Czech University of Life Sciences, Prague

Faculty of Agrobiology, Food and Natural Resources

Department of Soil Science and Soil Protection

Extractability of selected risk elements from soils with anthropogenic pollution

Bachelor's Diploma Thesis

Karel Šlanc

Supervisor: Ing. Ondřej Drábek Ph.D

Consultant: MSc. Christopher Ash

2014

Declaration

I declare that the Bachelor Thesis Extractability of selected risk elements from soils with anthropogenic pollution is my own work and all the sources I cited in it are listed in Bibliography.

Prague   Signature ……………………………………

Acknowledgement

I wish to express my gratitude to my supervisor Ing. Ondřej Drábek Ph.D. for his tireless and patient support and guidance of the thesis. I wish to acknowledge my great friend and consultant of my thesis MSc Christopher Ash who gave me the courage to finish the paper. I am also grateful to RNDr. Václav Tejnecký Ph.D and doc. RNDr. Aleš Vaněk Ph.D. for their help, time and contributions in laboratory. Finally I would like to thank to my family and my girlfriend Markéta for their support and love during my studies.

Keywords: soil contamination, smelting slag, extracting agents, heavy metals

Table of contents:

1. Introduction

In the study and assessment of soils contaminated with inorganic pollution, there exist a number of extraction methods which are operationally defined, but which are synonymous to environmental binding phases of toxic metals. Two such extractions are the standardized aqua regia extraction, and the commonly used 2M HNO_3 extraction methods; both aim to represent the pseudo total content of metal elements within a sample. The aim of this thesis was 1) to evaluate the importance of shaking time on the extractability of metals from a range of contaminated soils when applying the nitric acid extraction procedure. 2) to compare the nitric acid and aqua regia extractions to each other, and to the true total metal content in our studied soils. 3) to assess the ability of a coca-cola soft drink to solubilise metals from a set of contaminated soil samples by comparison with the nitric acid extraction. This study made use of samples from an area of known contamination due to historic smelting practices used for the segregation of silver and copper. With respect to Czech agricultural soil guideline values, our results showed that the studied samples were strongly contaminated, local forest soils which were used as a background sample showed to little or no contamination. The difference in shaking time for 2M HNO_3 extraction dictates the rate of release of PTE. Cadmium release occurred mostly within the first 10 minutes of extraction. The most significant differences occurred for Cu and for Zn. A reduction in shaking time can result in a gross underestimation of the true releasable content of PTE. Correlation between PTE extractable by 2M HNO_3 and the total content revealed large differences in the concentrations obtainable by these two extraction methods; nitric acid extraction does not represent near to total content in many cases. Correlation between aqua regia and nitric acid showed that on average, aqua regia extracted far more PTE than did nitric acid, particularly in the case of Pb and Zn. Results from correlation between aqua regia extract and the total PTE content showed that aqua regia gives a good approximation of total Cd in a sample. However, the total content as determined by the used method was underestimated when compared to amount of Cu and Pb extractable by aqua regia. Zinc was underestimated in aqua regia compared to the total. Six hours of shaking samples with coca cola gave varying rates of extraction between different PTE and different soil samples.

2. Aims and objectives

2.1 Aims:

- To compare the closeness of an aqua regia extraction to a commonly used nitric acid extraction and to the total content of potentially toxic elements in contaminated soils
- To determine the importance of shaking time during a 2M HNO_3 extraction procedure with regards to the final concentrations of potentially toxic elements which are released from soil

2.2 objectives

To make use of standardized methodologies for the comparison of 2M HNO_3 extraction, aqua regia extraction, and total potentially toxic element content from a range of soil samples with varying properties and varying degrees of contamination by a known source of inorganic pollution.

3. Literature Review

3.1 Potentially toxic elements and heavy metals

In correlation with industrial development, in recent decades there has been increased interest in the protection of the environment. It is primarily due to the development of greater knowledge within the field of contamination and environmental protection. In particular, since the end of the communist regime there has been a conceivable effort to clean up the environment which had been disregarded over many years of unregulated industrial activities. Moreover, public has expressed their discontent with respect to the urgency and necessity to protect the environment. This phenomenon is typical for developed countries where people are willing to invest money into research that deals with contamination. Therefore, a significant volume of papers, books, studies and reports dealing with contamination have been published since the second half of the 20th century. Different authors have used a different terms to describe the contaminants.

As the contamination was typically associated with industry, connected with mining, metallurgy and other heavy industries dealing with metals, authors extensively used the term "heavy metals". The term "heavy metals" (HM) was commonly used for the last few decades.

It is mainly used for a group of metals and can sometimes refer to metalloids (semimetals) that have been associated with contamination, potential toxicity, or ecotoxicity (Duffus 2002). However this term has never been established and defined by any world authority or organization such as IUPAC (International Union of Pure and Applied Chemistry). The term HM. is commonly used for metals with relative atomic mass greater than sodium (Bennet 1986). Other authors described HM as a collective term for metals of high atomic mass, particularly those transition metals that are toxic and cannot be processed by living organisms, such as lead, mercury, and cadmium (Harrison 1998). The second characteristic typical for compounds called HM is density. Van der Perk (2006) defined HM as metals with the density ranging from 3.5 to 6 $g.cm^{-3}$, while Sparks (2003) states that HM are elements with greater density than 5 $g.cm^{-3}$. The term HM is widely considered to be inaccurate, unscientific and meaningless. van der Perk (2006) states that HM such as Zn, Cu, Pb, Cd, Hg, Cr, Ni, Sn, and Ag are subset of trace elements.

Trace elements (sometimes including these referred to as HM) are essential for plant growth and many living organisms, however if they exceed natural concentrations due to anthropogenic sources of contamination they can become strongly toxic to plants and even lethal to animals. In particular, such elements as Co, Cr, Cu, Mn, Mo and Zn are indispensible for organisms, their growth and metabolism. However, they are generally considered to be toxic and harmful to the environment if they exceed an acceptable level that is standardized. This phenomenon is visual at the study area on the slag heap near to Kutná Hora (see section 4.5).

The titles heavy metals, toxic metals or trace elements seem to be unsatisfactory and misrepresenting a large and diversified group of elements (Bhat et al 2011). A more convenient term of Potentially Toxic Elements (PTE) in concurrent practice is adopted as an alternative (Wilkinson et al 2001).

The species in which PTE may occur in soil is governed by many properties, particularly pH, humus content and quality, and clay content (Borůvka and Vacha 2006). Concentration is one of the most important factors that dictate the toxicity and danger that PTE represent to organisms and plants. However, their speciation is also an essential factor.

Assuming all modifications of a given PTE to have the same or similar toxicity, physical and chemical properties and behavior in the soil and the environment would be a fundamental mistake. It is generally known that this is not the case as the toxicity of a given element is

largely controlled by its speciation. For example: the comparison of metallic sodium and NaCl. The danger associated with improper storage of metallic sodium is a generally accepted statement. There is a significant risk toward human health associated with the consumption of metallic sodium. The aforementioned consumption can even be fatal without medical treatment. However, NaCl is essential for human biological cell functions and we all accept it in our nutrition (Duffus 2002).

The bioaccumulation paradigm in a particular organism in relation to toxicity effects is complex, being influenced by biological processes, chemistry of the growth media, multiple routes of exposure and different strategies of organisms to regulate PTE (Luoma and Rainbow 2005). Ionic speciation is a key factor in controlling mobility (solubility), recalcitrance or persistence and corresponding bioaccumulation of metals and metalloids and their compounds in natural environments since most PTE in the environment are present as cations; although some semi-metals may occur as oxy-anions, e.g., arsenate, AsO_4^{3-} (Bhat et al 2011). It is assumed that the toxicity is a complex function of the element itself and its chemical properties, its speciation, its concentration and the variables of the environment it is in interaction with such as soil properties (Ph, clay content, organic matter content, particle size, …etc), climate (temperatures, precipitation, wind, air humidity …etc) and other factors, e,g., flora and fauna occupying the location. Therefore there is not an existence of a general unified formula for PTE behavior in the soil and the environment due to so many variables and their possible modifications and combinations. Scientists can only suppose partial behavior such as copper and lead being elements with a strong binding affinity toward soil organic matter. However there is not any scheme of behavior valid for all polluted areas.

3.2 Behavior of PTE in soils with anthropogenic pollution

Materials, such as mined ore, smelter slag or other industrial by-products or wastes are a source of potentially toxic elements and heavy metals (Ash et al. 2013). Inappropriate handling, management and storage of materials can result in damage and harm toward human health and to the health of the environment. Therefore state authorities set environmental legislation which includes guideline values for different risk elements.

Soil guideline limits are typical for developed countries. Values differ among countries. Czech guideline values (Table 3.1) for example are more strict and conservative compared to

those in U.K. Czech authorities have also given values for elements Be, Co, Cu, Mo, V and Zn where they are absent from the U.K guideline values (Ash, 2012).

Table 3.1 Soil Guideline Values for risk elements extractable with Aqua Regia ("Pseudo-total content") and those extractable with 2M HNO$_3$ (simulating an extreme environmental scenario in which elements are mobilized in the environment) in Czech soils (Podlešakova *et al.* 1990)

Contaminant	Total content (mg/kg)		2M HNO$_3$ (mg/kg)	
	Sandy soils	Other soils	Sandy soils	Other soils
As	30	30	4.5	4.5
Be	7	7	2	2
Cd	0.4	1	0.4	1
Co	25	50	10	25
Cr	100	200	20	40
Cu	60	100	30	50
Hg	0.6	0.8	-	-
Mo	5	5	5	5
Ni	60	80	15	25
Pb	100	140	50	80
V	150	220	20	50
Zn	130	200	50	100

The behavior and associated problems with PTE in the soils of Kutná Hora have been studied and described by colleagues from CULS (e.g., Ash *et al* 2013, Székova *et al* 2009). According to their research PTE exceeded agricultural guideline limits by the following: Pb 30; Cd 86.7; Cu 103; Zn 121; and As 39 times. These values were compared to the levels of PTE acceptable in agricultural soil values according to Beneš (1993).

PTE mobility and availability to the environment radically differs among the soil properties. The mineralogy of the slag and the soil will be a considerable determining factor on the mobility of metals (Navarro *et al.* 2008). According to the paper by Ash *et al* (2013) there is a significant negative correlation between clay content and PTE availability.

Organic matter as well as dissolved organic matter has an important but not an unambiguous influence on the concentration, availability and transport of metal ions in the environment (Quevauviller, 2002). Prior studies (Ash, 2013) indicate that a greater amount of organic

matter generally lowers the bioavailable concentration of PTE, most likely due to the metals strong affinity for the many ligands associated with organic substances. However in the case that the organic matter fraction is dissolved in water, there can exist a phenomenon of increased total concentration of soluble metals. Studies by Quevauviller (2002) prooved that it is the soluble fraction of the organic matter that can lead to an increased mobility of the metal ions, while the metal ion activity in solution is low to very low. A general characteristic for natural organic matter is the high heterogeneity of its affinity for ions and its complex composition. Once in soils, bioavailability and mobility of many PTE are limited by adsorption onto mineral surfaces (Fe and/or Mn oxides, clay minerals), complexation to organic compounds, and incorporation into newly formed minerals (phyllosilicates and/or layered double hydroxides) (Juillot et al. 2011).Medium strong positive correlation was found by Ash *et al*, (2013) between availability of arsenic and manganese and content of organic carbon at the 95% confidence level, while copper and lead (elements known for their strong binding affinity to soil organic matter) showed low availability when organic matter increased.

3.3 Problems caused by PTE in soil and the environment

Unsecured dumps, mining materials, smelter slag and other sources of potentially toxic elements (PTE) are present in countries all around the world. The Czech Republic is not an exception. A rich history and high level of development in the middle ages (such as at Kutná Hora) as well as intensive exploitation and mining during the communist regime (Uranium mines in Příbram and Stráž pod Ralskem) led to a substantial number of places, from where PTE could be exposed to the environment. Such sources of potentially toxic elements with different properties are dangerous for both the aquatic and soil environment. For soil contamination, the typical symptoms include soil texture destruction, reduced fertility, ecological landscape destruction, and decrease in biological diversity (Deng *et al.* 2013). Some of these features were observed in the surroundings of the slag heap of the Vrchlice river valley where our study took place. Ecological landscape destruction and a decrease in biodiversity were visible and obvious to the eye without any research, for example, the poor health and stunted growth of trees at the edge of the slag and the complete absence of vegetation on the main slag heap. Even though the heap was deposited many centuries ago, its toxic effects are still evident.

