ASSESSMENT AND POTENTIAL FOR PHYTO-AND-MYCORREMEDIATION OF SOIL

HEAVY METAL POLLTUION IN SOUTHERN NEW JERSEY (USA)

BY

ZACHARY COOK

A thesis submitted to the

Graduate School-Camden

Rutgers, The State University of New Jersey

In Partial fulfillment of the requirements

For the degree of Master of Science

Graduate Program in Biology

Written under the direction of

Dr. John Dighton

And approved by

_____

Dr. John Dighton

_____

Dr. Amy Savage

_____

Dr. Jennifer Oberle

Camden, New Jersey

January 2019

THESIS ABSTRACT

ASSESSMENT AND POTENTIAL FOR PHYTO-AND-MYCORREMEDIATION OF SOIL

HEAVY METAL POLLTUION IN SOUTHERN NEW JERSEY (USA)

By ZACHARY COOK

Thesis Director:

Dr. John Dighton

Two studies were performed and are presented in this thesis. The first study (Chapter I) involves

the characterization of soils in Southern New Jersey. This was performed with the creation of an

urban-rural sampling gradient which starts in city of Camden and extends to Salem county. The

physiochemical properties and four heavy metal concentrations Zinc (Zn), Copper (Cu), Lead

(Pb), and Iron (Fe) were determined from soil samples collected along this urban-rural sampling

gradient. The urban soil Zn, Cu, Pb, and Fe concentrations were averaged and used to conduct

the second study (Chapter II). The second study tests the phytoremediation potential of two plant

species, *Trifolium repens* (white clover) and *Panicum virgatum* (switch grass) with a mycorrhizal

inoculum.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

First, I would like to thank my family and friends. For my parents Jeffery and Barbara and my sister Hannah for all of the support they provided over my college career and propelling me to excel in my studies. I would also like to thank my best friend Jaime Wright and her family for all of their support since the beginning of my college experience at Rutgers University. To Katherine Shawaryn, who helped edit my various papers throughout my college career. And to my grandparents, Antoinette Carlin-Banach and Joseph Banach who passed away in my last years of study. My grandparents provided me with immense support, love, and advice. They will be forever missed as they play such a key role in my life.

Next, I would like to thank my committee members Dr. John Dighton, Dr. Amy Savage, and Dr. Jennifer Oberle. To Dr. John Dighton, who gave me a great experience and knowledge under his direction with the teaching assistantship. To Dr. Savage, who let me start my research career as a under graduate. And to Dr. Oberle whose classes provided me a greater understanding of microbiology and invertebrate zoology.

Lastly, I would like to thank my fellow colleagues, professors, and Rutgers university. To Alexa Martinelli, who I worked with during my first project and our friendship. To my fellow lab mates, who provided support and knowledge for my experiments that I conducted. To the many other professors at Rutgers University who provided a great deal of knowledge and support over the years. To Rutgers University, for the degrees that it allowed me to achieve.

dry weight soil from urban, suburban, rural soils. There were no significant differences observed (Tukey's range test).

Figure 4: The white bars (left) indicate the mean (± Standard error) bioavailable Pb concentrations ($\mu g\ g^{-1}$) of dry weight soil from urban, suburban, rural soils. While the black bars (right) indicate the mean (± Standard error) total Pb (± Standard error) concentrations ($\mu g\ g^{-1}$) of dry weight soil from urban, suburban, rural soils. There were no significant differences observed (Tukey's range test).

Figure 5: The white bars (left) indicate the mean (± Standard error) bioavailable Fe concentrations ($\mu g\ g^{-1}$) of dry weight soil from urban, suburban, rural soils. While the black bars (right) indicate the mean (± Standard error) total Fe (± Standard error) concentrations ($\mu g\ g^{-1}$) of dry weight soil from urban, suburban, rural soils. There were no significant differences observed (Tukey's range test).

Figure 6: PCA analysis of soil microbial carbon utilization using ECOLOG microtiter plates. Axis 1 accounts for 24.32% of the total variation; $F_{(4,27)} = 4.50$, $P = 0.021$. Axis 2 accounts for 16.56% of the total variation. Results of the MANOVA are as follows; $F_{(4,27)} = 1.07$, $P = 0.365$. $\lambda = 0.678$, $F = 2.79$, $P = 0.0356$. Values presented after the enzymes in parentheses are Pearson Correlations.

CHAPTER II

Figure 3: The concentration of Pb (μg g^{-1}dry weight, mean ± standard error, n =3) of *T. repens* and *P. virgatum* shoots and roots, grown in increasing Pb soil concentrations (μg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.

Figure 4: The concentration of Fe (μg g^{-1}dry weight, mean ± standard error, n =3) of *T. repens* and *P. virgatum* shoots and roots, grown in increasing Fe soil concentrations (μg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.

Figure 5: The total concentration of Zn (μg pot^{-1}, mean ± standard error, n =3) in *T. repens* and *P. virgatum* shoots and roots, grown in increasing Zn soil concentrations (μg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.

Figure 6: The total concentration of Cu (μg pot^{-1}, mean ± standard error, n =3) in *T. repens* and *P. virgatum* shoots and roots, grown in increasing Cu soil concentrations (μg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.

Figure 7: The total concentration of Pb (μg pot^{-1}, mean ± standard error, n =3) in *T. repens* and *P. virgatum* shoots and roots, grown in increasing Pb soil concentrations (μg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.

Figure 8: The total concentration of Fe (μg pot^{-1}, mean ± standard error, n =3) in *T. repens* and *P. virgatum* shoots and roots, grown in increasing Fe soil concentrations (μg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.

CHAPTER I

SOIL HEAVY METAL POLLUTION ALONG AN URBAN-RURAL SAMPLING GRADIENT
IN SOUTHERN NEW JERSEY

1. INTRODUCTION

The process of urbanizing and industrializing natural habitats dramatically transforms landscapes and creates ecological disturbances. Heavy metals are useful tracers of environmental pollution. Considered "sinks," urban soils accumulate large amounts of heavy metals over time because of industrial, commercial, and residential use (Manta et al., 2002; Paterson et al., 1996). Soil contamination by heavy metals is of great environmental concern, since metals can be transferred to water and crops, posing potential risks for human health (Mielke et al., 1999; Sansalone and Buchberger, 1997; Zhang et al., 2015). Accumulation of heavy metals has long lasting effects on soil habitats, organisms, and the surrounding environment (Chander et al., 2001; Mapanda et al., 2005). Determining the concentrations of heavy metals in soils can provide further insight on how urbanization and industrialization are chemically altering environments.

Heavy metals are introduced into urban environments through anthropogenic activities, such as; automobile emissions, degradation of automobile parts, factory emissions and runoff, combustion of fossil fuels, construction materials, and illegal dumping (Li et al., 2013; Al-Khashman and Shawabkeh, 2006; Hu et al., 2015; Johansson et al., 2009). Because of large populations and densities, urban areas experience these anthropogenic processes more than rural counterparts (Norman et al., 2006; Dodman, 2009). Numerous factories, metal scrap yards, recycling facilities, and waste incinerating plants in urban areas pose a serious risk of introducing heavy metals into urban soils (Heinonen and Junnila, 2011; Schuhmacher et al., 1997; Tang et al., 2010; Chicharromartin et al., 1998). This risk increases without proper regulation, recycling, and disposal of heavy metals.

Urban landscapes are dominated by impervious surfaces that further exacerbates heavy metal pollution of soils. These surfaces include roads, buildings, and sidewalks that allow for the accumulation of heavy metal particulate matter within urban environments (Faiz et al., 2009; Gromaire et al., 2001; Zheng et al., 2010). Washing heavy metal particulate matter accumulating

on these surfaces by atmospheric deposition can expose the surrounding soil to increased local concentrations (Christoforidis and Stamatis, 2009; Kondo et al., 2016; Ouyang et al., 2015). Heavy foot traffic and landscaping in dense populations frequently alters urban soils. In turn, this alters plant communities and increases the risk of heavy metal exposure (Jan et al., 2015; McDonald, 2008; Vakhlamova et al., 2016).

Soil microbes play a crucial role in ecosystem processes as they are responsible for nutrient cycling, acquisition, and soil formation which overall affect ecosystem quality (van der Heijden et al., 2008). Soil microbes are extremely sensitive to heavy metals and the effects of heavy metals on soil microbes varies between species (Giller et al., 2009). The effects are also dependent on the type and speciation of metals and soil concentrations (Nies, 1999). Alterations to microbial biomass, respiration, mineralization, and survival rates have all been observed in soils with increased heavy metal concentrations (Chander et al., 2001; Nwuche and Ugoji, 2008). Higher soil metal concentrations do not always correlate to reduced microbial activities. Several soil microbial species have been shown to prosper in heavy metal polluted conditions (Lazzaro et al., 2008; Sandaa et al., 2001). The effects of heavy metals on soil microbes is complex and research is sparse, thus, more observations are needed on how heavy metals affect soil microbes and their processes.

To our knowledge, heavy metal contamination in southeast New Jersey in urban, suburban, or rural areas (an urban-rural gradient) has not been previously investigated. New Jersey is the most densely populated state in the United States. Therefore, New Jersey is worthwhile to study in respect to urbanizations effects on ecosystems. An urban-rural gradient is useful in assessing the impacts of urban and industrial development on soil pollutant accumulation and soil quality (Lu et al., 2009; Wagrowski and Hites, 1997; Steinberg et al., 1997; Zhao et al., 2007). Urban-rural gradients are useful in the comparison of environments outside urban environments, as many environmental conditions are shared locally. Currently, urbanized

ecosystems and heavy metal concentrations in soils along an urban to rural gradient are

significantly unrepresented in literature (Decker et al., 2000; Lu et al., 2009).

Along an urban to rural gradient the objectives of this study are to 1) determine the

concentrations of four heavy metals Zinc (Zn), Copper (Cu), Lead (Pb), and Iron (Fe) in urban,

suburban, and rural areas soil's, 2) determine physicochemical properties (pH, moisture content,

total organic carbon, soil particle size distribution, micronutrients; nitrate, ammonium,

phosphate); and 3) to establish soil microbial carbon utilization using ECOLOG microtiter plates.

I hypothesize that as urbanization and industrialization increases heavy metal pollutant loadings

in the soils will increase respectively toward the city of Camden in New Jersey. I expect that

towards the rural end of the study area heavy metal pollutant loadings will decline as a result of

being less urbanized and industrialized.

2. MATERIALS AND METHODS

2.1. Study area

The study took place in southeast New Jersey. A southwest transect of 30 sampling locations were chosen according to soil maps (Tedrow, 1986). All of the sampling locations had the same soil type being that of the Upper Coastal Plain which are characterized as being sandy nutrient-poor soils. A total of 30 soil samples were collected across the urban – rural gradient. 10 urban sites were chosen in the city of Camden. 10 suburban sites were chosen further south in Gloucester County; and l0 rural sites were chosen in southern-most sampling region in Salem County. These 30 sites are termed the "urban-rural gradient." Longitudinal and latitudinal coordinates of each sampling location were recorded (Table 1). The 30 sampling locations are presented in Fig. 1.

2.2. Soil sampling strategy and treatment

Road verges are the grassy area between the sidewalk and street and were the sampling location in the urban and suburban areas. Road verges do not exist in the rural sampling area, so an average area of the road verges in the urban and suburban sampling areas were calculated and then superimposed on rural sampling locations. For each sampling location, a minimum of two trees were required. If more than two trees were present at a sampling location, trees were assigned numbers and were randomly picked with a random number generator. At each site three sub-samples were collected from the top soil (0-10 cm) measured one meter from the randomly chosen tree. These three sub-samples were combined to form a bulk sample.

A stainless-steel hand auger (LaMotte soil sampling tube Model EP code 1055) was used to collect soil samples for heavy metal analysis. The Model EP Soil Sampling Tube has a diameter of 2.5 cm and is 30 cm in length. Bulk samples for heavy metal analysis were immediately placed in polyethylene bags for transport.

For heavy metal analysis, the collected soil was transferred to paper bags and placed into a dryer at 60°C. The soil samples were then allowed to dry for 16 hours or until a constant mass was achieved. After drying, the masses were recorded. Samples were then sifted using a Fieldmaster 78-700 soil sampling sieve set to remove any larger debris, such as rocks, twigs, and roots. Samples were initially sifted through a 2-mm mesh and then finally through a final 1-mm mesh. Between samples, the sifter was cleaned thoroughly with a stiff cleaning brush followed by a finer brush to remove soil particles. The dried sifted soil samples were then placed into polyethylene bags and stored in a cool, dry, dark container until the extraction process.

2.3. Soil physicochemical properties

Soil pH was determined by using a 1:2 soil: water (w/v) suspension that was allowed to shake on a reciprocal shaker for 30 minutes and settle for 15 minutes before analysis with an Oakton Ion 700 pH meter and WD-35811-71 Oakton pH electrode. Total organic carbon was determined as in (Salehi et al., 2011). In short, 2.0 g of dried sifted soil was added to a pre-dried (550°C for one hour) crucible and placed into a muffle furnace to heat at 550°C for two hours. After heating, samples were allowed to cool and then the masses were recorded. Soil particle size distribution was determined by using a Fieldmaster 78-700 soil sampling sieve set.

Water extracts created for analysis of bioavailable metals were used for the analysis of the soil nutrients ammonium, nitrate, and phosphate. Analysis of ammonium and nitrate were conducted using microplate spectrophotometry as described in (Hood-Nowotny et al., 2010). For the analysis of ammonium, 8.5 g sodium salicylate was mixed with 63.9 mg sodium nitroprusside dehydrate in 50 ml deionized water (prepared fresh), to this resulting solution 0.3 M Sodium hydroxide was added 1:1 (v/v) to create the colorant. The oxidation reagent was prepared in which 0.1 g dichloroisocynauric acid was added to 100 ml of deionized water. A standard solution of $(NH_4)_2SO_4$ (1000 µg ml^{-1}) was created and used to produce a calibration curve with standard concentrations ranging from 0.1 – 4 µg ml^{-1}. For all the soil nutrient analyses the first

column of each microplate was devoted to standards while successive columns were devoted to sample extracts. To each well 100 µl of sample extract or blank, 50 µl of colorant, and 20 µl of the oxidant was added. The microplate was allowed to stand at room temperature for 20 minutes and was then read at 650 nm with an accuSkan FC microplate photometer by Fisher Scientific.

For the analysis of nitrate, three reagents were prepared in advance. The first reagent was created by mixing 400 mg Vanadium chloride with 50 ml $1M$ HCl. The second reagent was created by mixing 50 mg N-napthylethylenediamine dihydrochlorine in 250 ml of deionized water (Griess reagent I). The last reagent was created by mixing 5 g sulphanilamide in 500 ml 3 M solution of HCl (Griess reagent II). A standard stock solution was created of KNO_3 (1000 µg ml^{-1}) this was used to create a calibration curve with standard concentrations ranging from $0.1 - 3$ mg ml^{-1}. Each well received 100 µl of sample extract or blank, 100 µl VCl_2, 50 µl Griess I, and 50 µl Griess II were added. The microplate was then read at 540 nm with an accuSkan FC microplate reader photometer by Fisher Scientific.

 Analysis of phosphate was also conducted using microplate spectrophotometry as described in (D'Angelo et al., 2001) (Jeannotte et al., 2004). Two reagents were prepared in advanced for analysis. The first was created by mixing 14.2 mmol L^{-1} ammonium molybdate tetrahydrate in 3.1 M H_2SO_4. The second reagent was created by mixing 3.5 g L^{-1} aqueous polyvinyl alcohol (molecular weight 50,000) with deionized water at 80°C until fully dissolved. After the polyvinyl alcohol solution cooled, 0.35 g L^{-1} malachite green carbinol hexachloride was added. A standard stock solution was created of KH_2PO_4 (1000 µg ml^{-1}) with standard concentrations ranging from $0 - 2$ mg ml^{-1}. 200 µl of soil extract was added to each well followed by 40 µl of reagent one and allowed to mix for 10 minutes on an orbital shaker. Then 40 µl of reagent two was added to each well and allowed to mix for 20 minutes on an orbital shake at high speed. The microplate was then read at 630nm with an accuSkan FC microplate reader photometer by Fisher Scientific.

2.4. Heavy metals (Zn, Cu, Pb, Fe) extractions

2.4.1. Acid Extraction (Total heavy metals)

The soil samples were analyzed for total heavy metal concentrations using the acid digestion method (McGrath and Cunliffe, 1985). Approximately 0.50 grams of dried, sifted soil samples were weighed and placed into clean Foss 100 ml digestion tubes. Eight milliliters of concentrated trace metal grade hydrochloric acid and 2 ml of trace metal grade nitric acid (Fisher Scientific) were added to the test tubes in the fume hood and allowed to digest overnight at room temperature. The tubes were then placed into a Tecator digestion system 40 1016 digestion block and heated to 105°C for one hour and then increased to 140°C until the samples were dry. Once the dried samples were cool, 12.5 ml of 20% (by vol.) hydrochloric acid was added and then was reheated to 80°C for 20 minutes. The digests were then filtered through a Whatman #2 filter into a 50 ml volumetric flask and were made up to 50 ml with deionized water.

2.4.2. Water Extraction (Bioavailable heavy metals)

The water extractable metals were extracted with slightly modified methods in (Séguin et al., 2004) because they are readily available to plants, deeming them as bioavailable. In turn, 5.0 g of dried sifted soil was weighed and was suspended in 100 ml of deionized water in a container and allowed to shake on a reciprocal shaker for 30 minutes. The soil suspension was then filtered through a Whatman #2 filter by vacuum filtration. Extracted samples were then transferred to a clean sealed plastic container for storage until metal analysis with flame atomic absorption spectrophotometry.

2.5. Analysis of extracts using flame atomic absorption spectrophotometry

Concentrations of heavy metals; Zn, Cu, Fe, and Pb were determined by using a 211 Accusys flame atomic absorption spectrophotometer from Buck Scientific. The bulbs associated with the metals to be analyzed (Zn, Cu, and Pb) were purchased from Buck Scientific. A

calibration curve for each metal was established from standards (1000 µg ml^{-1}) purchased from Ricca and Inorganic Ventures. The Fe standard was created according to the methods in the Standard methods for the examination of water and waste water (American Public Health Association, 1992). The range of concentrations for the standards for Zn, Cu, Pb, and Fe was; 0 – 5 µg ml^{-1}, 0 – 0.7 µg ml^{-1}, 0 – 3 µg ml^{-1}, and 0 – 500 µg ml^{-1} respectively. Before analysis of the extracted samples, they were allowed to reach room temperature and shaken for 30 minutes to ensure proper homogenization. Between analysis of samples, a 5% nitric acid solution was used to clean the nebulizer and prevent cross contamination of samples. Absorbance values were converted to concentration values, using the appropriate calibration curve.

2.6. Soil microbial carbon utilization using ECOLOG microtiter plates

ECOLOG plates (Biolog, Inc.) were used to investigate the substrate utilization of the microbial community (Garland, 1996a; Garland, 1996b; Garland and Mills, 1991). ECOLOG plates contain 31 carbon substrates in three sets of replicates on each plate. A soil suspension was prepared by shaking 50 ml of sterile deionized water to 0.5 grams of fresh soil on a reciprocal shaker for 30 minutes. 100 µl of soil suspension solution was pipetted into each well of the ECOLOG plate. If a substrate is degraded it turns color and the optical density can be read with a plate reader at 593 nm. The inoculated plates were read with a Fisher Scientific accuSkan FC plate reader every three days to produce a total three readings.

2.7. Statistical analysis

Statistical analyses were performed with GraphPad Prism 8, R studio 1.1.414, PC-Ord 7 for Windows. The site map was generated with R Studio package "ggmap". Data were checked for normality using Shapiro-Wilk test, and soil physiochemical properties and heavy metal concentrations were analyzed by performing ANOVAs in GraphPad Prism 8. Analysis of the results of the ECOLOG plates were conducted using a PCA analysis using PC-Ord.

