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Stabilization of zinc in the soil profile after the application of biochar and amorphous

manganese oxide

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DIPLOMA THESIS ASSIGNMENT

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Thesis title

Stabilization of zinc in the soil profile after the application of biochar and amorphous manganese oxide

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Objectives of thesis

Aim of this study is to optimize a dose of applied biosorbent using batch experiment and to compare the application of biochar and its modified form as a thin layer against the well-mixing of biochar with the soil. Additional aim is to provide geochemical modelling of zinc speciation and its potential precipitation in the leachate.

Methodology

First, review of the biochar presence to metal(loid)s behaviour in the soil will be provided.

Next, a batch experiment will then be realized in order to test optimal dose of tested biosorbents (biochar vs. AMOchar, respectively). Selected biosorbent at optimized dose will then be used for column leaching test in order to compare the application separately (as a thin layer) against the mixing together with soil. Continuously, pore water and leachate will be analysed for Zn and other elements concentration, pH, Eh and EC. Finally, all obtained data will then be used for geochemical modelling using Visual Minteq software package.

The proposed extent of the thesis

50-60

Keywords

biosorption, column leaching, biochar, AMOchar, modelling

Recommended information sources

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Prague on 17. 04. 2017

Diploma Thesis Author's Declaration:

I, Cody Shepard, hereby declare that I have written the following diploma thesis as an independent piece of scholarship by myself under the direction of Mgr. Lukáš Trakal, Ph.D. Every publication and piece of literature used to acquire information for the purpose of constructing this diploma thesis is listed in the Works Cited section of this diploma thesis.

Signature: _____

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Abstract

English: It is well documented that zinc and other metal contaminants pose ecological and human health risks. There is a need for cost-effective *in situ* remediation of metal contaminants. This study uses soil contaminated by a lead smelting plant near Příbram, Czech Republic with an amendment that combines synthetic amorphous manganese oxide with biochar derived from grape stalk agricultural waste (2:1) as an amendment called AMOchar to stabilize zinc in the soil profile. The biochar was pyrolysed at 600 °C under nitrogen flow and the amorphous manganese oxide was synthesized according to a modified procedure for synthesizing birnessite. The results of the experiment show that a 2% treatment by weight of AMOchar in the soil profile significantly stabilized zinc by lowering soil solution concentrations and leachability of zinc. The presence of AMOchar also significantly raises the pH level of soil solution and leachate. Applying the 2% AMOchar treatment distributed throughout the soil profile rather than in one discrete layer also significantly raised the pH of the soil solution.

Keywords: Biochar, AMOchar, Remediation, Sorption, Zinc

Česky: Je dobře zdokumentováno, že zinek a další kovy a metaloidy představují ekologické a zdravotní riziko. Existuje tak potřeba cenově efektivních *in situ* sanačních metod pro odstraňování kovů a metaloidů. V této studii je využita kontaminovaná půda z okolí olověné hutě u Příbrami s použitím půdního sorbentu, který kombinuje výhody syntetického amorfního oxidu manganu s biocharem (pocházejícího z vinných hroznů tedy zemědělského odpadu) a to v poměru (2: 1) nazývaný jako AMOchar pomocí něhož je zde stabilizován zinek. Biochar byl zde pyrolyzován při teplotě 600°C za dusíkového proudění a pro výrobu amorfního oxidu manganu byla použita modifikovaná výroba birnesitu. Výsledky pokusu ukazují, že ošetření dvěma hmotnostními procenty AMOcharu byl významně stabilizován zinek v půdě snížením koncentrace půdního roztoku a vyluhovatelností zinku. Aplikace AMOcharu distribuovaného v půdě byla vhodnější než použití sorbentu jako samostatné vrstvy s významným zvýšením půdního pH.

Keywords: Biochar, AMOchar, Remediace, Sorpce, Zinek

1. Introduction

The quality of our soil has a direct impact on the lives of humans and is constantly subjected to degradation and harmful pollution. The impact of metal contamination in soil is considered to be one of the most pressing matters regarding food security and food safety in Europe and the rest of the world (Toth *et al* 2016). Metal concentrations in our soils are exceeding background levels due to anthropogenic activities specifically that of the industrial, mining and agricultural sectors (Guan *et al* 2014). Soil pollution from metal and metalloid deposition tends to be a difficult yet very important focus of remediation because of the non-degradable characteristics associated with these pollutants and the risks that they present to groundwater reserves through leaching and adjacent land, including agricultural areas that provide an entry point for metals into the trophic chain where they may eventually present health risks to humans (Madejon *et al* 2010). Because soil pollution can affect the availability of resources to humans, it is very important to make sure our soils are of high enough quality to sustain present ecosystems for the benefit and well-being of future generations of humans (Adriano 2001). As soil is a medium for interaction between various biospheres, metals and metalloids may have adverse effects on biological systems, which ultimately affects humans, within several environmental compartments including groundwater, surface water, atmospheric air, and microbial, plant and animal communities (Komárek *et al* 2013). The extent of the pollution in the European Union alone is daunting, annual remediation costs are estimated to exceed €17 billion annually (Toth *et al* 2016). Crops grown on polluted soils may suffer from decreased crop yield and quality (Adriano 2001). Therefore, the quality of the soil can indirectly affect the economic activities of an area and also how well nourished the people of an affected area are. Although most of these elements are necessary for the survival of animals and plants at small levels, they become toxic once they have reached a certain concentration, which can be especially harmful to farmers who are cultivating crops as it has an effect on biomass

production and, in turn, crop yield.

Determining the optimal level of stabilizing material necessary to adsorb and thus stabilize zinc in soil can allow us to use the treatment more effectively, efficiently and increase the efficacy of other treatments to remove the metal from the soil by other means. By determining the most appropriate levels of biochar application in soil and which material is best used in combination with biochar a more cost-effective method for treating metal contaminated soil can be developed, which may impact the ease at which this technology is available. Stabilizing metal contaminants, such as zinc, in soil is an important first step in several contaminated soil remediation techniques (Mulligan *et al* 2001), which is why perfecting the technique through further research is important.

2. Aims of Study

The aims of this diploma thesis include determining the most efficient, cost-effective stabilizing material with regards to zinc stabilization and other inorganic metal pollutants in the soil profile, determine the most appropriate dosage of zinc stabilizing sorbent in the soil profile and determining the best method of application for this remediation technique. The best material and best dosage were determined by comparing several dosages of different materials for zinc sorption during the optimization experiment and then a comparison between two different applications of the same treatment, a layered and a distributed amendment approach, was carried out by examining zinc sorption in various depths of the soil profile and by analyzing leachate and soil solution samples.

3. Literature Research

3.1 Basic characterization of zinc in the context of soil and the implications on ecological and human health

Zinc, atomic number 30, is commonly produced on a global scale from ores

containing zinc sulfide minerals (Adriano 2001), such as sphalerite, wurtzite, smithsonite and hemimorphite. Zinc can be found as five stable isotopes, listed in order of descending relative abundances: ^{64}Zn, ^{66}Zn, ^{68}Zn, ^{67}Zn and ^{70}Zn. The relative abundances of those isotopes are 48.89%, 27.81%, 18.56%, 4.11% and 0.62%, respectively (Adriano 2001). The Zn^{2+} ion exists in an aqueous form in neutral and acidic solutions but zinc precipitates out of solution in alkaline conditions (Adriano 2001), which is important from the perspective of immobilizing zinc in soil environments. Zinc has been reported in the past of having a mean concentration in global soils of 50 ppm (Vinogradov 1959) but more recently Berrow and Reaves (1984) have reported that same concentration level to be approximately 40 ppm.

In general, the presence of metals in soil can pose a threat to the health of plants and microorganisms residing in the soil as well as the organisms downstream in the trophic chain due to the bioaccumulation of these metals (Kabata-Pendias 2004). One issue with metal contamination is that, while the metals are toxic when present in higher concentrations, most metals are considered to be essential for the growth of living organisms, including humans (Rzymski *et al* 2013). These metals are resistant to decomposition, which is why they are subject to bioaccumulation and the reason that metal contamination can be present not only in the soil but also in the ecosystem that the soil is a part of (Miloškovic *et al* 2013). This bioaccumulation of metals, including zinc, has the potential to affect human health by moving through the trophic chain and is responsible for higher concentrations in agricultural meat products than fruit and vegetable products (Toth *et al* 2016). The mobility of an element, which affects whether the metal will have the tendency to stay in the soil column or leach into groundwater, has implications on how dangerous the metal can be but what's perhaps more dangerous is the bioavailability of metals as this is what will initially introduce the metal into the trophic chain and subject the metal to bioaccumulation. In some cases, the presence of excess concentrations of metals in soils can also result in the loss of vegetation due to phytotoxicity (Kabata-Pendias 2004). This is the reason that the danger posed by a metal

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is largely a product of its mobility and phytotoxicity. The toxicological threshold and EU guideline limits regarding metal concentrations can be seen in Table 1.

Zinc is essential for humans, animals and plants but it is toxic in excess quantities (Toth *et al* 2016, Fosmire 1990), which is why it is important for management in agricultural soils. Zinc toxicity in humans may cause problems with the digestive and immune system and also can inhibit copper absorption, inducing copper deficiencies (Toth *et al* 2016). Zinc toxicity is not regarded as a serious health risk in Europe because only 0.45% of all samples exceeded recommended soil concentrations in a study carried out with over 20,000 agricultural soil samples from EU countries (Toth *et al* 2016). Zinc can be absorbed at different rates depending on the speciation, which can also affect the health risks of zinc contaminated soils (Toth *et al* 2016).

Substance (symbol)	Threshold value mg/kg	Lower guideline value mg/kg	Higher guideline value mg/kg
Antimony (Sb) (p)	2	10 (t)	50 (e)
Arsenic (As) (p)	5	50 (e)	100 (e)
Mercury (Hg)	0.5	2 (e)	5 (e)
Cadmium (Cd)	1	10 (e)	20 (e)
Cobalt (Co) (p)	20	100 (e)	250 (e)
Chrome (Cr)	100	200 (e)	300 (e)
Copper (Cu)	100	150 (e)	200 (e)
Lead (Pb)	60	200 (t)	750 (e)
Nickel (Ni)	50	100 (e)	150 (e)
Zinc (Zn)	200	250 (e)	400 (e)
Vanadium (V)	100	150 (e)	250 (e)

The guideline values have been defined on the basis of either ecological risks (e) or health risks (t). If the risk of groundwater contamination is higher than normal in concentrations below the lower guideline value, the substances are marked with the letter p.

Table 1: This table shows toxicological threshold values along with lower and upper guideline values for each metal(loid) based on human health and environmental risks. (Adapted from Toth *et al* 2016).

3.2 Factors Affecting Zinc Mobility in Soil

Zinc, among other metals, is very mobile depending on the conditions of the soil (Kabata-Pendias 2004). One thing that provides zinc with a relatively high mobility is the decreased affinity for sorption sites when compared to other metals, resulting in zinc being easily outcompeted for sorption sites (Kumpiene *et al* 2008). There is the potential for zinc to be sorbed on an individual basis at efficient levels, depending on the sorbent, but the presence of other metals with relatively high affinity for the sorbent will hinder that efficiency. The relatively low affinity for sorbents of zinc increases the ability of other metals to outcompete zinc for sorption sites and ultimately leads to the increased mobility of zinc in soils. Because other metals with a relatively high sorption affinity displace zinc at binding sites, zinc will not be held as efficiently and therefore the potential for zinc to be leached as a free ion is increased.

The mobility of zinc is largely affected by the pH level of the soil. Increasing the pH of soil solution is one of the most effective measures of immobilizing zinc, especially in soils with high organic matter content (Adriano 2001). Liming of soils to increase pH levels can immobilize and reduce plant uptake of potentially toxic contaminants, such as zinc. (Albasel and Cottenie 1985). This method of immobilization has its downfalls as plants also require small amounts of some metals in order to survive. By limiting plant uptake of metals, plants may be unable to obtain the concentrations of metals necessary for survival, resulting in nutrient deficiency. Numerous reports serve as evidence of plants not being able to obtain optimal amounts of zinc in soils with a high pH level (Adriano, 1986). It has been shown that a pH of above approximately 5.5 is likely to induce zinc deficiencies at some degree in the crops grown in these soils (Friesen *et al*, 1980; Gupta *et al*, 1971; Pepper *et al*, 1983) as well as a reduction in the amount of exchangeable zinc in extracted soil solution (MacLean, 1974). Further evidence supporting the claim that zinc deficiencies are correlated with high pH levels in soil can be seen studies carried out in Asian rice paddies by Forno *et al,* 1975 and Yoshida *et al*, 1973. The results from these experiments showed that zinc

uptake in rice plant, under both aerobic and anaerobic conditions, continuously decreased with increasing pH levels in the soil. In such cases where the pH is too high, it may be necessary to slightly acidify the soil in order to mobilize a small portion of the metal in order to create a bioavailable fraction of the metal for plant uptake (Fenn *et al* 1990). The challenge of this process is to limit the acidification of the soil to only the area where it affects the bioavailable level of metals where it may be obtained by plants. When soil conditions are more acidic, zinc has been found to be present in higher concentrations in its exchangeable and bioavailable form (Chlopecka *et al*, 1996; Liang *et al*, 1990). Payne *et al* (1988) showed that when the pH of a soil is above 6.5, most of the zinc in soil will revert to a non-exchangeable form, which may induce zinc deficiencies in plants. Doing so below this level may result in unwanted leaching of metal contaminants into groundwater and ultimately into our drinking water. Speciation of zinc as a function of pH can be seen in Figure 1.

