Amelioration of Metal Contaminated Soils by Biochars

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Sveučilište u Zagrebu
Agronomski fakultet

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MASTER'S THESIS

Jonti Evan Shepherd

Zagreb, June 2022

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Graduate study program:

Environment, Agriculture and Resource Management (INTER-EnAgro)

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Jonti Evan Shepherd

Supervisor:

Prof. Gabrijel Ondrašek, PhD

Zagreb, June 2022

STUDENT'S STATEMENT

ON ACADEMIC RECTITUDE

I, **Jonti Evan Shepherd**, JMBAG: 0178126853-4, born on the 17th of August 1992 in Johannesburg, South Africa, declare that I have independently written the master's thesis under the title of

Amelioration of Metal Contaminated Soils by Biochars

With my signature, I guarantee:

- that I am the sole author of this thesis;
- that all literature references, published or unpublished, are adequately cited or paraphrased, and listed at the end of this paper;
- that this thesis does not contain parts of other papers submitted at the Faculty of Agriculture or other higher education institutes, for the reason of completing studies;
- that electronic version of this thesis is identical to the printed one approved by the mentor; Professor Gabrijel Ondrasek, PhD
- That I am familiar with the regulations of the Ethical Code of Students of the University of Zagreb (Art. 19).

In Zagreb, June 15th 2022, *Students' signature,*________________

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REPORT

ON EVALUATION AND MASTER THESIS DEFENSE

Master thesis written by Jonti Evan Shepherd, JMBAG: 0178126853, under the title of

Amelioration of Metal Contaminated Soils by Biochars

Is defended and evaluated with the grade 5 (Excellent) on July 15th, 2022.

Committee for thesis evaluation and defense:

1. Mentor Prof. Gabrijel Ondrašek, Ph.D.______________________________
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Acknowledgement

I would like to express my gratitude to my supervisor, Prof Dr. Gabrijel Ondrašek, and Filip Kranjčec Ph.D., for their assistance and guidance in not just completing this master's thesis but obtaining the necessary materials, lab space, and greenhouses used to carry out the research. I would also like to thank Boris Lazarević for establishing the connection with Marta Gold the director of Sista d.o.o. who provided the industrial hemp seeds used in the study. I would like to express further gratitude to the Institute of Rijeka for the characterization of biochar particles by scanning electron microscopy – SEM, the AgroLab Group for the biogeochemical composition of the biochar types, as well as the University of Zagreb, Faculty of Agriculture for the chemical speciation and composition of the metal-contaminated soils, of which enabled accurate data measurements necessary for this successful experiment. To professors, Vilim Filipović, Mario Njvaro, Sanja Sikora, Hrvoje Kutnjak, Snjezana Topolovec-Pintarić, Kristina Krklec, Marina Bubalo Kovačić, Vedran Rubinić, Barbara Holzman, Robert Patterson, Snjezana Kreša, Lana Filipović, Monika Zavko, Edyta Đermic, Marko Petek, Irina Tanuwidjaja, Darko Vončina, and Marko Vinceković, I would whole heartedly like to thank each one of them for their teachings, compassion, and support.

Lastly, I want to thank my parents David and Lisa Shepherd, my sister, and brother-in-law Rio and David Shamula, as well as my grandparents Izzy and Bernice Rimer, Roy and Silvia Shepherd along with my aunts and uncles Martin and Jill Epstein, Anthony Rimer, Dana Mondry, Michelle and Roy Ellison, Debbie and David Jankelow, along with my cousins and the rest of my extended family and friends for all their support in me to conquer graduate school and write this thesis. Thanks to their encouragement, acceptance, and love, throughout my entire life, education, career, and chosen path, I truly would not be the person I am proud to be today. I will continue to strive forward towards success, and achieve every goal I have set before myself, not just for me but for my family and friends.

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ABSTRACT

Of the Master's Thesis – Student **Jonti Evan Shepherd**, entitled

Amelioration of Metal Contaminated Soils by Biochars

Metal contamination of soil represents a severe threat to human health via natural resources and food production practices due to the accumulation of heavy metals and metalloids via emissions displaced from rapidly expanding industrial sources. The addition of organic matter in the form of biochar to metal-contaminated soils could significantly impact the mobility and chemistry of three commonly found metals Cadmium (Cd), Zinc (Zn), and Lead (Pb), while creating suitable conditions for non-edible agricultural plants to be utilized for bioremediation practices. This study utilized two types of biochar 1) derived from wood chips (BC1), and 2) derived from horse manure (BC2), at two rates (1% and 4% w/w), as an amendment to metal-contaminated soil (MCS) to provide an in-depth physicochemical investigation of the MCS and tested biochars, comparing the metal chemistry in the MCS after the addition of biochar. Before the influence of the applied biochar treatments, all three metals exceeded the global average values for metal in soil; Cd 25-fold, Zn 56-fold, and Pb 127-fold. It was also confirmed that the addition of tested biochar types decreased the levels of high metal concentrations within the contaminated soil matrix by approximately 3% for Cd, 34% for Zn, and 93% for Pb. SEM analysis confirmed that micro to nano pore structure, surface area, and viable space for gas exchange in the amended soil was positively altered from the addition of the biochar types. Lab analysis confirmed that both biochar types enriched the soil with organic matter, N, C, P, K, S, and dropped the heavy-metal concentration within the test-crop by 1-3%. Thus, sowing of the test crop with biochar derived from wood chips and horse manure within metal-contaminated soil, leads to the amelioration of the soil, from metals such as Cd, Zn, and Pb. The amelioration of metal-contaminated soil with a particular biochar type could significantly affect the transfer of metals from a contaminated matrix to food production and water systems, thus allowing the chemical amelioration, and bioremediation of natural or artificial ecosystems globally that have been contaminated by heavy metals.

