

**CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE**

**FACULTY OF ENVIRONMENTAL SCIENCES**



**STABILITY AND IMMOBILIZING POTENTIAL OF MANGANESE OXIDE  
BASED ADSORBENTS IN CONTAMINATED SOILS UNDER CHANGING  
REDOX CONDITIONS**

**DIPLOMA THESIS**

**MILLER OWUSU AGYEI**

**THESIS SUPERVISOR: Ing. Zuzana Michálková, Ph.D**

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# CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

## DIPLOMA THESIS ASSIGNMENT

BcA. Miller Owusu Agyei

Environmental Geosciences

### Thesis title

Stability and immobilizing potential of manganese oxide based adsorbents in contaminated soils under changing redox conditions

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### Objectives of thesis

The aim of the review part of the study is to summarize basic knowledge concerning risky metals in the environment, i.e., their properties, occurrence and behavior in the environment with respect to soil conditions like pH or redox. Methods of remediation feasible for soils contaminated with metals will be reviewed too with special interest in chemical stabilization.

In the experimental part of the thesis, two novel manganese oxide based adsorbents will be applied to soil contaminated with metals. Their stabilizing potential towards contaminating elements will be evaluated with respect to changing redox conditions.

### Methodology

Review part is based on appropriate literature sources, eg., mainly scientific books and articles.

In the experimental part of the thesis, two novel manganese oxide based stabilizing amendments will be incubated in soil contaminated with risky metals under various redox conditions. The stabilizing potential of both amendments towards targeted elements and their stability in soil conditions will be evaluated.

Obtained data will be processed, summarized and statistically evaluated. Student will discuss the results with existing literature and summarize main conclusions of the work.

The proposed extent of the thesis  
approximately 50

**Keywords**

cadmium; lead; zinc; soil; stabilization; remediation; redox

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**Recommended information sources**

- Adriano, D. C. 2001. Trace elements in the terrestrial environments: Biogeochemistry, bioavailability, and risks of metals. Springer-Verlag. New York. Berlin. Heidelberg. 867 p. ISBN: 0-387-98678-2
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**The Diploma Thesis Supervisor**

Ing. Zuzana Michálková, Ph.D.

**Supervising department**

Department of Environmental Geosciences

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prof. RNDr. Michael Komárek, Ph.D.

Head of department

Electronic approval: 17. 3. 2017

prof. RNDr. Vladimír Bejček, CSc.

Dean

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## **Declaration**

I hereby declare that this Diploma Thesis, titled “Stability and immobilizing potential of manganese oxide based adsorbents in contaminated soils under changing redox conditions” was independently written, under the direction of Ing. Zuzana Micháľková, Ph.D. All literature and publications from which information was acquired have been listed.

In Prague, 15<sup>th</sup> of December 2017



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Miller Owusu Agyei

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## **Abstract**

The immobilizing efficiency of two novel engineered materials towards metals in contaminated soil has been studied. Metal(loid)s contamination of agricultural soils liable to flood presents a challenge in their remediation as redox conditions may change with seasonal floods. To this end, amorphous manganese oxide (AMO) and amorphous manganese oxide modified biochar (AMO/BC) were synthesized and tested for their stabilizing efficiency towards zinc (Zn), cadmium (Cd), lead (Pb) and arsenic (As) under oxic conditions (soil moisture of 70% water holding capacity) and over-saturated conditions (soil over-saturated with water) over time using an incubation experiment. A modified sol-gel procedure (without the heating step) was adopted in synthesizing the AMO whiles grape stalks were used in the biochar synthesis (600 °C). The results of the experiment determined that metal(loid)s dissolution and soil organic matter (SOM) degradation (suggested by increased concentrations of dissolved organic carbon (DOC)) were at least an order of magnitude higher in over-saturated conditions than more oxic conditions. Also, the application of both amendments increased metal(loid)s and DOC release in over-saturated conditions whiles being effective in immobilizing Zn, Cd and Pb in oxidic conditions. Both AMO and AMO/BC promoted increased arsenic (As), manganese (Mn) and DOC dissolution under both water regimes suggesting the influence of other factors such pH and amendment degradation. Not much significant difference was observed between the immobilizing efficiency of AMO and AMO/BC in both water regimes.

## **Keywords:**

cadmium; lead; zinc; arsenic; soi; stabilization; remediation; redox; contamination

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## 1. Introduction

The rate of soil pollution by metal(loid)s since the industrial revolution has become a major environmental and toxicological concern to authorities in most part of the world. Substantial amounts of metal(loid)s such as cadmium (Cd), lead (Pb), zinc (Zn), chromium (Cr), arsenic (As) and mercury (Hg) have gotten into the environment posing serious threat to microorganisms, plants, animals and humans (Dudka and Adriano, 1997; Mulligan *et al.*, 2001; Nolan, 2003; Cui *et al.*, 2005). Metal(loid)s can enter the food chain from the soil environment depending on factors such as amount of metal(loid)s available, source of metal(loid)s, rate and magnitude of uptake or adsorption chain (Adriano, 2001; Wang *et al.*, 2003; Wuana and Okieimen, 2011).

Due to these, a cost effective, less environmentally disruptive and long-term techniques for remediating contaminated soils are required. Stabilization techniques such as chemical stabilization (use of chemical amendments to reduce mobility, bio-availability and bio-accessibility of soil contaminants), phyto-stabilization (using living plants and microorganisms for immobilizing contaminants in the interface of roots and soil) and their combined application have come across as viable alternative(s). The efficiency of stabilizing techniques far outweighs that of the traditional methods including excavation and landfilling, soil washing, vitrification and ground freezing (Mench *et al.*, 2003, 2009; Adriano *et al.*, 2004).

The use of stabilizing amendments are often not to reduce the total amount of metal(loid)s available but to immobilize their fractions that pose significant risk to the soil environment including mobile fraction, soluble fraction, bioavailable and bioaccessible fraction (Komárek *et al.*, 2013). Stabilization of metal(loid)s fractions can occur through organic ligand complexation, adsorption, ion exchange or surface precipitation (Kumpiene *et al.*, 2008).

Soil oxides are examples of chemical amendments used in stabilizing soil contaminants. They are minerals occurring naturally in soils and sediments. Although not abundantly present, they form a major part of the soil chemistry and as well known for their sorption properties (Post, 1999; Sparks, 2003). One of such oxides is Manganese (Mn) oxide which has proven to be effective in reducing mobility, bio-availability and bio-accessibility of metal(loid)s fractions taking away the possible risk of soil environment contamination (Post, 1999; Feng *et al.*, 2007; Michálková *et al.*, 2014). The amorphous nature of Mn oxide has also proven in various studies to be an



efficient, less costly and environmentally friendly amendment in remediating metal(loid)s contaminated soils (Della Puppa *et al.*, 2013; Ettler *et al.*, 2014, 2015; Micháľková *et al.*, 2016a; 2017)

Furthermore, the use of biochar as an amendment has gained interest in recent times. Though its efficiency is low when used independently, some studies indicate that the sorption capacity of biochar with well-developed structures improve significantly after modification with soil oxides (Komárek *et al.*, 2013; Trakal *et al.*, 2014, 2016).

## **2. Aims of the thesis**

The aims of this study are to assess the effectiveness of an amorphous manganese oxide and its modified biochar in immobilizing metals and As fraction in soil solution, under changing redox conditions over time. The two redox conditions were chosen to ascertain the efficiency of the amendment in areas liable to flood.

In this regard, an incubation experiment will be performed with metal(loid)s-contaminated Fluvisol from the Příbram (Czech Republic) district. Two novel manganese oxide based adsorbents (AMO and AMO/BC) will be applied to this soil to determine their stabilizing potential towards contaminating elements with respect to changing redox conditions

### 3. Literature Review

#### 3.1. Environmental Contamination by Risk Elements

Generally, contaminants are those substances that enter the environment from anthropogenic sources in amounts exceeding their natural content (Motuzova *et al.*, 2014). Heavy metals are contaminants threatening the world today due to the rapid advancement in industrial, agricultural and urban activities (He *et al.*, 2015). These metal(loid)s do not degrade biologically or chemically and their concentration is quite permanent in the environment (Science Communication Unit, 2013). As shown in Figure 1, heavy metals are the most frequently occurring contaminants in Europe.

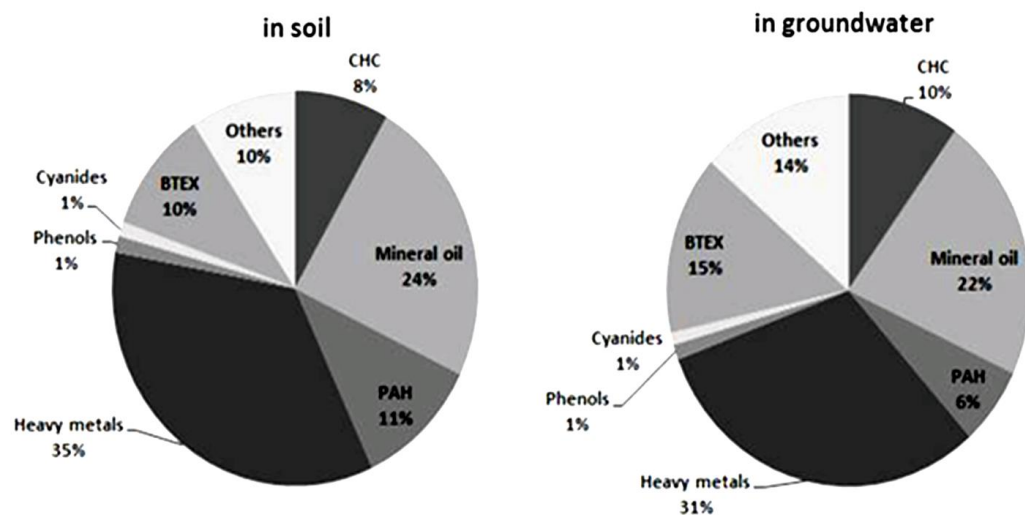


Figure 1. Most frequently occurring contaminants in soil and groundwater (Huber and Prokop, 2012)

Globally, more than 10 million soil contaminated sites have been reported, with more than 50% of these sites contaminated with metals and/or metalloids (see Table 1). The economic impact of this is estimated to be in excess of US\$ 10 billion per year worldwide (He *et al.*, 2015).

Table 1. Share of heavy metals in world soil pollution

Number of polluted sites (%) of metal(loid)s pollution		
<b>Global</b>	>10,000,000	>50
<b>USA</b>	>100,000	>70
<b>European Union</b>	>80,000	>37
<b>Australia</b>	>50,000	>60
<b>China</b>	1.0 million km <sup>2</sup>	>80

(European Environmental Agency, 2007; Environmental Protection Ministry of China, 2014; United States Environmental Protection Agency, 2014)

The terminology “heavy metal” has generated some controversy in the field of environmental soil chemistry. It has been argued that the description “heavy” has not been accurately established scientifically (Science Communication Unit, 2013). Wuana and Okieimen (2011) defines heavy metals as “an ill-defined group of inorganic chemical hazards”. Descriptions such as “light”, “heavy”, “semi-metal”, “trace” and “toxic” have been used in qualifying metals based on some chemical and physical principals (Roberts *et al.*, 2005). Other classification of metals include weight, density, level of toxicity and atomic number (Roberts *et al.*, 2005). For instance (Fergusson, 1990; Tamunobereton-ari *et al.*, 2011; He *et al.*, 2015) describe heavy metals by their atomic mass (higher than 20) and density (5times greater than density of water). On the basis of toxicity, heavy metals include metalloids which induce toxicity with least exposure (Duffus, 2002). As trace elements, metals are present in trace concentrations (ppb range to less than 10ppm) in soils and sediments (Kabata- Pendia, 2001).