Zinc mobility in the soil column can also be affected by the amount of organic matter in the soil. Zinc deficiencies are more likely to develop in plants that are grown in soils with high organic matter, particularly in muck, peat soils and even old agricultural sites (Adriano, 2001). The presence of organic matter in soil decreases the bioavailability and exchangeability of zinc in soil by forming insoluble compounds with zinc or by serving as a site for zinc adsorption (Adriano, 2001). In a study conducted by comparing two soils with varying organic matter concentrations, it was found that organic matter was more effective at decreasing the bioavailability of zinc to soybeans than iron and manganese oxides. Because soil organic matter becomes more soluble with increasing pH (Adriano, 2001), increasing the amount of organic matter in soil can compound with liming of soil to immobilize zinc but, as stated previously, this treatment should be implemented with caution as increasing the pH too high may result in zinc deficiencies in plants. This has major implications regarding the use of biochar as a soil amelioration for controlling zinc concentrations as the application of biochar, which is composed of pyrolysed organic matter, has the potential to affect the organic matter

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content in different regions of the soil column, depending on the application process.

Redox potential has an effect on zinc speciation also, although not to the same degree as that from pH or organic matter (Adriano 2001). Zinc itself can not be reduced under low redox conditions but under strongly reductive conditions (less than 150mV) zinc can form insoluble zinc sulfide, which affects its bioavailability and mobility (Adriano 2001, Connell and Patrick 1968). Redox potential is much less influential on the behavior of zinc than soil pH and soil organic matter and would only become influential in heavily flooded soils and rice paddies (Adriano 2001).
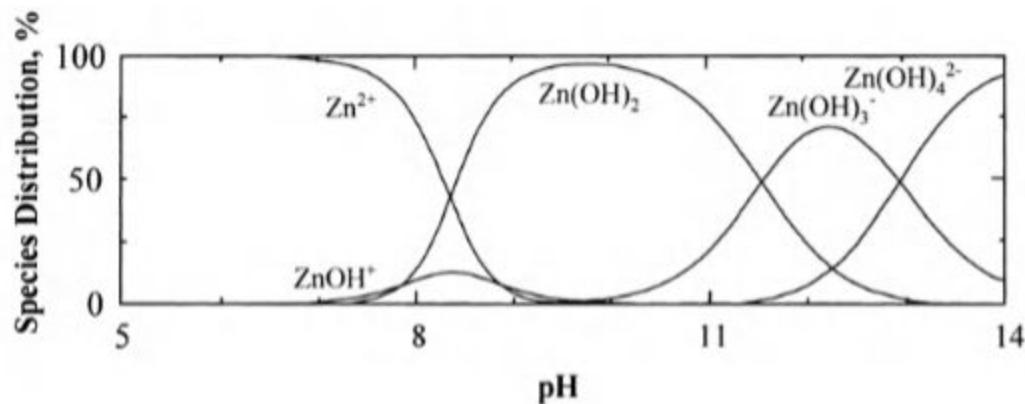
Figure 1: This figure shows zinc species distribution as a function of pH (adapted from Adriano 2001).

3.3 Remediation of zinc in soil

Zinc and other metal contaminants can be removed from the soil with a number of different methods. An overview of the main methods that can be used to remediate soils contaminated with metals can be seen in Table 2. The applicability and efficacy of these treatments vary depending on factors including the contaminant concentrations and the geologic setting of the contaminated site.

Type of Technology		Description
Containment/ Isolation	Physical Barrier/Capping	Prevention of movement or leaching with impermeable barrier
	Cementation	Injected chemicals solidify contaminant to create inert waste
	Vitrification	Application of heat or electrical energy to create glassy material
Ex situ Treatment	Physical Separation	Using froth flotation, screening, gravity separation or another method to separate contaminant from soil
	Pyrometallurgical	Extract metal contaminants through melting and metallurgical extraction and processing
	Soil Washing	Addition of another substance to dissolve contaminant and wash from soil
In situ Treatment	Reactive Permeable Barrier	Permeable material that reacts with dissolved contaminant to stabilize or immobilize
	Soil Flushing	Flushing with water to leach water-soluble contaminants
	Electrokinetic	Applying an electrical current to remove contaminant
	Phytoremediation	Using plants to stabilize or extract contaminants

Table 2: This table summarizes remediation technologies applicable to soil contaminated with metals (adapted from Mulligan *et al* 2001).

3.3.1 Physical Barriers and Capping

Another method of containing contamination *in situ*, which was mentioned previously, is the use of a physical barrier that is meant to limit or completely inhibit the movement of groundwater and soil water that could potentially move contaminants that are within the soil. These physical barriers consist of materials such as cement, bentonite, which is a clay developed from volcanic ash, and steel (Mulligan *et al* 2001). Techniques for the implementation of these contaminant containing barriers include

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capping, which inhibits surface penetration of water and thus reduces the amount of water infiltration in an attempt to avoid metal contaminant leaching, horizontal barriers, which disallow or severely limit vertical water infiltration past the point of the barrier, and vertical barriers, which prevents water movement from spreading metal contaminants horizontally in an attempt to keep the contamination within the same area (Mulligan *et al* 2001). These methods are often used in combination with extraction wells as a means to remove the contamination, as physical barriers only serve to contain the contamination rather than removing it (Anderson and Mesa 2006).

Capping as a means to keep contamination from spreading is an effective way to limit the amount of water within the soil column. Caps used to cover contaminated soils may be designed with uncomplicated materials such as clays, soil or gravel but may also consist of more complex or synthetic materials such as geotextiles, liners or even multiple layers of aforementioned materials (Palermo 1998). Using capping techniques decreases water infiltration in an attempt to decrease contaminant leaching and also decreases the amount of biological activity present in the contaminated sediment, which is another way that metal contaminants can become mobile in the soil column (Zoumis *et al* 2001). Incorporating a reactive material into the cap is another way of decreasing metal contamination within the underlying sediments but does little to aid in the remediation of soil that already has metal contamination present (Jacobs and Förstner 1999). One thing to consider before implementing this method is the potential for flooding events as capping cannot prevent any influx of water that moves through the soil horizontally. Flooding events in contaminated soils have the potential to oxidise metal contaminants, specifically zinc, and mobilize them this way (Zoumis *et al* 2001).

For contaminated sites with horizontal groundwater flow presenting a risk to the mobilization and transport of contaminants, a vertical barrier may be an effective option. Two types of vertical barriers exist: open design and circumferential. Open barrier designs serve to redirect groundwater flow around a specific area, such as extraction wells (Anderson and Mesa 2006). Circumferential barriers, which completely surround

and enclose a contaminated area, are most widely used with landfills and hazardous waste sites, such as that of the Superfund Site of Nashua, New Hampshire in the United States. (Anderson and Mesa 2006). Open barrier designs of miscellaneous composition have also been used at various sites in the United States in an attempt to halt groundwater flow to a specific region to reduce the amount of groundwater flow moving through a contaminated area and thus reduce the amount of contamination reaching groundwater reserves that are used for drinking water (Anderson and Mesa 2006). Vertical barrier designs are largely dependant on the local geology and groundwater flow and therefore vary greatly at each enactment (Anderson and Mesa 2006). Because zinc is a water soluble metal contaminant that is often organically bound (Mulligan *et al* 2001), the implementation of a vertical contaminant containment barrier could prove to be a very effective measure in the ultimate remediation of zinc contamination in sediments and soils.

Horizontal barriers, which are not as utilized and are still in the developmental stages of use, are often designed to limit the downward flow of contaminants within the soil and soil water columns (Dada *et al* 2015). Rather than completely restrict the movement of water, often times these barriers are implemented as reactive and permeable in an attempt to filter metal contaminants before they reach aquifers and groundwater supplies, such as that which has been trialed in Casey Station, Antarctica in 2005-2006 (Mumford *et al* 2014). Implementation of a horizontal barrier can be very costly due to the fact that excavation is often required for the installation of said barrier, which can be attributed to the lack of existing technology regarding the implementation of horizontal barriers without excavation. Although this technology does exist, it can only be used with certain materials and also requires a combination of vertical and horizontal boreholes (Kim and Corapcioglu 2002).

3.3.2 Solidification

Technology also exists to stabilize an environmental contaminant and reduce

contaminant mobility by solidification (Dada *et al* 2015). Solidifying the soil contaminants involves mixing a binding agent, such as cement, bitumen and asphalt, with the contaminated soil to create a crystalline, glassy or polymeric mass that reduces the rate of leaching (Dada *et al* 2015). The choice of remediation technique should be based on compounds other than zinc in the soil, since zinc can be found in several forms depending on the presence of P, Ca, Al, Mn and Fe oxides, pH as well as organic matter, as zinc is easily outcompeted by other cations for sorption sites (Kumpiene *et al* 2008). Contrarily, it has been found that the presence of certain anions, such as As anions, in soil solution increase Zn sorption with Fe oxides, such as goethite, by approximately 10 times (Kumpiene *et al* 2008). It is possible for zinc to precipitate with oxides, carbonates, phosphates, sulfides, molybdates and other anions and zinc also has the tendency to form complexes with organic ligands (Kumpiene *et al* 2008), further complicating the selection process for the most appropriate remediation technique.

3.3.3 Electrokinetic Treatment

Electrokinetic treatment of zinc contaminated soils requires a low intensity electrical current passing from a cathode to an anode within the contaminated soil (Mulligan *et al* 2001). This transports ions and other charged particles, including water, between the two electrodes (Mulligan *et al* 2001). Anions will move towards the cathode and cations will move towards the anode due to electromigration (charged chemical movement), electro-osmosis (movement of fluid), electrophoresis (charged particle movement) or electrolysis (chemical reactions resulting from an electric field) caused by an electrical gradient (Mulligan *et al* 2001). Metal contaminants that approach the electrodes can be removed from soil by electrodeposition, (co)precipitation, adsorption, complexing or soil flushing near electrodes (Dada *et al* 2015). This process can be carried out *in situ* or *ex situ* with the excavated soil (Mulligan *et al* 2001). This treatment is the most suitable when zinc and other metals are soluble ions or when they exist as oxides, hydroxides and carbonates (Mulligan *et al* 2001). This treatment is only

possible with fully saturated soils with a permeable soil matrix, low groundwater flow velocity and no obstructions to the electrical current such as a large boulder or anthropogenic structure (Mulligan *et al* 2001). More recently, enhancements have improved the efficacy of electrokinetic soil remediation of zinc by utilizing several anodes, known as the approaching anodes technique, instead of the conventional approach with only one anode (Cai *et al* 2016). A study carried out by Cai *et al* (2016) in soil contaminated by zinc from mining activities revealed that employing this approaching anode technique over the conventional fixed anode (single anode) technique increased Zn removal from 37.3% to 63.4%.

3.3.4 Soil Flushing

Soil flushing is a remediation treatment that attempts to remove the contaminant from the soil profile by passing water, or some other solution with an enhancing additive, through the soil matrix (Martin and Ruby 2004). This liquid accesses the soil matrix through infiltration at the surface, via flooding, sprinkler application, basin infiltration systems and surface trench drainage systems, or by injection (Mulligan *et al* 2001). Once the liquid has passed through the contaminated zone it will be recovered and treated to remove the contaminant, allowing for the possibility of recycling the soil flushing extraction fluid multiple times and improving the feasibility of large-scale treatment prospects (Martin and Ruby 2004). The liquid is often treated for metal removal in existing wastewater treatment plants between uses but may be directly recycled multiple times for flushing before treatment at a wastewater treatment facility (Mulligan *et al* 2001). Soil flushing requires little handling or excavation of soil and may be viewed as a relatively cost-effective option for metal contamination. Limitations of *in situ* soil flushing include the high relative cost of implementing, hydraulic conductivity and infiltration permeability of the soil profile that the extracting solution must travel through and recovering soil flushing with extractant solution and the high specificity of extractant solutions for particular contaminants (Martin and Ruby 2004,

Mulligan *et al* 2001). These limitations place a strong emphasis on the hydrogeological settings of a contaminated site, the solubility of the contaminant and the properties of the desired chemical binding reaction when deciding if this treatment is appropriate. Removal efficiency of this treatment is affected by pH of soil, soil type/texture, clay and organic matter content in the soil, cation exchange capacity of the soil, particle size, permeability, contaminant solubility and the amount of contact between the extraction fluid and the contaminated soil matrix (Martin and Ruby 2004, Mulligan *et al* 2001). According to the United States Environmental Protection Agency (1997), this treatment is best employed in situations with a homogenous and highly permeable soil and inconsistencies in soil texture can lead to incomplete treatment. The management of the extracting solution used for the soil flushing treatment is very important as it can lead to increased contamination and increased mobility of existing contaminants in the soil profile, which can lead to increased leaching of contaminants (Martin and Ruby 2004). Due to the heterogeneity of real-world soil profiles, this treatment has been used on a limited basis. According to a report on soil remediation for metals at several Superfund sites in the United States, soil flushing was the preferred treatment option for seven sites but was only used in two of the sites due to the complexities surrounding the recovery of soil flushing extraction fluids (Martin and Ruby 2004).