Keywords: Metal Contaminated Soil; Biochar; Metal Sorption, Metal Leaching; Soil Amelioration; Phytoremediation; *Cannabis sativa* L.

1.INTRODUCTION

Metal contamination of soil represents a severe threat to human health, soils, plants, aquatic life, soil biota, natural resources, and food production practices. This common threat of toxic metal insurgence in soils began 11,000 years ago, with the most notable negative changes beginning in the late 1780s during the Anthropocene era, which has affected our entire planet, growing in potency as time continues (Méndez, et al. 2012; WHO, 2021). Soils have since become contaminated by the accumulation of heavy metals and metalloids via emissions displaced from rapidly expanding industrial sources such as manufacturing plants, pesticides, wastewater irrigation, coal combustion residues, release or spillage of petrochemicals, atmospheric deposition, mine activities of all kinds including tailings and closed source points, disposal of high metal wastes, Pb products of which most notably are gasoline and paints, land application of fertilizers, animal manures most notably factory farm settings, and sewage sludge (Ernst, et al. 2000; Ju, et al. 2008; WHO, 2021). Heavy metals constitute both a poorly designated and under-regulated group of inorganic chemical hazards, of which Pb, Zn, and Cd, are some of those most commonly found within contaminated sites, while other commonly identified metals are arsenic (As), copper (Cu), mercury (Hg), and nickel (Ni) (Méndez, et al. 2012; Ondrasek, et al. 2021). Among many factors that determine the chemistry of metals, organic matter (OM) is one of the most important, for example, increased OM content causes metal mobility to decrease due to an organa-complexation (Ondrasek, et al. 2022).

Biochar is a charcoal-like substance that is created by burning organic material drawn primarily from agricultural and forestry wastes (also called biomass) in a controlled process called pyrolysis (Haider, et al. 2022). By adding biochar of varying concentrations into metal-contaminated soils, the mobility of metals will not only decrease, but a plethora of other physiochemical changes will occur (Ondrasek, et al. 2019; Ondrasek, et al. 2021). The perception of biochar may be that it looks a lot like common charcoal, but biochar is actually produced using a specific pyrolysis process that greatly reduces all types of contaminants entering the environment as well as allows the safe storage of carbon (Gao, et al. 2016). During pyrolysis, organic materials such as plant and animal biomass made up of; wood chips, multiple types of residual plant trimmings, food processing residues, as well as forestry cuttings, are essentially burned in a container that has been deprived of oxygen, resulting in the release of little to no polluting fumes (Mohan, et al. 2006; Mukherjee, et al. 2013; Mukherjee, et al. 2011). During this process, the above-stated types of organic materials are quickly converted into biochar, which is an extremely stable form of carbon that can’t easily escape into the atmosphere (Mukherjee, et al. 2013). It should be noted that the make-up of biochar will differ quite heavily depending on the type of OM used during the pyrolysis process, while this study utilizes biochar composed of wood chips and horse manure. It should also be noted that the energy or heat created during pyrolysis can be captured and used as a form of clean energy, making biochar the most energy-efficient product that is also able to convert carbon into a stable form of an applicable soil amendment (Kong, et al. 2014).

Sowing industrial hemp (*Cannabis sativa* L., cv. Henola) within toxic soils, causes the amelioration of the soil, leading to the successful remediation of metals such as Cadmium (Cd), Zinc (Zn), and Lead (Pb) (Kumar, et al. 2017). Hyper-accumulating plants to extract or mine heavy metals from contaminated soils have been employed in many studies globally, however, industrial hemp varieties have not been utilized in this manner proportionality, due to legal implications that are slowly been eased globally (Kong, et al. 2014). Research goals that may be able to be achieved are the potential industrial uses for the biomass of the hemp after the completion of a remediation project. This would be determined by future testing of fiber strength, chemical compositions, and food and safety guidelines that may play a large role in the restrictions of *Cannabis sativa* that had been formerly used for remediation practices (USA EPA, et al. 2020; USA USDA, et al. 2021). Although there is still much to learn and research, progress has been made in the understanding of mechanisms that govern Cd, Zn, and Pb, accumulation, and detoxification in accumulating plants. There has been quite a lot of recent progress in both soil biogeochemistry and plant physiology of Cd as stated by both (Wuana, et al. 2011; Wang, et al. 2020). The range of mechanisms of hyper-accumulation of Cd stretches through remediation strategies including both chemical and microbiological enhancement, as well as the optimization of field management practices (Wang, et al. 2020). The movement of Zn to plant roots has been noted as a hot topic in recent years due to its dependency on intensity factors like concentration, and on capacity factors like replenishment (Fellet, et al. 2011). Increasing the pH decreases the solubility of Zn in soils, which reduces not only the concentration, but the concentration gradient, and, hence, the uptake and availability of Zn to plants (Puga, et al.(2015). This is an important factor to take note of due to Zn's important role in auxin formation as well as in other enzyme systems (Wang, et al. 2020). Presently, Zn is recognized as an essential component in several dehydrogenases, proteinases, and peptidases, making it a vital part of the composition of soils and soil amendments (Haanstra, et al. 2003). This shines a greater light on Zn's potential, as it could possibly be up-taken by plants in areas of Zn toxicity and redistributed as plant biomass in areas of Zn deprivation. In addition to the challenges faced by Cd and Zn, Pb poses many risks to plants, however, there are many species of plants that are able to hyper-accumulate Pb in their leaves and roots, with some plants having the ability to do both (Wuana, et al. 2011). Phytostabilization has been accredited for the remediation of soils contaminated with high concentrations of Pb based on the bioaccumulation coefficient bioconcentration factor, and translocation factor (Haanstra, et al. 2003). Thus, providing high hopes, that the right combination of plants, soil, microorganisms, and soil amendments will be able to ameliorate heavy metal contaminated sites.