3. RESULTS

3.1. Soil physiochemical properties

Selected soil physiochemical properties are summarized in Table 2. pH values of soils ranged from 3.80 in the rural sampling area to 7.76 in the urban area. The mean pH values of the urban, suburban, and rural soils were 6.26, 5.15, and 5.73 respectively. Overall, the urban soils were significantly less acidic than rural soils, which were more acidic ($F = 5.29$, $P = 0.0083$). Soil moisture ranged from 5.70% within the suburban area to 63.83% in the rural sampling area. The mean moisture content of the urban, suburban, and rural soils was 9.99%, 21.92%, and 24.15%. Overall, the rural soils had a higher moisture content (24%) as compared to the moisture content of the urban soils (10%) ($F = 4.20$, $P = 0.03$). Soil organic matter ranged from 1.58% in the urban sampling area to 78% in the rural sampling area. The mean organic matter of the urban, suburban, and rural soils was 5.09%, 8.36%, and 20.88%. No significant differences were observed between the sampling areas with regards to soil organic matter.

Soil particle size distribution of each site is summarized in Table 3. The urban soils had less granule gravel (6.24%) when compared to the rural soils which had significantly more (18.97%) ($F = 6.69$, $P = 0.003$). The urban soils had a larger content of medium sand (24.75%) when compared to the rural area which had 14.33% ($F = 5.27$, $P = 0.02$). The suburban soils had a larger content of medium sand (25.08%) when compared to the rural area soils ($F = 5.27$, $P = 0.02$). The urban soils had higher contents of fine sand (22.72%) as compared to the rural soils which had significantly less (9.39%) ($F = 9.57$, $P = 0.0005$). The urban soils had a larger content of very fine sand (4.33%) as compared to the rural soils which had significantly less (2.36%) ($F = 3.68$, $P = 0.03$). There were no significant differences with regards to pebble gravel, very coarse sand, and silt across the urban – rural gradient.

3.2. Heavy metal contents along the urban-rural gradient

The soil concentrations of water (bioavailable) and acid extractable (total) Zn, Cu, Pb, and Fe along the urban-rural gradient are summarized in the graphs in Figures 2 – 4. Soil water extractable heavy metals are low and homogeneous across the urban-rural gradient. The following bioavailable metal concentrations were obtained along the urban – rural gradient. In the case bioavailable Zn (urban, 0.30 µg g^{-1}; suburban, 0.26 µg g^{-1}; rural, 0.23 µg g^{-1}), Cu (urban, 0.28 µg g^{-1}; suburban, 0.49 µg g^{-1}; rural, 0.28 µg g^{-1}), Pb (urban, 1.03 µg g^{-1}; suburban, 1.09 µg g^{-1}; rural, 0.97 µg g^{-1}), and Fe (urban, 12.87 µg g^{-1}; suburban, 15.17 µg g^{-1}; rural,15.63 µg g^{-1}). There were no significant differences between sampling areas with regards to soil water extractable heavy metal concentrations. The low concentrations of these heavy metals are based on the speciation of the metals ability to dissociate into water, thus making them bioavailable to organisms.

The highest concentrations of the acid extractable/total of Zn, Cu, and Pb were found in the urban (326.3 µg g^{-1}, 60.9 µg g^{-1}, 376.3 µg g^{-1}) and rural (320 µg g^{-1}, 78.8 µg g^{-1}, 321.8 µg g^{-1}) soils of the study. In the case of the maximum concentrations in the suburban soils for Zn, Cu, and Pb were 89.1 µg g^{-1}, 35.8 µg g^{-1}, and 206.6 µg g^{-1}, respectively. There is a unique pattern with total Zn concentrations, in which urban and rural soils exhibit similar averaged concentrations (178.6 µg g^{-1} and 160.8 µg g^{-1}) as compared to the suburban soils which had significantly lower concentrations (57.1 µg g^{-1}) (F = 4.3, P = 0.0296). A similar pattern is seen with acid extractable Cu and Pb soil concentrations though it is not significant, it should not be overlooked. The metal concentrations data reject the hypothesis that heavy metal concentrations will decline in rural regions. The results indicate the opposite; urban are rural areas have similar heavy metal concentrations.

3.3. ECOLOG microtiter plates for microbial carbon utilization PCA analysis

The results of the ECOLOG are summarized by a principal component analysis in Figure 4. Axis 1 accounts for 24.32% of the variance and axis 2 accounts for 16.56% of the variance.

Urban and rural have greater utilization of D-xylose substrate. While the suburban area has a greater utilization of phenylethylamine, i-Erythritol, and Glycyl-L-Glutamic Acid. A similar pattern regarding total zinc concentrations is displayed by the PCA analysis of the ECOLOG data. Urban and rural areas exhibit the same substrate utilization while the suburban area is significantly different (F = 4.50, P = 0.03).

4. DISCUSSION

Overall, the pH values of the soils across the urban – rural gradient were acidic (pH < 7), even though the urban soils were significantly higher (pH = 6.26) than the rural soils, with no difference between suburban soils and urban/rural soils. A common characteristic of soils that are sandy and nutrient poor is that they are more acidic in nature, which could explain the relatively low pH values seen across the urban – rural gradient. The elevated pH of urban soils is commonly related to the presence of construction materials like brick, cement, plaster, concrete, and mortar which all contain calcium. When these construction materials are weathered and degraded, they release leachates that contain calcium which increase the alkalinity of urban soils (Jim, 1998a).

The moisture content of the urban area's soils was lower than the rural soils, 10% as compared to 24%. The reduced moisture content of urban soils is most likely attributed to the urban landscape (impervious surfaces) and mechanical activities. The most notable consequence of urbanization is the artificial sealing of soils with impervious surfaces. Impervious surfaces deviate water to storm water infrastructure and away from soils which prevents the accumulation of water by urban soils. The compaction of urban soils from machinery, foot traffic, and landscaping reduces the permeability of soils thus affecting water infiltration rates (Scalenghe and Marsan, 2009).

The urban area of this study has soils that are poorly maintained and lacked low lying vegetation (i.e. grasses). Without above ground vegetation in urban areas, exposed soils are directly subjected to natural and anthropogenic physical disturbances. Rain, wind, vehicular traffic, and human trampling are examples of how urban soils are disturbed. Depending on the type and severity of a disturbance soils are ultimately damaged and soil aggregate size is drastically reduced. The urban area of this study has a larger composition of finer soil particles (i.e. medium, fine, and very fine sand) than rural soils.

Pollutants, specifically heavy metals, tend to accumulate in finer soil particles due to their high surface areas and negative charges (Ajmone-Marsan et al., 2008). Particularly Zn in this study was found to be at the highest concentrations in urban and rural soils. Soil heavy metal pollution is more concentrated in urban areas due to large human populations, automobile traffic/wear, and industries which emit heavy metal particulate matter into urban environments. A study conducted in 2011 by Yu et. al. found that Zn, Cu, Pb, and Fe, analyzed in this study, were being emitted by a cement factory in the Water Front South area of Camden. The heavy metal particulate matter released from the cement factory was atmospherically deposited on to surrounding urban soils and impervious surfaces (Yu et al., 2011). Urban soil heavy metal pollution is then exacerbated by urban impervious surfaces in which heavy metal particulate matter accumulates on and then is transferred to surrounding exposed soil (Jim, 1998b; McDonald, 2008; Weng et al., 2004).

Rural soils are often recipients of heavy metal contamination from agriculture. Fertilizers such as P-fertilizers, composts, manure, and sewage sludge not only contain essential nutrients, but also contain heavy metals (Nicholson et al., 2003). The rural sampling sites of this study are separated by land that was/is used for agriculture dating back to the 17th century. Agriculture land-use is one explanation why rural soils exhibit similar heavy metal concentrations to the urban soils in relation to this study. Another explanation of the high concentration of Zn in rural soils is the deposition of atmospherically transported heavy metal particulate matter. Heavy metal particulate matter has been shown to be transported large geographical distances within air masses, resulting in the enrichment of metal concentrations far from emission sources; e.g. in the Artic, California, and Hong Kong (Blumenthal et al., 1978; Wang et al., 1998; Węgrzyn et al., 2016).

The suburban soils of the study had lower concentrations of Zn as compared to the urban and rural soils. In general, suburban areas are heavily maintained for aesthetics. Lawn clippings

and leaves are removed in suburban areas which may provide a heavy metal phytoremediation effect (Krogmann, 1999). Non-native plant species are introduced in suburban areas, which are known to produce a large amount of biomass that is able to sequester heavy metals; e.g. sunflowers (*Helianthus Annus)*, Indian mustard (*Brassica juncea*), white willow (*Salix alba*), and poplar trees (*Populus deltoides)* (Di Baccio et al., 2003; Mleczek et al., 2010; Salido et al., 2003; Turgut et al., 2004). The natural bioaccumulation of heavy metals by plants can result in significant differences in soil concentrations between geographical locations (Weber and Karczewska, 2004).

The microbial enzyme analysis via ECOLOG microplate assay shows a similar trend to total Zn soil concentrations across the urban – rural gradient. Likewise, in which urban and rural soils had similar Zn concentrations; the urban and rural microbial community had a similar utilization of carbon substrates, as compared to the soil microbes of the suburban soils which were significantly different. The differences between the urban/rural and suburban microbial substrate utilization is most likely due to landscape practices in the suburban study area.

The urban and rural soils of this study lack inputs of foreign nutrients, as urban soils are poorly maintained and rural soils are secluded from the introduction of nutrients from humans. Suburban landscaping regimes introduce foreign nutrients to suburban soils. Nutrients that are commonly introduced to suburban soils are mulches, composts, and fertilizers (N and P). The introduction of foreign nutrients has shown to cause a shift in the substrate utilization potential of soil microbial communities (Raciti et al., 2012; Schutter and Dick, 2001). The introduction of foreign nutrients is a possible explanation on why there was significant differences between the carbon utilization potential of microbes from urban/rural and suburban soils.

5.   CONCLUSIONS

It was hypothesized that soil heavy metal concentrations would decline away from the urban area, but the results show the contrary.   Urban and rural soils had elevated concentrations of Zn when compared to suburban soils which had significantly lower concentrations. Between the different area's (urban, suburban, rural) soils, Cu and Pb soil concentrations followed a similar trend to Zn, though they were not significant. The trend seen with total Zn soil concentrations is further exhibited by the microbial carbon substrate analysis (ECOLOG).

Heavy metal concentrations in contaminated soils are not homogenous as there were numerous point-sources and environmental factors that influence heavy metals deposition and accumulation rates. Soils that are contaminated by heavy metals are problematic, as contaminated soil particles can be resuspended by natural and artificial airflows generated by foot and automobile traffic. Once the contaminated soil particles are resuspended, exposure risks increase as well as the possibility of environmental recontamination. Heavy metals persist and accumulate in environments; thus, cost-effective and environmentally friendly remediation methods need to be explored. The next appropriate step for continuation of this study would be to examine microbial diversity through next generation sequencing methods. Genetic sequencing data would be useful in deciphering the significant differences between the microbial carbon substrate utilization results between the different geographical locations. The urban soils Zn, Cu, Pb, and Fe concentrations collected from this study will be used in a future experiment to test the phytoremediation potential of *Trifolium repens* and *Panicum virgatum* with Arbuscular Mycorrhizal Fungi (AMF).

6. Tables

Site Code	Latitude	Longitude
Urban		
U1	39.95421	-75.1148
U2	39.94933	-75.1167
U3	39.94896	-75.1228
U4	39.94106	-75.1221
U5	39.94018	-75.1223
U6	39.93014	-75.1238
U7	39.93578	-75.1189
U8	39.93222	-75.124
U9	39.92488	-75.1215
U10	39.92238	-75.1227
Suburban		
S1	39.75364	-75.3122
S2	39.7456	-75.2569
S3	39.77782	-75.2162
S4	39.80001	-75.2506
S5	39.8106	-75.2098
S6	39.79191	-75.1477
S7	39.80968	-75.1433
S8	39.82707	-75.1498
S9	39.82908	-75.1757
S10	39.82423	-75.1184
Rural		
R1	39.64152	-75.4566
R2	39.60445	-75.554
R3	39.5289	-75.4875
R4	39.48613	-75.3992
R5	39.56403	-75.3621
R6	39.57072	-75.4137
R7	39.60778	-75.408
R8	39.64248	-75.3139
R9	39.68301	-75.4374
R10	39.59245	-75.2969

Table 1: Latitudinal and longitudinal coordinates of the 30 soil sampling locations of the urban, suburban, and rural areas

Site Code	pH	Soil moisture, %	SOM, %	ISA, %
Urban				
U1	5.99	9.40	3.24	75
U2	6.37	9.05	5.47	100
U3	6.40	10.83	3.96	80
U4	6.09	10.37	3.18	45
U5	6.47	8.20	4.63	45
U6	6.51	12.96	5.83	50
U7	5.29	6.52	1.58	95
U8	7.76	8.92	5.95	45
U9	5.59	10.78	8.64	90
U10	6.22	12.89	8.42	85
Mean ± S.D.	6.27(0.66)[a]	9.99(2.01)[a]	5.09(2.26)[a]	71(22.46)
Suburban				
S1	5.97	5.70	5.11	35
S2	6.18	49.26	12.76	20
S3	5.62	38.71	21.75	15
S4	5.03	17.12	5.84	25
S5	5.70	17.34	5.99	25
S6	6.46	30.78	13.40	25
S7	5.30	9.03	2.71	30
S8	5.10	13.18	4.66	40
S9	5.03	20.22	6.74	30
S10	6.97	17.83	4.65	35
Mean ± S.D.	5.77(0.66)[ab]	21.92(13.7)[ab]	8.36(5.86)[a]	28(7.53)
Rural				
R1	6.19	25.80	7.57	0
R2	4.79	12.16	4.91	0
R3	3.89	18.56	5.89	0
R4	3.80	22.60	78.00	0
R5	6.63	63.83	12.57	0
R6	4.80	23.58	7.41	0
R7	5.06	28.52	8.91	0
R8	5.26	17.41	76.31	0
R9	6.15	14.79	2.80	0
R10	5.00	14.21	4.42	0
Mean ± S.D.	5.16(0.94)[b]	24.15(14.9)[b]	20.88(29.8)[a]	0

Table 2: Properties of soil according to sampling locations, the mean (± standard deviation) is also reported of each area (urban, suburban, rural). Significance is indicated by the mean superscripts that are different letters (Tukey's range test). SOM: Soil Organic Matter, ISA: Impervious Surface Area.

Site Code	PG, %	GG, %	VCS, %	MS, %	FS, %	VFS, %	S, %
Urban							
U1	2.83	3.87	23.29	28.57	34.17	5.71	1.56
U2	16.15	7.73	27.94	23.07	20.12	3.83	1.16
U3	4.22	15.91	40.18	18.07	16.76	3.54	1.32
U4	1.14	2.97	41.97	34.19	17.66	1.78	0.29
U5	11.40	7.12	44.37	14.42	17.49	3.82	1.38
U6	1.43	2.69	21.92	31.25	36.29	5.63	0.79
U7	0.89	0.97	37.98	34.67	20.60	3.55	1.34
U8	4.28	12.86	37.20	20.91	18.73	4.48	1.55
U9	17.10	4.98	23.07	18.04	26.11	7.46	3.24
U10	13.68	3.26	34.78	24.26	19.25	3.53	1.24
Mean ± S.D.	7.31(6.3)[a]	6.24(4.80)[a]	33.27(8.48)[a]	24.74(7.14)[a]	22.72(7.11)[a]	4.33(1.57)[a]	1.39(0.76)[a]
Suburban							
S1	3.49	2.24	35.63	27.67	22.96	6.03	1.98
S2	1.81	1.00	42.33	35.77	16.61	1.79	0.68
S3	21.39	22.71	38.62	12.00	4.06	0.86	0.36
S4	4.28	26.22	44.63	10.94	8.96	3.28	1.69
S5	2.64	1.63	34.49	33.39	21.67	4.30	1.87
S6	2.88	18.28	31.06	26.50	17.69	2.66	0.95
S7	1.63	1.95	20.22	47.02	25.19	3.30	0.70
S8	0.89	10.54	35.60	23.78	23.61	4.34	1.24
S9	2.57	5.75	57.82	18.48	11.42	2.79	1.17
S10	5.91	11.93	53.36	15.26	11.53	1.83	0.19
Mean ± S.D.	4.75(6.02)[a]	10.23(9.37)[ab]	39.38(10.86)[a]	25.08(11.48)[ac]	16.37(7.13)[ab]	3.12(1.50)[a]	1.08(0.62)[a]
Rural							
R1	10.71	19.27	47.06	13.28	8.00	1.19	0.50
R2	0.13	4.77	34.14	27.20	23.21	7.38	3.17
R3	0.37	21.82	47.20	15.79	10.96	2.08	1.79
R4	19.33	22.39	35.21	11.75	8.79	1.70	0.82
R5	31.83	9.24	40.80	10.79	5.69	1.24	0.41
R6	19.06	23.51	35.23	9.74	7.71	2.50	2.25
R7	1.25	37.66	38.46	10.85	7.17	2.36	2.25
R8	29.94	19.00	34.89	9.04	5.34	1.17	0.62
R9	8.44	13.92	45.50	16.93	12.16	1.99	1.05
R10	1.69	18.15	47.40	17.89	10.36	2.00	2.51
Mean ± S.D.	12.28(12.16)[a]	18.97(8.90)[b]	40.59(5.70)[a]	14.33(5.46)[b]	9.94(5.16)[b]	2.36(1.83)[b]	1.53(0.98)[a]

Table 3: Percentage of soil particle size distribution according to sampling locations, the mean (± standard deviation) is also reported of each area (urban, suburban, rural). Significance is indicated by the mean superscripts that are different letters (Tukey's range test). PG: Pebble Gravel, GG: Granule Gravel, VCS: Very Coarse Sand, MS: Medium Sand, FS: Fine Sand, VFS: Very Fine Sand, S: Silt.