3.3.5 Phytoremediation

Phytoremediation in soil remediation is the remediation of contaminated soil with plants via *in situ* stabilization or extraction. This method of remediation is considered to be one of the most environmentally friendly options and is very cost-effective (Dada *et al* 2015). This remediation method offers the opportunity to remove, degrade, metabolize or immobilize many organic and inorganic contaminants, metals (Dada *et al* 2015). Phytoremediation is limited by the depth of roots in most applications and climatic conditions at contamination site and this method takes a longer amount of time relative to other methods (Mulligan *et al* 2001). The use of plant soil

amendments as remediation also reduces erosion that could potentially spread the contaminated soil and reduces infiltration of water and therefore leaching of metals (Martin and Ruby 2004).

3.3.5.1 Phytostabilization

Phytostabilization is carried out in the soil matrix by plant roots. Phytostabilization can be done by the plant in the rhizosphere by secreting a substance to form metal complexes or manipulate the soil pH to reduce mobility and also by adsorbing the contaminant onto the biomass of the plant (Mulligan *et al* 2001). Contrary to phytoextraction, phytostabilization does not involve the actual uptake of metals into the plant and therefore it may be used for agricultural crops without introducing contaminants to the trophic chain (Martin and Ruby 2004). It is very common to use this technique in combination with other amendments that make stabilization more favorable (Martin and Ruby 2004). Phytostabilization has been successfully implemented to reduce the bioavailability and mobility of zinc and other metals in soils (Martin and Ruby 2004). An experiment performed by Perez-de-Mora *et al* (2007) showed that stabilization of zinc is possible and significant when compared to soil without plants with *Agrostis stolonifera*, commonly known as creeping bentgrass, and other species.

3.3.5.2 Phytoextraction

Phytoextraction, sometimes referred to as phytomining, in the context of soil remediation utilizes trees, herbs, grasses and agricultural crops that are able to tolerate and accumulate metal contaminants and be harvested to remove the contaminants from the system. (Mulligan *et al* 2001, Martin and Ruby 2004). Ideal plants will tolerate metals and metal contaminated soil, move metals to above-ground portions of the plant for easy harvesting and have high biomass production of harvestable compartments (Martin and Ruby 2004). It is advantageous to use vascular plants because of their natural ability to take up metals through their roots and transport them to their vascular

tissues, which makes harvesting plant organs with concentrated metals easier (Dada *et al* 2015). Phytoextraction can be paired with other soil amendments to increase their bioavailability and therefore increase the efficiency of metal uptake by remediating plants from the soil (Komárek *et al* 2008). Shen *et al* (2015) showed that biochar can be used with phytoextraction to increase extraction of zinc from soils. The best species for phytoextraction are hyperaccumulators, which are plants that can tolerate and accumulate very high levels of metal contaminants, Phytoextraction treatment requires careful disposal of contaminated plants used for extracting metals (Mulligan *et al* 2001) but these plants can be incinerated in order to recover the metal contaminant (Dada *et al* 2015). *Sedum plumbizincicola* has been shown as an excellent hyperaccumulator of zinc in soils with repeated use and extraction can be increased by intercropping with other remediation or agricultural crop plant species, such as celery (*Apium graveolens*) (Luo *et al* 2015).

3.3.6 Stabilization

Stabilizing zinc and other metal contaminants in soil is usually a crucial first step in metal-contaminated soil remediation techniques as it may affect the efficacy of steps within the remediation process further along (Mulligan *et al* 2001). This type of treatment is preferable to *ex situ* methods of metal extraction or isolation with physical barriers because it causes less environmental disruption or damage and is much less expensive (Perez-de-Mora *et al* 2007). Chemical stabilization of soil contaminants involves a stabilizing agent chemically reacting with the metal contaminant in order to bind the contaminant to some surface or substrate or to reduce the mobile fraction of the metal contaminant (Dada *et al* 2015). Common materials used as stabilizing agents are able to adsorb, complex or (co)precipitate metal pollutants, such as lime, apatite, zeolites, Mn and Fe oxides, materials with high organic matter content (Bolan *et al* 2003). This treatment is meant to reduce both the mobility and bioavailability of metal contaminants (Madejon *et al* 2010). Unlike solidification, stabilization does not always

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yield a solid mass but rather results in the formation of a chemically stable compound that is less mobile or soluble and therefore less likely to leach into surrounding groundwater reserves or adjacent surface waters and less likely to be uptaken by plants and introduced into the trophic chain, which ultimately poses a health risk to humans. After stabilization, these compounds can be further treated or removed from the soil if desired but stabilized compounds can also be left in the soil as they usually are no longer a threat for environmental contamination due to their immobility and non-reactivity (Dada *et al* 2015).

Because there is a high diversity of compounds containing Zn in soils (Manceau *et al* 2004), the most effective stabilization technique can vary. The selection of the best method for stabilization of metals also can be affected by the presence of other metal contaminants in soils. This can be attributed to competition for sorption sites and the effects of certain stabilization techniques on metals other than zinc present in the contaminated site (Kumpiene *et al* 2008). Some methods for immobilizing or isolation zinc may be effective but also have negative effects on the other metals in the soil. Applying soil amendments that result in the reduction of zinc mobility or bioavailability may increase mobility or bioavailability of other elements and therefore increase the risk to the environment and humans. This is why selecting the most appropriate soil amendment is important and why you must consider all contaminants that are present rather than treating each metal on an individual basis. Stabilizing metal contaminants in soil with *in situ* methods, such as the application of biochar, is often the most cost-effective method of soil remediation as it involves less costs involved with excavation and transportation of contaminated soils and less time spent on the treatment of the soil leading to decreased labor costs (Mulligan *et al* 2001). The application of stabilizing agents that function through adsorption of metal contaminants are more cost-effective options for remediating contaminated soils because they are more specific to the contaminants. In one study, researchers only target the contaminant, rather than attempting to contain the entire contaminated area, which is what other stabilizing

techniques, such as physical barriers or excavation with *ex situ* treatment, attempt to do. Another advantage that stabilization has over other *in situ* methods of remediation is that it prevents the contamination from leaching into groundwater reserves due to the fact that the contaminant will be immobilized where it is adsorbed to the stabilizing agent (Mulligan *et al* 2001).

Of course, the use of stabilizing agents that adsorb metal contaminants has drawbacks. The efficacy of this remediation technique is largely dependant on the geological situation of the remediation site as well as the contamination depth (Mulligan *et al* 2001). Because this technique is usually applied with the use of tools that are meant to mix the application material with the soil, such as vertical auger mixers, clamshell buckets, backhoes and draglines, it becomes quite difficult to target contaminants in deeper layers of the soil profile (Jasperse and Ryan 1992). With these standard techniques, it is widely accepted that the maximum treatment depth, which is reached with vertical auger mixers, is around 13 meters (Mulligan *et al* 2001). The means by which *in situ* remediation materials can be applied is limited by large, rigid objects or clays that are present within the soil column because it makes mixing the remediation treatment material with the soil difficult and more costly (Mulligan *et al* 2001). A less expensive alternative to excavation of the treated contaminated soil involves the stabilized pollutants to remain at the site, which although essentially immobilized and non-reactive remains in the same content in the soil, which will not decrease over time (Madejon *et al* 2010). It is for this reason as well as a lack of knowledge and large-scale field studies regarding *in situ* that the sustainability of stabilizing applications has been questioned (Madejon *et al* 2010).

3.3.6.1 Stabilization with Clay Particles

Although clay within the soil profile may impede the application of certain *in situ* remediation processes, the presence of certain clay particles and other similar soil amendments within the soil may actually be quite effective at immobilizing and

stabilizing zinc. Alvarez-Ayuso and Garcia-Sanchez (2003) showed that magnesium aluminum silicate clays were extremely efficient at retaining Zn. Incorporating a 4% dose of palygorskite clay particles and sepiolite clay particles in polluted mining soils resulted in a reduction of exchangeable Zn by 76% and 99%, respectively (Alvarez-Ayuso and Garcia-Sanchez 2003). Other alkaline materials with high specific surface areas are also able to effectively bind to Zn. Phosphorous has also been spotlighted as a Zn immobilizing agent in soil by creating metal-phosphate precipitates with low solubility (Kumpiene *et al* 2008). Ciccu *et al* (2003) have shown that coal fly ash incorporated into soil decreased Zn leaching by 99.7%. It should be noted that implementing remediation techniques that involve the input of an alkaline material or acidic material should be accompanied by pH monitoring, as the presence of these materials could affect the soil pH, which could have consequences regarding the retention of Zn and other metal contaminants (Kumpiene *et al* 2008). Zn has been shown to be the least mobile around neutral to slightly alkaline pH levels, which is why extra attention should be paid to pH monitoring when implementing *in situ* remediation of zinc and other similar metals such as Pb and Cu (Kumpiene *et al* 2008).

3.3.6.2 Stabilization with Manganese Oxides and Other Oxides

Manganese oxides, which occur naturally in soils, have also been known to be effective sorbent materials for soil remediation processes of stabilizing contaminants (Ettler *et al* 2014). Although much is known about the sorption affinity for many metals with crystalline Mn oxides, there are relatively few studies done to show the efficacy of synthetic amorphous Mn oxides as a chemical stabilization agent (Ettler *et al* 2014). These amorphous manganese oxides, also known as AMO, are poorly crystalline structures, making it amorphous, and are prepared with a modified sol-gel technique that focuses only on precipitation and washing (Ettler *et al* 2014). The addition of manganese oxides to soil can reduce metal mobility and bioavailability in contaminated soils (Komárek *et al* 2013). They are relatively fast and easy to synthesize in a

laboratory setting making it a feasible remediation option for *in situ* application (Della Puppa *et al* 2013). With AMO synthesis, small (<10 nm) crystalline domains are formed, providing the material with a very high surface area increasing its capacity to adsorb materials (Ettler *et al* 2014). Manganese oxides usually exists within the soil matrix as a coating of other materials or fine-grained aggregates and is also considered to be ubiquitous within the soil matrix (Della Puppa *et al* 2013). It has been observed by Ettler *et al* (2014) with AMO treatment in plant pot experiments that pore water pH in soil is relatively unaltered under oxidizing conditions and only exhibited slight increases when compared to controls, likely resulting from proton sorption by the AMO material. These results indicate a certain acidity buffering ability of the AMO and therefore could have pH controlling implications in soil remediation circumstances, which improves its potential for remediating zinc and other metal contaminants in soil because the adsorption of zinc increases with pH (Della Puppa *et al* 2013). Although this treatment has been shown as an effective immobilization treatment for zinc, it is best suited for immobilization of Pb and Cu (Della Puppa *et al* 2013). This same experiment also showed that the concentration of AMO in pore water solution decreased over time, which could indicate that the AMO either has the potential to dissolve in solution. Della Puppa *et al* (2013) showed that in pH 4 solution, the AMO lost roughly 5% of its total mass to dissolution.This dissolution of AMO in solution was found to be pH-dependent (Ettler *et al* 2014), which could suggest that this treatment is best implemented in combination with pH-controlling treatments and best reserved for less acidic soils. Additionally, manganese oxides have been shown to increase soil pH, which can increase cation sorption but have undesirable effects on soil such as dissolving organic matter and consequently mobilizing metal contaminants (Komárek *et al* 2013). It was shown in a study by Della Puppa *et al* (2013) that adsorption of zinc by AMO increased with pH, indicating that this practice could be enhanced by pairing amo application with liming or some other pH controlling treatment. regarding This material is also considered to be a cost-effective soil remediation option due to the potential for

repurposing of organic waste products to be used as source materials (Ettler *et al 20*14).