This study will analyze the soil and plant conditions that have been altered by biochar types derived from wood chips and horse manure, to determine if the combination has the ability to positively impact metal-contaminated soil. A controlled plant-pot-based experiment was conducted to study the effect of both biochar types (derived from wood chips – (BC1), and horse manure – (BC2)), and their rates of (1% and 4% w/w) on the release of metals from the MCS matrix collected near a former mine and metallurgical plant in the town of Zerjav, Slovenia, as well as their effect on the Henola's seed germination, that was tested in a lab utilizing the water leachate after a 175 day incubation period. The reason why industrial hemp was selected for this study is due to its high affinity of absorption toward all types of macro and micro elements found within soils (Kumar, et al. 2017). This combined with its strong resistance to toxins, ability to grow in small amounts of soil, and fast growth rate allows a direct correlation between *Cannabis sativa* L. and effective phytoremediation practices (Kamnev, et al. 2000; Kumar, et al. 2017). The germination testing utilizing leachate derived from the contaminated soil allowed further understanding of the effects and implications caused by the biochar types. Furthermore, the cellular composition of the plants' biomass from both treated and non-treated soils will be determined to ensure the accuracy of the ideal combination between the applied biochar percentage, biochar make-up, plant type, and the ratio of the contaminated soil matrix, which will be supported by the metal chemistry in the soil, vegetation parameters, and mineral composition of the Polish industrial hemp (Henola). This study will provide the necessary insight into the applicability of biochar and industrial hemp for their potential to remediate contaminated soils globally while allowing the remaining byproducts of plant mass to be further engaged by industry leaders for a plethora of non-edible purposes, ranging from textiles and building materials to fuel and paper substitutes.

1.1 Hypothesis and Aims

The hypothesis of this research is that the addition of organic matter in the form of biochar to metal-contaminated soils could significantly impact the mobility and chemistry of three commonly found metals in the examined soil (Cd, Zn and Pb), which will be tested under controlled conditions.

The study will be performed utilizing two different types of biochar; 1) derived from wood chips (BC1), and 2) derived from horse manure (BC2), at two rates (1% and 4% w/w) to metal-contaminated soil. The main goal of the study is to investigate the effect of the biochar types and rates on metal-contaminated soil, while utilizing *Cannabis sativa* L. (Polish industrial hemp, Henola) as the test crop for phytoremediation practices and data.

This study aims to:

- provide an in-depth physicochemical investigation of metal-contaminated soil and tested biochars,

- evaluate and compare the metal chemistry in the metal-contaminated soil after the addition of biochar,
- evaluate vegetation parameters such as seed germination, plant height, and dry matter yield of the test crop after the addition of biochar, and

- examine the mineral composition of the test crop after the addition of biochar.

2. Literature Review

2.1. Metal Contaminated Soils - A global Environmental Issue

Historically, on a global scale, agriculture was the first major anthropogenic influence on the soil, however, it was far from the last. Since the start of agriculture some 11,000 thousand odd years ago, soils along with the surrounding environment have been receiving varying amounts of pollution (Hass, et al. 2012). It should be noted that the first major notable implications of the demise of soils began around the 1780s within the Anthropocene era due to the rise of the industrial revolution (Méndez, et al. 2012). It is well known that soils are a major sink for heavy metals that have been released into the environment by the before-mentioned anthropogenic activities, however, unfortunately unlike organic contaminants that are oxidized into carbon (IV) oxide by processes undertaken via microbial action, the vast majority of metals do not undergo chemical or microbial degradation, meaning that their total concentration in soils is able to persist for a long period of time after each metals initial introduction (Simona, et al. 2004; Steinbeiss, et al. 2009). Changes in metals' chemical forms (speciation) and their bioavailability are, however, possible and have been studied within other research opportunities (Méndez, et al. 2012). Due to the presence of toxic metals in soil, the biodegradation process of organic contaminants can be severely inhibited (Maslin, et al. 2000; Lal, et al. 2015). Heavy metal contaminants within the soil, generally pose a plethora of both risks and hazards to humans and the larger ecosystem as a whole due to: direct ingestion or contact with the contaminated soil, within the food chain (soil-plant-human or soil-plant-animal-human), drinking of contaminated groundwater as well as leeching from aggregated areas, heavy reductions in food quality via phytotoxicity employing testing for safety and marketability, however, in some cases the food will go towards food waste as it cannot be utilized for either human or animal consumption, reduction in land usability requiring some sort of remediation project as well as a potential agricultural production loss leading to food insecurity, and land tenure problems (Harvey, et al. 2011; Inyang, et al. 2016). In order for both adequate protection and restoration of soil ecosystems that have been contaminated by heavy metals, there are initial requirements that are necessary for either remediation or other solutions to take place, being the characterization, source, range of affected distance, and affected depth (Houben, et al. 2013).

