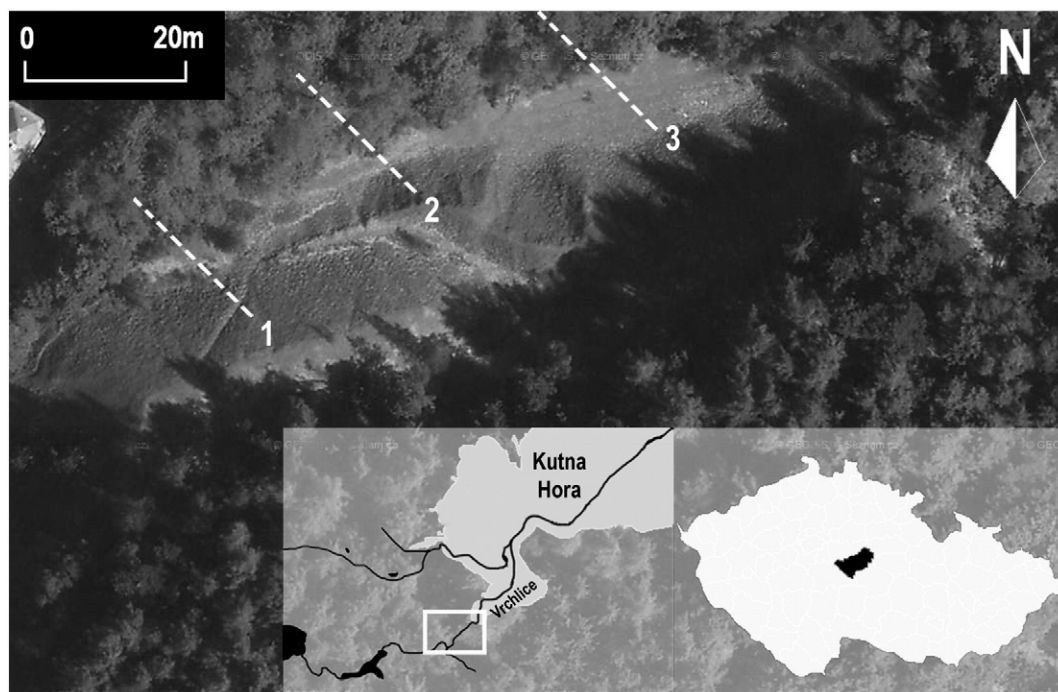

Fig. 1. Satellite image of slag deposit and mixed forest with transect positions.
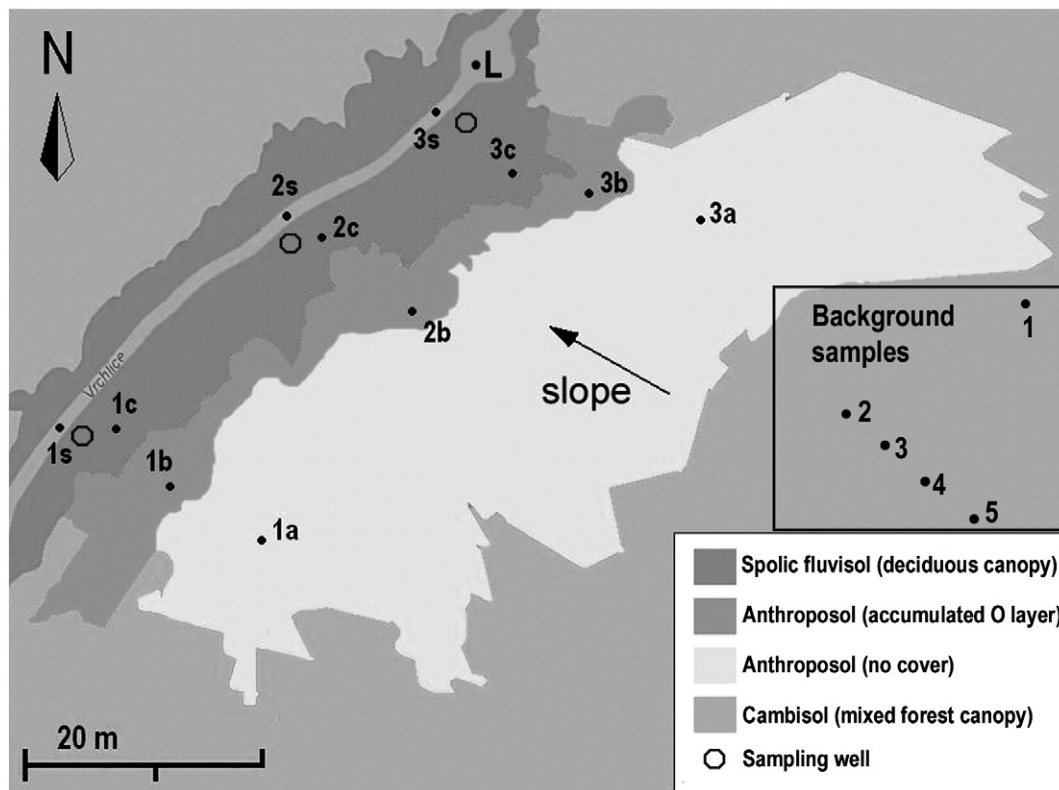
Fig. 2. Soil characterization map of study area with sampling positions and location of background samples.

mineral horizons of 5 pits were sampled in the mixed forest (background samples 1–5 in Fig. 2). River sediment samples were collected from the river bed by filtering surface sediment (~upper 5 cm) at points 1 s, 2 s and 3 s, and from the shallow lagoon formation marked L in Fig. 2. At the same aforementioned locations, water was sampled by collecting at the river surface, and from ~20 cm below the water table from a riverbank depression which is separated from the flowing surface water of the river. Vegetation samples were collected in the areas that correspond to the position of transects from the dominant species in those areas (ash *Fraxinus excelsior*, birch *Betula pendula* and grass *Apera spica-venti*). Stem and leaf samples of tree species were separated.

Samples were air dried then slag pieces were separated from soil. Slag pieces were washed and ground in a mill to <2 mm. A 2 M HNO_3 extraction was applied to sediment, soil and slag samples (5 g sample: 50 ml HNO_3, 6 h of shaking) and a 1 M NH_4NO_3 extraction to slag and soil samples (20 g sample: 50 ml NH_4NO_3, 2 h of shaking). Both extracts were centrifuged and filtered then analysed for Pb, Cd, Mn, Cu, Ag, Zn by Varian 280 FS FAAS with SIPS 20 using Analytika s.r.o standards. For the detection of As, an Istran Ecaflow 150 coulometre was utilized. All samples were analysed in duplicate with a procedural blank for each extraction. Soil pH was determined by preparing a 1:5 soil:liquid (w:v) ratio using water (pH_{H2O}) then measuring after agitation using an Inolab WTW pH metre. Organic carbon content (C_{org}) was

Table 1
Mean soil PTE contents in 2 M HNO₃ extract with standard deviations, mean plant available PTE and mean plant available as percentage of 2 M HNO₃ soil extract with comparison to agricultural limit value of Czech Republic, and background PTE content of the study area.

Position		Pb	Cd	Mn	Cu	Ag	Zn	As
Limit value		80	1	n.d.	50	n.d.	100	4.5
Background*		318 ± 73.0	0.50 ± 0.61	143 ± 51.4	26.8 ± 3.85	1.15 ± 0.57	74.1 ± 62.9	42.9 ± 25.4
2 M HNO₃ extractable PTE in soil								
'a'	A	903 ± 738	7.08 ± 9.17	461 ± 501	2085 ± 2627	3.59 ± 0.88	4412 ± 5336	97.2 ± 23.9
	B	1377 ± 152	14.9 ± 3.53	664 ± 204	2742 ± 877	4.11 ± 0.68	11,042 ± 1545	112 ± 86.8
'b'	A	743 ± 126	5.86 ± 2.90	912 ± 298	1962 ± 1363	3.95 ± 0.29	5423 ± 4501	84.7 ± 12.5
	B	1721 ± 662	33.7 ± 46.0	883 ± 273	3525 ± 1580	4.41 ± 0.55	8263 ± 2868	80.2 ± 65.8
'c'	A	390 ± 353	3.11 ± 3.10	551 ± 312	876 ± 1040	3.42 ± 1.54	2826 ± 2229	65.3 ± 11.8
	B	547 ± 544	4.21 ± 4.44	820 ± 655	1302 ± 1571	4.05 ± 1.24	3452 ± 2778	68.1 ± 26.8
Plant available PTE in soil (% of 2 M HNO₃)								
'a'	A	9.27 (2.18)	1.53 (31.9)	18.3 (11.2)	64.4 (2.67)	0.06 (1.67)	48.9 (2.61)	0.28 (0.34)
	B	1.13 (0.09)	2.04 (13.3)	12.8 (2.02)	27.9 (0.90)	0.05 (1.13)	93.1 (0.86)	0.29 (0.56)
'b'	A	1.68 (0.24)	0.65 (10.8)	72.9 (9.42)	18.4 (0.71)	0.03 (0.53)	54.3 (1.11)	0.52 (0.60)
	B	7.15 (0.41)	1.64 (14.6)	28.1 (3.81)	67.4 (2.44)	0.03 (0.80)	67.8 (0.87)	0.10 (0.19)
'c'	A	0.51 (0.26)	0.25 (10.9)	93.9 (23.7)	3.12 (0.41)	0.03 (0.73)	24.8 (1.46)	0.55 (1.07)
	B	0.81 (0.30)	0.46 (17.1)	171 (25.6)	8.62 (0.60)	0.02 (0.43)	42.1 (2.02)	0.51 (1.42)

Values in mg/kg except for those in parentheses.

* Background content (n = 5).
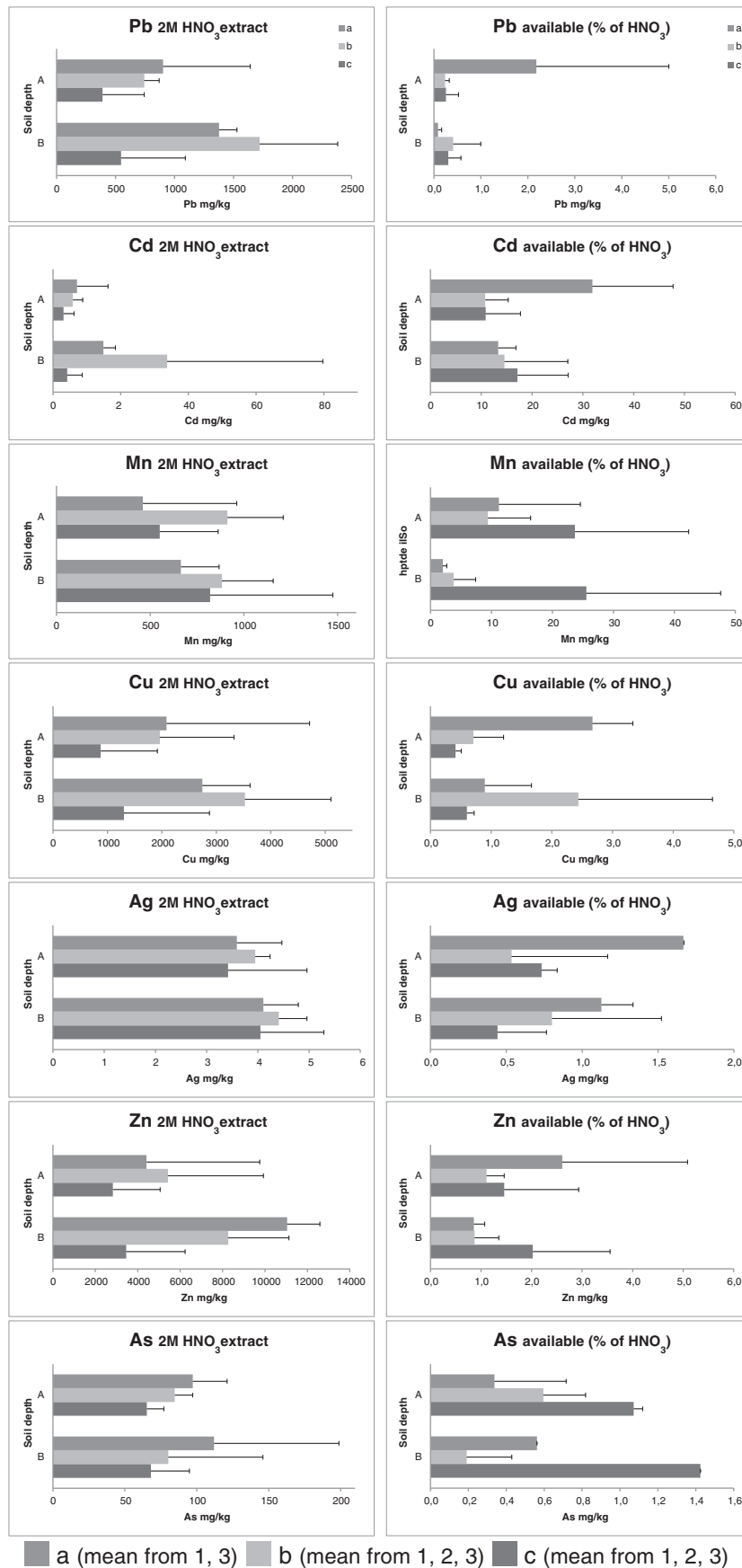
Fig. 3. Change in 2 M HNO$_3$ extractable and plant available PTE concentrations between sample depths.

Table 2
Kruskal–Wallis test for different 2 M HNO_3 extractable PTE concentrations (mean from depths A and B) between transect positions a, b, c.

		Pb	Cd	Mn	Cu	Ag	Zn	As
	n	Rank	Rank	Rank	Rank	Rank	Rank	Rank
Position a	4	11.00	10.75	6.50	9.50	7.50	10.50	9.50
Position b	6	10.50	9.83	10.83	10.67	9.33	10.33	7.00
Position c	6	4.83	5.67	7.50	5.67	8.33	5.33	6.25
P		0.057	0.175	0.299	0.170	0.832	0.119	0.507
P (a–b)		1.000	0.522	0.088	0.831	0.522	0.670	0.394
P (b–c)		0.055	0.078	0.337	0.055	0.749	0.037*	0.831
P (a–c)		0.033*	0.201	1.000	0.286	0.831	0.201	0.284

* Statistically significant difference at 95% confidence level.

derived by the loss-on-ignition method, as described by Schumacher (2002). The content of clay (<0.002 mm) expressed as fraction percentage and soil cation exchange capacity was determined according to methods described in Carter and Gregorich (2008) and Gee and Or (2002) respectively. Vegetation samples were washed, dried at ~30 °C then ground in a mill. Certified reference material (NCS DC 73351, Beijing China) was included in the sample batch. The samples were digested in Teflon tubs using 65% HNO_3 (w:v 1:10) on a hotplate at 130 °C, 40 W for 16 h. After heating, the digest was diluted by decanting into a 100 ml conical flask and making up to the mark with D.I. water. The solution was filtered (Filtrak 390) then analysed for PTE by means of FAAS.
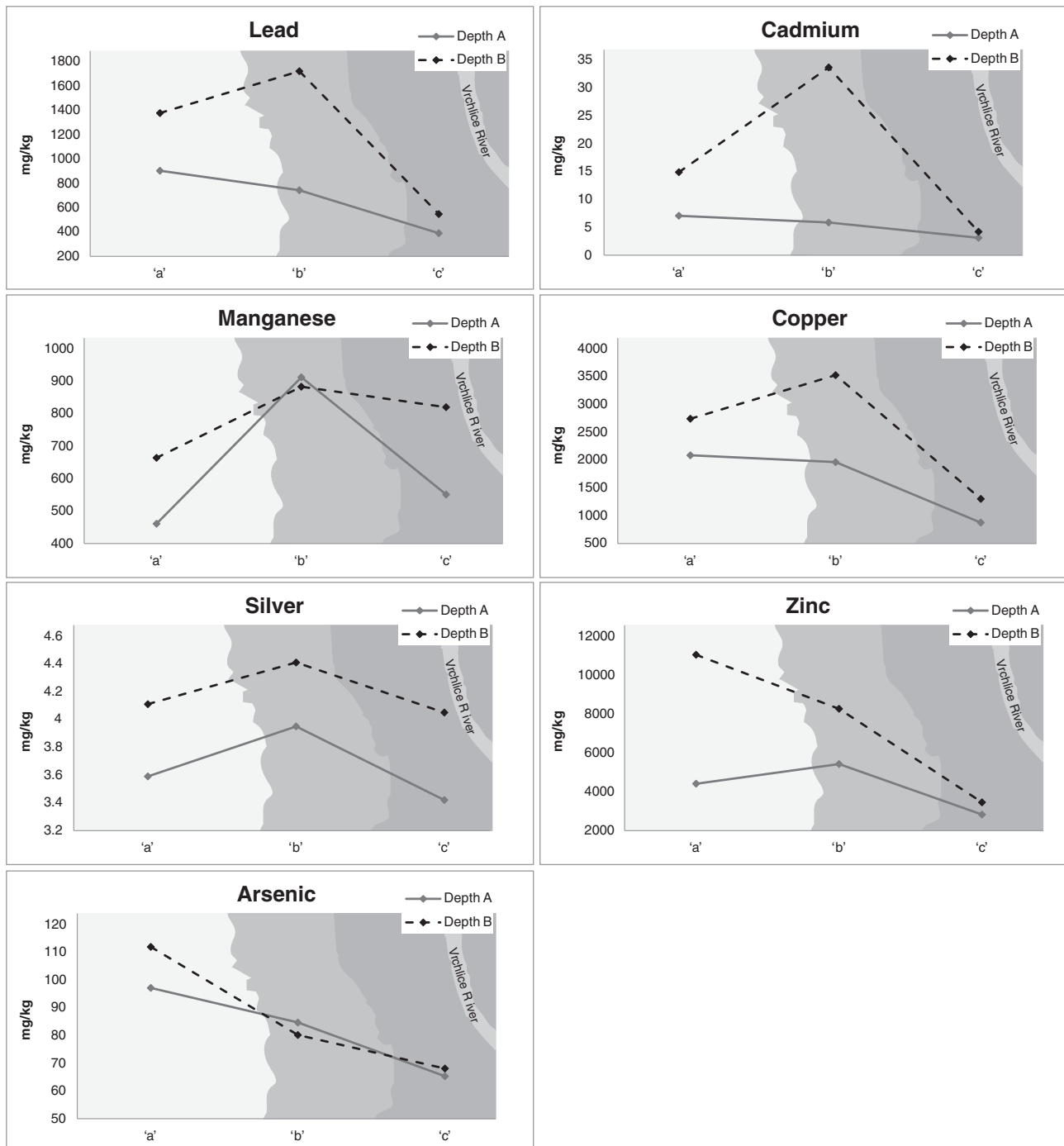
Fig. 4. Change in mean 2 M HNO_3 extractable PTE concentrations between slag heap and river bank soils a–b–c.

IBM — SPSS PASW statistics 20.0 was used for statistical analyses. Graphs were prepared in Microsoft Excel 2007.

4. Results and discussion

4.1. Potentially toxic elements

A 2 M HNO$_3$ extraction was applied to soil samples (Table 1). When compared to both the limit values for PTE in agricultural soils (Beneš, 1993) and to background contents, it is evident that the studied samples are highly enriched in PTE. Following are the highest observed values for each PTE respective to the limit value: Pb 30; Cd 86.7; Cu 103; Zn 121; and As 39 times above the agricultural limit. Only Pb and As were more highly concentrated in the control soils than the limit values dictate, however, the concentrations in study area soils which are influenced by the intrusion of slag are generally much higher. For the majority of soil pits, the higher mean PTE concentrations were observed in depth B (Table 1). To better illustrate the observation, the mean PTE contents from a, b and c sample pits at depths A and B were plotted (Fig. 3). Fig. 3 shows that for Pb, Cd, Cu and Zn there is a trend of increasing content from depth A to B. The difference in plant available PTE content between depths A and B is not consistent with the results from the 2 M HNO$_3$ extract. In the 'a' samples (those from the upper slag heap) there is a significantly higher availability in the upper layer (0–10 cm) for all PTE except for As. For 'b' samples (anthroposol with organic matter layer), Cu availability is increasing with depth while Mn and As availability are greater at the surface. In the 'c' samples (spolic fluvisol under tree canopy), there is little to differentiate availability between depths for all elements. Soil parameters which may be controlling PTE availability are discussed further in Section 5.

The results showed that the studied soils are highly enriched with PTE in terms of their overall contents. However, in most cases the plant available portion (represented by 1 M NH$_4$NO$_3$ extract) proved to be only a small fraction of the total amount that is present in both slag and soil samples. Cadmium and Mn showed the highest bioavailability respective of their 'total' amounts, whereas Pb and As have very low availability in the studied samples.

A Kruskal–Wallis test was applied to data to identify trends in PTE concentrations along transects (Table 2). Lead and Zn showed the only significant differences. Statistical significance was proven between a–c and b–c for Pb and Zn respectively whereby these elements are less concentrated in riverbank soils. Manganese and Ag concentrations were greater in riverbank soils than within the main slag heap, suggesting non-anthropogenic occurrence, however, the difference is not statistically proven beyond the 0.05 significance level. Fig. 4 illustrates the mean PTE concentration differences between sampling positions a–c for depths A and B independently.

Table 3
Soil C$_{org}$ and clay content (%) and soil pH.

Sample	Depth	C$_{org}$	pH	Clay content (<0.002 mm)	CEC*
1a	A	44.0	3.92	n.d.	95.3
	B	6.57	5.90	4.79	23.5
1b	A	41.6	5.08	n.d.	88.0
	B	11.8	4.78	0.38	32.7
1c	A	67.2	4.92	n.d.	148
	B	49.0	4.38	3.26	108
2b	A	11.4	5.51	2.25	29.3
	B	6.06	6.14	7.60	18.8
2c	A	68.6	3.98	2.41	191
	B	63.8	4.03	3.55	168
3a	A	6.80	5.42	2.35	19.3
	B	5.84	5.83	2.88	21.3
3b	A	52.4	4.93	4.05	38.5
	B	7.87	5.68	4.03	22.0
3c	A	25.8	5.56	3.81	50.5
	B	9.46	5.54	4.76	27.8

* CEC = cation exchange capacity, n.d. = no data.

4.2. Soil properties relating to plant availability of PTE

As previously described, the 1 M NH$_4$NO$_3$ extractable PTE content was taken as a proportion of the PTE concentration in the 2 M HNO$_3$ extract. A Mann Whitney U test was applied to data to determine any influence that vegetation cover may have on PTE availability. According to the statistical output (Table 4) a significantly greater content of plant available Mn exists in soils which are covered by a tree canopy. Copper availability is shown to be far lower in soils under the deciduous trees, however, this could not be confirmed at the 95% confidence level.

Correlation of plant available PTE (% of HNO$_3$ extract) with soil properties (C$_{org}$ content, soil pH, clay content, as shown in Table 3) revealed clear trends for some PTE (Table 5). Availability of As and Mn showed a medium–strong positive correlation with organic carbon content (C$_{org}$) at the 95% confidence level, whereas Cu and Pb (known for their strong binding affinity to organic matter) show poor availability when C$_{org}$ increases, although the trends are not significant at the 95% confidence level. In all cases, except for Ag, an increase in soil sample pH corresponds to a decrease in PTE availability; however, the correlation is only significant for Mn and Zn. The observed relationship between plant available PTE and clay fraction percentage is negative in almost every case (significant at 95% confidence level for Pb and Cu).

4.3. Potentially toxic element source to soil analysis

Crushed slag was analysed for 2 M HNO$_3$ extractable PTE concentrations to determine the potential source concentration of PTE into soil. A comparison was made between the slag PTE concentration and the soil PTE concentration from where the slag was collected, and a ratio was assigned to determine whether soils are accumulating or losing metals respective to the source. Data for the percentage of PTE in soil as a proportion of PTE concentration in slag are presented in Table 6. Shaded cells indicate samples where the soils contain a lesser content of PTE than the slag fragments which were collected in the same sample, whereas non-shaded cells indicate that the soil contains a greater content of PTE than what was extractable in the slag. By making these highlights a picture becomes clear as to which elements are retained in the soil (respective to their sources). Lead, Mn, Cu and As are highly concentrated in soils. Zinc and Ag show particularly low concentrations in soil indicating that they are either a) easily removed from soil by leaching and/or physical weathering and b) these elements are more easily released from the slag into the 2 M HNO$_3$ extract, thus decreasing the respective percentage observed for soil.

After determining which soils accumulate PTE and which appear to be losing PTE respective of their source, statistical tests were applied to relate these findings to soil properties. Chi-square analysis was used to determine whether soil pH or soil C$_{org}$ are associated with the accumulation of PTE in soils. In the case of pH being the independent variable, only Cu was shown to have statistical dependence (higher pH = greater accumulation of Cu). Copper was also the only element to have statistical dependence on C$_{org}$ content (organically rich soil = greater accumulation of Cu).

The soil PTE concentrations as a proportion of slag PTE concentrations (values in parentheses in Table 6) were plotted against soil pH in a scatter chart (Fig. 5). By delineating the line at which the soil: slag

Table 4
Mann Whitney U-test for difference in PTE plant availability in soils with a) tree canopy and b) no vegetation cover.

		n	Pb	Cd	Mn	Cu	Zn
Mean rank	Tree canopy	6	8.50	8.65	12.00	5.67	8.58
	No cover	10	8.50	8.25	6.40	10.20	8.45
	Z		0.000	−0.163	−2.278	−1.844	−0.054
	P		1.000	0.871	0.023*	0.065	0.957

* Statistically significant at 95% confidence level.

Table 5
Pearson correlation of soil organic carbon content, soil pH and soil clay content with plant available PTE percentage.

	Pb	Cd	Mn	Cu	Ag	Zn	As
Soil C$_{org}$ content							
n	17	17	17	17	17	17	12
R	−0.108	−0.033	0.871	−0.404	−0.226	0.469	0.683
P	0.680	0.900	0.000*	0.108	0.383	0.058	0.014*
Soil pH							
n	17	17	17	17	17	17	12
R	−0.288	−0.462	−0.860	−0.012	0.159	−0.814	−0.376
P	0.263	0.062	0.000*	0.965	0.542	0.000*	0.228
Soil clay content							
n	13	13	13	13	13	13	n.d.
R	−0.661	−0.512	−0.207	−0.601	0.144	−0.263	n.d.
P	0.014*	0.074	0.498	0.030*	0.639	0.385	n.d.

* Statistically sig. at 95% confidence level, R = correlation coefficient, n.d. = no data.

PTE concentrations are equal (1:1 or 100%), it is possible to interpolate the pH value at which selected PTE are predicted to accumulate in soil samples. According to Fig. 5, Pb starts to accumulate in soil at relatively low pH (~4.5), at pH 5.5 twice as much Pb is expected to be present in the soil than in the slag and at pH 6 up to 3 times the amount of Pb in the soil than is in the slag. Cd and Cu start to accumulate in the soil at a

higher pH range (~5.1–5.2) and Zn in soil does not exceed its source concentration in the slag until a pH of >6 is reached.

When the plant available contents of PTE in slag were determined (Table 6), a similar trend to that of the soil was observed (generally low availability; Cd and Mn were the most available elements).

4.4. River sediment and water analysis

The following Table 7 gives the concentrations of PTE in riverbed sediments extractable by 2 M HNO$_3$ and in river water collected at the surface and from river bank wells. Elevated concentrations of PTE were present in all river sediment samples, with slightly greater contents of Cd, Mn, Cu, Ag and Zn in the lagoon sample. A comparison to background concentrations is given in the discussion section. In water samples only Mn and Zn were detectable.

4.5. Vegetation analysis

Vegetation samples were collected from three species along the edges of the slag heap (positions corresponding to transect locations). The mean PTE content from the three samples of each species is given in Table 8. The measured values of certified reference material corresponded well to actual values, Zn is slightly underestimated. Values for Ag, As, Cd and Pb were all below the detection limit indicating insignificant or no uptake of non-nutritional elements.

Table 6
PTE concentration in slag sample by 2 M HNO$_3$ extraction and by 1 M NH$_4$NO$_3$ extraction (plant available fraction).