AMO as a remediation treatment also can be influenced by the soil biological system (Ettler *et al* 2014). One experiment showed that the amount of Mn released in abiotic treatment scenarios was very low compared to the implementation of Mn oxides in the presence of indigenous bacteria (Quantin *et al* 2002). Oppositely, Dissolved Mn^{2+} has been shown to become oxidized more rapidly and Mn oxides can be sourced from biological systems too (Ettler *et al* 2014). The application of AMO requires careful monitoring of species fractionation, as certain soil conditions favoring the dissolution of AMO can result in high fractions of mobile Mn^{2+}, which has been shown to be toxic for plants (Ettler *et al* 2014). It is because of this that further research regarding the best conditions for AMO application and conditions that favor the accumulation of free Mn^{2+}ions is required before widespread application of AMO as a chemical stabilization agent in soil remediation becomes acceptable. Although roughly 10-18% of the mass of the applied amorphous manganese oxide was lost in a study regarding amorphous manganese oxide stability in solution by Ettler *et al* (2014), that mass loss was stabilized after 60 days. This stabilization process is likely attributed to the formation of new manganese phases, including rhodochrosite ($MnCO_3$), kutnahorite ($CaMnCO_3$) and scacchite ($MnCl_2$) (Ettler *et al* 2014). This dissolution process is also pH dependant in the soil with more neutral and slightly alkaline favoring lower mass loss rates (Ettler e*t al* 2014). Since manganese oxide is already ubiquitous in soil systems, this mass loss is not an environmental concern and could be considered a less invasive option for soil remediation.

Other metal oxides that are commonly used for soil remediation include iron oxides and aluminum oxides (Komárek *et al* 2013). These oxides vary in their suitability for different sorbates but overall exhibit a wide range of applications in soil remediation for metal contamination (Komárek *et al* 2013). Several studies have demonstrated the efficacy of iron oxides and aluminum oxides for stabilization of zinc in soil (Vitkova *et al* 2017). An interesting prospect related to iron oxide use in remediation of zinc

contaminated soil is nano zero-valent iron because it may significantly decreases leachability at certain pH ranges, specifically those near neutral and slightly alkaline, while also improving certain soil characteristics such as organic matter content and microbial activity (Vitkova *et al* 2017).

2.3.6.3 Stabilization with Biochar

The use of biochar, which is a carbon-rich material created by the pyrolysis of organic material, as a stabilizing agent is quite an attractive remediation option because it can be produced with organic waste materials, such as unused agricultural plant residues, leftover feedstock, some organic industrial wastes and some forms of municipal solid waste (Beesley *et al* 2011, Trakal *et al* 2014). This has implications regarding the overall sustainability of some agricultural practices because the waste can be repurposed for the production of biochar. Organic matter is pyrolysed at high temperatures, typically between 400°C and 800°C, in the absence of oxygen to produce biochar (Sohi *et al* 2010). Ideally, about 40-50% of the original mass of the biowaste used as feedstock for the biochar can be recovered in the form of char but it's largely dependent on the type of feedstock used and the speed of pyrolysis (Sohi *et al* 2010). Biochar characteristics are quite variable depending on the composition of the source material used, which also includes various mineral components that influence sorption or complexation, the pyrolysis process with pyrolysis temperature being one of the more influential factors, post modification or post activation of the biochar and, perhaps most importantly, the environmental conditions that the different sorption processes take place in, such as pH, exposure time, initial concentration of metal and dose of biochar implemented (Trakal *et al* 2014). Perhaps the most important of these factors is pH, as it was found by Trakal *et al* (2014) that the sorption efficiency of biochar with regards to lead and cadmium was more dependant on pH than characteristics including specific surface and biochar morphology. The pyrolysis of agricultural waste into biochar also serves as a potential carbon sink for forms of carbon that may eventually become

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atmospheric greenhouse gases such as CO_2 and CH_4 (Sohi *et al* 2010).

This material is excellent for contaminant stabilization because it has a very high surface area, high cation exchange capacity, high pH and active functional groups, allowing for stabilization through precipitation, ion exchange, surface complexation and adsorption (Beesley *et al* 2011). Biochar is advantageous over other forms of stabilization, including physical barriers, cementation, addition of lime or clay, etc, because it increases the organic matter in the soil which facilitates improved nutrient cycling and has the capacity to aid with plant revegetation at contaminated sites (Beesley *et al* 2011). The application of biochar mimics natural attenuation processes that adsorb, (co)precipitate and complex metal contaminants to reduce mobility and bioavailability instead of reducing overall concentration (Perez-de-Mora *et al* 2007). Biochar application facilitates plant growth on degraded land and improves the activity of soil microorganisms (Perez-de-Mora *et al* 2007), allowing for the restoration of natural ecosystems or increased utility of agricultural lands. Biochar application has been shown in studies to significantly improve biomass production in certain plants in the first year after application (Perez-de-Mora *et al* 2007).

Past studies have shown that biochar application can reduce extractable zinc, as well as other harmful contaminants, as quickly as one week after application (Rees *et al* 2014). Similar results have been shown by Uchimiya *et al* (2012) and Houben *et al* (2013) who demonstrated that extractable zinc can be reduced within the timespan of one week to two months by application of cottonseed hull biochar and *Miscanthus* straw biochar, respectively. A study lasting three years carried out by Shen *et al* (2016) demonstrated that biochar applied in doses of 2% and below can be a long-term solution for stabilizing zinc as well. The results of this field study, which took place on a pre-WWII industrial site in the UK, showed that all plots contained exchangeable zinc amounts reduced by 83%-97%. Shen *et al* (2016) also showed that three years after biochar application the leachability of zinc was reduced from 0.12% to 0.01% and an increase of residue fraction of zinc from 7% to 27-35%. These results are indicative that

the evaluation of biochar efficacy should be more focused on the speciation of metal contaminants rather than the overall concentration. A study conducted by Trakal *et al* (2014) showed that biochar from various source materials is very capable of adsorbing lead and cadmium contamination with efficiencies ranging from 43.8%-100%.

4. Methodology

4.1 Contaminated Soil Sampling Location

The contaminated soil sample used for this study was taken from a contaminated area surrounding a lead smelting factory in Příbram, Czech Republic along the Litavka River. The soil used in this study was taken from sampling point P2, as seen in Figure 2.

Příbram is approximately 60 kilometers southwest of Prague and has been, and still is to a lesser degree, subjected to heavy industrial pollution due to fly ash emissions and slag dumping near a local lead smelting plant. Lead smelting activity first began in the area in 1311 and the current lead smelting plant was established in 1786 (Komárek 2016). Although currently the plant produces 1.3 tons of Pb annually, it has produced annual lead emissions above 600 tons in the past (Komárek 2016). The lead smelting plant is located on the Litavka river, which is subject to occasional flooding that furthers the spread of metal contamination.
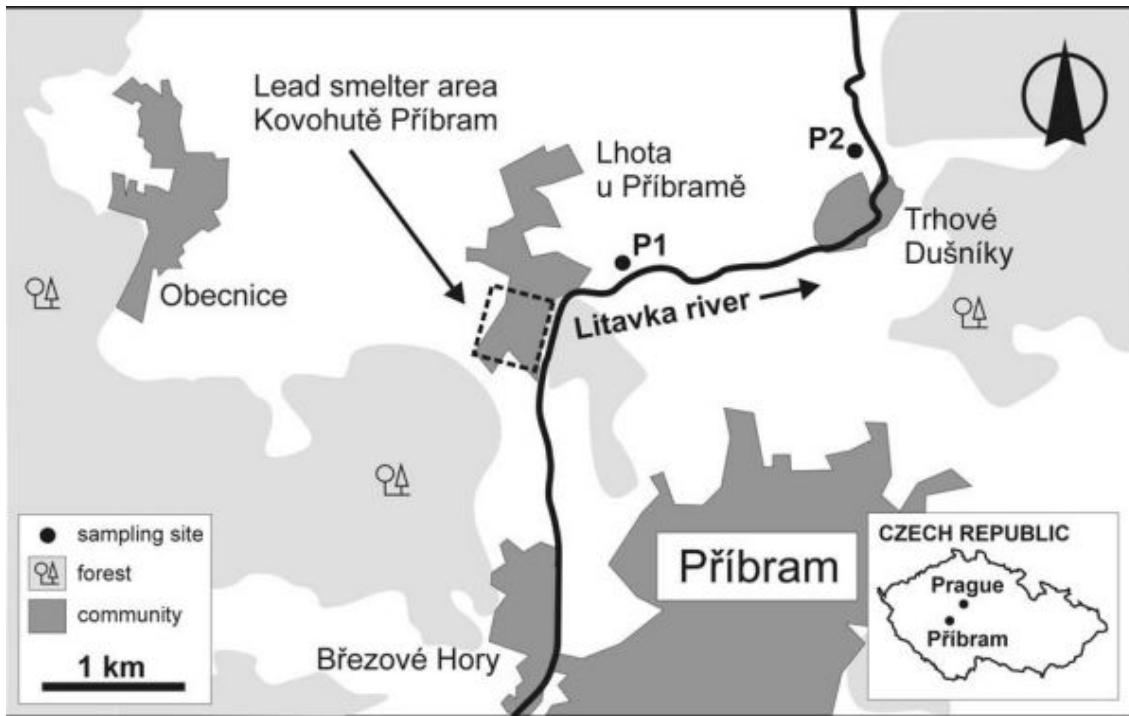
Figure 2: A map of the soil sampling location along the Litavka river in Příbram, Czech Republic. The soil samples were collected at sampling point P2, northeast of a lead smelting site. Adapted from (Vaněk *et al* 2005).

4.2 Soil Sample collection and Preparation

The soil was collected from the site by removing the topsoil and collecting from a depth 10 - 25 cm. The soil was then dried to a constant weight in the greenhouse at the Czech University of Life Sciences, homogenized and sieved to 2 mm.

4.3 Characterization of Contaminated Soil Sample

The soil sample used for the purposes of this study can be characterized as sandy loam (75% sand, 20% silt and 5% clay). The contaminated soil has a mean pH 5.95 (SD 0.01) when dissolved in water and 5.14 (SD 0.03) with 0.1 M KCl solution. The soil cation exchange capacity was 9.08 mmol/100g soil (SD 0.52). Metal concentrations from the contaminated soil sample obtained through analysis by inductively coupled

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plasma optical emission spectrometry, or ICP-OES, which is an elemental analytical technique using inductively coupled plasma to excite atoms and measures the electromagnetic emission to determine the compound present, after digestion of the sample with hydrofluoric acid show high concentrations of several metal contaminants. Notable values from this initial determination can be seen in Table 5.

4.4 Characterization and Preparation of AMOchar

AMOchar is a mixture containing a ratio of 2:1 amorphous manganese oxide and biochar. The AMOchar was prepared by weighing out the two materials in a 2:1 ratio to create a stock and then was added to the soil as needed by mixing with a stainless-steel pan.

4.4.1 Characterization and Preparation of Biochar

The biochar that was used in this experiment was made from grape stalks that would be considered a common agricultural waste in Central Europe. The pyrolysis temperature and organic material used for biochar synthesis affects certain characteristics of the biochar including the pH level, cation exchange capacity and ash percentage (Trakal *et al* 2014). The biochar used in this experiment was characterized by Trakal *et al* (2014) as having a pH of 10.0, a point of zero charge pH of 9.92, a cation exchange capacity of 402 mmol/kg, a BET surface area of 72 m^2/g and weight percentage of ash of 16.1%. The biochar is considered to be a more effective sorbent at increasing pH levels because functional groups are more likely to be deprotonated, thus creating more negatively charged sites for cation sorption (Trakal *et al* 2014).

The biochar used in this experiment was synthesized according the biochar preparation from Trakal *et al* (2014). This procedure involves pyrolysis of the organic material at 600 °C in a muffle furnace under 16.7 mL/min nitrogen flow rate at atmospheric pressure and then cooled in the same nitrogen atmosphere overnight. The pyrolyzed material was then ground, homogenized, sieved to 0.25 mm, washed and then

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dried at 60 °C for 24 hours until constant weight was achieved.

4.4.2 Characterization and Preparation of Amorphous Manganese Oxide

The amorphous manganese oxide consisted primarily of poorly crystalline/amorphous grains (Ettler *et al* 2014). A picture of the amorphous manganese oxide can be seen in Figure 3. The presence of amorphous manganese oxide in soil may cause slight increases in soil solution pH, with a larger effect in acidic soils (Ettler *et al* 2014). Amorphous manganese oxide has a pH of 8.1, a zero point of charge pH of 8.3, a cation exchange capacity of 34 meq/100g and a specific surface area of 14.8 m^2/g (Della Puppa *et al* 2013). Amorphous manganese oxide has been shown in laboratory settings to exhibit slight mass loss due to dissolution and phase change (Ettler *et al* 2014). This mass loss is pH dependant, with more acidic conditions favoring higher mass loss (Ettler *et al* 2014). The application of amorphous manganese oxide in soil has the potential to increase soil pH values (Komárek *et al* 2013), thereby facilitating increased efficacy of other stabilization techniques. Amorphous manganese oxide as a soil treatment also has the potential to form inner-sphere complexes with As(III) and Cr(III), oxidizing them to As(V) and Cr(VI) respectively (Komárek *et al* 2013).