7. FIGURES
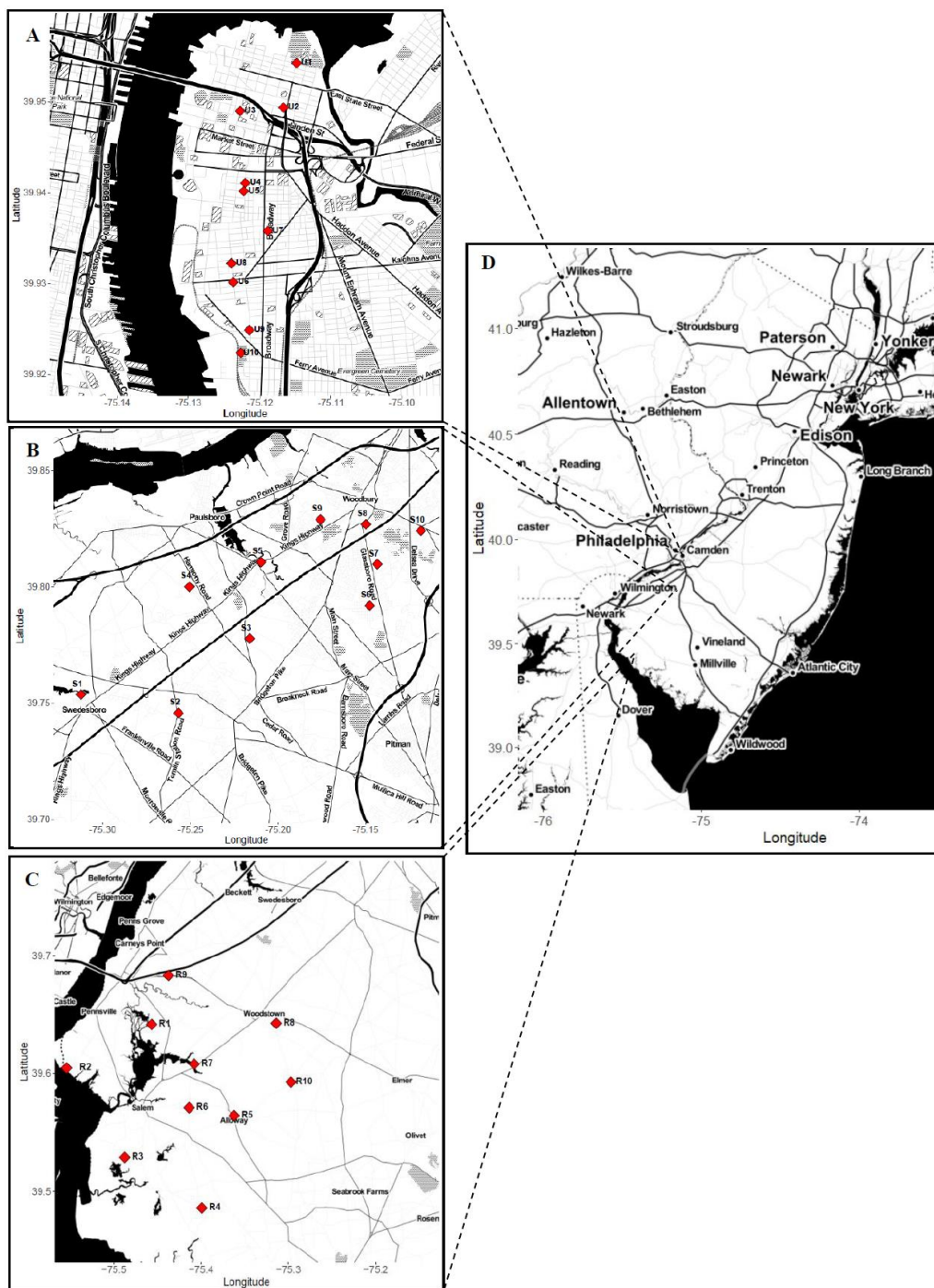
Figure 1: Location maps of the 30 soil sampling locations (solid red diamonds) along the urban-rural transect in the eastern part of New Jersey. A: Urban soil sampling locations, B: Suburban soil sampling locations, C: Rural soil sampling locations, D: New Jersey map
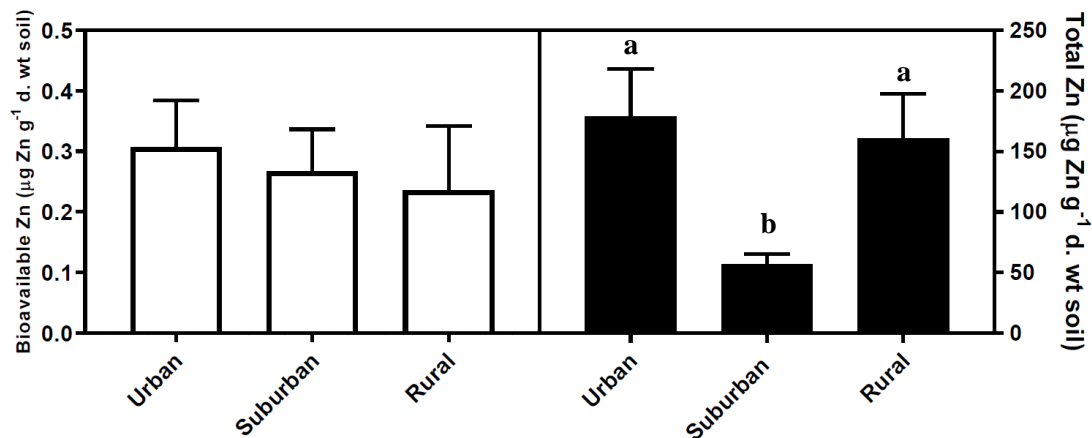
Figure 2: The white bars (left) indicate the mean (± Standard error) bioavailable Zn concentrations (µg g⁻¹) of dry weight soil from urban, suburban, rural soils. While the black bars (right) indicate the mean (± Standard error) total Zn (± Standard error) concentrations (µg g⁻¹) of dry weight soil from urban, suburban, rural soils. Significance is indicated by letters that are different (Tukey's range test).
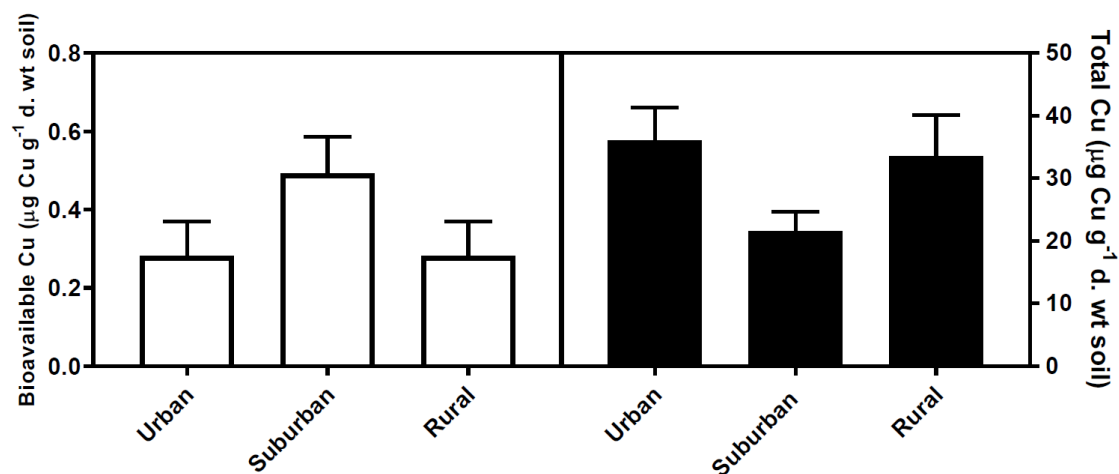
Figure 3: The white bars (left) indicate the mean (± Standard error) bioavailable Cu concentrations (µg g^{-1}) of dry weight soil from urban, suburban, rural soils. While the black bars (right) indicate the mean (± Standard error) total Cu (± Standard error) concentrations (µg g^{-1}) of dry weight soil from urban, suburban, rural soils. There were no significant differences observed (Tukey's range test).

Figure 4: The white bars (left) indicate the mean (± Standard error) bioavailable Pb concentrations ($\mu g \, g^{-1}$) of dry weight soil from urban, suburban, rural soils. While the black bars (right) indicate the mean (± Standard error) total Pb (± Standard error) concentrations ($\mu g \, g^{-1}$) of dry weight soil from urban, suburban, rural soils. There were no significant differences observed (Tukey's range test).

Figure 5: The white bars (left) indicate the mean (± Standard error) bioavailable Fe concentrations ($\mu g\ g^{-1}$) of dry weight soil from urban, suburban, rural soils. While the black bars (right) indicate the mean (± Standard error) total Fe (± Standard error) concentrations ($\mu g\ g^{-1}$) of dry weight soil from urban, suburban, rural soils. There were no significant differences observed (Tukey's range test).

Figure 6: PCA analysis of soil microbial carbon utilization using ECOLOG microtiter plates. Axis 1 accounts for 24.32% of the total variation; $F_{(4,27)} = 4.50$, $P = 0.021$. Axis 2 accounts for 16.56% of the total variation. Results of the MANOVA are as follows; $F_{(4,27)} = 1.07$, $P = 0.365$. $\lambda = 0.678$, $F = 2.79$, $P = 0.0356$. Values presented after the enzymes in parentheses are Pearson Correlations.

8. REFERENCES

Al-Khashman, O.A., and Shawabkeh, R.A. (2006). Metals distribution in soils around the cement factory in southern Jordan. Environ. Pollut. *140*, 387–394.

Ajmone-Marsan, F., Biasioli, M., Kralj, T., Grčman, H., Davidson, C.M., Hursthouse, A.S., Madrid, L., and Rodrigues, S. (2008). Metals in particle-size fractions of the soils of five European cities. Environmental Pollution *152*, 73–81.

American Public Health Association (1992). Standard methods for the examination of water and wastewater (Washington: American Public Health Ass).

Blumenthal, D.L., White, W.H., and Smith, T.B. (1978). Anatomy of a Los Angeles smog episode: Pollutant transport in the daytime sea breeze regime. Atmospheric Environment (1967) *12*, 893–907.

Chander, K., Dyckmans, J., Joergensen, R., Meyer, B., and Raubuch, M. (2001). Different sources of heavy metals and their long-term effects on soil microbial properties. Biol. Fertil. Soils *34*, 241–247.

Chicharromartin, A., Calarivero, V., and Larreamarin, M. (1998). Contamination by heavy metals in soils in the neighbourhood of a scrapyard of discarded vehicles. Sci. Total Environ. *212*, 145–152.

Christoforidis, A., and Stamatis, N. (2009). Heavy metal contamination in street dust and roadside soil along the major national road in Kavala's region, Greece. Geoderma *151*, 257–263.

D'Angelo, E., Crutchfield, J., and Vandiviere, M. (2001). Rapid, sensitive, microscale determination of phosphate in water and soil. J. Environ. Qual. *30*, 2206–2209.

Di Baccio, D., Tognetti, R., Sebastiani, L., and Vitagliano, C. (2003). Responses of Populus deltoides x Populus nigra (Populus x euramericana) clone I-214 to high zinc concentrations. New Phytologist *159*, 443–452.

Decker, E.H., Elliott, S., Smith, F.A., Blake, D.R., and Rowland, F.S. (2000). Energy and material flow through the urban ecosystem. Annu. Rev. Energy Environ. *25*, 685–740.

Dodman, D. (2009). Blaming cities for climate change? An analysis of urban greenhouse gas emissions inventories. Environ. Urban. *21*, 185–201.

Faiz, Y., Tufail, M., Javed, M.T., Chaudhry, M.M., and Naila-Siddique (2009). Road dust pollution of Cd, Cu, Ni, Pb and Zn along Islamabad Expressway, Pakistan. Microchem. J. *92*, 186–192.

Garland, J.L. (1996a). Patterns of potential C source utilization by rhizosphere communities. Soil Biol. Biochem. *28*, 223–230.

Garland, J.L. (1996b). Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biol. Biochem. *28*, 213–221.

Garland, J.L., and Mills, A.L. (1991). Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. *57*, 2351–2359.

Giller, K.E., Witter, E., and McGrath, S.P. (2009). Heavy metals and soil microbes. Soil Biology and Biochemistry *41*, 2031–2037.

Gromaire, M.., Garnaud, S., Saad, M., and Chebbo, G. (2001). Contribution of different sources to the pollution of wet weather flows in combined sewers. Water Res. *35*, 521–533.

van der Heijden, M.G.A., Bardgett, R.D., and van Straalen, N.M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters *11*, 296–310.

Heinonen, J., and Junnila, S. (2011). Implications of urban structure on carbon consumption in metropolitan areas. Environ. Res. Lett. *6*, 014018.

Hood-Nowotny, R., Umana, N.H.-N., Inselbacher, E., Oswald- Lachouani, P., and Wanek, W. (2010). Alternative Methods for Measuring Inorganic, Organic, and Total Dissolved Nitrogen in Soil. Soil Sci. Soc. Am. J. *74*, 1018.

Hu, X., He, M., and Li, S. (2015). Antimony leaching release from brake pads: Effect of pH, temperature and organic acids. J. Environ. Sci. *29*, 11–17.

Jeannotte, R., Sommerville, D., Hamel, C., and Whalen, J. (2004). A microplate assay to measure soil microbial biomass phosphorus. Biol. Fertil. Soils *40*.

Jim, C.. (1998a). Urban soil characteristics and limitations for landscape planting in Hong Kong. Landsc. Urban Plan. *40*, 235–249.

Jim, C.Y. (1998b). Soil Characteristics and Management in an Urban Park in Hong Kong. Environ. Manage. *22*, 683–695.

Johansson, C., Norman, M., and Burman, L. (2009). Road traffic emission factors for heavy metals. Atmos. Environ. *43*, 4681–4688.

Kondo, M.C., Sharma, R., Plante, A.F., Yang, Y., and Burstyn, I. (2016). Elemental Concentrations in Urban Green Stormwater Infrastructure Soils. J. Environ. Qual. *45*, 107.

Krogmann, U. (1999). Effects of season and population density on source-separated waste composts. Waste Manag. Res. *17*, 109–123.

Lazzaro, A., Widmer, F., Sperisen, C., and Frey, B. (2008). Identification of dominant bacterial phylotypes in a cadmium-treated forest soil: Cadmium-tolerant Burkholderia sp. in soil. FEMS Microbiology Ecology *63*, 143–155.

Li, H., Qian, X., and Wang, Q. (2013). Heavy Metals in Atmospheric Particulate Matter: A Comprehensive Understanding Is Needed for Monitoring and Risk Mitigation. Environ. Sci. Technol. *47*, 13210–13211.

Lu, S., Wang, H., and Bai, S. (2009). Heavy metal contents and magnetic susceptibility of soils along an urban–rural gradient in rapidly growing city of eastern China. Environ. Monit. Assess. *155*, 91–101.

Manta, D.S., Angelone, M., Bellanca, A., Neri, R., and Sprovieri, M. (2002). Heavy metals in urban soils: a case study from the city of Palermo (Sicily), Italy. Sci. Total Environ. *300*, 229–243.

Mapanda, F., Mangwayana, E.N., Nyamangara, J., and Giller, K.E. (2005). The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. Agric. Ecosyst. Environ. *107*, 151–165.

McDonald, K.W. (2008). Soil response model verification: A multi-year study of foot traffic impact. Environ. Impact Assess. Rev. *28*, 321–327.

McGrath, S.P., and Cunliffe, C.H. (1985). A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Cd, Pb, Cr, Co and Mn from soils and sewage sludges. J. Sci. Food Agric. *36*, 794–798.

Mielke, H.W., Gonzales, C.R., Smith, M.K., and Mielke, P.W. (1999). The Urban Environment and Children's Health: Soils as an Integrator of Lead, Zinc, and Cadmium in New Orleans, Louisiana, U.S.A. Environ. Res. *81*, 117–129.

Mleczek, M., Rutkowski, P., Rissmann, I., Kaczmarek, Z., Golinski, P., Szentner, K., Strażyńska, K., and Stachowiak, A. (2010). Biomass productivity and phytoremediation potential of Salix alba and Salix viminalis. Biomass and Bioenergy *34*, 1410–1418.

Nicholson, F.A., Smith, S.R., Alloway, B.J., Carlton-Smith, C., and Chambers, B.J. (2003). An inventory of heavy metals inputs to agricultural soils in England and Wales. Sci. Total Environ. *311*, 205–219.

Nies, D.H. (1999). Microbial heavy-metal resistance. Applied Microbiology and Biotechnology *51*, 730–750.

Norman, J., MacLean, H.L., and Kennedy, C.A. (2006). Comparing High and Low Residential Density: Life-Cycle Analysis of Energy Use and Greenhouse Gas Emissions. J. Urban Plan. Dev. *132*, 10–21.

Nwuche, C.O., and Ugoji, E.O. (2008). Effects of heavy metal pollution on the soil microbial activity. International Journal of Environmental Science & Technology *5*, 409–414.

Ouyang, W., Guo, B., Cai, G., Li, Q., Han, S., Liu, B., and Liu, X. (2015). The washing effect of precipitation on particulate matter and the pollution dynamics of rainwater in downtown Beijing. Sci. Total Environ. *505*, 306–314.

Paterson, E., Sanka, M., and Clark, L. (1996). Urban soils as pollutant sinks — a case study from Aberdeen, Scotland. Appl. Geochem. *11*, 129–131.

Raciti, S.M., Hutyra, L.R., and Finzi, A.C. (2012). Depleted soil carbon and nitrogen pools beneath impervious surfaces. Environmental Pollution *164*, 248–251.

Salehi, M.H., Beni, O.H., Harchegani, H.B., Borujeni, I.E., and Motaghian, H.R. (2011). Refining soil organic matter determination by loss-on-ignition. Pedosphere *21*, 473–482.

Salido, A.L., Hasty, K.L., Lim, J.-M., and Butcher, D.J. (2003). Phytoremediation of Arsenic and Lead in Contaminated Soil Using Chinese Brake Ferns (Pteris vittata) and Indian Mustard (Brassica juncea). International Journal of Phytoremediation *5*, 89–103.

Sandaa, R.-A., Torsvik, V., and Enger, Ø. (2001). Influence of long-term heavy-metal contamination on microbial communities in soil. Soil Biology and Biochemistry *33*, 287–295.

Sansalone, J.J., and Buchberger, S.G. (1997). Partitioning and First Flush of Metals in Urban Roadway Storm Water. J. Environ. Eng. *123*, 134–143.

Scalenghe, R., and Marsan, F.A. (2009). The anthropogenic sealing of soils in urban areas. Landscape and Urban Planning *90*, 1–10.

Schuhmacher, M., Meneses, M., Granero, S., Llobet, J.M., and Domingo, J.L. (1997). Trace element pollution of soils collected near a municipal solid waste incinerator: human health risk. Bull. Environ. Contam. Toxicol. *59*, 861–867.

Schutter, M., and Dick, R. (2001). Shifts in substrate utilization potential and structure of soil microbial communities in response to carbon substrates. Soil Biology and Biochemistry *33*, 1481–1491.

Séguin, V., Gagnon, C., and Courchesne, F. (2004). Changes in water extractable metals, pH and organic carbon concentrations at the soil-root interface of forested soils. Plant Soil *260*, 1–17.

Steinberg, D.A., Pouyat, R.V., Parmelee, R.W., and Groffman, P.M. (1997). Earthworm abundance and nitrogen mineralization rates along an urban-rural land use gradient. Soil Biol. Biochem. *29*, 427–430.

Tang, X., Shen, C., Shi, D., Cheema, S.A., Khan, M.I., Zhang, C., and Chen, Y. (2010). Heavy metal and persistent organic compound contamination in soil from Wenling: An emerging e-waste recycling city in Taizhou area, China. J. Hazard. Mater. *173*, 653–660.

Tedrow, J.C.F. (1986). Soils of New Jersey (Malabar, Fla: R.E. Krieger Pub. Co).

Turgut, C., Katie Pepe, M., and Cutright, T.J. (2004). The effect of EDTA and citric acid on phytoremediation of Cd, Cr, and Ni from soil using Helianthus annuus. Environmental Pollution *131*, 147–154.

Vakhlamova, T., Rusterholz, H.-P., Kamkin, V., and Baur, B. (2016). Recreational use of urban and suburban forests affects plant diversity in a Western Siberian city. Urban For. Urban Green. *17*, 92–103.

Wagrowski, D.M., and Hites, R.A. (1997). Polycyclic Aromatic Hydrocarbon Accumulation in Urban, Suburban, and Rural Vegetation. Environ. Sci. Technol. *31*, 279–282.

Wang, T., Lam, K.S., Lee, A.S.Y., Pang, S.W., and Tsui, W.S. (1998). Meteorological and Chemical Characteristics of the Photochemical Ozone Episodes Observed at Cape D'Aguilar in Hong Kong. Journal of Applied Meteorology *37*, 1167–1178.

Weber, J., and Karczewska, A. (2004). Biogeochemical processes and the role of heavy metals in the soil environment. Geoderma *122*, 105–107.

Węgrzyn, M., Wietrzyk, P., Lisowska, M., Klimek, B., and Nicia, P. (2016). What influences heavy metals accumulation in arctic lichen Cetrariella delisei in Svalbard? Polar Science *10*, 532–540.

Weng, Q., Lu, D., and Schubring, J. (2004). Estimation of land surface temperature–vegetation abundance relationship for urban heat island studies. Remote Sens. Environ. *89*, 467–483.

Yu, C.H., Fan, Z. (Tina), McCandlish, E., Stern, A.H., and Lioy, P.J. (2011). Characterization of Spatial Impact of Particles Emitted from a Cement Material Production Facility on Outdoor Particle Deposition in the Surrounding Community. J. Air Waste Manag. Assoc. *61*, 1015–1025.

Zhang, X., Zhong, T., Liu, L., and Ouyang, X. (2015). Impact of Soil Heavy Metal Pollution on Food Safety in China. PLOS ONE *10*, e0135182.

Zhao, Y.-G., Zhang, G.-L., Zepp, H., and Yang, J.-L. (2007). Establishing a spatial grouping base for surface soil properties along urban–rural gradient—A case study in Nanjing, China. CATENA *69*, 74–81.

Zheng, N., Liu, J., Wang, Q., and Liang, Z. (2010). Heavy metals exposure of children from stairway and sidewalk dust in the smelting district, northeast of China. Atmos. Environ. *44*, 3239–3245.

CHAPTER II

THE EFFECTS OF ARBUSCULAR MYCORRHIZAE FUNGI ON THE HEAVY METAL

UPTAKE ABILITY OF *TRIFOLIUM REPENS* AND *PANICUM VIRGATUM*

1. INTRODUCTION

As the foundation for terrestrial ecosystems, soils sustain food security and economic viability. They also serve as a medium that humans interact with daily, either directly or indirectly, through food material (Blum, 2005; Herrick, 2000). Soil contamination by heavy metals today is frequent because of the sheer variety and abundance of point sources, such as automobiles, industries, factories, agriculture, mining, and illegal dumping operations (Hu et al., 2013; Micó et al., 2006; Ozaki et al., 2004; Wei and Yang, 2010; Zhou et al., 2007). Coupled with modern urban architecture, such as impervious surfaces, and the increased amount of heavy metal point sources within environments, facilitate the transference of heavy metals to surrounding soils (Li et al., 2001).