The term “Heavy metal” has therefore become an umbrella name for metal(loid)s in soils and sediments connected to contamination and toxicity. However, as indicated above, neither the definition nor the types of heavy metals or metalloids have been agreed on universally by researchers. For this study, the term “risk metals” would be used in place of heavy metals to qualify the metals of interest.

### 3.2. Sources of Metal(loid)s

Risk metals occur naturally and from anthropogenic sources. Natural sources include parent material (natural geological processes) and volcanic eruptions (Wuana &

Okieimen, 2011; Alloway, 2013). The process involves the weathering of parent material resulting in the release of some metals at levels regarded as trace ( $<1000 \text{ mg kg}^{-1}$ ) (Kabata-Pendias and Pendias, 2001). Metal-enriched rocks such as serpentine and black shale are typical parent rocks noted for releasing metals into soils (He et al., 2015).

The largest source of heavy metal availability in both agricultural and urban soils is however anthropogenic including mining, smelting, industry, agricultural activities, and fossil fuel burning (Xue et al., 2005; Khan, *et al.*, 2007; Wuana and Okieimen, 2011; Alloway, 2013; Jovanović *et al.*, 2015). However, contamination from a principal, localized source such as a metal smelter, can be striking in soils, vegetation and the health of the local populace (Alloway, 2013; Science Communication Unit, 2013). According to Nriagu and Pacyna (1988), the median value as estimated for worldwide emissions of cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) in soils were 22, 954, 796 and  $1,372 * 10^6 \text{ kg yr}^{-1}$ , respectively. They added that a greater number of these metals related to base metal mining and smelting activities.

Other major sources of risk metals in soils and sediments include inorganic fertilizers, compost, industrial waste, atmospheric deposition, sewage sludge, and agrochemicals. Among these, atmospheric deposition was rated as the major source of risk metals in soils and sediments (Nicholson *et al.*, 2003; He *et al.*, 2015).

In a multivariate and GIS analysis of heavy metal sources in soils, Facchinelli et al. (2001) noted that the irregularity of chromium (Cr), cobalt (Co) and nickel (Ni) concentrations in soils are due to the peculiar development of ultramafic rocks. Again, long term anthropogenic activities such as grape cultivation have been proven to control Cu and Zn contents in soils while Pb is associated with road transport in urban and industrial areas (Facchinelli *et al.*, 2001; Xue *et al.*, 2005).

Metals found at industrial or mechanized sites are also a distinct group of metal(loid)s. Their composition often depends on the raw materials and products produced by the industry (Alloway, 2013). Wong *et al.* (2002) in their study of the agricultural soil in the Pearl River delta attributed the high concentration of risk metals in the crop soils compared with those of the paddy and natural soils to high usage of agrochemicals. The significance of anthropogenic-led contamination of soils by risk metals can therefore not be downplayed although, metal(loid) composition of agricultural soil is closely tied to the parent material (Alloway, 2013). It must be said however that not

every load of heavy metal contamination is a recipe for disaster (Science Communication Unit, 2013).

There are many pathways of metals in the environment. They are transported by water into soils or from atmospheric deposition. Plants, animals and other organisms absorb them by ingesting the contaminated water or soil which is in-turn transferred to humans after ingesting these plants and animals along the food chain (Mulligan *et al.*, 2001 Wang *et al.*, 2003; Wuana and Okieimen, 2011; He *et al.*, 2015). Figure 2 illustrates the various pathways and destination of risk metals in the environment once they get into the soil.

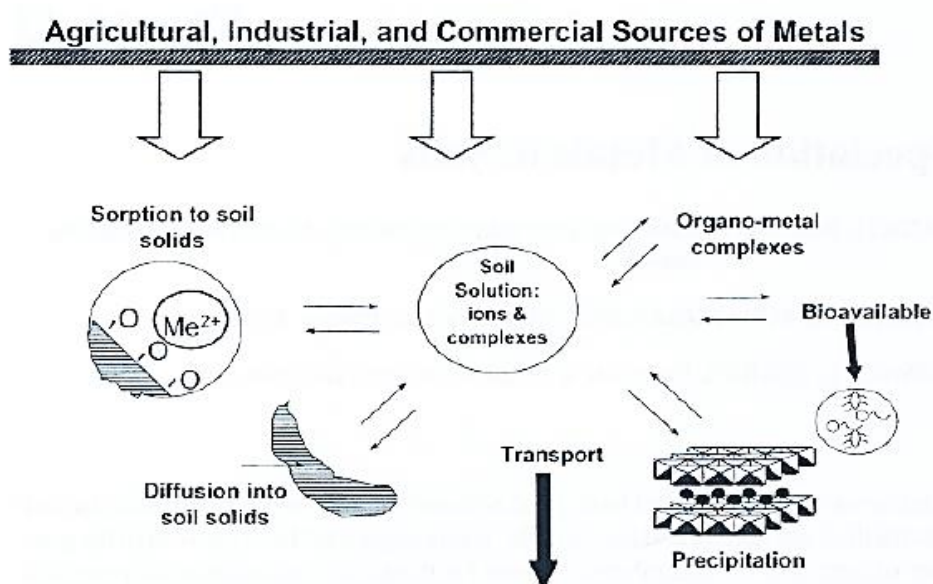


Figure 2: Pathways and destination of risk metals in soils and sediments (Roberts *et al.*, 2005)

### 3.3. Toxicity of metal(loid)s

Heavy metals exist extensively in all soils but under different limits of concentration (Jovanović *et al.*, 2015). As indicated under the sources of risk metals session, there are substantial amounts of risk metals entering the environment from various sources. Hence, potentially toxic elements end up accumulating in the soil and sediments in water bodies over lengthy periods posing a great threat upon the existence of some favorable condition.

The danger connected with increasing concentrations of risk metals is such that their influence on chemical processes is not manifested in the landscape, however, their effects on the biosphere are universal and of extreme importance (Motuzova *et al.*,

2014 ex. Weber and Karczewska, 2004). This includes phytotoxicity, degradation of agricultural soils, reduced performance of soil microbes and human poisoning in the distant future. (Nicholson *et al.*, 2003).

Metals are grouped under essential and non-essential metals based on their importance to human physiological development and microorganism activities. Pb, Hg and Cd are prime examples of non-essential metals because they have no known benefit for the physiology of humans. On the other hand, manganese (Mn), iron (Fe), magnesium (Mg), zinc (Zn) for example, are needed by humans and microorganisms as essential nutrients to function. Others including aluminium (Al), gold (Au), gallium (Ga), lithium (Li) and silver (Ag) are used therapeutically in medicine as part of the medical armamentarium (Adal and Wiener, 2016).

Nevertheless, an essential or non-essential metal(loid)s may to toxic depending on factors such as their chemical form, duration and route of exposure and amount absorbed (Adal and Wiener, 2016). Both essential and non-essential metal(loid)s are concentration-dependent such that in very low concentrations, some metal(loid)s are essential and beneficial, but they become toxic when they go beyond a particular limit of concentration (Jaishankar *et al.*, 2014). In the same vein, some non-essential metal(loid)s may be extremely low in concentration or even rare such that their toxicity is very unlikely. Nevertheless, due to their ability to bio-accumulate along food chains, risk metal(loid)s exposure, even at low concentrations may be very dangerous (Lenntech, 2004).

In addition, Hg may be less harmful when ingested, yet it can be deadly when inhaled or injected (Hoffman *et al.*, 2015). Also, barium sulfate ( $\text{BaSO}_4$ ) is basically non-toxic, whereas barium salts like barium chloride ( $\text{BaCl}_2$ ) are rapidly absorbed and may cause intense or deadly hypokalemia (Adal and Wiener, 2016). Again, reproductive functions of men are influenced by Zn, however, excess intake can cause growth and reproduction disorder although it is relatively non-toxic when ingested compared to Hg (Nolan, 2003).

Furthermore, chemical coordination and oxidation-reduction properties of metal(loid)s also determine their availability and toxicity (Jaishankar *et al.*, 2014). For example, As(III) is more toxic than As(V) and can in-activate enzymes when bounded to sulfhydryl groups on proteins (Cohen *et al.*, 1993). Also, equal amount of Cr(VI) compounds, which is a corrosive element become less toxic when reduced to Cr(III)

(Cohen *et al.*, 1993). This is because outside cell walls, Cr(III) is less soluble and therefore less likely to be adsorbed. Again, Cr(III) amplifies the activity of insulin in humans and animals thereby influencing glucose, fat and protein metabolism (Goyer, 2001).

Cu and Fe are essential co-factors for stress-related enzymes such as cytochrome oxidase which is beneficial to microorganisms (Tchounwou *et al.*, 2012). Fe, Zn, K and Mg are also cofactors for enzymes such as carbonic anhydrase, pyruvate kinase and hemoglobin beneficial to the red blood cells of humans and animals (Prohaska and Gybina, 2004; Stern, 2010). Nevertheless, in the case of copper,  $\text{Cu}^+$  ions readily reacts with hydrogen peroxide creating hydroxyl radical which is harmful (Prohaska and Gybina, 2004; Stern, 2010). Wilson disease has been connected to extreme exposure to Cu (Tchounwou *et al.*, 2008).

Some oxidation states of metals like Pb, Cd, Cu and Cr are able to bind with protein sites in organisms by displacing metals originally made for those sites resulting in cell malfunctioning and ultimate toxicity (Jaishankar *et al.*, 2014). Chromosomal abnormalities and deoxyribonucleic acid (DNA) strand breaks are the results of risk metals binding with DNA and nuclear proteins (Flora *et al.*, 2008).

Several long-term diseases in humans have been linked to risk metal exposure, even at lower levels. For example, neuro-behavioral disorders, hearing loss, diabetes, anemia among others recorded in some countries in Asia were associated to the exposure of As in drinking water (National Research Council, 2001; Tchounwou *et al.*, 2004). Also, exposure to Cd in the workplace has been connected to osteoporosis and low bone mineral density (Åkesson *et al.*, 2006; Gallagher *et al.*, 2008).

In addition, Pb attack several organs of the body (liver, kidney, central nervous system, reproductive system) when adsorbed (ATSDR, 1999). The risk associated with Pb exposure is very high in that pregnant women exposed to Pb can easily pass it on to the fetus affecting its development (Huel *et al.*, 1992; Andrews *et al.*, 1994). It is also known that gene mutation can be induced by Pb exposure (Yang *et al.*, 1999). “Itai-itai” and “minamata” are examples of diseases known to have resulted from the production of paddy rice in soil contaminated with Hg and Cd in Japan (Järup, 2003). The level of exposure, especially to non-essential metals, i.e. acute or chronic exposure, could also determine the extent of harm. Acute Hg exposure may damage



the lungs damage whilst chronic exposure to Hg results in neurological effects including nervous system damage (Järup, 2003; Jaishankar et al., 2014). Again, acute exposure to Pb leads symptoms including abdominal pain and headache, chronic exposure to Pb may lead to memory deterioration (Järup, 2003; Jaishankar *et al.*, 2014). The effect of acute exposure to As include serious malfunction of the central nervous system, however, long-term exposure can cause chronic diseases as mentioned in earlier paragraphs (Järup, 2003; Jaishankar *et al.*, 2014).