The amorphous manganese oxide material used in this experiment was synthesized at the Czech University of Life Sciences Prague according to a modified sol-gel procedure, which is commonly used to synthesize birnessite, but with the omission of heating the gel after the final drying of the sol-gel. This was omitted to avoid crystallization of the manganese oxide, thus preserving its amorphous structure. The synthesis involves the conversion of biowaste into amorphous manganese oxides, making it an environmentally friendly option for soil remediation. The amorphous manganese oxide was synthesized according to the methods that can be found in Ettler *et al* (2014).
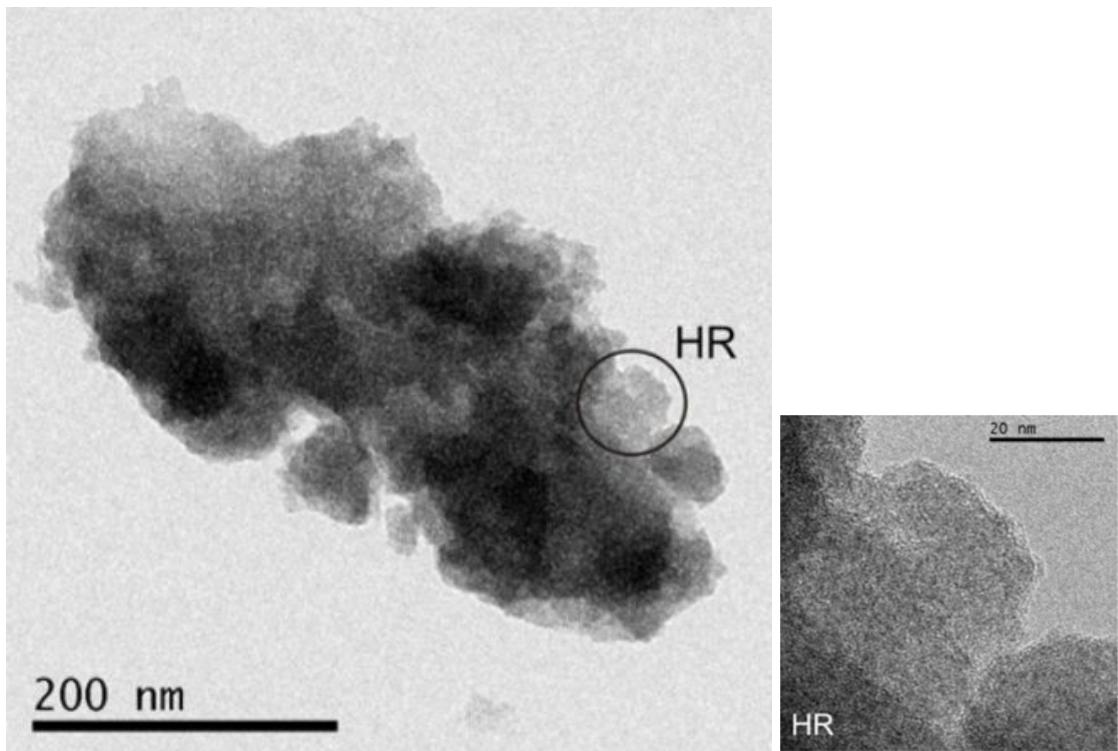
Figure 3: This figure shows a TEM image of amorphous manganese oxide. A high resolution image of the circled section from the left can be seen on the right. Adapted from Ettler *et al* (2014).

4.5 Batch Adsorption Kinetics Experiment Setup and Procedure

The first part of the experiment involved determining sorption efficiencies of the biochar and AMOchar material. 1 gram of the sorption material, either biochar or AMOchar, was mixed with 1.04 mM of zinc. The solution was mixed and the pH was kept around pH of 5 by the addition of HCl acid. At each timestep, a sample was taken for analysis of zinc content. Each sample was filtered to 0.45 μm and diluted 10 times. pH levels were determined with a pH probe. The concentration of zinc was then determined through ICP-OES. The trial was carried out for 120 minutes wherein sampling took place at 3, 5, 10, 15, 40, 60, 90, and 120 minutes. The sorption efficiency

at each sampling timestep was calculated with the following equation:

Sorption[Zn] = $((C_i - C_r) / C_i) * 100[\%]$

Where:

C_i = Initial concentration of zinc

C_r = Remaining concentration of zinc after trial

4.6 Optimization of Dosage for Zinc Leachate Experiment Setup and Procedure

The dosage optimization experiment was meant to optimize the dosage of sorbent treatment and select a sorbent material for the column leachate experiment. The dosages of biochar and AMOchar, which is amorphous manganese oxide mixed with the biochar at in a 2:1 ratio, that were tested in this initial batch experiment were 1%, 2% and 5% by weight mixed with soil. Each mixture was shaken with demi water for 10 minutes and dried for four days at 45℃.

This soil was then put into columns composed of plexiglass measuring 12 cm in length with a diameter of 2.5 cm with replicates at each dosage. Each column was 50 g of the soil mixed with a percentage of either biochar or AMOchar mixture. A control replicate with demi water mixed solely with the contaminated soil sample was also included. Effort was made to insure that there was no air pocket left in each column that would potentially affect water flow through the column. A filter was placed at the bottom and top of each column. Demi water was percolated from the bottom until the column was fully saturated and then the trial was conducted with an automatic sampling machine. The schedule of sampling that was used in the initial optimization experiment can be seen in Table 3. The automatic sampling machine took samples according to a sampling schedule while water was pumped through the column at 0.6 ml/minute and water that was pushed all the way through was collected in a beaker underneath each column, which was covered with a plastic wrap to prevent evaporation. After the six hour trial period the leachate outflow was analyzed for pH with pH probe, filtered to

0.45 μm, diluted 5 times and finally zinc concentrations were determined by ICP-OES.

Timeframe [hours]	Frequency of sample collection
0-1	Every 15 minutes
1-3	Every 30 minutes
3-6	Every hour

Table 3: This table shows how often sampling took place during the dosage optimization experiment with an automatic sampling machine.

4.7 Column Leachate Experiment Setup and Procedure

Two soil columns were used in this experiment and the difference between them is the application of the 2% AMOchar treatment. This AMOchar material was chosen for the column leachate experiment because the amorphous manganese oxide is very effective at adsorbing other harmful metal contaminants such as Pb, Co, Cu and Cd, and because of the ability of manganese oxides to alter the speciation of redox-sensitive species, such as As and Cr (Komárek *et al* 2013). Although the biochar sorbed more zinc than the AMOchar in the dosage optimization experiment, it was not enough to justify using biochar rather than the AMOchar. The 2% dosage of the AMOchar treatment was chosen because it was seen as less environmentally invasive as it is less than half of the material used in the 5% treatment with similar sorption efficiencies. The columns used were composed of plexiglass and measured 15 cm in length and 6 cm in diameter. The setup of each column for this experiment can be seen in Figure 4. The first column, the layered treatment, has a 2% AMOchar treatment mixed with sand and applied as a narrow layer (Layer B) and the second column, the distributed treatment, has the 2% AMOchar treatment distributed evenly throughout the contaminated soil sample (Layer A). The synthetic soil used in each column was synthesized to mimic the texture of the soil while excluding metal contaminants. The first column contains 340g

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of the contaminated soil sample as the top layer, the second layer contains a mixture of 7g of AMOchar (approximately 2% of the sample weight) and 7g of quartz sand and the last layer of the column contains 110g of synthetic soil. The second column also contains 340g of the contaminated soil sample but with 7g of AMOchar mixed evenly throughout, a second layer with 14g of quartz sand and a third layer with 110g of synthetic soil. Between each layer there is a filter in the two columns with 2% AMOchar treatment in order to keep the layers distinct from one another. The filters in the columns were porous enough as to not impede the water flowing through but disallowed soil aggregates from moving between layers. The third column represents the control for the experiment and the data was collected during a different experiment. This column was set up with rhizons at the same depth as the other two columns but with only one layer of soil. This layer was 450g of the contaminated soil sample.

Layered	**Distributed**	**Control**
340g contaminated soil sample	340g contaminated soil sample + 7g AMOchar	
		450g contaminated soil sample
7g AMOchar + 7g sand	14g sand	
110g synthetic soil	110g synthetic soil	
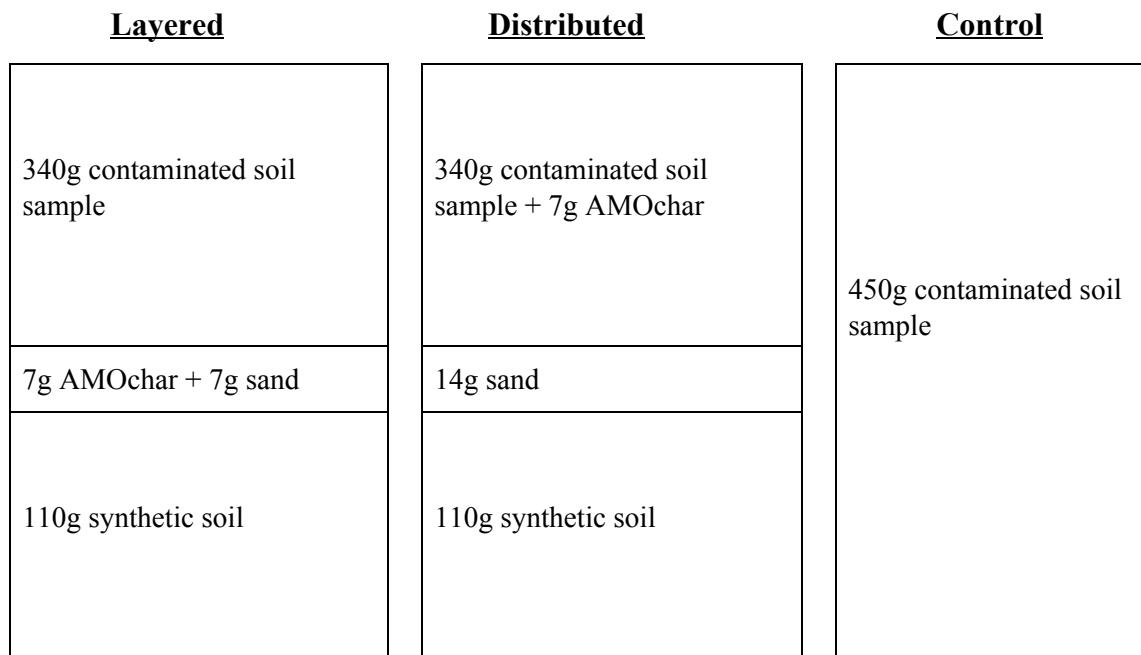
Figure 4: This figure shows the setup of each column used in the Column Leachate Experiment

4.7.1 Rhizon Sampling

Each column in the experiment contained a rhizon sampler, which uses a vacuum to draw pore water samples from the column, in each soil layer (Layer A, Layer B and Layer C). The relative location of each soil layer can be seen in Figure 4. The sampling was done several times throughout the entire experiment, which lasted for 71 hours. The time that each sample was collected can be seen in Table 4. The rhizon samples that were collected were held in a refrigerator and the pH was taken by pH probe after all rhizon samples were collected from the experiment. The samples were then filtered to 0.45 μm and diluted 5 times. These rhizon samples then were analyzed with ICP-OES to obtain metal concentrations.

Sampling Timestep	Time [hrs] - Experiment	Time [hrs] - Control
1	0	3
2	9	6
3	20.5	12
4	24.5	24
5	47.5	48
6	71	72

Table 4: This table shows the time [hours] that each sampling timestep was carried out both for this experiment and for the values obtained for the control column.

4.7.2 Leachate Sampling

After the rhizon samples were collected, the volume and pH of the leachate outflow, which is the water that completely passed through the column and exited at the bottom, was measured. The pH was measured directly in the collection container with pH probe at each sampling step and then 2 sample replicates of 2 mL were made for each column and timestep. The samples were then filtered to 0.45 μm and then were diluted 5 times and approximately 1-2 drops of nitric acid (HNO_3) was added to each sample. The samples were then analyzed with ICP-OES for metal concentrations.

4.8 Statistical Analysis of Results

The results of these experiments were analyzed for statistical differences using Microsoft Excel. The data were tested for normality and then analyzed with One-Way ANOVA and Tukey-Kramer pairwise comparison test.

5. Results

5.1 Notable Metal Concentrations in Sample Soil Results

Table 5 displays the characterization of the contaminated soil sample that was carried out by ICP-OES analysis after hydrofluoric acid digestion. All of the metal concentrations in Table 5 are at levels considered dangerous to humans (Toth *et al* 2016).

Metal	Zn	Pb	Cr	Cd	Cu
Concentration [ppm]	4002	3539	61.3	39.1	68.3
Standard Deviation	67.9	375	1.94	1.11	3.16

Table 5: This table contains notable metal concentrations obtained during the initial characterization of the contaminated soil sample in ppm.