Sample	Pb	Cd	Mn	Cu	Ag	Zn	As
2M HNO$_3$ extractable PTE in slag (proportion of PTE in soil of the same sample pit)							
1a B	683 (217)	14.1 (87.5)	297 (174)	1821 (116)	4.05 (89.7)	13580 (73.3)	56.3 (309)
1b A	656 (131)	2.57 (129)	423 (137)	1249 (83.1)	3.66 (99.9)	8420 (33.5)	27.6 (310)
1b B	1130 (153)	7.85 (59.3)	387 (163)	1777 (113)	6.41 (64.9)	11271 (50.5)	22.9 (213)
1c A	403 (71.8)	4.56 (32.0)	322 (227)	775 (39.2)	3.20 (149)	10951 (17.5)	25.6 (223)
1c B	302 (119)	3.99 (57.9)	371 (403)	703 (59.0)	4.26 (122)	10611 (23.1)	22.5 (219)
2b A	339 (224)	5.58 (162)	712 (163)	2141 (165)	4.05 (97.7)	14716 (72.2)	20.8 (345)
2b B	358 (295)	4.19 (231)	941 (125)	2714 (126)	5.08 (79.6)	19178 (59.2)	18.1 (862)
2c B	217 (57.2)	3.60 (28.9)	441 (42.3)	1107 (33.9)	5.44 (50.3)	12315 (10.6)	n.d
3a A	568 (251)	6.20 (219)	942 (86.7)	2915 (135)	5.13 (82.0)	12831 (63.8)	17.2 (664)
3a B	502 (253)	7.17 (242)	858 (94.3)	1770 (190)	5.33 (86.2)	16701 (72.7)	7.64 (668)
3b A	357 (171)	2.94 (178)	679 (147)	1076 (123)	5.07 (83.4)	9422 (30.0)	27.7 (349)
3b B	511 (466)	7.04 (1232)	771 (110)	1835 (281)	4.59 (110)	12964 (59.7)	17.1 (210)
3c A	452 (173)	7.63 (87.6)	701 (104)	1877 (111)	4.50 (83.4)	15066 (35.6)	8.96 (821)
3c B	471 (247)	6.13 (151)	715 (109)	1581 (197)	3.80 (111)	14146 (46.6)	20.9 (416)
	Pb	Cd	Mn	Cu	Ag	Zn	As
Plant available PTE in slag (proportion of 2M HNO$_3$ PTE in slag)							
1a B	3.65 (0.53)	1.00 (7.09)	12.8 (4.32)	4.67 (0.26)	0.03 (0.80)	369 (2.72)	b.d.l
1b A	54.8 (8.30)	1.20 (46.7)	21.5 (5.09)	12.6 (1.01)	0.03 (0.68)	542 (6.44)	b.d.l
1b B	56.3 (5.00)	1.12 (14.3)	19.7 (5.09)	19.2 (1.08)	0.03 (0.43)	398 (3.53)	b.d.l
1c A	0.28 (0.07)	0.01 (0.22)	5.41 (1.68)	0.14 (0.02)	0.02 (0.47)	83.1 (0.76)	b.d.l
1c B	0.28 (0.09)	0.02 (0.50)	30.9 (8.33)	0.28 (0.04)	0.02 (0.35)	77.0 (0.72)	b.d.l
2b A	0.78 (0.22)	0.29 (5.20)	13.7 (1.92)	2.26 (0.11)	0.01 (0.31)	308 (2.09)	b.d.l
2b B	3.88 (1.08)	0.35 (8.35)	16.6 (1.76)	4.02 (0.15)	0.02 (0.30)	324 (1.69)	b.d.l
2c B	0.10 (0.05)	b.d.l	11.9 (2.69)	0.14 (0.01)	0.02 (0.32)	134 (1.09)	b.d.l
3a A	4.38 (0.77)	0.50 (8.03)	13.6 (1.44)	10.7 (0.37)	0.01 (0.24)	218 (1.70)	b.d.l
3a B	0.95 (0.19)	0.44 (6.10)	8.73 (1.02)	1.25 (0.07)	0.02 (0.28)	256 (1.53)	b.d.l
3b A	0.13 (0.04)	0.01 (0.26)	3.08 (0.45)	0.11 (0.01)	0.02 (0.30)	38.3 (0.41)	b.d.l
3b B	b.d.l	0.65 (9.16)	9.53 (1.24)	13.4 (0.73)	0.03 (0.55)	49.1 (0.37)	b.d.l
3c A	0.10 (0.02)	0.35 (4.59)	10.6 (1.51)	1.92 (0.10)	0.02 (0.39)	126 (0.83)	b.d.l
3c B	0.78 (0.17)	0.29 (4.73)	6.48 (0.91)	0.86 (0.05)	0.01 (0.33)	96.0 (0.68)	b.d.l

PTE values by extractions are in mg/kg, values in parentheses are in percentage, n.d. = no data, b.d.l = below detection limit.
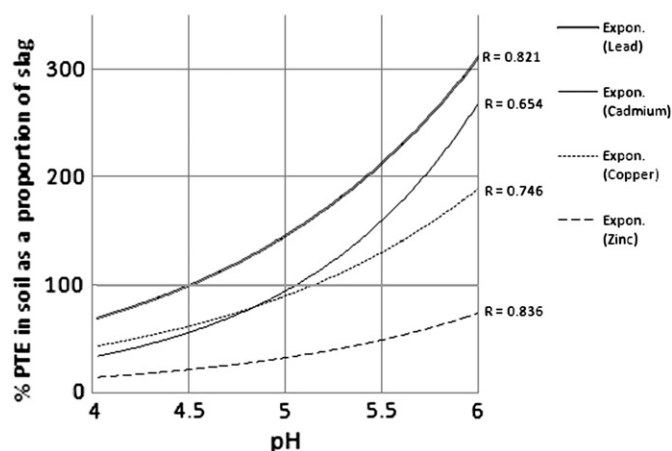
Fig. 5. Relationship between soil pH and percentage of Pb, Cd, Cu and Zn in soil proportional to slag content.

5. Discussion

The study determined the soils in and around the KH slag heap to be polluted by PTE, ranging from mild to extreme contamination, with particularly high values observed for Cu and Zn. Slag samples at the study area were previously analysed by means of X-ray diffraction as part of a thesis work (Rubešová, 2011). Slag was shown to contain significant quantities of lead feldspars and lead oxides, wurzite and olivines (source of Zn) and Mn bearing minerals such as fayalite and manganoan calcite. Fluxes such as PbS, vitrol (Fe, Cu Zn sulphates) as well as charcoal were commonly added to the medieval smelting process (Helm-Clark, 2001), thus accounting for the enrichment of these elements in the by-produced slag. When compared to the control soils, the severity of the contamination becomes clear. In the case of Pb and As, the control soils are also more concentrated by these elements than would be expected in a non-contaminated soil. Although there is no influence from the presence of slag in the control soils, higher than usual values for Pb and As can be expected in the KH region due to the deposition of particulates from the smelting of lead rich ores and arsenopyrites. Despite the strong enrichment of studied PTE in the soils on and around the slag heap, relatively low values were obtained in the extract which represents the plant available fraction. The propensity for soils enriched in PTE weathering from slag to exhibit high 2 M HNO_3 extractable concentrations but to express diminutive bioavailable concentrations is a trend that was also observed by Králová et al. (2010) in a nearby area with a similar contamination source. Low extractability of metals by NH_4NO_3 solution may be attributable to a buffering effect that the slag exhibits toward solvents which react with mineral components of the slag fragments as described in slag leaching studies (Ash et al., 2013; Ettler et al., 2004). The observed mean concentrations of Cu, Mn and Zn which are available to plants (29.6 ± 37, 69.2 ± 95 and 52.6 ± 28 mg/kg respectively) may well be expressed as nutritionally beneficial rather than toxic (McCauley

et al., 2009). Except for Ag, an increase in the soil sample pH corresponded to a decrease in availability of PTE. This is to be expected, but the decrease in availability cannot be directly attributed to pH as the soil organic matter has a strong negative correlation with pH in our studied samples (R = −0.786, P = <0.001). Availability of PTE was also shown to decrease with increasing clay in samples; this could be experimented with further as a possible amendment for mitigating the toxicity of PTE by reducing their uptake. The plant availability of PTE was shown to generally decrease with depth. Copper was an exception to this trend and is likely to be a function of its relationship to the organic matter at the surface. This was shown further by the fact that Cu availability is much decreased in samples where the tree canopy is present and by its negative correlation with C_{org} in all samples. In terms of their overall contents, the studied PTE in soil generally decreased with distance from the main slag heap. It could be anticipated that PTE would be accumulating in the valley bottom as they are deposited there due to weathering by wind and transport by surface water. On the other hand, samples nearer to the river are more highly enriched by vegetation and inputs of organic matter, the pH is generally lower in these positions and therefore the transport of PTE to the downstream positions could be increased. With respect to background contents usually expected in non-polluted alluvial sediments (Beneš, 1993), it is evident that PTE have been migrating toward and settling in the Vrchlice River. Mean values of Pb, Mn, Cu, Zn and As from sediment samples exceed the background value by 1.8, 1.5, 3.4, 9.8 and 1.3 times respectively, therefore there is strong evidence that PTE are entering the river in sufficient quantities to be precipitated and settled in sediments. Lagoon samples also contained unnaturally high concentrations of the studied PTE. Only Pb and As had a higher mean concentration in the river channel sediments than the lagoon sample. Complexation of Zn predominates in polluted waters (Callahan et al., 1979) and a distinct property of Mn is its precipitation into aerobic waters (Kabata-Pendias, 2001), thus, the observed elevated presence of these elements in the Vrchlice River sediments is a good indicator of metal transport from slag to river. Approximately 7 km downstream of River Vrchlice to the east, Horák and Hejcman (2013) were able to correlate the deposition of certain trace metals (including Cu, Pb and Zn) in alluvial sediment with the historical records of mining and smelting activity. Today the dissolution and transport of metals from mining wastes are still likely to result in their deposition in alluvial planes but concentrations are probably a fraction of those during operation of the mines and smelters. The analysis of river water did not reveal any high concentrations of the studied elements. However, in water samples from river bank depressions Zn was detected (max. 14.5 mg/L). According to World Health Organization (2003), in natural surface waters, the concentration of zinc is usually below 10 μg/L, and in groundwaters, 10–40 μg/L, but in certain places, for example in Finnish water resources, public drinking water supplies contained up to 24 mg/L Zn. The uptake and accumulation of micronutrient elements Cu, Mn and Zn in vegetation growing on slag impacted substrates were expressed by their presence in grass, and leaf and stem samples of ash and birch. Concentrations of Zn were particularly high in birch *B. pendula* samples. Although the study revealed excessive concentrations of Ag, As, Cd and Pb in studied soils,

Table 7
PTE in river sediment and PTE concentration in river water and river bank well water.

Sample	pH	Pb	Cd	Mn	Cu	Ag	Zn	As
River sediment[*] mg/kg	n.d.	96.7 ± 15.2	0.70 ± 0.22	1058 ± 479	73.4 ± 24.8	2.02 ± 0.00	1090 ± 275	10.8 ± 4.95
L sediment mg/kg	n.d.	90.1	1.13	1118	61.2	2.17	1556	8.76
River water[**] mg/L	7.58 ± 0.16	b.d.l	b.d.l	b.d.l	b.d.l	b.d.l	0.01 ± 0.008	b.d.l
Well water[**] mg/L	7.25 ± 0.46	b.d.l	b.d.l	0.12 ± 0.08	b.d.l	b.d.l	7.30 ± 10.21	b.d.l

n.d. = no data, b.d.l. = below detection limit, L = lagoon sample.
[*] Mean from S1, S2, S3.
[**] Mean from positions 1, 2, 3.

Table 8
Analysis of plant samples grass, ash and birch, collected at three positions on slag.

	CRM	Grass *Apera spica-venti*	Ash *Fraxinus excelsior*		Birch *Betula pendula*	
			Leaf	Stem	Leaf	Stem
Cu µg/g	24 17.3[*]	21.2 ± 5.05	21.8 ± 4.10	21.6 ± 6.10	20.9 ± 2.39	18.1 ± 4.82
Mn µg/g	1251 1240[*]	140 ± 18.2	44.3 ± 9.8	24.7 ± 7.71	190 ± 62.3	69.5 ± 40.6
Zn µg/g	42 29[*]	304 ± 136	93.5 ± 59.9	99.4 ± 47.5	878 ± 138	1074 ± 619

CRM = certified reference material.

[*] Measured value.

their low availability is reflected in the vegetation samples as all of these PTE were below the detection limit of the atomic absorption spectrometer.

6. Conclusions

This study aimed to characterize the slag and soils of the Kutná Hora smelter waste deposit in terms of PTE content and their distribution and mobility in the surrounding environment. Respective of background contents and agricultural limit values, highly elevated concentrations of PTE were identified in soils on both the main slag heap and in the adjacent river bank, with a generally increased enrichment below the surface. Particularly extreme values were observed for Cu and Zn. Despite determining high overall contents, low availability of PTE was observed. Cadmium and Mn were the most mobile elements. Statistical analysis showed that there was low availability of Cu in soils formed under tree cover, whereas Mn availability was significantly increased under a deciduous tree canopy. Likewise, when correlated with C_{org}, available Mn (and As) showed positive correlation, while Cu (and Pb) had negative correlation. With the exception of Ag, all elements showed negative correlation with pH, while Pb and Cu availability showed negative correlation with the soil clay content. By determining the PTE in the soil as a proportion of its source (PTE content in slag) it was possible to predict points at which PTE accumulate according to a given soil parameter. We achieved statistically significant trends for Pb, Cd, Cu and Zn when plotted in this way with pH as the independent variable, and it was possible to interpolate the threshold pH value at which PTE accumulate in soil. The model is limited to the assumption that all slag samples are weathered at a consistent rate and that the extracting agent reveals the total PTE concentration in both slag and soil; hence a total digestion should be applied in further investigation. Analysis of river sediment revealed high 2 M HNO_3 extractable PTE values which exceed natural background concentrations. It is evident that risk metals are being mobilised from the slag and settling in surrounding surface waters which are regularly used for irrigation of vegetable plots. However, analysis of the river water did not reveal the presence of PTE, only in the river bank depression where water flow is restricted did we detect Mn and Zn in the water column. Micronutrient elements were present in tissue samples of grass, ash and birch which grow on the slag impacted substrates but, PTE which have no function in plants (Cd, Pb, Ag, As) were not observed in the vegetation samples.

The slag deposits along the Vrchlice River in Kutná Hora are a source of high levels of PTE in surrounding soils and surface waters. However, low availability limits the threat to vegetation and other environmental receptors. Healthy vegetation in the surrounding soils and no detection of PTE in tissue samples suggest that the absence of plants on the main slag heap may be dependent on other factors such as slope, lack of substrate, poor water holding potential and availability of key nutrients etc. rather than due to toxicity by PTE. Although no PTE were detected in the flowing river water, intrusion of slag and deposition of PTE in stagnant wells may result in chronic persistence of Mn and Zn in the water.

Acknowledgements

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Contents of Potentially Toxic Elements in Forest Soils of the Jizera Mountains Region

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Abstract In central Europe, the region termed 'the Black Triangle' corresponds to an area of concentrated burning of fossil fuels during the period between early 1950s up to the mid 1980s. Although major polluting activities have ceased, effects of atmospheric deposition are still evident. The aims of this study were to determine potentially toxic element (PTE) concentrations in organic and mineral horizons of forest soils in the Jizera Mountains region and to assess the relationship between their distributions and stand factors (altitude, grass cover, forest type). One hundred thirty-eight samples were taken from 98 positions, comprising O, A, E and B horizon samples. Target elements (Cu, Cd, Pb, Zn, Mn) were extracted using 2 M HNO_3 and then analysed by FAAS. Basic statistical parameters were determined, and correlation, t test and ANOVA were used to assess the relationship to stand factors. Maps were created using ArcMap 9.1. Two map creation techniques were used—kriging and inverse distance weighting. Highest mean metal contents (except for Mn) were found in organic horizons, with Cu and Pb being particularly concentrated in the upper layers. Strong positive correlation exists between Cd content and altitude, while negative correlation exists for Mn. Cadmium concentration was also significantly higher in areas of grass cover and under spruce canopy as opposed to beech. Surface maps indicated a similar source of pollution with highest concentrations corresponding also to relief and the prevailing wind direction. Overall, mean PTE contents were not excessive. Top enrichment factors proved the anthropogenic origin of Cd, Cu, Pb and Zn, whereas Mn is probably of geologic origin. Analysis of stand factors showed a statistically significant influence of altitude, grass cover and forest type on cadmium concentration in forest soils. Spatial distribution of metals showed a similar pattern for Cd, Cu, Pb and Zn in O horizon soil, which points toward a single source of pollution.

Keywords Forest stand · Forest soils · Atmospheric deposition · Distribution mapping · Heavy metals

1 Introduction

As a consequence of poorly regulated industrial development during the Communist era and extensive coal burning for power generation, the Jizera Mountains region in the Czech Republic is recognised as being one of the most severely affected areas by atmospheric deposition in Europe [16, 27]. Being at the centre of what is referred to as 'the Black Triangle', the Jizera Mountains experienced heavy loads of acid deposition which peaked in the mid-1980s and resulted in the manifestation of several forest degradation processes, including decreasing rate of humification, decrease in quality of soil microbial activity and the formation of aluminium toxic forms in the soil [18, 20, 26]. Irrespective of acid rain, fossil fuel combustion and pyrometallurgic industry are responsible for anthropogenic loads of potentially toxic elements (PTEs), in the form of wet and dry deposition [1]. In areas of known atmospheric pollution due to the aforementioned industrial activities, a positive correlation exists with the increased presence of PTE in soil such as As, Cd, Cr, Cu, Mn, Ni, Pb, Sb and Zn [22]. As part of a spatial interpretation of ambient air quality for the Czech Republic, Hůnová [14] identified the Jizera Mountains as one of the regions exhibiting the poorest air quality in terms of both the ambient air quality and wet atmospheric deposition of pollutants.

When present in excessive concentrations, PTEs exhibit a range of effects, which are detrimental to the growth and reproduction of plants, and have a negative effect on the

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activity of microorganisms, disrupting important biological processes such as nitrogen fixation, adenosine triphosphate production, soil enzyme activity and subsequently microbial biomass production [3]. Additionally, studies have shown that high metal concentrations alter litter decomposition due to impaired biological functions [4, 10], and the distribution of metals is dependent on litter turnover mostly due to the binding affinity between metals and humified substances [8, 30]. Following political revolution at the end of the 1980s, industrial emissions and the associated atmospheric concentrations of harmful products including PTE have shown a gradually declining trend [25]. However, heavy metals and metalloids persist in the environment indefinitely; therefore, their presence and effects should be monitored. Generally, in soils formed under a cool humid climate, the leaching of trace elements downwards through the profiles is greater than their accumulation, unless there is a high input of these elements into the soils [15]. The forest floor is relatively consistent in its composition, with a litter and humic horizon featuring high cation exchange, overlaying a mineral soil which has little mixing with the upper organic layers [27], highlighting the need for vertical analysis of these soils.

A detailed survey of the Jizera Mountain forests was performed by Borůvka et al. [5, 6], with soil sample analysis focusing on Al distribution and forms. The study identified highest available Al_{KCl} contents in organic surface horizons (related to low pH, high S and N and low Ca and Mg).

Organically bound aluminium ($Al_{Na4P2O7}$) was more abundant under spruce compared to beech, with increased concentrations in clear cut areas covered with grass. In a recent publication, Harmens et al. [12] present temporal changes of heavy metals from atmospheric deposition in Europe through the use of mosses as bio-indicators, noting the greatest decrease since 1990 in the concentrations of As, Cd, Pb and V.

The main aim of this study was to determine the level of PTE (Cd, Cu, Mn, Pb, Zn) pollution in samples from the organic and mineral horizons of forest soils in the Jizera Mountains region. The distribution of PTE and their relationship to stand factors (vegetation cover type and altitude) were assessed, and surface mapping was used for the visual projection of PTE distribution.

2 Materials and Methods

2.1 Area Description

The Jizera Mountains region is a protected area in the northern Czech Republic. Altitudes of the sampling sites ranged from 400 to 1000 m (Fig. 1a). Mean annual rainfall in the region ranges from 900 to 1600 mm/year (Fig. 1b). Soils were identified as Podzols (Haplic or Entic) and Cambisols (mainly Dystric). Soil units generally correlate to altitude with Cambisols prevailing in lower positions and Podzols in the
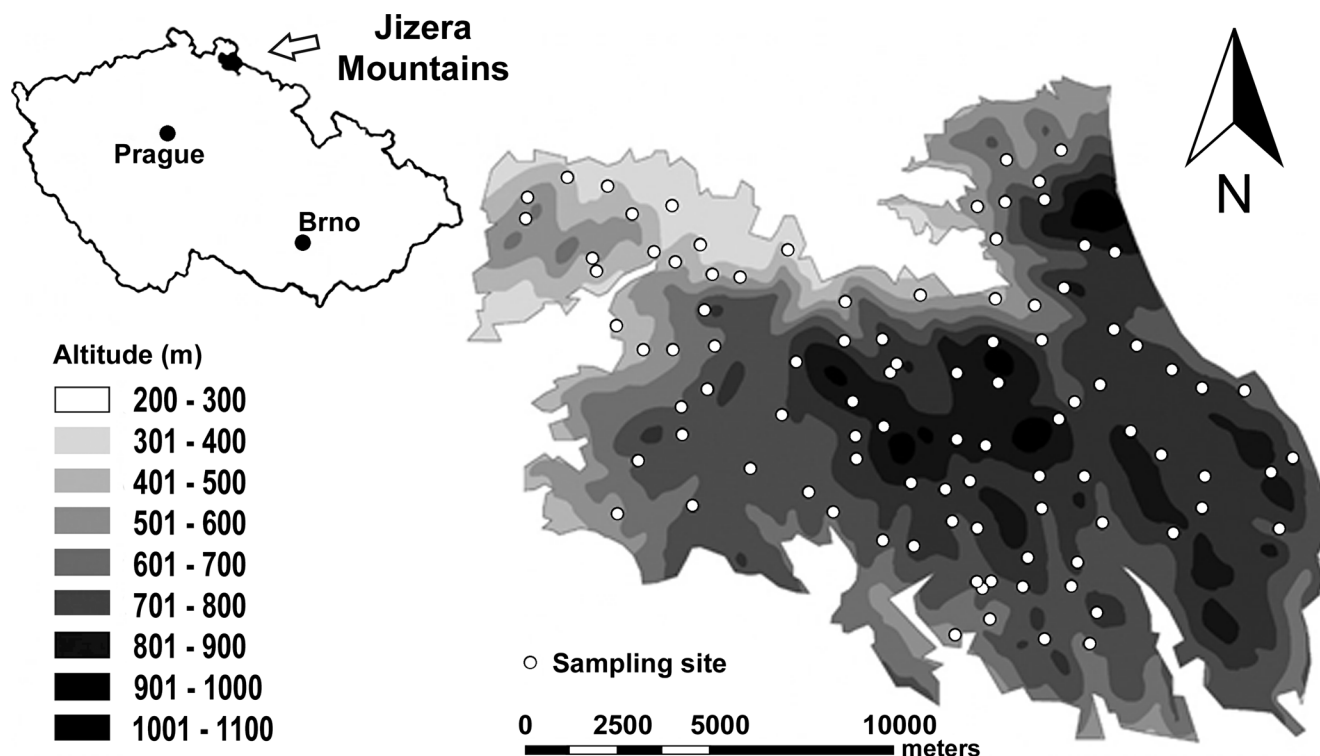
Fig. 1 a Sampling region in the Jizera Mountains and corresponding altitude. b Mean annual precipitation of the Jizera Mountains region in 2001 [24]. c Map of Jizera Mountains region showing tree age and type
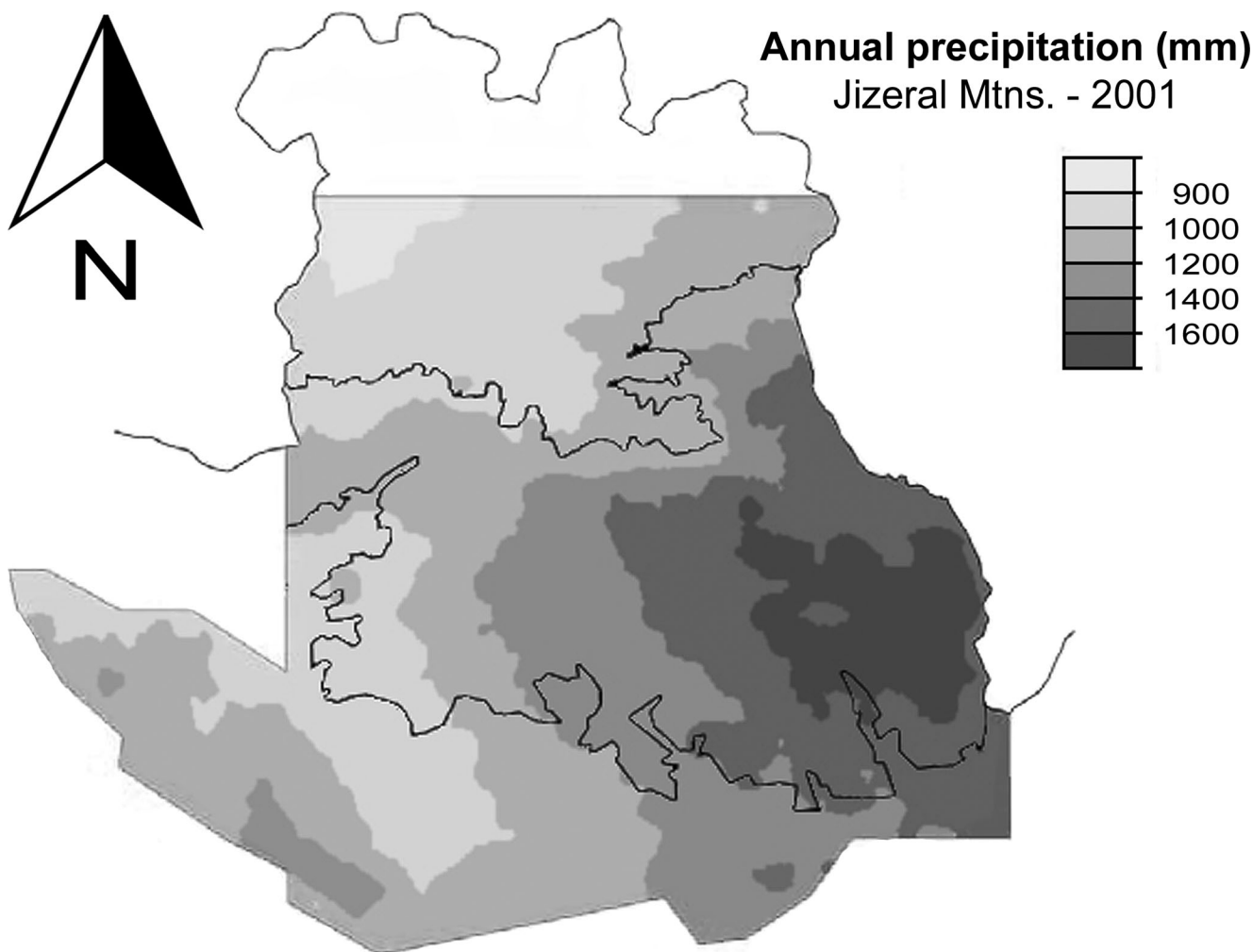
Fig. 1 (continued)

higher positions. All soils were formed on granite bedrock. The prevailing forest type was Norway spruce (*Picea abies*) monoculture, with areas of European beech (*Fagus sylvatica*) forest concentrated in the north-western and northern edge. A large part of the cover in the higher positions consists of open grass area (*Calamagrostis villosa*). Forest stand type and age are illustrated in Fig. 1c.

2.2 Soil Sampling

From an original sample set (e.g. Mládková et al. and Mládková et al. [18, 19]), 138 samples were selected from 98 positions, comprising 23 O horizon (mixture of F and H horizons), 18 A horizon, 27 E horizon and 70 B horizon samples. Samples were taken according to an irregular grid covering the area with an average sampling density of approximately 1 site/2 km^2. With respect to grass cover, 30 % of samples were from fully covered sites, 9 % from partially covered and 61 % from sites with no grass. Samples were air-dried and sieved to 2 mm prior to analysis.

2.3 Analysis Methodology

Concentrations of target elements in soil samples were determined by continuous shaking of 5 g of soil in 50 ml of 2 M HNO_3 for 6 h. Extracts were then filtered (Filtrak 390) and analysed for Cd, Cu, Mn, Pb and Zn by FAAS (Varian 200 with SIPS 20 using Analytika s.r.o standards). Samples and standards were matrix matched, and all analyses were performed in triplicates.

Statgraphics Plus 4.0 was used for the determination of basic statistical parameters, and for assessment of statistical significant differences between variables, correlation, *t* test for independent, normally distributed samples and analysis of variance (ANOVA) were used to assess at the 0.05 significance level, the relationship to stand factors, altitude, forest type and grass cover, respectively.

Top enrichment factor (TEF) was calculated as the ratio between O horizon and B horizon contents of PTE.

Final maps were created using ArcMap 9.1 software. Interpolation was used as a basis of the maps formation. Two
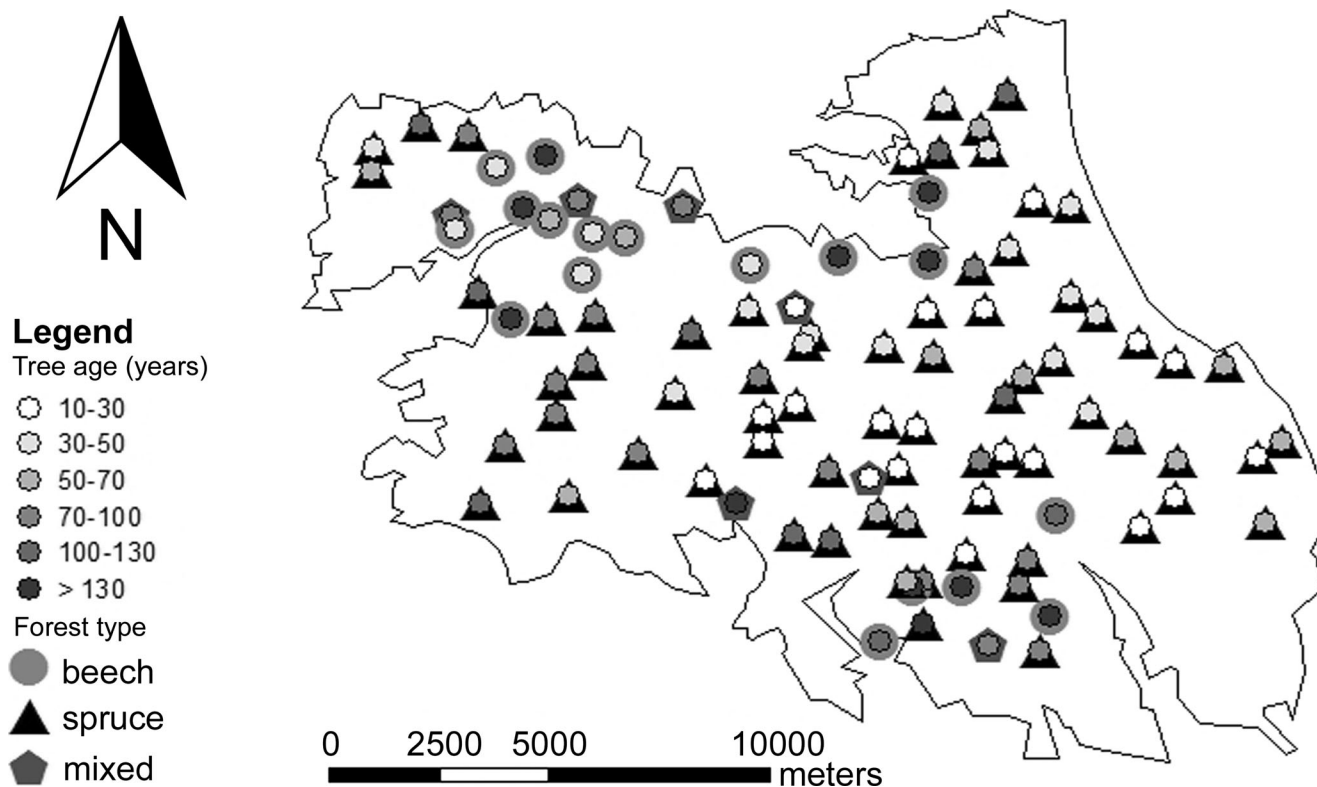
Fig. 1 (continued)

map creation techniques were used; ordinary kriging was applied in cases where spatial dependence among variables was observed. Inverse distance weighting was used in four cases: Cu and Zn in O horizon and Cu and Mn in A + E horizons. Due to a relatively small number of sampling points, maps show only general spatial trends of values, not detailed spatial distribution. In particular, the values close to the area border have higher uncertainty due to extrapolation. For the purpose of mapping, A and E horizons were combined in some cases, the A horizon in Podzols was either missing or was very shallow, and in other cases, the A horizon showed some eluviation. O and B horizons are presented individually.

3 Results

3.1 Basic Statistical Parameters

Mean and standard deviation were determined for O, A, E and B horizons (Table 1). PTE concentrations were measured in the following ranges: Cd 0.02–1.9, Cu 0.91–29.2, Mn 0.50–481.6, Pb 10.9–326.3 and Zn 3.38–27.2 mg/kg. Highest mean concentrations of PTE were found in O horizons for all elements with the exception of Mn where the highest mean was observed in the B horizon. One-way ANOVA was applied

to data to determine the significance of PTE distribution between horizons. Although significant differences among sample means exist for all PTE, the most pronounced is for Cu and Pb with strongest retention in the organic horizon. The probable geologic source of Mn as opposed to anthropogenic input is evident due to higher contents with increased depth. The likely origin of PTE into forest soils is also expressed by the TEF in Table 2.