### **3.4. Bio-availability, Bio-accessibility, Speciation and Fractionation**

Bio-availability and bio-accessibility are essential in the transport of metals to plant tissues. The definition of these terms has often been intermixed in literature. There is a general understanding that the meaning addresses the potential for a substance to interact with an organism. In fact, there may be no universally acceptable definition of bio-availability or bio-accessibility. The lack of a common view of bio-availability has however been addressed by a recent report from the National Research Council (NRC), (2003) in the context of soil and sediment contaminants, and summarized by Ehlers and Luthy (2003). The NRC recommended the adoption of a process-based view, with bio-availability processes being defined as “the individual physical, chemical, and biological interactions that determine the exposure of plants and animals to chemicals associated with soils and sediments”.

Semple *et al.* (2004), also suggested a temporal and/or spatial distinctions between bioavailable and bioaccessible compounds. A compound is bioavailable where the compound “is freely available to cross an organism’s cellular membrane from the medium the organism inhabits at a given time”. Also, bio-accessibility of a compound is where the compound “is available to cross an organism’s cellular membrane from the environment, if the organism has access to the chemical”. This shows the possibility of the compound becoming bio-available as far as the proximity of the compound to the organism allows.

Bio-availability/bio-accessibility of a given metal(loid) is closely linked to its speciation as it shows the distribution of an element amongst distinct chemical species (Templeton *et al.*, 2000). The total concentration plus speciation of a metal(loid) may influence its transport and risk in environment (Pagnanelli *et al.*, 2004; Clozel *et al.*, 2006; Wuana and Okieimen, 2011).

Speciation refers to the various chemical forms in which an element can be found. Metal(loid)s can occur in several chemical forms such as a free ion or complex ligand in various soil phases (Roberts *et al.*, 2005). Successful determination of metal species together with the identification of solubility and/or precipitation can be useful in determining the activities of metals in soil solutions (Roberts *et al.*, 2005).

Speciation involves the determination of many facets of the element such as isotopic composition, the oxidation state, inorganic and organic associations and complexes (Templeton *et al.*, 2000). However, determining these parameters can be very cumbersome if not impossible due to the specific characteristic of the various species of an element. Hence, the concept of fractionation was introduced which is suitable for simplifying the activity of metal(loid)s in the soil.

Fractionation includes an actual physical separation (e.g., filtration, size-exclusion chromatography) of the chemical species of an element or separation based on their chemical properties (e.g., bonding, reactivity) (Templeton *et al.*, 2000). While total concentration only indicates the amount of metal(loid)s present in the soil, the determination of the association of risk metals with various soil constituents (through fractionation) determines how risk metals behave in the environment thereby providing better understanding about risk metal contamination (Umoren and Udousoro, 2009; Mahanta and Bhattacharyya, 2011).

These soil associations or fractions mentioned include: i. reducible (bound to Al, Fe and Mn oxides); ii. oxidizable (bound to organic matter); iii. exchangeable (freely available); and iv. residual fraction (not available). Thus, fractionation studies determine possible mobility and bio-availability of risk metal(loid)s and their consequential impact in soils and sediments. (Akçay *et al.*, 2003; Cuong and Obbar, 2006).

To this end, sequential extraction method is used in determining which fraction of the soil a particular element associates (Sutherland and Tack, 2003). Tessier's procedure is often the method employed in sequential extraction process (Tessier *et al.*, 1979; Li *et al.*, 2001; Sutherland and Tack, 2003). The extraction procedure by Wenzel *et al.* (2001) is also an example of fractionation method used for metalloids like As.

Mulligan *et al.* (2001) ex Bourg (1995) indicated that the more mobile a metal is, the greater the risk associated with it. Thus, understanding the environmental

characteristics through fractionation of metal contaminant is the most crucial step in formulating appropriate methods for soil remediation.

### **3.5. Factors influencing metal(loid)s mobility in soils**

Evanko and Dzombak (1997) identified three forms by which contaminants appear in the soil matrix; i) dissolved in the soil solution, ii) adsorbed onto solid phases and iii) chemically fixed in the soil matrix as compounds. The mobility and availability of these contaminants, for instance risk metal(loid)s, are influenced by soil properties such as adsorption and desorption (Krishnamurti et al., 1999). Adsorption and desorption characteristics of soils are linked to their chemical properties as well physical properties. These properties include redox (Eh), pH, cation/ anion exchange capacity (CEC/ANC), organic matter (OM), oxides among others (Antoniadis et al., 2008; Usman et al., 2008; Kumpiene et al., 2008).

#### **3.5.1. Chemical Properties**

Among the various factors, pH is considered as the strongest determinant of metal speciation, mobility and bio-availability (Zhao *et al.*, 2010). High pH reduces metal(loid) mobility whereas low pH results in increased metal(loid) mobility. For instance, at low soil pH, Cd, Pb and Zn attached to solid phases dissolved in the soil solution (Bang and Hesterberg, 2004). This is also supported by Speir *et al.* (2003) in his study on the mobility of Cr, Zn and Pb.

Metal cations and anions are also removed from the soil solution in the presence of oxides via adsorption, ion exchange and precipitation (Dzombak and Morel, 1987). It is also important to mention clay as an effective ion exchange material for metals (Sposito, 2008). The affinity of soils to adsorb cations (CEC) and anions (ANC) on their solid surfaces is also another factor influencing metal(loid) adsorption or desorption.

Again, soil organic matter (SOM) content influences metal(loid)s mobility and bio-availability. Soil organic matter enhances the soils ability to retain highly mobile metal(loid)s. Decrease in SOM content in soils may lead to a decrease in adsorption of metals onto soil constituents (Antoniadis *et al.*, 2008). Despite this, the degradation of SOM releases metal(loid)s attached to it hence, increased mobility of metal(loid)s (Du Laing *et al.*, 2009). For example, it is reported that the mobility and up-take of Cd by rye grass was enhanced by the increasing soil organic matter content (Almås and

Singh, 2001). In the analysis of Dai *et al.* (2004), the contents of Pb, Cd and Zn were found to be positively correlated with the contents of organic matter present in the soil. As adsorption by organic matter has also been proven in agricultural soils (Cao and Ma, 2004). However, the contribution of organic matter to metal(loid)s mobility has been shown to be pH dependent. While As dissolution decreased with decreasing pH and SOM adsorption, reductive dissolution of As occurred at high pH (neutral levels) with SOM inducing further leaching (Shiralipour *et al.*, 2002). Studies by Grafe *et al.* (2002) also showed that aside pH, the type of SOM can influence metal(loid) mobility.

### **3.5.2. Physical Properties**

The rate of metal(loid) contamination is affected by the size of soil particles. Soil particles of sizes <100µm have higher surface area and are more reactive than coarser particles. Another major factor that control contaminated soil chemistry is the soil moisture content. The soil moisture content impacts redox and pH processes as well as the volume of dissolved minerals (Evanko and Dzombak, 1997).

Furthermore, soil structure details its physical characteristics (shape, size, arrangement etc.) which determines the possibility and rate of risk metal(loid) mobility. The structure of the soil for example can limit the degree of contact between groundwater and contaminants, thereby determining contaminant mobility (Evanko and Dzombak, 1997).

### **3.6. Soil Remediation Techniques**

Owing to the rapid build-up and near-permanency of risk metal(loid)s, with its associated toxicity in agricultural soils, developing unorthodox and appropriate techniques for soil remediation are essential (Khalid *et al.*, 2016). Various remediation technologies have been developed over the period (Sims, 1990; Cunningham *et al.*, 1995; Virkutyte *et al.*, 2002; Khalid *et al.*, 2016). However, it is unlikely to have a single technique capable of remediating contaminated soils effectively and efficiently. Therefore, the two or more of the three conventional methods (physical, chemical and biological methods) are often used in-tandem (Fig. 3). In addition, appropriate methods are chosen based on the soil type, risk metal(loid), scope of contamination as well as environmental products affected. Though most methods are effective, they can be costly, environmentally-destructive and time consuming, making soil clean-up difficult (see appendix).

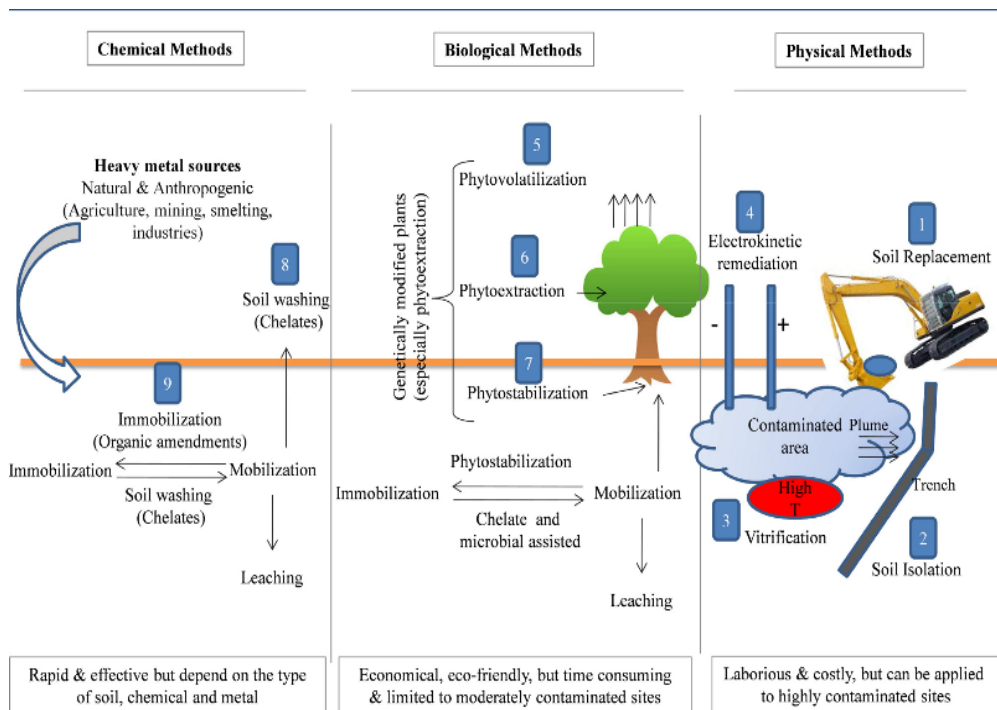


Figure. 3: Comparison of soil remediating techniques under the three convention methods (Khalid *et al.*, 2016).

For this study, immobilization by chemical method was employed due to its efficiency and effectiveness compared to other methods (described in appendix).

### 3.7. Chemical Immobilization

Immobilization techniques acts to either restrict the movement of metal(loid) contaminants physically or change their chemical nature from soluble forms to more stable forms in soils and sediments (Evanko and Dzombak, 1997). Physical processes may include solidifying contaminated soil with high temperatures whilst chemical treatment involves the addition of a chemical reagent or amendment (organic or inorganic) to change metal(loid) phases from soluble to stable forms (Wuana and Okieimen, 2011; Khalid *et al.*, 2016; Michálková *et al.*, 2016a).