Contemporary legislation designated to the protection of public and environmental health, at all government levels between local, national, and international levels, is purely based on the characterization data that allots the chemical properties allowance within each microsystem, whether it be that of the environment, our food chain, or the garden in which a school uses for children to play in (Glass, et al. 2000). Even with the above-mentioned data based on soil characterization, the policy would still need to provide funding (in most cases) for the heavy metal speciation testing and actual bioavailability, while attempting the strategies of remediation that may entail high costs due to the amount of required knowledge about the source of contamination, basic chemistry, fixation strategies, environmental and associated health effects that the heavy metals pose (Maslin, et al. 2000; Mulligan, et al. 2001). Because of the high potency of health threats, decision makers generally look towards solutions that can guarantee the removal of the metals causing the contaminations in the most cost-effective manner while still preserving both public and ecosystem health (Shah, et al. 2020). Some of the most effective solutions are immobilization, soil washing, and bioremediation which can be composed of combinations of phytoremediation, and microbial-remediation, which are all techniques that are frequently utilized and noted for their ability to ameliorate heavy metal-contaminated sites (Mench, et al. 2000; Pilon-Smith, et al. 2005; Shah, et al. 2020). Unfortunately, the majority of developing countries are not financing these techniques in spite of their cost-effectiveness and environmental friendliness, instead, they are mostly reliant on outside organizations for aiding the people within affected communities instead of addressing the main issues. Secondly, it should be noted that many developing countries do not possess the required technologies due to no commercial availability which may possibly be from an inadequate amount of awareness of each technology's inherent advantages and the simplistic principles of operation (Shah, et al. 2020). Luckily due to greater awareness by governments but more so by the public as to what the actual implications of contaminated soils are to both human and animal welfare, there remains a steady increase of interest amongst the scientific community, that is finding greater funding allowing the development of the above-listed technologies and some new options to remediate the growing number of contaminated sites.

Dating back almost 400 years is the common practice of applying both municipal and industrial wastewater as well as related effluents to the land which takes place in most parts of the world. Globally, it is estimated that 20-25 million hectares of arable land are irrigated with wastewater (Salleh, et al. 2022). However, in several Asian and African cities, studies suggest wastewater irrigation within agriculture actually accounts for roughly fifty percent of all vegetable supplies to urban centers (Zaman, et al. 2004). This is due to the fact that farmers in these regions are generally not concerned with benefiting or harming the environment, instead, they are primarily interested in maximizing their profits, which are directly linked to their yields (WHO, 2021). Notably, heavy metal concentrations in wastewater effluents are quite often relatively low, meaning that it will take an extensive period of time utilizing wastewater irrigation on the land to eventually result in the accumulation of heavy metals within the soil (Ondrasek, et al. 2021). Due to this large time frame, there are fewer funded studies within the scientific community, leading to research developments within other areas, that are producing a much more drastic situation of environmental toxicology, namely the processes of mining and milling metal ores (WHO, 2021). These activities have caused the widest distribution of metal contamination in soil, especially if held accountable for the products they have produced over the centuries.

The reason that these industries are so detrimental to the surrounding environment is that, during the mining process, tailings (heavier and larger particles settled at the bottom of the flotation cell during mining) are directly discharged into catchments which are generally natural depressions within surrounding landscapes (WHO, 2021). The reason that this happens is that the above-mentioned flotation cells were actually developed in order to both separate and recover sulfide ores (which are high in value) from low-grade ore bodies (Zaman, et al. 2004). The way in which this takes place is by the ore being crushed and then milled within the concentrator during a process known as comminution, which entails the mineral in a suspension of slurry (Blaylock, et al. 2000). Extensive ore mining in conjunction with smelting activities have resulted in the contamination of soils globally, of which are characterized as posing a serious threat to human and ecological health (WHO, 2007). Due to assimilation pathways being linked to the bioavailability metals have with plants, our major inputs are coming from foods, however because of our global emission rates we are also inhaling and drinking these same metals, moreover there are no places left on the planet that has not been affected (Blaylock, et al. 2000).

Airborne sources of metals include but are not limited to both stack and duct emissions of air, gas, or vapor streams, as well as fugitive emissions resulting from dust derived from soil mixing and chemical storage areas or waste piles (WHO, 2007). Most of the metals that are stemming from airborne sources are generally released as particulates which are contained in the gas stream (Li, et al. 2018). However, there are some metals such as Cd, Pb, and As, that can volatilize during high-temperature processing, causing unfavorable conditions for the environment (Beyersmann, et al. 2016). This is because these metals have undertaken a conversion into oxides, which causes them to condense into fine particulates unless a reducing atmosphere is maintained (Li, et al. 2018). Stack emissions are unfortunately able to be distributed over an extremely wide area due to natural air currents, however, once these emissions come into contact with dry and/or wet precipitation mechanisms they will be removed from the gas stream (Inyang, et al. 2012).