The history of heavy metal pollution dates back to the early use of fire by humans where the combustion of wood altered trace heavy metal concentrations of cave environments (Nriagu, 1996). Today, heavy metals are being released into environments at a rate unprecedented in history because of rapid industrialization/urbanization, technological advancement, and an exponential increase in population (McConnell and Edwards, 2008).

Six metals Lead (Pb), Zinc (Zn), Cadmium (Cd), Chromium (Cr), Copper (Cu), and Nickle (Ni) are commonly classified as heavy metals and occur at low concentrations in the Earth's crust (Callender, 2003; Duffus, 2002). The term "heavy metal" refers to a high-density metallic element that is toxic to organisms at low concentrations. Heavy metals persist in environments, unlike radionucleotides and organics that decay with time (Järup, 2003).

Soil contamination by heavy metals is a serious environmental concern. Toxic because of their subsequent bioaccumulation and biomagnification, heavy metals pose a serious ecological threat globally (Lindqvist, 1995; Nriagu, 1990). Physical and chemical remediation are two

conventional methods to remediating soil of heavy metal pollution (Khalid et al., 2017; Yao et al., 2012). Physical remediation exploits discrepancies with heavy metals and soil through soil filtering, vitrification, and electrokinetic remediation. Formally known as soil washing, chemical remediation uses highly reactive chemicals, such as acids and surfactants. Other methods include immobilization and encapsulation.

Depending on the severity of contamination, soil is treated ex situ or in situ. Unfortunately, both require removing above ground vegetation to access polluted soil. Conventional remediation methods are expensive and require a large amount of energy to remove heavy metals from soil. Though conventional methods are effective, they have serious adverse effects on the physical and chemical composition of soils. In turn, this affects the habitat of soil micro/macrofauna and the corresponding aboveground ecosystems.

The use of plants to remove pollutants from soil is formally known as phytoremediation. This process can be done in situ. It is inexpensive, environmentally friendly, prevents re-exposure via revegetation, and limits disturbances to the surrounding ecosystem (Wan et al., 2016). Plant species that are candidates for removing inorganic pollutants from soils have two key characteristics. One, they have the ability to produce a large amount of biomass. Two, they have a high metal accumulating capacity (McGrath and Zhao, 2003). The efficacy of phytoremediation depends on the initial establishment of vegetation and metal tolerance of phytoremediator species (Remon et al., 2005; Smith and Bradshaw, 1979).

There are two main strategies that are involved in the phytoremediation of heavy metal from contaminated soils, they are phytoextraction and phytostabilization (Sarwar et al., 2017). Phytoextraction accumulates metals in the above ground tissues of plants which can be harvested thus removing heavy metals from a environment. Alternatively, phytostabilization immobilizes metals in the soil, thus preventing the potential for re-exposure events.

In this work, two different plant species were studied, *Trifolium repens* (white clover) and *Panicum virgatum* (switch grass). *Trifolium repens* is herbaceous perennial plant that belongs to the family Fabaceae. *Trifolium repens* bears nodules that house N-fixing bacterium called *Rhizobium trifolii* (Burdon, 1983). The transference of micronutrients especially Nitrogen is the central focus in the literature regarding this symbiotic relationship. There are only a few studies on *T.repens* metal uptake ability (Bidar et al., 2007, 2009; Mcgrath et al., 1988).

Panicum virgatum is a high-yielding biomass perennial grass that has been extensively studied. Most of the literature focuses on biomass production for feedstock and biofuel. Few studies focus on metal uptake (Di Virgilio et al., 2007; McLaughlin and Adams Kszos, 2005; Porter, 1966; Shen et al., 2012, 2013). *Panicum virgatum* produces a complex, deep penetrating root system that may be beneficial for metal acquisition because it's larger root architecture, biomass, and length enhance the acquisition of minerals (Lambers et al., 2006).

In addition to gaps in literature regarding the metal uptake ability of *T. repens* and *P. virgatum*, there is even less literature on the contributions of symbiotic rhizosphere organisms to heavy metal remediation. Arbuscular Mycorrhizae Fungi (AMF) are rhizosphere plant symbionts that are known to increase the transference of water and minerals to host plant species (Al-Karaki, 1998, 2000). Colonization by AMF has also shown to alleviate heavy metal induced stress to their host plants (Galli et al., 1994; Khan, 2005).

The underlying mechanisms of the enhancement of plants to tolerate heavy metal contamination is thought to be the binding capacity of fungal hyphae to metals in the roots or in the rhizosphere (Hetrick et al., 1994; Joner et al., 2000). Studies involving AMF have focused on heavy metal stress tolerance and nutrient uptake (Hildebrandt et al., 2007; Marschner and Dell, 1994). They have ignored the heavy metal uptake ability to host plants. AMF are major contributors to ecosystems and may provide a significant impact on remediation efforts (Jeffries et al., 2003; Leyval et al., 2002).

In this study, heavy metal concentrations were experimentally manipulated based on the findings collected from an urban to rural gradient field study conducted in south western New Jersey in 2017 (Chapter I). Soils collected in this urban to rural gradient study were analyzed for their concentration of zinc, copper, lead, and iron. The metal concentrations of the urban location soil samples were averaged and used for this study to simulate real-world metal concentrations.

The main objective of this study was to determine the effects of AMF on the heavy metal uptake ability of *T. repens* and *P. virgatum*. This was achieved by (i) determining metal (Zn, Cu, Pb, Fe) concentrations in the shoots and roots of *T. repens* and *P. virgatum;* and (ii) then statistically analyzing the concentrations to determine if the mycorrhizal inoculated *T. repens* and *P. virgatum* have a significant difference in the metal remediation potential versus non-inoculated plants.

The results of the study helped address the following questions. Are *T. repens* and *P. virgatum* phytoextractors, phytostabilizers, or neither? Does AMF increase or decrease heavy metal accumulation of *T. repens* and *P. virgatum*? Does AMF increase metal concentrations in the shoots, roots, both, or neither of *T. repens* and *P. virgatum*? Does Zn, Cu, Pb, and Fe accumulate at the same rate and in similar plant tissues (shoots and roots) or does each metal vary in the accumulation in *T. repens* and *P. virgatum* tissues? Do increasing soil concentrations of zinc, copper, lead, and iron correspond to higher concentrations in *T. repens* and *P. virgatum?*

2. MATERIALS AND METHODS

This study was conducted in the greenhouse at the Rutgers University Pinelands Field Station in New Lisbon, New Jersey. During the duration of the experiment, the greenhouse's temperature ranged from 15°C to 48°C, and the average temperature was 30°C (S.E. ± 2.13). The relative humidity of the green house ranged from 56 to 76 percent, the average relative humidity was 68 (S.E. ± 0.70) percent.

2.2 Soil preparation

An unsterilized soil/sand substrate (4:1) was created by mixing topsoil and sand. The topsoil was obtained from Roork's Farm Supply, Inc located in Elmer, NJ. The sand was collected at a depth of 0-25 cm from the Pinelands Preserve. Copper, zinc, lead, and iron were added to this soil substrate. The previous study's average urban soil locations heavy metal (Zn, Cu, Pb, and Fe) concentrations were 200 $\mu g\ g^1$, 40 $\mu g\ g^1$, 200 $\mu g\ g^1$, and 15,000 $\mu g\ g^1$ respectively. These average metal concentrations were increased two and three-fold to achieve a total of three different metal treatment levels. Sulphate forms of metals were used to prevent excess nitrogen from altering the growth of the plants and changing the results. Copper (II) sulfate pentahydrate ($CuSO_4,5H_2O$), Zinc sulphate heptahydrate ($ZnSO_4,7H_2O$), Lead (II) sulfate ($PbSO_4$), and Iron (II) sulfate heptahydrate ($FeSO_4, 7H_2O$) aqueous solutions (1000 μg ml^{-1}) were created with deionized water and diluted to the appropriate concentrations with deionized water and then added to the soil to simulate metal contamination.

2.3 Planting of T. repens and P. virgatum

Two different kinds of growing tubes were used to accommodate the small biomass plant species *T. repens* and larger biomass specie *P. virgatum.* *T. repens* plants were grown in plastic tubes of 3.81 cm and 20.95 cm of height. *P. virgatum* plants were grown in polyvinyl chloride tubes of 5.08 cm of diameter and 60.96 cm of height. *P. virgatum* and *T. repens* growth tubes

have a capacity 130 g and 600 g of soil respectively. The water retention capacity for each tube was determined to be 20 ml for *T. repens* and 100 ml for *P. virgatum*. *T. repens* plants were grown from seed and reduced to 10 plants per tube. *P. virgatum* seeds were allowed to germinate in trays in advance. One plant was then transplanted to each growth tube. Before seeding and transplantation of growth tubes, mycorrhizal inoculum was added to the surrounding soil of corresponding mycorrhizal treated replicates. Plants were grown for four months. They were watered once every two days. Excess watering was avoided to prevent leaching of applied metals for the duration of the experiment.

2.4 Plant and soil sampling

Immediately after the growing period, shoots were cut at the soil surface and washed with tap water to remove large soil particles and dust. They were then rinsed with deionized water three times. Next, the soil cores within the tubes were carefully extracted onto waxed paper and the roots were removed. The roots were removed from the soil cores by hand, and then gently washed with tap water to remove soil particles. They were subsequently washed with deionized water three times.

The roots were dried by blotting them with paper to remove excess water and then weighed. Samples from the cleaned, dry, and weighed roots were randomly chosen and removed for determination of AMF root colonization. Plant shoots and roots for analysis of heavy metals were placed into paper bags and dried at 60°C for 16 hours until completely dry. Soil cores removed from the growing tubes were transferred to paper bags and allowed to air dry for approximately three weeks until constant weight was achieved.

2.5 Plant and soil heavy metal analysis

Plant and soil heavy metal concentrations were determined by flame atomic absorption spectroscopy (FAAS, Buck Scientific, 211 Accusys). After plant shoots and roots were cleaned,

dried, and weighed, they were ground to homogenize large samples. The plant shoots and roots that were above the recommend sample weight for digestion (0.5 g) were ground. Those that were below the recommended mass were not ground to prevent loss of sample mass. Both plant and root samples were digested using a solution of trace metal grade HCl: HNO_3 4:1 (v/v) overnight at room temperature in clean, dry 100 ml Foss digestion tubes. The samples were then placed into a Tecator digestion system 40 1016 digestion block and heated to 140°C for one hour or until samples were dry. Once the dried samples were cool, 10 ml of 20% HCl (by vol.) was added to each tube. The remaining digest solutions were filtered through a Whatman #2 filter by vacuum filtration and placed into labeled clean plastic containers for metal analysis. Soil bioavailable water extractable metal concentrations were determined by creating a soil suspension in deionized water. The soil solution was shaken on a reciprocal shaker for 30 minutes and then filtered through a Whatman #2 filter by vacuum filtration. It was then placed into labeled, clean plastic containers for metal analysis. Standards of 1000 ppm were created by using the sulfate forms of the metals added to the soil.

2.6 Determination of AMF root colonization

To visualize AMF colonization, roots were first cleared in 10% KOH for one week in 20 ml plastic scintillation vials. After the initial clearing with KOH, roots were then washed with tap water to remove KOH and particulates. Roots were then acidified with a 1% HCl solution for 24 hours. After the 24-hour acidification period, the HCl solution was removed and the remaining roots were stained with a 0.05% Trypan blue solution in lactoglycerol (v/v). Approximately five 1 cm pieces of the stained roots were mounted on glass slides for viewing with an optical microscope. One hundred and fifty-six roots samples from non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* were prepared and viewed with an optical microscope to identify mycorrhizal colonization.

2.7 Statistical analyses

The data were statistically analyzed with analysis of variance using both two-way and three-way ANOVAs. The program used to perform data analysis was Prism 8. To compare heavy metal uptake of non-mycorrhizal and mycorrhizal plants grown in varying heavy metal soil concentrations Šidák *post-hoc* test was performed. Two different indices were used to assess plants for phytoextraction purposes. Bioconcentration factor (BCF) was calculated with the following equation:

$$\text{BCF} = C_{\text{harvested tissue}} / C_{\text{soil}}$$

where $C_{\text{harvested tissue}}$ is a metal (Zn, Cu, Pb, or Fe) concentration in either the shoot or roots of *T. repens* and *P. virgatum* and C_{soil} is the metal concentration in the soil. The phytoextraction rate (PR) was also calculated for *T. repens* and *P. virgatum* with the following equation:

$$\text{PR} = (C_{\text{harvested tissue}} \times M_{\text{plant}} / C_{\text{soil}} \times M_{\text{rooted zone}}) \times 100$$

where $C_{\text{harvested tissue}}$ is a metal (Zn, Cu, Pb, or Fe) concentration in the shoots of *T. repens* or *P. virgatum,* M_{plant} is the mass of the shoots at the time of harvest, C_{soil} is the concentration of a metal in the soil, and $M_{\text{rooted zone}}$ is the mass of soil rooted by either *T. repens* or *P. virgatum*.

A mass-balance analysis was performed to calculate the percentage of each metal within the shoots, roots, and soils (water extractable and soil bound) of non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum.* The mass-balance for each metal for *T. repens* and *P. virgatum* with the following equation:

$$\% = (C_{\text{harvested tissue/soil}} / CI_{\text{soil}}) \times 100$$

where $C_{\text{harvested tissue/soil}}$ is a metal (Zn, Cu, Pb, or Fe) concentration in the shoots, roots, or soil of non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum*, CI_{soil} is the initial experimental metal treatment concentration.

Mass-balance analyses were performed to determine the final distribution of Zn, Cu, Pb, and Fe in non-mycorrhizal and mycorrhizal *T. repens, P. virgatum,* and corresponding soils. The initial metal soil concentrations were used to calculate the final percentages of metals in the shoots, roots, and soils (water extractable and soil bound) of non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* after the four-month growing period (Table 6). Pie charts were created by averaging mass balance percentages across all soil treatment concentrations to better visualize the large amount of data (Figures 9 – 12).

3. RESULTS

3.1. Zn, Cu, Pb, and Fe concentrations of T. repens and P. virgatum

Figures 1 - 4 summarize the mean concentrations of Zn, Cu, Pb, and Fe in the shoots and roots of non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* which were grown in increasing soil metal concentrations ($\mu g\ g^{-1}$). All plant metal tissue concentrations are expressed as $\mu g\ g^{-1}$ dry plant tissue weight. The corresponding three-way ANOVA summary for the experimental variables is presented in Table 1.

3.1.1. Zn concentrations of T. repens and P. virgatum tissues

Comparing Zn (Fig. 1) concentrations of non-mycorrhizal *T. repens* and *P. virgatum* shoot tissues, non-mycorrhizal *T. repens* shoots overall had higher concentrations than non-mycorrhizal *P. virgatum* shoots, 25.96 $\mu g\ g^{-1}$ as compared to 4.39 $\mu g\ g^{-1}$ (P < 0.005). Non-mycorrhizal *P. virgatum* root tissues had higher concentrations of Zn than non-mycorrhizal *T. repens* roots at the soil concentration of 600 $\mu g\ g^{-1}$ of Zn, 151.5 $\mu g\ g^{-1}$ compared to 4.3 $\mu g\ g^{-1}$ *(P = 0.006).*

There was no significant difference when comparing Zn concentrations of both mycorrhizal *T. repens* and *P. virgatum* shoots. However, when comparing between mycorrhizal *T. repens* and *P. virgatum* roots, there was one significant difference in terms of Zn concentrations. At a soil concentration of 200 $\mu g\ g^{-1}$ of Zn, mycorrhizal *P. virgatum* roots had higher concentrations than mycorrhizal *T. repens* roots, 413.1 $\mu g\ g^{-1}$ as compared to 6.9 $\mu g\ g^{-1}$ (P = 0.022).

Examining intraspecies interactions, non-mycorrhizal *T. repens* shoots had higher concentrations when compared to mycorrhizal *T. repens* shoots at two different Zn soil concentrations (P < 0.017). At the soil concentration of 200 $\mu g\ g^{-1}$ of Zn, non-mycorrhizal *T. repens* shoots had a mean concentration of 0.5 $\mu g\ g^{-1}$ as compared to the mycorrhizal *T. repens*

shoots mean of 10.2 µg g^{-1}. Mycorrhizal *T. repens* roots at the highest Zn soil concentration (600

µg g^{-1}) accumulated more than their non-mycorrhizal counterparts, 259.1 µg g^{-1} compared to 4.3

µg g^{-1}. Non-mycorrhizal *P. virgatum* shoots exhibited no differences between mycorrhizal *P.*

virgatum shoots. Mycorrhizal *P. virgatum* roots had higher concentrations of Zn than non-

mycorrhizal *P. virgatum* roots at the soil concentration of 200 µg g^{-1} (P = 0.022).

3.1.2. Cu concentrations of T. repens and P. virgatum tissues

No significant differences were observed with Cu (Fig. 2) between non-mycorrhizal *T.*

repens and non-mycorrhizal *P. virgatum* shoot and root tissues. Even though mycorrhizal *T.*

repens shoots had higher concentrations of Cu than mycorrhizal *P. virgatum* shoots at the highest

Cu soil concentration (120 µg g^{-1}) (P < 0.0001).

There was a significant difference when examining intraspecies interactions with Cu of

non-mycorrhizal and mycorrhizal *T. repens* shoots at the highest Cu soil concentration (120 µg g^{-1}) (p < 0.0001).   Mycorrhizal *T. repens* shoots had elevated Cu concentrations compared to non-

mycorrhizal *T. repens* shoots, 34.8 µg g^{-1} as compared to 0 µg g^{-1}. There were no significant

differences that were observed with *P. virgatum* shoots or roots regardless of mycorrhizal

treatment.

3.1.3. Pb *concentrations of T. repens and P. virgatum tissues*

Non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* shoot and root Pb

concentrations are displayed in Figure 3. At soil concentrations of 200 µg g^{-1} and 400 µg g^{-1} of

Pb, non-mycorrhizal *P. virgatum* shoots had higher concentrations of Pb as compared to non-

mycorrhizal *T. repens* shoots, which were significantly lower, 81.39 µg g^{-1} and 80.59 µg g^{-1} as

compared to 27.67 µg g^{-1} and 48.43 µg g^{-1} respectively (P < 0.004). No significant differences

were observed between non-mycorrhizal *T. repens* and *P. virgatum* roots and between

mycorrhizal *T. repens* and *P. virgatum* shoots regarding Pb concentrations. However, a Pb soil

concentration of 600 µg g^{-1} mycorrhizal *T. repens* roots accumulated more than mycorrhizal *P. virgatum* roots, 382.7 µg g^{-1} as compared to 126 µg g^{-1}.

3.1.4. Fe concentrations of T. repens and P. virgatum tissues

For Fe (Fig. 4), no significant differences were observed between the non-mycorrhizal shoots of *T. repens* and *P. virgatum.* A very different trend exists when comparing non-mycorrhizal roots of *T. repens* and *P. virgatum.* At all Fe soil concentrations, non-mycorrhizal *P. virgatum* roots had higher concentrations than non-mycorrhizal *T. repens* roots (P < 0.0032). Mycorrhizal *T. repens* shoot concentrations were higher than the concentrations of mycorrhizal *P. virgatum* shoots. The concentrations were toward the higher end (>30,000 µg g^{-1}) of the soil Fe treatments (P <0.01). There were no significant differences when comparing mycorrhizal *T. repens* and *P. virgatum* root concentrations. Fe intraspecies interactions were not observed with *T. repens* and *P. virgatum*.

3.2. Total biomass accumulation of Zn, Cu, Pb, and Fe of T. repens and P. virgatum tissues

Figures 5 – 8 summarize the mean total biomass accumulation of Zn, Cu, Pb, and Fe of non-mycorrhizal and mycorrhizal inoculated *T. repens, P. virgatum,* shoots, and roots for the four-month growing period grown expressed in µg per pot. Increasing soil metal treatments are expressed in µg g^{-1}. The three-way ANOVA summary for the total biomass accumulation of metals by *T. repens* and *P. virgatum* is summarized in Table 2.