The reactions involved in chemical immobilization of risk metals include adsorption, precipitation/dissolution and redox reactions (oxidation/reduction). Metal(loid)s are often influenced by microbial oxidation/reduction reaction causing a change in their speciation and mobility. For instance, under oxic conditions As would be present as As(V) whereas under reducing conditions, As will be present as As(III) or As(0) or arsine ( $H_2As$ ). Again, reducing conditions may cause the reductive dissolution of  $Mn^{4+}$  and  $Fe^{3+}$ .

Redox reactions can be either assimilatory or dissimilatory. In assimilatory reactions, metabolic functioning of species responsible for the reaction is influenced by the substrate of the metal whereas the no identifiable effect on the metabolic functioning of the organism by metal(loid) substrate is observed in dissimilatory reactions (Bolan *et al.*, 2014).

Precipitation is the principal mechanism involved when there is high soil pH, high concentration of metal(loids), and when anions ( $\text{SO}_4^{2-}$ ,  $\text{CO}_3^{2-}$  etc.) are present (Hong *et al.*, 2007). Cu, Cd, Zn and Pb are examples of metal(loids) immobilized through the precipitation of phosphates/carbonates (McGowen *et al.*, 2001).

Adsorption is the major process involved in chemical stabilization of compounds in the soil (Michálková *et al.*, 2016b). It involves the retention of charged dissolved compounds on charged surfaces of soil components (Bolan *et al.*, 2014). This retention process can happen either through electrostatic attraction between the charge on the ions and the charge on the soil particles i.e, non-specific or physical adsorption, or through a chemical bond between ions in the solution and ions on the soil surface, i.e., specific or chemical adsorption (Sposito, 2008; Zenteno *et al.*, 2013). Specific adsorption is strongly pH-dependent and linked to hydrolysis of metal ions.

Adsorption in general is a dynamic process subject to changes in time and concentration of metals (Roberts *et al.*, 2005). Both amorphous and crystalline materials are significant in the metal sorption process. Although limited minerals present in soils, Mn (birnessite, pyrolusite), Al (gibbsite, boehmite) and Fe (goethite, hematite, ferrihydrite) are very significant in removing risk metal(loid)s from soil solution (Manceau *et al.*, 2002). This is due to their high reactive surfaces and large specific surface areas (SSA). In several instances, these metal oxides act as initial reactive phases in metal adsorption (Manceau *et al.*, 2002).

One of the tools used in determining the various adsorption mechanisms is adsorption isotherms (Roberts *et al.*, 2005; Limousin *et al.*, 2007). Adsorption isotherms primarily describes the relation between the amount in equilibrium of a dissolved compound and the amount of the compound adsorbed to a solid surface (Roberts *et al.*, 2005). The four types of isotherms used are S-shaped, Langmuir, high affinity with steep slope, and linear isotherms (Sparks, 2003). However, these isotherms are non-mechanistic and used only for macroscopic observations.

### 3.8. Metal Oxides

Various materials can be employed for chemical stabilization. These include alkaline materials (milled limestone, dolomite), clay minerals and zeolites, organic matter and organic-based industrial waste products (compost, sludge), inorganic based industrial waste (by)products (by-products from lime and gypsum, by-products from aluminum and iron industry) and metal (Fe, Mn and Al) oxides (Kumpiene *et al.*, 2008; Michálková *et al.*, 2016b).

Among these materials, metal oxides are widely used because of their large surface area and amphoteric nature making them effective for sorption and immobilization of various risk metal(loid)s (Komárek *et al.*, 2013; Bolan *et al.*, 2014; Michálková *et al.*, 2016b). Oxides (hydroxides, hydrous oxides, oxyhydroxides etc.) occur naturally in all soil types, synthesized artificially or as industrial by-products (Bolan *et al.*, 2014). They develop in soils as disjunct crystals covering the surface of other particles or as mixed gels (Komárek *et al.*, 2013; Michálková *et al.*, 2016b). Risk metals are effectively retained by oxides through sorption, co-precipitation and formation of inner-sphere complexes (Roberts *et al.*, 2005).

Arsenic has a high affinity for Fe oxides and they therefore play a significant role in its retention. Goethite successfully reduced exchangeable fraction of As and its bio-availability in the study conducted by Bagherifam *et al.* (2014). In addition, goethite is capable of reducing the amount of Zn, Cu and As uptake by spinach (*Spinacea oleracea L.*). The total reduced concentrations of Cu, As and Zn in the plant biomass was 95, 99 and 97%, respectively (Hartley and Lepp, 2008).

Another effective and cheap way of immobilizing risk metals with Fe oxides is by using them in their precursor form such as Fe sulfates and nano-zerovalent iron (nZVI) (Komárek *et al.*, 2013). For instance, Fe(0) oxidation to ferrihydrite in soils makes it efficient in the long term with no negative impact on pH (Komárek *et al.*, 2013). Nonetheless, the application of Fe sulfates could acidify soils leading to the remobilization of Cd, Pb and other metallic cations (Komárek *et al.*, 2013; Bolan *et al.*, 2014). Liming is needed in such situations to control the soil pH (Warren and Alloway, 2003; Contin *et al.*, 2008).

Manganese oxides are also efficient in scavenging metal(loid)s (McCann *et al.*, 2015). Although available in small quantities as compared to Fe oxides, they are one of the

foremost driving forces responsible for separating metal(loid)s into solid and liquid phases of the soil (Post, 1999). Manganese oxides have a negative surface charge due to their large SSA and low point of zero charge (pHpzc) (usually between a pH of 2 and 5) making them powerful sorbents of metal(loid)s (Essington, 2004; Feng *et al.*, 2007).

They normally exist in the form of coatings and fine-grained aggregates (Post, 1999). Biogenic oxidation of Mn(II) is the main path of forming Mn oxides in soil (Droz *et al.*, 2015). This is because the kinetics of microbe-aided oxidation of Mn(II) is several orders of magnitude higher than in thermodynamic reactions (Morgan, 2000).

Due to their properties, Mn oxides can form inner-sphere complexes with metal(loid)s and also partake in redox reactions to actively reduce bio-availability and bio-accessibility of risk metals (Hettiarachchi *et al.*, 2000; Qin *et al.*, 2011). For example, the more mobile As(III) was oxidized to As(V) using Mn oxides (Manning *et al.*, 2002). On the other hand, Mn oxide is not suitable for stabilizing Cr due to the possible oxidation of stable Cr(III) to more mobile and toxic Cr(VI) (Feng *et al.*, 2007; Landrot *et al.*, 2012).

The various Mn oxides which occur in soils include birnessite, todorokite, cryptomelane, and hausmannite. Among these, the most available oxide is birnessite ((Na,Ca,K)<sub>x</sub>Mn<sub>2</sub>O<sub>4.1</sub>\*5H<sub>2</sub>O), and has the highest adsorption capacity on Pb, Cu, Co, Cd, and Zn. According to Bolan *et al.* (2014), the order of adsorption capacity by birnessite from the highest is Pb(II) > Cu(II) > Zn(II) > Co(II) > Cd(II).

### **3.9. Amorphous Manganese Oxide and Biochar**

In general, relatively little work has been done on the application of Mn oxides as an amendment for risk metal immobilization compared to Fe oxides (Komárek *et al.*, 2013). Nonetheless, growing efforts are being made to bring more focus on the synthesis and application of crystalline Mn oxides (Chen *et al.*, 2006; Feng *et al.*, 2007; Komárek *et al.*, 2013; Michálková *et al.*, 2014) including also, the development of amorphous Mn oxide as an alternative immobilization material (Landrot *et al.*, 2012; Ettler *et al.*, 2014, 2015, Michálková *et al.*, 2014, 2016a; 2017).

Amorphous manganese oxide (called AMO in the studies dealing with this material) is a poorly crystalline Mn oxide synthesized by modifying the sol-gel technique employed in birnessite synthesis (without the heating crystallization step) (Ching *et*



*al.*, 1997; Della Puppa *et al.*, 2013). The sol-gel technique is a wet-chemical technique involving precipitation and successive washing.

The adsorption capacity of AMO has been determined to be an order of magnitude higher than some Fe-nano oxides (nano-maghemite,  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> and nano-magnetite, Fe<sub>3</sub>O<sub>4</sub>), reaching 0.52, 0.46, 4.02, 1.79 and 2.24 mmol g<sup>-1</sup> for Cu, Zn, Pb, As and Cd, respectively (Micháľková *et al.*, 2014; 2016a). Nonetheless, the material has a relatively lower stability (Della Puppa *et al.*, 2013; Micháľková *et al.*, 2016a). In the experiment determining the stability of AMO, it was observed that about 10-18% of AMO dissolved in 90 days of exposure in contrasting soils (Ettler *et al.*, 2014). This dissolution was attributed to the high dependency of AMO leaching on pH. When AMO is applied at lower pH, Mn<sup>2+</sup> is released at increased level in soils which may be toxic to plants (Ettler *et al.*, 2014; 2015; Micháľková *et al.*, 2014; 2017). Again, SOM oxidation and degradation has also been observed when AMO is applied in low pH values; consistent with the studies conducted by McBride and Martínez (2000) dealing with hydrous Mn oxide. Studies are therefore ongoing to further test the efficient remediation potential of AMO (Ettler *et al.*, 2015; Micháľková *et al.*, 2016).

The potential of biochar (BC) as an environmentally-friendly remediation tool has also been critically studied in recent years. Biochar is a solid material obtained from the pyrolysis of biomass (International Biochar Initiative, 2017). Some sources of biomass for BC include residues of crops and wood, animal droppings-based manure (Tang *et al.*, 2013).

Various sources of biomass and degrees of pyrolysis produce an infinite variety of biochars with different sorption capacity (Mohan *et al.*, 2014). The laboratory study by Melo *et al.* (2013) revealed BC sorption capacity increased for Cd and Zn when the temperature of sugar cane-straw-based BC was increased during pyrolysis. The influence of biochars on sorption process also depend on the charge of their surfaces (Trakal *et al.*, 2014). With a large active surface, BC are able to adsorb both transition and non-metals (Amonette and Joseph, 2009).

The review by Mohan *et al.* (2014) indicates that when independently used, the efficiency of biochar to remove metal(loid)s is a few milligrams per gram of activated carbon. Nevertheless, other studies showed that the modification of biochar with soil oxides (Fe, Mn) greatly improves its sorption capacity (Komárek *et al.*, 2013; Trakal

*et al.*, 2014; 2016). For instance (Wang *et al.*, 2015) determined the potential of soil oxides to increase the sorption capacity of biochars for As(V), Cd(II) and Pb(II).

For this study, the removal efficiency of original AMO and AMO modified biochar (AMO/BC) for metal(loid)s is tested under oxic and reducing conditions.

## 4. Materials and Methods

### 4.1. Synthesis and Characterization of AMO and AMO/BC

For this study, two amendments were synthesized in order to ascertain their ability to immobilize Zn, Cd, Pb and As in the studied soil: (i) Amorphous Manganese Oxide (AMO) and (ii) Amorphous Manganese Oxide/Biochar Composite (AMO/BC). The synthesis of the novel amorphous manganese oxide (AMO) was based on Della Puppa *et al.* (2013), using the modified sol-gel procedure for the preparation of birnessite (Ching *et al.*, 1997).