Fugitive emissions are easier to trace due to the distribution patterns taking place over a much smaller area, simply because these emissions are released near to the ground (WHO, 2007). It should also be noted that in most cases, contaminant concentrations stemming from fugitive emissions are lower in comparison to those stemming from stack emissions (Kızılkaya, et al. 2008). Each source of both of these types of emissions will determine its own composition of metals being emitted, with rarely any of them being identical due to a variety of reasons ranging from what is being produced or destroyed, to the type of filters if any that are being utilized to capture emissions (Inyang, et al. 2012). It is a fact that all solid particles within smoke stemming from fires or from other emission sources like factory chimneys will eventually be deposited on either land or sea (Beyersmann, et al. 2016). Most notably and famously recognized are fossil fuels which contain multiple forms of some heavy metals, which have been causing widespread contamination along with countless other extremely large-scale global issues since the industrial revolution began. A great example of this is the extraordinarily high concentration of Cd, Pb, and Zn that have been located within plants, soils, and waterways that are adjacent to smelting works (Xu, et al. 2013). Another major aerial source of soil contamination is the emissions related to the combustion of petrol/gasoline which contains tetraethyl Pb; this majorly contributes to the substantial issues of high Pb concentrations within soils in both urban areas and in areas that are directly adjacent to major roadways (Li, et al. 2018). Zn and Cd should also be added to the list of contaminants in soils adjacent to roads because one of the major sources for both metals is tyres/tires, and lubricant oils (Kizilkaya, et al. 2008).

The most commonly located heavy metals within contaminated sites, written in order of their abundance are Pb, Cr, As, Zn, Cd, Cu, and Hg (Kizilkaya, et al. 2008). These listed metals are extremely important due to their capability of them to decrease crop production because of issues stemming from bioaccumulation and biomagnification occurring within the food chain (WHO, 2021). Both the fate and transport of heavy metals in soils depend significantly on each individual's chemical speciation and form (Xu, et al. 2013). This can be further acknowledged once in the soil because heavy metals are adsorbed initially by fast reactions that can take place over the course of minutes or hours followed by the slower adsorption reactions which will take place over the course of days, weeks, months, or years, and are, therefore, redistributed back into different chemical speciation having varying mobility, toxicity, and bioavailability (Inyang, et al. 2012). This distribution is linked to the control entailed by multitudes of reoccurring reactions that take place amongst heavy metals in soils such as (i) ion exchange, adsorption, and desorption, (ii) mineral precipitation and dissolution, (iii) plant uptake, (iv) biological mobilization and immobilization, and (v) aqueous complexation (Xu, et al. 2013).

Finally, in biological systems, heavy metals are known to heavily affect cellular organelles as well as other cellular components such as the endoplasmic reticulum, cell membrane, lysosome, mitochondrial, nuclei, as well as some processes that involve enzymes involved in metabolism, damage repair and, detoxification (Tchounwou, et al. 2001; Yedjou, et al. 2008). Metal ions have been identified on multiple occasions to interact with multitudes of cell components such as DNA as well as nuclear proteins, which causes DNA damage and conformational changes which have been shown to lead to cell cycle carcinogenesis, modulation, or apoptosis (Yedjou, et al. 2008). Multitudes of studies have revealed that within reactive oxygen species, both production and oxidative stress play a major supporting role in the actual toxicity and carcinogenicity of metals such as As, Cd, Cr, Pb, and Hg (Beyersmann, et al. 2016). Due to their high degree of toxicity, these five metals have continually ranked among the priority metals that need to be monitored and further studied due to their public health significance (Yedjou, et al. 2008). The reason is, that the above-listed metals are known systemic toxicants that can induce damage to multiple organs, at low levels of exposure, thus justifying concern. Both the United States Environmental Protection Agency, and the International Agency for Research on Cancer, have declared and classified these metals as human and animal carcinogens (Beyersmann, et al. 2016). Heavy metal-induced toxicity should be considered a major threat to the well-being of all living organisms on this planet. (Beyersmann, et al. 2016) Thus, this paper will continue to explore the solutions that we have been researching to ameliorate soils in order for the well-being of current and future generations.

2.2. Amelioration of Metal Contaminated Soils by Biochar

Biochar as a highly porous, carbon-rich organic material is currently receiving enormous amounts of attention due to its significant beneficial uses. It has been tested and proven to remediate heavy metal-contaminated soils in multitudes of studies and is continuing to be researched in order for greater gains and relationships to be established (Enders, et al. 2012). Applying and amending a vast array of soils with biochar has shown the reduction of both bioavailability and leachability of heavy metals as well as the inhibition of the accumulation of heavy metals within the edible portion of plants (Li, et al. 2014). Multiple types of biochar derived from a variety of organic materials ranging from horse manure to wood chips and even plant biomass have been utilized to greatly reduce the bioavailability of Cd, Pb, and Zn (Du, et al. 2011). In a study conducted by (Puga, et al. 2015) these three metals decreased by 56.5%, 50.0%, and 54.0%, respectively with an application rate of 5%. Other research studies have proven that biochar derived from bamboo and rice straw were able to significantly reduce both the $CaCl_2$-and diethylenetriaminepentaacetic (DTPA)-extractable heavy metals in soil, as well as the ability for organic-bound fractions to form for many heavy metals, increased at a significant level (Xue, et al. 2012; Lu, et al. 2017). There have also been considerable studies performed in both the lab and field to verify the ability of biochar to stabilize heavy metals within soils, however, it has not been deemed as an efficient enough method to be utilized for the purposes of practical application, although ongoing research may soon alter that fact (Alpaslan, et al. 2002).