5.2 Batch Adsorption Kinetics Experiment Results

The sorption efficiencies of AMOchar and biochar as a function of time can be seen in Figure 5. Both materials appear to have reached an equilibrium by the end of the trial. The results of the batch experiment show that the zinc sorption efficiency of AMOchar, which was approximately 45%, was much higher than that of biochar, which had a zinc sorption efficiency of roughly 15%.
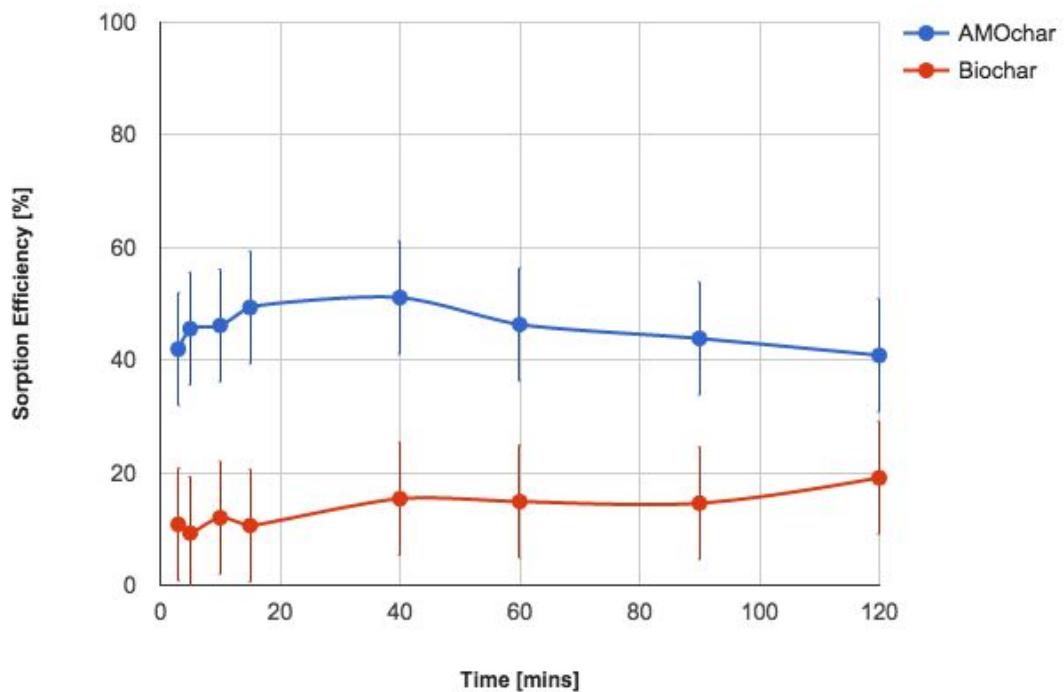
Figure 5: This figure shows sorption efficiencies of AMOchar and biochar in a demi water solution with pH of 5.

5.3 Dosage Optimization Experiment Results

The results of the optimization experiment were obtained by analyzing zinc concentrations from the leachate outflow of each column with ICP-OES to determine sorption of zinc and other metals. Sorption of zinc increased with dosage percentage in all three (1%, 2% and 5%) for both the biochar and AMOchar and it was statistically significant with one-way ANOVA. The biochar stabilized the zinc more effectively than the AMOchar but the AMOchar effectively stabilized a wider range of metal contaminants than the biochar, including lead and arsenic (Komárek *et al* 2013), due to the inclusion of amorphous manganese oxides. The sorption efficiency of AMOchar was much higher when apparent equilibrium was reached, as seen in Figure 5, with

efficiency percentages of AMOchar being almost double that of biochar. It was for these reasons and the reason of being less environmentally invasive by using less of the sorbent as a treatment that the 2% AMOchar treatment was chosen for the column leachate experiment. The sorption efficiency results can be seen in Figures 5 and 6.
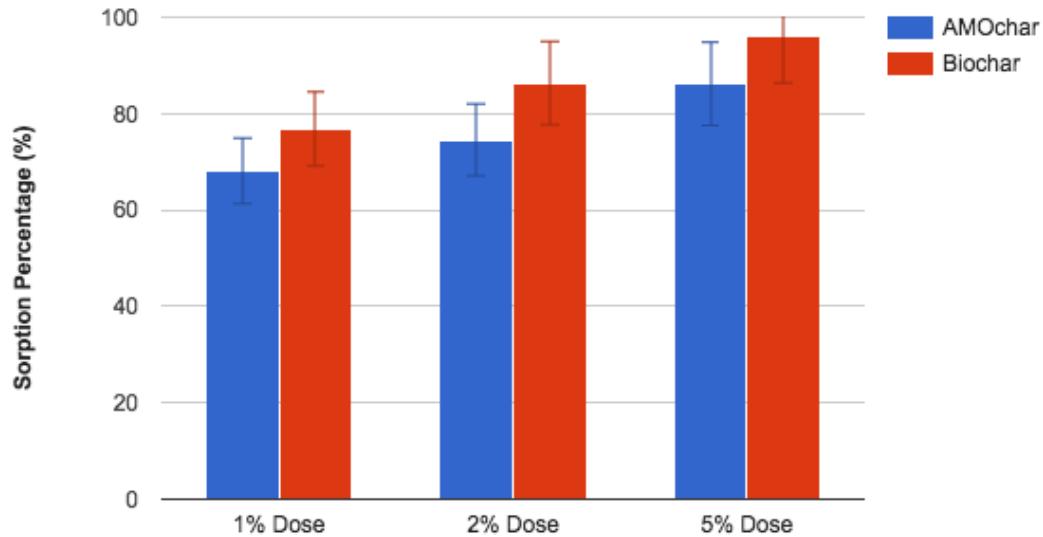
Figure 6: This figure shows the sorption percentages of zinc with varying dosages of AMOchar and biochar from the dosage optimization experiment.

5.4 Column Leachate Experiment Results

Figure 7 clearly displays the large difference in the accumulation of zinc in the leachate between both AMOchar applications and the control column. In both cases, this difference was statistically significant while the difference between applying the 2% AMOchar treatment by layering or distributing was not statistically significant. There was also a significant difference between the two treatments and the control with regard to the pH level of the leachate outflow, which can be seen in Figure 8. The leachate pH

level of each treated column seemed to reach equilibrium after 30 hours. The pH levels of both 2% AMOchar treatment columns were slightly alkaline (7-9) while the control was in a pH range of 6-7.
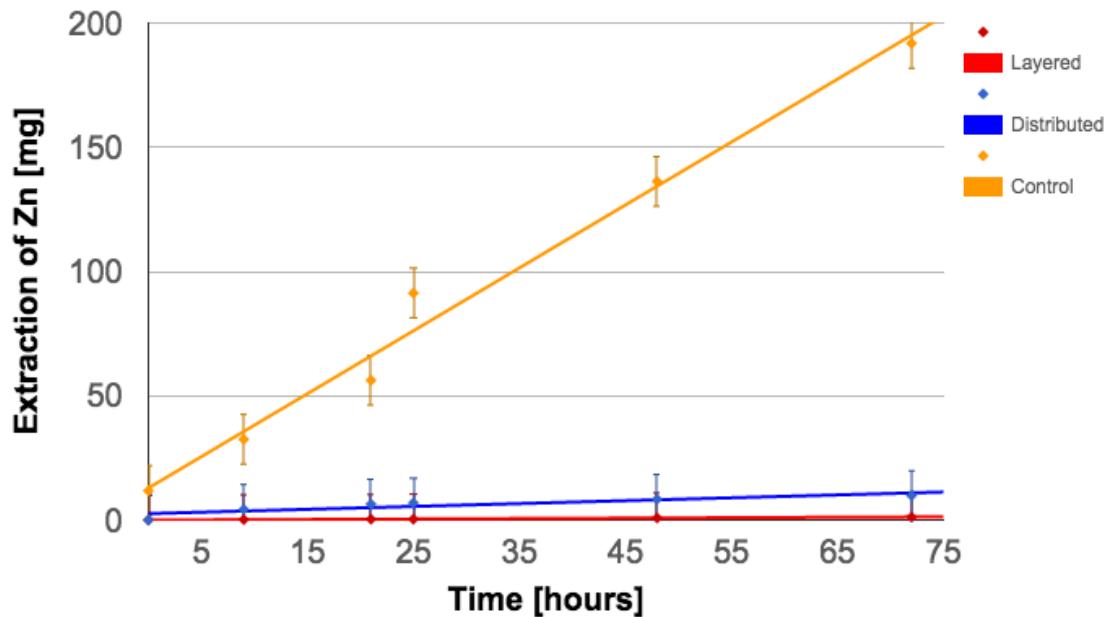
Figure 7: This figure displays the cumulative amount of zinc extracted in mg from the leachate for layered AMOchar (2%), distributed AMOchar (2%) and the control.
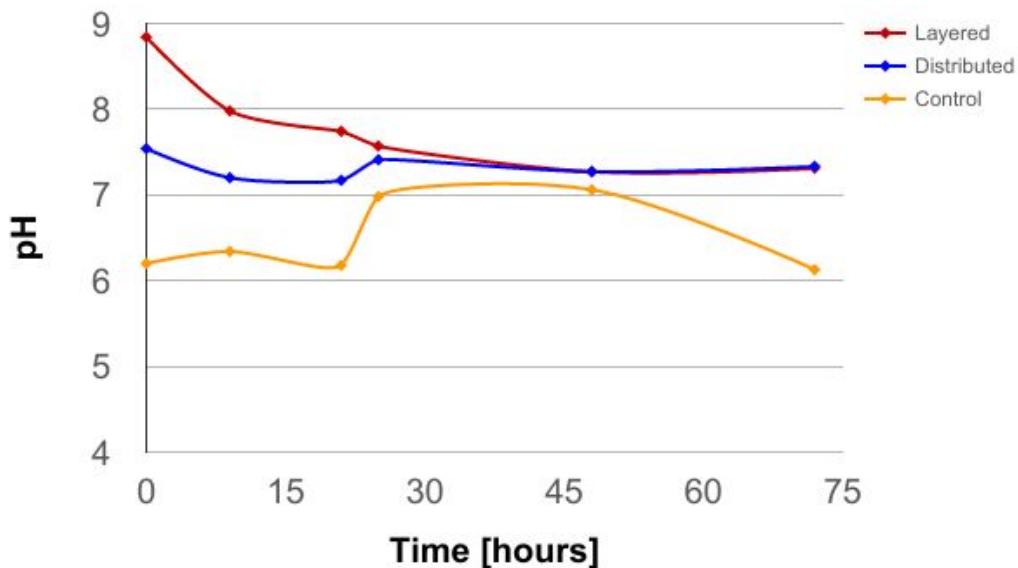
Figure 8: This figure displays the pH of leachate outflow solutions collected for layered AMOchar (2%), distributed AMOchar (2%) and the control.

Although pH levels appear to behave somewhat similarly in all three treatments, statistically significant differences were found. The pH levels obtained from rhizon samples of the soil solution in different soil layers of the leachate columns can be seen in Figure 9. Overall, the pH of the two columns with 2% AMOchar treatments were nearly neutral and slightly alkaline while the control column was always slightly acidic (pH of approximately 5.5-6.5). In the top layer of the soil, Layer A, both treatments with 2% AMOchar, layered and distributed, were significantly different from the control and from one another with regards to the soil solution pH level. This indicates that applying the 2% AMOchar treatment by layering or distributing evenly can have a significant effect on the pH of the soil solution and overall the presence of 2% AMOchar treatment, regardless of application technique, significantly affects the soil pH. In the middle layer, Layer B, there was a significant difference between the distributed and layered as well as between the distributed and control. In the bottom layer, Layer C, both applications of

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the 2% AMOchar treatment were significantly different.
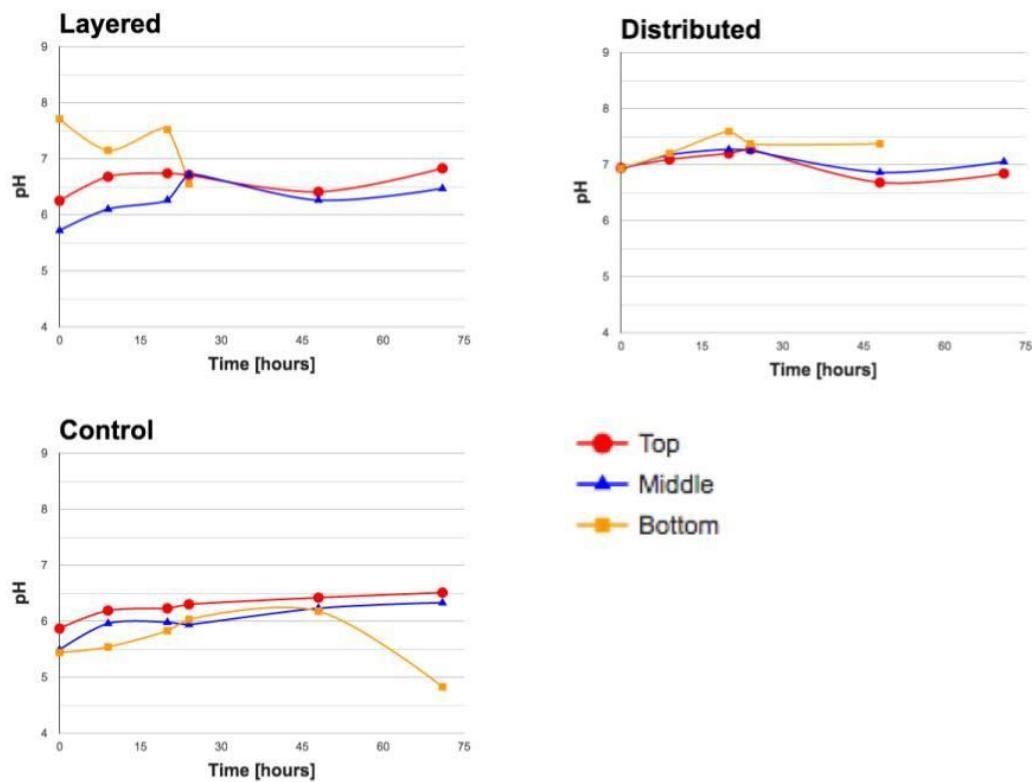
Figure 9: This figure shows the pH of the soil solution obtained from rhizon samples from layered AMOchar (2%), distributed AMOchar (2%) and the control.