Specifically, 0.5 L of a 1.4 M glucose solution was added to 0.5 L of a 0.4 M  $\text{KMnO}_4$  solution. The suspension was mixed and subsequently allowed to settle for 30mins. The resulting gel was filtered, washed with 2 L of deionized water to remove excess reactants and dried at room temperature. It was subsequently milled in a mortar into fine powder to make it more reactive. The modification to the birnessite synthesis was the omission of the crystallization step (i.e., heating to 400°C).

As part of the characterization of the AMO amendment, the pH of the AMO synthesized was determined after 24-hour shaking in deionized water (1:10, w/w). A Sorptomatic 1990 Thermo Electron instrument (ThermoFisher Scientific, USA) was used to also measure the specific surface area (SSA) of the AMO by Brunauer Emmett-Teller (BET) method at 105°C.

In addition to the SSA, the average oxidation state of Mn was measured according to (Murray *et al.*, 1984), by iodometric titration. Further, the XRD analysis conducted in the study of Ettler *et al.* (2014) was employed in the solid phase characterization of the AMO including granulometry.

The second material, AMO-biochar composite (AMO/BC), was newly synthesized to serve as an alternative agent where AMO fall shorts. The effectiveness of AMO in stabilizing metal(loid)s in soils has been well proven together with its instability after application over time (Della Puppa *et al.*, 2013; Ettler *et al.*, 2014; 2015, Michálková *et al.*, 2014, 2016a; 2017).

For AMO/BC synthesis, the same grape stalks biochar used by (Trakal *et al.*, 2014) was also used for this study as a pure biochar due to its highest metal sorption capacity. This pure biochar was mixed directly with AMO during its synthesis to form the composite (AMO/BC). Specifically, biochar was first mixed with 0.5 L of a 1.4 M

glucose solution and subsequently, 0.5 L of a 0.4 M  $\text{KMnO}_4$  solution was added (Della Puppa *et al.* 2013).

The resulting gel was filtered and washed several times with deionized water. Eventually, it was dried at laboratory temperature and milled in a mortar. The composition of the AMO/BC synthesized and used for this study was 33% biochar and 67% Mn oxide.

In terms of characterization, the pH,  $\text{pH}_{\text{zpc}}$ , and cation exchange capacity (CEC) of AMO/BC were measured. The pH was measured with an inoLab® pH meter (pH 7310, WTW, Germany) while the immersion technique (IT) was used to determine the  $\text{pH}_{\text{zpc}}$  (Fiol & Villaescusa 2009). The 0.1M  $\text{BaCl}_2$  (1:50 w/V) procedure described in Trakal *et al.* (2014) was used in determining the cation exchange capacity. The X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS: Omicron Nanotechnology, Ltd.) analysis performed in the study of Trakal *et al.* (2014) was used to identify the structure of the AMO/BC.

#### **4.2. Sampled Soil Characteristics**

The soil used for this study is Fluvisol collected at 0-20cm depth from the Litavka river alluvium. The soil in this area is contaminated to a great degree with metal(loid)s due to the historical activity of a Pb-processing smelter located in the Příbram (Czech Republic) district, close to the study area. The samples used were air-dried in the greenhouse and subsequently sieved using a clean 2mm stainless steel sieve. The distribution of the particle size was established by the hydrometric method (Gee and Or, 2002).

The pH of the soil was measured by suspending the sample in a 1:2.5 (w/v) ratio of soil and deionized water or 1 M KCl (ISO 10390:1994). The 0.1M  $\text{BaCl}_2$  procedure was used in determining the cation exchange capacity (Carter and Gregorich, 2008). The samples were digested in *aqua regia* (US EPA method 3051a) under microwave conditions (SPD-Discover, CEM, USA) and ICP-OES (Agilent 730, Agilent Technologies, USA) analysis was performed to ascertain the pseudo total concentrations of the various metal(loid)s in the soil.

To understand the distribution of metal(loid)s in the soil and the potential for toxicity, fractionation of metal(loid)s in the sampled soil was determined. The modified BCR extraction of metals by Rauret *et al.* (2000) and As fractionation according to (Wenzel

*et al.*, 2001) was utilized in determining the metal fractions in the soil. Chemicals of analytical grade were used in all the processes.

### **4.3. Incubation Experiment**

To evaluate the interactions of the AMO and AMO/BC with metals in the soil solution, an incubation batch experiment was conducted. This experiment was done in triplicates at two humidity levels simulating different redox regimes (i.e., more oxic and more reducing environment) using both the AMO and AMO/BC. The purpose of this set-up was to ascertain the influence of AMO and AMO/BC on soil solution characteristics and the mobility of metal(loid)s under oxic and reducing conditions.

To determine this, 150 g of the soil sample was weighed and mixed with AMO and AMO/BC each at a concentration of 1% (w/w) and placed into plastic pots with rhizon samplers attached. Control variants without any AMO or AMO/BC were included. The pots were watered with pure water to maintain ~70% humidity for oxic conditions and ~90% of the water holding capacity just before the sampling.

The experiment was performed in separate pots for time intervals of 1, 4 and 10 weeks. After these sets of duration, the soil solution was collected with the rhizon samplers (mean pore volume size 0.15  $\mu\text{m}$ ; Rhizosphere Research Products, Netherlands) and the concentrations of Mn, Zn, As, Cd and Pb were analyzed without filtration (soil solution is filtered through the rhizon samplers) using ICP-OES.

The content of dissolved organic carbon (DOC) in the sampled soil solutions were determined using a Total Organic Carbon/Dissolved Organic Carbon (TOC/DOC) analyzer to determine the likelihood of the amendments' influence on SOM dissolution. In addition, the pH and Eh of the soil solutions collected were measured.



Figure 4: Measurement of dry sampled soil mixed with amendment in pot for incubation experiment



Figure 5: Pore water collection with rhizon samplers after incubation period

#### 4.4. Statistical Analysis

Statistical analysis was performed for all the data plotted using SigmaPlot 14.0 software (Systat Software Inc., USA) except pH and Eh. The data distribution and significance were determined with analysis of variance (ANOVA) at  $P < 0.05$  using the Tukey test.

## 5. Results

### 5.1. Synthesis and Characterization of AMO and AMO/BC

The physico-chemical properties of the AMO and AMO/BC are summarized in Table 2. The pH and  $\text{pH}_{\text{zpc}}$  of AMO/BC was higher than that of the original AMO. The average oxidation state of Mn obtained was 2.52, which indicates that one Mn atom was linked to 1.26 oxygen (O) atoms ( $\text{MnO}_{1.26}$ ) in the AMO (Della Puppa *et al.*, 2013). The XRD analysis (see Ettler *et al.*, 2014) suggests that several small Mn-oxalate hydrates crystals could be present in the structure of AMO. The granulometry analysis of the AMO sample showed the following distribution:  $<1 \mu\text{m}$  (3.28%), 1-2.6  $\mu\text{m}$  (22.01%), 2.6-5  $\mu\text{m}$  (34.25), 5-10  $\mu\text{m}$  (28.69%) and  $>10 \mu\text{m}$  (11.77%) (Ettler *et al.*, 2014).

The XRD analysis of the AMO/BC showed the presence of Mn-oxalate hydrate ( $\text{C}_2\text{MnO}_4 \cdot \text{H}_2\text{O}$ ) and carbon which are the best reflections of the AMO (Ettler *et al.*, 2014) and the presence of the biochar (Trakal *et al.*, 2014), respectively. The XPS analysis of the AMO-biochar composite indicated the presence of Mn2p in the amount of 6.60% (atomic) which corresponds with the presence of Mn-oxalate.

Table 2: Physico-chemical properties of the amorphous manganese oxide (AMO) and AMO/biochar (AMO/BC)

Amendment	pH	$\text{pH}_{\text{zpc}}$	BET ( $\text{m}^2 \text{g}^{-1}$ )	CEC ( $\text{cmol kg}^{-1}$ )
AMO	$6.46 \pm 0.02$	$6.97 \pm 0.02$	135	$60.75 \pm 2.5$
AMO/BC	$8.47 \pm 0.03$	$7.88 \pm 0.10$	-	$78.9 \pm 1.0$

### 5.2. Studied Soil Characteristics

The basic physico-chemical properties of the studied soil are summarized in Table 3. The soil is slightly acidic and well developed with high content of organic matter. It has a slightly coarse texture and heavily contaminated with metal(loid)s due to occasional floods in the area which carries contaminants from slag piles generated from the Pb-processing smelter. All the metal(loid)s (As, Cd, Pb and Zn) exceeded the limit values for metal(loid)s in agricultural soils set by the Ministry of the Environment of the Czech Republic (Act No. 13/1994) (Table 4).

Metal(loid)s fractionation in the studied soil (Table 5) shows the majority of As concentration (72%) was in the fraction associated with amorphous and poorly crystalline hydrous oxides of Fe, Mn and Al. Also, the largest part of Cd and Zn were in the exchangeable fraction (61% and 46%, respectively) which is the most mobile fraction whilst, Pb was mainly found in the reducible fraction (61%), usually associated with metals bound to Fe, Mn and Al oxides.

Table 3: Basic physico-chemical characteristics of the studied soils.

<b>pH H<sub>2</sub>O</b>	5.95 ± 0.02
<b>pH KCl</b>	5.14 ± 0.04
<b>CEC (cmol kg<sup>-1</sup>)</b>	9.08 ± 0.52
<b>TOC (%)</b>	2.15 ± 0.05
<b>Particle size distribution (%)</b>	
<b>Clay (%)</b>	7
<b>Silt (%)</b>	31
<b>Sand (%)</b>	62
<b>Texture</b>	Sandy loam

Table 4: Pseudo-total content of metals in studied soil. Limit concentrations of metal(loid)s in agricultural soils are set according to the Ministry of the Environment of the Czech Republic (Act No. 13/1994)

<b>Pseudo-total concentrations (mg kg<sup>-1</sup>)</b> (n= 3)		<b>Limit concentrations (mg kg<sup>-1</sup>)</b>
<b>As</b>	296 ± 6	30
<b>Pb</b>	3539 ± 375	140
<b>Cd</b>	39 ± 1	1
<b>Zn</b>	4002 ± 68	200
<b>Cu</b>	68 ± 3	100
<b>Mn</b>	4276 ± 34	No limit



Table 5: Fractionation of Zn, Cd, Pb and As in the studied soil.