In more recent studies, researchers have begun increasing not only the application rate of biochar but the variety of feedstock with the level of pyrolysis in order to achieve the desired level of stabilization efficiency towards heavy metals (Sun, et al. 2014). Although the maximum application rate of biochar can reach up to 20%, this approach is rarely used and studied for non-practical purposes, due to the cost associated with the creation and application of biochar (Wang, et al. 2020). In the future, it may be possible that the creation costs can become significantly decreased, however it should be noted that there are ulterior options for the amelioration of contaminated sites, that cost similar amounts of funding (Lu, et al. 2017). These application rates are unfeasible for agronomic operations by farmers or environmental engineers, which inhibits the large-scale practical application of biochar in agricultural soils (Wang, et al. 2020). The downside factor that must be taken into account lies in the potential of an overdose of biochar that has the ability to increase the risk of soil alkalinity issues occurring as well as decrease the biomass of crops (Cui, et al. 2012; Khan, et al. 2015). Secondly as stated by (Beesley, et al. 2013), due to the unbalanced nutrient that lies within the content of biochar, there is a potential for it to lead to unfavorable conditions for the germination of seeds. That is why this current research is underway, in the hopes of locating an alternative method that can enhance the stabilization efficiency of biochar without simply increasing the actual application rate to the necessary allotments that would alleviate the burden caused by heavy metals (Wang, et al. 2020).

However, it must be noted that great strides of success have been accomplished via the modification process of biochar due to the increased number of oxygen-containing functional groups like, -OH, and –COOH, as well as thiol groups like, -SH, which occur on the surface of biochar (Uchimiya, et al. 2011). This rapid increase in functional groups has a direct effect on both the surface area as well as the total pore volume, which has shown significant increases (Uchimiya, et al. 2011). After completion, it has been concluded that the stabilization efficiency of heavy metals will increase drastically in comparison to that of the original biochar (Cobbett, et al. 2002). This is important because it is indicating that the surface complexation linked to functional groups can actually be the main mechanism for the stabilization of Cd (O’Connor, et al. 2018; Wang, et al. 2018). In summary, the modification process has been proven to increase not just pore volume, but both surface area and surface functional groups as well (O’Connor, et al. 2018). Indicating that biochar can in fact be utilized to efficiently remediate not just Cd, but Pb, and Zn contamination in multitudes of different soils, effectively reducing both the bioavailability and leachability (Chaperon, et al. 2008). Thus, the mobility of many species of heavy metals can effectively be transformed into a more stable fraction of themselves, decreasing the harsh soil conditions initially caused by the metals (D’Amore, et al. 2005). Not to be taken for granted is the fact that biochar is able to improve all studied soil properties to some extent while simultaneously enhancing soil enzyme activity (O'Connor, et al. 2018). These results have proven that biochar is an extraordinary amendment for soils suffering from metal contamination, as well as that, it has still unsolved characteristics that may be able to form greater qualities of the necessary traits for remediation practices.

2.2.1 The composition, production, and physiochemical characterization of Biochar

Considering the properties that have been previously explored, detailing the significance of biochar in remediation practices, it is necessary to understand more about the product itself. The simplest way to begin is in terms of the physical attributes of biochar being that it is black, highly porous, extremely lightweight, generally fine-grained, and has quite a large surface area (Spears, Stefanie. 2018). Some of the greatest attributes in terms of functionality is that it is composed of approximately seventy percent carbon, while the remaining percentage consists of varying percentages of nitrogen, hydrogen, and oxygen among some other elements depending on its feedstock type (Cornelissen, et al. 2016). Generally, the carbon content of biochar will range between 380 and 800 g kg-1 and can easily be characterized by both its aromatic and alkyl structures (O’Connor, et al. 2018). The other notable inorganic elements that biochar contains are, Si, K, Al, Ca, and P, although that depends on its feedstock type, its chemical composition can still vary quite greatly containing other elements that may potentially affect the methods utilized during the pyrolysis process (Cornelissen, et al. 2016). However, within this study, the biochar types are composed of manure and wood chips. It should be noted that the general pH value of biochar ranges between 5 through 12 according to (Xu, et al. 2015), which is directly linked to the pyrolysis temperature which tends to increase the pH with increasing temperature mostly due to the decomposition of bionic acid found with biochar (Beesley, et al. 2014). Secondary to this the temperature increase also leads to increases in the concentrations of mineral alkali elements such as Na, Ca, K, and Mg (Clemente, et al. 2006).