TEFs were calculated for the studied PTE (Table 2). TEF values greater than 1 indicate accumulation of PTE in the upper horizon, whereas a TEF value less than 1 generally indicates geological origin or the possibility of leaching from top soil. High TEF values were observed for Cu and Pb, which reflects the binding affinity that these elements have towards organic matter [2]. TEF values >1 were observed for Cd and Zn. However, Mn has a value less than 1, indicating that this element either originates from parent material or is highly mobile.

3.2 Spatial Relationship with Stand Factors

3.2.1 Altitude

A highly significant positive correlation between altitude and Cd concentration was identified in the O horizon and, to a lesser extent, in the B horizon, while a significant negative correlation was determined for Mn in almost all horizons

Table 1 Basic statistical parameters of PTE in soil horizons

Horizon	Parameter	Cd	Cu	Mn	Pb	Zn
O (23)	Mean (mg/kg)	0.82	15.2	37.1	163	14.6
	Standard deviation	0.44	5.09	74.5	52.9	3.62
A (18)	Mean (mg/kg)	0.34	4.75	12.3	52.7	8.44
	Standard deviation	0.19	3.04	8.60	16.0	2.81
E (27)	Mean (mg/kg)	0.26	4.04	15.1	32.6	6.25
	Standard deviation	0.21	2.23	47.9	14.7	1.72
B (70)	Mean (mg/kg)	0.49	3.13	81.4	38.2	13.6
	Standard deviation	0.15	1.85	83.1	19.3	4.46
F ratio of residual square mean		25.0	102	8.66	132	33.2
P value of significance		<0.001	<0.001	<0.001	<0.001	<0.001

Value in parenthesis refers to number of samples

(Table 3). Other elements showed mostly weak and non-significant dependencies on altitude with the exception of Cu in the B horizon.

3.2.2 Grass Cover

ANOVA and multiple range tests were applied to individual data sets by horizons for comparing forest vegetation zones. In the O horizon, a statistically significant difference among the variables was determined at the 95 % confidence level for Cd (Table 4), with mean values decreasing as follows: full grass cover (1.26 mg/kg) > partial grass cover (1.01 mg/kg) > no grass cover (0.58 mg/kg). Cu, Mn, Pb and Zn did not show any statistically significant difference with respect to grass cover, and no significant difference between grass cover categories was determined in mineral horizons.

3.2.3 Forest Type

t Test was used to determine differences among PTE contents under spruce and beech forest (Table 5). Given that P value is <0.05, there is a significant difference between the two types of mono-species forest at the 95 % confidence level in the case of Cd. Although not statistically significant, there was also an observed higher mean content of Pb and Cu under spruce forest compared to beech, whereas Zn and Mn were higher under beech.

3.3 Map Projection

PTE spatial distribution maps are given in Fig. 2. The maps showed a similar distribution of Cd, Cu, Pb and Zn in the O horizon, indicating a common source of pollution between these elements. Such similarity was not observed for the lower horizons. Maximum O horizon contents of Cd, Cu, Pb and Zn were found in the central western half of the region, corresponding to the relief and main windward direction. Kriging errors are given in Fig. 2d for Cd in all horizons. The errors were similar for all studied PTEs, so for the purpose of simplicity of presentation, only Cd is shown.

The spatial distributions of TEF (calculated as the ratio between PTE concentrations in O and B horizons) are illustrated in Fig. 3. No significant common pattern was observed for TEF value distribution or relationships between particular elements.

4 Discussion

A broad study concerning the distribution of PTE in forest floor humus in the Czech Republic was carried out by Suchara and Sucharová [27] only a decade ago. Humus samples taken from the Jizera Mountains contained elevated levels of Al,

Table 2 Top enrichment factor (mean values)

	Cd	Cu	Mn	Pb	Zn
TEF	1.46	7.80	0.69	5.53	1.20

Table 3 Correlation coefficients between PTE content and altitude in different horizons

Horizon	Cd	Cu	Mn	Pb	Zn
O	0.67***	−0.01	−0.45*	0.33	0.05
A	−0.39	0.06	0.07	−0.09	−0.08
E	0.10	0.32	−0.52**	0.09	0.03
B	0.25*	0.37**	−0.43***	0.06	−0.31*

*,**,***Significant at 5, 1 and 0.01 probability level, respectively

Table 4 Multiple range test and ANOVA for grass cover effects (O horizon)

Parameter	Cd	Cu	Mn	Pb	Zn
Full grass cover (mg/kg)	1.26 b	13.76	7.92	191.53	16.32
Partial grass cover (mg/kg)	1.01ab	17.56	7.98	148.88	12.14
No grass cover (mg/kg)	0.58 a	15.54	55.89	150.71	14.16
F ratio of residual square mean	10.32	0.48	1.10	1.46	1.33
P value of significance	0.001	0.627	0.353	0.255	0.287

Within the columns, values followed by the same letter are not significantly different at $P \leq 0.05$

most likely present as $Al(OH)_3$ which is dispersed throughout the water phase of coal [21]. As, Cr and Mo occurred from the combustion of lignite containing arsenopyrite, Cr- and Mo-bearing compounds, mostly in the north-western Bohemia region [27] and V from chemical and oil industries. Considerably lower concentrations of Cd, Cu, Pb and Zn were observed in the forests of northern Czech Republic within the Black Triangle. Contamination with Cd and Cu generally corresponds with the operation of steel and engineering industries with increased soil concentrations found largely in the northern Slovak Republic. Some increased humus concentrations of Cu may be attributed to the combustion of lignite. Lead contamination in the Czech Republic correlates most strongly with smelting and Pb processing operations, found mostly in southern Bohemia, particularly in Příbram. Hot spots of Zn generally correlate with steel and engineering industries and processing of polymetallic ores, which were concentrated in central and south-west Bohemia [27] as opposed to in the Black Triangle area, which was impacted mostly by coal burning. A similar trend of PTE occurrence was observed in our research whereby the studied elements did not prove to be particularly high when compared to Czech guidelines concerning PTE contents in agricultural soils [17] (no guidelines have yet been published for forest soils of the Czech Republic). Following are the maximum aforementioned elements in the O horizon as a percentage of the Czech agricultural guideline value (AGV) for comparison: Cd (191 %), Cu (58.3 %), Pb (407 %) and Zn (21.7 %). Only Cd and Pb exceed the AGV for a 2 M HNO_3 extraction. However, these high values do not necessarily have to present a danger, as the nature of the forest floor with respect to soil organic matter content and sorption ability is incomparable to the top soil of arable land.

Table 5 t test for PTE content and forest type (O horizon)

Parameter	Cd	Cu	Mn	Pb	Zn
Beech (mg/kg)	0.53	14.78	108.80	139.68	16.65
Spruce (mg/kg)	1.02	15.90	10.76	178.93	14.14
Independent t	−3.42	−0.42	1.84	−1.49	1.35
P value of significance	0.003	0.680	0.125	0.155	0.193

The differentiation between geological enrichment and anthropogenic pollution in weakly polluted soil samples from northern and north-eastern Czech Republic was studied by Borůvka et al. [7]; their results suggest that Cu and Zn are mainly of geological origin and that Pb and Cd are a considerable contribution of anthropogenic pollution. In this study, the TEF identified higher proportions of all PTE in the O horizon compared to predominantly mineral subsoil, with the exception of Mn which is likely to be originating from parent material. Map projection shows the greatest occurrence of Mn in all horizons in the north-western portion of the studied area (Fig. 2c). Geologic composition of the northern part of the studied locality includes orthogneiss, which has a high variable content of MnO (0.01 to 0.27 %), and phonolite of the Paleogene-Neogene age with 0.20 % MnO. Homogenous porphyritic granites in the southern part contain manganese-bearing compounds to a lesser extent of 0.04–0.09 % [9]. When forest types were compared, the mean value for Mn in beech forest which was considerably higher than in spruce can be attributed to two aspects: geological origin and/or deeper root systems of beech, which can transport Mn up from the subsoil. Hernandez et al. [13] investigated the heavy metal distribution in 11 selected forest sites in France with different types of atmospheric deposition. The abundance of heavy metals measured in these soils decreased as follows: Zn > Pb > Cu > Cd. In the case of Cu and Zn, some surface soil horizons presented enrichment factors ranging between 2 and 4, whereas Pb and Cd enrichment factors were far higher in surface horizons of forest soils, which were expected to be derived from long range transportation of anthropogenic atmospheric pollutants. In this study, we identified the highest TEF in the case of Cu (7.8) and Pb (5.5). As organic matter is one of the most influential factors controlling the movement of Cu and Pb, it is logical that TEF is highest for these elements in the forest floor where there is a great deal of surface humic material. This hypothesis was confirmed, to some extent, in the Polish Karkonosze National Park, a mountainous forest region, which neighbours our studied Jizera Mountains region. Szopka et al. [29] identified lead concentrations in upper (0–10 cm) forest floor layers in the range of 19–248 mg/kg. Calculated pools of Pb correlated strongly with organic matter; however, spatial distributions were
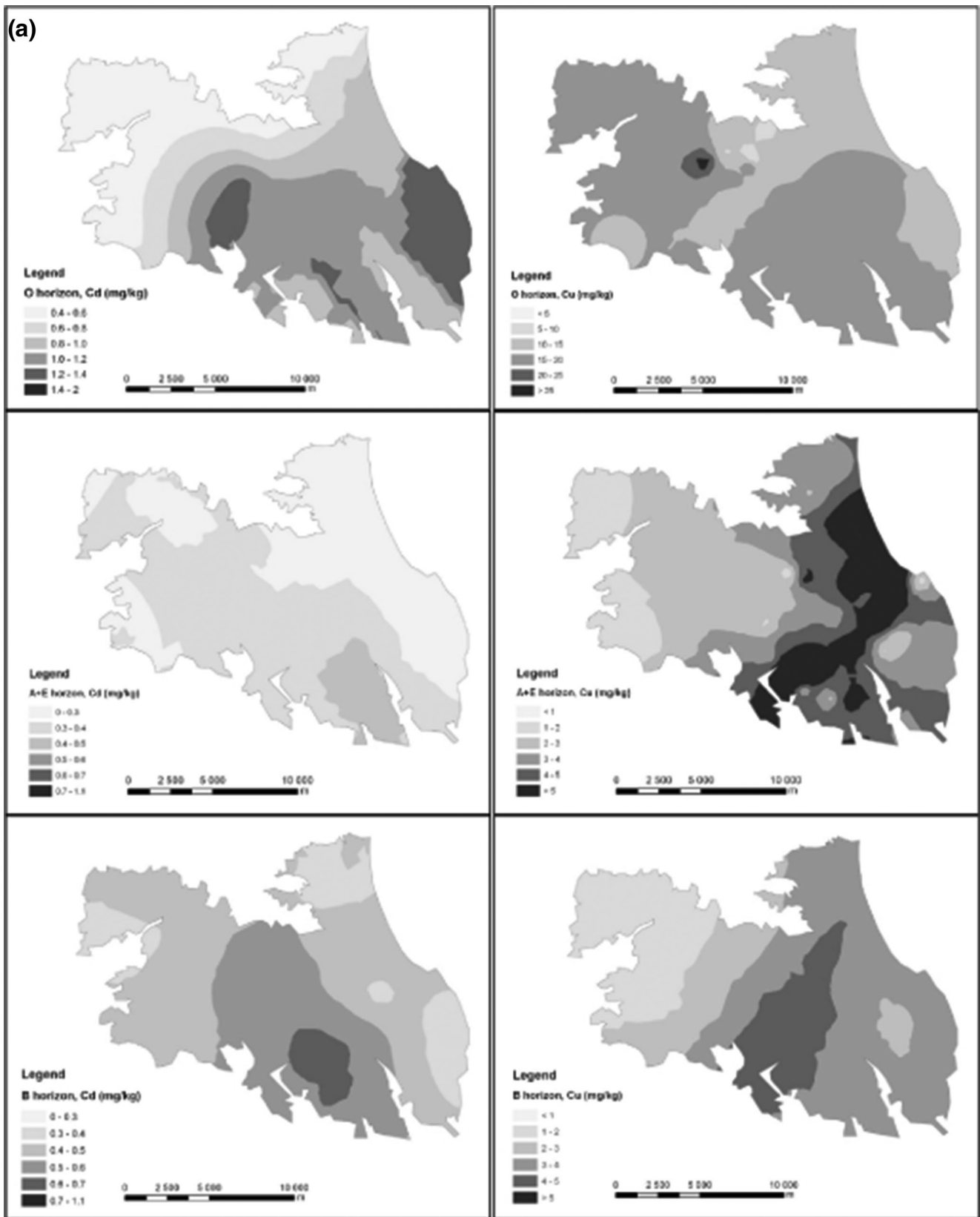
Fig. 2 Interpolated maps of **a** PTE (Cd *left*, Cu *right*), **b** PTE (Pb *left*, Zn *right*) and **c** Mn distribution in different soil horizons of the Jizera Mountains region. **d** Kriging errors for Cd in O, AE and B horizons
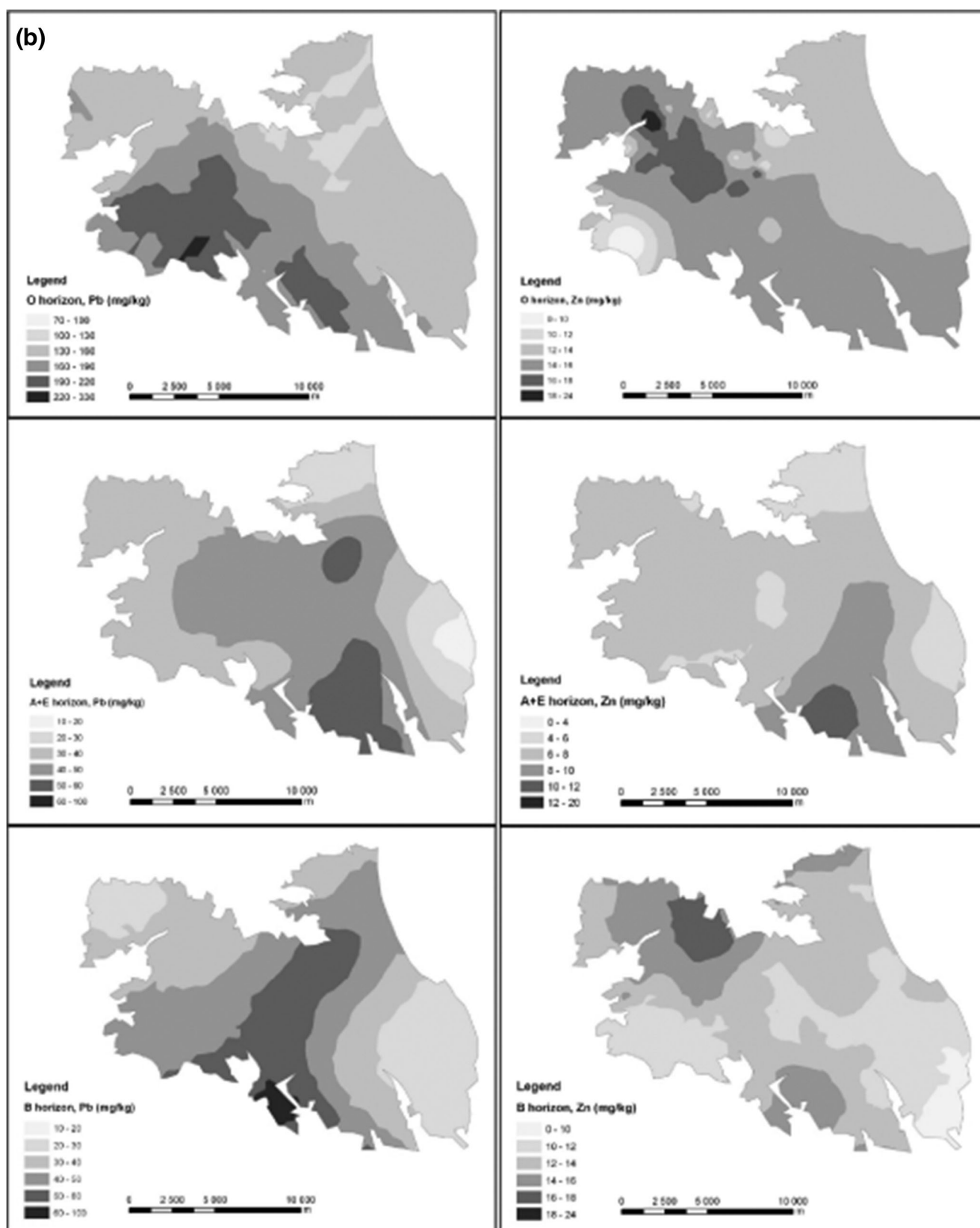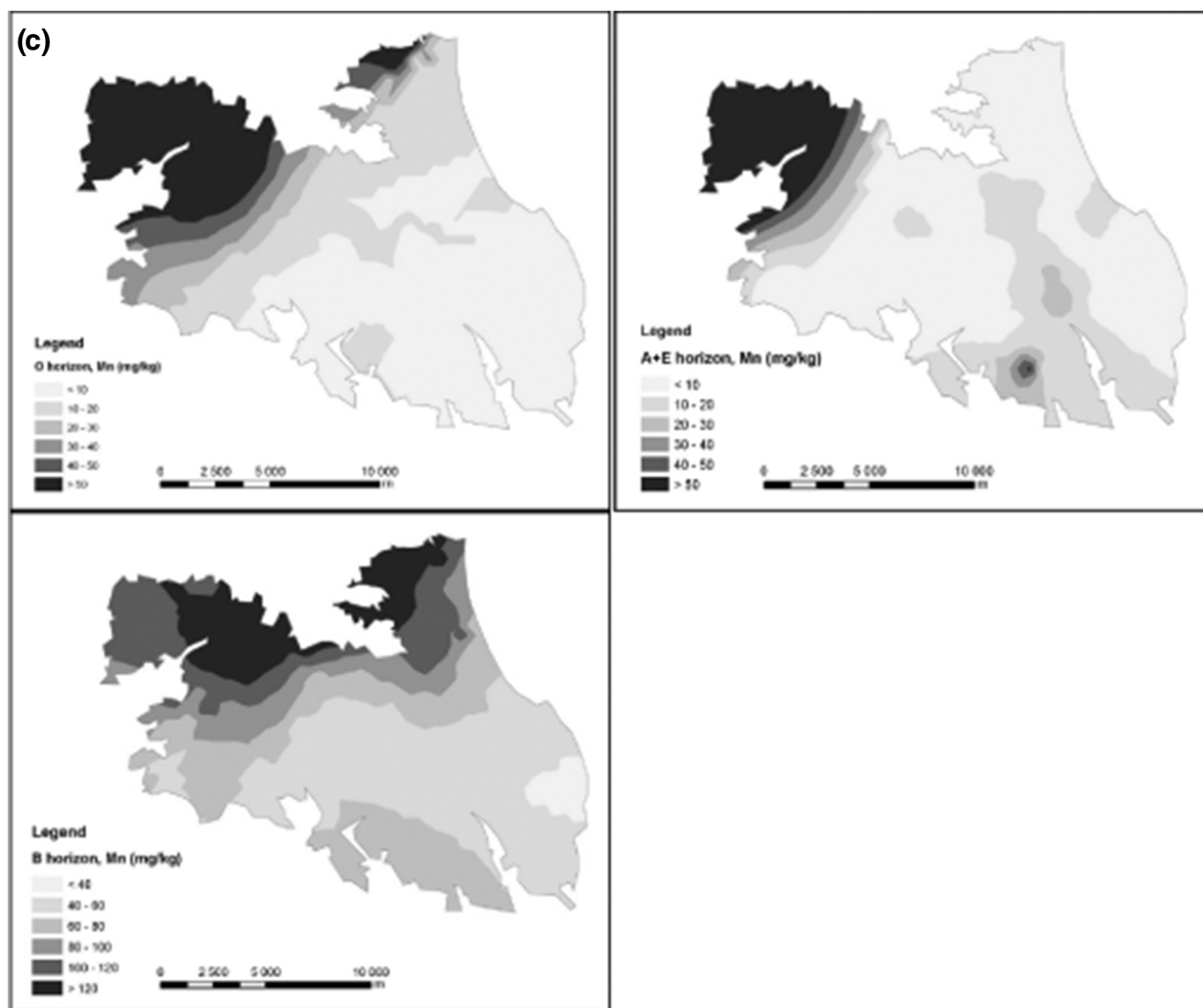
Fig. 2 (continued)

(c)

Legend
O horizon, Mn (mg/kg)
- < 10
- 10 - 20
- 20 - 30
- 30 - 40
- 40 - 50
- > 50

Legend
A+E horizon, Mn (mg/kg)
- < 10
- 10 - 20
- 20 - 30
- 30 - 40
- 40 - 50
- > 50

Legend
B horizon, Mn (mg/kg)
- < 40
- 40 - 60
- 60 - 80
- 80 - 100
- 100 - 120
- > 120

Fig. 2 (continued)

highly variable and, for this reason, were difficult to attribute to other factors such as altitude.

Shparyk and Parpan [23] studied the Ukrainian Carpathians which are characterised by high air pollution caused by emissions from numerous industries. Risk metals were identified as entering the forest system via wet and dry deposition, becoming fixed in soil and accumulating in leaves, needles and mosses. In the Jizera Mountains region, a significant positive correlation between altitude and O horizon Cd concentration was identified. Lead content in the O horizon also increased with altitude, although the trend is not statistically significant and the relationship may be indirect. At higher altitude, there is an increase in precipitation; therefore, there exists a greater input of PTE via wet deposition. The Jizera Mountains region is characterised by overall high annual precipitation (900–1,600 mm); although highest inputs of rainfall occur at the central and central eastern portion of the sampled area (Fig. 1b), this data does not reflect individual

peaks of higher altitude. It was noted, previously concerning the map projection, that greater concentrations of PTE in upper horizons generally occurred in the western portion of the study area. Szopka et al. [29] and Suchara and Sucharová [28] made similar observations that the westerly wind is the reason for greatest deposition of atmospheric pollutants on the western side of the mountains which act as a barrier to many airborne pollutants. There are other considerations that can account for varying concentrations with altitude that were not factored into this study, such as proximity to pollution sources, soil pH and slope; therefore, interpretation of data should consider other such variables.

ANOVA statistics showed a significant trend of decreasing soil Cd concentration with decreasing grass cover. Reasons for this occurrence have been proposed by [11]; grass occurs mainly on open areas which occurred as a result of damage to trees, mostly due to acid deposition. As this was the place of most severe deposition, a greater share of PTE occurs in these
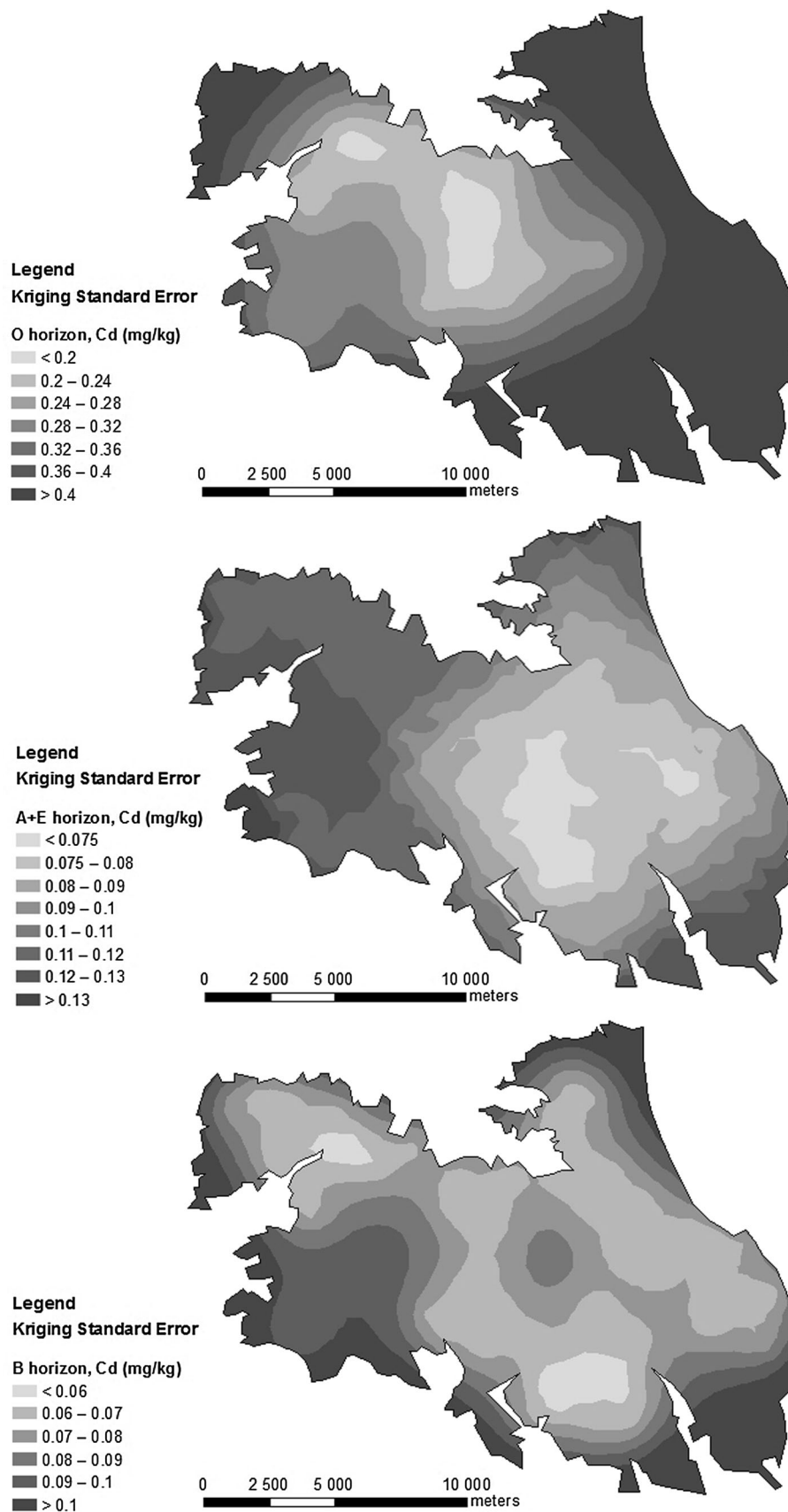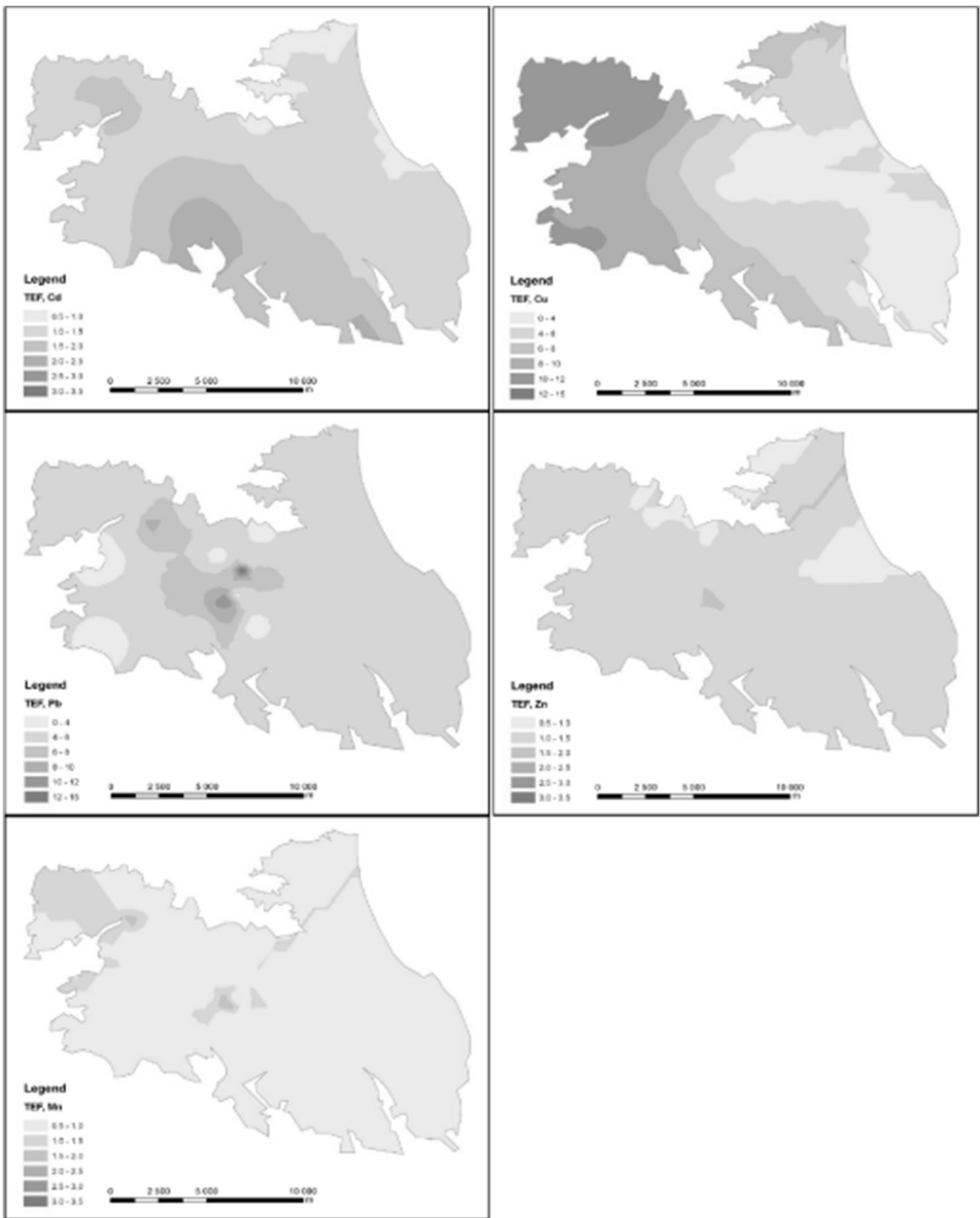
Fig. 2 (continued)

Fig. 3 Top enrichment factor of PTE

zones. Another reason for greater occurrence of PTE in the areas populated by grass can be partly attributed to the fact that these areas were limed in response to the high deposition of acidic compounds [5], which increases the retention of cations at the surface. However, this phenomenon requires further research, as liming could also increase the rate of mineralisation of soil organic matter, consequently reducing the number of sorption sites for PTE in soils. The effect of grass on PTE distribution is principally indirect, as the occurrence of grass only reflects the deposition and consequent management practices that followed. A map was created for tree age data (Fig. 1c). Very young forest is on areas which were afforested in the 1970s and 1980s, the period of highest atmospheric deposition. Lower interception of dry atmospheric deposition during this period can be expected on sites where there were new trees planted at that time; i.e. where the forest is 30–50 years old now. Correlation analysis was made between tree age and PTE content, but no significant trends were shown in any of the horizons. However, there was a significant difference between the two types of monoculture forest, with higher contents of Cd being present under spruce. Likewise, Pb and Cu were found to be more abundant under spruce forest, although this was not statistically proven. Higher PTE accumulation under spruce can be explained by the fact that (1) spruce has a higher specific surface on its needles as opposed to beech and (2) beech is a deciduous tree species. Thus, spruce exhibits increased year-round interception via rain, fog and winter rime and therefore a stronger input of particulate matter to soil. Despite the observed trends, we must consider that there are many variables concerned with such large-scale sampling and more detailed analysis is required to relate stand factors in detail.