Concentrations of zinc in the soil solution of columns with 2% AMOchar treatment, which was sampled through rhizons in each soil layer, were significantly lower than concentrations of zinc obtained from the control column soil solution. This difference can be seen in Figure 10. Zinc concentrations in soil solution for the distributed 2% AMOchar was comparable to the control for the first few hours of the trial but decreased dramatically and stayed at low concentrations (>5 ppm) for the remainder of the trial. The layered 2% AMOchar treatment was below 10 ppm for the entirety of the trial and stayed below 5 ppm for the whole trial after 15 hours. There was

no clear pattern to the control zinc concentration distribution aside from the fact that it was dynamic throughout the trial.
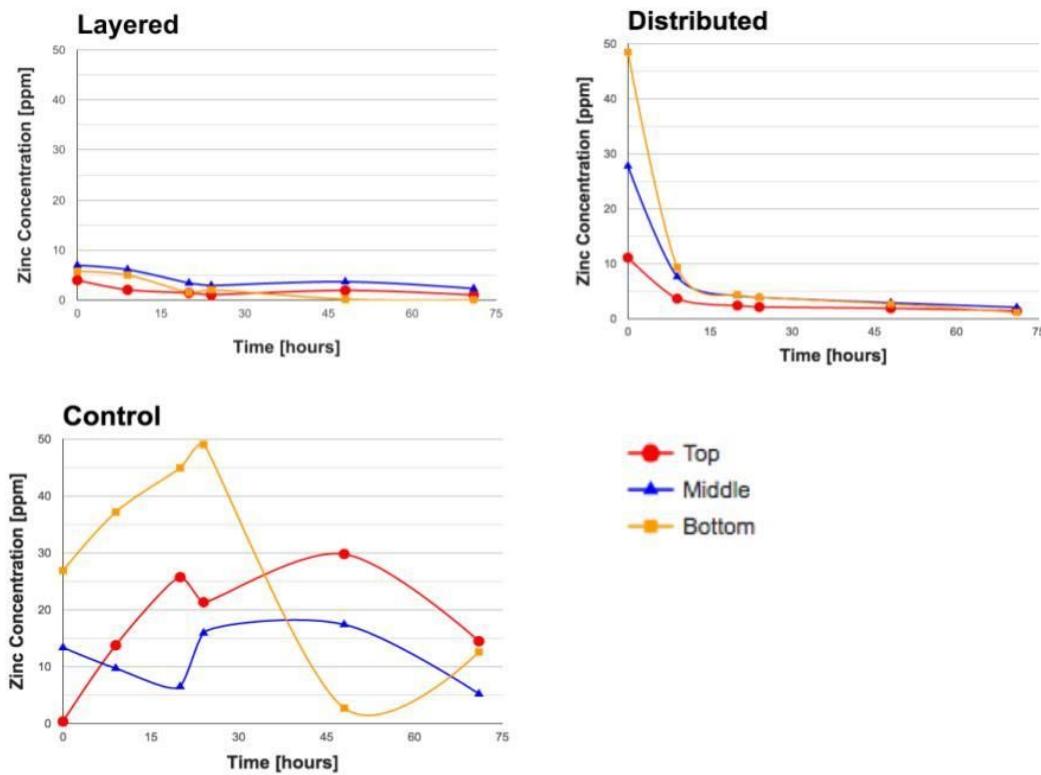
Figure 10: This figure displays zinc concentrations obtained from each rhizon layer in ppm for the layered AMOchar (2%), distributed AMOchar (2%) and the control.

Table 6 displays the results of statistical tests that were used to analyze the results of the column leachate experiment. A Tukey-Kramer Pairwise Comparison was used when significance was indicated by One-Way ANOVA. The highest amount of significance was observed when comparing the zinc concentrations and pH levels of the leachate outflow but there was also a high amount of significance surrounding the pH level between the distributed application, layered application and control in each soil layer.

Comparison	p-value/ level of significance	Pairwise Comparison (Tukey-Kramer)
Zn concentration of leachate between treatment applications (Figure 7)	p=0.00003 (p<0.001)***	Layered vs. Control (p=0.0001)*** Distributed vs. Control (p=0.0002)***
Leachate pH between treatment applications (Figure 8)	p=0.0002 (p<0.001)***	Layered vs. Control (p=0.0002)*** Distributed vs. Control (p=0.0097)***
Zn concentration of Layer A rhizon samples between treatment applications (Figure 9)	p=0.0133 (p<0.05)*	Layered vs. Control (p=0.0102)*
Zn concentration of Layer C rhizon samples between treatment applications (Figure 9)	p=0.0242 (p<0.05)*	Layered vs. Control (p=0.0206)*
Pore water pH of Layer *A* between treatments (Figure 10)	p=0.0001 (p<0.001)***	Layered vs. Distributed (p=0.0177)* Layered vs. Control (p=0.0231)* Distributed vs. Control (p=0.0002)***
Pore water pH of *Layer B* between treatments (Figure 10)	p=0.00001 (p<0.001)***	Layered vs. Distributed (p=0.0003)*** Distributed vs. Control (p=0.00009)***
Pore water pH of *Layer C* between treatments (Figure 10)	p=0.00006 (p<0.001)***	Layered vs. Control (p=0.0003)*** Distributed vs. Control (p=0.0001)***
Level of significance codes: *** p<0.001, ** p<0.01, * p<0.05.		

Table 6: This table displays results of One-Way ANOVA statistical tests with significant results and a Tukey-Kramer pairwise comparison statistical test between treatments.

6. Discussion
6.1 Batch Adsorption Kinetics Experiment

The sorption rate of zinc with biochar was similar to what was found by Bogusz *et al* (2015), where sorption efficiency of zinc was roughly 50%-70% at pH 7, depending on characteristics of the material such as surface area. The decreased efficiency could be due to a slightly lower pH level than that used by Bogusz *et al* (2015) during our batch experiment, a claim which is also supported by Chen *et al* (2011) who show in an experimental study that the adsorption of zinc was very pH dependent, with sorption percentages of zinc increasing as pH increases from 2 to 5. When conducting adsorption kinetic experiments with biochar sorption of zinc, Chen *et al* (2011) showed that equilibrium can be reached within the first 120 minutes of contact time, which is aligned with the findings of this study. The decreased affinity of zinc and biochar with lower pH was also shown by Bogusz *et al* (2015), which could explain why the sorption values obtained in this experiment were lower. The sorption rates of the AMOchar could be explained by differences in surface area and concentration of the sorbent as Tani *et al* (2004) shows that sorption of zinc increases with increases in surface area and concentration of synthetic manganese oxides.

6.2 Dosage Optimization Experiment

It was observed that there was a significant difference in the amount of sorption between the different dosages of both biochar and AMOchar. The fact that the sorption efficiency of AMOchar was much higher at a slightly acidic pH of 5 during the batch experiment also played a role in the decision to use a 2% AMOchar treatment. The use of the 2% dosage consumes less than half of the material required for the 5% treatment and is able to stabilize zinc almost as effectively. Although the difference between the 2% and the 5% dosage turned out to be statistically significant, it was decided that the values were not high enough to justify using more than double of the material for the

experiment. AMOchar was selected instead of just biochar also to minimize environmental impacts of this hypothetical *in situ* soil remediation treatment by introducing minimal amounts of treatment material, making the 2% treatment a more attractive and practical sorbent for typical soil remediation scenarios. These findings are consistent with that of Shehzad *et al* (2016), who optimized the dosage a biochar derived from sea mango that was meant to be applied to remove inorganic pollutants and found that approximately 7% was the ideal dosage and that sorption efficiency increased until this dosage. Chen *et al* (2011) found that two different biochars tested, one synthesized from hardwood at 450 °C and the other from corn straw at 600 °C, exhibited different optimum doses, further stressing the importance of feedstock material and pyrolysis temperature with regards to the sorption kinetics. The previously mentioned study by Chen *et al* (2011) demonstrated that zinc sorption efficiency of zinc increased with dosage with biochar derived from corn straw until about 10% dosage but also found that sorption efficiency of zinc with biochar derived from hardwood decreased with dosage after 5% treatment and was roughly just as efficient with dosages between 1% and 5%. Differences in sorption in these studies could be caused by a difference in the source material of the biochar or temperature of pyrolysis, as the biochar used in this study was derived from grape stalks at a pyrolysis temperature of 600 °C. The sorption percentages of zinc when compared by dosage are very similar to findings of Sanyang *et al* (2014) who showed that although the amount of zinc sorption increased with dosage, the efficiency began to level off around 1%. There are slight differences regarding where the sorption efficiency begins to level off but the concept is the same and it is possible that the slight difference in sorption efficiency could be attributed to the source material, which in the case of Sanyang *et al* (2014) was rice husk.

6.3 Comparison of Application of AMOchar Treatment

This experiment showed that the use of AMOchar in soil may significantly increase the soil pH and significantly reduce the mobility of zinc, which is supported by

findings from other studies such as Shen *et al* (2016). It was also demonstrated that the application of the AMOchar treatment can also have significant effects on the soil pH and zinc sorption, which is consistent with Trakal *et al* (2014) and Komárek *et al* (2013). Because the results of this study indicate that the method of application of AMOchar in soils can significantly alter the pH of soil solution and soil leachate while effectively immobilizing zinc, using AMOchar as a stabilization agent in soil for *in situ* remediation could be an effective way to prevent zinc and other hazardous metal(loid) contaminants from becoming mobile and also aid in stabilization of metals, including zinc, and facilitate increased efficacy of other remediation techniques that perform best in alkaline or neutral pH conditions (Kumpiene *et al* 2008). The prospect of using amorphous manganese oxides and biochar as a stabilizing remediation technique is also supported by the fact that this material has a natural tendency to increase the pH of acidic solutions that zinc and other metals are most likely to be present as a mobile form within (Komárek *et al* 2013, Trakal *et al* 2014). When analyzing the results of zinc concentration in the rhizon samples, interesting differences were observed with the layered AMOchar application. In Figure 10, it can be seen that although both applications appear to be effective in reducing zinc concentrations in the pore water, the distributed application seems to be slightly less effective at stabilizing zinc, especially near the beginning of the trial period. Statistical analysis via one-way ANOVA revealed that the difference of volume of water that was able to move through the column was not statistically significant, which can be seen in Table 1a in the appendices. Further research and more replicates would be required to support any claim that applying *in situ* remediation of zinc with AMOchar as a layer is more effective than mixing it with the soil but this study provides an indication that the presence of AMOchar in soil can stabilize zinc in solution and significantly alter pH levels in soil solution and leachate.

6.3.1 pH of Leachate

The treatment involving amorphous manganese oxide and biochar increasing soil solution pH is aligned with the findings of Komárek *et al* (2013), Trakal *et al* (2014), Chen *et al* (2011) and Lu *et al* (2012), as both materials are alkaline in solution. This may facilitate higher rates of zinc sorption as zinc sorption is shown to increase to a certain extent as soil solution pH increases, but this increase also may lead to problems because the alkaline soil may dissolve organic matter and therein release zinc that it was bound with (Komárek *et al* 2013). In Figure 10 it can be seen that both AMOchar treatments stayed around pH 7-7.5 while the pH of the control remained between 6-7. The results indicate that the difference in application of the AMOchar does not significantly affect the pH of the leachate. On the other hand, the presence of the AMOchar does significantly affect the pH of the leachate.