Fractionation of metals (sequential extraction by Rauret <i>et al.</i> , 2000) (mg kg <sup>-1</sup> ) (n = 3)					
	<b>FA: exchangeable</b>	<b>FB: reducible</b>	<b>FC: oxidizable</b>	<b>FD: residual phase</b>	
<b>Zn</b>	1822 ± 50	816 ± 45	298 ± 32	1066	
<b>Cd</b>	24.0 ± 0.3	8.0 ± 1.1	1.3 ± 0.2	6	
<b>Pb</b>	281 ± 11	2165 ± 176	705 ± 93	388	
Fractionation of As (sequential extraction by Wenzel <i>et al.</i> , 2001) (mg kg <sup>-1</sup> ) (n= 3)					
	<b>FA: non-specifically sorbed</b>	<b>FB: Specifically adsorbed</b>	<b>FC: bound to poorly crystalline hydrous oxides of Fe, Al and Mn</b>	<b>FD: bound to well crystallized hydrous oxides of Fe, Al and Mn</b>	<b>FE: Residual phase</b>
<b>As</b>	0.16 ± 0.02	20.2 ± 0.1	214 ± 4	45.9 ± 8.4	16

### 5.3. Incubation Experiment

From Figure 6, the application of both the AMO and AMO/BC increased the pH significantly from control values in the more oxic condition. However, the influence of the AMO/BC started reducing gradually after one week of application to control values (from 7.2 to 5.6), whilst the effect from the AMO was still seen after 10 weeks. Under the over-saturated conditions, the pH was generally higher for all variants. Although both amendments effectively increased the pH after 1 week, not much influence was observed for both amendments after 4 weeks. Nonetheless, the AMO/BC seemed promising in increasing the pH further after 10 weeks similar to control values. The Eh-diagrams confirms the drop of Eh in variants over-saturated with water compared to those with common humidity (Fig. 7). Under the over-saturated condition, the rate of reduction of Eh in AMO/BC variants was drastic in all periods than the AMO variants in comparison with the control. Nonetheless, the drop occurring in AMO amended variants was consistent in contrast to the AMO/BC amended variants which seemed to normalize after 4 weeks.

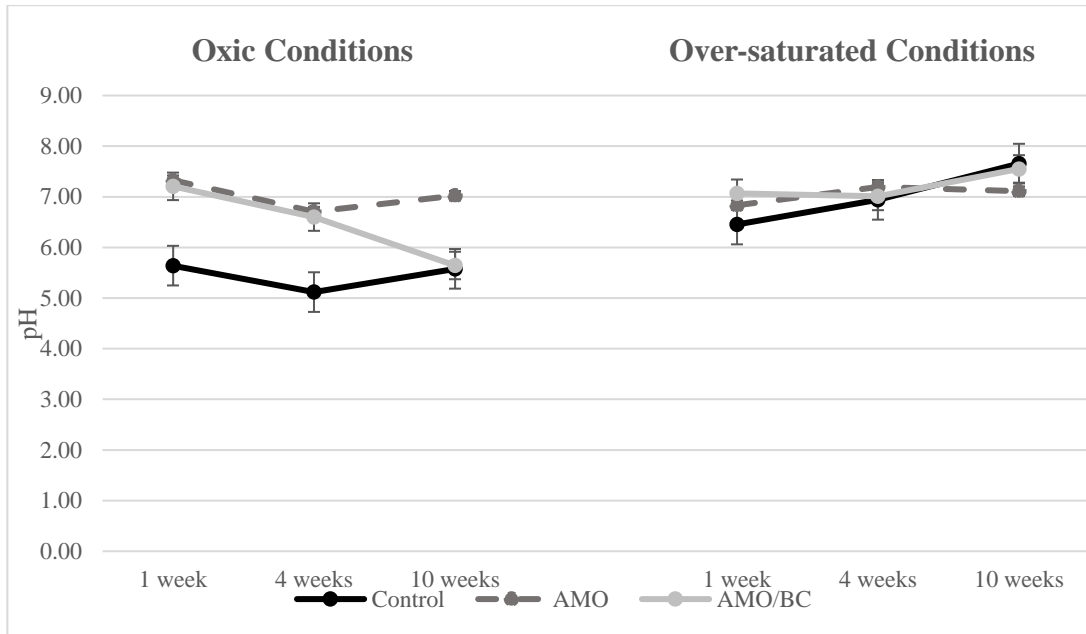


Figure 6: pH of soil solution from incubation experiment for both redox regimes. AMO: amorphous manganese oxide (1%, w/w), AMO/BC: amorphous manganese oxide/biochar (1%, w/w), (n=2).

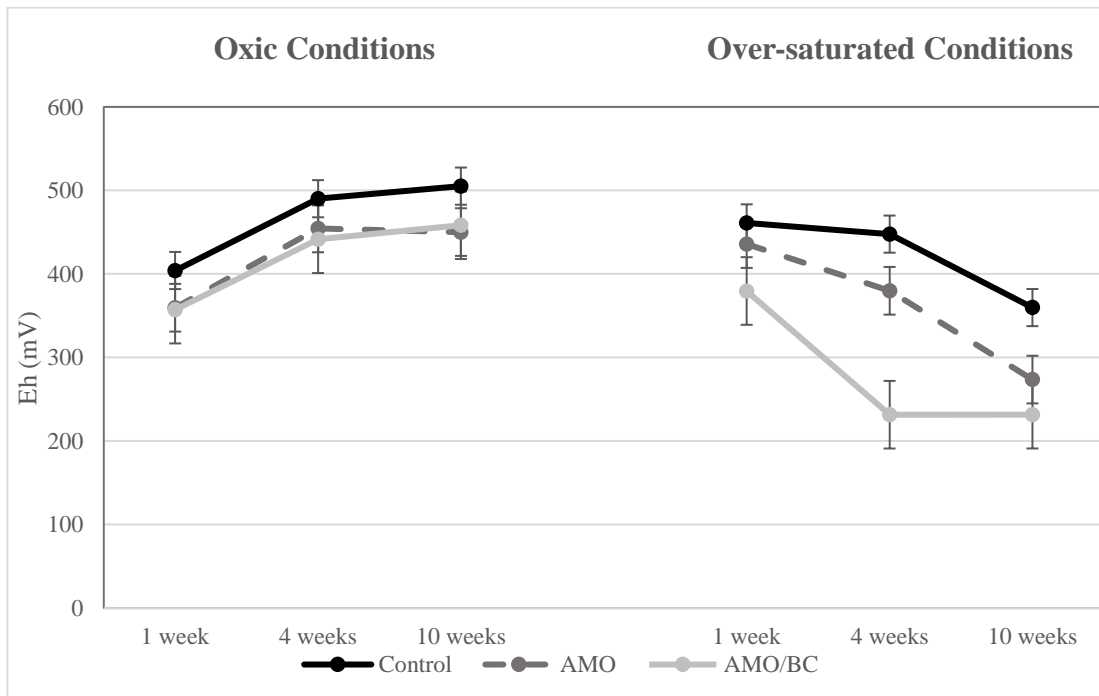


Figure 7: Eh of soil solution from incubation experiment. AMO: amorphous manganese oxide (1%, w/w), AMO/BC: amorphous manganese oxide/biochar (1%, w/w), (n= 2).

The influence of pH and Eh is clearly seen in the metal availability and sorption capacity of both amendments with increased incubation time. For instance, the concentration of all the elements except Zn were at least an order of magnitude higher in the over-saturated variants than in oxic conditions (see Figs. 8 and 9). In the case of

Mn for example, the concentration under oxic conditions increased rapidly in the presence of both amendments whereas the amount for the control samples reduced with increased incubation time (Fig. 8a). The higher concentration of dissolved Mn was recorded in AMO treatment than the AMO/BC with the peak concentration recorded in 4 weeks.

A different phenomenon was however observed under over-saturated conditions (Fig. 8b). After the 1 week and 4 weeks of incubation, the concentration of Mn under the influence AMO and AMO/BC reduced below the control, with the lowest being AMO. Nonetheless, after 10 weeks, the concentration of Mn increased for all variants but significantly under the amended variants.

Both amendments effectively reduced the concentration of Zn, Cd and Pb throughout the incubation period under more oxic conditions (Figs. 8c, 9a, 9c). The most significant reduction after amendment application occurred in 1 week. The AMO seem more efficient in the reduction of Zn than AMO/BC after the entire incubation period (1, 4 and 10 weeks). Same can be said for Cd but only after 4 weeks. On the other hand, the AMO/BC was more efficient in the reduction of Pb than the AMO for the entire incubation time.

In the over-saturated conditions, low concentrations were observed for Zn, Cd and Pb after the AMO and AMO/BC application in 1 week and 4 weeks although the amounts were generally an order of magnitude higher than concentrations in more oxic conditions (except for Zn). Furthermore, the concentrations of all three elements (Cd, Pb and Zn) after the 10 weeks, increased sharply beyond the control for both amendments. This is similar to the observation made with Mn under the same condition (see Figs. 8d, 9b and 9d). In addition, no significant difference was realized between the adsorption capacity of both AMO and AMO/BC in 1, 4 and 10 weeks under over-saturated condition except for Cd.

For As dissolution, the amount in all the variants increased with time in both water conditions although the concentrations in over-saturated condition is an order of magnitude higher than more oxic condition. No significant difference was observed between both amendments in As dissolution under both redox regimes.

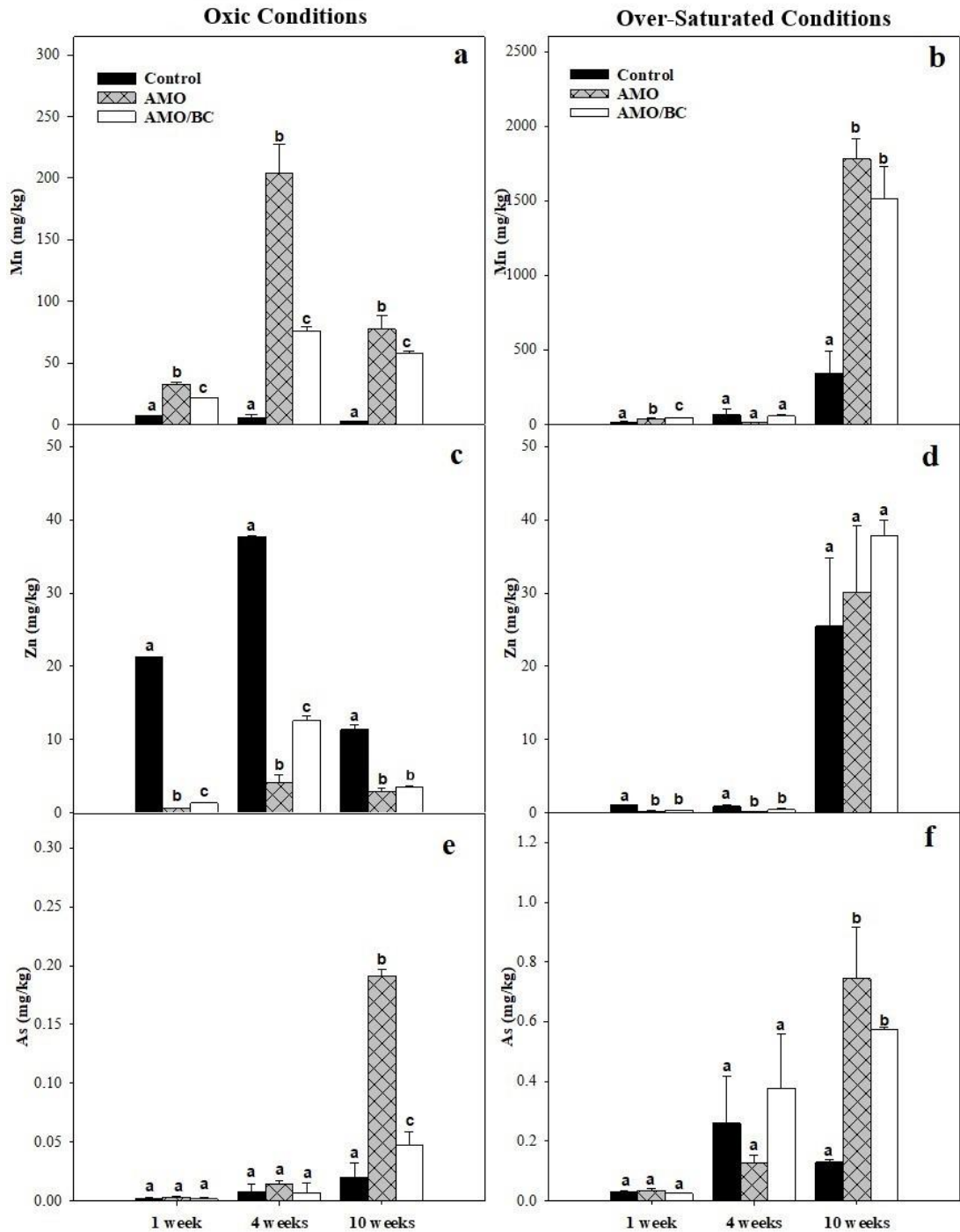


Figure 8: Metal and As concentrations observed after amendment application over time in changing redox: concentrations of Mn (a), Zn (c) and As (e) solutions under oxic conditions, concentrations of Mn (b), Zn (d) and As (f) in solutions under over-saturated conditions (n = 2); C: control, AMO: amorphous manganese oxide (1%, w/w), AMO/BC: amorphous manganese oxide biochar (1%, w/w). Statistical evaluation was performed to compare data for various variants in each time interval separately, data with the same letter represent statistically identical values (P < 0.05).