5 Conclusions

The aim of the study was to determine the level of forest soil pollution in the Jizera Mountains, an area strongly affected by atmospheric deposition. Compared to AGVs, mean values of PTEs do not appear to be excessive. The only mean value that surpassed the guideline value was lead in the O horizon (two times the guideline limit). TEF proved the anthropogenic origin of cadmium, copper, lead and zinc, whereas manganese appears to be of geologic origin. An analysis of stand factors showed a statistically significant influence of altitude, grass cover and forest type (spruce vs. beech) on cadmium concentration in forest soils. Maps with spatial distribution and TEFs of potentially toxic elements were created. Maps generated for spatial distribution of potentially toxic elements showed a similar pattern for Cd, Cu, Pb and Zn distribution in O horizon soil, which points towards a single source of pollution.

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Redistribution of cadmium and lead fractions in contaminated soil samples due to experimental leaching

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ARTICLE INFO

ABSTRACT

The sequential extraction procedure (SEP) is often applied to soils for identification of binding phases but rarely is it used to monitor binding dynamics temporally. In this study, contaminated soils were subjected to two different leaching scenarios, one analogous to a hydrostatic or stagnant water exposure, and one that represents a hydrodynamic event such as heavy precipitation. A four step SEP was applied before and after each stage of leaching and the adsorption dynamics of cadmium and lead were studied. In the hydrostatic experiment, removal of Cd and Pb occurred largely from the residual fraction (from initially >30%–zero Cd after the 720 h experiment, 40%–<20% Pb, and from 50%–20% Cd, >50%–40% Pb, from organic and mineral soil horizons respectively). There was a significant redistribution onto the Fe/Mn oxides (from initially 30%–60% Cd after 720 h, 40%–65% Pb, and from 15%–30% Cd, 45%–60% Pb for organic and mineral soils respectively). A significant redistribution of Cd onto the organic matter was observed (from initially zero, to 20% after 720 h in organic soil, and from 1%–15% in mineral soil). After the experiment, fraction distribution of the organic soil sample closely matched the distribution pattern of a slightly contaminated fluvisol sample that was taken in a river valley adjacent to the studied soils. When a CaCO₃ amendment was applied, dissolution of both elements decreased, >50% less Cd and Pb released from organic soil, and ~80% less Cd released from mineral soil with amendment. However, the fraction distributions remained similar to that of a non-amended sample. In the hydrodynamic leaching experiment, important binding phases of Cd were identified as organic matter (from initially 0%–15% after leaching and from ~2%–~10% in organic and mineral soils respectively) and metal oxides (from initially 40%–50% after leaching in organic soil). Residual Pb increased after leaching, and losses occurred from the Fe/Mn oxides (>50% decrease in organic soil). The CaCO₃ amendment had little influence on both element distribution and concentrations in hydrodynamic column leaching. It is anticipated that this study could be a precursor for modelling heavy metal fractionation of contaminated soils based on water regime in the environment.

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1. Introduction

The transfer of potentially toxic elements (PTE), in gaseous, particulate, aqueous or solid phase, from anthropogenic sources to the pedosphere can result in persistent contamination of surface and subsurface soil. Such occurrences of PTE present a challenge for authorities and landowners to maintain PTE values below soil guideline limits. A diverse array of remedial strategies has evolved in response to the demand for the mitigation of polluted soils,

and many studies have focused on the physical chemistry of soil dynamics. This serves to increase understanding of the processes controlling the pollutant mobility and toxicity, often as a precursor to remediation technology development. Amendments aimed at immobilizing inorganic contaminants decrease trace metal leaching and reduce their bioavailability by inducing a range of sorption processes, e.g., adsorption to mineral surfaces, formation of stable complexes with organic ligands, and surface precipitation (Kumpiene et al., 2008). A number of studies have focused on the immobilisation of PTE with the addition of basic amendments (Alvarenga et al., 2008; Lin and Zhou, 2009; Kim et al., 2013). For example, the addition of calcium carbonate to a soil strongly reduces metal scavenging by the solution from all soil fractions. Houben et al. (2012) studied CaCO₃, and other amendments, for the potential

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immobilisation of Cd and Pb in contaminated soil. A reduction in Cd leaching was observed mainly due to increases in pH caused by amendment additions. However, only $CaCO_3$ addition resulted in a strong reduction of metal plant uptake. The addition of calcium carbonate during production of card products inspired recent research by Prica et al. (2013), who focused on the potential for cardboard mill pulp as an immobilizing amendment to Cd enriched sediments. The study showed promising results, with significant reductions in Cd mobility when pulp was incorporated with the sediments. The sequential extraction procedure (SEP) was developed for the purpose of characterizing soil binding fractions, by selectively targeting and releasing metals bound in certain geochemical phases (Larner et al., 2006). Identification of the main binding sites and pedochemical phase associations of trace metals in soils helps toward predicting their mobilisation potential and the likely risks they pose to the environment (Gleyzes et al., 2002). Although the SEP is utilized frequently as a descriptive tool in the study of contaminated soils (Padmavathiamma and Li, 2010; Tang et al., 2008; Yang et al., 2011), little consideration has been given to the dynamics of PTE binding with time, across the spectrum of different soil fractions. For example in leaching or extraction experiments that are synonymous to a particular leaching scenario. Over a prolonged exposure of contaminated soil to a given solvent, a question can be formulated as to which of the operationally defined fractions contribute to the "soluble" portion of metal during dissolution, and what are the dynamics between the metal fractions that are being leached. To date there exists a gap in the literature concerning the temporal changes to the PTE distribution in contaminated soils.

The aim of this study is to provide a description of the temporal changes in Cd and Pb concentrations between binding phases of contaminated soil samples after leaching in hydro-static and hydro-dynamic scenarios. Furthermore, we compare the binding fractions of a contaminated organic soil sample after leaching with a soil that is frequently saturated due to flooding. The study also seeks to determine the effects of a lime treatment ($CaCO_3$) on the Cd and Pb binding dynamics in the studied soils. It is intended that results from this study will provide new insight into the behaviour of Cd and Pb in contaminated soils that are subjected to periodic and prolonged saturation.

2. Materials and methods

2.1. Study site and sampling

Soil was collected from the edge of a smelter waste heap in the historic mining district of Kutná Hora, approximately 60 km to the east of Prague in the Czech Republic (Fig. 1). The area is situated on the Kutná Hora Crystalline Unit, represented by micaschist and orthogneiss zones. An extensive description of the geology of KH can be found in Kachlík (1999). Mining operations here followed the path of Ag-enriched quartz veins which formed from metamorphosed igneous material (Pauliš and Mikuš, 1998). Soils around the mining wastes in this area are highly enriched in Cd and Pb (plus other PTE) as evidenced in previous studies (Ash et al., 2014). The presence of metal bearing minerals (e.g., arsenopyrite, chalcopyrite, tetrahedrite, galena and sphalerite) in the material that was mined and smelted, plus fluxes used in the smelting process, resulted in a metal rich slag. Fragments of slag at the studied site were shown to be reactive in water; Cd exhibited increasing dissolution with contact time, Pb showed lesser solubility but was still extractable from the material (Ash et al., 2013). Furthermore, Cd and Pb, which may exist in elemental form or bound with sulphates and carbonates, are readily dissolved in soil solution. Hence, chemical weathering of slag is a likely source of PTE into the studied soils. By means of isotopic composition analysis, Ettler et al. (2004) confirmed that metallurgy and smelter activities play a predominant role in severely contaminating local soils, and that downward migration of Pb in smelter-impacted soils can occur at a rate of approximately 0.3 to 0.36 cm/yr. Although the operation of smelters at our studied vicinity ceased sometime in the 1800s, mining and ore processing continued in the nearby locality of Kaňk (approx. 3 km north) up until 1991 (Vrátný, 1998). In addition to any atmospheric fallout, physical weathering and aeolian deposition of metallic dust from the slag heap are likely to compound the contamination of the studied soils.

One pit was dug on a flat area adjacent to the smelter waste heap (marked A in Fig. 1) where a thin layer of leaf litter and vegetation debris has accumulated and grass is growing sparsely. The pit was dug to sufficient depth so as to expose the mineral layer which underlies the organic horizon. Soils at the edge of the waste heap have developed over the last
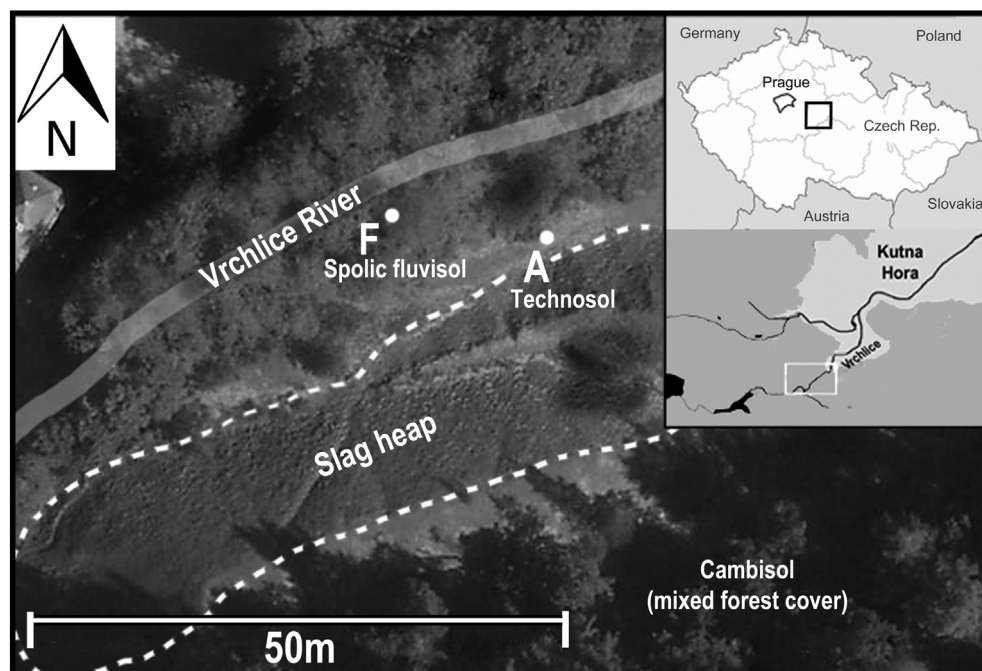

Fig. 1. Map of study area to the south west of Kutná Hora showing soil type, Vrchlice River, and position of sample pits.

few centuries, since the final deposition of slag at this location. Plant material and debris were removed, then the organic (0–6 cm) and mineral (6–20 cm) layers were collected separately. Hereinafter, samples collected from organic and mineral layers are referred to as samples O and B respectively. A second pit was dug in a river bank soil underneath a deciduous canopy (marked F in Fig. 1), soil was collected here from the upper 15 cm. The purpose of collecting this sample was to compare a frequently flooded fluvisol to the experimental samples after leaching. Soils pits can be seen in Fig. 2. Fragments of slag mixed into the lower parts of the pits were manually separated from soils. On return to the laboratory soil samples were air dried then sieved to <2 mm.

2.2. Soil analyses

Soil pH was measured by preparing a 1:5 soil:liquid (w:v) ratio using water (pH_{H_2O}) or KCl solution (pH_{KCl}) then measuring after agitation using a Denver Instrument UB-5 pH meter. Elemental analysis of N, C, and S was made using a Thermo Scientific Flash 2000 NCS Analyser. Total cation exchange capacity (CEC) was determined according to the Bower method as described by Hesse (1998). A four step BCR sequential extraction procedure (SEP) was performed according to a modified method described by Tokalioglu et al. (2003), whereby target metals are extracted from soil samples in the following successive stages: weakly bound metals (exchangeable) are released into 0.11 mol/L acetic acid. Metals bound to iron and manganese oxides (reducible) are extracted using 0.1 mol/L hydroxylammonium chloride. Metals bound to organic matter and sulphides (oxidizable) are liberated by oxidizing the sample in 8.8 mol/L hydrogen peroxide, followed by dissolution into 1.0 mol/L ammonium acetate. Metals bound in residual soil (residual) are dissolved in a heated mixture of concentrated HCl and HNO_3. Analysis of extracts from the sequential extraction was made using Varian Spectra AA280 FS (fast sequence, Mulgrave, Australia) FAAS under standard analytical conditions. Calibration was matrix-matched with standards from Analytika s.r.o. (Prague, Czech Republic). Pseudo-total content of elements was determined by stirring 1.00 g of soil with 7.00 mL of 35% HCl and 2.33 mL of 65% HNO_3, then heating at ~190 °C on a hot plate for 6 h. The suspension was then filtered (Filtrak 390) and diluted before analysing for target elements using an ICP-OES (Thermo Scientific iCAP 6500 Radial ICP, Cambridge, U.K.). All analyses

Fig. 2. Photographs of soil pits: Young soil (~200 yrs old) developed on technosol mining waste with distinct O and B horizons (left). River bank fluvisol underneath a mature deciduous tree canopy (right).

Table 1
Basic soil properties and pseudo-total Cd, Pb content in samples.

	O horizon	B horizon	Fluvisol
pH_{H_2O}	5.46 ± 0.021	4.91 ± 0.007	4.30 ± 0.021
pH_{KCl}	5.35 ± 0.078	4.44 ± 0.078	4.27 ± 0.028
N_{tot} %	2.52 ± 0.001	0.43 ± 0.010	1.85 ± 0.008
C_{tot} %	34.2 ± 0.044	7.59 ± 0.044	25.6 ± 0.038
S_{tot} %	0.40 ± 0.002	0.58 ± 0.038	0.24 ± 0.004
$CEC_{mmol(+)/100\,g}$	90.8 ± 5.66	21.8 ± 0.35	89.9 ± 1.95
$Cd_{mg/kg}$	2.97 ± 0.361	5.83 ± 0.205	0.81 ± 0.113
$Pb_{mg/kg}$	205 ± 19.1	699 ± 50.2	59.7 ± 1.03

were made in triplicate. X-ray diffraction (XRD) was used to determine the semi-quantitative content of minerals in random powder samples using a PANalytical X'Pert Pro diffractometer (Almelo, Netherlands).
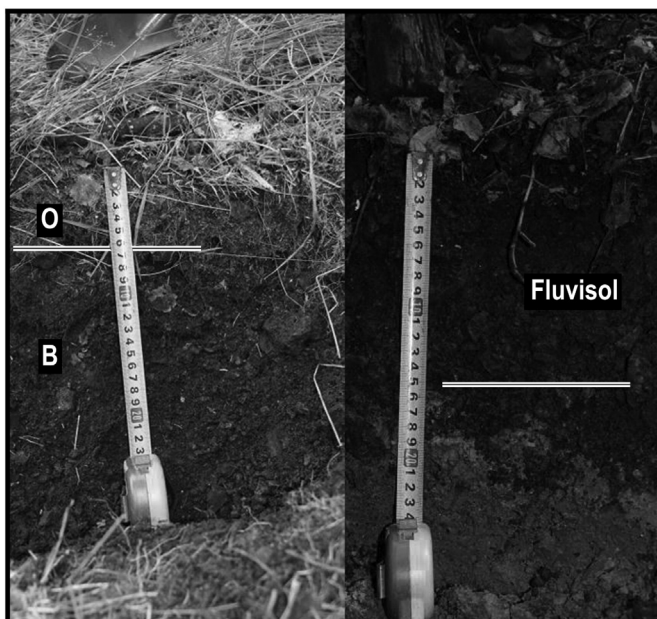
2.3. Leaching experiments

Samples were prepared in a batch leaching experiment to determine the release of Cd and Pb from soil fractions over time in a hydrostatic situation. Samples were prepared in a 1:20 (w:v) soil:deionised water ratio in opaque 250 mL PE bottles. Reaction vessels were then shaken for 2 min and stored in the dark at 21 °C. Analyses were made at intervals of 1 h, 24 h, 168 h and 720 h. Reaction vessels were prepared in triplicate, with a procedural blank included. At the predetermined analysis times, vessels were decanted through Whatmann 390 filter paper into a 50 mL PE tube, the solution was then centrifuged and further filtered through a nylon 0.45 μm syringe filter before analysing for target elements by means of ICP-OES. Residual soil was laid out on filter paper and dried in the oven at ~30 °C to constant weight. 1.00 g of the soil was then used for SEP.

Column experiments were conducted to compare differences in Cd and Pb fraction distribution before and after leaching, analogous to a short-term hydrodynamic situation such as precipitation events. Acid washed glass columns (18 × 3 cm) were used to contain the sample material in a sealed vertical flow-through system. Either side of the sample (8 cm soil layer with a volume of 56.55 cm^3) was a 2 cm layer of fine inert sand to stop movement of soil particles within the column. Small glass marbles were used to fill the space between the sand and column outlet, separated by a layer of Filtrak 390 filter paper. Deionised water was pumped in from the bottom of the column using a peristaltic pump (Gilson, miniplus 3) set to flow at 200 mL/h and leachate was collected hourly for 12 h. Column experiments were performed in duplicate. Collected leachate was immediately tested for pH, then frozen. After further filtering through a 0.45 μm nylon syringe filter, samples were analysed for target elements, by means of ICP spectrometry, and for the determination of water extractable low molecular mass organic acids (LMMOA). Content of anions in aqueous extract was measured using an ion chromatograph ICS 1600 equipped with IonPac AS11-HC.

Table 2
Mineralogy of samples according to X-ray diffraction analysis.

O horizon	B horizon	Fluvisol
Mineral	Mineral	Mineral
Quartz	Muscovite/illite	Quartz
SiO_2	$KAl_2(AlSi_3O_{10})(F,OH)_2$	SiO_2
Muscovite/illite	Quartz	Orthoclase
$KAl_2(AlSi_3O_{10})(F,OH)_2$	SiO_2	$KAlSi_3O_8$
Albite	Magnetite	Muscovite/illite
$NaAlSi_3O_8$	Fe_3O_4	$KAl_2(AlSi_3O_{10})(F,OH)_2$
Magnetite	Hematite	Kaolinite
Fe_3O_4	Fe_2O_3	$Al_2Si_2O_5(OH)_4$
–	Anorthite	Magnetite
	$CaAl_2Si_2O_8$	Fe_3O_4
–	Clinochlore	–
	$(Mg_5Al)(AlSi_3)O_{10}(OH)_8$	

Quality control and assurance of ion chromatography analysis are described in a paper by Tejnecký et al. (2013). As in batch experiments, after leaching, residual soil was laid out on a filter paper and dried at ~30 °C to constant weight. 1.00 g of the soil was then used for SEP.

For comparison in both experiments, an amended sample was prepared by mixing 40 mg/g of powdered CaCO₃ (Lachema s.r.o p.a, f.w 100.09), into the homogenized soil.

2.4. Statistical analysis

Independent t-test and Kruskal–Wallis test were used (statistical software IBM SPSS Statistics 20) to determine the significance of mean changes in fraction Cd and Pb concentrations between experiment times.

3. Results and discussion

3.1. Basic soil properties

Some properties of samples are given in Table 1. All soil samples are acidic, pH of the mineral horizon (B) is lower than that of the overlaying organic horizon (O). A greater number of cation exchange sites in the O horizon and fluvisol sample than in the B horizon reflect the high content of C and N in these samples. Both horizons contain a high amount of Cd and Pb. In the Czech Republic, soils considered non-polluted with PTE and suitable for agricultural production contain no more than 1 mg/kg and 70 mg/kg of Cd and Pb respectively in a 2 M HNO₃

extract (Czech Ministry of Environment, 1994). The fluvisol sample falls marginally within this guideline value.

3.2. Mineralogy of samples

Table 2 lists mineral components that were detectable in the studied samples. Mineral phases in both the organic layer and fluvisol sample were dominated by quartz, with an occurrence of micas and a presence of iron oxides. The B horizon sample was composed mainly of muscovite and quartz, with an occurrence of iron oxides magnetite and hematite.

3.3. Batch leaching experiment

In this research, the effects of prolonged submersion in water of contaminated O and B horizon soils were studied, and compared to a mature spolic fluvisol with respect to the PTE soil fraction distributions (Fig. 3; Cd and Fig. 4; Pb).

Results of the batch experiment showed that prolonged saturation causes an increasing portion of Cd to be retained on the metal oxides (Fig. 3), particularly in the O horizon sample (increased from 38% to 59% of total after 720 h). After 1 h of experiment time, Cd fractionation of experimental soil is characterised by somewhat evenly distributed exchangeable, reducible and residual fractions with little (~1%) or no Cd evident in the oxidizable fraction in B and O horizons respectively. However, after one month in reaction vessels, the distribution of Cd has changed markedly, with an increase in organically bound Cd (~20% increase in O) and a significant loss of residual Cd (total removal
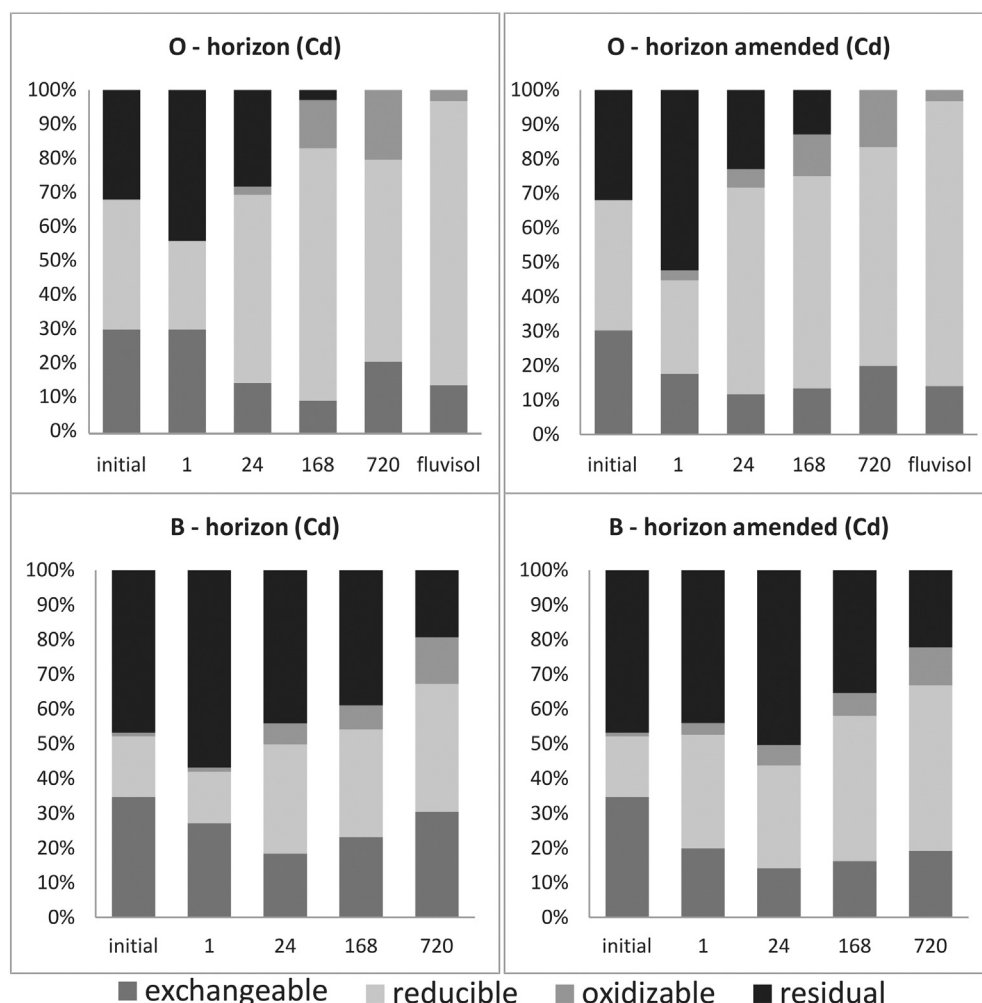

Fig. 3. Cadmium fraction distribution dynamics with batch experiment time and fluvisol sample for comparison.

from O). Furthermore, after 720 h of saturation, the fraction signature of the O horizon soil has approached that of a well established fluvisol soil (Fig. 3). Characteristic features at this point are the dominant metal oxide phase and an increased amount of organically bound Cd. In the B horizon, the most notable change in both amended and untreated soils was the significantly increased share of organically bound Cd (up to 14% and 11% respectively after 720 h) and losses from exchangeable and residual fractions. Our results are in accordance with findings by Vaněk et al. (2008). Cadmium speciation in polluted alluvial profiles is characterised by a significant binding to the exchangeable fraction, a large portion bound to Fe/Mn oxides, and little Cd present in the residual fraction. It is also stated by Vaněk et al. (2008) that numerous studies on mining-affected river systems have shown that sorption and co-precipitation of metals with Fe and Mn oxides are amongst the most important chemical processes of contaminant (Pb, Zn, Cd and Cu) binding in stream sediments and alluvial soils. Sahuquillo et al. (2003) suggest that high leachability of Cd can be explained by the fact that this metal is adsorbed normally in the cation-exchange positions, from which it can be easily removed by divalent ions. However, our results do not support this, as it is evident, particularly in the O horizon, that after 720 h when the majority of leaching had already occurred, the most significant removal of Cd from soil is not from the easily exchangeable positions (~50% decrease), but from residual Cd compounds where we observed complete removal (Fig. 3).

Despite the fact that the B horizon contained much greater concentrations of Cd (and Pb), and has more than four times less cation exchange

capacity (Table 1), it was the O horizon soil which released the larger amount of PTE to the solution (Fig. 5). This may be an effect of the concentrations of extractable LMMOA that were leached from the respective samples, as observed during the column experiment (see Section 3.4).

In the O horizon, a greater portion of Cd is retained in the residual fraction after 168 h in the $CaCO_3$ amended sample compared to the untreated sample (Fig. 3). In the B horizon, samples amended with $CaCO_3$ had generally less Cd sorbed to the exchangeable fraction; possibly the easily exchanged metals (outer-sphere and ternary complexes) are dissolved and quickly precipitated in the solution, thus appearing in the residual step of the SEP. Although the overall pattern of contaminant redistribution is mostly similar to that of non-amended samples, the amount of Cd released into the solution was significantly less (Fig. 5, Table 4). In a comprehensive review of Cd behaviour in soil by Kabata-Pendias (2000), it is stated that in acidic soils the organic matter and sesquioxides may largely control Cd solubility, whereas in alkaline soil, precipitation of Cd compounds is more important. In the O horizon, dissolution of Cd continued with experiment time, from 0.0–0.4, and 0.0–0.15 mg/kg in untreated and amended samples respectively (Fig. 5), despite a significant increase in solution pH to >7.0 in both untreated and amended samples. Cadmium is assumed to be most mobile in acidic soils within the range of pH 4.5–5.5, whereas in alkaline soil Cd is rather immobile. However, as pH increases into the alkaline range, monovalent hydroxy ion species are likely to occur (e.g., $CdOH^+$), which cannot easily occupy the sites on cationic exchange complexes (Kabata-Pendias, 2000). It is also supposed by Laxen (1985) that as
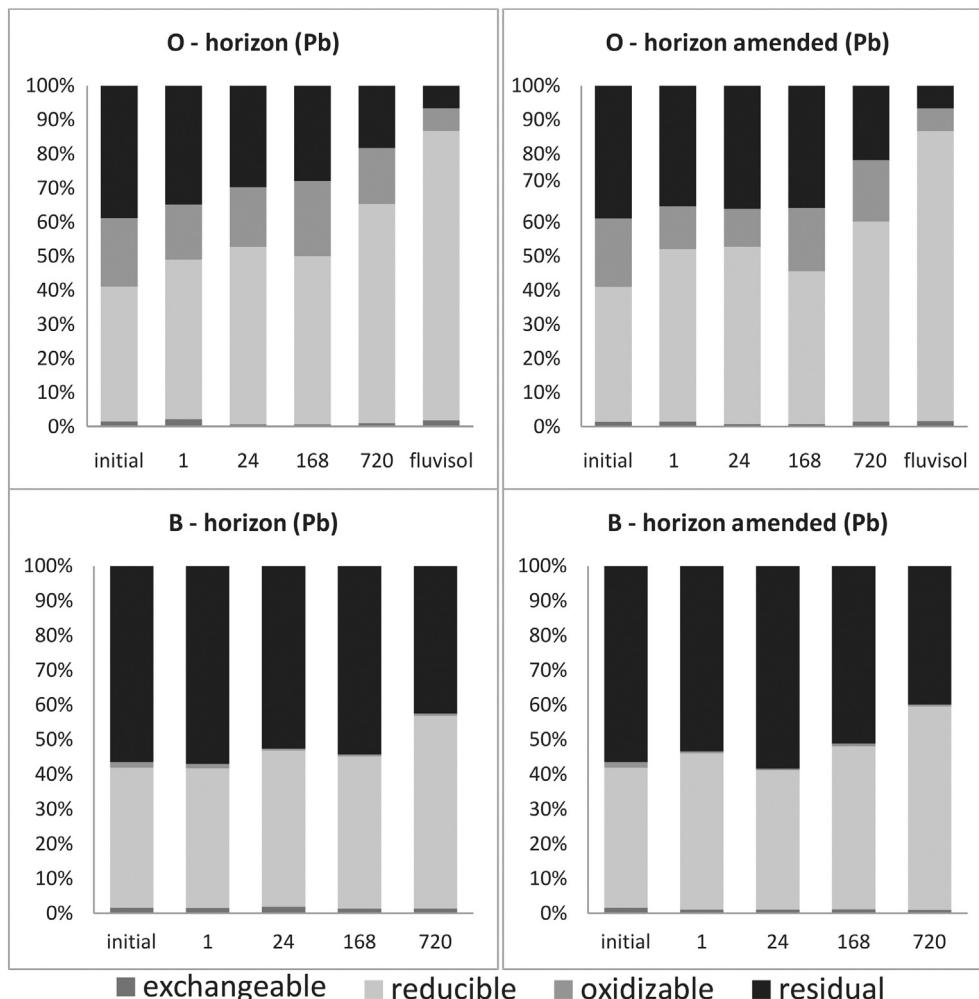

Fig. 4. Lead fraction distribution dynamics with batch experiment time and fluvisol sample for comparison.
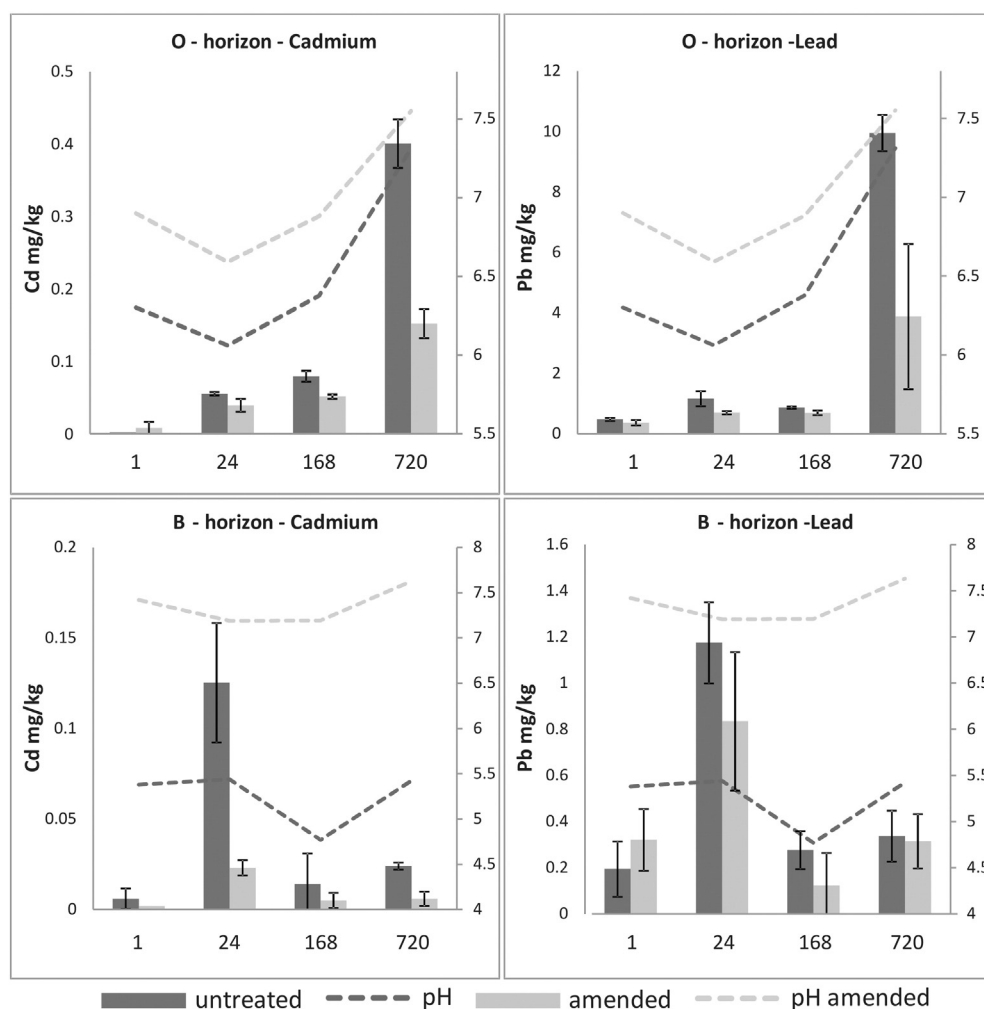
Fig. 5. Changes in extract solution content of cadmium (left) and lead (right) from O and B horizons for amended and non-amended samples in batch experiment (pH on right axis).

alkalinity increases, Cd adsorption decreases, which is likely to be a result of competition from Ca^{2+} and Mg^{2+} ions.