6.3.2 Zinc Concentrations in Leachate

Figure 10 shows differences that indicate that the presence of the AMOchar in the soil, regardless of the application, was significant in stabilizing zinc in the soil profile. By the end of the trial, the control leachate accumulated approximately 200 mg of zinc while both treatments accumulated less than 25 mg of zinc. This could have implications for significantly reducing zinc leachability. These findings are supported by Shen *et al* (2016) who showed an increased residual fraction of zinc from 7% to the range of 27% to 37% and a decrease in leachability of zinc from 0.12% to 0.01% with the application of biochar. This would also be supported by the findings of Chen *et al* (2011) who showed that zinc is successfully stabilized and therefore immobile in the soil profile after varying dosages of different biochar derived from hardwood and corn straw that were pyrolysed at different temperatures. The decreased leachability of zinc with the application of AMOchar would also align with studies from Mench *et al* (1994a,b,1997) and Sappin-Didier *et al* (1997) showing that manganese oxides have the

capacity to effectively stabilize zinc in the soil profile, significantly reducing the bioavailability and mobility of zinc and other cationic metal contaminants.

6.3.3 pH of Pore Water

It can be seen in Figure 9 that the distributed application of AMOchar was consistently the most alkaline of the three pH measurements. The results of a One-Way ANOVA with all rhizon samples, regardless of soil layer, showed that all three columns, layered, distributed and the control, were significantly different from one another. This is what prompted a more precise investigation regarding the pH of each specific soil layer and how they compared between different applications of the 2% AMOchar treatment. Not only do the results indicate that both of the treatments significantly raised the pH of the pore water but also it shows that the distributed application was even significantly higher than the layered application. This is very interesting because sometimes raising the pH of the soil solution can facilitate increased adsorption of metal contaminants, including zinc, and offers a prospective amendment that can be paired with remediation methods that are effective in setting with increased pH levels. This increase in pH by application of biochar and manganese oxides is supported by Trakal *et al* (2014), Chen *et al* (2011) and Komárek *et al* (2013).

A further investigation into differences between the soil layers showed that in Layer A, which was the top soil layer that contained the contaminated soil in all columns and a mixture with 7g of AMOchar in the distributed column, showed significant differences in pH between all three treatments but the most significant difference in the pairwise comparison was between the distributed and the control. This indicates that not only does the presence of AMOchar in the uppermost layers may have an effect on the pH of the topsoil or application layer, which is important for agricultural biomass production, but also that the application method can significantly affect the pH of the soil layer in which it exists. This could be due to the increased interaction between solutes, specifically zinc, and the AMOchar in the distributed application resulting from

the sorbent being more evenly spread throughout the soil and therefore more immediately available for reactions and providing greater access to sorption sites via soil solution and less immediate competition for sorption.

The layered treatment and control being statistically the same pH is inconsistent with what would be expected based on the concentrations of AMOchar in that layer in the layered treatment and the alkalinity of both the biochar and amorphous manganese oxides (Trakal *et al* 2014, Komárek *et al* 2013). This may have been caused by an air bubble that formed in the middle layer of the 2% AMOchar layered application column.

The findings from Layer C indicating that the 2% AMOchar treatment was able to significantly alter the pH level are consistent with the findings of Shen *et al* (2016), Chen *et al* (2011) and Bian *et al* (2014) who all show that the mobility of zinc is significantly decreased by biochar application, a process that is facilitated by neutral and alkaline pH conditions, and also with Komárek *et al* (2013) who states that the application of manganese oxides in soils has the potential to significantly increase the pH of the soil solution.

6.3.4 Zinc Concentrations in Pore Water

The differences of the control could be attributed to the solubility of zinc in the first two layers (A and B) while the decrease of zinc in Layer C could be due to the soluble zinc being leached within the first few timesteps of the trial. This leachability of zinc is also reflected in the cumulative amount of zinc collected from the leachate (Figure 7). The findings that these materials have significantly affected the amount of zinc concentrations in soil solution is consistent with that of Shen *et al* (2016) and Chen *et al* (2011) who showed that biochar can decrease zinc mobility and plant availability.

When comparing the two applications of AMOchar in the soil profile the most notable difference in zinc concentrations is at the beginning of the trial. It seems that the layered application was able to stabilize zinc more rapidly in the soil profile but by the end of the trial both treatments stabilized zinc effectively as the concentrations of zinc

were less than 5 ppm in both applications. These results indicate that the layered application is quicker to adsorb zinc in the soil profile but the difference could be considered negligible as it was only pronounced in the first 9 hours of the trial period (Figures 10). Adsorption rates between different types of biochar were found to be variable in the first 10 hour period of experiments by Bolusz *et al* (2015) and all treatments reached equilibrium by the end of this time period. The first 10 hour period is usually considered to be less important for remediation purposes and therefore the results of this study regarding the difference in adsorption rates between the two periods is less significant. Chen *et al* (2011) also showed that zinc adsorption is able to reach equilibrium quite rapidly, with results also showing approximate equilibrium being reached in the first few hours of the trial, accounting for the variability in sorption near the beginning of the trial in this study. Chen *et al* (2011) also found that zinc concentrations were significantly decreased in aqueous solution with the application of biochar in various dosages, a result that strongly supports the findings of this study.

6.4 Possible Sources of Error

With regards to the collection of the leachate outflow sample, some clogging that occurred at the base of the layered column between the first and second sampling timestep could have affected the amount of zinc leached during this timestep and could possibly affect the zinc concentrations and pH levels during the second sampling timestep. It is also possible that there was a brief period of time between the fifth and sixth sampling step that there was no water inflow as was intended for the duration of the trial period. This would be due to the water supply for the pump running out in the approximately 24 hours between sampling. Additionally, a large air bubble was formed during the experiment that affected the sampling of soil solution from Layer B of the Layered column. This was a problem because it affected our ability to retrieve soil solution samples with the vacuum sampling tube because the rhizon needle was not in contact with the soil solution at all times. This could have been prevented by wetting the

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soil and AMOchar before putting it into the column but wetting the soil also increases the complexity of the experiment by initiating zinc and metal dissolution before the trial period has begun, thus potentially creating more problems than it solves, which is why we opted not to do this in the first place although it could be plausible in highly controlled lab scenarios.

7. Conclusion

This study showed that the application of AMOchar in soils can significantly stabilize zinc in the soil profile, reducing its mobility and bioavailability, thus reducing its leachability and therefore it can be said that it reduces the risk of groundwater and food contamination presented by plant and microbial introduction of metal contaminants into the trophic chain. Distributing the 2% AMOchar remediation treatment evenly in the topsoil has the potential to significantly increase the soil pH when compared with a single layer of 2% AMOchar in the soil profile and no treatment. Zinc was shown as immobile in the soil solution as well as the soil water leachate. The application of 2% AMOchar to stabilize zinc in the soil profile is a cost-effective and non-environmentally intrusive solution to reduce the risk of zinc and other metals contaminating groundwater and food supplies. It is highly recommended that the experiment be carried out again with more replicates in order to confirm the findings of this experiment by increasing the size of the data set and thus increasing the reliability of the results and following statistical analysis.

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9. Appendices

| Time [hrs]	Layered AMOchar 2%				Distributed AMOchar 2%			
	Layer A	Layer B	Layer C	Leachate	Layer A	Layer B	Layer C	Leachate
0	6.25	5.72	7.71	8.84	6.94	6.91	6.93	7.54
9	6.68	6.1	7.15	7.98	7.09	7.17	7.2	7.2
20.5	6.74	6.26	7.52	7.74	7.2	7.27	7.59	7.17
24.5	6.71	6.72	6.55	7.57	7.27	7.25	7.37	7.41
47.5	6.41	6.26	*NA*	7.27	6.68	6.86	7.37	7.27
71	6.83	6.47	*NA*	7.31	6.84	7.05	*NA*	7.33

Table 1a: This table displays the pH values obtained from soil solution that was obtained from one of the three rhizon layers or the leachate outflow from both columns. *NA* indicates that not enough of the solution was collected to obtain a measurement.

Time [hrs]	Layered AMOchar 2%				Distributed AMOchar 2%			
	Layer A [ppm]	Layer B [ppm]	Layer C [ppm]	Leachate [mg]	Layer A [ppm]	Layer B [ppm]	Layer C [ppm]	Leachate [mg]
0	3.957	6.914	5.753	0.001	11.132	27.779	48.497	0.0247
9	2.025	6.082	4.971	0.1619	3.679	7.639	9.371	4.2620
20.5	1.416	3.387	1.504	0.0948	2.421	4.233	4.375	2.1207
24.5	1.024	2.889	2.056	0.1237	2.133	3.870	3.893	0.3999
47.5	1.912	3.656	0.150	0.5123	1.905	2.911	2.701	1.4955
71	0.980	2.278	0.028	0.3271	1.455	2.087	1.166	1.5140

Table 2a: This table displays the amount of zinc extracted from samples of each rhizon layer and leachate outflow in ppm for both AMOchar treatments.

Time [hrs]	pH				Zinc Concentration			
	Layer A	Layer B	Layer C	Leachate Outflow	Layer A [ppm]	Layer B [ppm]	Layer C [ppm]	Leachate Outflow [mg]
3	5.87	5.49	5.44	-	0.4028	13.3759	26.9120	-
6	6.19	5.96	5.54	6.2	13.7842	9.7295	37.1763	11.8812
12	6.23	5.98	5.83	6.34	25.7323	6.5350	44.9288	20.6267
24	6.3	5.94	6.03	6.18	21.3474	15.9564	49.0803	23.7313
48	6.42	6.23	6.18	6.98	29.7997	17.4266	2.7435	35.1420
72	6.51	6.33	4.83	7.06	14.4940	5.2414	12.6059	44.8486

Table 3a: This table displays the pH and zinc extracted from samples of each rhizon layer and leachate outflow that was used as a control for the experiment. These data were obtained from a previously completed experiment.
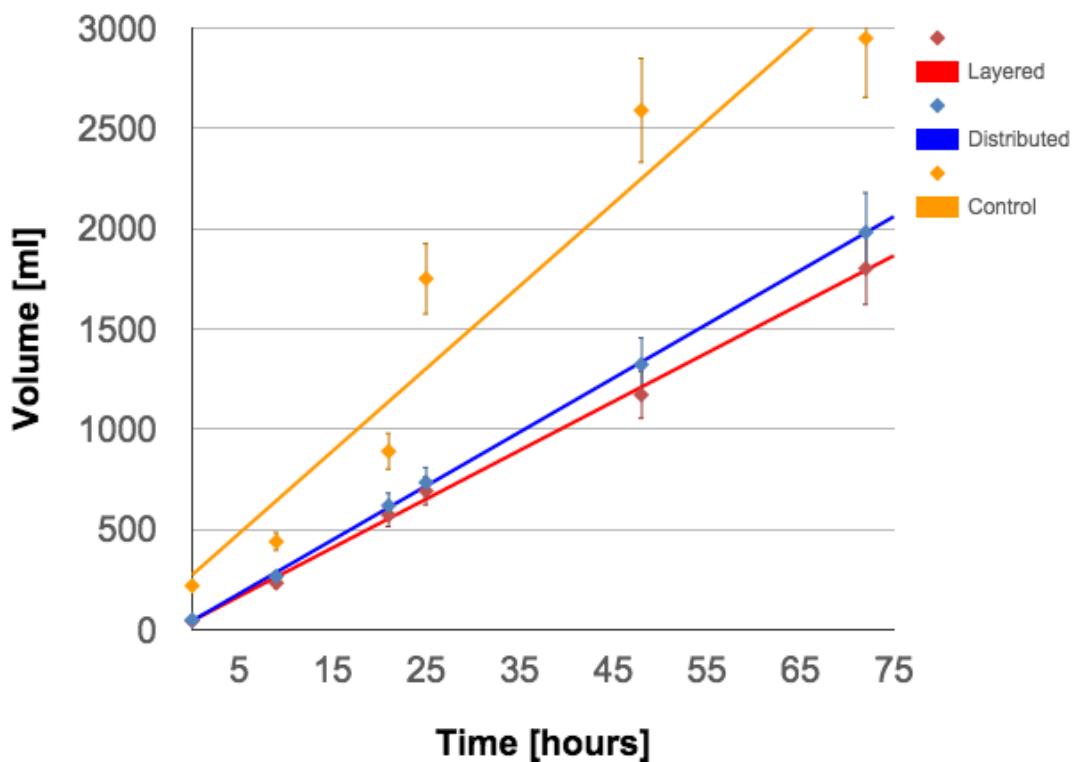
Figure 1a: This figure displays the volume of solution collected from the leachate outflow of the columns with layered AMOchar and distributed AMOchar treatments at 2% dosage.