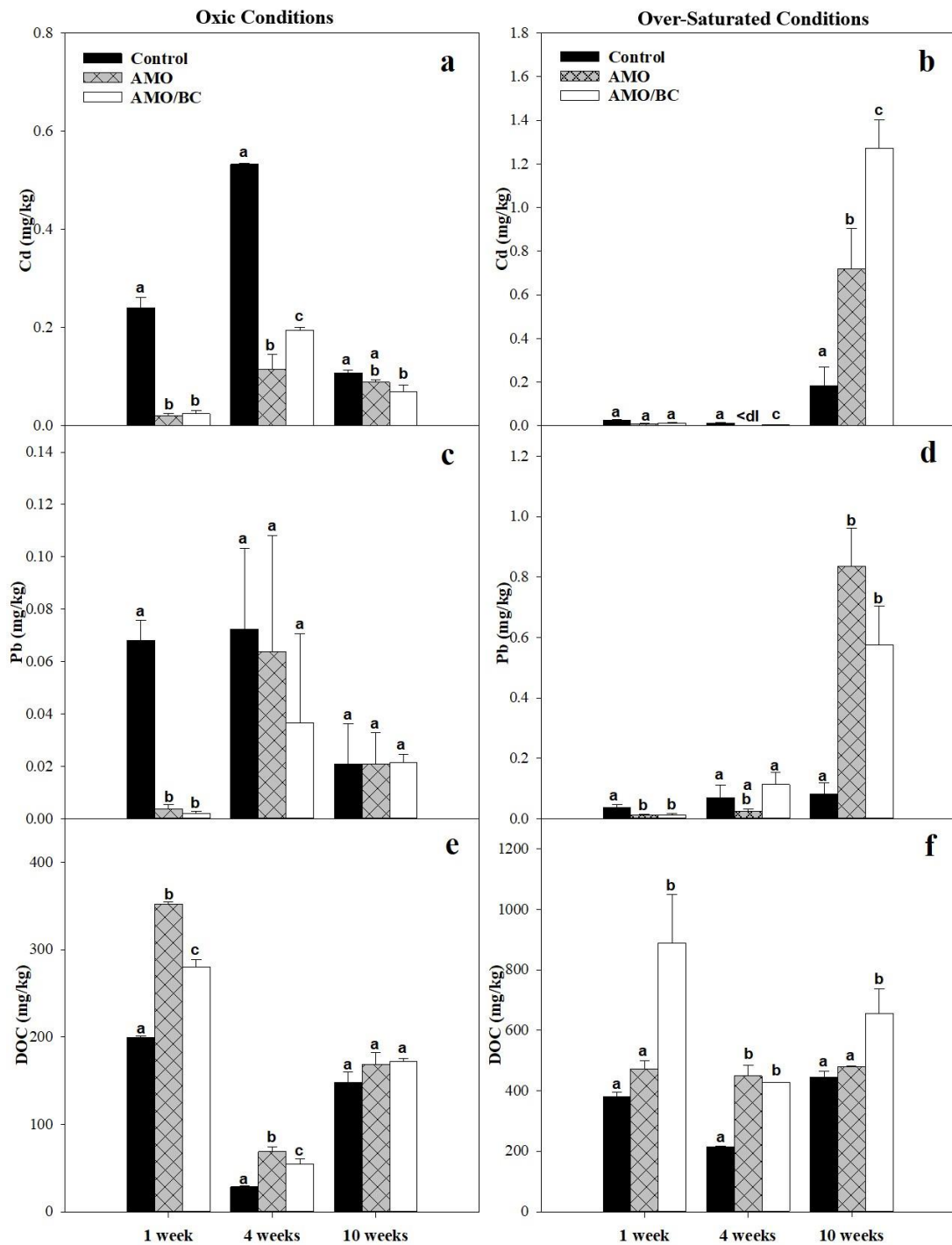


Figure 9: Metals and DOC concentrations observed after amendment application over time in changing redox: concentrations of Cd (a), Pb (c) and DOC (e) in solutions under oxic conditions, concentrations of Cd (b), Pb (d) and DOC (f) in solutions under over-saturated conditions (n = 2); C: control, AMO: amorphous manganese oxide (1%, w/w), AMO/BC: amorphous manganese oxide biochar (1%, w/w), <dl: values below the limit of detection. Statistical evaluation was performed to compare data for various variants in each time interval separately, data with the same letter represent statistically identical values (P < 0.05).

The increased concentrations of dissolved organic carbon (DOC) compared to control were pronounced for both AMO and AMO/BC in 1 and 4 weeks under oxic conditions. However, no significant difference was seen in amended variants compared to the control in 10 weeks under oxic conditions. In contrast, DOC dissolution under over-saturated conditions was significant in AMO/BC variants for 1, 4 and 10 weeks and only 4 weeks for AMO compared to control. Again, concentration of DOC was an order of magnitude higher in over-saturated conditions than oxic conditions (see Figs. 9e, 9f).

## 6. Discussion

### 6.1. Synthesis and Characterization of AMO and AMO/BC

The higher pH and  $\text{pH}_{\text{zpc}}$  values recorded for the AMO/BC as compared to AMO may be due to the biochar added to the synthesis. Biochar is typically an alkaline material (Lehmann, 2007) and a positive correlation has been found between the pH and pyrolysis temperature of biochar (Jin *et al.*, 2016). Increasing temperature in biochar synthesis leads to higher ash-component which positively correlates with high pH of biochar as it is in this study (600°C) (Trakal *et al.*, 2014). The high pH of AMO/BC could also be a result of oxygen-containing organic functional groups and carbonate minerals that might be present in the biochar (Yuan *et al.*, 2011).

As indicated in the study of Della Puppa *et al.* (2013), sorption properties for metal(loid)s by amendments correlate with their physicochemical properties (pH,  $\text{pH}_{\text{zpc}}$ , SSA and CEC). Whereas high CEC, high SSA, low  $\text{pH}_{\text{zpc}}$  and high pH indicates higher sorption of metals cations, a high  $\text{pH}_{\text{zpc}}$  indicates a higher sorption of metalloids like As. However, as indicated in Hongbo *et al.* (2017), when  $\text{pH}_{(\text{solution})} > \text{pH}_{\text{zpc}}$ , the material has a negatively charged surface and may be efficient in binding metal cations and vice versa.

Based on this assertion, the AMO/BC in this study was positively charged throughout the incubation period under both water regimes whilst the AMO charge varied with increasing incubation period. Regardless, considering the adsorption pattern in the incubation experiment other factors such as redox (Eh) and soil organic matter (SOM) may have played a role in adsorbing or desorbing metal(loid)s. The surface functional groups (carboxyl and hydroxyl groups) of the biochar could also be a factor in AMO/BC sorption capacity (Chen *et al.*, 2008). In addition, the sorption properties of Mn-oxide coated biochars have been tested and proven in (Trakal *et al.*, 2014, 2017; Ren *et al.*, 2015; Tan *et al.*, 2015; Zhou *et al.*, 2017). Further, the value of the average oxidation state of the AMO may reflect the detachment of Mn bound weakly bound on the surface of the AMO (Della Puppa *et al.*, 2013). The formation of Mn-oxalate hydrates crystals in both amendments also suggest the transformation of glucose during the AMO synthesis (Ettler *et al.*, 2014).

## 6.2. Sampled Studied Soil

The high amount of metal(loid)s found in the studied soil is consequent of historical activities of the Pb-processing smelter in the soil location (Komárek *et al.*, 2013). These concentrations may explain the slightly acidic condition of the soil leading to high metal(loid)s mobility (Ashraf *et al.*, 2012). For instance, a high amount of Zn and Cd were in more mobile fraction (exchangeable) reflected in the control values found for the concentration of these metals during the incubation experiment (Figs. 8c and 9a respectively).

The highest amount of Pb in reducible fraction of the soil also indicates a potential remobilization of Pb under reduced conditions as a result of oxide degradation or dissolution and vice versa (Frohne *et al.*, 2011). Similarly, though a slightly acidic soil, the huge fraction of As associated with amorphous and poorly crystalline hydrous oxides of Fe, Al and Mn may result in the dissolution of As in the soil when the oxides dissolves or As adsorption when these oxides are stable (Zhang *et al.*, 2017).

## 6.3. Incubation Experiment

The increase in pH of the soil solution during the incubation experiment may be due to the high pH of both amendments and the redox conditions as previously mentioned. Generally, redox conditions affect soil pH, DOC, microorganism activity and redox chemistry of oxides like those of Mn (Huang, 2014; Schulz-Zunkel *et al.*, 2015; Rinklebe *et al.*, 2016). Increase in Eh is known to facilitate oxidation processes which generates more H<sup>+</sup> thereby reducing pH (Yu *et al.*, 2007). Thus, as pH increases, there is a linear decrease in Eh. This is consistent with the pH and Eh results observed (see Fig. 6 and Fig. 7) although the sharp decrease in pH of AMO/BC after 4 weeks may not be the result of oxidation.

A similar observation was also made in over-saturated conditions for AMO/BC where a sharp decrease in Eh resulted in a slight increase in pH after 1 week. This difficulty in describing redox processes on pH may be because of the interplay between microorganism activities, availability of oxygen and changes in DOC in the presence of redox sensitive species like Mn (Chen *et al.*, 2006; Ying *et al.*, 2011).

Except for Zn, the concentration of all the elements in soil solution, including DOC, were at least an order of magnitude higher in over-saturated conditions than in more oxic conditions (see Figs. 8 and 9). This may be consequential to increasing levels of



Mn in soil solution under over-saturated conditions, with increased incubation time (Fig. 8b). Reductive dissolution of Mn oxides (from more stable  $Mn^{4+}$  to a more mobile  $Mn^{2+}$ ) occurs under reducing Eh conditions or decrease in pH thereby releasing metal(loid)s bound on their surfaces (Frohne *et al.*, 2011; Rinklebe *et al.*, 2016; Beiyuan *et al.*, 2017).