3.2.1. Total biomass accumulation of Zn of T. repens and P. virgatum

The total biomass accumulation of Zn in the non-mycorrhizal and mycorrhizal shoots and roots of *T. repens* and *P. virgatum* is summarized in Figure 5. Non-mycorrhizal *T. repens* shoots had higher concentrations of Zn at the soil concentration of 400 µg g^{-1}, 8.99 µg compare to 0 µg (P = 0.0461). When comparing the total biomass accumulation of Zn by non-mycorrhizal roots, *P. virgatum* had higher concentrations than non-mycorrhizal *T. repens* roots at every Zn soil

treatment ($P < 0.039$). No significant differences were observed when comparing the total biomass accumulation of Zn between mycorrhizal *T. repens* and *P. virgatum* shoots. At the Zn soil concentrations of 200 and 400 µg g^{-1}, mycorrhizal *P. virgatum* roots accumulated more Zn than mycorrhizal *T. repens* roots, 42.40 µg and 61.77 µg as compared to 0.41 µg and 0 µg for mycorrhizal *T. repens* roots ($P < 0.033$).

The Zn concentration of non-mycorrhizal *T. repens* shoots was greater than that of mycorrhizal *T. repens* shoots at a soil concentration of 400 µg g^{-1} ($P = 0.014$). Mycorrhizal *T. repens* roots at the highest Zn soil concentration (600 µg g^{-1}) accumulated more Zn than non-mycorrhizal *T. repens* roots ($P < 0.0001$). There were no significant differences between non-mycorrhizal and mycorrhizal *P. virgatum* shoots. At the Zn soil concentration of 400 µg g^{-1}, mycorrhizal *P. virgatum* roots accumulated more Zn than non-mycorrhizal *P. virgatum* roots ($p = 0.0082$).

3.2.2. Total biomass accumulation of Cu of T. repens and P. virgatum

Figure 6 summarizes the mean total biomass accumulation of Cu in non-mycorrhizal and mycorrhizal shoots and roots of *T. repens* and *P. virgatum*. There were not significant differences between non-mycorrhizal *T. repens* and *P. virgatum* shoots with regards to the total biomass accumulation of Cu. Non-mycorrhizal *P. virgatum* roots had higher concentrations of Cu than non-mycorrhizal *T. repens* roots at the soil concentrations of 80 µg g^{-1} and 120 µg g^{-1}, 8.99 µg and 8.38 µg as compared to 0.41 µg and 0.06 µg ($P < 0.0187$).

There were no significant differences between mycorrhizal *T. repens* and *P. virgatum* shoot Cu concentrations. Only one significant difference existed between *T. repens* and *P. virgatum* mycorrhizal root concentrations. At a soil concentration of 80 µg g^{-1}of Cu, mycorrhizal *P. virgatum* roots had greater concentrations than mycorrhizal *T. repens* roots, 45.43 µg as compared to 4.41 µg ($P = 0.0205$).

At the highest Cu soil concentration (120 µg g^{-1}), mycorrhizal *T. repens* shoots had higher concentrations than their non-mycorrhizal counterparts, 3.79 µg as compared to 0 µg (P = 0.0046). There were no significant differences between non-mycorrhizal and mycorrhizal *T. repens* roots or *P. virgatum* shoots. At the Cu soil concentration of 80 µg g^{-1}, mycorrhizal *P. virgatum* roots had approximately five times greater accumulation of Cu (P = 0.0448).

3.2.3. Total biomass accumulation of Pb of T. repens and P. virgatum

The total biomass accumulation of Pb of non-mycorrhizal and mycorrhizal shoots and roots of *T. repens* and *P. virgatum* is summarized in Figure 7. Non-mycorrhizal *P. virgatum* shoots accumulated higher concentrations of Pb than non-mycorrhizal *T. repens* shoots at the highest Pb soil concentration, 284.9 µg as compared to 8.35 µg (P =0.0206). Non-mycorrhizal *P. virgatum* roots accumulated more than non-mycorrhizal *T. repens* roots at the soil Pb concentration of 400 µg g^{-1}, 44.8 µg as compared to 0.17 µg (P = 0.0292). At the highest Pb soil concentration (600 µg g^{-1}), mycorrhizal *P. virgatum* shoots and roots had higher Pb concentrations when compared to the mycorrhizal *T. repens* shoots and roots (P < 0.005).

Non-mycorrhizal *T. repens* shoots at all Pb soil concentrations had higher Pb concentrations when compared to mycorrhizal *T. repens* shoots (P < 0.0311). Also, at the Pb soil concentration of 600 µg g^{-1}, mycorrhizal *T. repens* roots had higher Pb concentrations than non-mycorrhizal *T. repens* roots (P = 0.0482). Non-mycorrhizal *P. virgatum* shoots at the Pb soil concentration of 600 µg g^{-1} had higher concentrations than mycorrhizal *P. virgatum* shoots (P = 0.0482). Mycorrhizal *P. virgatum* roots at the Pb soil concentration of 600 µg g^{-1} were higher than non-mycorrhizal *P. virgatum* roots (P = 0.0166).

3.2.4. Total biomass accumulation of Fe of T. repens and P. virgatum

Figure 8 summarizes the mean total biomass accumulation of Fe by non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* shoots and roots. Both non-mycorrhizal *P. virgatum*

shoots and roots at the Fe soil concentrations of 15,000 and 30,000 µg g^{-1} had higher tissue concentrations than non-mycorrhizal *T. repens* shoots and roots (P < 0.0006). There were no significant differences between mycorrhizal *T. repens* and *P. virgatum* shoots. However, at the Fe soil concentrations of 15,000 µg g^{-1} and 40,000 µg g^{-1}, mycorrhizal *P. virgatum* roots exhibited higher concentrations than mycorrhizal *T. repens* roots (P < 0.0327).

There were no significant differences observed between the non-mycorrhizal and mycorrhizal *T. repens* shoots and roots. Also, no significant differences were observed between non-mycorrhizal and mycorrhizal *P. virgatum* shoots. However, at the Fe soil concentration of 30,000 µg g^{-1}, non-mycorrhizal *P. virgatum* roots were higher in Fe concentrations than mycorrhizal *P. virgatum* roots, 2961 µg as compared to 407.6 µg (P = 0.003).

3.3. Bioconcentration factor (BCF)

Table 3 shows the bioconcentration factors (BCF) of non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* shoots and roots. The BCF values for Zn of non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* shoots and roots varied between 0.27 and 212.02 with the lowest BCF in non-mycorrhizal *T. repens* roots and the highest in mycorrhizal *P. virgatum* roots. The BCF values for Cu of non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* shoots and roots varied between 0.52 and 580.42, with the lowest BCF in non-mycorrhizal *T. repens* roots and the highest in mycorrhizal *P. virgatum* roots. The BCF values for Pb varied between 0.43 and 474.91 with lowest BCF in non-mycorrhizal *T. repens* roots and the highest in non-mycorrhizal *P. virgatum* shoots. The BCF values for Fe ranged from 8.01 and 293.31 with the lowest BCF in mycorrhizal *T. repens* shoots and the highest in non-mycorrhizal *P. virgatum* shoots.

3.4. Phytoextraction rate (PR)

The phytoextraction rates (PRs) of Zn, Cu, Pb, and Fe for non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* shoots and roots for the four-month growing period are summarized in Table 5. Mycorrhizal *P. virgatum* roots had the highest PR for Zn (21.2%). The lowest PR for Zn was for mycorrhizal *T. repens* shoots (0.02%). Mycorrhizal *P. virgatum* roots had the highest PR for Cu (58%). The lowest PR for Cu was for non-mycorrhizal *T. repens* roots (0.05%). Non-mycorrhizal *P. virgatum* shoots had the highest PR for Pb (47.5%). The lowest PR for Pb was for non-mycorrhizal *T. repens* roots (0.04%). The highest PR for Fe was for non-mycorrhizal *T. repens* shoots (29.3%). The lowest PR for Fe was for mycorrhizal *T. repens* shoots (0.8%).

3.5. Mass-balance analysis of Zn, Cu, Pb, and Fe

Figure 9 displays the percentage of Zn in non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum.* The percentage of water extractable Zn from the soil for non-mycorrhizal *T. repens* and *P. virgatum* was 7.9% and 0.9% respectively. For mycorrhizal plants, the percentage of water extractable Zn was 8.4% and 7.7% for *T. repens* and *P. virgatum*. Non-mycorrhizal *T. repens* shoots had a higher percentage of Zn 2.6%, compared to 1.7% Zn in non-mycorrhizal *P. virgatum* shoots. A comparison of mycorrhizal shoot percentage of Zn *P. virgatum* shoots had a higher percentage at 1.1% compared to 0.6% in *T. repens*. Mycorrhizal *P. virgatum* roots had the highest percentage of Zn, consisting of 14.5% compared to both non-mycorrhizal and mycorrhizal *T. repens* roots at 0.01% and 0.3% respectively. The average percentage of soil bound Zn ranged from 76.6% - 94.7% with the lowest in mycorrhizal *P. virgatum* and the highest in non-mycorrhizal *P. virgatum*.

Figure 10 displays the percentage of Cu in non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum*. The percentage of water extractable Cu from non-mycorrhizal and mycorrhizal *T. repens* soils was 9.9% and 3.1% respectively. For both non-mycorrhizal and mycorrhizal *P. virgatum,* there was no water extractable Cu from the soils. Mycorrhizal *P. virgatum* shoots had

the highest percentage of Cu at 4.5% as compared to non-mycorrhizal and mycorrhizal *T. repens* 1.1% and 0.6% respectively. Mycorrhizal *P. virgatum* roots had the highest percentage of Cu 34% when compared to their no-mycorrhizal counterpart and both non-mycorrhizal and mycorrhizal *T. repens* roots at 9%, 0.7%, and 1.1%. The percentage of Cu bound to the soils ranged from 63.3% - 92% with the lowest in mycorrhizal *P. virgatum* and the highest in mycorrhizal *T. repens*.

Figure 11 displays the percentage of Pb in non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum*. There was no soil water extractable Pb in both no-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* soils. Non-mycorrhizal *P. virgatum* shoots had the highest percentage 43.3% of Pb when compared to mycorrhizal *P. virgatum* shoots and both non-mycorrhizal and mycorrhizal *T. repens* shoots, 4.7%, 3.6%, and 0.4% respectively. Mycorrhizal *P. virgatum* roots had the highest percentage 14.7% of Pb compared to their non-mycorrhizal counterparts 6.8% and both non-mycorrhizal 0.1% and mycorrhizal 1.5% *T. repens*. The percentage of Pb bound to the soils ranged from 50% - 98% with the lowest percentage in non-mycorrhizal *P. virgatum* and the highest in mycorrhizal *T. repens*.

Figure 12 displays the percentage of Fe in non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum*. The percentage of soil water extractable Fe was similar between non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* and ranged from 1.6% - 2.6%. The percentage of Fe was higher in both non-mycorrhizal 15.4% and mycorrhizal *P. virgatum* 11% shoots and when compared to 1.3% in both non-mycorrhizal and mycorrhizal *T. repens* shoots. Non-mycorrhizal and mycorrhizal *T. repens* roots had no Fe. Non-mycorrhizal *P. virgatum* roots had a higher percentage 9.7% of Fe as compared to mycorrhizal *P. virgatum* roots at 3.6%. The percentage of soil bound Fe ranged from 72.4% - 97% with the lowest percentage in non-mycorrhizal *P. virgatum* soils and the highest in mycorrhizal *T. repens* soils.

3.6. AMF root colonization

The results pertaining to colonization rates by mycorrhiza was inconclusive. Little to no colonization was observed by mycorrhizae regarding structures such as: arbuscules, coils, vesicles, spores, and hyphae. There has been evidence in the literature that supports this peculiar observation of the absence of mycorrhizal structures. Frankland and Harrison (1985) observed that in absence of mycorrhizal structures there was still mycorrhizal growth effects on infected plant species in regard to micronutrients.

4. DISCUSSION

In this work, the primary interest was to determine the effects of AMF on the uptake of Zn, Cu, Pb, and Fe by *T. repens* and *P. virgatum*. The results showed that the capacity of metal uptake by *T. repens* and *P. virgatum* depended on the type of metal, soil metal concentrations, and the presence of AMF.

The concentrations of Zn, Cu, Pb, and Fe in *T. repens* and *P. virgatum* tissues varied significantly. The variations were influenced by the addition of the mycorrhizal inoculum and the soil metal treatment concentrations. For example, when comparing tissue concentrations of Zn, non-mycorrhizal *T. repens* shoots had higher concentrations than non-mycorrhizal *P. virgatum* shoots at 25.96 µg g^{-1} compared to 4.39 µg g^{-1}, across all Zn soil concentrations (Figure 1). However, when comparing Cu shoots concentrations, mycorrhizal *T. repens* shoots only had higher concentrations than *P. virgatum* shoots at the highest Cu soil concentration of 120 µg g^{-1} (Figure 2). Based on the additional results provided from Pb and Fe, not all metals and corresponding soil concentrations behave the same regarding the remediation by *T. repens* and *P. virgatum*.

Mycorrhizal inoculum and varying soil metal concentrations influenced the remediation potential of these two species. This difference was further noted when the total biomass accumulation of Zn, Cu, Pb, and Fe were compared. For example, depending on the Cu soil metal treatment concentration, both non-mycorrhizal and mycorrhizal *P. virgatum* roots accumulated more Cu than *T. repens* roots (Figure 6). At the Cu soil concentration of 40 µg g^{-1}, however, there was no significant differences between or amongst species.

This trend of the metal remediation potential of different plant species by altering soil concentrations and using chemical mobilizing agents or rhizobacteria is found throughout the literature. Examples of different plant species includes maize (*Zea mays*), Sunflowers (*Helianthus*

annuus) in combined with EDTA and citric acid, and European yellow lupine (*Lupinus luteus*) with metal resistant rhizobacterium *Serratia Sp. MSMC541* (Aafi et al., 2012; Szabó and Fodor, 2006; Turgut et al., 2004).

The bioconcentration factor (BCF) is defined as the ratio of a metal concentration in the plant shoots or roots to the metal concentration in the soil. BCF values are important in evaluating the potential of a plant species for phytoremediation (McGrath and Zhao, 2003). BCF values greater than one in the shoots or roots determine if a plant is eligible for phytoextraction or phytostabilization of metals in soil.

Depending on the type of metal and the addition of the mycorrhizal inoculum, BCFs varied greatly between the shoots and roots of *T. repens* and *P. virgatum* (Table 3). Without the addition of the mycorrhizal inoculum shoot, BCFs for *T. repens* and *P. virgatum* were higher, suggesting that both species could be used for phytoextraction. When comparing species that had the addition of the mycorrhizal inoculum, both had higher root BCFs except for Fe for *T. repens*. This finding suggests that adding the mycorrhizal inoculum to *T. repens* and *P. virgatum* creates a favorable condition for phytostabilization instead of phytoextraction. The higher root BCFs for mycorrhizal *T. repens* and *P. virgatum* may be explained by various mechanisms, such as the storage of metals in root tissues to prevent foliar toxicity, the low mobility of metals because of their binding to xylem within root tissues, and the immobilization of metals by rhizosphere organisms (Alloway, 2013; Meier et al., 2017; Raskin et al., 1997).

The immobilization of metals by rhizosphere organisms is the most probable explanation for the findings of this experiment, given the differences in metal accumulations between non-mycorrhizal and mycorrhizal treatments. As described earlier, the results of the root colonization by AMF were inconclusive, even though mycorrhizal growth effects still can be observed without classical structures (Frankland and Harrison, 1985). AMF have been known to facilitate the transfer of nutrients, increase root water absorption, and sequester radioactive

isotopes of host plant species (Berreck and Haselwandter, 2001; Johansen et al., 1992; Wu and Xia, 2006).

The effect of AMF on plant uptake of metals during phytoremediation is not always clear. A limited number of studies show that at high soil metal concentrations, AMF have been able to increase the uptake by host plants (Cooper and Tinker, 1978; Gildon and Tinker, 1983; Killham and Firestone, 1983) . In other studies, however, AMF significantly reduced metal concentration in plants or shoots (Berreck and Haselwandter, 2001; Kaldorf et al., 1999; Vogel-Mikuš et al., 2005, 2005). Jankong and Visoottiviseth (2008) provided evidence that AMF prevented the translocation of Arsenic (As) to the aerial parts of host plants. AMF metal uptake is also dependent on soil concentrations as demonstrated by Schüepp et al. AMF decreased uptake of Cd and increased uptake of Zn in the same plant species (Galli et al., 1994). Evidence also shows that AMF has no effect on shoots and roots concentrations and uptake ability of metals (Weissenhorn et al., 1994). This is only a few examples of the complexity involving the role of AMF in phytoremediation.

From the AMF phytoremediation metal strategies listed above, two results from this study involving non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* are the most telling. Based on the results, it can be concluded the foremost strategy employed by AMF is that it prevents the translocation of Zn, Cu, Pb, and Fe to the aerial parts of *T. repens* and *P. virgatum*. The prevention of metal translocation by AMF is best displayed by the results between the non-mycorrhizal and mycorrhizal plant BCFs, where the mycorrhizal root BCFs were higher than non-mycorrhizal shoots and roots. The second AMF metal strategy indicates that metal uptake is dependent on soil concentrations and type of metal as observed by the differences in BCF of plants between metals and soil concentrations.

5. CONCLUSIONS

Unlike organic pollutants, heavy metals cannot be degraded. Therefore, soil heavy metal remediation efforts are limited to stabilization or extraction. Unlike conventional remediation methods, phytoremediation is an effective and sustainable alternative to removing metals from soils. When applying phytoremediation strategies to polluted soils, it is important to have a defined objective for the result of metals in environments. If the objective is to remove metals, phytoextraction techniques should be applied over phytostabilization.

This study provides useful data pertaining to *T. repens* and *P. virgatum* metal tissue concentrations and accumulation rates. It also provides data on the effects of adding mycorrhizal inoculum on metal uptake of *T. repens* and *P. virgatum*. For the application of these two species in *in-situ* metal remediation efforts, it is critical that the end goal be defined first. In relation to these two species, if they were used for in-situ metal remediation, and the goal is to remove metals from a soil, it is important not to add a mycorrhizal inoculum, to increase the likelihood of metals being transferred to above ground plant tissues for removal (phytoextraction).

If the end goal for the metals is to prevent leaching and re-exposure or re-contamination (phytostabilization), then a mycorrhizal inoculum can be added to *T. repens* and *P. virgatum*. In-situ experiments would have to be created to test if non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum* are applicable for phytoremediation or phytostabilization. After completion of an in-situ metal remediation operation it is important that both the above ground shoots and below ground roots of the phytoremediator plant are removed to maximize the metal remediation effect. This study provides data pertaining to phytoremediation, along with a biological understanding of how organisms survive in extreme environments.

6. TABLES

Element	Zn				Cu				Pb				Fe			
Tissue	Shoot		Root		Shoot		Root		Shoot		Root		Shoot		Root	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Explanatory variables																
Soil metal treatment concentration	3.34	0.0313	8.08	0.0004	4.72	0.0077	3.99	0.0161	102.86	0.0001	7.35	0.0007	39.27	0.0001	1.01	0.3996
Plant species	1.97	0.1703	19.1	0.0001	1.04	0.316	0	0.9467	28.29	0.0001	0.05	0.4263	7.24	0.0112	1.66	0.2068
Mycorrhizal Inoculum	0.04	0.8354	26.27	0.0001	1.65	0.2087	0.2	0.6546	317.66	0.0001	4.18	0.0491	0.1	0.7507	0.88	0.3552
Plant species + Soil metal treatment concentration	1.71	0.1847	2.04	0.1273	5.92	0.0025	0.37	0.7786	7.15	0.0008	0.18	0.9092	4.08	0.0146	1.01	0.3996
Mycorrhizal Inoculum + Soil metal treatment concentration	1.4	0.2618	3.58	0.0243	7.84	0.0005	0.34	0.7956	57.64	0.0001	0.49	0.6894	0.55	6513	1.02	0.3964
Plant species + Mycorrhizal Inoculum	7.06	0.0122	9.88	0.0036	6.81	0.0136	0.07	0.7959	21.35	0.0001	6.19	0.0183	5.23	0.029	0.88	0.3552
Plant species + Mycorrhizal Inoculum + Soil metal treatment concentration	1.82	0.1632	2.14	0.115	4.96	0.0061	0.78	0.5127	5.09	0.0054	3.91	0.00174	1.35	0.2746	1.02	0.3964

Table 1: Three-Way ANOVA summary table for *T. repens* and *P. virgatum* shoot and root concentrations of Zn, Cu, Pb, and Fe (μg g^{-1}dry weight).