Biochar technology has been considered a more recent strategy for carbon sequestration, the practice of adding charred biomass to improve soil quality is not new at all (Gil-Sotres, et al. 2005). This process is modeled after its origins that date back to a 2,000-year-old practice from the Amazonian basin, where indigenous people created areas of rich, fertile soils called Terra Preta which literally means dark earth (Novak, et al. 2009). It should be noted that scientists are still unsure about whether these soils were intentionally made or are simply a by-product of different farming and/or cooking practices. Due to the production of biochar revolving around the process of pyrolysis, which can be easily understood as the thermal decomposition of biomass within an oxygen-limited environment, biochar can be claimed as a carbon sequestration source that releases clean and renewable energy as a byproduct (Qian, et al. 2015). Not only this but due to the above-stated properties of biochar, it also plays a large part in benefiting the environment due to its ability to decrease groundwater pollution, as well as cause the reduction of agricultural wastes that in turn provide an extra profit bracket for farmers (Toková, et al. 2020). Along with those benefits are the contributions biochar makes toward food security by simply increasing most crop yields while simultaneously retaining water within areas that are prone to drought (Choppala, et al. 2012).The production process of biochar in general is considered a carbon-negative process due to it reducing the amount of available carbon dioxide within the atmosphere (Kumar, et al. 2011). Because biochar is composed of approximately seventy percent carbon, the application of biochar to soils can be considered a carbon sink that has the potential to secure a place that can store carbon for potentially hundreds or thousands of years, alleviating some of the main stresses surrounding climate change (Hu, et al. 2010). Biochar can also be considered as a product that is a direct correlation to the mitigation of climate change by reducing the need for chemical fertilizers as well as for shortening irrigation times, which are both linked to the reduction of greenhouse gas emissions (Toková, et al. 2020).

Although the small-scale operations are mostly utilizing biochar for its rich sources of carbon, due to biochar having an intricate microstructure that contains numerous pore spaces, it is able to contribute to enhancing its own specific surface area, that in turn lands up benefiting the soils that it is applied to (Lee, et al. 2010). This is notable because biochar types for a general average typically have large surface areas, that range between 100 to about 460 m2 g-1 (Brewer, et al. 2014). Not only this but due to the diverse functional groups, which are, carbonyl, carboxylic, hydroxyl, and phenolic groups, the actual porosity along with the specific surface area of each individual biochar is easily altered by the increase or decrease in pyrolysis temperature (Chen, et al. 2018). The way that porosity and surface area are actually increased is due to the increases in temperature as stated previously, which force volatile substances out of the char, which in turn creates higher levels of porosity as well as expands the surface area (Peng, et al. 2011; Pandey, et al. 2020). The result of the increased porosity along with the increased surface area contribute to the high water-holding capacity that biochar encompasses (Chen, et al., 2018). Lastly, it should be noted that the previously mentioned physicochemical characteristics that define biochar generally led to subtle changes in soil pH, as well as in the water-holding capacity, base saturation, and even the cation exchange capacity (Pandey, et al. 2020). Now that a keen understanding of biochar has been accomplished, the rest of this research will focus on how the aforementioned principles were utilized to aid the study.

3. Materials and Methods

3.1. Experimental setup

The two types of biochar; wood chip-derived biochar (BC1) and horse manure-derived biochar (BC2), with a detailed physicochemical characterization were provided by PYREG GmbH, Germany (Figure 1). In addition, both types of biochar were analyzed using scanning electron microscopy (SEM) (Jeol JSM-7800F, Tokyo, Japan) at the Department of Physics and Center for Micro and Nano-sciences and Technologies, University of Rijeka, Croatia.

Figure 1. Presentation of wood chip-derived biochar (BC1, left) and horse manure-derived biochar (BC2, right)

Figure 2. Contaminated soil after being sieved to < 2 mm and before being mixed into pots

The metal-contaminated soil was sampled in 2019 from a landfill near the former Pb and Zn smelter in Žerjav municipality, Slovenia. The soil was sampled in a destroyed state from a depth of 0-30 cm and stored in plastic bags and delivered to the analytical laboratory of the Department of Soil Amelioration (MELILAB) at the Faculty of Agriculture Zagreb.

The soil was air-dried, manually homogenized, and then sieved through a sieve with a wire mesh density of 2000 μm. Preparation of samples for analysis was made according to the standardized procedure of soil preparation for physical and chemical analyzes (HRN ISO 11464: 2004). From this prepared soil, an average sample was taken in triplicates to be then examined on physicochemical parameters utilizing standard methods (Table 1).

Table 1. Physicochemical parameters and methods used for soil characterization

Parameter	Method
pH	HRN ISO 10390:2005
Electrical conductivity (dS/m)	HRN ISO 11265:2004
Organic matter (%)	HRN ISO 14235:2004
Available P and K (mg/100 g)	AL method (Egner et al., 1960)
Total Cd, Co, Cr, Cu, Ni, Pb, Zn, Mo, Al, K, Ca, Na, Fe, Mg, Mn, P, S, V (mg/g)	HRN ISO 11466:2004 HRN ISO 22036:2011
Moisture (%)	HRN ISO 11465:2004
Mechanical composition (%)	HRN ISO 11277:2004

The study was conducted in a greenhouse at the Department of Vegetable Crops, Faculty of Agriculture, and within an analytical laboratory within the Department of Soil Amelioration (MELILAB) at the Faculty of Agriculture, both part of the University of Zagreb. Applied treatments were organized in a completely randomized block design as presented in (Table 2). In brief, the weighted values of the metal-contaminated soil and particular biochar type were placed in the PVC pot (3 L) in combination as follows; Control - 2500 g of metal-contaminated soil, (BC1 1%) – 2475 g contaminated soil, 25 g biochar 1, (BC1 4%) - 2400 g contaminated soil, 100 g biochar 1, (BC2 1%) - 2475 g contaminated soil, 25 g biochar 2, (BC2 4%) - 2400 g contaminated soil, 100 g biochar 2 (Table 2).