Disposal of mine wastes and other materials which can act as sources of mobile PTE should be done so with consideration of the composition and discharge of their leachates (Ash *et al.* 2013). Antecedent studies of the smelter slag heap in the Vrchlice river valley and its neighboring ecosystems have shown the amounts of PTE exceeding World Health Organization limits for soil heavy metal concentrations. Leaching is one of many possible ways for these inorganic pollutants to spread into the environment. Another possible pathway for PTE movement is by plant uptake. The majority of plant species is intolerant to heavy metals and cannot subsist in soils which contain PTE levels exceeding the level originating from natural circumstances. However, there are some plants known as hyper-accumulators which can persist under the presence of high concentrations of certain metals in soil. These plants are even able to uptake and accumulate metals in their cells. Approximately 450 plants species have been classified as hyper-accumulators of heavy metals (Boularbah *et al.* 2006).

Zinc and lead are some of the most excessive PTE in the studied area. Lead was present in metallic ores in a small concentration. Nevertheless it was also added during the smelting process for the segregation of silver from roasted ores (Pauliš and Mikuš, 1998). These were the main factors resulting in an excess of lead in smelter slag at the site of this study.

3.4 Potentially toxic element composition of smelter slag

Smelting of the silver ores and overall silver production increased during the middle ages in the vicinity of Kutná Hora town. Ores were mined and processed (smelted) mainly in the Vrchlice river. The energy of the river was used for stampers to crush the ore. The smelter slag (a by-product of smelting) was commonly used for road constructions or piled and left (Pauliš and Mikuš 1998). The knowledge of detailed mineralogical and chemical compositions of solid phases is crucial for the assessment of hazardous properties in solid waste materials (Ettler *et al.* 2012). The composition of historical smelting slags depends on the composition of the ore and the fluxes used (Lottermoser 2002). Slag heap is exposed to atmospheric agents such as precipitation, oxygen, carbon dioxide, wind that lead to the erosion of the smelters slag. Crucial leaching of PTE to the environment is mainly due to the erosion of this material. Intensive infusion containing significant amounts of contaminants is extracted on the surface of the slag heap and run-off into the deeper parts of the heap. This mechanism leads to the formation of solutions containing a concentration of PTE (Trdlička 1963).

3.5 Potentially toxic elements and Plants

Bare plains and dumps which remain non-colonized by plants are a common feature among industrial areas; areas with active mining history, or places which served as a storage of waste materials. One of the reasons for plant environment devastation is the excess of PTE released into the soil, water and surroundings of the waste. In addition to the negative implications that PTE have on the vegetation with regards to phytotoxicity of elements, consideration must be given to the other physical and chemical conditions within the slag which may inhibit the successful growth and reproduction of plants (Ash 2012).

Some other problems connected mainly with smelter slag exposure were observed in the study area of this paper. Depositing of the smelter slag material could dramatically influence and change the pH character of the soil. Most of the organic matter is washed away from ores during the process; the rest is destroyed during the smelting. Lack of organic matter and essential nutrients such as nitrogen and phosphorous are a result of this process. Particle size of smelter slag is commonly greater than few tens of millimeters which cause rapid seepage and thus the immediate outflow of water and nutrients. Therefore, the nature and size of smelter slag particles makes water holding capacity and nutrient availability negligible. Absence of vegetation means no input of plant material and organic matter. Thus, there will be little mineralization and degradation of compounds which would otherwise serve as a source of nutrients for a microorganism community (Ash 2012).

However, there are plant species which are tolerant to some metals in soils. *Thlaspi* species naturally adapted to metal rich soils in areas of Pb-Zn mineralization in Europe and are hyper-accumulators of Pb and Zn (Alloway 1990). Zinc is an important trace element, essential for the majority of living organisms. However at higher concentration it is toxic toward animals and mainly plants. Inhibition of growth of the plants can be observed in areas where content of zinc in the soil was escalated by industry or storage of unsecured waste which was rich in zinc. Excess accumulation of Zn in plant tissues causes alterations in vital growth processes such as photosynthesis and chlorophyll biosynthesis (Stoyanova and Doncheva 2002).

Thlaspi species have been found to accumulate concentrations of over 3% Zn and 0.8% Pb (Alloway 1990). Increasingly, the spontaneous or assisted establishment of vegetation on contaminated soils is regarded as a commonsense mitigation measure since it helps reduce the

dispersion of contaminants by wind or water erosion, runoff and water percolation through the substrate (Vangronsveld et al., 1995). Purposeful and controlled planting and growth of *Thlaspi* species could help to reduce amount of zinc and lead available into the environment.

3.6 History of mining in the Kutná Hora region

Some theories suggest that mining in Kutná Hora started in the 10th century. The mining operations were located near to the village of Malin where the Slavnikovecian mint functioned. The real expansion of exploitation started in the second half of the 13th century, probably due to contributions of Cistercians from the monastery in Sedlec (Pauliš and Mikuš 1998). The town of Kutná Hora became one of the richest cities in the Czech Lands and was set to equal Prague in the beginning of 14th century. These circumstances were due to advanced technology and good organization of mining of silver ore. Czech Lands flourished thanks to the wealth generated from silver mines. The amount of silver was sufficient enough to allow the minting of Prague Groschens. Shafts became deeper and exploitation greater during the 14th and 15th century. These shafts were from 50 to 80 meters long and up to 75,100 meters deep. In special cases the depth reached to 180 meters. Some shafts reached the depth of 450 meters and the production of silver ranged from 5-6 tons per year in the beginning of the 14th century. This industry employed from 1000 to 2000 people. Significant enlargement was recorded at the end of 14th century. At the peak of production, 6 to7 tons of silver were smelted annually. Also 50-100 tons of copper was gained per year.

Processing of crushed ores took place in smelters. Rivers and streams were an important factor and source of power for the smelting process, therefore the largest smelters were located in the Vrchlice and Bylanka river valley. Smelter slag, the unwanted by-product of the smelting process was accumulated on heaps in surrounding areas. The dump of smelter slag in the Vrchlice river valley is a point of particular interest with regard to it being a source of high levels of risk elements, and serves as the study site in this research paper (Figure 3.1). The operation of smelters required the use of pyritic sulphides (from northern zones) and lead, which served for the segregation of silver from roasted ores or from raw "black" copper, which contained 1-2% of silver (Pauliš and Mikuš 1998).

Evidence of mining and heavy industry are present all around Kutná Hora. Tons of ore and other material were mined and processed in this region. A lack of knowledge in the field of

organic chemistry, risk elements, toxicity and environmental sciences resulted in dumping and depositing of processed material and industrial waste without any treatment or care. Smelter slag was piled up near to smelters or used as a material for construction of medieval roads or spread around or incorporated into the fields. Therefore scientists from the Czech Republic have collected samples, examined and analyzed the geological, hydrological, biological, pedological and other aspects of human behavior from the past (e.g. Szaková et al. 2009; Kierczak et al. 2013).
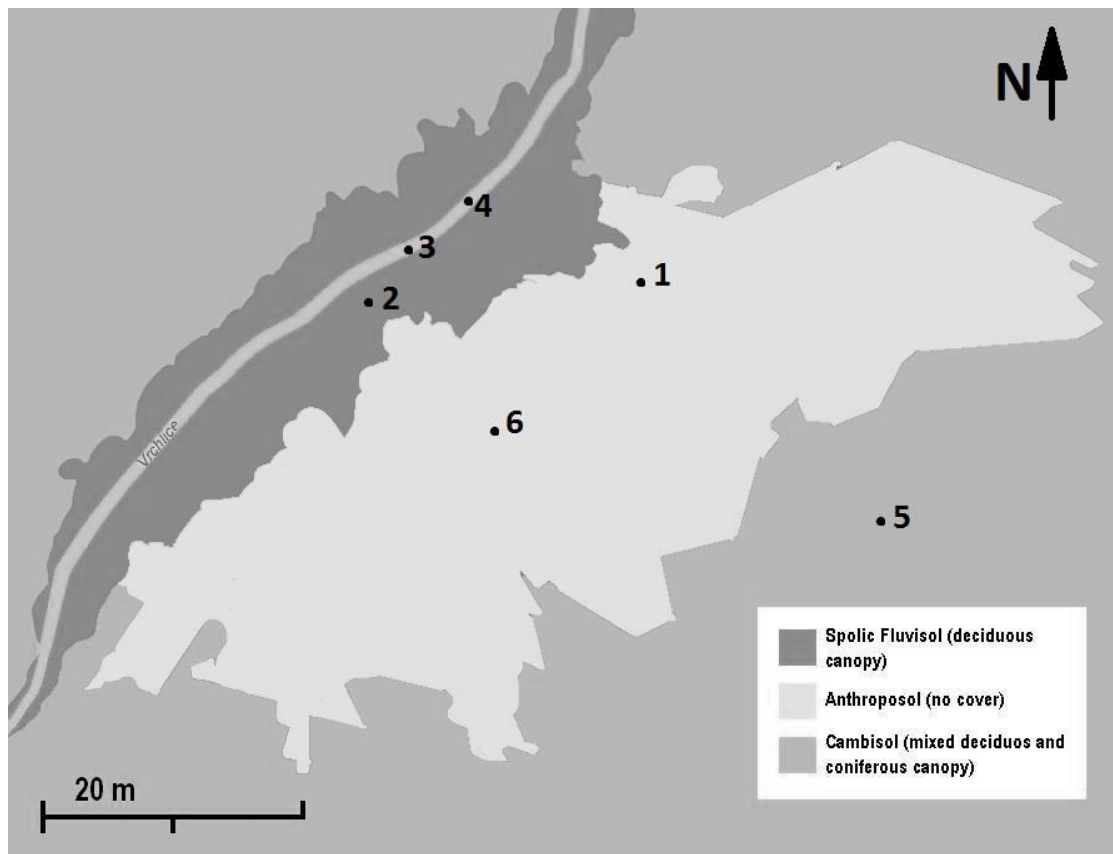

Figure 3.1 Map of sample site showing sample collection points 1: slag, 2: river bank soils, 3 + 4: river sediment, 5: cambisol forest soil, 6: weathered slag

Figure 3.2 View of the studied slag heap from the eastern side

3.7 Possibilities of PTE extraction from polluted soils

The improper management of sources of PTE contamination such as smelter slag can result in their transport into the environment through a variety of pathways. Some of the most typical pathways are leaching into the soil, erosion of the smelter slag, wind transport and deposition of eroded particles, direct run-off …etc. Due to the different sources of contaminants, different pathways of entrance to the soil and different forms of presented PTE in the soil possess different mobility and availability to organisms. From the environmental point of view more important are exchangeable values of the PTE than their total amount. The metals

bound in silicates and other compounds with low mobility and exchangeability can be assumed to be relatively harmless (Kabata-Pendias 2001).

3.7.1. Nitric acid extraction and aqua regia digestion

The first step of monitoring the contaminated area is to determine whether the total metal content is within the range of background levels or over the concentration limits according to the national legislation. In the latter case, a further step is to assess the mobility rate of the PTE in the soil-plant system for a better evaluation of the environmental impact of a contamination event (Kubota 1983). Digestion with 2M HNO_3 is a method commonly used by soil researchers to approximate the "releasable" metal content in soils. This does not include the metals fixed within the minerals e.g diffused metal species and those bound in the silicates. Use of hydrofluoric acid is essential for determining the total metal content according to Sastre *et al* (2002). However, the use of HF leads to long, dangerous and cumbersome schemes and its use is not recommended for routine analyses (Sastre *et al* 2002). Thus aqua regia seems to be a better alternative.

3.7.2. Total digestion

Total digestion is one of the most important procedures for assessment of the studied contaminated area. However, it does not describe the availability and exchangeability of PTE, which have key implications for the environment. Total digestion exposes the total amount of PTE present in the soil sample, which is an essential piece of knowledge for further studies of the sample. There exist three common but different methods used by scientists to obtain total metal content. The first possibility is acid digestion which is used when conversion of the sample from solid to solution is mandatory for further analyse. Strong inorganic acids are used for this purpose. Sastre *et al* (2002) state that the use of HF is essential for the total extraction of PTE. HF is usually mixed with other inorganic acids such as H_2SO_4, H_3BO_3, HNO_3, HCl, Krakovská and Kuss (2001). This solution is heated for several hours.