Element	Zn				Cu				Pb				Fe			
Tissue	Shoot		Root		Shoot		Root		Shoot		Root		Shoot		Root	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Explanatory variables																
Soil metal treatment concentration	3.34	0.0313	7.93	0.0004	4.72	0.0077	3.99	0.0161	102.90	<0.0001	7.35	0.0007	39.27	<0.0001	1.01	0.3996
Plant species	1.97	0.1703	18.78	0.0001	1.04	0.316	0.00	0.9467	28.29	<0.0001	0.65	0.4263	7.24	0.0112	1.66	0.2068
Mycorrhizal Inoculum	0.04	0.8354	26.16	<0.0001	1.65	0.2087	0.20	0.6546	317.70	<0.0001	4.18	0.0491	0.10	0.7507	0.88	0.3552
Plant species + Soil metal treatment concentration	1.71	0.1847	2.01	0.1324	5.92	0.0025	0.37	0.7786	7.15	0.0008	0.18	0.9092	4.08	0.0146	1.01	0.3996
Mycorrhizal Inoculum + Soil metal treatment concentration	1.40	0.2618	3.60	0.024	7.84	0.0005	0.34	0.7956	57.64	<0.0001	0.49	0.6894	0.55	0.6513	1.02	0.3964
Plant species + Mycorrhizal Inoculum	7.06	0.0122	9.89	0.0036	6.81	0.0136	0.07	0.7989	21.35	<0.0001	6.19	0.0183	5.23	0.029	0.88	0.3552
Plant species + Mycorrhizal Inoculum + Soil metal treatment concentration	1.82	0.1632	2.10	0.12	4.96	0.0061	0.78	0.5127	5.09	0.0054	3.91	0.0174	1.35	0.2746	1.02	0.3964

Table 2: Three-Way ANOVA summary table for *T. repens* and *P. virgatum* total biomass accumulation of Zn, Cu, Pb, and Fe (μg pot^{-1}).

Plant species			*T. repens*				*P. virgatum*	
Mycorrhizal Inoculum		-		+		-		+
Tissue BCF	BCF_{shoot}	BCF_{roots}	BCF_{shoot}	BCF_{roots}	BCF_{shoot}	BCF_{roots}	BCF_{shoot}	BCF_{roots}
Soil metal treatment ($\mu g\ g^{-1}$)								
Zn								
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
200	46.60±12.37	0.00±0.00	14.45±6.47	2.07±2.07	35.45±35.45	29.74±15.72	34.68±19.34	212.02±106.09
400	22.48±4.79	0.00±0.00	2.33±0.91	0.00±0.00	14.99±12.03	0.00±0.00	0.00±0.00	154.43±4.01
600	9.91±5.18	0.00±0.00	0.27±0.27	7.35±1.93	0.72±0.72	48.76±30.69	0.00±0.00	68.42±29.93
Cu								
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
40	0.00±0.00	16.78±0.13	1.66±1.66	0.00±0.00	78.19±62.14	88.93±88.93	64.79±37.40	343.75±175.09
80	17.90±17.90	5.21±5.21	0.00±0.00	55.15±45.20	35.60±12.81	112.46±41.19	43.75±25.26	580.42±306.79
120	0.00±0.00	0.52±0.52	31.65±10.72	39.02±7.24	18.06±2.70	69.86±11.93	14.38±8.30	97.48±23.49
Pb								
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
200	45.59±8.22	1.70±0.85	1.65±1.08	19.73±8.76	405.03±134.64	54.18±26.45	47.79±8.62	89.37±44.88
400	48.43±3.69	0.43±0.38	7.37±2.53	12.44±5.49	419.78±161.46	112.17±69.96	23.13±2.23	80.66±6.27
600	13.92±4.45	1.72±0.81	3.85±2.75	14.44±3.77	474.91±260.04	37.96±10.30	70.82±27.83	272.32±130.84
Fe								
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
15,000	17.15±7.43	0.00±0.00	21.14±3.13	0.00±0.00	293.31±45.99	169.35±59.03	181.73±111.72	69.56±35.49
30,000	10.06±1.98	0.00±0.00	11.14±2.13	0.00±0.00	122.17±28.45	98.70±14.42	75.27±38.96	13.59±3.33
40,000	12.68±2.46	0.00±0.00	8.01±0.92	0.00±0.00	47.38±10.06	24.51±3.77	63.34±7.80	25.46±3.48

Table 3: Bioconcentration factor (BCF) of *T. repens* and *P. virgatum* shoots and roots (mean ± standard error, n =3) of non-mycorrhizal and mycorrhizal inoculated plants grown in increasing Zn, Cu, Pb, and Fe soil concentrations.

Element	Zn				Cu				Pb				Fe			
Tissue BCF	BCF_{Shoot}		BCF_{Root}		BCF_{Shoot}		BCF_{Root}		BCF_{Shoot}		BCF_{Root}		BCF_{Shoot}		BCF_{Root}	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Explanatory variables																
Soil metal treatment concentration	7.019	0.0009	3.041	0.0431	2.104	0.1192	3.066	0.0419	2.437	0.0827	2.913	0.0494	11.05	<0.0001	8.537	0.0003
Plant species	0.05158	0.8218	18.96	0.0001	7.11	0.0119	10.08	0.0033	15.46	0.0004	14.22	0.0007	28.79	<0.0001	32.25	<0.0001
Mycorrhizal Inoculum	3.043	0.0907	9.984	0.0034	0.05599	0.8145	4.926	0.0337	13.92	0.0007	3.155	0.0852	1.177	0.2862	6.781	0.0139
Plant species + Soil metal treatment concentration	0.1599	0.9225	2.982	0.0459	2.298	0.0963	2.366	0.0893	1.857	0.1568	2.398	0.0863	8.26	0.0003	8.537	0.0003
Mycorrhizal Inoculum + Soil metal treatment concentration	0.5861	0.6285	2.512	0.0761	0.3825	0.7663	1.558	0.2187	1.555	0.2194	2.377	0.0883	0.6799	0.5708	2.336	0.0923
Plant species + Mycorrhizal Inoculum	1.023	0.3194	9.034	0.0051	0.02055	0.8869	3.358	0.0762	10.02	0.0034	1.526	0.2257	1.19	0.2835	6.781	0.0139
Plant species + Mycorrhizal Inoculum + Soil metal treatment concentration	0.3787	0.769	2.624	0.0674	0.4019	0.7526	1.297	0.2924	1.152	0.3434	2.229	0.1039	0.8526	0.4756	2.336	0.0923

Table 4: Three-Way ANOVA summary table for the bioconcentration factors of *T. repens* and *P. virgatum*.

Plant species		T. repens				P. virgatum			
Mycorrhizal Inoculum		-		+		-		+	
Tissue		Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Soil metal treatment ($\mu g\ g^{-1}$)									
Zn									
0		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
200		4.66±1.24	0.00±0.00	1.45±0.65	0.21±0.21	3.55±3.55	2.97±1.57	3.47±1.93	21.20±10.61
400		2.25±0.48	0.00±0.00	0.23±0.09	0.00±0.00	1.50±1.20	0.00±0.00	0.00±0.00	15.44±0.40
600		0.99±0.52	0.00±0.00	0.03±0.03	0.73±0.19	0.07±0.07	4.88±3.07	0.00±0.00	6.84±2.99
Cu									
0		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
40		0.00±0.00	1.68±0.01	0.17±0.17	0.00±0.00	7.82±6.21	8.89±8.89	5.86±3.74	34.38±17.51
80		1.79±1.79	0.52±0.52	0.00±0.00	5.52±4.52	3.56±1.28	11.25±4.12	5.01±2.53	58.04±30.68
120		0.00±0.00	0.05±0.05	3.17±1.07	2.58±1.48	1.81±0.27	6.99±1.19	2.70±0.83	9.75±2.35
Pb									
0		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
200		4.56±0.82	0.17±0.09	0.17±0.11	1.97±0.88	40.50±13.46	5.42±2.64	4.78±0.86	8.94±4.49
400		4.84±0.37	0.04±0.04	0.74±0.25	1.24±0.55	41.98±16.15	11.22±7.00	2.31±0.22	8.07±0.63
600		1.39±0.45	0.17±0.08	0.39±0.28	1.44±0.38	47.49±26.00	3.80±1.03	7.08±2.78	27.23±13.08
Fe									
0		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
15,000		1.72±0.74	0.00±0.00	2.11±0.31	0.00±0.00	29.33±4.60	16.93±5.90	18.17±11.17	6.96±3.55
30,000		1.01±0.20	0.00±0.00	1.11±0.21	0.00±0.00	12.22±2.84	9.87±1.44	7.53±3.90	1.36±0.33
60,000		1.27±0.25	0.00±0.00	0.80±0.09	0.00±0.00	4.74±1.01	2.45±0.38	6.33±0.78	2.55±0.35

Table 5: Phytoextraction rate (PR) % of *T. repens* and *P. virgatum* shoots and roots (mean ± standard error, n =3) of non-mycorrhizal and mycorrhizal inoculated plants grown in increasing Zn, Cu, Pb, and Fe soil concentrations.

Plant species	T. repens								P. virgatum							
Mycorrhizal inoculum	−				+				−				+			
% Metal distribution	% Extractable	%Shoot	%Root	%Soil	% Extractable	%Shoot	%Root	%Soil	% Extractable	%Shoot	%Root	%Soil	% Extractable	%Shoot	%Root	%Soil
Soil metal treatment ($\mu g\ g^{-1}$)																
Zn																
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
200	19.95±18.88	4.66±1.24	0.00±0.00	75.39±19.41	19.95±18.88	1.45±0.65	0.21±0.21	78.40±19.42	0.00±0.00	3.55±3.55	2.97±1.57	93.48±2.25	12.81±12.81	3.47±1.93	21.20±10.61	62.52±21.58
400	3.65±1.85	2.25±0.48	0.00±0.00	94.10±2.27	3.65±1.85	0.23±0.09	0.00±0.00	96.12±1.78	1.66±1.66	1.50±1.20	0.00±0.00	96.84±1.31	8.07±5.76	0.00±0.00	15.44±0.40	76.49±5.63
600	0.24±0.24	0.99±0.52	0.05±0.01	98.72±0.75	1.75±0.59	0.03±0.03	0.73±0.19	97.49±0.51	1.11±1.11	0.07±0.07	4.88±3.07	93.94±3.18	2.21±1.11	0.00±0.00	6.84±2.99	90.94±4.10
Cu																
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
40	16.92±7.83	0.00±0.00	1.68±0.01	81.40±7.83	3.25±1.71	0.17±0.17	0.00±0.00	96.59±1.87	0.00±0.00	7.82±6.21	8.89±8.89	83.29±8.84	0.00±0.00	5.86±3.74	34.38±17.51	59.77±20.90
80	12.73±7.45	1.79±1.79	0.52±0.52	84.96±5.76	3.33±0.00	0.00±0.00	5.52±4.52	91.15±4.52	0.00±0.00	3.56±1.28	11.25±4.12	85.19±3.59	0.00±0.00	5.01±2.53	58.04±30.68	42.76±28.50
120	0.17±0.17	0.00±0.00	0.05±0.05	99.78±0.15	2.79±0.57	3.17±1.07	3.90±0.72	90.14±0.95	0.00±0.00	1.81±0.27	6.99±1.19	91.21±1.18	0.00±0.00	2.70±0.83	9.75±2.35	87.55±2.54
Pb																
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
200	0.00±0.00	4.56±0.82	0.17±0.09	95.27±0.75	0.00±0.00	0.17±0.11	1.97±0.88	97.86±0.88	0.00±0.00	40.50±13.46	5.42±2.64	54.08±15.87	0.00±0.00	4.78±0.86	8.94±4.49	86.28±3.63
400	0.00±0.00	4.84±0.37	0.04±0.04	95.11±0.40	0.00±0.00	0.74±0.25	1.24±0.55	98.02±0.36	0.00±0.00	41.98±16.15	11.22±7.00	46.80±23.14	0.00±0.00	2.31±0.22	8.07±0.63	89.62±0.66
600	0.00±0.00	1.39±0.45	0.17±0.08	98.44±0.41	0.00±0.00	0.39±0.28	1.44±0.38	98.17±0.29	0.00±0.00	47.49±26.00	3.80±1.03	48.82±24.97	0.00±0.00	7.08±2.78	27.23±13.08	65.69±15.77
Fe																
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
15,000	5.25±1.96	1.72±0.74	0.00±0.00	93.03±2.31	2.74±0.00	2.11±0.31	0.00±0.00	95.14±0.31	3.74±0.00	29.33±4.60	16.93±5.90	49.99±9.33	3.74±0.00	18.17±11.17	6.96±3.55	71.13±14.48
30,000	1.43±0.26	1.01±0.20	0.00±0.00	97.56±0.23	1.50±0.06	1.11±0.21	0.00±0.00	97.39±0.24	2.17±0.15	12.22±2.84	9.87±1.44	75.75±4.20	1.43±0.00	7.53±3.90	1.36±0.33	89.69±4.20
40,000	1.27±0.24	1.27±0.25	0.00±0.00	97.46±0.25	0.59±0.04	0.80±0.09	0.00±0.00	98.61±0.05	1.29±0.11	4.74±1.01	2.45±0.38	91.52±1.09	1.07±0.00	6.33±0.78	2.55±0.35	90.05±0.43

Table 6: Mass-balance for Zn, Cu, Pb, and Fe in *T. repens* and *P. virgatum* shoots and roots, water extractable, and remaining in soil (percentage % ± standard error, n =3).
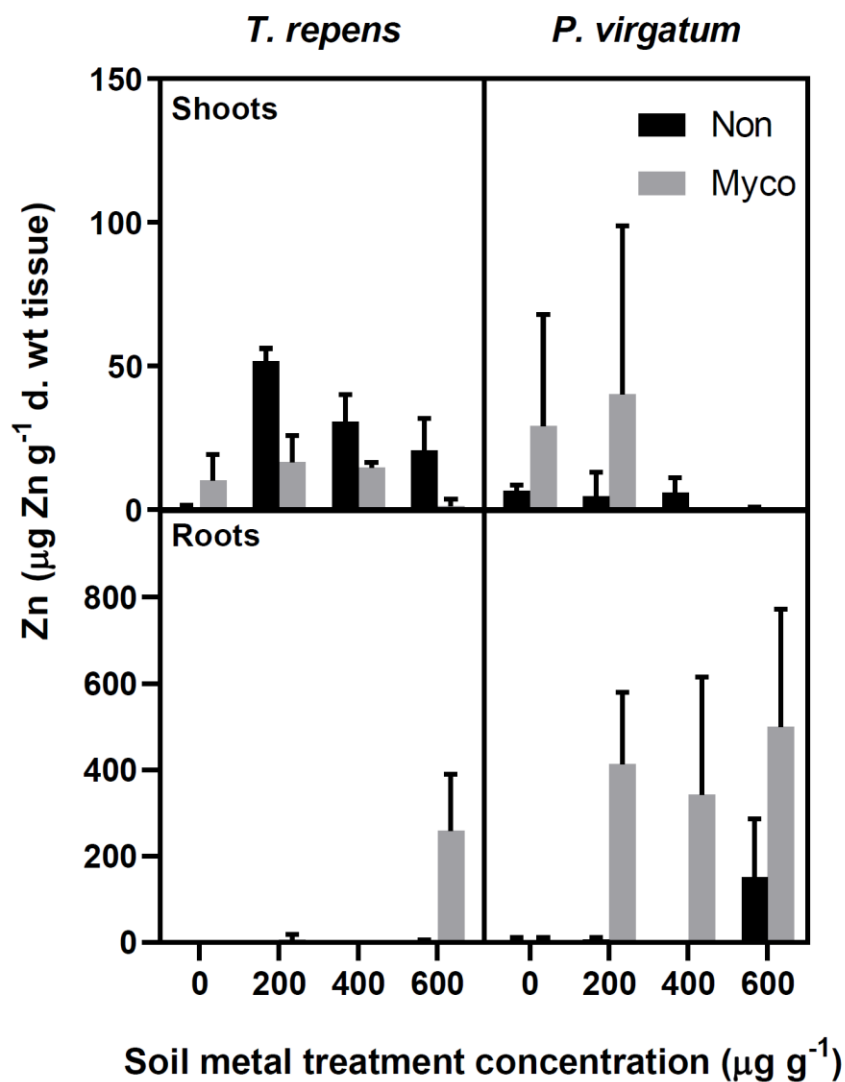
7. FIGURES