Similar to the observations for Cd, a redistribution of Pb occurred during the batch leaching experiment, developing a distinctly new fractionation pattern after 720 h (Fig. 4). Moreover, the pattern is not very dissimilar to the distribution pattern of the fluvisol soil; in the O horizon, after 720 h the majority of Pb is bound to Fe/Mn oxides, residual Pb has significantly decreased, and the organic constituents of the soil remain an important phase for Pb binding. A slight, but significant (Table 3) increase in Pb occurred only on the Fe/Mn oxides (reducible fraction). Oxides, hydroxides and oxyhydroxides of Al (gibbsite, boehmite), Mn (birnessite, pyrolusite), and Fe (goethite, hematite, ferrihydrite) are not the most abundant phases in soils, but they do typically possess high surface reactivity and have large surface areas, so they are important in the sequestration of metals in soils, and in many cases can be the primary reactive phases with respect to metal sorption (Manceau et al., 2002). A relatively quick reaction which promotes the increase in trace metal bound to metal oxide surface has been described by Ford et al. (2001), whereby an unstable mineral surface is dissolving as metal ions are sorbed, yielding a mixed metal precipitate phase without the requirement for the newly formed precipitate to have a structural link to the substrate. It is suggested by Sauvé and Parker (2005) that, as soils are under a continuous state of weathering and are never truly at equilibrium, this reaction may be common in contaminated soils. Furthermore, inner-sphere adsorption complexes have been directly

observed and established as quantitatively important species in soils contaminated with Pb (Morin et al., 1999), thus high rates of Pb removal seldom occur from the organic matter and metal oxides. Complexation with humus is also said to limit migration of Pb in surface waters (Berkowitz et al., 2008), this may explain in part why there is no significant decrease in oxidizable Pb from the initial sample to 720 h (Fig. 4, Table 3).

As a proportion of the total content in the studied soils, the amount of soluble Pb in leachates was significantly lower than that for Cd (Table 4). This is analogous to the typical behaviour of these metals in soil; Cd is highly soluble when compared to Pb which is commonly reported to be the least mobile amongst the other heavy metals (Kabata-Pendias, 2000).

In O horizon samples, solution pH showed a firm overall increase, >1 pH scale unit increase after 720 h (Fig. 5), even without the addition of amendment. However, despite the addition of $CaCO_3$, a virtually indistinguishable fractionation pattern exists in the untreated samples when compared to amended samples for both O and B horizons (Fig. 4). Ahmad et al. (2012) observed significant differences in the redistribution of Pb in a contaminated soil depending on the addition of calcareous material. Amendment with high alkalinity and solubility dissolved pre-existing Pb in the residual fraction and decreased exchangeable Pb to <1%. The redistribution occurred primarily and significantly in humus associated and Fe/Mn mineral phases (Ahmad et al., 2012). Although our experiment shows a similar trend, the same pattern also

Table 3
Significant changes in Cd and Pb fractions between analysis times according to Kruskal–Wallis test (P-values). A = exchangeable, B = reducible, C = oxidizable, D = residual.

	O-horizon (Cd)				O-horizon amended (Cd)			
	A	B	C	D	A	B	C	D
Initial — 1	0.653	0.046*	1.000	0.037*	0.127	0.513	0.121	0.184
Initial — 24	0.050*	0.037*	0.317	0.184	0.050*	0.050*	0.037*	0.050*
Initial — 168	0.046*	0.050*	0.037*	0.050*	0.050*	0.050*	0.034*	0.050*
Initial — 720	0.050*	0.050*	0.034*	0.037*	0.050*	0.050*	0.037*	0.037*

	B-horizon (Cd)				B-horizon amended (Cd)			
	A	B	C	D	A	B	C	D
Initial — 1	0.034*	0.184	0.197	0.050*	0.037*	0.050*	0.046*	0.376
Initial — 24	0.037*	0.050*	0.043*	0.275	0.037*	0.050*	0.043*	0.513
Initial — 168	0.037*	0.050*	0.046*	0.275	0.034*	0.050*	0.046*	0.050*
Initial — 720	0.487	0.050*	0.046*	0.050*	0.034*	0.050*	0.046*	0.046*

	O-horizon (Pb)				O-horizon amended (Pb)			
	A	B	C	D	A	B	C	D
Initial — 1	0.197	0.046*	0.148	0.275	0.814	0.046*	0.050*	0.376
Initial — 24	0.068	0.043*	0.513	0.050*	0.099	0.046*	0.050*	0.513
Initial — 168	0.099	0.043*	0.658	0.050*	0.114	0.369	0.658	0.376
Initial — 720	0.114	0.046*	0.376	0.050*	1.000	0.046*	0.487	0.050*

	B-horizon (Pb)				B-horizon amended (Pb)			
	A	B	C	D	A	B	C	D
Initial — 1	0.456	0.658	0.114	0.507	0.317	0.376	0.099	0.513
Initial — 24	0.114	0.184	0.068	0.275	0.317	0.513	0.114	0.513
Initial — 168	0.317	0.050*	0.114	0.077	0.317	0.050*	0.114	0.050*
Initial — 720	0.456	0.050*	0.099	0.046*	0.317	0.050*	0.114	0.050*

* Sgnificant change at 95% confidence level, where P = ≤0.05 (lower value = greater level of significance).

occurred in the untreated samples (Fig. 4). In the B horizon there is a considerably more significant difference in the solution pH between amended samples and untreated samples (Fig. 5). It is evident that the amendment was effective in significantly reducing the leaching of both Cd and Pb from soil compared to untreated samples (Fig. 5). As mentioned, the amendment was particularly effective in raising leachate solution pH for the B horizon sample, and consequently a significantly reduced dissolution of Cd occurred (38% and 25% of untreated sample after 720 h of leaching for O and B respectively). In the O horizon soil, the amendment had a lesser effect on raising pH than in B, and the difference in solution pH between amended and untreated samples also lessens as experiment time progresses. In both amended and untreated samples, most of the Cd and Pb were released to the solution at 24 h, after which time the observed content in leachates was much lower. This may be attributable to the release of weakly bound metals into the solution within the first 24 h, after which more stable inner-sphere complexes are forming, resulting in the release of H^+ as indicated by the decrease in solution pH at this time. The effects of liming as a remedial response may also have consequences that should not be overlooked. Increasing pH may facilitate increased leaching of metals

Table 4
Maximum observed PTE content in solution as a percentage of the total content in soil sample.

Sample horizon/type	% Cd	% Pb
O	10.3	5.40
O$_{Amended}$	3.92	2.10
B	1.91	0.15
B$_{Amended}$	0.35	0.11

and losses of organic carbon compounds as solubility is enhanced. Furthermore, raising pH creates a more favourable environment for microbiological organisms, which can increase the rate of mineralization of organic matter.

3.4. Column leaching experiment

Leachates from 12 hour column experiments were collected at hourly intervals then analysed for target elements and LMMOA (Fig. 7). However, despite an observed re-arrangement of the PTE binding fractions within the soil samples before and after leaching (Fig. 6), Cd and Pb occurred below the detection limit in all collected leachates. Without the possibility to detect Cd and Pb in leaching solutions, the alternative was to compare the sum of all fractions before and after leaching (Fig. 6).

The content of water extractable LMMOA was generally higher in O horizon samples, as evident in the column leaching experiment (Fig. 7). Particularly higher concentrations were observed for acetic and oxalic acids; LMMOA that are commonly associated with rhizosphere secretions (Fox and Comerford, 1990; Van Hees et al., 2005). Cadmium goes readily into solution and, although known to occur as Cd^{2+} (depending on pH and oxidation potential), it may also form several complex ions ($CdCl^+$, $CdOH^+$, $CdHCO_3^+$, $CdCl_3^-$, $CdCl_4^{2-}$, $Cd(OH)_3^-$ and $Cd(OH)_4^{2-}$) and organic chelates (Kabata-Pendias, 2000). The chelating effect of organic acids is likely to contribute strongly to the higher rate of release of Cd in the O sample, as observed in the batch experiment (Fig. 5).

Fig. 6 shows that after 12 h of column leaching, the Cd distribution pattern in the O horizon soil was altered from its initial state and reflects the situation for the batch experiment, whereby there is a loss of Cd from easily exchangeable positions (>50% decrease), an overall decrease in residual Cd (from ~30 to ~20%), and a significant redistribution of labile Cd onto the organic matter (from 0 initially to ~15% after leaching). In the case of the B horizon, residual Cd increased slightly and the reducible fraction was a lesser contributor to the retention of Cd. Similarly, the oxidizable portion of Cd increased after leaching (Fig. 6). There is little to distinguish between untreated and amended samples in the O horizon soil. In the B horizon, there is a notable decrease in residual Cd and an increase in reducible Cd in amended samples. However, these differences were not determined to be significant (Table 5).

For both O and B samples the Cd soil content (sum of fractions) after 12 h of leaching is lower than the initial for untreated and amended samples (Fig. 6). The amount of Cd lost is difficult to determine with confidence given the relatively high level of error in the initial sample. Possibly no more than 1 mg/kg of Cd was leached. Furthermore, we cannot conclude that the amendment made any significant difference to the amount of Cd leached after 12 h.

Considerable differences were observed between Pb fraction distribution dynamics after batch leaching (Fig. 4) compared to column leaching (Fig. 6). In O horizon samples, after 12 h of leaching, both residual and oxidizable fractions increased, whereas the reducible fraction decreased (Fig. 6). The same can be seen in B horizon samples, but with less emphasis on the redistribution to the oxidizable fraction (not significantly different). One of the most notable features is that of the increased redistribution of Pb onto the organic matter in the O horizon soil. In nature the organic matter is a likely short-term sink for Pb during rain events. The experiments highlighted a tendency of the metals in the hydrostatic system (Figs. 3, 4) to be redistributed to more labile fractions (significantly increased portions bound to oxidizable and reducible fractions), while in the hydrodynamic system (Fig. 6) the more stable fractions become more relevant (a higher proportion of residual metal, particularly for Pb). In a study by Kumar et al. (2013), a similar leaching column experiment was performed to detect changes in Pb binding on soil and sediment fractions before and after the introduction of artificial road runoff. Although the experiment

was for a much greater duration (first analysis after 10 days), and includes the introduction of metal in the leaching solution, similar patterns to that of our month long batch experiment were described, whereby there is an increase in reducible Pb and a marked decrease in the residual fraction with time for both surface and underlying soils.

The results showed an unexpected increase in total Pb content (sum of fractions) from the initial sample to those after leaching. This may be explained in part by gleaming the historical records for the sources of Pb in the smelter slag (e.g., Pauliš and Mikuš, 1998; Helm-Clark, 2001). To reduce the melting temperature of silver, Pb sulphides, charcoal, vitrol and calcite were often added during smelting, resulting in a mixture of slag and argentiferous metals bearing significant quantities of Pb. Small fragments of lead, which are identified in the residual fraction, are heterogeneously distributed within the studied samples and consequently can increase total Pb contents between sample aliquots.

4. Conclusions

Operationally defined fractions which correspond to weakly bound (exchangeable), bound to Fe/Mn oxides (reducible), bound to organic matter (oxidizable), and residual metals (residual) were monitored in contaminated soil samples throughout a series of leaching experiments.

During one month of saturation (analogous to flooding events), the removal of Cd and Pb from contaminated O and B horizon soil samples occurred primarily from the residual fraction, with a significant redistribution onto the Fe/Mn oxides (identified mainly as magnetite and hematite) and partially onto the organic matter. After 720 h, the distribution signature of Cd and Pb in contaminated O horizon soil closely matched the distribution pattern of that in a well established spolic fluvisol sample. In batch experiments, the addition of a CaCO$_3$ amendment resulted in decreased dissolution, and a lower portion of Cd and Pb on the exchangeable fraction (decreased mobility) with increasing time. Although CaCO$_3$ amendment successfully reduced the amount of Cd and Pb being leached from soil, the fraction distribution pattern was practically indistinguishable from the untreated samples.

In a hydrodynamic situation (analogous to a 12 hour heavy rain event), metal oxides and organic matter were shown to be important phases of Cd retention. Pb was leached mainly from the reducible fraction whereas the oxidizable and residual fractions were of greater importance for Pb retention.

Based on the observations in this study, further research could focus on the climatic and spatial environmental parameters which control PTE soil speciation dynamics with time. Further research may enable the creation of a model for predicting PTE fractions in contaminated soils based on water regime.
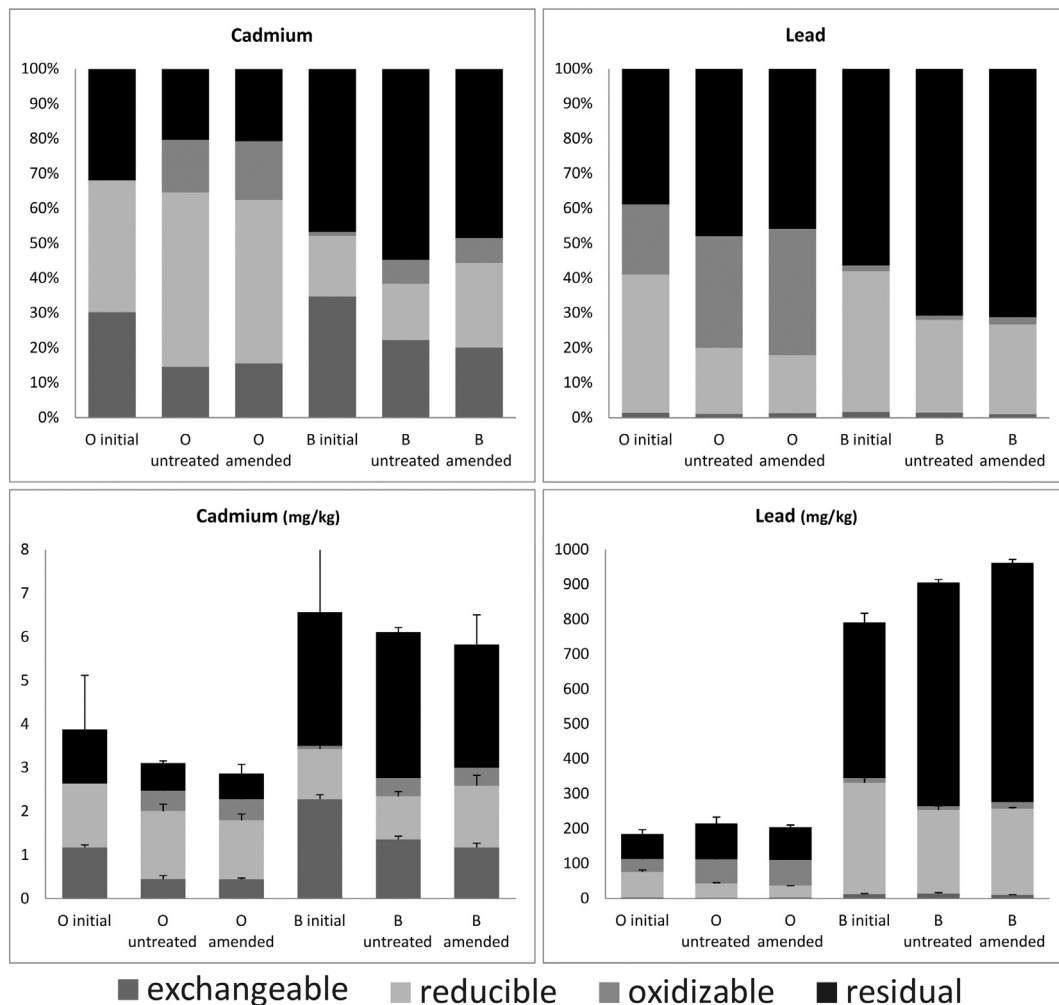

Fig. 6. Cadmium and lead fraction distribution dynamics before and after column leaching for 12 h.
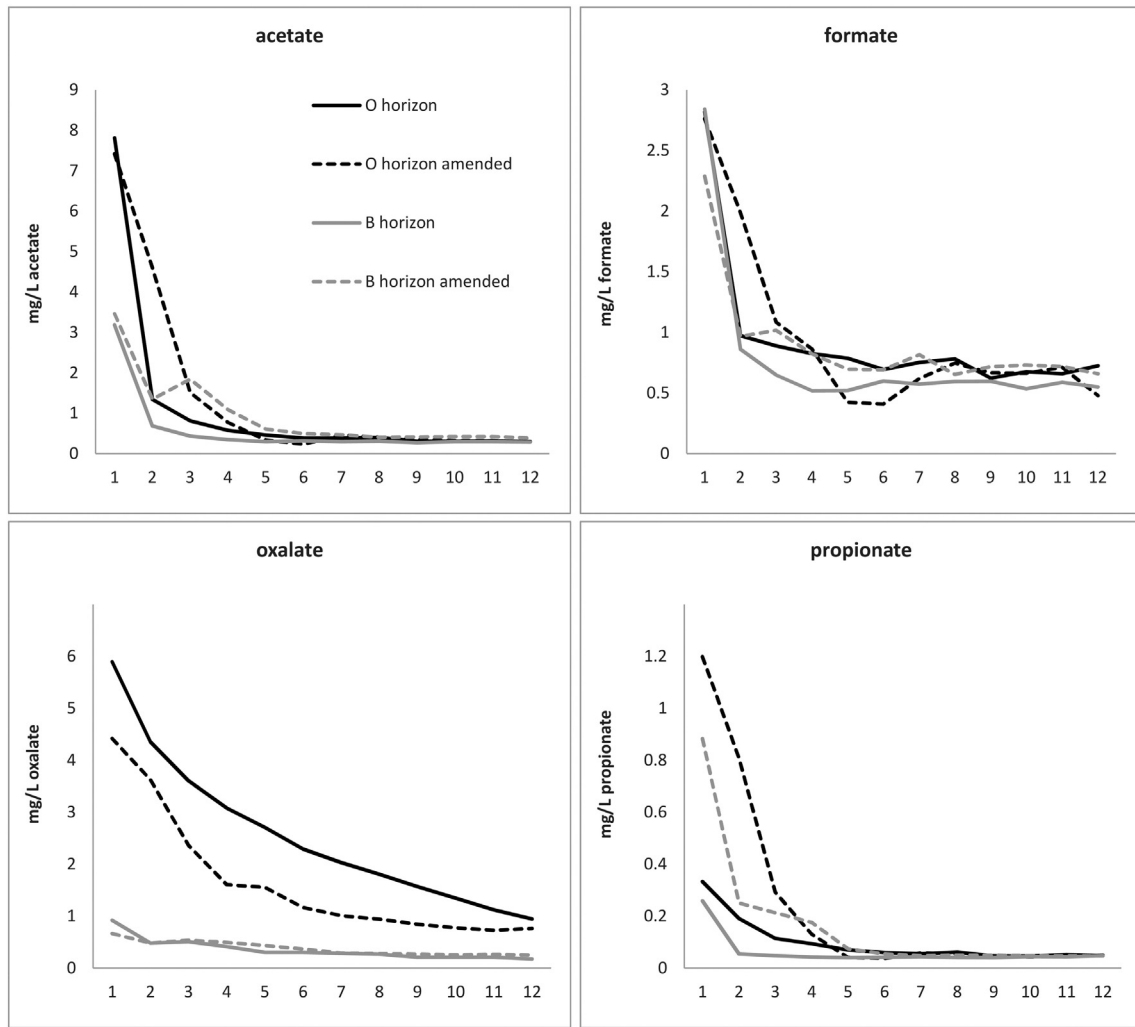
Fig. 7. Detected concentrations of selected low molecular mass organic acids in leachate solutions over 12 h of column leaching.

Acknowledgement

Table 5
Independent t-test for significant mean changes before and after column leaching, and between amended and non-amended samples after column leaching (P-values), A = exchangeable, B = reducible, C = oxidizable, D = residual.

	Cadmium				Lead			
	A	B	C	D	A	B	C	D
Oi — Ou	0.001*	0.616	0.001*	0.011*	0.703	0.007*	0.006*	0.099
Oi — Oa	0.001*	0.050*	<0.001*	0.025*	0.998	0.004*	0.007*	0.111
Ou — Oa	0.925	0.315	0.706	0.832	0.750	0.050*	0.515	0.557
Bi — Bu	0.002*	0.489	0.003*	0.162	0.676	0.009*	0.287	0.002*
Bi — Ba	<0.001*	0.265	0.002*	0.585	0.105	0.001*	0.140	<0.001*
Bu — Ba	0.115	0.113	0.979	0.386	0.114	0.383	0.149	0.016*

* significant change at 95% confidence level, where p = ≤0.05 (lower value = higher significance). i = initial, u = untreated, a = amended.

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Sustainable Soil Washing: Shredded Card Filtration of Potentially Toxic Elements after Leaching from Soil Using Organic Acid Solutions

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Abstract

Shredded card (SC) was assessed for use as a sorbent of potentially toxic elements (PTE) carried from contaminated soil in various leachates (oxalic acid, formic acid, $CaCl_2$, water). We further assessed SC for retention of PTE, using acidified water (pH 3.4). Vertical columns and a peristaltic pump were used to leach PTE from soils (O and A/B horizons) before passing through SC. Sorption onto SC was studied by comparing leachates, and by monitoring total PTE contents on SC before and after leaching. SC buffers against acidic soil conditions that promote metals solubility; considerable increases in solution pH (+4.49) were observed. Greatest differences in solution PTE content after leaching with/without SC occurred for Pb. In oxalic acid, As, Cd, Pb showed a high level of sorption (25, 15, and 58x more of the respective PTE in leachates without SC). In formic acid, Pb sorption was highly efficient (219x more Pb in leachate without SC). In water, only Pb showed high sorption (191x more Pb in leachate without SC). In desorption experiments, release of PTE from SC varied according to the source of PTE (organic/mineral soil), and type of solvent used. Arsenic was the PTE most readily leached in desorption experiments. Low As sorption from water was followed by fast release (70% As released from SC). A high rate of Cd sorption from organic acid solutions was followed by strong retention (~12% Cd desorption). SC also retained Pb after sorption from water, with subsequent losses of $\leq$8.5% of total bound Pb. The proposed use of this material is for the filtration of PTE from extract solution following soil washing. Low-molecular-mass organic acids offer a less destructive, biodegradable alternative to strong inorganic acids for soil washing.

Introduction

Contamination of the environment with potentially toxic elements (PTE) is a persistent threat in the industrialized world. Remedial responses to soils polluted with PTE vary according to many factors, such as the contamination source, and the pathways or environmental media through which PTE must pass before reaching a target [1].

Column leaching experiments are a commonly used tool for the simulation of a given solution percolating through a porous medium [2]. To a certain extent, they allow the user to determine the level of binding and mobility of PTE through applied leaching of the solution of interest. Metals dissolved in the extract solution represent an environmental hazard, and metals left in the soil following leaching are likely to be present in chemically stable mineral forms and bound to non-labile fractions [3].

In soils, conventional remedial techniques are typically based on containment of contaminants, or removal of contaminated soil for treatment on or off site. Certain agents are chosen to enhance the solubilization of inorganic contaminants in soil; following decontamination, the selected agent must be recovered, and possibly treated. In general, bases and complexing agents are rarely applied because of the difficulties of treating the wastewater [4]. Strong mineral acids such as HCl, and HNO_3 can be used to leach PTE from soil, however, these strong acids can compromise soil structure. Alternatively, weaker organic acids may be equally as effective in the removal of PTE, whilst at the same time being easily decomposed by microorganisms following washing, and maintaining desirable soil properties. Many studies have focused on the use of amendments to immobilize and reduce availability of PTE through their incorporation into the soil. Amendments can be mixed into the contaminated soil or into a layer that forms a barrier between the pollution source and the environmental receptor. Houben et al. [5] studied several cost effective amendments for their potential immobilization of Cd, Pb and Zn. Calcium carbonate, iron grit, fly ash, manure, bentonite and bone meal all proved effective at reducing Cd and Zn leaching, mainly due to the increase in pH that was caused by amendment additions. However, only $CaCO_3$ addition resulted in a strong reduction of plant uptake for the considered metals. Recycling of materials with adsorbent properties for the immobilization of toxic metals offers obvious benefits. Consequently, studies have focused on using a range of materials for this purpose, including simple amendments such as sawdust [6],[7],[8] and brewers' draff [9], and more comparatively hi-tech materials such as recycled activated alumina and recycled collagen fiber [10].

A relatively high anionic charge density of mechanical pulp fibers is consistent with the presence of high levels of resin acids, fatty acids, hemicellulose, and certain degradation products of lignin [11]. Furthermore, the addition of calcium carbonate as a binding agent during production gives paper and card products a highly alkaline property (pH > 8.0). Research by Prica et al. [12] focused on the potential for cardboard mill pulp as an immobilization amendment to Cd enriched sediments, making use of standardized leaching tests using weak acetic acid and humic acid solutions. In earlier works, Battaglia et al. [13] and Calace et al. [14] also experimented with paper mill sludge for the treatment of metal polluted soils.

The aim of this study was to analyze different leaching solutions (oxalic acid, formic acid, $CaCl_2$, deionized water) for content of As, Cd, and Pb after passing through columns of contaminated soil with/without a shredded cardboard (SC) filter. We then further assess SC for its subsequent effectiveness at retaining PTE after leaching with acidified deionized water. We propose that SC could be applied as a filter to remove PTE from the extract solution following soil washing.

Methodology

Sample collection and pre-treatment

Soil samples were collected from a forested area near to Příbram in the Czech Republic (49.7°N, 14.0°E) that has been heavily affected by atmospheric deposition of Cd, Pb, and As [15]. A map of the area can be seen in S1 Fig. No specific permissions were required for the access and sampling of the location used, nor did the field study involve endangered or protected species. Two separate samples were collected from a single soil pit. The upper layer (3–11.5 cm), a mixture of F and H organic horizons was labeled as 'soil O'. This represents a strongly contaminated soil rich in organic matter. In the underlying layers, a shallow organo-mineral A horizon (11.5–13.5 cm) and mineral B horizon (13.5–20 cm) were taken as a mixed sample and labeled as 'soil AB'. This sample represents a well-developed mineral soil with some organic constituents, which is also contaminated. On return to the laboratory, samples were air dried (~25°C) to constant weight.

Soil analyses

Soil pH was measured by preparing a 1:5 soil:liquid (w:v) ratio using water (pH_{H_2O}) or 2 M KCl solution (pH_{KCl}) then measuring after agitation using a Denver Instrument UB-5 pH meter. Content of combustible soil organic matter (Corg) was measured by the loss on ignition method. Quality of humic substances was calculated from spectrophotometric analysis, taken as the ratio of a pyrophosphate soil extract absorbance at wavelengths 400 and 600 nm ($_{A400/A600}$) [16]. Cation exchange capacity (CEC) was determined according to the Bower method as described by Hesse [17], using Varian Spectra AA280 FS (Mulgrave, Australia). Elemental analysis of N, C, S was made using a Thermo Scientific Flash 2000 NCS Analyzer. All analyses were made in triplicates.

Total contents of PTE in 1g of soil and SC samples was determined by digestion in a mixture of concentrated acids (HF, HNO_3), with heating on a hot plate for 16 hrs at 190°C. After evaporation, 1 ml of 65% HNO_3 and 49 ml of deionized water (DI_{H_2O}) were added to the residue before filtering (Whatman 390). Extracts were analyzed by inductively coupled plasma-optical emission spectrometer (ICP-OES, iCAP 7000 Duo ICP; Thermo Scientific) under standard conditions. Blanks, and standard reference material "Montana II" and "San Joaquin" by NIST, which represent polluted and baseline soils respectively, were used to control analytical performance.

Leaching experiment

Adsorption. This study focuses on the possible use of shredded cardboard (SC) as a sorbent material to capture PTE following soil washing. Cardboard used was salvaged generic brown packaging card that was free of plastic tape, ink or print. Cardboard was ground in a mill to obtain a light porous shred. Some physicochemical properties of the SC are given in Table 1.