The second option is melting. This type of digestion represents an effective method for the analysis of geo materials. Na_2O_2, Na_2CO_3, K_2CO_3, NaOH, and KOH are some of the most frequently used chemical agents in melting (Šulcek and Povondra 1989).

The use of microwave radiation for direct increase of pressure and temperature in the mineralization vessel is a relatively new method, which demands investment in equipment. The sample and reacting agents (usually acids) are placed into a compressive vessel and heated by microwave radiation. This technique is mostly used to analyse samples rich in organic matter.

3.7.3. Other possibilities for PTE extraction

A common and useful method is known as the 4 stage BCR extraction. This method allows scientists to determine the percentage of operationally defined binding phases in which the PTE are present in the soil. According to Tokalioglu et al. (2003) the first stage is focused on exchangeable metals and the soil is extracted by 0.11M acetic acid. The second stage exposes the iron and manganese oxide bound metals. The residue of the first stage is extracted by 0.1M hydroxylammonium chloride usually adjusted to pH 2 with nitric acid. The third stage examines metals bound to organic matter and sulphides. 8.8M hydrogen peroxide digestion is applied in this step at the temperature of ~100°C. The fourth stage exposes the residual metals. The soil sample is digested by a mixture of 35% HCl and 65% nitric acid in the ratio 2.334 to 7 at the temperature ~150°C Smeda and Zyrnicki (2002).

4. Sampling, materials and methods

4.1 Sampling

4.1.1. Study area description and sampling strategy

The heap of slag, deposited for centuries to the southern side of the Vrchlice River valley near Vrbuv mill, represents a source of contaminants into the local environment. PTE are distributed within the soil profiles, the river, and present a threat to surrounding vegetation due to water erosion, weathering, water run-off, infiltration, wind deposition and other natural mobilizing processes. All these aspects lead to uneven distribution of contaminants within the soil profiles and the slag in general; this was consideration was made during the formation of a sampling strategy.

In order to be able to compare extracting agent's efficiency different soil samples with different soil properties and level of contamination were needed. Therefore the samples were

collected from the slag heap, mixed forest above the heap, deciduous forest below the heap and river sediment.

Sampling pits were dug on the slag heap as well as in the slope between the heap and the river and the slope above the heap. These pits were made by digging with a shovel to the depth necessary to expose the B horizon then a sample of soil without slag fragments was taken. Soil samples were collected from 6 different positions within the study area. Samples No. 1 and No. 2 were collected from the first pit (see figures in appendix). Sample No. 1 was taken from the thin organic level, basically grasses and mosses growing on the bottom of the slag heap. Soil sample was shaken from the root system of grasses and mosses into the plastic bag. Sample No. 2 was taken from level below, where was a mixture of the slag and decayed residues of grass roots, wind deposited material and eroded slag eventually. Pieces of slag were removed by hand and the soil sample was kept in a labeled plastic bag. Sample No.3 was collected from the slope below the slag heap. The slope is covered by deciduous canopy. Soil was visibly rich in organic matter and interlaced by tree roots. Samples No. 4 and No. 5 were collected in the river itself; river sediment was collected by sifting surface deposits with a trowel. Samples No. 6 and No. 7 were taken from the pit No. 5. This pit was dug in the mixed forest above the slag heap. Sample No. 6 was taken from the organic layer of the soil by shovel. Sample No. 7 was taken from Bv horizon. Samples No. 8 and No.9 were taken from the pit No. 6 which was located on the slag heap, specifically on the heap pathway. Digging the pit exposed 2 horizons. Sample No. 8 was taken from the upper horizon and sample No.9 was collected from the horizon B.

Figure 4.1 Sample pit of the path (left) and sample pit of the slope (right)

Figure 4.2 River Vrchlice where sediment samples were taken (left) and the forest sample pit showing horizons L, O (F +H), A and B (right)

Figure 4.3 Sample pit which was prepared on the main slag heap

4.1.2. Sample preparation

On return to the laboratory samples which were transported in plastic bags were opened to air dry until constant weight. The samples were sieved to 2 mm, and then relabeled. 10 grams of each sample were ground to a fine powder in an agate grinding mill. This fine powder was used for total digestion and determination of total metal content.

4.2. Methods

The samples were examined to obtain basic soil properties. It was decided to examine soil pH and soil organic matter as some of the most important factors influencing the mobility and exchangeability of PTE in the soil. Extractions were focused on cadmium, cooper, lead and zinc, the elements suspected of their toxicity. Values of these PTE were found exceeding already in previous studies of this site.

Two samples were collected from the river sediment to examine the potential toxicity of the slag heap to the aquatic environment. The slag heap is situated in the drainage basin of the Vrchlice river. A foot of the heap is located just a few meters from the river. Although one

might expect the surface sediments to be laden with elevated concentrations of PTE, the distribution of elements in a sediment profile are not uniform, but consist of a series of peaks and troughs in elemental compositions, corresponding with periods of industrial activity, abnormally high atmospheric depositions, and in certain cases, weapons testing (Ciszewski *et al.* 2008). As the slag heap was piled centuries ago, the most radically polluted sediments were probably buried. Nevertheless elevated concentration of PTE in the surface of the river sediment would confirm constant leaching and potential harm of the PTE to the environment.

4.2.1. Soil pH

10 g of soil sample was weigh into a beaker. Deionised water was boiled for 10 minutes to get rid of carbon dioxide. 50 ml of boiled deionised water was added to each beaker with soil sample. Suspension was stirred by a glass stick for 5 minutes. Electrode was submersed into the blend and pH was recorded after reaching of equilibrium point. Measurement was made by WTW inolab pH level 1, pH probe.

4.2.2. Organic matter content

Tyurin's method (1931, 1936) is one of the modifications of the volumetric method for determination of the organic carbon in the soil by oxidation with $K_2Cr_2O_7$ in strong acid medium until formation of CO_2 according to the equation:

$$2 K_2Cr_2O_7 + 8 H_2SO_4 = 2K_2SO_4 + 2Cr_2(SO_4)_3 + 8H_2O + 3O_2$$

$$3C + 3O_2 = 3CO_2$$
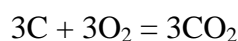
The mixture is then titrated with Mohr salt to determine the amount of $K_2Cr_2O_7$ which did not react with soil organic matter. It is possible to then deduce the amount of soil organic matter which was present in the sample (the amount which was oxidized in the oven).

Soil samples were sieved through 0.2 mm sieve. Sieved samples were stored in labeled plastic bottles. 0.3 g (in the case of organic soils 0.2 g) of a sample was weight into a 150 ml beaker and 10 ml of $K_2Cr_2O_7$ was added. The suspension was stirred by a glass stick. Beakers were covered by a watch glass and put into the oven for 45 minutes at the temperature of 125 degrees Celsius. The beakers with samples were removed from the oven cooled and sides of

the beaker as well as the watch glass with distilled water. Level of water was adjusted by distilled water and the conduct meter was immersed into the solution. Solution was titrated with Mohr salt $(NH_4)_2Fe(SO_4)_2$ till the equilibrium was reached. Amount of Mohr salt was recorded and organic matter was calculated according to following formulas:

1. Factor of Mohr salt:
$$f = \frac{40}{a}$$

Where a is determined by titration of acid dichromate (amount needed to reach equilibrium when blank sample was titrated.

2. Cox- oxidizable carbon:
$$Cox = (12 - 0,3 \cdot S \cdot f) \cdot \frac{100}{n}$$

Where S is amount of Mohr salt used in the titration in ml and n is amount of soil sample used in mg.

3. Humus content:
$$\% humus = Cox \cdot 1,724$$

4.2.3. PTE content determination

4.2.3.1. 2M nitric acid digestion

280 ml of 65% nitric acid was measured by measuring cylinder and poured into 2 liter measuring flask. The flask was filled to the line by the deionised water resulting in a 2M HNO_3.

Five grams of soil sample was weigh into a plastic beaker. Fifty milliliters of 2M nitric acid was added. A common procedure of 6 hours shaking of the soil sample and nitric acid was implemented. The suspension was centrifuged for 11000 rotations per minute for 5 minutes. Supernatant was filtrated into a plastic bottles labeled and kept for ASS measurement. 6 hours shaking is generally accepted method to extract all available PTE that are able to pass into the nitric acid. However the experiment compared the results for the half of a time and for 10 minutes. The same amount of soil was weigh again into plastic beaker with 50 ml of 2M nitric acid and shaken for 3 hours and 10 minutes to approximate speed of PTE extraction. Each procedure was repeated 3 times. Result is an average of these three values.

Concentration of following PTE was measured: cadmium, lead, cooper and zinc. Concentration of zinc exceeded measureable values therefore the filtered supernatant had to be diluted. 0.5 ml of supernatant was diluted with 50 ml of deionised water and zinc concentration was measured again.

4.2.3.2 Aqua regia digestion

Aqua regia digestion is a procedure which allows discovering "pseudo" total PTE content. However some authors (Borůvka and Drábek 2000, Tlustoš et al. 1994) did not find a significantly higher ability of aqua regia to extract PTE from the soil compared to 2M nitric acid. Therefore the comparison of these two methods is one of the goals of this thesis.

1 gram of soil sample was weigh into a Teflon crucible. 7 ml of 35% hydrochloric acid and 2.334 ml of 65% nitric acid was added. Suspension was stirred with a glass stick and closed. The teflon crucible was put on the stove and heated to 190 degrees Celsius for 6 hours. The crucible was opened and cooled after 6 hours heating and suspension was filtered thru filtering paper. Obtained extract was too concentrated therefore was diluted. One ml of extract was pipetted into a plastic bottle and 49 ml of distilled water was added. This solution was labelled and kept for measurement.

4.2.3.3 Total PTE content

Extraction by a mixture of hydrofluoric acid and nitric acid is a method used for discovering total metal content. This strong and aggressive solution breaks all bounds in the soil and extracts the highest possible percentage of PTE.

Soil samples were ground in agate mill till very fine powder. Powder was stored in plastic bottles and labelled. Ground soil sample was weight into a teflon crucible at the mass of 0.5 gram. Two ml of 40% hydrofluoric acid and 3 ml of nitric acid was added to the sample into the crucible. The crucible was closed and put to the stove to heat for 4 hours at the temperature of 190 degrees Celsius. The samples were open and the next day were heated opened on the stove at the temperature of 190 degrees Celsius under the extractor till the

slurry was accomplished. One ml of nitric acid was added to the slurry to dissolve all possible remaining fragments of soil. 49 ml of deionised water was added into the crucible and the whole volume was filtered thru the filtering paper into a plastic bottle, labelled and stored for measurement.

4.2.3.4. Coca Cola extraction

The Coca Cola extraction was chosen rather by curiosity than by any scientific background. Coca Cola is generally considered to be unhealthy in the human nutrition, being known for low pH and content of phosphates. There in not much information in scientific publications about Coca Cola extraction of PTE from polluted soils. Therefore the methodology was adjusted and done similarly to the 2M nitric acid digestion.

Soil samples were weigh into a plastic beaker at the mass of 5 grams. Coca Cola was added to the beakers at the volume of 50 ml. The beaker was closed and put into the shaker to be shaken for 6 hours. Plastic beakers were centrifuged at 11000 rotations per minute for 5 minutes after the shaking. The supernatant was filtered thru filtering paper into the plastic bottle, labelled and kept for measurement. The process was repeated 3 times and the result is an average of 3 values. The amount of zinc was to high to be measured on that account the solution had to be diluted. Volume of 0.5 ml of Coca Cola extract was measured into a plastic bottle and diluted by 50 ml of deionised water. This plastic bottle was labelled and kept for measurement of zinc concentration.

5. Results

5.1 Basic soil properties

Table 5.1 soil pH and oxidizable carbon percentage of soil and sediment samples

	pH	C_{ox} (%)
1 path A	5.28 ± 0.03	3.70
1 path B	5.11 ± 0.01	6.45
2 slope	4.49 ± 0.04	a.d.l
3 sediment	7.37 ± 0.06	0.30
4 sediment	7.36 ± 0.07	0.27
5 forest A	3.98 ± 0.02	a.d.l
5 forest B	3.77 ± 0.03	1.89
6 slag A	5.67 ± 0.02	1.43
6 slag B	5.33 ± 0.07	2.98

a.d.l = above determination limit. Note: C_{ox} are single determinations

Table 5.1 lists the values obtained for pH and content of oxidizable carbon (C_{ox}). Despite reducing the mass of soil used, some values of C_{ox} were unobtainable, particularly for samples which contain a high content of organic matter. This problem generally applies to forest soils and is due to the sensitivity of the oxidimetric titration method; the endpoint of the titration occurs before the titration has effectively begun, due to excess reaction of the acid dichromate with organic matter.