In addition, the higher levels of DOC (suggesting SOM degradation) in over-saturated conditions compared to oxic conditions, is another very important factor that may influence the higher concentration of metal(loid)s recorded (Du Laing *et al.*, 2009). The concentration of DOC decreases with increasing Eh resulting from microbial carbon consumption (Yu *et al.*, 2007) and increases under reducing conditions (Grybos *et al.*, 2009).

Increasing degradation of SOM contributes to degradation of AMO/AMO-BC (suggested by increased Mn concentrations) thus making metal(loid)s more mobile. Further, metals bound to SOM may be released into soil solution as SOM degrades. The strong oxidation properties and the glucose residue in the structure of the AMO even after washing may result in increasing DOC concentrations (Della Puppa *et al.*, 2013; Trakal *et al.*, 2014; Michálková *et al.*, 2016a).

The decrease of Zn and Cd concentrations in the amended variants under oxic conditions confirm the effectiveness of AMO and AMO/BC to adsorb metals onto its surface as proven in other studies (Della Puppa *et al.*, 2013; Trakal *et al.*, 2017). The high amount of Mn and DOC released into the soil solution upon the application of the amendments did not reduce their sorption capacity for both metals under more oxic conditions in this study. On the other hand, the concentration of Zn and Cd increased as Mn dissolves from the amendments and DOC increases with decreasing Eh from 1-10 weeks under over-saturated conditions.

Significant difference was realized between AMO and AMO/BC adsorption in 1 and 4 weeks for Zn under oxidic conditions, 4 weeks for Cd under oxic as well as 4 and 10 weeks for Cd in over-saturated conditions. In these situations, concentration was higher under AMO/BC than AMO.

Like Zn and Cd, very small amounts of Pb was recorded under oxidic conditions compared to over-saturated conditions where the concentration is an order of magnitude higher. The affinity of Pb towards SOM was highlighted in the study of

(Orsetti *et al.*, 2013). In this study, SOM was a major sorbent for Pb in flood plain soils. Also, Pb affinity to Mn oxides was confirmed in (Rinklebe *et al.*, 2016 ex. Mekenzie, 1980). Here, Mn oxides adsorption of Pb was 40 times greater than Fe oxides. Similar experiment (Micháľková *et al.*, 2014) also showed a faster adsorption of Pb onto Mn oxides than Fe oxides. Thus, SOM decomposition and/or Mn oxide degradation may lead to Pb dissolution and vice versa in soil solution.

These may well explain the gradual increase in Pb dissolution over time in over-saturated conditions, most especially, the spike in concentrations in 10 weeks as well as the gradual decrease of Pb in oxidic conditions from 1 to 10 weeks. Although, DOC dissolution was high in 1 week under oxic conditions and may influence Pb dissolution, Mn was relatively stable after amendment application in the same period under same water condition. This could explain the high efficiency of both amendments in decreasing Pb concentrations compared to control values in 1 week of oxic conditions than 4 and 10 weeks.

The insignificant difference between both amendments for Pb in all periods, except 4 weeks under over-saturated conditions, may be attributed to the high pH values recorded for both amendments since Pb is highly pH dependent. This difference observed in 4 weeks under over-saturated conditions where AMO adsorption was more efficient than AMO/BC ( $p < 0.05$ ) may be because, whilst Mn dissolution was low after AMO application, DOC was high for both amendments. Therefore, degradation of the biochar in AMO/BC may be occurring faster and releasing Pb bound to its surface under over-saturated conditions.

Arsenic(As) is a redox sensitive element and a decrease in redox which correlates with an increase in pH may increase As mobility (Manning *et al.*, 2002). As mobility occurs when a more stable As(V) is reduced to a more mobile and toxic As(III) under reducing conditions (Frohne *et al.*, 2011). The addition of biochar, which is generally alkaline and may increase pH of acidic soils may therefore contribute to As dissolution when added to soils (Choppala *et al.*, 2016).

The study by Micháľková *et al.* (2016b) also proved that the adsorption of As onto AMO generally decreases with decreasing pH. In this study, although very negligible and statistically insignificant in 1 and 4 weeks, the As availability or mobility seem to increase with increasing Eh and decreasing pH for all variants under oxic conditions, especially for AMO (Fig. 8e) in line with the observation made in (Micháľková *et al.*,

2016b). In 10 weeks however, a slight increase in pH from the previous week significantly increased As concentration for AMO variants. This is in line with other studies (Dixit and Hering, 2003; Zhang *et al.*, 2017) in which As concentration was noted to increase with increasing pH and contrary to the finding made in Michálková *et al.* (2016b).

Similarly, in over-saturated conditions, the As solubility seemed to respond to the Eh and pH dynamics although the difference between the control and the amended variants are not statistically significant in 1 and 4 weeks. In 10 weeks however, As dissolution was significantly higher in amended variants for the two amendments ( $p < 0.05$ ) compared to control values (Fig. 8f). No significant difference was seen between the amendments. This situation may be attributed to the very low Eh values recorded for amended variants in the period coupled with rising pH values compared to with control values. Again, this is consistent with the study of Zhang *et al.* (2017) where As adsorption was noted to decrease when the pH increases.

In addition, majority of As concentration present in the studied soil was in the fraction bound to amorphous and poorly crystalline hydrous oxides of Fe, Al and Mn (Table 4). Therefore, a reduction in Eh leading to high dissolution of Mn in amended variants may contribute to high As dissolution. The complexation with biochar functional groups (carboxyl and hydroxyl groups) could also explain As sorption by the AMO/BC (Samsuri *et al.*, 2013).

## 7. Conclusions

The efficiency of amorphous Mn oxide (AMO) and AMO/BC (1% wt.) in stabilizing metals in contaminated soil was evaluated under two soil moisture regimes – 70% water holding capacity and over-saturation with water and over a period of 10 weeks using an incubation experiment. After the experiment, it was observed that metal(loid)s and DOC dissolution was at least an order of magnitude higher in over-saturated conditions than more oxic conditions. Also, increased Mn and DOC release occurred under both water regimes in amended variants while being more pronounced in over-saturated conditions.

Both amendments were effective in reducing Zn, Cd and Pb concentrations in soil solution over time in more oxic conditions while the concentrations of these risk metals increased over time in over-saturated condition in amended variants. Again, both amendments were ineffective in stabilizing As over time as As concentration increased with increasing incubation period under both moisture regimes.

Overall, not much significant difference was recorded between the immobilizing efficiency of AMO and AMO/BC in both water regimes. Both amendments degraded under the two redox conditions (suggested by Mn and DOC dissolution) but highly under over-saturated conditions. Nevertheless, the degradation of the amendments did not hinder its adsorption capacity for Zn, Cd and Pb in more oxic conditions than in over-saturated conditions.

It can therefore be concluded that both amendments are comparably efficient in metal adsorption under normal humidity but poorly efficient under over-saturated conditions over time. Also, both amendments proved ineffective in As stabilization under both redox regimes. Lastly, metal(loid)s and DOC dissolution are higher under decreasing Eh.

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**Appendix: Comparison of Environmental Remediation Methods (Khalid *et al.*, 2016)**

Techniques	Process	Applicability	Acceptance	Multi-metal sites	Time required	Advantages	Limitations
<b>Physical remediation</b>							
<b>Soil replacement</b>	Excavating contaminated soil and replacing with non-contaminated soil	Small scale but long term	Very low: limited to highly contaminated soils	Effective	Comparatively very less	Can effectively isolate risk metals from contaminated site. Effective for highly contaminated soils.	Large in working volume, costly, production of dangerous waste and negative effect on soil
<b>Soil isolation</b>	Isolating the contaminated soil from the uncontaminated soil using subsurface barriers	Small scale, and short to long term	Very low: limited to highly contaminated soils	Effective	Comparatively very less	Prevent off-site transport of heavy metals, Effective for highly contaminated soils	Costly, soil clean up still needs further engineering measures, effectiveness varies with the type of subsurface barrier
<b>Vitrification</b>	Using high-temperature to vitrify contaminated soil thereby reducing risk metal bioavailability	Small scale but long term	Very low	Effective	Comparatively very less	Easy application, applicable to variety of contaminants	High cost due to energy requirement
<b>Electrokinetic Remediation</b>	Applying DC voltage via electrophoresis, or electro-migration to reduce risk metals in soil	Small scale but long term	Very low	Effective	Comparatively very less	Easy application, economically effective, do not destroy the nature of soil	Requires soil with low permeability, pH needs to be controlled
<b>Chemical remediation</b>							
<b>Immobilization</b>	Reduction in metal mobility and bioavailability by applying immobilizing amendment, and forming stable and immobile complexes via adsorption	Small to medium scale and short-term	High public acceptability	Can be effective. Depends on the type of soil, metal and immobilizing amendment	Less to medium	Fast and easy applicability, relatively low costs, covers a broad spectrum of inorganic pollutants	Temporary solution and permanent monitoring is necessary

<b>Soil washing</b>	Applying extractants (organic or inorganic) and forming stable and mobile complexes	Small scale but can be long term	Medium to high public acceptability	Can be effective. Depends on the type of soil, metal and immobilizing amendment	Less to medium	Cost-effective, completely removes metals, meets specific criteria and reduces long-term liability	Washing extractants may cause environmental issue, effectiveness varies with soil, metal and extractant type
<b>Biological remediation</b>							
<b>Phytovolatilization</b>	Risk metal uptake by plants from soil and release in vapor form to atmosphere	Small to medium scale and long-term	Low-medium public acceptability	No	Very high	Economical and less disruptive	Restricted to volatile metals, may cause other environmental issues, no control after metal release to atmosphere
<b>Phytostabilization</b>	Use of plants to decrease metal bioavailability and mobility in soils via sequestration in plant roots	Small to medium scale and short-term	Medium public acceptability	Very low	Very high	Economical, less Disruptive	Temporary solution, effectiveness varies with soil, plant and metal type
<b>Phytoextraction</b>	Use of hyperaccumulator plants to uptake, translocate, and concentrate risk metals from soil to the aboveground harvestable plant parts	Large-scale and long-term	Highest public acceptability	Very low except for some plants	Very high	Highly economical, ecofriendly, less disruptive	Effectiveness depends on growing conditions, tolerance of the plant, bioavailability of metals in soil. Metal accumulator plants are generally very less in number
<b>Chelate assisted Phytoextraction</b>	Use of organic and inorganic ligands to enhance phytoextraction capacity of plants	Small to medium scale and long-term, low to moderate levels of metal	Very high public acceptability	Very low but more effective than phytoextraction alone	Very high but less than phytoextraction alone	Low time of remediation, enhance metal uptake and translocation	Costly, can be disruptive, effective for low-moderately contaminated soils, groundwater contamination risk
<b>Microbial assisted phytoextraction</b>	Use of microorganisms to enhance phytoextraction capacity of plants	Large-scale and long-term	Very high public acceptability	Very low but more effective than phytoextraction	Very high but less than phytoextraction alone	Economical, low time of remediation, enhance plant growth and metal uptake and translocation	Depends on microorganism, soil, plant and metal type