For use as the mobilizing solvent, this study focuses on the following low-molecular-mass organic acids (LMMOA): 1 mM formic acid (pKa 3.75) and 1 mM oxalic acid (pKa_1 1.25, pKa_2 3.81). LMMOA were produced by Lach-Ner, s.r.o. Czech Republic. An unbuffered salt solution of 0.1 M $CaCl_2$, a solvent commonly used to represent availability and mobility of metals in polluted soils evaluation [18], and DI_{H_2O} (not acidified), which represents a control solution, were also included in the leaching study.

Soil samples were ground and sieved (< 2 mm) to obtain homogeneous material. Vertical glass columns (18 x 3 cm) were used to contain soil. Either side of the sample (layer equal to 5

Table 1. Physicochemical properties and total PTE content of soil and SC samples.

	pH		Organic matter		CEC	N	C	Total content mg/kg		
	pH_{H2O}	pH_{KCl}	Corg (humus)	$Q_{A400/A600}$	cmol/kg	%		As	Cd	Pb
Soil O	4.17±0.01	3.66±0.04	77.0%(nd)	8.10	95±13.3	1.70±0.02	28.1±0.05	266±16.6	14.4±0.05	24962±1401
Soil AB	4.27±0.01	3.61±<0.01	22.4%(5.80%)	7.26	46.5±11.7	0.31±0.01	4.60±0.10	327±20.0	5.86±0.24	5046±255
SC	8.27±0.03	8.03±0.01	-	-	16.3±0.40	0.18±<0.01	38.6±0.05	0.42±0.36	0.14±0.02	7.06±5.40

Corg = combustible organic matter, $Q_{4/6}$ = ratio of a pyrophosphate soil extract absorbance at wavelengths 400 and 600 nm (lower $Q_{4/6}$ value corresponds to a greater degree of humification). CEC = cation exchange capacity. nd = no data. Analyses were performed in triplicates

doi:10.1371/journal.pone.0149882.t001

g of soil) was a filter disk. A layer of small glass marbles was used to separate soil sample from SC, which was positioned in the column ~2 cm above the soil sample. SC was also added on a weight basis (5 g). An illustration of the experimental layout can be viewed (S2 Fig). Experimental solutions were pumped vertically upward through the column; solution mobility was provided by a peristaltic pump (Kouřil PCD 83.4S) set to flow at 100 mL/hr, giving an hourly solution:soil (LS) ratio of 20:1 (v:w). Leaching experiments ran continuously for 24 hrs. After 24 hrs, collected solutions were filtered through nylon 0.45 mm syringe filters, and then target elements were measured using ICP-OES. Content of anions were also determined using an ion chromatograph ICS 1600 equipped with IonPac AS11-HC. Tejnecký et al. [19] describe quality control and assurance of IC analysis. After the experiment, samples of soil and SC were air dried to constant weight. A part was then analyzed for PTE contents by total digestion. For comparison, a blank experiment was performed for each leaching (as described but without SC sorbent).

The following equation was applied to determine:

Sorption ratio between PTE on SC and PTE in solution after leaching =

$$\frac{Aa - Ab}{sol1 - sol0} \tag{1}$$

Where **Aa** is PTE content in SC after leaching, **Ab** is PTE content in SC before leaching, **sol^1** is PTE content in solution after leaching, **sol^0** is PTE content in a blank solution.

Desorption. After drying, a part of SC (1g) was analyzed for total PTE content, and 3 g was prepared in a second column experiment. The arrangement was similar to the first column leaching experiments except that the soil sample was absent. The purpose of the second leaching was to test the performance of SC at retaining PTE after leaching with an acidified DI_{H2O} solution (adjusted to pH 3.4 using HNO_3). Experimental solvent was introduced by a peristaltic pump set to flow at 100 mL/hr for 5 continuous hours. Leachate was collected every 30 minutes, and then later analyzed for PTE content.

Results and Discussion

Properties of solid samples

Soil samples O and AB were both acidic, with little difference in pH between these two layers (Table 1). The amount of combustible organic matter was considerably higher in soil O (77.0%) than in soil AB (22.4%). NCS analysis estimates the percentage of C in soil O as 28.1%, and 4.6% in soil AB. Content of N was also higher in soil O, a probable reflection of the amine groups associated with humic and fulvic acids. Both soils contained relatively poorly polymerized organic substances. CEC of soil O was considerably higher than that of soil AB.

Acid digestion of soil showed that heavy metals Cd and Pb were most enriched in soil O, whereas As was more concentrated in soil AB (Table 1). The observed soil PTE concentrations were well in excess of typical guideline values for these elements. The content of PTE in SC was measured in order to obtain values for Ab Eq 1; traces of As, Cd and Pb were observed on the shredded card. These values are likely to vary according to the source of cardboard, but are negligible compared to the concentrations in contaminated soil.

## Leaching experiments (adsorption)

pH dynamics.   The alkaline nature of SC (pH >8.0), as well as its light open structure, and ion exchange surfaces are reasons for its application in this study as a potential sorbent of metal ions. pH measurements before and after leaching (Fig 1) showed that significant changes occurred to solution pH when SC was present in the column. Largest pH shifts occurred in oxalic acid (+4.49 and +4.15 pH units for AB and O samples respectively). Highest solution pH values after leaching were observed for AB samples. This is a probable reflection of the initially lower pH of soil O, and a higher content of reactive humus. There was a greater water extractable content of organic acids from soil O than for soil AB (Table 2), which can buffer solution against further pH increases.

Anion content in leachates.   In some cases, there was an increase in LMMOA (oxalate, lactate, and acetate) and NO_3^- and SO_4^- concentration in leachate for samples with SC present (Table 2). A simple extraction was made on SC to determine the content of water extractable anions (1 g SC: 100 mL DI_{H2O}, with 4 hrs shaking). Results showed that SC is a source of trace amounts of the following anions: oxalate (130 mg/kg), lactate (540 mg/kg), acetate (592 mg/kg), NO_3^- (140 mg/kg), SO_4^{2-} (427 mg/kg). Consequently, under certain leaching conditions, small increases in the aforementioned anions could be expected along with the studied PTE.

Comparison of PTE content in solution after leaching with and without SC.   Fig 2 illustrates the data for measured PTE concentrations in solution after leaching with and without SC present in the column. Ratios of PTE content in solution without SC to those with SC are also shown in Table 3.

Arsenic: Comparison of As content in solution for columns with/without SC indicates a high level of As sorption by SC from some of the studied solutions (Fig 2). The sorbent is particularly effective at immobilizing As from solutions of oxalic acid and $CaCl_2$. Far less As sorption was observed when formic acid and DI_{H2O} were applied. Table 3 confirms the difference between As content in solution after leaching with/without SC between oxalic acid and formic acid. Formic acid is a simple mono-carboxylic acid of approximately half the molecular mass of oxalic acid (di-carboxylic), which may contribute to lesser interception of As-formate complexes. Regarding differences between As sorption in O or AB samples, it is evident from columns without SC that, with the exception of DI_{H2O}, experimental solvents released a greater amount of As from soil AB than from soil O. Moreover, the sorption efficiency was greater for As released from soil AB than for soil O. Studies have shown that As is highly reactive with natural organic matter, and that soil organic matter and dissolved organic carbon (DOC) content correlate strongly with As sorption [20]. This has led to conflicting theories that organic matter can either immobilize As due to strong retention at the stationary solid phase, or can in fact enhance As transport via soluble DOC-As complexes. Our results suggest the latter. A greater source of fulvic, humic, and simple organic acids is likely to aid As transport from soil O. The higher portion of As interception by SC for AB samples may be partly pH dependent. The difference in solution pH before and after leaching was higher in every case for AB samples; pH of DI_{H2O} reached 8.28 after leaching with SC present. Arsenic forms a variety of inorganic and organic compounds in soils, but most of them are in the inorganic forms of As(III) and As(V)
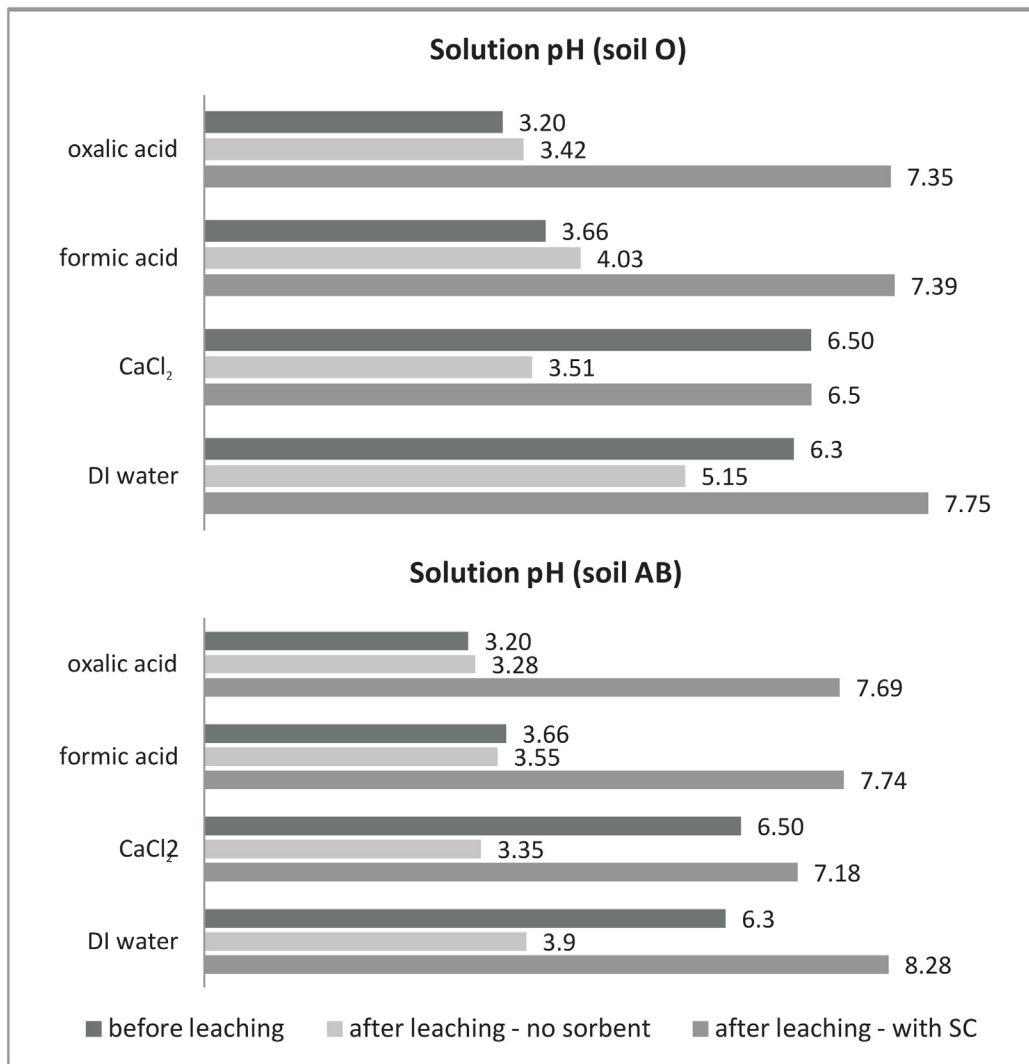
Fig 1. Change in solution pH before and after first leaching when shredded card sorbent was either present or absent in the leaching column.

doi:10.1371/journal.pone.0149882.g001

[21], and although complex anions (AsO_2^-, AsO_4^{3-}, $HAsO_4^{2-}$, and $H_2AsO_3^-$) are the most common mobile forms of As, sorption occurs in the pH range from 7–9 [22].

The order of As sorption (Table 4) was the same for both soil samples ($CaCl_2$ > oxalic > formic > DI_{H2O}). As previously noted, sorption of As by SC is much greater in oxalic acid than for formic acid. Arsenic was most strongly sorbed from $CaCl_2$, with a SC to solution ratio of >14:1 in the case of soil AB. This also reflects the direct observations, whereby experimental solution without SC contained up to 28x more As. In contrast, for metals Cd and Pb, $CaCl_2$ was the most effective solvent with very little of these elements bound by the SC. The reason may be attributable to the release mechanism of heavy metals by $CaCl_2$, which is the exchange between Ca^{2+} and divalent cations, whereas As exists primarily as complex anions. Arsenic may react with $CaCl_2$ to form calcium arsenate, a soluble polar compound that could bind to SC via the positive portion. In DI_{H2O}, a considerably smaller amount of As was sorbed onto SC; very low ratios were obtained for both soil samples. Weak sorption of As from DI_{H2O} could be due to the low ionic strength of DI_{H2O}.

Table 2. Content of main anions in leachate solution (mg/L) after leaching.

| Leaching experiment	formate	oxalate	lactate	acetate	NO_3^-	SO_4^{2-}	PO_4^{3-}
Soil O				mg/L			
oxalic—no sorbent	0.099	87.08	0.246	0.154	0.179	1.235	2.855
oxalic—with SC	0.080	1.499	0.613	0.418	0.241	1.833	b.d.l
formic—no sorbent	43.22	0.708	0.160	0.185	0.324	0.549	0.663
formic—with SC	41.64	1.015	0.570	0.660	0.065	1.170	b.d.l
$CaCl_2$—no sorbent	b.d.l	0.146	b.d.l	b.d.l	0.920	0.619	0.284
$CaCl_2$—with SC	b.d.l	0.375	b.d.l	b.d.l	0.420	1.105	b.d.l
DI_{H2O} - no sorbent	b.d.l	0.238	0.109	0.034	0.290	0.677	0.613
DI_{H2O} - with SC	0.045	0.931	0.140	0.057	0.052	1.151	0.157
Soil AB							
oxalic—no sorbent	0.109	84.74	0.126	0.085	2.962	0.942	2.625
oxalic—with SC	b.d.l	0.526	0.245	0.050	4.895	1.713	b.d.l
formic—no sorbent	43.65	0.415	0.206	0.126	3.719	0.381	0.619
formic—with SC	41.53	0.242	b.d.l	b.d.l	5.610	0.939	b.d.l
$CaCl_2$—no sorbent	b.d.l	0.064	b.d.l	b.d.l	10.63	0.517	0.183
$CaCl_2$—with SC	b.d.l	0.255	b.d.l	b.d.l	5.014	1.092	b.d.l
DI_{H2O} - no sorbent	b.d.l	0.075	b.d.l	b.d.l	15.86	0.278	0.411
DI_{H2O} - with SC	b.d.l	0.028	b.d.l	b.d.l	6.659	1.129	b.d.l

b.d.l = below determination limit

Cadmium: A considerable amount of Cd was retained on SC after leaching for experiments involving oxalic and formic acids; $\leq$ 3.18 and 2.98 mg/kg Cd respectively (Table 4). This is not just a reflection of the amount of Cd that was mobilized by the organic acids, because $CaCl_2$ leached a comparable amount of Cd (Fig 2). SC sorbent can be considered particularly effective at immobilizing Cd from the studied LMMOA as shown by the ratio between Cd concentration in solutions with and without SC present in the column (Table 3). The greatest difference was observed for oxalic acid with soil AB, whereby 15.9x more Cd was detected in leachate from the column without SC. Similar concentrations of Cd were released into oxalic acid from soils O and AB (5.82 and 5.35 mg/kg respectively), but greater interception of Cd by SC occurred for soil AB. Of the studied PTE, sorption from $CaCl_2$ was the lowest for Cd. Lodenius [23] studied the leaching of Cd using salt solution (pH 7.0); strong leaching was achieved by neutral solution containing Ca^{2+}. It is also stated by Kabata-Pendias [22], that in some cases, Cd adsorption can decrease in the alkaline range due to the competition from Ca^{2+} ions.

Cd Sorption ratios (Table 4) reflect the direct observations for the most part. In soil O, the order of SC sorption was repeated; oxalic > formic > DI_{H2O} > $CaCl_2$. In AB sample there is some ambiguity. The largest difference between leachate Cd concentration for experiments with/without SC was observed for oxalic acid. However, the SC sorption ratio suggests that sorption of Cd was slightly higher in formic acid. Nevertheless, we can state that SC was effective at immobilizing Cd in both oxalic and formic acid solutions. Similar to the direct observations, sorption of Cd onto SC was very weak when $CaCl_2$ was used (SC:leachate ratios of 0.03:1 and 0.01:1 for O and AB samples respectively). A small proportion of Cd was bound to SC in the DI_{H2O} experiment (ratio < 1:1). In addition to the low ionic strength of DI_{H2O}, poor sorption could result from generally low concentrations of Cd in solution.

Lead: Total Pb soil content averaged 24,962 and 5,046 mg/kg in O and AB samples respectively. The extremely high loading with Pb means that its distribution is likely to be heterogeneous. This
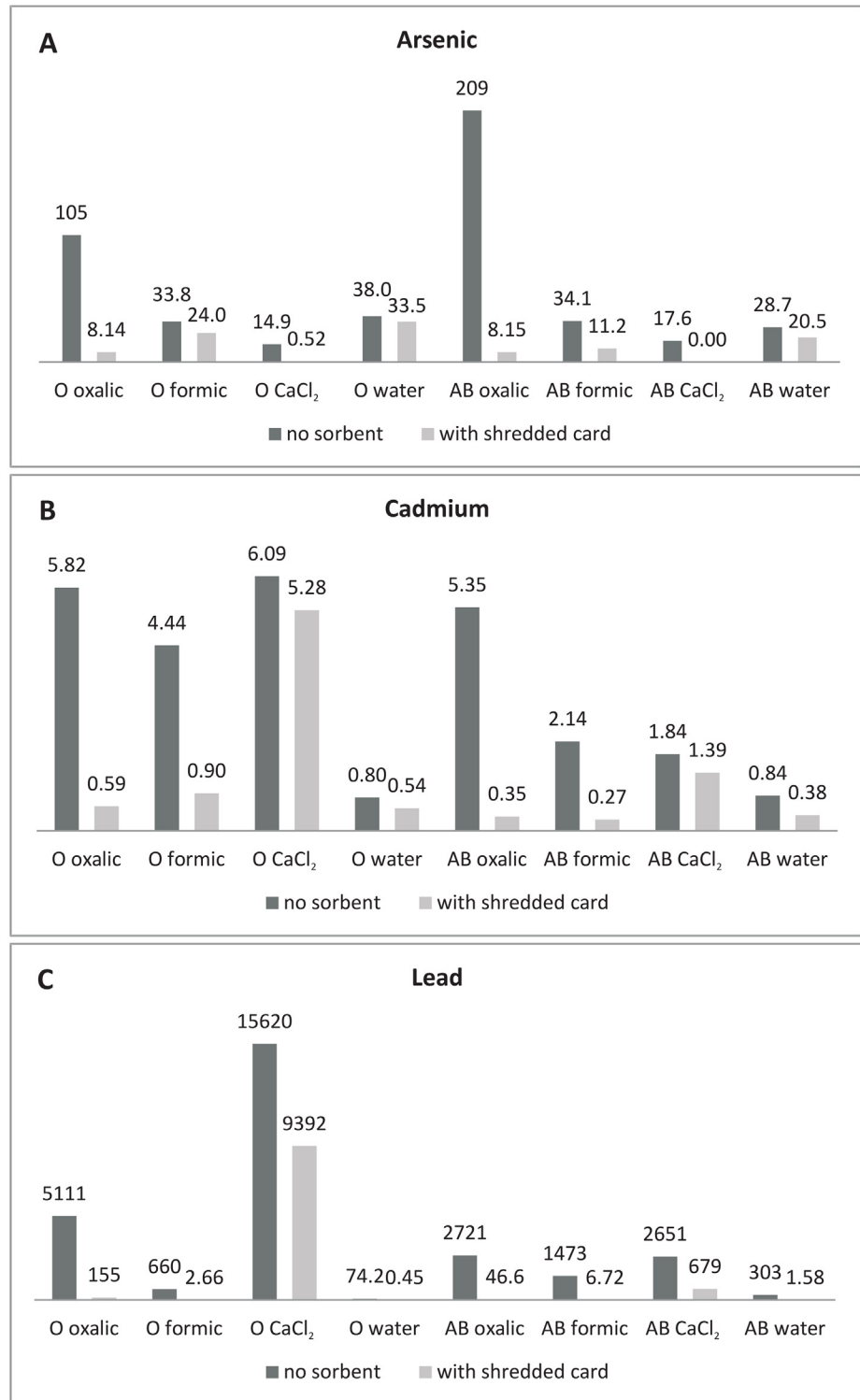
Fig 2. Difference in solution concentrations of As (A), Cd (B), Pb (C) after 24hrs of leaching (mg/kg) when shredded card sorbent was either present or absent in the leaching column.

Table 3. PTE content in solution without shredded card filter as a ratio of the PTE content in solution with shredded card filter.

	As	Cd	Pb
soil O		PTE content ratio	
oxalic with SC < oxalic—no sorbent	12.9	9.86	33.0
formic with SC < formic—no sorbent	1.41	4.93	248.1
CaCl$_2$ with SC < CaCl$_2$—no sorbent	28.7	1.15	1.66
DI$_{H2O}$ with SC < DI$_{H2O}$ - no sorbent	1.13	1.48	164.9
soil AB			
oxalic with SC < oxalic—no sorbent	25.6	15.3	58.4
formic with SC < formic—no sorbent	3.05	7.93	219.2
CaCl$_2$ with SC < CaCl$_2$—no sorbent	*b.d.l	1.32	3.90
DI$_{H2O}$ with SC < DI$_{H2O}$ - no sorbent	1.39	2.21	191.8

*b.d.l = below determination limit for the sample with added SC sorbent

doi:10.1371/journal.pone.0149882.t003

was evident even at the sample level. Consequently, it is difficult to draw conclusions based on differences in soil concentrations before and after leaching. However, despite the deviations in total Pb soil concentrations, there is a clear difference in solution Pb content after leaching when SC sorbent was present (Fig 2). Compared to As and Cd, the difference in Pb solution concentration with and without SC was much higher, except for in CaCl$_2$ (Table 3). Although the amount of Pb released from O and AB samples into formic acid was far lower than that of oxalic acid, the proportional difference of Pb in solution between samples with or without SC was much greater (SC was more effective at immobilizing Pb in formic acid). Lead reacts with oxalic acid to produce lead oxalate, which is insoluble, however, with excess oxalate ions the soluble complex ion Pb(C$_2$O$_4$)$_2^{2-}$ is formed. The anionic nature of Pb(C$_2$O$_4$)$_2^{2-}$ means that it is unable to form electrostatic bonds with the negatively charged surface of SC, thus as experiment time progresses there is less interception of Pb-oxalate by SC. This may go some way to explaining why the sorption of Pb was less for oxalic acid. The result may also be a reflection of the dissociation of these acids (oxalic acid = 1.25$_{pKa1}$, 3.81$_{pKa2}$, formic acid = 3.75$_{pKa}$). Moreover, formic acid is mono-carboxylic and two formate ions are needed to form a neutral complex with Pb^{2+}, which may explain the increased sorption onto negatively charged SC. Perelomov et al. [24] suggest that such binding is attributable to the formation of heavy metal-organic acid anion complexes and the simultaneous sorption of acids at positively charged sites with the formation of mineral–organic compounds. Table 2 shows that in samples with SC, almost no oxalate is present in leachates, whereas in the case of formate the amount is almost equal to that of experiments without SC. This prompts the suggestion that formate is acting as a Pb carrier and donor to the SC as it passes, rather than forming a stable organo-Pb complex. We also observed a substantial decrease of Pb in the DI$_{H2O}$ leachate of column experiments with SC present; 164 and 191x more Pb was in DI$_{H2O}$ leachate without SC for O and AB samples respectively. Results were comparable to those of Battaglia et al. [25] who studied Cd and Pb sorption in a pH independent batch experiment using paper mill sludge admixtures with soil sample. After 15 days of wetting, the amount of Pb retained by soil-paper mill sludge had doubled compared to a control. Cadmium on the other hand was not overly influenced by the addition of paper mill sludge. Of all the studied solvents, the highest pH after leaching with and without SC was observed for DI$_{H2O}$. He et al. [26] also observed considerable increases in Pb^{2+} sorption in a paper sludge amended soil, which correlated with the rise of pH during the sorption process. This has quite significant implications.

Table 4. As, Cd, Pb concentrations in SC after leaching (top), and ratio between PTE on SC and PTE in solution after leaching (bottom).

	As		Cd		Pb	
	O	**AB**	**O**	**AB**	**O**	**AB**
	PTE content in SC after leaching					
	**Aa–Ab (mg/kg)**					
Oxalic acid	58.1	43.7	3.18	1.32	2459	647
Formic acid	7.35	3.69	2.98	1.11	456	700
CaCl$_2$	6.17	14.4	0.32	0.16	1415	484
DI$_{H2O}$	0.80	1.07	0.25	0.31	25.2	14.3
	Ratio between PTE on SC and PTE in solution after leaching					
	(higher ratio = greater sorption)					
	As		Cd		Pb	
	O	AB	O	AB	O	AB
Oxalic acid	7.09	5.31	5.15	3.37	15.8	13.7
Formic acid	0.29	0.29	3.16	3.59	169	103
CaCl$_2$	11.1	14.0	0.03	0.01	0.15	0.70
DI$_{H2O}$	0.01	0.03	0.20	0.45	40.3	4.58

Aa is PTE content in SC after leaching (mg/kg), **Ab** is PTE content in SC before leaching (mg/kg) Eq 1

doi:10.1371/journal.pone.0149882.t004

Studies have shown increasing fiber surface charge with increasing pH, consistent with the expected dissociation of carboxylic groups [10]; hence, an increased sorption of cations.

Lead sorption ratios (Table 4) confirm the high interception rate of Pb in formic acid, a high removal of Pb also in DI$_{H2O}$, followed by oxalic acid, and poor sorption of Pb from CaCl$_2$. There are some discrepancies concerning the order of Pb sorption in the studied solutions for AB sample. The SC sorption ratio suggests that in AB sample, greater Pb sorption efficiency occurred in oxalic acid than in DI$_{H2O}$.

Leaching experiments (desorption)

The ability of SC to retain the studied PTE varied widely, as indicated in Fig 3. Solution (leachate) concentrations were converted from mg/L to mg/kg based on hourly extraction ratios. PTE solution concentrations (mg/kg) at collection intervals were then taken as a proportion of total PTE content on SC prior to desorption.

Arsenic: Arsenic was the PTE most readily released from SC for all sorption pathways except for CaCl$_2$, whereby Cd desorption was slightly higher. Significant differences in the extent of release were observed according to the type of initial sorption and the soil layer from which As originated. Significant differences between amounts of PTE released from SC, when initial source of the PTE was from either O or AB soil samples, were validated by t-test (Table 5). Arsenic desorption from SC was between 10–20% for samples with As initially released from soil sample O, and sorbed from oxalic and formic acid (Fig 3A and 3B). However, for AB samples, desorption was > 50% for samples initially sorbed from formic acid. This confirms the stronger binding of As to SC from oxalic acid compared to formic acid, as identified during the adsorption experiment (Tables 3 and 4). Because the sorption efficiency was high for CaCl$_2$, it is therefore also expected that desorption would be relatively low. This is certainly the case for AB sample (~2%), but for soil O, a greater release of As occurred (~18%). The highest release of all PTE was observed for As with initial sorption from DI$_{H2O}$ (>70% desorption). This also reflects the low level of initial sorption observed in adsorption
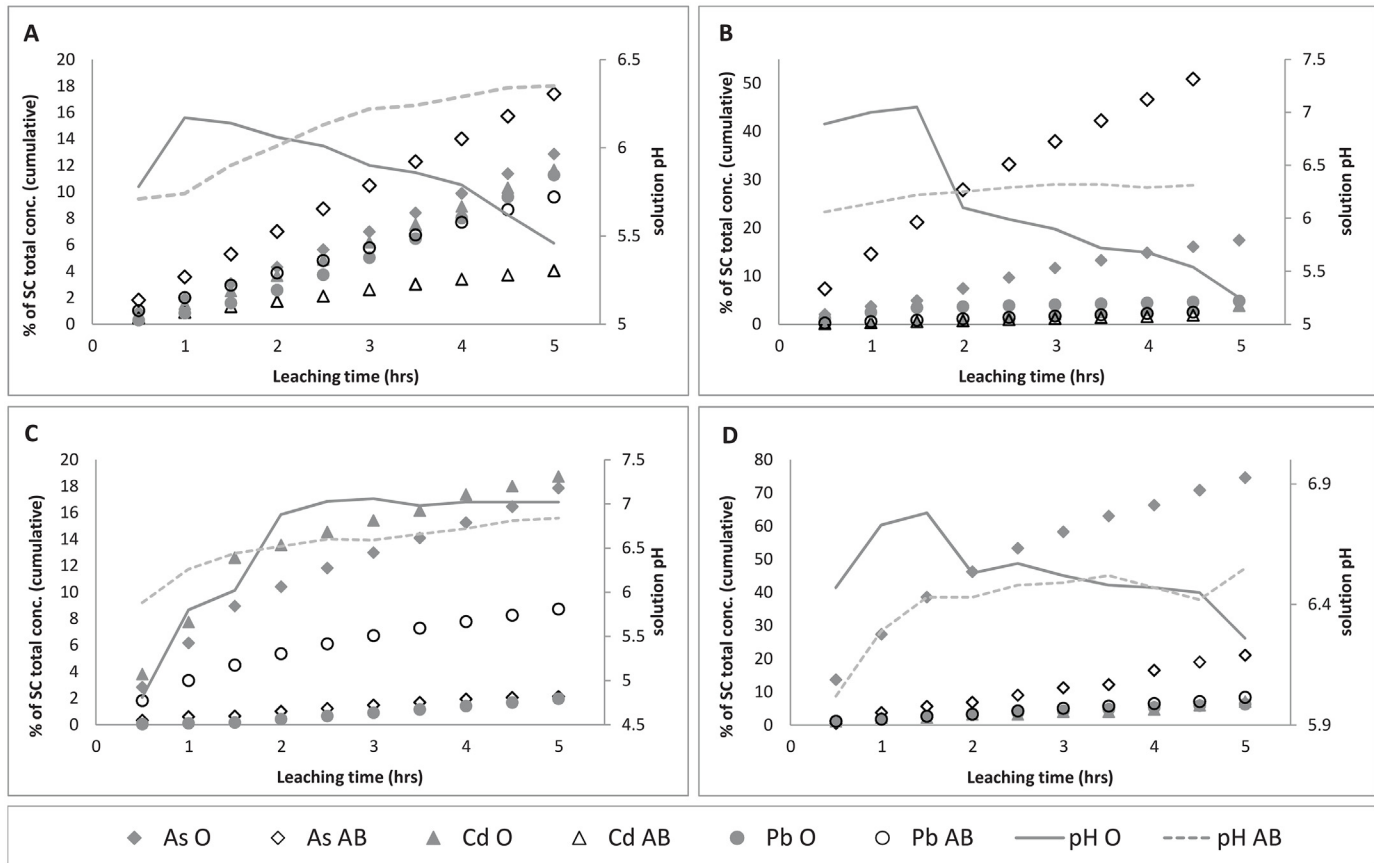
Fig 3. Desorption of As, Cd, Pb from SC after leaching with acidified (pH 3.4) deionized water. Desorption trends from SC, where initial sorption of studied PTE onto SC was in solutions of oxalic acid (A), formic acid (B), CaCl₂ (C), deionized water (D).

experiments. It leads to the inference that SC may not be effective at retaining As if left exposed to the elements and not immediately disposed of.