5.2 Content of PTE in studied extractions

Table 5.2a. Extraction results (means from 3 replicates) for samples 1 - 4

		Cd		Cu		Pb		Zn	
Sample	Extraction	Mean	blank	Mean	blank	Mean	blank	Mean	blank
1 path A	2M HNO$_3$ 10 min.	4.40 ±0.30	b.d.l	1377 ±72.9	b.d.l	315 ±17.9	b.d.l	1777 ±45.2	b.d.l
	2M HNO$_3$ 3 hr.	4.18 ±0.13	b.d.l	1358 ±23.1	0.03	368 ±10.4	b.d.l	2351 ±22.3	b.d.l
	2M HNO$_3$ 6 hr.	4.99 ±0.57	n.d	1715 ±170	n.d	447 ±46.1	n.d	2457 ±148	n.d
	aqua regia	2.37	n.d	206	n.d	413	n.d	517	n.d
	total digest	14.6	b.d.l	2580	b.d.l	1177	b.d.l	13327	b.d.l
	coca-cola	1.21 ± 0.04	b.d.l	134 ±9.22	0.04	9.97 ±1.50	4.16	653 ±36.2	b.d.l
1 path B	2M HNO$_3$ 10 min.	3.37 ±0.51	b.d.l	1307 ±156	b.d.l	250 ±19.0	b.d.l	1665 ±201	b.d.l
	2M HNO$_3$ 3 hr.	4.40 ±1.31	b.d.l	1328 ±47.2	0.03	359 ±55.8	b.d.l	2478 ±93.0	b.d.l
	2M HNO$_3$ 6 hr.	3.89 ±0.14	n.d	1892 ±59.0	n.d	405 ±12.7	n.d	2704 ±39.1	n.d
	aqua regia	12.3	n.d	4913	n.d	1997	n.d	9679	n.d
	total digest	11.2	b.d.l	2834	b.d.l	1258	b.d.l	17823	b.d.l
	coca-cola	1.47 ± 0.18	b.d.l	591 ±155	0.04	27.2 ±7.51	4.16	850 ±138	b.d.l
2 slope	2M HNO$_3$ 10 min.	1.05 ±0.23	b.d.l	116 ±10.3	b.d.l	59.7 ±4.39	b.d.l	546 ±112	b.d.l
	2M HNO$_3$ 3 hr.	0.45 ±0.09	b.d.l	112 ±0.93	0.03	57.1 ±1.40	b.d.l	548 ±28.1	b.d.l
	2M HNO$_3$ 6 hr.	1.54 ±0.02	n.d	421 ±37.2	n.d	124 ±2.61	n.d	1036 ±28.3	n.d
	aqua regia	2.85	n.d	628	n.d	251	n.d	3098	n.d
	total digest	1.84	b.d.l	307	b.d.l	135	b.d.l	1726	b.d.l
	coca-cola	0.21 ±0.07	b.d.l	7.05 ±0.45	0.04	15.1 ±5.02	4.16	64.0 ±86.5	b.d.l
3 sediment	2M HNO$_3$ 10 min.	1.98 ±0.02	b.d.l	40.8 ±5.79	b.d.l	62.0 ±14.9	b.d.l	560 ±204	b.d.l
	2M HNO$_3$ 3 hr.	1.49 ±0.14	b.d.l	50.5 ±3.62	0.03	72.0 ±12.6	b.d.l	984 ±96.5	b.d.l
	2M HNO$_3$ 6 hr.	1.89 ±0.06	n.d	222 ±62.7	n.d	84.6 ±7.00	n.d	866 ±18.7	n.d
	aqua regia	1.90	n.d	152	n.d	52.2	n.d	1850	n.d
	total digest	3.06	b.d.l	188	b.d.l	184	b.d.l	3478	b.d.l
	coca-cola	1.13 ±0.08	b.d.l	14.0 ±1.29	0.04	7.90 ±1.57	4.16	400 ±22.2	b.d.l
4 sediment	2M HNO$_3$ 10 min.	1.49 ±0.11	b.d.l	25.8 ±3.33	b.d.l	42.9 ±6.86	b.d.l	705 ±57.8	b.d.l
	2M HNO$_3$ 3 hr.	1.00 ±0.07	b.d.l	33.0 ±3.88	0.03	61.6 ±12.3	b.d.l	1002 ±31.5	b.d.l
	2M HNO$_3$ 6 hr.	1.56 ±0.05	n.d	127 ±49.0	n.d	82.1 ±15.2	n.d	940 ±79.9	n.d
	aqua regia	0.95	n.d	532	n.d	123	n.d	2926	n.d
	total digest	2.65	b.d.l	225	b.d.l	219	b.d.l	5123	b.d.l
	coca-cola	0.83 ±0.11	b.d.l	9.53 ±4.15	0.04	9.43 ±5.54	4.16	374 ±113	b.d.l

b.d.l = below determination limit, n.d = no data

Tables 5.2a and 5.2b show the data for all of the studied PTE in each extraction for each sample. The obtained results are generally highly satisfactory with small standard deviations around 3 replicates for each extraction. For the most part, the blank samples reflect good laboratory practice. For the 2M HNO$_3$ extractions only 1 of the blank sample appears to be contaminated (0.03 mg/L of Cu at 3hrs shaking). However, the small value does not impose a significant bias on the results. For 2 of the blank coca-cola samples we observed 0.04 mg/L Cu and 4.16 mg/L Pb. The content of Pb was unexpected and initially it was suspected that it

was due to contamination by the lab equipment. However, all three of our blank replicates confirmed the presence of small amounts of Pb in the cola (4.16 ± 1.29).

Table 5.2b. Extraction results (means from 3 replicates) for samples 5 − 6

Sample	Extraction	Cd Mean	Cd blank	Cu Mean	Cu blank	Pb Mean	Pb blank	Zn Mean	Zn blank
5 forest A	2M HNO$_3$ 10 min.	1.90 ±0.08	b.d.l	13.3 ±3.48	b.d.l	133 ±5.07	b.d.l	b.d.l ±0	b.d.l
	2M HNO$_3$ 3 hr.	1.88 ±0.09	b.d.l	14.9 ±0.94	0.03	134 ±4.70	b.d.l	b.d.l ±0	b.d.l
	2M HNO$_3$ 6 hr.	2.19 ±0.10	n.d	7.26 ±0.75	n.d	163 ±3.99	n.d	32.3 ±5.62	n.d
	aqua regia	2.85	n.d	26.1	n.d	294	n.d	379	n.d
	total digest	3.16	b.d.l	62.3	b.d.l	170	b.d.l	167	b.d.l
	coca-cola	0.43 ±0.26	b.d.l	5.43 ±0.50	0.04	4.13 ±0.21	4.16	b.d.l ±0	b.d.l
5 forest B	2M HNO$_3$ 10 min.	0.16 ±0.14	b.d.l	14.4 ±0.48	b.d.l	287 ±11.4	b.d.l	b.d.l ±0	b.d.l
	2M HNO$_3$ 3 hr.	0.16 ±0.05	b.d.l	14.5 ±0.12	0.03	292 ±3.52	b.d.l	b.d.l ±0	b.d.l
	2M HNO$_3$ 6 hr.	0.14 ±0.01	n.d	7.18 ±0.96	n.d	319 ±4.73	n.d	b.d.l ±0	n.d
	aqua regia	b.d.l	n.d	164	n.d	465	n.d	359	n.d
	total digest	1.43	b.d.l	48.6	b.d.l	341	b.d.l	178	b.d.l
	coca-cola	0.12 ±0.11	b.d.l	6.76 ±1.03	0.04	150 ±63.9	4.16	b.d.l ±0	b.d.l
6 slag A	2M HNO$_3$ 10 min.	3.56 ±0.19	b.d.l	561 ±31.1	b.d.l	391 ±10.5	b.d.l	2298 ±288	b.d.l
	2M HNO$_3$ 3 hr.	5.20 ±0.01	b.d.l	760 ±23.7	0.03	332 ±5.73	b.d.l	4852 ±189	b.d.l
	2M HNO$_3$ 6 hr.	5.64 ±0.10	n.d	951 ±51.2	n.d	395 ±9.46	n.d	5642 ±121	n.d
	aqua regia	11.4	n.d	1818	n.d	854	n.d	13796	n.d
	total digest	11.3	b.d.l	1615	b.d.l	798	b.d.l	23095	b.d.l
	coca-cola	3.22 ±0.19	b.d.l	499 ±66.0	0.04	38.6 ±17.0	4.16	2936 ±119	b.d.l
6 slag B	2M HNO$_3$ 10 min.	5.17 ±0.44	b.d.l	753 ±60.6	b.d.l	259 ±19.8	b.d.l	3388 ±66.4	b.d.l
	2M HNO$_3$ 3 hr.	3.61 ±0.04	b.d.l	588 ±8.65	0.03	438 ±11.6	b.d.l	3847 ±548	b.d.l
	2M HNO$_3$ 6 hr.	3.80 ±0.09	n.d	742 ±47.9	n.d	511 ±7.78	n.d	3111 ±31.0	n.d
	aqua regia	9.01	n.d	1632	n.d	1100	n.d	10262	n.d
	total digest	9.28	b.d.l	1245	b.d.l	885	b.d.l	18640	b.d.l
	coca-cola	2.00 ±0.04	b.d.l	337 ±5.04	0.04	73.8 ±7.40	4.16	1264 ±18.0	b.d.l

b.d.l = below determination limit, n.d = no data

6. Discussion

6.1 PTE in the studied samples

According to soil guideline values (SGV) for non-sandy type agricultural soils (Ministry of Environment 13/1994 sb), in a 2M HNO$_3$ extract after 6 hours of shaking we can see that the majority of the samples are strongly contaminated with all of the studied PTE. The general exception is the slope samples (2) and the river sediments (3, 4), which were only lightly contaminated, compared to other samples which are directly impacted by the slag. The forest

samples (5) which were taken uphill at a much greater elevation than the other samples are much less contaminated according to the guideline values.

Cadmium: As expected, samples most contaminated with Cd were the samples taken on the path and slag (as much as 5 x the SGV). The slope samples were only slightly contaminated beyond the SGV after 6 hrs of shaking. Despite the closeness of these samples to the slag (and actual intrusion of slag pieces within the soils on the slope) the levels of Cd are relatively low in comparison to other samples. This is a probable reflection of the low pH (4.49 ± 0.02) of these samples and the permanently moist conditions which would contribute to enhanced leaching of this element. The sediments showed some evidence of contamination by the slag with Cd (up to 190% of the SGV). The deeper horizon of the forest sample (5 B) contained Cd well below the advisable levels however the upper organic layer (5 A) showed an accumulation of Cd up to > 220% of the SGV.

Copper: A similar trend as with Cd can be seen for Cu. The path (1) and slag (6) samples contain excessive concentrations of Cu, > 1892 mg/kg and > 951 mg/kg of Cu respectively. According to 6hrs extract by 2M HNO_3, the slope is contaminated by more than 800% with Cu, a likely response to the strong binding affinity which copper has for organic matter (Kabata-Pendias 2001) (see Table 5.1). The sediments contained not excessive, but above the limit concentrations of Cu, possibly settled with dissolved organic carbon. In even the uppermost layer there was no Cu which occurred above the SGV for the forest samples.

Lead: Pb is one of the most prevalent PTE at the study area; it occurs on average above the SGV in all of our studied samples. Compared to a SGV of 80 mg/kg, the path samples contained Pb > 447 mg/kg, and slag samples contained Pb > 511 mg/kg in the deeper soil layers. After 6 hrs of shaking the slope samples which are relatively unpolluted also contained Pb above the SGV (> 124 mg/kg). It is debatable as to whether or not the sediments can be considered contaminated as the mean value of Pb is on a level with the SGV. However, the SGV is set for agricultural soils. According to Beneš (1993) the concentration of Pb in our sediments exceed the value for uncontaminated sediments by around 1.7 times. Unlike the other studied PTE, the forest samples were considerably polluted with Pb, suggesting a high mobility and deposition of this element by wind.