Figure 1: The concentration of Zn (µg g^{-1}dry weight, mean ± standard error, n =3) of *T. repens* and *P. virgatum* shoots and roots, grown in increasing Zn soil concentrations (µg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.
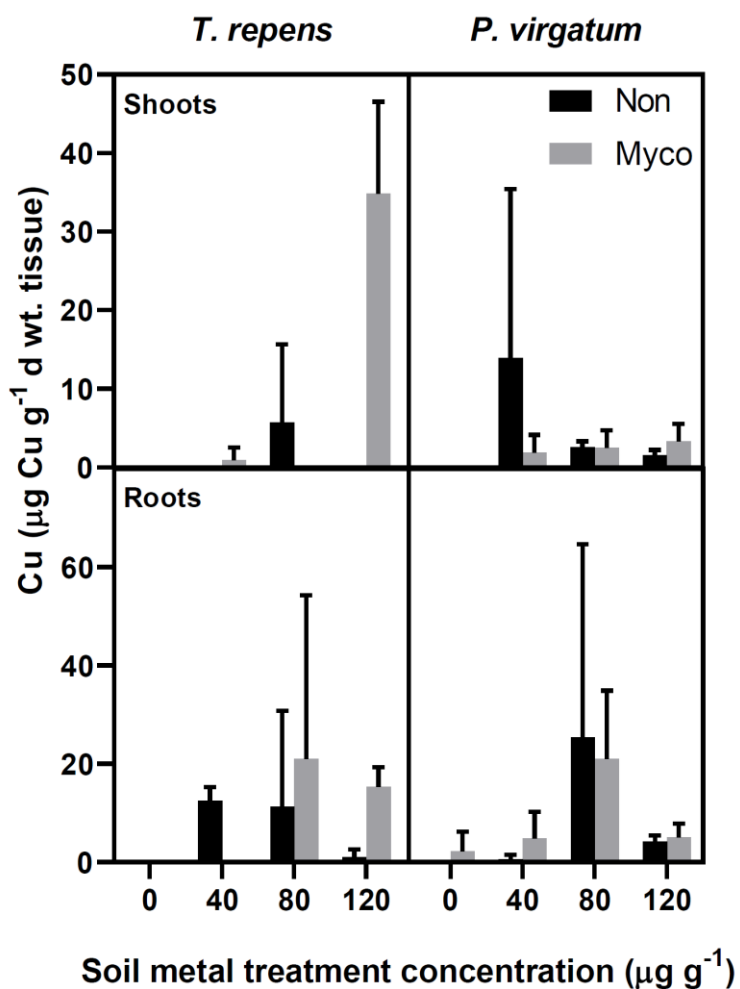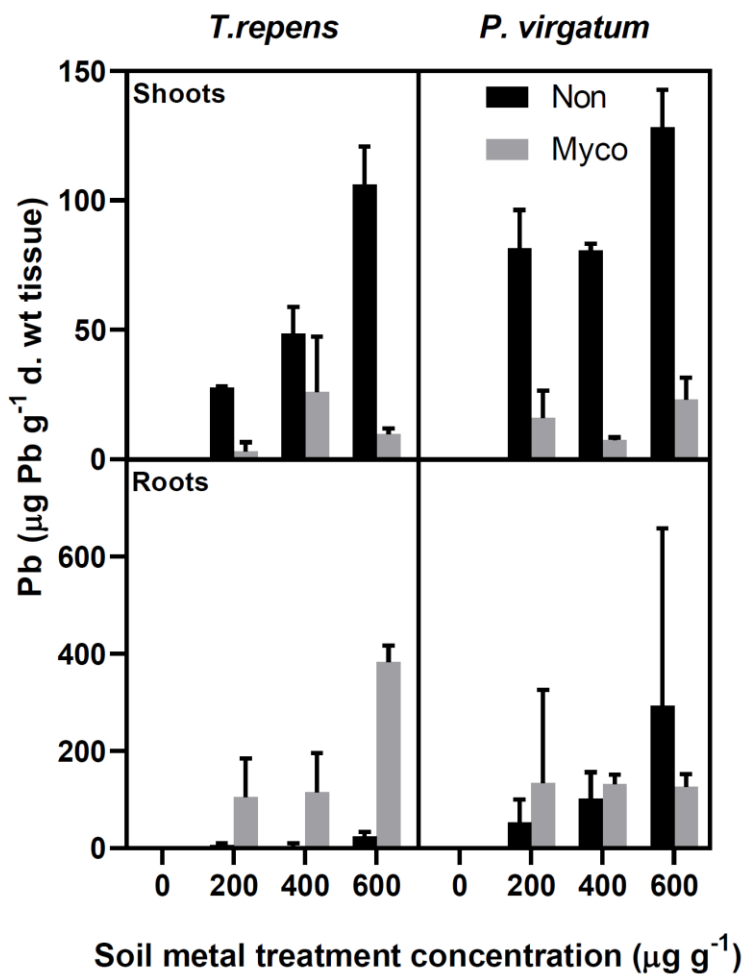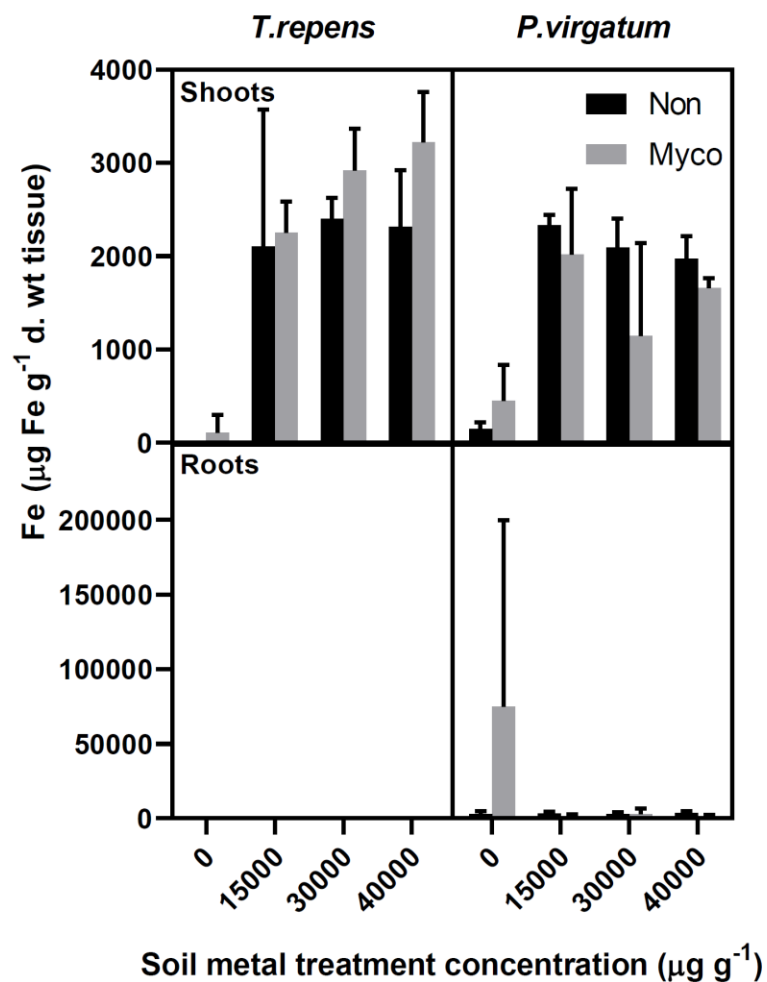
Figure 2: The concentration of Cu (μg g^{-1}dry weight, mean $\pm$ standard error, n =3) of *T. repens* and *P. virgatum* shoots and roots, grown in increasing Cu soil concentrations (μg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.
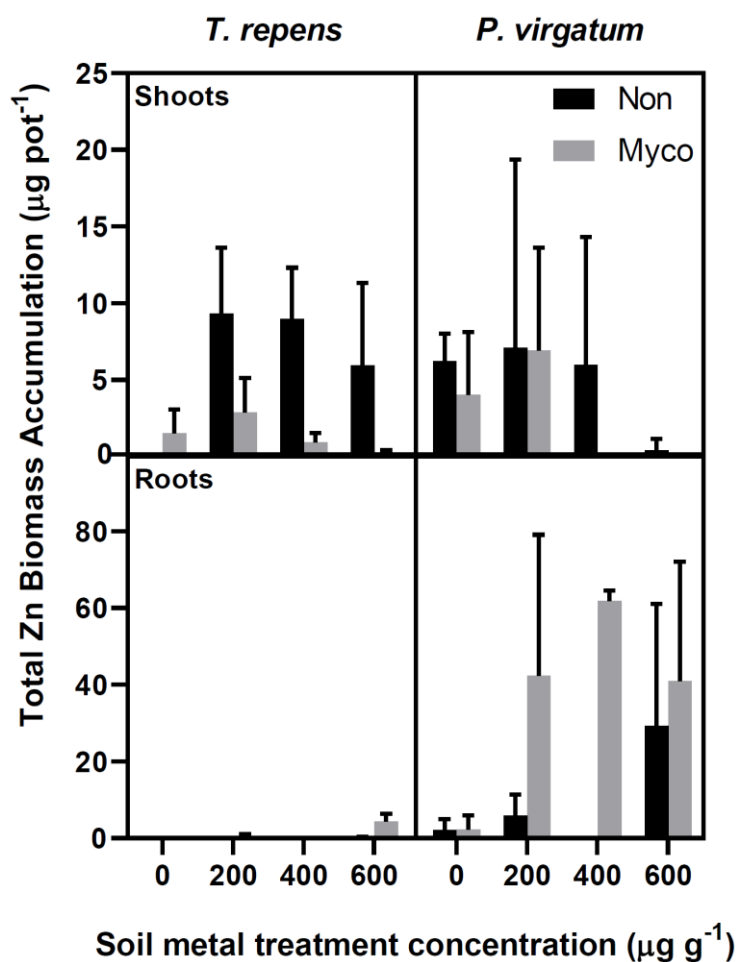
Figure 3: The concentration of Pb (μg g^{-1}dry weight, mean $\pm$ standard error, n =3) of *T. repens* and *P. virgatum* shoots and roots, grown in increasing Pb soil concentrations (μg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.
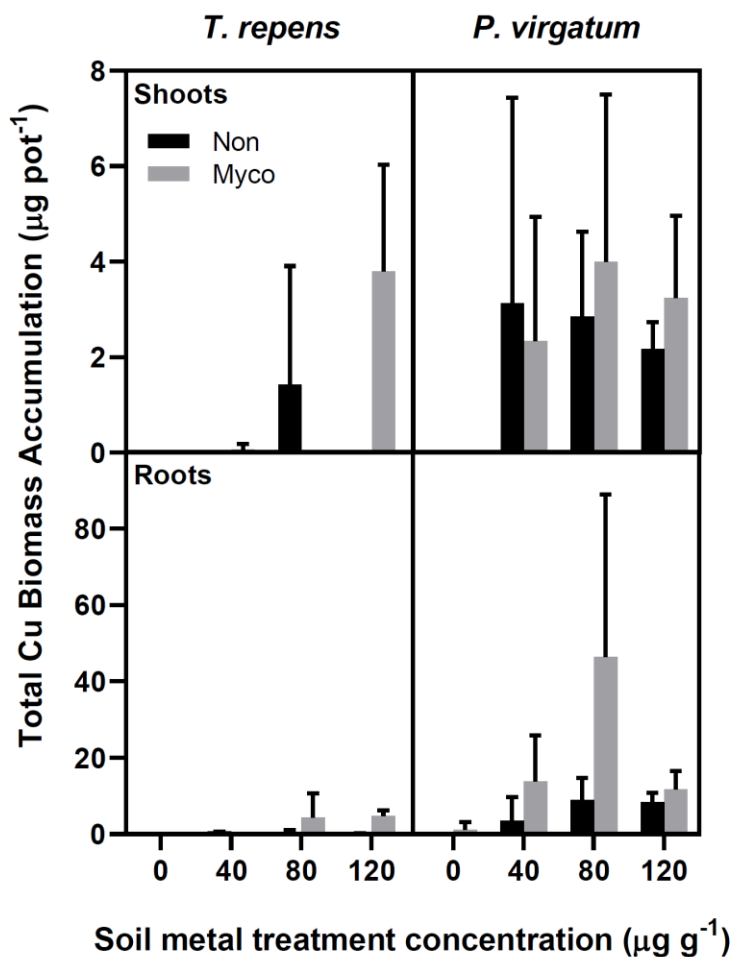
Figure 4: The concentration of Fe (μg g^{-1}dry weight, mean $\pm$ standard error, n =3) of *T. repens* and *P. virgatum* shoots and roots, grown in increasing Fe soil concentrations (μg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.

Figure 5: The total concentration of Zn (µg pot^{-1}, mean ± standard error, n =3) in *T. repens* and *P. virgatum* shoots and roots, grown in increasing Zn soil concentrations (µg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.
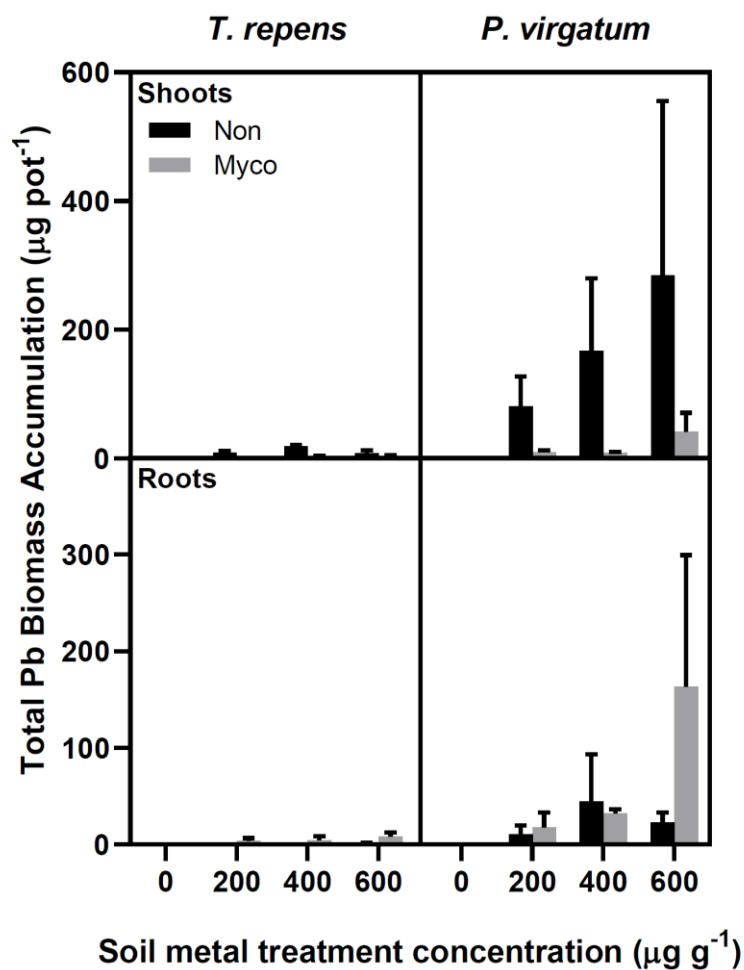
Figure 6: The total concentration of Cu (μg pot^{-1}, mean ± standard error, n =3) in *T. repens* and *P. virgatum* shoots and roots, grown in increasing Cu soil concentrations (μg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.
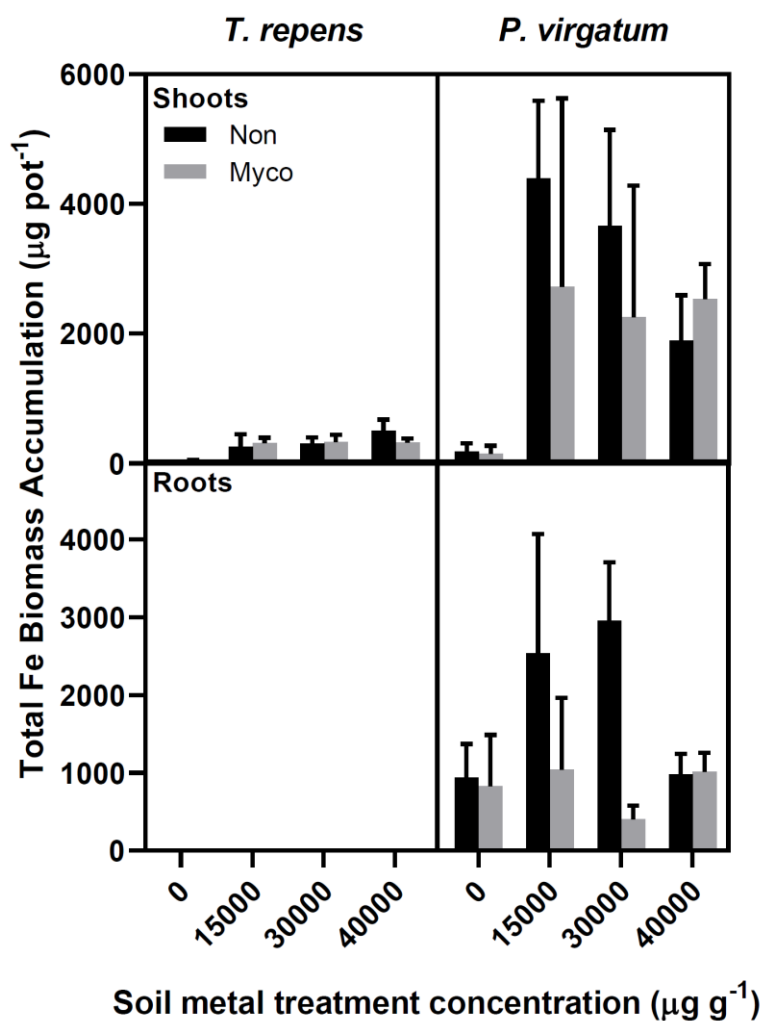
Figure 7: The total concentration of Pb (µg pot^{-1}, mean ± standard error, n =3) in *T. repens* and *P. virgatum* shoots and roots, grown in increasing Pb soil concentrations (µg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.