Cadmium: For SC samples with initial sorption by oxalic acid, Cd desorption was low (soil AB) to moderate (soil O), with ~4% and ~11% Cd released respectively. Furthermore, Cd release for soil O was linear, whereas for AB samples Cd release decreased after approximately 3 hrs. For SC samples with initial Cd sorption in formic acid, greater Cd desorption was again from O samples, but only marginally. After 5 hrs of leaching, Cd release for both O and AB

Table 5. Significant differences (independent t-test) between PTE released from SC after sorption from sample O or sample AB in different leachates.

	As		Cd		Pb	
	t	p	t	p	t	p
Oxalic acid	6.979	<0.001*	9.296	<0.001*	1.123	0.276
Formic acid	8.549	<0.001*	4.635	<0.001*	1.377	0.185
CaCl₂	5.822	<0.001*	-	-	4.383	<0.001*
DI$_{H2O}$	4.068	<0.001*	-	-	2.110	0.049

* Significant at 95% confidence level

samples was relatively small, 3.8 and 2.0% respectively. Again, we suggest that because sorption onto SC was generally higher for Cd from soil AB, the subsequent rate of release was lower (Fig 3A and 3B). Solution pH seems to have little influence on the release of Cd in samples with initial sorption from formic acid. The released Cd remained low for both samples. For SC with initial sorption from $CaCl_2$, Cd was the element most readily released, and again reflects the very low initial sorption. For soil AB, the concentrations were below detection limit. For SC with initial sorption from DI_{H2O}, desorption of Cd remained low for the duration of the experiment (<5% released), and for soil AB, Cd was below detection level.

Lead: Pb featured among the least leached elements in the majority of desorption experiments, which is promising for the potential application of SC as a post soil washing filter. For SC samples with initial Pb sorption from oxalic acid, a modest release of Pb occurred, up to ~6% and 11% after 5 hrs of leaching for soils AB and O respectively. Release of Pb from samples with initial sorption in formic acid was low overall for both O and AB samples (~4.8% and 2.5% for O and AB respectively). The retention of Pb for these samples is highly significant, as shown by the adsorption efficiency (Table 3). During the adsorption experiment, SC intercepted a high proportion of Pb, despite the extracting strength of $CaCl_2$ toward divalent cations. In the desorption experiment, approximately 2% and 8.7% of Pb was released from SC for soil O and soil AB respectively. This limited leaching of Pb may be a reflection of the buffering against pH decrease in this sample (Fig 3C). With regard to the leaching of Pb from SC that was initially adsorbed in DI_{H2O}, the results indicate good retention (Fig 3D). After 5 hrs of leaching, approximately 8.5 and 6.5% of total Pb was released from SC for AB and O samples respectively.

Conclusions

Increases in solution pH (as much as pH +4.49) were observed after leaching through SC. Sorption of PTE onto SC varied between the studied PTE, and according to type of leaching solution. In oxalic acid, all of the studied PTE were effectively sorbed. In formic acid, Pb sorption was very efficient, but As and Cd were adsorbed to a lesser extent. In DI_{H2O}, only Pb showed a high rate of sorption. A generally greater degree of sorption was achieved for PTE that had leached from a mineral soil layer. Further attention is needed to clarify the role of DOC, and the influence that degree of humification of source soil has on PTE sorption. Desorption experiments showed that release of PTE from SC varies according to the source of the PTE (organic vs. mineral soil), and the type of solvent. The soil layer from which PTE are washed not only dictates the amount of sorption (respective of total contents), but also has an effect on the subsequent release of the PTE; a greater adsorbed proportion of PTE generally results in lower release of that element. Arsenic was the PTE most readily leached in all desorption experiments. A high rate of Cd sorption from LMMOA solutions was followed by strong retention during desorption experiments. For sorption of Cd onto SC from DI_{H2O}, the desorption experiment indicated a good level of retention. For SC samples with initial Pb sorption from LMMOA and $CaCl_2$, desorption in acidified water was relatively low ($\leq$ 10% after 5 hrs of leaching). SC was able to retain Pb after adsorption in DI_{H2O}, with losses of no more than 8.5% of total lead. Shredded card showed good potential as a sorbent of toxic elements in various solutions.

Supporting Information

S1 Fig. Map showing where soil was collected, in the forested area adjacent to a lead processing plant.
(JPG)

S2 Fig. Image of shredded card as it was used in leaching experiments (top), and the experiment arrangement (bottom).
(JPG)

Acknowledgments

Author Contributions

Conceived and designed the experiments: CA OD. Performed the experiments: CA NM. Analyzed the data: CA OD VT. Contributed reagents/materials/analysis tools: CA OD NM VT JJ. Wrote the paper: CA LB.

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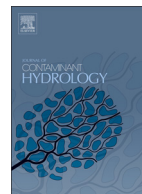
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Different low-molecular-mass organic acids specifically control leaching of arsenic and lead from contaminated soil

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ABSTRACT

Low-molecular-mass organic acids (LMMOA) are of key importance for mobilisation and fate of metals in soil, by functioning as ligands that increase the amount of dissolved metal in solution or by dissociation of metal binding minerals. Column leaching experiments were performed on soil polluted with As and Pb, in order to determine the specificity of LMMOA related release for individual elements, at varying organic acid concentrations. Acetic, citric and oxalic acids were applied in 12 h leaching experiments over a concentration range (0.5–25 mM) to soil samples that represent organic and mineral horizons. The leaching of As followed the order: oxalic > citric > acetic acid in both soils. Arsenic leaching was attributed primarily to ligand-enhanced dissolution of mineral oxides followed by As released into solution, as shown by significant correlation between oxalic and citric acids and content of Al and Fe in leaching solutions. Results suggest that subsurface mineral soil layers are more vulnerable to As toxicity. Leaching of Pb from both soils followed the order: citric > oxalic > acetic acid. Mineral soil samples were shown to be more susceptible to leaching of Pb than samples characterised by a high content of organic matter. The leaching efficiency of citric acid was attributed to formation of stable complexes with Pb ions, which other acids are not capable of. Results obtained in the study are evidence that the extent of As and Pb leaching in contaminated surface and subsurface soil depends significantly on the types of carboxylic acid involved. The implications of the type of acid and the specific element that can be mobilised become increasingly significant where LMMOA concentrations are highest, such as in rhizosphere soil.

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1. Introduction

Particulate depositions from fossil fuel combustion and the pyrometallurgical industry are major contributors to the high anthropogenic loads of potentially toxic elements (PTE) in soil, which are still largely detectable in many areas of northern Czech Republic (Sucharová et al., 2011). Although metals are natural components of many soils, toxicity is expressed when the metal is labile and present in concentrations that are deemed excessive for a given land use or soil type. A metal's lability can be assessed by means of continuous leaching in laboratory column experiments. Column leaching experiments

are commonly used for the simulation of a solution percolating through a contaminated soil, as the mobilisation mechanisms are suggested to be close to those found in the field (Jean-Soro et al., 2012). The experiment allows the researcher to determine the level of binding and mobility of metals through applied leaching of the solution of interest; metals dissolved in the extract represent an environmental hazard, while metals left in the soil following leaching are likely to be present in chemically-stable mineral forms and bound to non-labile soil fractions (Leštan et al., 2008). Low-molecular-mass organic acids (LMMOA) are simple carboxylic acids of low molecular weight. Their occurrence in the environment and in organisms is extensive; in soils, the conditions of release are largely provided by the slow decomposition of abundant soil organic matter and are usually confined to the upper portion of

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weathering profiles. LMMOA in soils function as ligands increasing the total amount of dissolved cations in soil solutions by chelation/complexation (Hue et al., 1986; Fox, 1995). Therefore, complexes of LMMOA with metals are readily transported in pores through the soil profile (Strobel, 2001). It has been suggested that LMMOA also promote desorption of metals by dissolving the minerals that adsorb the metal (Collins, 2004). In general, a complex metal ion is more soluble in water, less retained on the solid phase, and more easily transported through the porous medium (Berkowitz et al., 2008). Extractability of PTE in rhizosphere soil is influenced by the ionic species, which are dependent on the pH, and chemical compositions of root exudates, such as LMMOA (Chiang et al., 2006). The importance of organic acid exudates in the transport of metals is particularly evident in hyper-accumulating plant species, which contain high endogenous levels of organic acids (Boominathan and Doran, 2003). Levels reported for different LMMOA vary between nM to mM, and soil solution concentrations of the dominant organic acids can range from 1 to 100 mM (Van Hees et al., 2002, 2005). However, in common soil solutions, concentrations of LMMOA are suggested to be usually up to 1 mM (Ettler et al., 2004). Due to the potential of LMMOA to form soluble metal complexes, a number of researchers have focused on their application in soil leaching experiments (e.g., Qin et al., 2004; Ettler et al., 2009; Ash et al., 2013). However, the impact of rhizospheric processes on metal speciation and extractability is not well understood, and the impacts of rhizosphere chemistry on the behaviour of individual elements in soils require further attention.

The aim of this study was to describe trends of As and Pb release from soil by continuous leaching using different LMMOA (acetic, oxalic, and citric acid). Using the leaching data, it was possible to identify the most effective LMMOA (type and concentration) for the extraction of As and Pb in organic and mineral soil samples. The resulting data bring new insight into the roles that are played by LMMOA in polluted soils, with respect to the availability, mobility, and consequent toxicity of As and Pb. We test the hypotheses:

i. Cumulative amounts of As and Pb released from soil samples by LMMOA of differing molar concentration will not be linear but will plateau in the upper concentration range (up to 25 mM);

ii. Differences in the extent of As and Pb leaching will reflect the carboxylic structure of LMMOA; an organic acid with a greater number of carboxyl groups is a more effective extracting agent than an acid of simpler carboxylic structure (citric > oxalic > acetic);

iii. The major leaching mechanism for As and Pb from soil is similar for each of the studied LMMOA.

2. Material and methods

2.1. Sample collection and treatment

Soil samples were collected from a wooded area (approximately 0.4 km^2) to the north of Příbram in the Czech Republic (average annual temperature 7 °C, rainfall 651 mm). The sampling area is to the east of an adjacent lead smelter (downwind) that presently functions as a part of a lead battery processing plant. Due to the smelting activities, the land has been heavily contaminated with As, Cd, Pb, Sb and Zn, as identified in previous studies (Ettler et al., 2010; Rieuwerts and Farago, 1996). Particularly high concentrations of PTE are expected in forest soils downwind of smelting facilities due to interception of emissions by the canopy (Ettler et al., 2010). The tree stand is composed predominantly of spruce (Picea abies), with the occasional presence of oak (Quercus sp.). Because of the abundance of spruce trees, the litter layer consists almost exclusively of needles. The sampled region overlies a Cambrian sandstone and arkose sequence. Deep mineral ore veins (As, Pb, Zn) occur in dykes that intersect the Příbram bedrock (Cathro, 2006). Arsenic bearing minerals of the district are mainly arsenopyrite (FeAsS) and admixtures of common sulphides (galena PbS, sphalerite ZnS, and chalcopyrite CuFeS$_2$) (Ettler et al., 2010). Other PTE bearing minerals that were mined and smelted in Příbram, mainly for their silver content, include freibergite, pyrargyrite, and stephanite.

A trench approximately 50 cm deep and 1 m wide was excavated to reveal horizons for taxonomic description. Soil type was identified as toxic (Tx) Cambisol (Inceptisol), having in some layer within 50 cm of the soil surface toxic concentrations of organic or inorganic substances other than ions of Al, Fe, Na, Ca and Mg. A number of composite samples were collected by trowel along the trench from the two following soil layers: soil O (3–11.5 cm); mixture of F and H horizons (after litter layer removed), and soil AB (11.5–25 cm); mixture of A and B horizons. Samples were air dried at 21 °C to constant weight. A sample of the litter layer was collected for analysis of LMMOA content in a fresh state; soil solution was separated from samples by centrifuging (1509 g at 10,000 rpm) for 5 min in Maxi-Spin filter tubes. Soil solution was then measured immediately for content of anions by means of ion chromatography, ICS 1600 equipped with IonPac AS11-HC. After drying, soil samples were sieved (<2 mm). Prior to analysis, bulk soil was sterilised by gamma ray treatment to mitigate the possibility of microbial decomposition of LMMOA during the experiment. The procedure was performed according to the standard outlined by the Ministry of Health of the Czech Republic (Ministerstvo Zdravotnictví, 2009).

2.2. Determination of soil properties

Active (pHH2O) and exchangeable (pHKCl) soil pH were measured by preparing a 1:5 soil:liquid (w:v) ratio using deionised water (DI$_{H2O}$) or 1 M KCl solution respectively, then measuring the mixture using a calibrated Denver Instrument UB-5 pH meter after agitation. Salinity of soil DI$_{H2O}$ leachate was measured using a conductivity probe (inoLab WTW). Content of soil organic matter (SOM) was measured by the loss on ignition method. Quality of humic substances was calculated from spectrophotometric analysis (HP Agilent 8453 UV–visible spectroscopy system), taken as the ratio of a pyrophosphate soil extract absorbance at wavelengths 400 and 600 nm (A$_{400}$/A$_{600}$), according to Podlešáková et al. (1992). Amounts of amorphous Al and Fe were determined by an acid ammonium oxalate extraction method performed in the dark (Carter and Gregorich, 2008). Cation exchange capacity (CEC) was determined according to the Bower method as described by Hesse (1998). Concentration of Na in the final

extract was determined by Varian Spectra AA280 FS (fast sequence, Mulgrave, Australia) flame atomic absorption spectroscopy. All glassware used for analytical procedures were acid washed. Elemental analysis of N, C, and S was made using a Thermo Scientific Flash 2000 NCS Analyser. All analyses were made in triplicates. Mineralogical analysis of soil samples was performed by means of XRD phase analysis; X'Pert Pro, PANalytical B.V., Almelo, Netherlands.

2.3. Analysis of PTE in soil

Contents of As and Pb in 1 g of soil were determined by digestion in a mixture of concentrated acids (19.1 M HF and 14.4 M HNO_3 in a 2:3 ratio), with heating on a hot plate for 16 h at 190 °C. After evaporation, 1 mL of 14.4M HNO_3 and 49 mL of DI_{H2O} were added to the residue before filtering (Whatman 390 paper). The extract was analysed for PTE content by inductively coupled plasma-optical emission spectroscopy (Thermo Scientific iCAP 6500 Radial ICP, Cambridge, U.K.). Limit of detection (LD) was calculated according to the equation: LD = 3.29 σ0 (σ0 is blank sample standard deviation). Analyses were made in triplicates and procedural blanks were included. Standard reference material "Montana II" and "San Joaquin" by NIST, which represent polluted and baseline soils respectively, were used to control analytical performance.

A modified Commission of the European Communities Bureau of Reference (BCR) sequential extraction (SE) was performed according to a method described by Rauret et al. (1999): Target metals were extracted from soil samples in the following successive stages: weakly bound metals (exchangeable) were released into 0.11 mol/L acetic acid. Metals bound to Fe/Mn oxides (reducible) were extracted using 0.1 mol/L hydroxylammonium chloride. Metals bound to organic matter and sulphides (oxidisable) were liberated by oxidising the sample in 8.8 mol/L hydrogen peroxide, followed by dissolution into pH adjusted 1.0 mol/L ammonium acetate. Metals bound in residual soil (residual) were dissolved in a heated mixture of 12.0 M HCl and 14.4 M HNO_3. PTE in extracts were determined by ICP-OES.

2.4. Leaching experiment

LMMOA used in this experiment (acetic, oxalic, and citric acid) are representative of mono-, di- and tri-carboxylic acids respectively, with varying dissociation constants (pKa = acetic acid 4.76, oxalic acid 1.25; 4.19, citric acid 3.13; 4.76; 6.40). Acids were produced by Lach-Ner, s.r.o. Czech Republic (p.a. grade).

Vertical glass columns (18 × 3 cm) were used to contain soil samples. A filter disk (Filtrak 390) was fitted either side of the sample (soil layer equal to 10 g). Small inert glass marbles were used to fill space between the sample and the column outlet.

Solution mobility was provided by a peristaltic pump (Kouřil PCD 83.4S) set to flow upward through the column at 50 mL/h, thus giving an hourly liquid to solid (LS) ratio of 5 (v:w). Leaching time was limited to 12 h to mitigate the effects of LMMOA decomposition during the experiment. Leachate was collected at hourly intervals in polyehylene tubes.

Before LMMOA were applied, DI_{H2O} was pumped through the column at a rate of 100 mL/h for a predetermined amount of time (Fig. 1). This was to achieve a state of chemical equilibrium with respect to pH and conductivity (salinity) of aqueous extract, as inspired by the experiments of Jean-Soro et al. (2012). For both soils, pH and conductivity measurements reached an observable state of equilibrium at ~5 h. Accordingly, it was determined that this was to be the duration of DI_{H2O} leaching before exchanging for experimental solution.

Soil samples (soil O and soil AB) were each subjected to experimental leaching by solutions of acetic, oxalic, and citric acid, prepared to concentrations of 0.5, 1, 2.5, 5, 10, and 25 mM. A blank column leaching was made (no soil) for each LMMOA at 25 mM concentration. Leaching extracts were filtered (nylon 0.45 μm), and then analysed for As and Pb by means of ICP-OES.

2.5. Statistical analysis

Microsoft Excel (2007) was used for data processing, constructing graphs, and basic statistical applications. A polynomial function was applied on plotted leaching curves
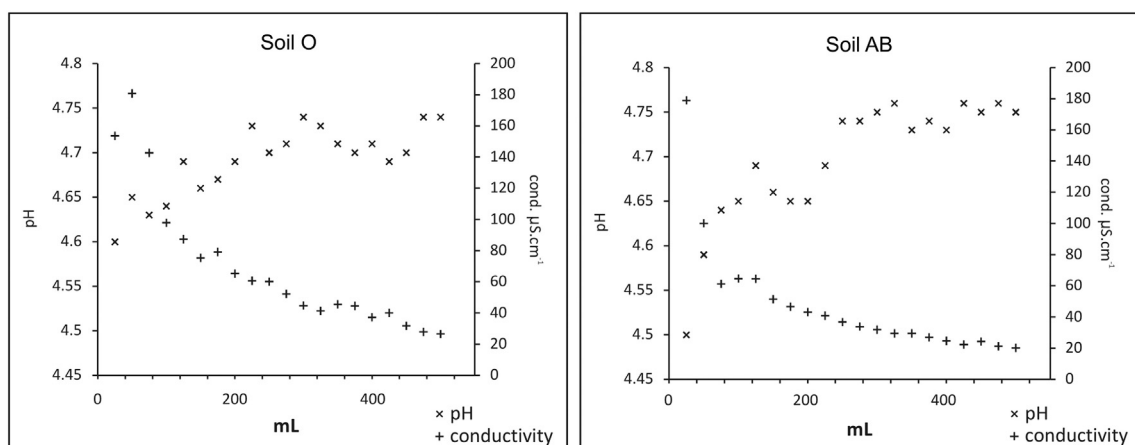

Fig. 1. Pre-leaching tests to determine the point of equilibrium of solution pH and electrical conductivity for soil O and AB. Experiment was performed at a rate of 100 mL/h.

to identify the maximal (peak) leaching by means of interpolating data beyond 12 h. One-way analysis of variance (ANOVA) and multiple range tests were calculated in Statgraphics Centurion XVI (Statpoint Technologies, Warrenton, VA).

3. Results and discussion

3.1. Basic soil properties

Both soil samples are acidic, with little difference in pH between the two layers (Table 1). Solution extraction of the litter layer (fresh soil) showed that the concentration of LMMOA occurred in the following order: acetic > oxalic > citric acid (10.6, 4.41, 0.59 mg/L respectively). Organic matter content was considerably higher in soil O (77.0%) than in soil AB (22.4%). NCS analysis estimates the percentage of C in soil O (28.1%) and soil AB (4.6%). Content of N was higher in soil O (1.70%) than soil AB (0.31%), due to amine groups associated with humic and fulvic acids in organic soils. Photospectral analysis of soil extracts showed that both soil samples contained poorly polymerised organic substances. Humic substances of soil AB were slightly more developed than that of soil O, as indicated by a lower $A_{400/}$ A_{600} value (Table 1). CEC of soil O was far greater than that of soil AB, due to abundant carboxyl, phenol, and alcohol groups associated with the organic molecules. XRD analysis indicated a strong presence of hematite in both samples, and other iron oxides (magnetite and goethite) in soil O. Quartz, muscovite and plagioclase were dominant minerals in both soil layers (Fig. 2).

3.2. Soil PTE contents

Lead was most highly enriched in soil O, whereas As was more highly concentrated in the underlying mineral layer soil AB (Table 1). Lead is known for its strong affinity to humic matter, and under circumstances of atmospheric deposition onto organic surface soils, it is suggested that the release of Pb to deeper horizons depends mainly on transport of dissolved Pb-organic complexes (Alloway, 2013). Arsenic on the other hand is readily leached from the surface of forest soils, and is more commonly known for its strong sorption to the reactive sites of variable charge minerals, e.g., metal oxides, alumino-silicates (Alloway, 2013; Kabata-Pendias, 2011), which explains its accumulation in the deeper mineral soil layers as shown by the total content (Table 1). However, for both elements, irrespective of total content, organic matter binding of the analytes was predominant in soil O, whereas binding to metal oxides was the major sink in soil AB, as shown by the soils PTE fraction distribution (Fig. 3).

3.3. Leaching experiment

3.3.1. Solution pH

pH of the studied solutions were characterised by an overall decreasing trend (Fig. 4). The acidity of leaching solution varied in terms of initial pH, and in the soils ability to buffer against further decrease. Leaching solution pH values remained generally highest in the organic sample (soil O), reflecting the greater CEC capacity of this sample. Conversely, at the stronger LMMOA concentration (10–25 mM) in oxalic acid, the mineral soil layer (soil AB) was able to buffer against a further drop in

Table 1
Basic properties of soil samples and total content of As and Pb.

	Organic matter		Soil pH		CEC mmol(+)/100 g	As mg/kg	Pb mg/kg	Al %*	Fe %	N %	C %	S %
	Combustible SOM	A_{400}/A_{600}	pH_{H2O}	pH_{KCl}								
Soil O	77.0% ± 1.72	7.04 ± 0.98	4.17 ± 0.01	3.66 ± 0.04	95 ± 13.3	266 ± 16.6	24,962 ± 1401	0.27 ± 0.01	0.85 ± 0.02	1.70 ± 0.02	28.1 ± 0.05	0.21 ± 0.01
Soil AB	22.4% ± 0.86	6.03 ± 0.48	4.27 ± 0.01	3.61 ± 0.01	46.5 ± 11.7	327 ± 20.0	5046 ± 255	0.35 ± 0.01	0.64 ± 0.01	0.31 ± 0.01	4.60 ± 0.10	0.08 ± 0.00

n.d. = no data.
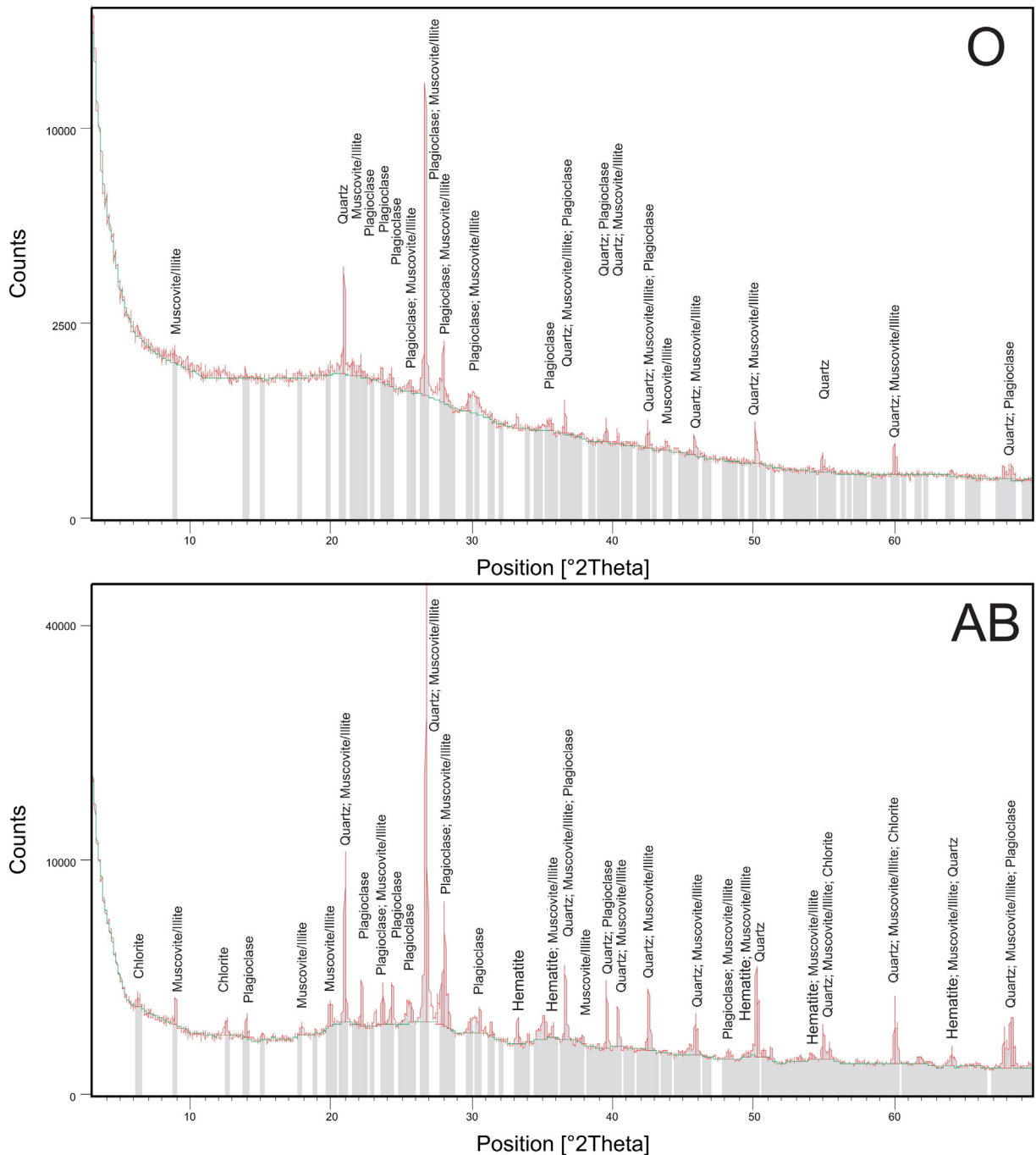* Percentage of <0.15 mm soil fraction.

Fig. 2. X-ray diffraction analysis of minerals in soil O and soil AB.

leaching solution pH more effectively; a probable reflection of more abundant Al compounds than in Soil O.

3.3.2. Arsenic

There are evident differences in both the shape of leaching curves and concentrations of released As for the studied LMMOA (Fig. 5). In acetic acid, the leaching curve is linear for most concentrations, reflecting a less effective extraction of As

over 12 h. For citric and oxalic acids, linear leaching is observed at lower concentrations, but at the higher concentration (25 mM), the leaching curve flattens; suggesting the maximum leaching potential of As in these acids.

Using the equation derived from the polynomial function, predicted maximum (peak) As leaching was made for LMMOA of 25 mM concentration (Table 2). In soil O, peak cumulative As leaching is not only higher for oxalic acid, but peak leaching
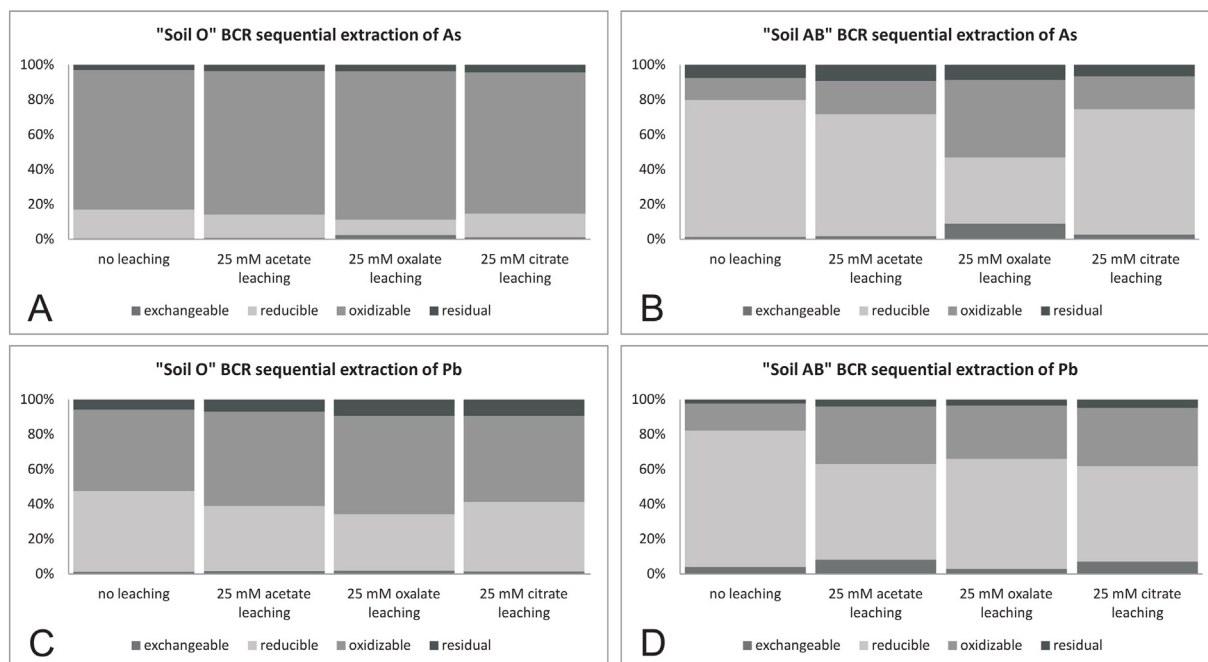
Fig. 3. Modified BCR sequential extraction of As (top) and Pb (bottom) for soil O and soil AB before and after leaching.

occurs much faster (107 mg/kg As after 15 h compared to 69.7 mg/kg As after 48 h for oxalic and citric acid respectively). Thus, oxalic acid is a superior and more efficient extracting agent of As from organic soil.

Similarly, oxalic acid proved to be the more effective LMMOA for the release of As in soil AB, with a peak leaching of 266 mg/kg As after just 13 h. This compares to 108 mg/kg As leached after 37 h for citric acid. Multiple range tests showed that in all cases, leaching of As was significantly greater in oxalic acid than in citric acid, with the exception of soil AB at 5 mM concentration, whereby citric acid released significantly more As over 12 h of leaching.