Zinc: With the exception of the forest samples in which Zn occurred mostly below the determination limit, Zn was also a highly prevalent contaminant at the study area. In the path samples (1) Zn was present in excess of 2700 mg/kg in the 2M HNO_3 extract. Considering a

SGV of 100 mg/kg for Zn in agricultural soils, the slope samples (2) can be considered as somewhat contaminated with Zn present at more than 10 times the guideline. Analysis of the river sediment also revealed a relatively high content of Zn (up to ~1000 mg/kg Zn). The upper layer of the slag sample was particularly enriched in Zn, with values exceeding 5000 mg/kg Zn in the 2M HNO_3 extract.

By inspecting Tables 5.2a and 5.2b we can observe clear differences in the concentrations of PTE extracted by 2M HNO_3 depending on the length of shaking time. These changes in concentration with extraction time are more pronounced for certain contaminants and vary between samples. These results are easier to interpret when illustrated, and so for discussion of the extraction time dynamics, refer to Table 6.1 and Figure 6.1.

6.2 Significance of shaking time for a 2M HNO_3 extract

As mentioned above, there are distinct differences in the extracting strength of the 2M HNO_3 according to the length of shaking time. To attempt to quantify these changes in a relevant format, the percentage change of PTE released from sample for 10 min – 3 hrs, 3 hrs – 6 hrs, and 10 min – 6 hrs is given in table 6.1.

Table 6.1 Mean percentage change of 2M HNO$_3$ extracted PTE between different shaking times

Sample	Extraction time	Cd	Cu	Pb	Zn
1 path A	10min – 3hr	95	99	117	132
	3hr – 6hr	120	126	121	104
	10min – 6hr	113	125	142	138
1 path B	10min – 3hr	131	102	144	149
	3hr – 6hr	88	142	113	109
	10min – 6hr	115	145	162	162
2 slope	10min – 3hr	42	96	96	100
	3hr – 6hr	342	377	216	189
	10min – 6hr	147	362	207	190
3 sediment	10min – 3hr	75	124	116	176
	3hr – 6hr	127	439	117	88
	10min – 6hr	96	544	137	155
4 sediment	10min – 3hr	67	128	144	142
	3hr – 6hr	156	387	133	94
	10min – 6hr	104	493	191	133
5 forest A	10min – 3hr	99	112	101	n.d
	3hr – 6hr	116	49	121	n.d
	10min – 6hr	115	55	122	n.d
5 forest B	10min – 3hr	100	101	102	n.d
	3hr – 6hr	90	50	109	n.d
	10min – 6hr	90	50	111	n.d
6 slag A	10min – 3hr	146	136	85	211
	3hr – 6hr	108	125	119	116
	10min – 6hr	158	170	101	245
6 slag B	10min – 3hr	70	78	169	114
	3hr – 6hr	105	126	117	81
	10min – 6hr	73	99	198	92

Note: percentages are rounded to nearest whole number. Light green refers to ≥ 200% increase, dark green refers to ≥ 200% increase, red refers to % decrease

For most of the samples there is a general increase in PTE dissolution from 10 minutes upwards, Cd is the exception whereby we can observe a slight decrease with extraction time. This is likely to be attributed to the fact that Cd is a relatively mobile element which is more easily released from the solid phase than other metals (Alloway 1990). Therefore, the majority of the Cd is released into the 2M HNO$_3$ solution within the first 10 minutes. Any release that occurs with additional shaking time is likely to be minimal, thus the final Cd concentration in the final extract is likely to be dictated by the heterogeneous distribution of this element in the sample, rather than the increased shaking time. The other somewhat obvious trend is that in the slope sample, there is a considerable increase in release of PTE when extraction time is increased, particularly Cu and Pb. We can attribute this to the locality

of the sample, which came from a permanently moist, acidic river bank; we propose that the fraction of weakly bound/easily exchangeable metals in this soil is small due to the continuous leaching within the river bank. Hence, 10 minutes of extraction time is probably too small to dissolve the majority of the metals, which are likely to be bound in the more stable fractions e.g. reducible and oxidizeable fractions which correspond to those metals bound to the metal oxides and organic matter respectively. Studies by Ash et al. (2014) on the same samples confirm this whereby the exchangeable fraction accounted for < 10% of Cd, and < 1% of Pb from the total fraction. A similar claim could be made about the Cu in the sediment sample; there are unlikely to be any loosely bound Cu in this sample as it is from a permanently submerged location with constant water flow. Consequently it takes more than 10 minutes for the 2M HNO_3 to desorb the more strongly bound Cu in the sediment.

Figures 6.1a to 6.1e illustrate the data for tables 5.1a and 5.1b in bar chart form.

Cadmium: It is difficult to deduce any solid conclusion from the graphs about the effect of extracting time on Cd release. There is a fairly inconsistent trend between 10 min and 3 hrs of shaking , the most significant increase was observed in the upper layer of the slag samples (> 1mg/kg Cd release between 10 min and 3 hrs). However, when we examine the figures between 10 min and 6 hrs, it can be generally concluded that there is an overall increase, with an exception of slag sample B, where there is a definite overall decrease. Again this is attributable to the mobile nature of Cd and to an extent the heterogeneity of Cd concentration in samples.

Copper: In the case of Cu we can identify a general trend that between 10 min and 3 hrs of shaking there is little to no significant change in the amount of Cu extracted. However, with the exception of sample slag B and the forest samples, which are not technically contaminated, we can say with confidence that there is a significant increase in extracted Cu between 10 min – 6 hrs, and also for between 3 hrs – 6 hrs. This was already shown by the mean percentage change in table 6.1 but the small error between the replicate samples confirm the strong difference between extraction times.

Lead: A trend of increasing Pb with increasing extraction time can be confirmed for the studied samples. In a similar overall dynamic to the Cu, there was little difference in the release between 10 min and 3 hrs, but between 3 hrs and 6 hrs there was a definite increase. In the case of Pb, we can conclude that this increased is more pronounced for the organic samples (path, slope and forest). Similar to Cu, lead is an element which has a strong affinity

for organic matter (Kabata-Pendias 2001), and so it is probable that a longer shaking/extraction time is needed for full dissolution of organically bound Pb to occur.

Zinc: The importance of extracting and shaking time is possibly most evident in the case of Zn. The results in Figures 6.1a – 6.1e show very strong evidence of increasing dissolution of Zn with increasing time. In the case of samples with an increased organic matter content, setting an extraction and shaking time of less than 6 hrs will result in an under-estimation of the amount of Zn in the sample.
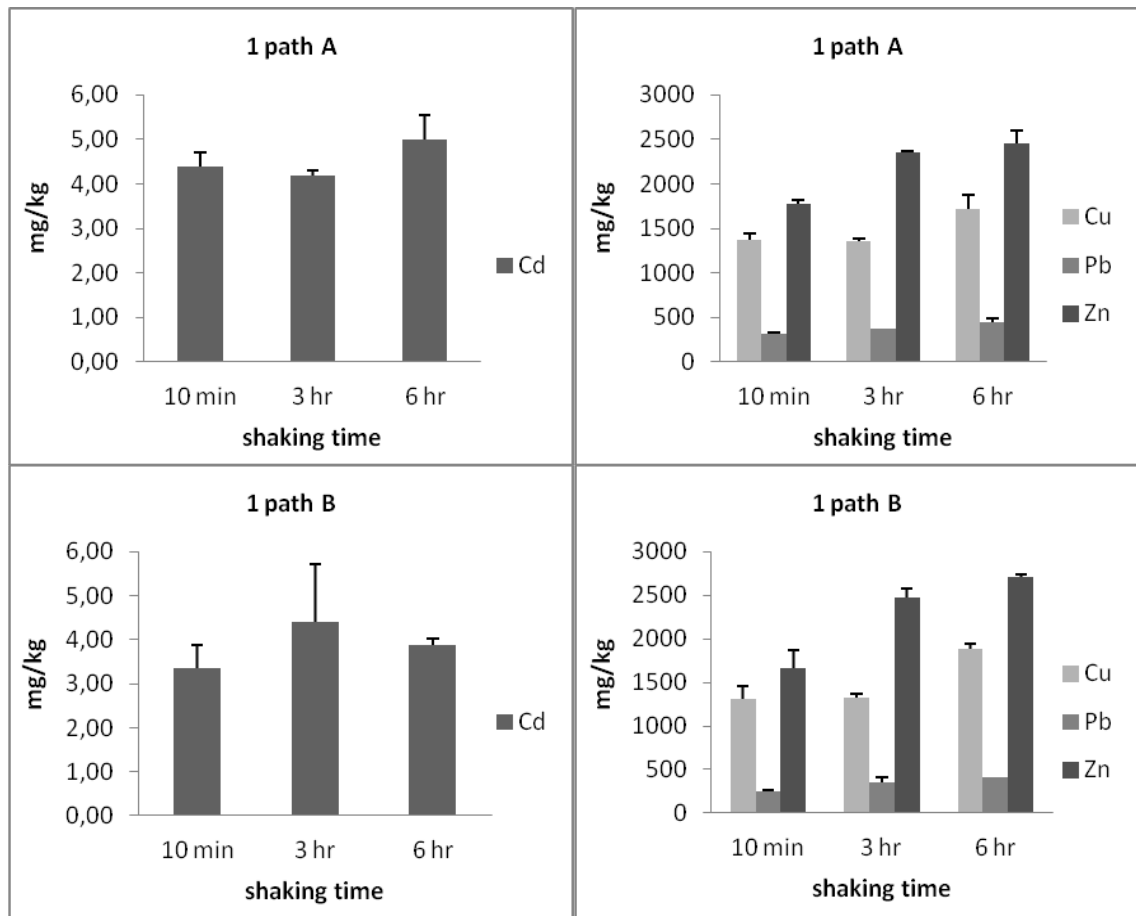
Figure 6.1a Difference in PTE extraction for a 2M HNO_3 solvent for 10 minutes, 3 hours and 6 hours of shaking - sample path (1)
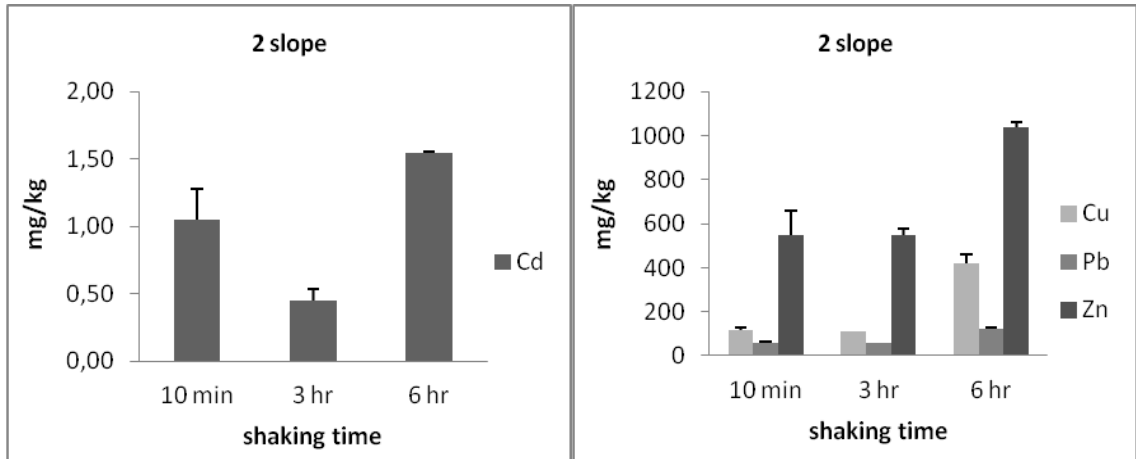
Figure 6.1b Difference in PTE extraction for a 2M HNO$_3$ solvent for 10 minutes, 3 hours and 6 hours of shaking - sample slope (2)
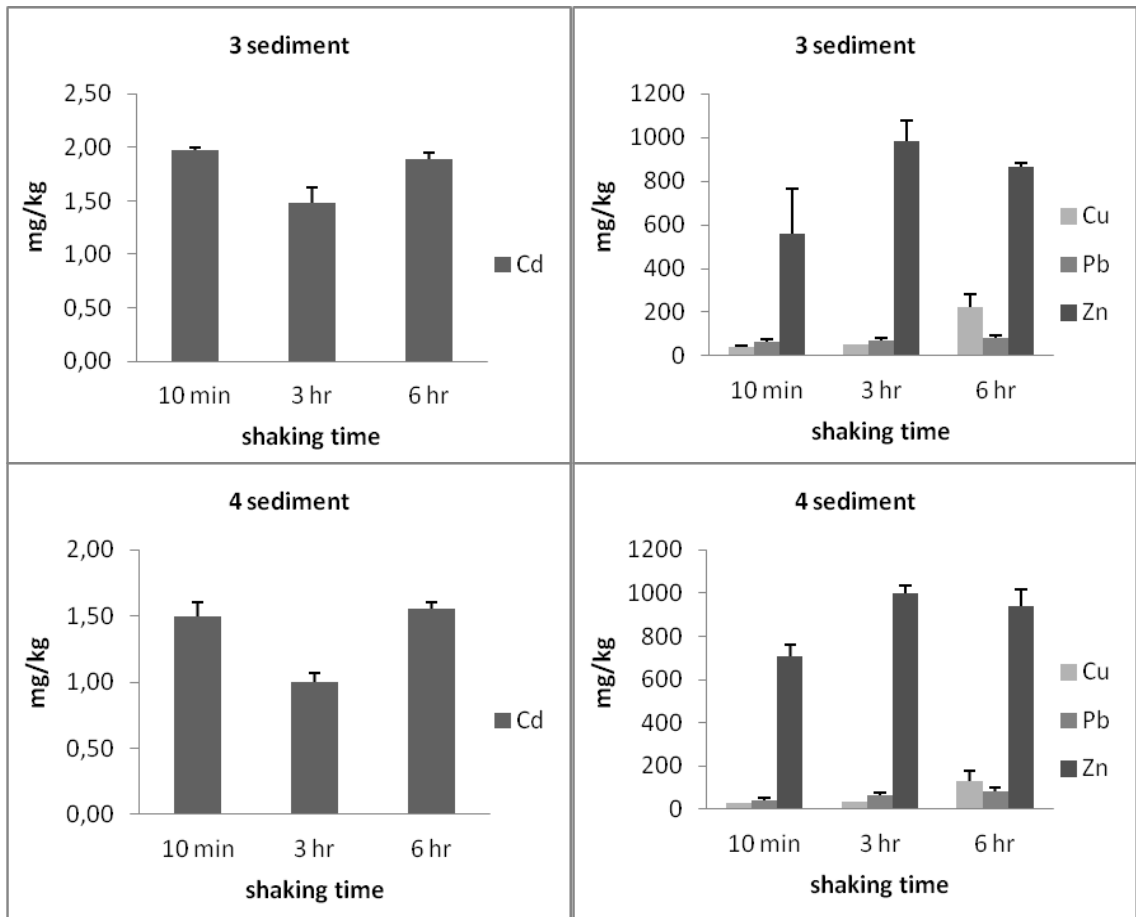