Figure 8: The total concentration of Fe (μg pot^{-1}, mean ± standard error, n =3) in *T. repens* and *P. virgatum* shoots and roots, grown in increasing Fe soil concentrations (μg g^{-1}). Black bars indicate plants not treated with mycorrhizal inoculum. Gray bars indicate plants treated with mycorrhizal inoculum.
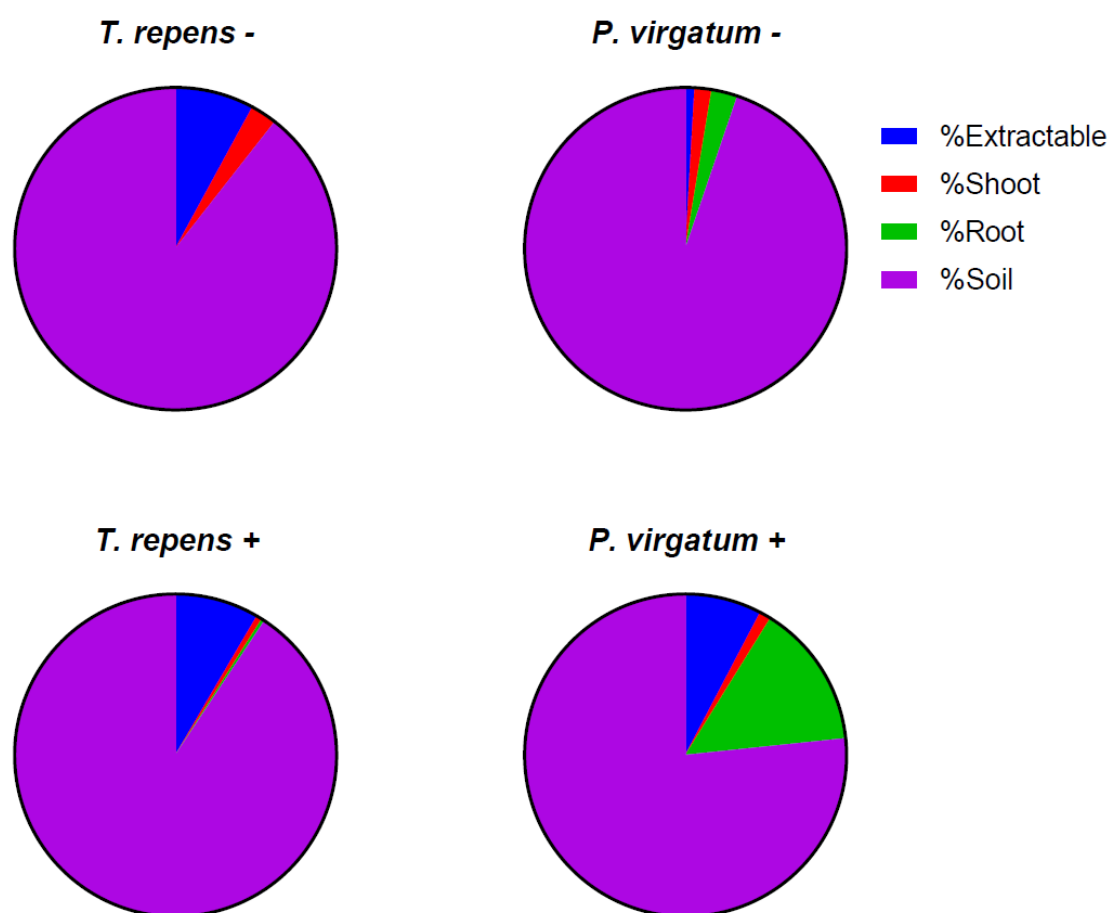
Figure 9: Pie chart showing % Zinc metal distribution in non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum*, - and + indicate mycorrhizal treatment
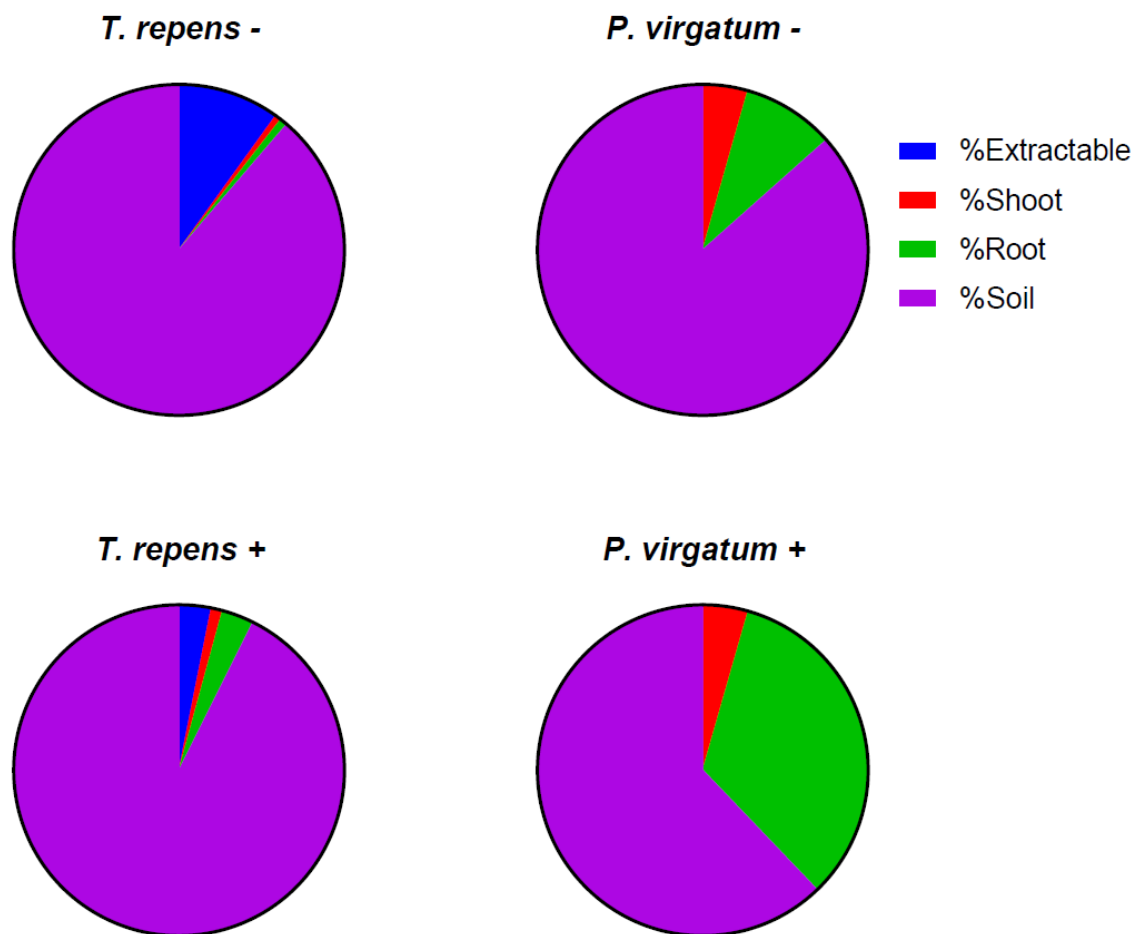
Figure 10: Pie chart showing %Copper metal distribution in non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum*, - and + indicate mycorrhizal treatment
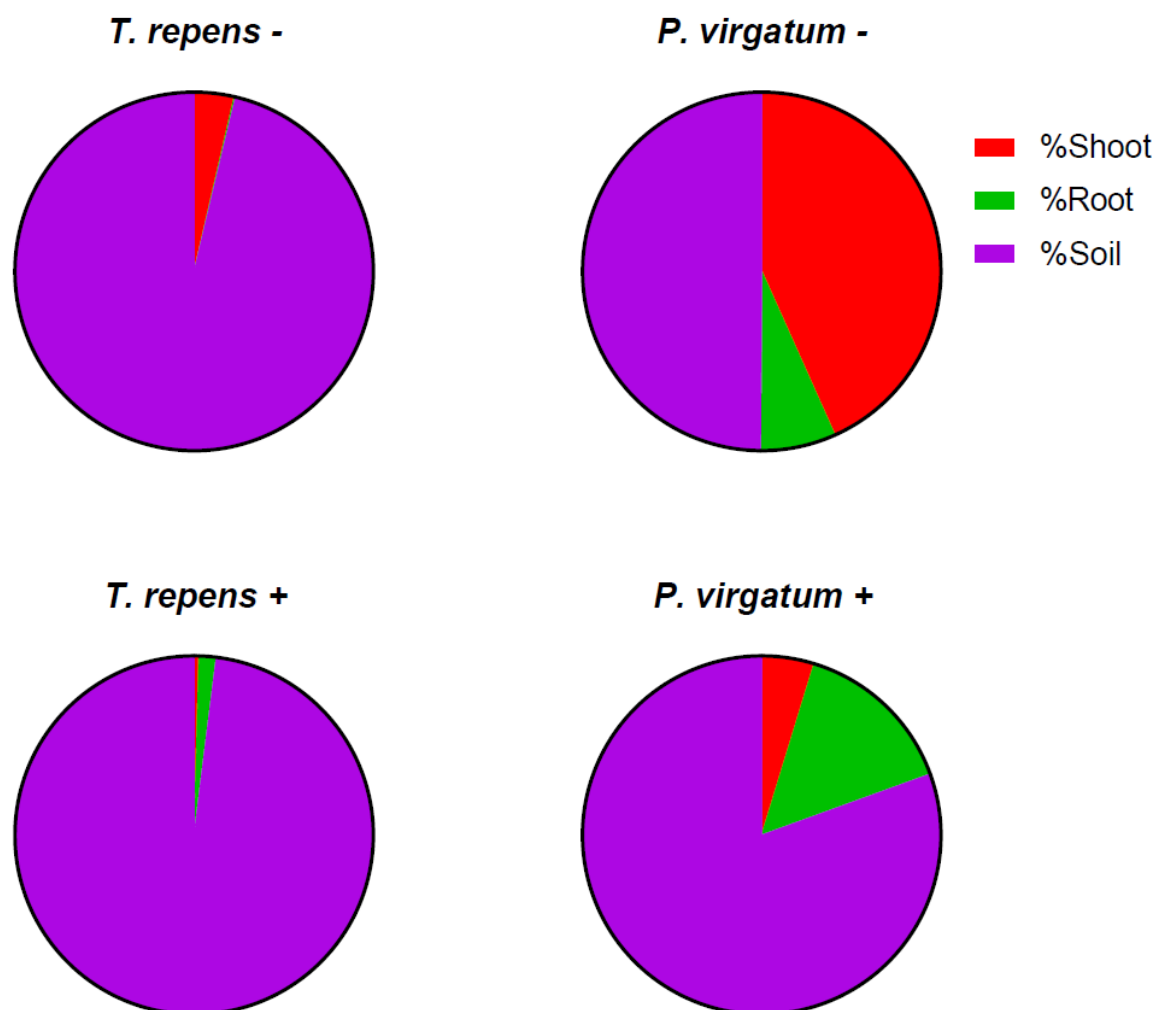
Figure 11: Pie chart showing %Lead metal distribution in non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum*, - and + indicate mycorrhizal treatment
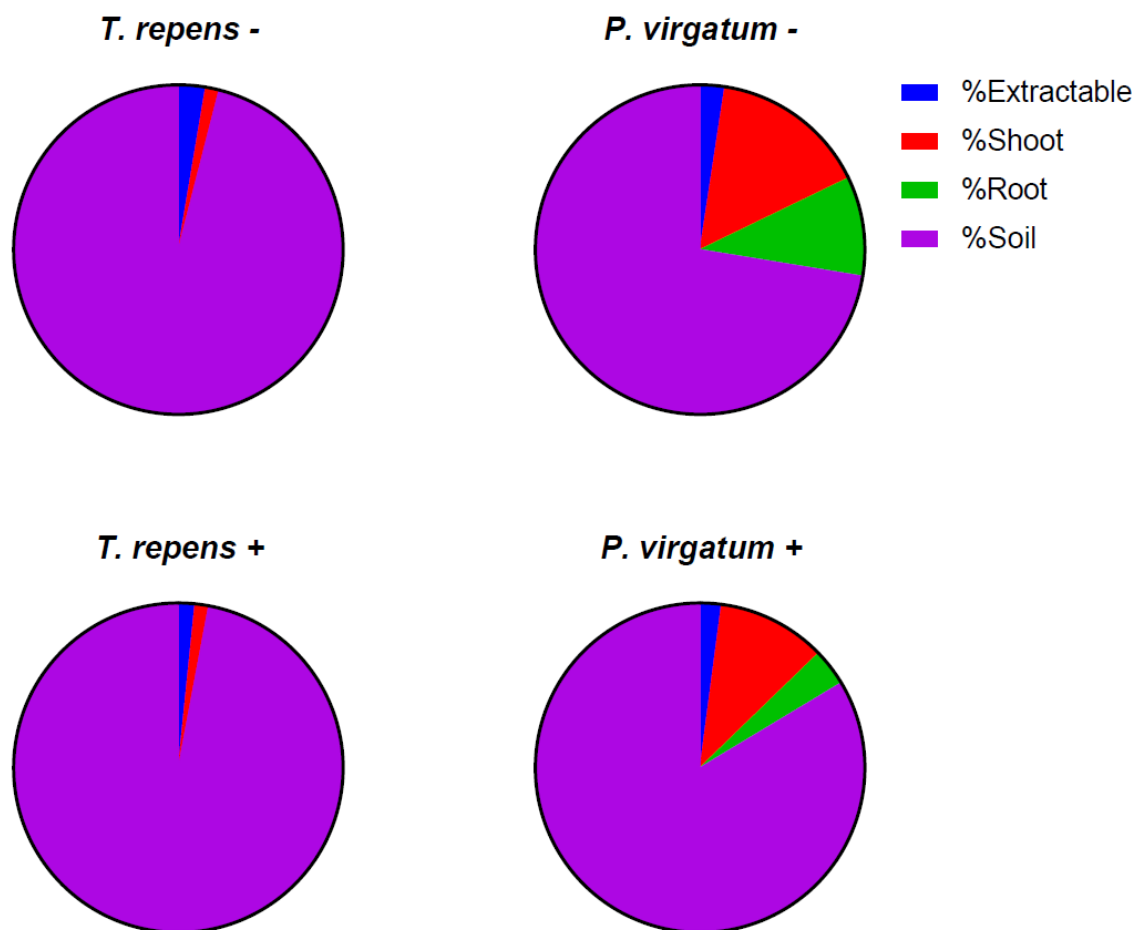
Figure 12: Pie chart showing %Iron metal distribution in non-mycorrhizal and mycorrhizal *T. repens* and *P. virgatum*, - and + indicate mycorrhizal treatment

8. REFERNCES

Alloway, Brian J. (2013). Heavy metals in soils: trace metals and metalloids in soils and their bioavailability. Dordrecht, Netherlands; New York: Springer.

Aafi, N.E., Brhada, F., Dary, M., Maltouf, A.F., and Pajuelo, E. (2012). Rhizostabilization of metals in soils using Lupinus Luteus inoculated withe metal resistant rhizobacterium Serratia Sp. MSMC541. Int. J. Phytoremediation *14*, 261–274.

Al-Karaki, G.N. (1998). Benefit, cost and water-use efficiency of arbuscular mycorrhizal durum wheat grown under drought stress. Mycorrhiza *8*, 41–45.

Al-Karaki, G.N. (2000). Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza *10*, 51–54.

Alloway, B.J. (2013). Heavy metals in soils: trace metals and metalloids in soils and their bioavailability (Dordrecht, Netherlands; New York: Springer).

Berreck, M., and Haselwandter, K. (2001). Effect of the arbuscular mycorrhizal symbiosis upon uptake of cesium and other cations by plants. Mycorrhiza *10*, 275–280.

Bidar, G., Garçon, G., Pruvot, C., Dewaele, D., Cazier, F., Douay, F., and Shirali, P. (2007). Behavior of Trifolium repens and Lolium perenne growing in a heavy metal contaminated field: Plant metal concentration and phytotoxicity. Environ. Pollut. *147*, 546–553.

Bidar, G., Pruvot, C., Garçon, G., Verdin, A., Shirali, P., and Douay, F. (2009). Seasonal and annual variations of metal uptake, bioaccumulation, and toxicity in Trifolium repens and Lolium perenne growing in a heavy metal-contaminated field. Environ. Sci. Pollut. Res. *16*, 42–53.

Blum, W.E.H. (2005). Functions of Soil for Society and the Environment. Rev. Environ. Sci. Biotechnol. *4*, 75–79.

Burdon, J.J. (1983). Trifolium Repens L. J. Ecol. *71*, 307.

Callender, E. (2003). Heavy Metals in the Environment—Historical Trends. In Treatise on Geochemistry, (Elsevier), pp. 67–105.

Cooper, K.M., and Tinker, P.B. (1978). Translocation and Transfer of Nutrients in Vesicular-Arbuscular Mycorrhizas II. Uptake and Translocation of Phosphorus, Zinc and Sulphur. New Phytol. *81*, 43–52.

Di Virgilio, N., Monti, A., and Venturi, G. (2007). Spatial variability of switchgrass (Panicum virgatum L.) yield as related to soil parameters in a small field. Field Crops Res. *101*, 232–239.

Duffus, J.H. (2002). "Heavy metals" a meaningless term? (IUPAC Technical Report). Pure Appl. Chem. *74*, 793–807.

Frankland, J.C., and Harrison, A.F. (1985). Mycorrhizal Infection of Betula pendula and Acer pseudoplatanus: Relationships with Seedling Growth and Soil Factors. New Phytol. *101*, 133–151.

Galli, U., Schuepp, H., and Brunold, C. (1994). Heavy metal binding by mycorrhizal fungi. Physiol. Plant. *92*, 364–368.

Gildon, A., and Tinker, P.B. (1983). Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants. New Phytol. *95*, 247–261.

Herrick, J.E. (2000). Soil quality: an indicator of sustainable land management? Appl. Soil Ecol. *15*, 75–83.

Hetrick, B.A.D., Wilson, G.W.T., and Figge, D.A.H. (1994). The influence of mycorrhizal symbiosis and fertilizer amendments on establishment of vegetation in heavy metal mine spoil. Environ. Pollut. *86*, 171–179.

Hildebrandt, U., Regvar, M., and Bothe, H. (2007). Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry *68*, 139–146.

Hu, Y., Liu, X., Bai, J., Shih, K., Zeng, E.Y., and Cheng, H. (2013). Assessing heavy metal pollution in the surface soils of a region that had undergone three decades of intense industrialization and urbanization. Environ. Sci. Pollut. Res. *20*, 6150–6159.

Jankong, P., and Visoottiviseth, P. (2008). Effects of arbuscular mycorrhizal inoculation on plants growing on arsenic contaminated soil. Chemosphere *72*, 1092–1097.

Järup, L. (2003). Hazards of heavy metal contamination. Br. Med. Bull. *68*, 167–182.

Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K., and Barea, J.-M. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biology and Fertility of Soils *37(1)*, 1–16.

Johansen, A., Jakobsen, I., and Jensen, E.S. (1992). Hyphal transport of 15N-labelled nitrogen by a vesicular-arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. New Phytol. *122*, 281–288.

Joner, E.J., Briones, R., and Leyval, C. (2000). Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant Soil *226*, 227–234.

Kaldorf, M., Kuhn, A.J., Schröder, W.H., Hildebrandt, U., and Bothe, H. (1999). Selective Element Deposits in Maize Colonized by a Heavy Metal Tolerance Conferring Arbuscular Mycorrhizal Fungus. J. Plant Physiol. *154*, 718–728.

Khalid, S., Shahid, M., Niazi, N.K., Murtaza, B., Bibi, I., and Dumat, C. (2017). A comparison of technologies for remediation of heavy metal contaminated soils. J. Geochem. Explor. *182*, 247–268.

Khan, A.G. (2005). Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J. Trace Elem. Med. Biol. *18*, 355–364.

Killham, K., and Firestone, M.K. (1983). Vesicular arbuscular mycorrhizal mediation of grass response to acidic and heavy metal depositions. Plant Soil *72*, 39–48.

Lambers, H., Shane, M.W., Cramer, M.D., Pearse, S.J., and Veneklaas, E.J. (2006). Root Structure and Functioning for Efficient Acquisition of Phosphorus: Matching Morphological and Physiological Traits. Ann. Bot. *98*, 693–713.

Leyval, C., Joner, E.J., del Val, C., and Haselwandter, K. (2002). Potential of arbuscular mycorrhizal fungi for bioremediation. In Mycorrhizal Technology in Agriculture, S. Gianinazzi, H. Schüepp, J.M. Barea, and K. Haselwandter, eds. (Basel: Birkhäuser Basel), pp. 175–186.

Li, X., Poon, C., and Liu, P.S. (2001). Heavy metal contamination of urban soils and street dusts in Hong Kong. Appl. Geochem. *16*, 1361–1368.

Lindqvist, O. (1995). Environmental impact of mercury and other heavy metals. J. Power Sources *57*, 3–7.

Marschner, H., and Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. Plant and Soil *159*, 89–102.

McConnell, J.R., and Edwards, R. (2008). Coal burning leaves toxic heavy metal legacy in the Arctic. Proc. Natl. Acad. Sci. *105*, 12140–12144.

McGrath, S.P., and Zhao, F.-J. (2003). Phytoextraction of metals and metalloids from contaminated soils. Curr. Opin. Biotechnol. *14*, 277–282.

Mcgrath, S., Brookes, P., and Giller, K. (1988). Effects of potentially toxic metals in soil derived from past applications of sewage sludge on nitrogen fixation by trifolium repens L. Soil Biol. Biochem. *20*, 415–424.

McLaughlin, S.B., and Adams Kszos, L. (2005). Development of switchgrass (Panicum virgatum) as a bioenergy feedstock in the United States. Biomass Bioenergy *28*, 515–535.

Meier, S., Curaqueo, G., Khan, N., Bolan, N., Rilling, J., Vidal, C., Fernández, N., Acuña, J., González, M.-E., Cornejo, P., et al. (2017). Effects of biochar on copper immobilization and soil microbial communities in a metal-contaminated soil. J. Soils Sediments *17*, 1237–1250.

Micó, C., Recatalá, L., Peris, M., and Sánchez, J. (2006). Assessing heavy metal sources in agricultural soils of an European Mediterranean area by multivariate analysis. Chemosphere *65*, 863–872.

Nriagu, J.O. (1990). Global Metal Pollution: Poisoning the Biosphere? Environ. Sci. Policy Sustain. Dev. *32*, 7–33.

Nriagu, J.O. (1996). A History of Global Metal Pollution. Science *272*, 223–0.

Ozaki, H., Watanabe, I., and Kuno, K. (2004). Investigation of the Heavy Metal Sources in Relation to Automobiles. Water. Air. Soil Pollut. *157*, 209–223.

Porter, C.L. (1966). An Analysis of Variation Between Upland and Lowland Switchgrass, Panicum Virgatum L., in Central Oklahoma. Ecology *47*, 980–992.

Raskin, I., Smith, R.D., and Salt, D.E. (1997). Phytoremediation of metals: using plants to remove pollutants from the environment. Curr. Opin. Biotechnol. *8*, 221–226.

Remon, E., Bouchardon, J.-L., Cornier, B., Guy, B., Leclerc, J.-C., and Faure, O. (2005). Soil characteristics, heavy metal availability and vegetation recovery at a former metallurgical landfill: Implications in risk assessment and site restoration. Environ. Pollut. *137*, 316–323.

Sarwar, N., Imran, M., Shaheen, M.R., Ishaque, W., Kamran, M.A., Matloob, A., Rehim, A., and Hussain, S. (2017). Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. Chemosphere *171*, 710–721.

Shen, H., He, X., Poovaiah, C.R., Wuddineh, W.A., Ma, J., Mann, D.G.J., Wang, H., Jackson, L., Tang, Y., Neal Stewart, C., et al. (2012). Functional characterization of the switchgrass (Panicum virgatum) R2R3-MYB transcription factor PvMYB4 for improvement of lignocellulosic feedstocks. New Phytol. *193*, 121–136.

Shen, H., Poovaiah, C.R., Ziebell, A., Tschaplinski, T.J., Pattathil, S., Gjersing, E., Engle, N.L., Katahira, R., Pu, Y., Sykes, R., et al. (2013). Enhanced characteristics of genetically modified switchgrass (Panicum virgatum L.) for high biofuel production. Biotechnol. Biofuels *6*, 71.

Smith, R.A.H., and Bradshaw, A.D. (1979). The Use of Metal Tolerant Plant Populations for the Reclamation of Metalliferous Wastes. J. Appl. Ecol. *16*, 595.

Szabó, L., and Fodor, L. (2006). Uptake of Microelements by Crops Grown on Heavy Metal–Amended Soil. Commun. Soil Sci. Plant Anal. *37*, 2679–2689.

Turgut, C., Katie Pepe, M., and Cutright, T.J. (2004). The effect of EDTA and citric acid on phytoremediation of Cd, Cr, and Ni from soil using Helianthus annuus. Environ. Pollut. *131*, 147–154.

Vogel-Mikuš, K., Drobne, D., and Regvar, M. (2005). Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress Thlaspi praecox Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. Environ. Pollut. *133*, 233–242.

Wan, X., Lei, M., and Chen, T. (2016). Cost–benefit calculation of phytoremediation technology for heavy-metal-contaminated soil. Sci. Total Environ. *563–564*, 796–802.

Wei, B., and Yang, L. (2010). A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. Microchem. J. *94*, 99–107.

Weissenhorn, I., Glashoff, A., Leyval, C., and Berthelin, J. (1994). Differential tolerance to Cd and Zn of arbuscular mycorrhizal (AM) fungal spores isolated from heavy metal-polluted and unpolluted soils. Plant Soil *167*, 189–196.

Wu, Q.-S., and Xia, R.-X. (2006). Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J. Plant Physiol. *163*, 417–425.

Yao, Z., Li, J., Xie, H., and Yu, C. (2012). Review on Remediation Technologies of Soil Contaminated by Heavy Metals. Procedia Environ. Sci. *16*, 722–729.

Zhou, J.-M., Dang, Z., Cai, M.-F., and Liu, C.-Q. (2007). Soil Heavy Metal Pollution Around the Dabaoshan Mine, Guangdong Province, China. Pedosphere *17*, 588–594.