In soil O, at 0.5 mM concentration, the cumulative amounts of As leached after 12 h were not so wide ranging for the three LMMOA; 5.75, 7.18, and 8.48 mg/kg As for acetic, citric, and oxalic acid respectively (range = 2.73 mg/kg). Whereas at 25 mM concentration, the range of cumulatively leached As is considerably wider; 9.30, 29.2, and 105 mg/kg As for acetic, citric, and oxalic acid respectively (range = 95.3 mg/kg). The difference between the three acids potential to mobilise As from soil becomes more considerable with increasing concentration, expressed as an exponentially increased difference in extraction of As after 12 h. Analogous trends were observed in soil AB. These differences in As release between LMMOA and their concentrations were validated by ANOVA and multiple range tests (Table 3). In bulk soil solutions, implications of this finding may not be so consequential, as authors (e.g., Collins, 2004; Ettler et al., 2004) have suggested that common soil solution concentrations rarely exceed 1 mM. However, in rhizosphere soil, differences in the types of LMMOA present may have significant implications in this respect, as LMMOA concentrations are expected to be much higher.

Our results tend towards the inference that acetic acid is an overall poor extractant of As, when compared to other LMMOA. Feng et al. (2005) found a strong correlation between plant roots PTE content and a rhizosphere-based extracting solution that consisted of acids: acetic, lactic, citric, malic, and formic in the ratio 4:2:1:1:1. The strong presence of acetic acid compared to other LMMOA also matched our analysis of fresh litter. However, it is evident that despite its predominant concentration in surface soil solutions, acetic acid alone is likely to have only a small contribution towards As (and other PTE) mobility and uptake. Chiang et al. (2006) found that the contents of LMMOA in temperate rainforest soil samples showed the following trend: oxalic acid > succinic acid > malonic acid > fumaric acid, with the content of oxalic acid accounting for more than 60% of the total LMMOA. This observation could have strong implications for any bound As. A study focusing on the extraction of LMMOA from podzol O and E horizons determined that citrate and isocitrate were the most abundant acids, respectively (Ali et al., 2011). Citric acid was also the most common acid to be found underneath six North American tree species, often occurring with lactic acid (Dijkstra et al., 2001).

With respect to the hypothesis that release of PTE will be dictated by the carboxylic structure of LMMOA, the data for As leaching in both soil O and AB disproves this presumption. The amount of leached As better reflects the aciddissociation properties of the studied LMMOA, as opposed to the number of carboxyl groups (di-oxalic$_{pKa\ 1.25;\ 4.19}$ > tri-citric$_{pKa\ 3.13;\ 4.76;\ 6.40}$ > mono-acetic$_{pKa\ 4.76}$); lower pKa value corresponds to greater acid dissociation.

Respective of total soil concentrations, peak As leaching in 25 mM citric acid was approximately 26% of total As after 48 h,
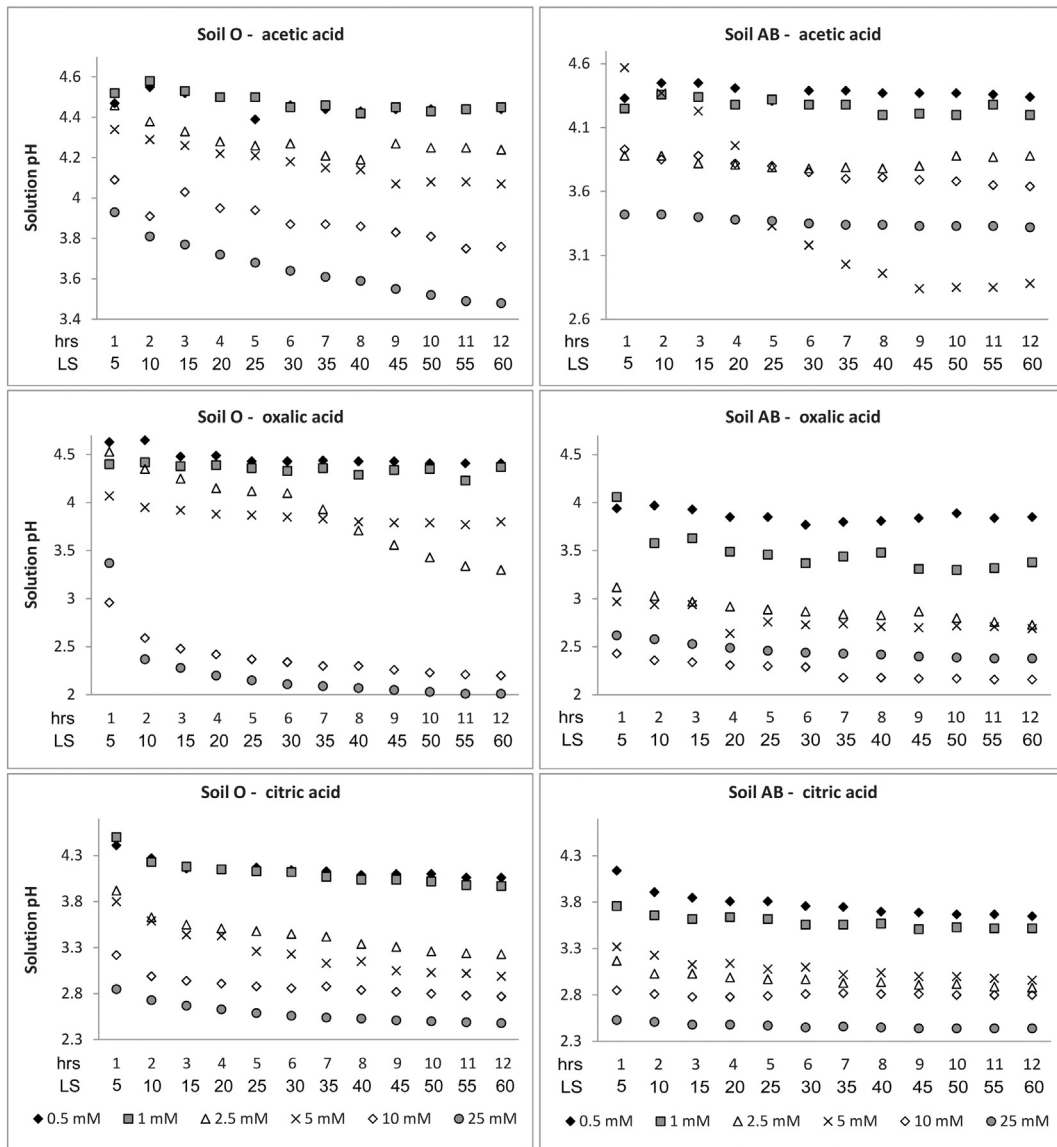
Fig. 4. Solution pH measured on leaching effluent between 1 and 12 h.

and 33% after 37 h for soil O and AB respectively. Peak As leaching in 25 mM oxalic acid was approximately 41% of total after 15 h, and 82% after 13 h for soil O and AB respectively. Hence, a greater proportion of As was leached in a shorter duration from soil AB for oxalic and citric acids. This suggests that underlying forest soil layers are more vulnerable to As contamination than surface organic horizons, especially in rhizosphere soil. The direct bonding of As onto SOM has been regarded as negligible by some authors. Wenzel et al. (2001) state that there is growing evidence that As is virtually not associated with SOM when in competition with other soil constituents such as hydrous Fe oxides, and that As solubility may even be enhanced in organic surface layers in reference to associated mineral horizons. Moreover, it is suggested by Motuzova (1999) that 27–90% of total As is fixed by the

clay granulometric soil fraction. Beesley et al. (2010) conducted a leaching study concerning As in rhizon pore water, and in applied column leaching. In pore water extract, very low leaching of As was determined (after 6 weeks). They observed greatest As leaching in the deeper soil layers (70–100 cm), where lower dissolved organic carbon content was measured, indicating that deeper soils contain more labile pools of As. Results from our BCR SE (Fig. 3) are in agreement with this, which show that As bonding in soil O was primarily to the oxidisable (organic matter bound) fraction (Fig. 3). After leaching with 25 mM LMMOA (especially oxalic acid), the metal oxide bound fraction decreased, whereas the organic matter bound fraction increased. This indicates that oxides of Fe/Al were the main fraction contributing to As leaching.
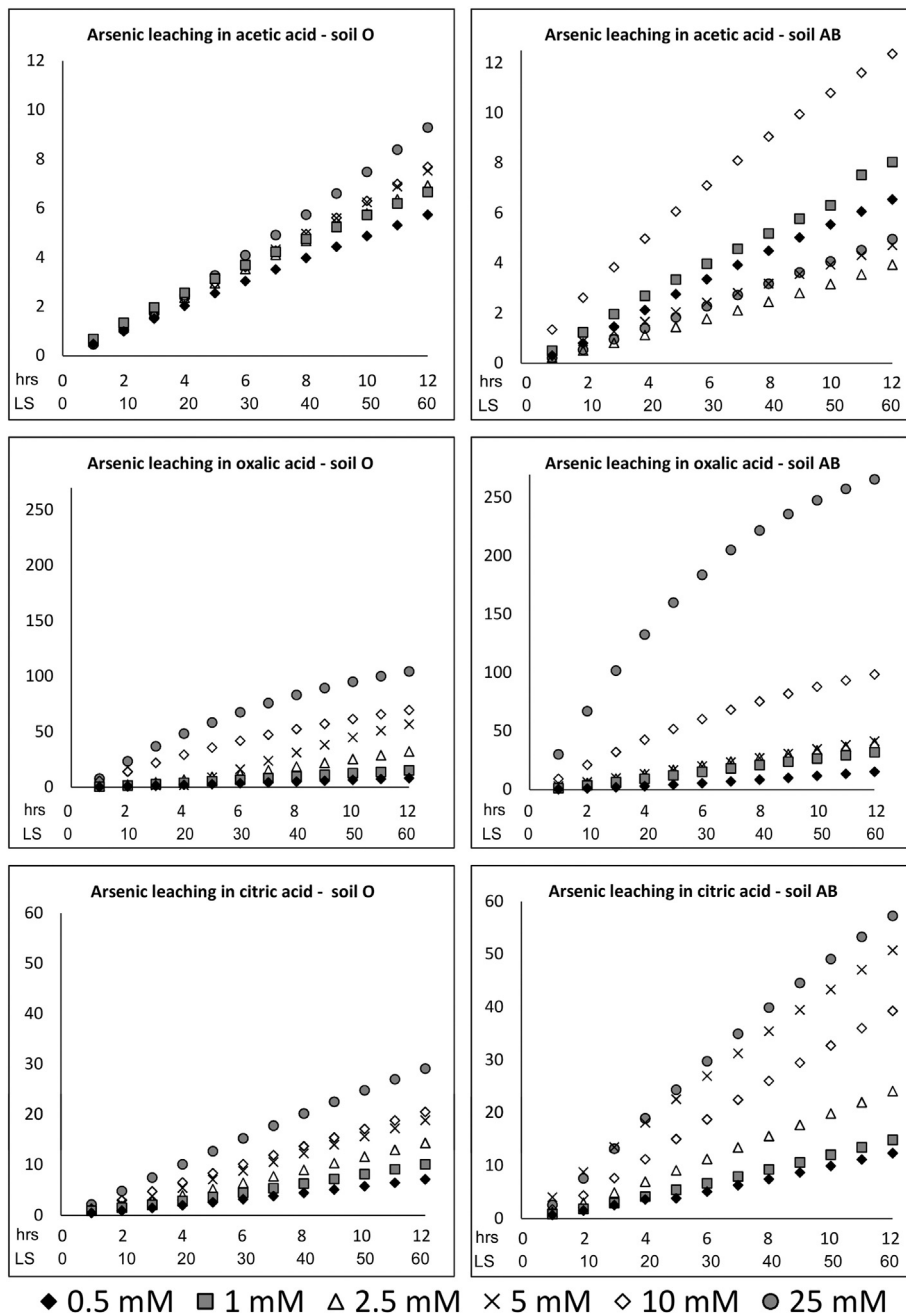
Fig. 5. Leaching of As over 12 h in acetic acid (top), oxalic acid (centre), and citric acid (bottom). Values of As are in mg/kg.

Researchers at Texas A&M University (2015) proposed two major As release mechanisms in soil involving oxalate; ligand exchange (LE), and ligand-enhanced dissolution (LED). The mechanism of LED (dissolution of structural cations of a mineral resulting in its dissolution) for As release from soil is also supported by findings in experiments by Tao et al. (2006), and by Zhang et al. (2005). In the present study, correlation was made between As and Fe/Al in collected leachate (Table 4). In oxalic acid (25 mM), very strong correlation was identified between As and Fe in both soils (R^2 = 0.995 and 0.984 (n =

12) for soil O and AB respectively). Significant correlation was also observed between As and Al (R^2 = 0.943 and 0.909 respectively). The BCR SE concurs with this observation (Fig. 3). In the case of citric acid, trends are evident, and although they are not completely linear, they do suggest some association between the release of As and Fe/Al. The non-linear part of the trend (not shown) represents the initial stages of leaching, whereas the linear portion represents the latter part of the leaching experiment. This suggests that citric acid was capable of releasing As by way of LED, but there is an initial time lag before

Table 2
Interpolated peak cumulative leaching of As, Pb in soil O and AB.

Arsenic

Soil O

	Acetic acid	Oxalic acid	Citric acid
Equation (poly.)	n.d.	$y = -0.519x^2 + 15.31x - 5.304$	$y = -0.0297x^2 + 2.8513x - 0.707$
R^2	–	0.999	1.000
Peak (hours)	–	15	48
As mg/kg	–	107 (40.5)	69.7 (26.2)
	Soil AB		
Equation (poly.)	$y = -0.0251x^2 + 1.3261x + 0.070$	$y = -1.5561x^2 + 41.384x - 8.619$	$y = -0.0833x^2 + 6.1328x - 4.023$
R^2	1.000	9.999	9.999
Peak (hours)	26	13	37
As mg/kg	17.6 (5.38)	266 (81.5)	108 (33.3)

Lead

Soil O

	Acetic acid	Oxalic acid	Citric acid
Equation (poly.)	$y = -0.0761x^2 + 19.793x - 4.0253$	n.d.	$y = -13.729x^2 + 489.98x + 136.67$
R^2	1.000	–	0.999
Peak (hours)	130	–	18
Pb mg/kg	1283 (5.14)	–	4508 (18.1)
	Soil AB		
Equation (poly.)	$y = -0.0957x^2 + 17.362x - 4.6896$	$y = -0.7886x^2 + 40.328x - 25.568$	$y = -3.5399x^2 + 155.76x - 55.828$
R^2	1.000	0.999	0.999
Peak (hours)	91	26	22
Pb mg/kg	783 (15.5)	490 (9.71)	1769 (35.1)

n.d. = no data, due to increasing leaching rate. Values in parenthesis are % of total content.

the dissociation of Fe/Al oxide–As complexes occurs. In the case of acetic acid, there was no observed correlation with Al or Fe for both soils.

The strength of linearity between As and Fe/Al for the studied LMMOA follows the same order as the overall As release by LMMOA: oxalic > citric > acetic, which supports the

Table 3
Multiple range tests (MRT) and analysis of variance (ANOVA) between mean hourly PTE (mg/kg) extraction during 12 h of leaching in acetic, oxalic, and citric acids.

Arsenic	Soil O					
MRT	0.5 mM	1 mM	2.5 mM	5 mM	10 mM	25 mM
Acetic–citric	0.48–0.60[*]	0.62–0.85[*]	0.58–1.20[*]	0.63–1.58	0.64–1.71[*]	0.78–2.43
Acetic–oxalic	0.48–0.71[*]	0.62–1.29[*]	0.58–2.70[*]	0.63–4.75[*]	0.64–5.81[*]	0.78–8.72[*]
Citric–oxalic	0.60–0.71[*]	0.85–1.29[*]	1.20–2.70[*]	1.58–4.75[*]	1.71–5.81[*]	2.43–8.72[*]
ANOVA F-ratio	27.4	39.4	66.9	16.1	124	52.8
Soil AB						
MRT	0.5 mM	1 mM	2.5 mM	5 mM	10 mM	25 mM
Acetic–citric	0.55–1.03[*]	0.67–1.24[*]	0.33–2.01[*]	0.39–4.23[*]	1.03–3.28[*]	0.41–4.78
Acetic–oxalic	0.55–1.29[*]	0.67–2.68[*]	0.33–3.34[*]	0.39–3.47[*]	1.03–8.24[*]	0.41–22.2[*]
Citric–oxalic	1.03–1.29	1.24–2.68[*]	2.01–3.34[*]	4.23–3.47[*]	3.28–8.24[*]	4.78–22.2[*]
ANOVA F-ratio	17.6	149	393	794	90.2	47.1
Lead	**Soil O**					
MRT	0.5 mM	1 mM	2.5 mM	5 mM	10 mM	25 mM
Acetic–citric	2.62–10.8[*]	3.48–19.1[*]	3.36–37.9[*]	5.12–63.8[*]	8.67–122[*]	18.5–340[*]
Acetic–oxalic	2.62–8.90[*]	3.48–17.8[*]	3.36–59.5[*]	5.12–25.3[*]	8.67–57.4[*]	18.5–33.9
Citric–oxalic	10.8–8.90[*]	19.1–17.8	37.9–59.5[*]	63.8–25.3[*]	122–57.4[*]	340–33.9[*]
ANOVA F-ratio	68.6	47.3	26.8	77.6	359	84.0
Soil AB						
MRT	0.5 mM	1 mM	2.5 mM	5 mM	10 mM	25 mM
Acetic–citric	2.43–18.9[*]	3.05–12.4[*]	4.02–22.3[*]	6.76–48.7[*]	13.7–53.8[*]	15.8–108[*]
Acetic–oxalic	2.43–8.68[*]	3.05–25.6[*]	4.02–47.0[*]	6.76–38.1[*]	13.7–38.9[*]	15.8–28.9[*]
Citric–oxalic	18.9–8.68[*]	12.4–25.6[*]	22.3–47.0[*]	48.7–38.1[*]	53.8–38.9[*]	108–28.9[*]
ANOVA F-ratio	3870	392	1075	247	152	175

[*] Denotes a statistically significant difference, p ≤ 0.001.

Table 4
Correlation (R^2) of As and Pb with Fe/Al in leaching effluent.

		Soil O			Soil AB		
		25 mM acetic	25 mM oxalic	25 mM citric	25 mM acetic	25 mM oxalic	25 mM citric
Arsenic	Al	−0.225	0.943[*]	0.825[*]	0.363	0.909[*]	0.813[*]
	Fe	0.352	0.995[*]	0.834[*]	6×10^{-5}	0.984[*]	0.893[*]
Lead	Al	0.055	0.259	0.938[*]	0.228	0.133	0.964[*]
	Fe	0.201	−0.034	0.855[*]	−0.142	0.372	0.919[*]

n = 12.
[*] Significant at 0.01 confidence level.

conclusion that oxides of Fe and Al are a significant source of As in the studied soils.

In the study by Tao et al. (2006) it was also shown that at the upper oxalic acid concentration, it was the soil with highest organic matter content (6.13%) that released the lowest amount of As. This is in agreement with our findings. Arsenic retention was generally greater in organic soil compared to predominantly mineral soil phases, which are subject to somewhat rapid dissolution and release of bound As.

3.3.3. Lead

Release of Pb into acetic acid from soil O was linear up to 12 h (Fig. 6), and corresponded to a steady decrease in pH with leaching time. Peak cumulative Pb extraction from soil O into 25 mM acetic acid was predicted to occur after 130 h (1283 mg/kg, or approximately 5% of total Pb). In 751 Scottish soil profiles, Reaves and Berrow (1984) reported a mean acetic acid extractable Pb content of 1.7% of the total, which is slightly lower than our predicted peak extraction. However, the study was performed on primarily uncontaminated soils. In comparison, 4508 mg/kg (~18%) of Pb was released after 18 h for 25 mM citric acid. Predicted peak leaching for oxalic acid was not possible due to the increasing trend of the leaching curve; however, it was possible to ascertain from Fig. 6 that leaching of Pb was considerably higher in oxalic acid than in acetic acid, but less than in citric acid. In contrast to observations for As leaching, extraction of Pb by LMMOA corresponds to the order citric > oxalic > acetic, reflecting the number of carboxylic groups on the acid. This conforms to hypothesis (ii).

The synthesis of lead citrate and its structural chemistry was studied in detail by Kourgiantakis et al. (2000); showing that in aqueous solutions, the presence of citrate ions invokes the hard metal ionic character of Pb^{2+}, which leads to the formation of a stable complex. The research confirmed the ability of the bio-ligand citrate to coordinate and solubilise the Pb ion at low pH values. A very stable complex forms in which further coordination of the central hydroxyl oxygen to Pb^{2+} promotes a chelate effect (Kourgiantakis et al., 2000). The tricarboxylate citrate ion acts as a doubly deprotonated ligand that coordinates to Pb^{2+} forming dimeric units (Fig. 7). These units are interconnected through the remaining carboxylate group leading to a polymer of Pb-citrate complexes. The ability of citrate to bind lead ions, and to form long stable chains may account for the observed results whereby citric acid was by far the most effective LMMOA at leaching Pb. In contrast, a single oxalate ion is capable of binding with one Pb ion in a monodentate complex. Furthermore, lead oxalate is sparingly soluble in water.

When comparing the pH of citric acid leaching from soil O and soil AB, it is apparent that the pH of soil AB leachates are consistently lower than of soil O (Fig. 4). The range of pH values observed for citric acid leaching from soil AB after 12 h at different concentrations was between 2.5 (25 mM) and 3.8 (0.5 mM). These pH values are comparable to those for oxalic acid in the same soil sample: 2.4 (25 mM)–3.9 (0.5 mM); and so, despite having closely matching pH, citric acid extracted considerably more Pb than oxalic acid (Fig. 6).
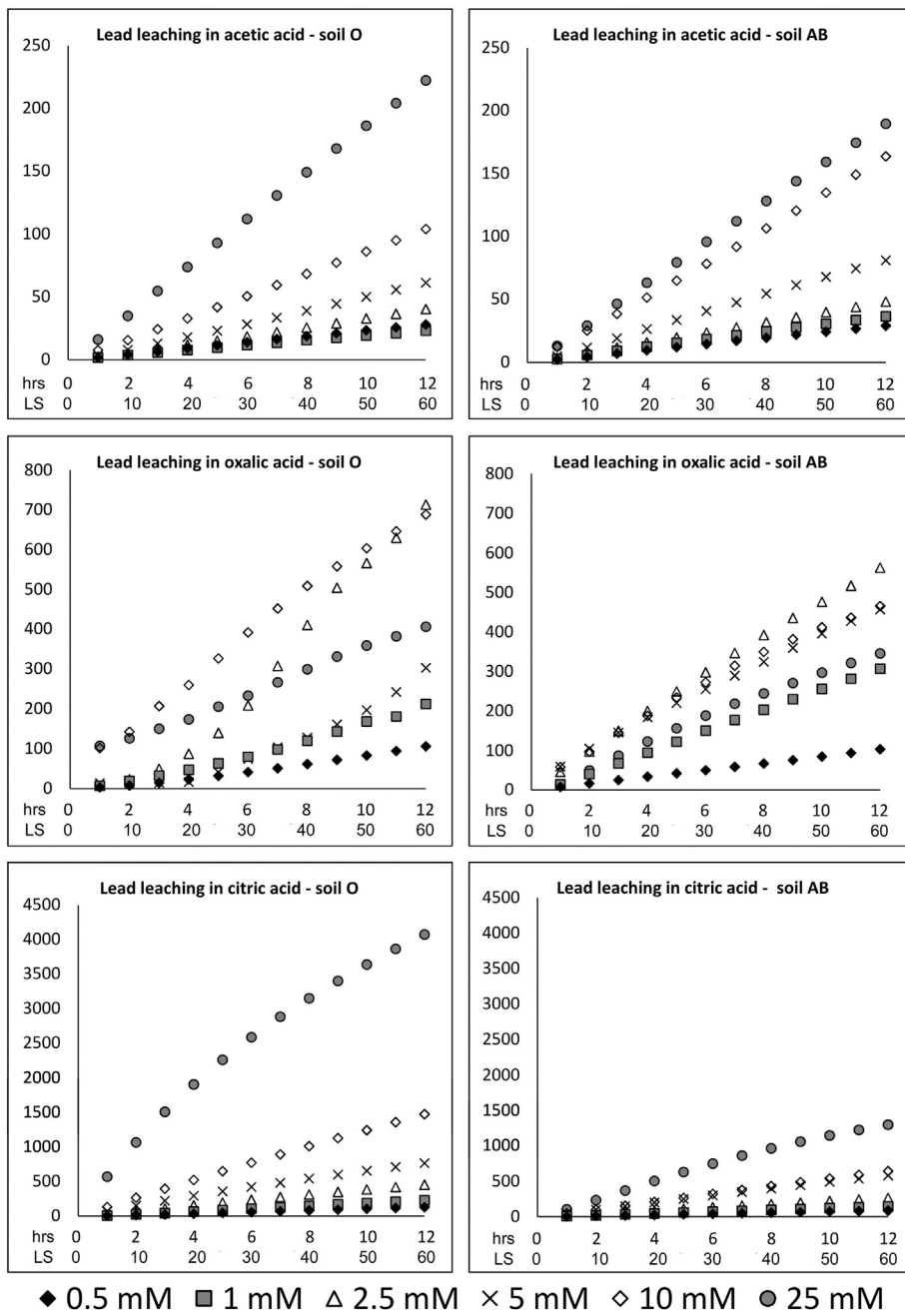
With respect to the hypothesis (i), citric acid leaching was the only case where the trend of Pb leaching was close to equilibrium within 12 h for both soil samples.

Analogous to the leaching from soil O, release of Pb from soil AB into acetic acid was linear up to 12 h. The decrease in solution pH for the corresponding acetic acid concentrations were not so evident for soil AB as for soil O; however, the overall pH values of acetic acid leaching through soil AB were consistently lower. pH values of leachate do reflect the amount of Pb being leached to some extent (more Pb leached from soil AB respective of total than in soil O). This could be a factor influencing the resilience of Pb leaching in the surface layer. Abundant organic matter and greater buffering against acidity contribute to lesser release of Pb into acetic acid. A peak release of 783 mg/kg Pb (~16% of total) into acetic acid is predicted after 91 h. This estimate is greater than that of Pb into oxalic acid at 25 mM (490 mg/kg Pb, ~10% of total). However, the peak cumulative leaching for oxalic acid occurred much faster (26 h) than for acetic acid.

The greatest amount of Pb, respective of total contents, was leached from soil AB into citric acid solution (35%). At the higher concentration, a non-linear curve was observed, with predicted cumulative peak Pb leaching expected to occur after 22 h. The range of Pb leached into citric acid after 12 h is 89–1300 mg/kg Pb between the lower (0.5 mM) and upper (25 mM) concentrations respectively. This implication is quite significant for the bioavailability and potential mobilisation of Pb in the soil environment, particularly in the rhizosphere where LMMOA concentrations are highest.

Results for leaching of Pb into oxalic acid from soil AB are somewhat ambiguous, as there was no logical order of Pb leaching with respect to the varying concentration; 2.5 mM > 10 mM > 5 mM > 25 mM > 1 mM > 0.5 mM. At the lower concentration range (0.5–2.5 mM), oxalic acid was an overall more effecting leaching agent of Pb, especially in soil AB, whereas in the upper range (5–25 mM), citric acid is clearly the superior extracting agent. This observation was also statistically validated by ANOVA.

Results of the research indicate that a greater proportion of Pb was released into LMMOA from soil AB than from soil O; thus supporting the opinion that organic matter is a long-term

Fig. 6. Leaching of Pb over 12 h in acetic acid (top), oxalic acid (centre), and citric acid (bottom). Values of Pb are in mg/kg.
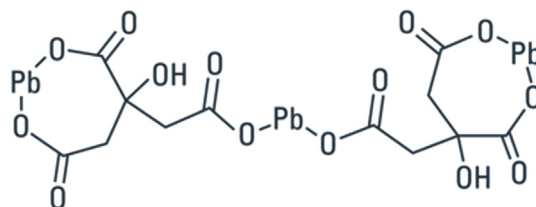

Fig. 7. Possible structural complex formation between Pb and citrate. Image adapted from Pandithage (2013).

sink for Pb in the environment. Alloway (2013) states that the chemical behaviour of Pb in soil depends very much on the organic matter content and that Pb is strongly adsorbed on humic matter at pH 4 and above. However, Stevenson and Welch (1979) observed that Pb moved from topsoil treated with Pb acetate into subsoil, even though the soil was shown to have a high capacity for binding Pb in non-exchangeable forms. This mobility was attributed to the metal leaching as soluble chelated complexes with organic matter. Although mineral subsoil appears to be more vulnerable to Pb leaching with LMMOA, surface soil Pb is also potentially mobile and can be subject to downward movement in the presence of LMMOA.

4. Conclusions

The leaching of As into LMMOA solutions followed the order oxalic acid > citric acid > acetic acid in both organic and mineral soils. The observed differences in extraction efficiency into the studied acids were validated by ANOVA and multiple range tests. The mechanism of ligand-enhanced dissolution of mineral oxides followed by As release into solutions of LMMOA has been recognised, and is supported by findings in this study. Highly significant correlation was observed between oxalic and citric acids and the content of Al and Fe in leaching solutions, which infers that hydrous oxides of Al and Fe are major sources of soluble As in the presence of LMMOA. BCR sequential extraction confirmed a significant decrease of metal oxide bound As after leaching for both soils. A greater portion of As was leached from the mineral soil sample in a much shorter duration than the surface organic soil by both oxalic and citric acids, suggesting that subsurface mineral soil layers are more vulnerable to As toxicity.

Significant releases of Pb into the studied LMMOA were observed, with extractability from both soils following the general order: citric > oxalic > acetic. Soil AB, which represents a mineral soil layer, was shown to be more susceptible to leaching of Pb than surface soil samples that were characterised by a high content of organic matter and greater ability to buffer against acidic solution pH changes. High affinity to humic matter is known to play a key role in the behaviour of Pb, and the release of Pb to deeper horizons is likely to depend on transport of dissolved Pb-organic complexes. Superior leaching efficiency of citric acid was attributed to stable complex formation structures with Pb ions, which are not demonstrated by the other acids.

The hypothesis that carboxylic structure is the key variable for effective leaching of metal/metalloids did not hold true for As. It is apparent that acid dissociation is the key controlling factor, releasing As by way of mineral dissolution. In the case of Pb, the carboxylic structure of the studied LMMOA reflected the amount of Pb released into, and held in solution (tri-carboxyl citric > di-carboxyl oxalic > mono-carboxyl acetic).

Results obtained in the study are evidence that the extent of As and Pb leaching in contaminated surface and subsurface soil depends significantly on the types of carboxylic acid involved, and that the mechanisms of As and Pb release differ between the studied LMMOA. Implications of the type of acid and the specific metal that can be mobilised most effectively become increasingly significant where LMMOA concentrations are highest, such as in rhizosphere soil.

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