Figure 6.1c Difference in PTE extraction for a 2M HNO$_3$ solvent for 10 minutes, 3 hours and 6 hours of shaking - sediment samples (3, 4)
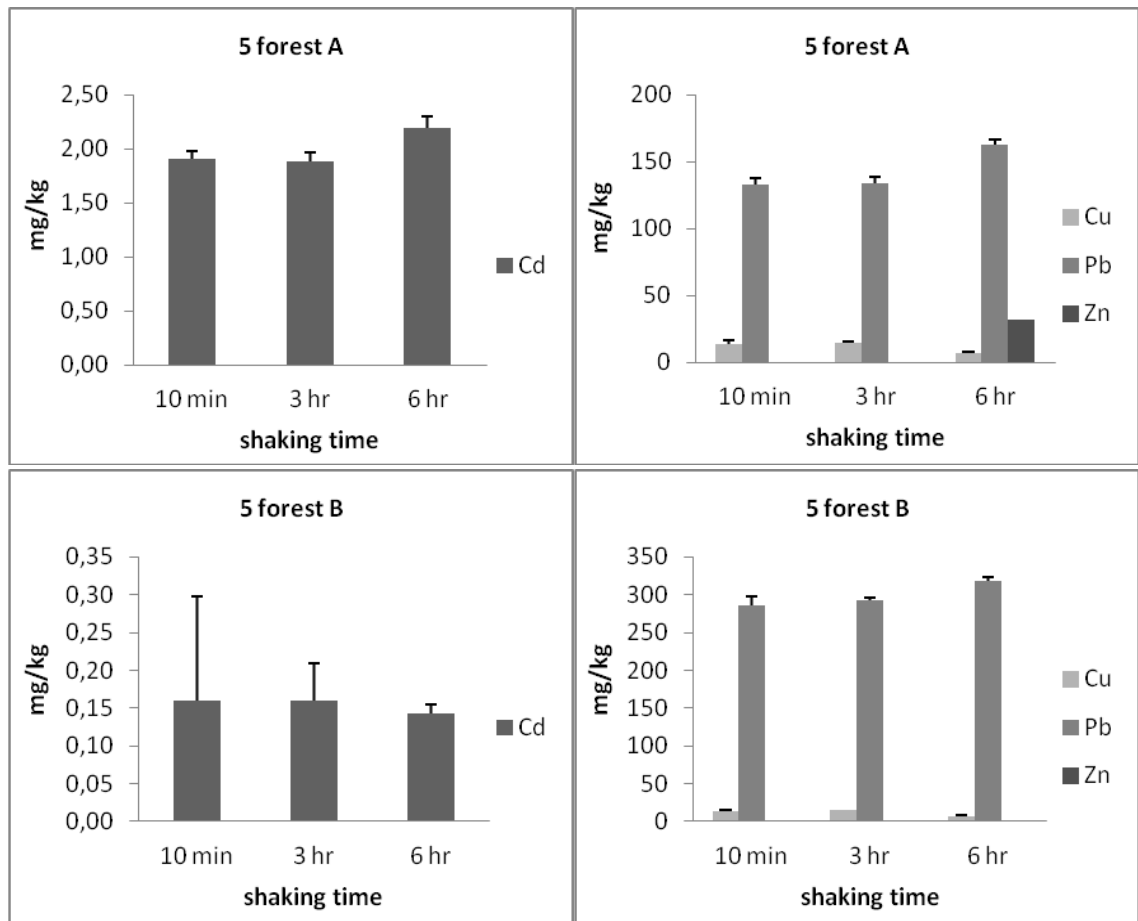
Figure 6.1d Difference in PTE extraction for a 2M HNO$_3$ solvent for 10 minutes, 3 hours and 6 hours of shaking - sample forest (5)
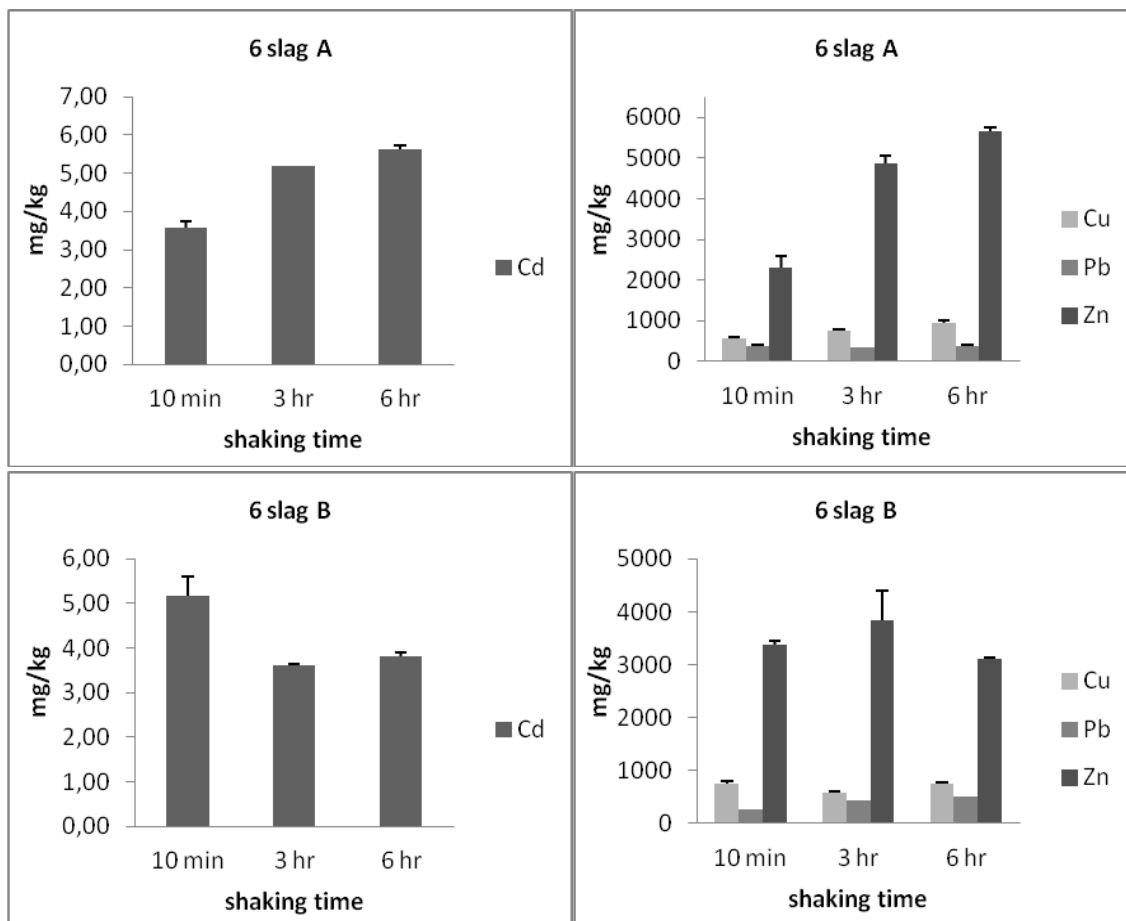
Figure 6.1e Difference in PTE extraction for a 2M HNO$_3$ solvent for 10 minutes, 3 hours and 6 hours of shaking - sample slag (6)

6.3 Comparison between 2M HNO$_3$, aqua regia and total extraction

Although the generally accepted solvent for determining the pseudo total metal content in a soil is aqua regia, a commonly used acid for the same purpose is 2M HNO$_3$. In this section we compare the real differences in extracting power for our four target elements Cd, Cu, Pb, Zn in a range of contaminated soil types between the two extracting agents, and also compare them both to the actual total as determined by heated digestion in a mixture of concentrated acids.
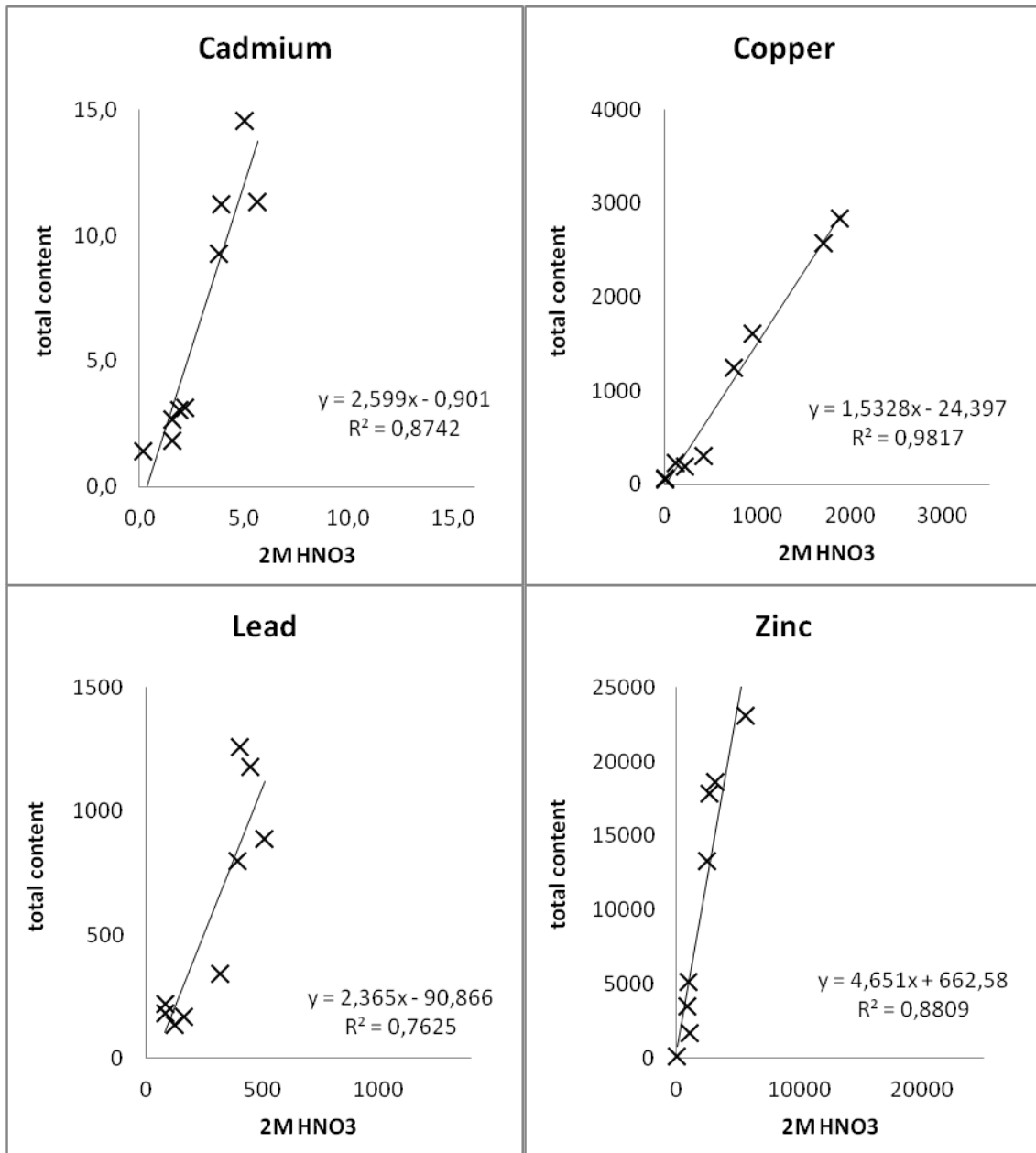
Figure 6.2 Correlation between concentration of PTE extractable by 2M HNO_3 and the total content of PTE in a range of soil sample types

Extraction by 2M HNO_3 is a commonly used method to approximate the content of releasable or reactive PTE within a soil sample. One of the aims of this thesis was to determine how closely this extraction method matches the total content of PTE in a sample. Figure 6.2 shows correlation between PTE in a 2M HNO_3 extraction and the total PTE content. The equation for a linear trend line shows how closely the two extractions fit; a correlation between two of the same extracting agents will theoretically produce y = 1.00x. The more this number increases, the more resistant the PTE is to dissolution in the 2M HNO_3. Our results showed that the studied metals are resistant to dissolving by the 2M HNO_3 extraction method in the

following order: Zn > Cd > Pb > Cu. When studying a particular metal in a soil sample by means of nitric acid extraction, the aforementioned information regarding the closeness to the real total should be considered.
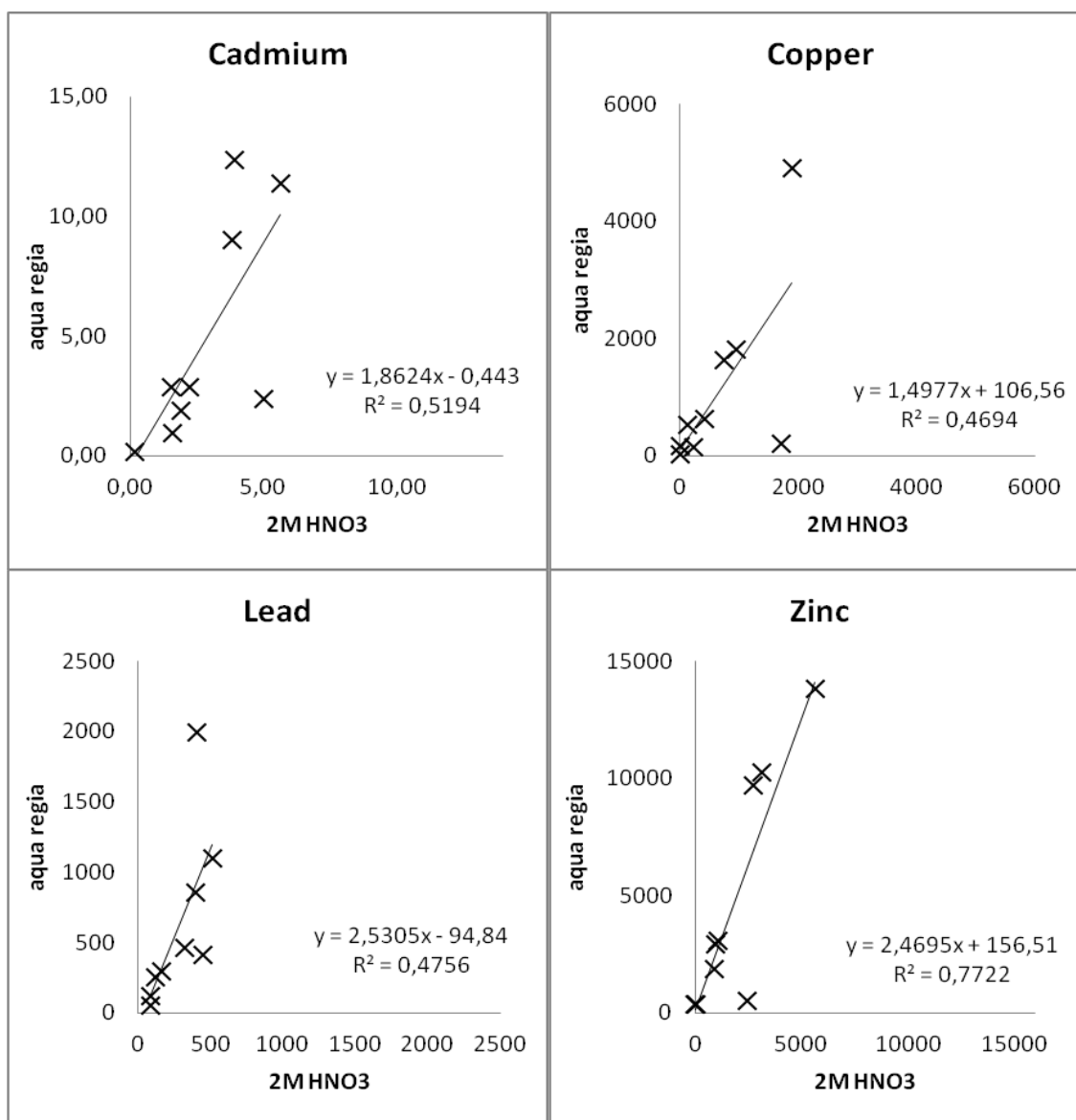

Figure 6.3 Correlation between concentration of PTE extractable by 2M HNO$_3$ and an aqua regia extract in a range of soil sample types

Some scientists consider the 2M HNO$_3$ extraction method as effective as aqua regia digestion. Borůvka and Drábek (2000) examined the pollution in the Litavka river alluvium (The Czech Republic) and compared the results from 2M nitric acid and aqua regia. These authors state that the metal content did not differ significantly between nitric acid extraction and aqua regia digestion for any of the elements under study (cadmium, lead and zinc) and in some cases the results of nitric acid extraction were even slightly higher than results of aqua regia digestion.

Similar results were given by Tlustoš et al. (1994). Tlustoš et al. (1994) attributed this result to the soil heterogeneity and Borůvka and Drábek (2000) indicated that another possibility could be tolerable analytical incorrectness (in the range of 10% maximum); in their case this was mainly due to multiple sample dilution before the measurement (Borůvka and Drábek 2000). The conclusions drawn from our results do not reflect the conclusions of Borůvka and Drábek (2000). In some cases we can say that there are irregularities or discrepancies in the data which may be attributed to inhomogeneous concentrations of PTE within the studied samples or could also be due to analytical incorrectness. However, there is a general and significant trend that the aqua regia method extracts a much greater share of PTE than the 2M HNO_3 method. This trend is expressed particularly strongly for elements Pb and Zn; aqua regia = 2.53 x nitric acid − 94 mg/kg, and aqua regia = 2.47 x nitric acid + 157 mg/kg respectively. Extraction of Cd and Cu was also greater in the aqua regia than in nitric acid but to a lesser extent: aqua regia = 1.86 x nitric acid − 0.44 mg/kg, and aqua regia = 1.5 x nitric acid + 107 mg/kg respectively.
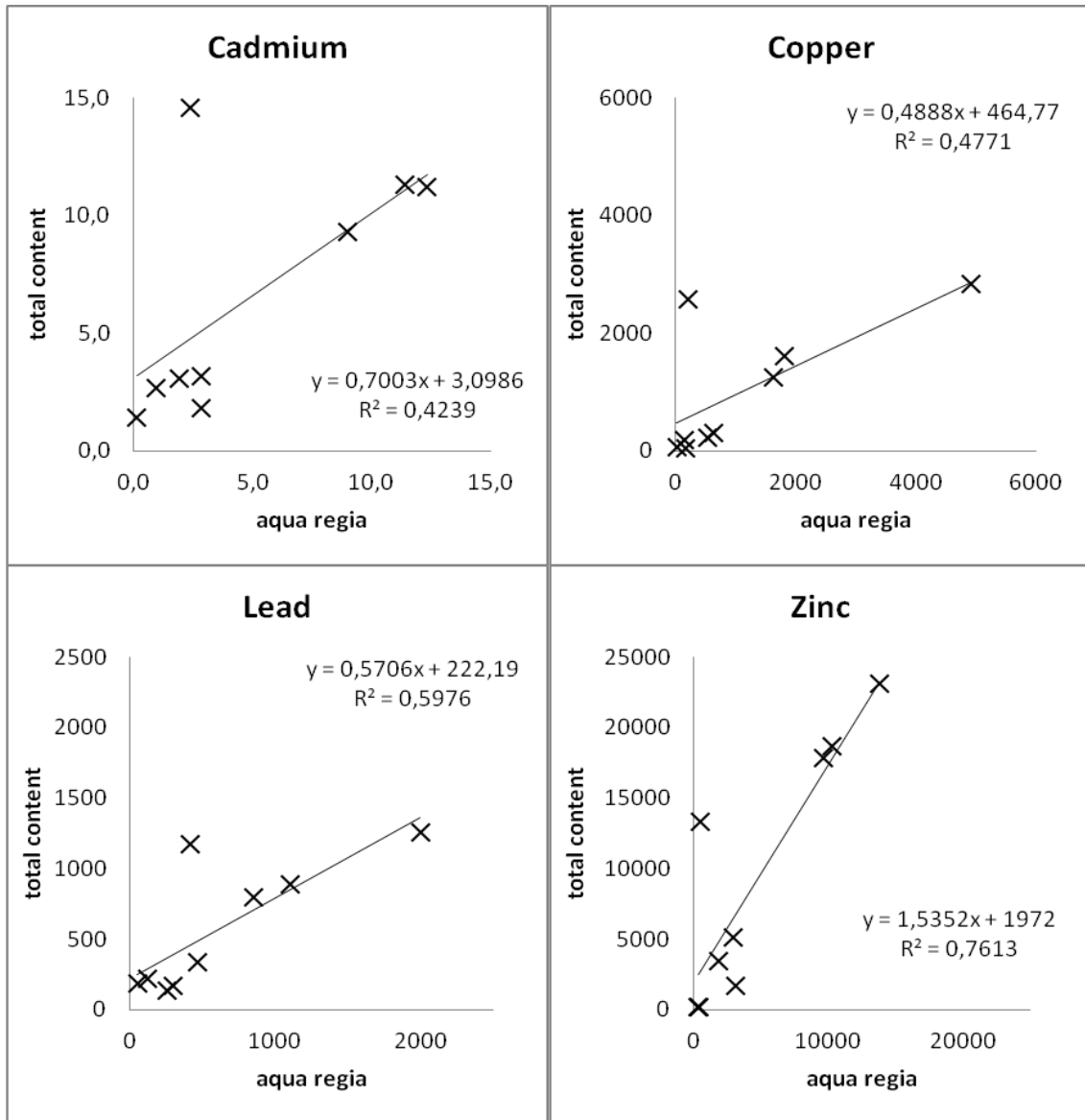
Figure 6.4 Correlation between concentration of PTE extractable by aqua regia and the total PTE content in a range of soil sample types

The comparison of aqua regia extract with the total PTE content in soil samples yields mixed conclusions. Contrary to our expectations, in the case of Cu and Pb it is evident that for the majority of our studied samples, the aqua regia extraction yielded a greater share of PTE into solution than did the procedure to determine total content: total content = 0.49 x aqua regia + 465 mg/kg, and total content = 0.57 x aqua regia + 222 mg/kg for Cu and Pb respectively. With the exception of an anomalous result (extremely low extraction by aqua regia) for sample 1 path A, Cd was extracted in equal amounts by both extracts (aqua regia is a good approximation of total Cd. Zinc on the other hand was far underestimated by the aqua regia extract when compared with the total: total = 1.5 x aqua regia + 1972 mg/kg of Zn.

6.4 Extraction with coca-cola

The extraction using coca-cola was done so mostly for novelty as there are no real implications for this in the natural environment. However, the results were interesting. Although the PTE release was not synonymous with the other solvents used in this experiment, we did see a significant extraction of some PTE with respect to the guideline value which is proposed for a 2M HNO_3 extract on agricultural soil, as can be seen in Table 6.2

Extraction of Cd and Zn by cola most closely matched the 2M HNO3 extract, with many values above 50% of the nitric acid extract. In the case of the forest samples there was an observed high extraction rate of Pb and Cu, particularly in the lower horizon. With respect to the SGV, a sufficient enough share of the PTE contained in the samples were released into the coca-cola extract at a level which in the case of Cu and Zn, often far exceeded the SGV. Lead and Cd were released to a lesser extent. It is apparent that in the slope samples, which have the increased content of organic matter in a constantly humid environment, coca-cola was able to desorb the studied PTE to only a small fraction of the 2M HNO_3 extract.

Sample	Cd	Cu	Pb	Zn
1 path A	24 (121)	8 (267)	2 (12)	27 (653)
1 path B	38 (147)	31 (1183)	7 (34)	31 (850)
2 slope	14 (21)	2 (14)	12 (19)	6 (64)
3 sediment	60 (113)	6 (28)	9 (10)	46 (400)
4 sediment	53 (83)	7 (19)	11 (12)	40 (374)
5 forest A	20 (43)	75 (11)	3 (5)	n.d
5 forest B	86 (12)	94 (14)	47 (188)	n.d
6 slag A	57 (322)	52 (998)	10 (48)	52 (2936)
6 slag B	53 (200)	45 (673)	14 (92)	41 (1264)

Table 6.2 coca-cola extraction of PTE from the studied samples as a percentage of the 2M HNO_3 extract (after 6 hrs shaking) and of the SGV (percentage of SGV are in parenthesis)

Lottermoser et al. (2011) made a similar experiment using a range of cola products to extract metals from uranium mining soils in Australia. They found that the classic cola extracted the most metals when compared to other cola products e.g diet coke and sugar free brands. Similarly to in our study, a reasonable amount of Cu and Zn was extracted, but when compared with a DTPA extraction, Cu and Zn did not correlate so strongly. However, other elements, which were not studied here, showed very close correlation, for example, U was extracted at almost the same rate in cola classic as by the DTPA (Lottermoser et al. 2011)

7. Conclusions

A 2M HNO_3 extraction that is representative of the extraction used to determine soil guideline values for agricultural soils revealed that path and slag soil samples were strongly contaminated, slope soil and river sediment were lightly contaminated, and that forest soils from above the slag were subjected to little or no contamination by potentially toxic elements.

The difference in shaking time of a 2M HNO_3 extraction dictates the rate of release of PTE. Cadmium release occurred mostly within the first 10 minutes of extraction. There was an overall increase in Pb extraction between the three extraction times. However, the most significant differences occurred for Cu (particularly in organic samples), and for Zn. A reduction in shaking time from 6 to 3 hours will result in a gross underestimation of the true releasable content of these elements in a contaminated sample.

Correlation between content of PTE extractable by 2M HNO_3 and the total content of PTE in a range of soil sample types showed that there are potentially very large differences in the concentrations obtainable by these two extraction methods. The closeness of PTE concentration extracted by 2M HNO_3 to the total content occurred in the following order: Cu > Pb > Cd > Zn. From the correlation between aqua regia and nitric acid, we can conclude that on average, aqua regia extracted far more PTE than did nitric acid, particularly in the case of Pb and Zn. Results from correlation between aqua regia extract and the total PTE content showed that aqua regia gives a good approximation of total Cd in a sample. The total content is underestimated for the used method when compared to extractable amount of Cu and Pb by aqua regia. Zinc was underestimated in aqua regia compared to the total.

A novelty extraction was made by shaking soil with coca-cola for 6 hours. We observed varying rates of extraction between different PTE and different soil samples. Lead and Zn were extracted by the coca-cola but at less than half the rate of the 2M HNO_3 extraction. Cadmium and Cu on the other hand were frequently released into the coca-cola at levels well above 50% of the rate of release by 2M HNO_3.

Reference

Alloway, B. J. 1990. *Heavy Metals in Soils*. Blackie and sons Ltd, London, U.K. ISBN: 0751401986.

Ash, C., 2012. Environmental Burden of Potentially Toxic Elements Originating from Silver Smelting Slag. MSc. Thesis defended at Czech University of Life Sciences

Ash,C., Borůvka, L., Tejnecký, V., Šebek, O., Nikodém, A., Drábek, O., 2013, 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., Nikodém, A., Šebek, O., Drábek, O. 2013. Potentially Toxic Element Distribution in Soils from the Ag-Smelting Slag of Kutná Hora (Czech Republic): Descriptive and Prediction Analyses, *Journal of Geochemical Exploration*, doi: 10.1016/j.gexplo.2013.11.010

Ash, C., Tejnecký, V., Šebek, O., Houška, J., Chala, A. T., Drahota, P., Drábek, O. 2014. Redistribution of cadmium and lead fractions in contaminated soil samples due to experimental leaching. (with publishers under review).

Beneš, S., 1993. Content and balance of elements in spheres of the environment, Ministry of Agriculture of the Czech Republic, Prague.

Bennet, H. (Ed.). *Concise Chemical and Technical Dictionary*, 4th enlarged ed., Edward Arnold, London (1986).

Bhat, N., U., Khan, A., B., 2011, Heavy metals: an Ambigous Category of Inorganic Contaminants, Nutrients and Toxins, *Research Journal of Environmental Science,* **9**

Borůvka, L., Drábek, O (2000) Extractability of some heavy metals from polluted soil Arbeitstagung Mengen- Und spurenelemente. 774 - 778

Borůvka, L., Vacha, R. 2006. Litavka River Alluvium as a Model Area Heavily Polluted with Potential Risk Elements, in *Phytoremediation of Metal-Contaminated Soils*, *Nato Science Series: IV*. Springer, Netherlands. 267 – 298.

Boularbah, A., Schwartz, C., Bitton, G., Aboudrar, W., Ouhammou, A., Moral, J. L. 2006. Heavy Metal Contamination from Mining Sites in South Morocco: 2. Assessment of Metal Accumulation and Toxicity in Plants. *Chemosphere*. **5:** 811-817.

Brady, N. C., Weil, R. R. 2002, *Elements of the nature and properties of soils, second edition*. Pearson, New Jersey. ISBN: 0-13-048038-X

Ciszewski, D., Czajka, A., Blazej, S. 2008. Rapid Migration of Heavy Metals and 137Cs in Alluvial Sediments, Upper Odra River Valley, Poland. *Environmental Geology*. **55:** 1577 – 1586.

Dang, X., Chai, L., Yang, Z., Tang, Ch., Wang, Y., Shi, Y., Bioleaching mechanism of heavy metals in the mixture of contaminated soil and slag by using indigenous Penicillium chrysogenum strain F1, *Journal of Hazardous Materials* 248– 249 107– 114

Duffus, J. H. 2002. ‖Heavy metals‖ – a meaningless term?. Pure Applied Chemistry. 74 (5). 793- 807.

Ettler, V., Johan, Z., Vítková, M., Skala, R., Kotrlý, M., Habler, G., Klementová, M., 2012, Reliability of chemical microanalyses for solid waste material, *Journal of Hazardous Materials,* **221– 222:** 298– 302

Harrison, P., Waites, G., *The Cassell Dictionary of Chemistry*, Cassell, London (1998).

Kabata-Pendias, A., 2001. Trace Elements in Soils and Plants, Third ed. CRC Press, U.S.A.

Kierczak, J., Potysz, A., Pietranik, A., Tyszka, R., Modelska, M., Neel, C., Ettler, V., Mihaljevi_c, M., 2013. Environmental impact of the historical Cu smelting in the Rudawy Janowickie Mountains (South-Western Poland). J. Geochem. Explor. 124, 183e194.

Krakovská E., Kuss H. M. (2001): Rozklady v analytickej chémii, Vienala Košice, SK, p 226.

Kubota, J., in: Thornton, I., (Ed.), Soils and Plants and the Geochemical Environment in Applied Environmental Geochemistry, Academic Press, London, 1983 (Chapter 4).

Lottermoser, B. G., Schnug, H., Haneklaus, S., 2011. Cola soft drinks for evaluating the bioaccessability of uranium in contaminated mine soils. *Science of the Total Environment*, **409, 18:** 3512 - 3519

Luama, S.,N., Rainbow, P.,S., 2005 Why is metal bioaccumulation so variable? Biodynamics as a unifying concept., *Environ. Sci. Technol.,* **39:** 1921-1931

Ministry of Environment. (1994). Act number 13/1994 Sb. Risk elements in soils belonging to the agricultural land fund. In Czech. http://eagri.cz/public/web/mze/legislativa/ostatni/100313828.html.

Navarro, A., Cardellach, A., Mendoza, J., Corbella, M., Domenech, L. M. 2008. Metal mobilization from base-metal smelting slag dumps in Sierra Almagrera (Almeria, Spain). *Applied Geochemistry.* **23:** 895 – 913.

Pauliš, P., Mikuš, M., 1998, Silver Pathway – Instructional Pathway in Kutná Hora, Vydavatelstí a nakladatelství Martin Bartoš- Kuttna, Kutná Hora,

Podlešáková, E., Němeček, J., Přístupy, K. 1990. Řěšení Limitních Hodnot Rizikových Prků v Půdach. Sbornik "Hygiena pod a jej monitoravanie", Frýdek-Místek, Pedohyg. Bratislava. p. 127 – 137.

Quevauviller, P., 2002, Methodologies for soil and sediment Fractionation studies, The Royal Society of Chemistry, Cambridge, UK, 0-85404-453-1

Sastre, J., Sahuquillo, A., Vidal, M., Rauret, G., 2002, Determination of Cd, Cu, P band Zn in environmental symplex: microwave-assisted total digestion versus aqua regia and nitric acid extraction, *Analytica Chimica Acta* **462:** 59-72

Smeda, A., Zyrnicki, W., 2002, Application of sequetial extraction and the ICP-AES Metod for study of the partioning of metals in fly ashes, *Microchemical Journal*, **72:** 9-16

Sparks, D. L. 2003. Environmental Soil Chemistry, Academic Press, London, Second Edition, p. 352. ISBN: 0-12-656446-9.

Stoyanova, Z., Doncheva, S. 2002. The Effect of Zinc Supply and Succinate Treatment on Plant Growth and Mineral Uptake in Pea Plant. *Brazilian Journal of Plant Physiology.* **14:** 111 – 116.

Száková, J., Tlustoš, P., Goessler, W., Frková, Z., Najmanová, J., 2009. Mobility of arsenic and its compounds in soil and soil solution: the effect of soil pretreatment and extraction methods. J. Hazard. Mater. 172, 1244e1251.

Šulcek Z., Povondra P. (1989): Methods of decomposition in inorganic analysis. CRC Press, Boca Raton, Florida, USA, p 325.

Tokalioglu, S., Kartal, S., Birol, G. 2003. Application of a Three-Stage Sequential Extraction Procedure for the Determination of Extractable Metal Contents in Highway Soils. *Turkish Journal of Chemistry*. **27:** 333–346.

van der Perk, M. 2006. Soil and water contamination. Taylor and Francis Group plc. London. p. 379. ISBN:0-415-40943-8.

Vangronsveld, J., Vanassche, F., Clijsters, H., 1995. Reclamation of a bare industrial area contaminated by nonferrous metals: In-situ metal immobilization and revegetation. Environ. Pollut. 87, 51–59.

Wilkinson, J.M., Hill, J., Livesey, C.T., 2001. Accumulation of potentially toxic elements in the body tissues of Wheel grazed on grassland given repeated application of sewage sludge., Anim. Sci., 72: 179-190