Table 2. Applied Treatments

1. Control – 100% metal-contaminated soil
2. Metal-contaminated soil (99%) + biochar BC1 (1%)
3. Metal-contaminated soil (96%) + biochar BC1 (4%)
4. Metal-contaminated soil (99%) + biochar BC2 (1%)
5. Metal-contaminated soil (96%) + biochar BC2 (4%)

After manual mixture and homogenization of the pot content, water moisture was gravimetrically maintained at 100% of water field capacity by adding distilled water coupled by regularly mixing for the next 175 days of the incubation. After the incubation period the simulation of precipitation over the PVC pots was performed by adding a certain volume of distilled water, in order to collect an aqueous percolate (50 mL) from the PVC pot. The percolate samples were filtered with a white tape filter and examined for specific chemical parameters shown in (Table 3).

Table 3. Detected Parameters in Water Percolates from the Contaminated Soil

Parameter	Method
pH	HRN ISO 10523:1998
Total content of Cd, Cu, Pb, Zn	HRN ISO 22036:2011
Dissolved organic carbon (mg/L)	HRN EN 1484:2002

In order to gather the required aqueous solution for the germination test, the collected percolates (4 ml) were pipetted into Petri dishes that contained five seeds/dish of the test crop and maintained under the lab conditions for true germination to occur. On the fifth day after pipetting the radical length of each seed was determined. This was done via two methods, one of which was utilizing standard measurement tools to determine the radical length by hand, while the second method consisted of photographing each petri-dish with measured grid lines in the background for the purpose of allowing the measurement software, Aequo, to determine the length of each radical.

After the incubation period the Henola seeds were sown (4 seeds/pot) and grown for next 126 days to fully maturity. Throughout this period water management was maintained from 80-100% field water capacity by refiling PVC pots with distilled water. At the end of experiment, the whole plants were sampled from each pot and then divided into, root, stem, leaf, and inflorescence. The root samples were carefully separated from the soil, then washed under running water and immersed in 5 mM $CaCl_2$ solution for 20 minutes, then washed and immersed in distilled water for 20 minutes, and carefully dried with paper towels. Such prepared samples of roots and remaining aboveground organs were then weighed in fresh condition and allowed to dry at 65°C for 24 hours. After 24 hours of drying, the samples of plant material were weighed again, to be then grounded and prepared for laboratory chemical analyses. Finally, from each PVC pot after the plant sampling, soil samples were taken and prepared for laboratory chemical analyses. The areas of vegetative performance that were analyzed are Stem – height, diameter (base, midway, top), biomass (fresh, dry), Leaf – total number, fallen off total, biomass (fresh, dry), Root – biomass (fresh, dry), Inflorescence – biomass (fresh, dry), and Total plant – biomass (fresh, dry). For each analysis, there are the captured values from the parameters listed above, as well as a standard error, and a fisher comparison test run, in order to accurately understand the data and determine if there are statistical differences within the observed data to support or reject the null hypothesis.

3.2. Data Processing and Analysis

The amelioration effects of the applied biochar type and its rates in metal-contaminated soil on observed variables were examined by analysis of variance (ANOVA), while the significance of differences among mean values of applied treatments was determined by the Fischer t-test at $p<0.05$. Complete data analyses were performed using the SAS 9.3 (SAS Institute Inc, et al. 2011) computer package.

4. Results and Discussion

4.1. Physiochemical Properties of Metal Contaminated Soil

The physiochemical properties of the contaminated soil utilized for this research are defined by a neutral soil pH (H_2O) averaging 7.3, allowing peak availability of the most necessary nutrients for plant uptake (Table 4). A soil electrical conductivity averaging 0.33 dS/m indicates a non-saline soil, with the organic matter averaging 7.09%, P_2O_5 105.3 mg/100kg, and K_2O 25.9 mg/100kg (Table 4). The particle size percentages of the soil were as follows 5% of clay (<0.002 mm), 27% of fine silt (0.02-0.002 mm), 25% of coarse silt (0.063-0.02 mm), 20% of fine sand (0.2-0.063 mm), and 23% of course sand (2-0.02 mm) (Table 4). The soil type can be classified as a silty loam.

Table 4. An average (n=3) chemical and physical properties of tested metal-contaminated soil

pH_{H2O}	EC dS / m	Organic matter %	P_2O_5	K_2O	Particle size distribution (%)				
			mg/100g		2 - 0.2 mm	0.2 -0.063 mm	0.063 -0.02 mm	0.02-0.002 mm	<0.002 mm
7.3	0.33	7	105	26	23	20	25	27	5

There is an extraordinarily high average level of Cd occurring at (25 mg/kg) in comparison to the global mean of (0.36 mg/kg) (according to the USA EPA soil health guidelines) confirming highly contaminated soil (Table 5). The concentrations of both Pb and Zn within the examined soil also confirmed that the soil matrix is highly contaminated, given that the average concentration of, Pb (5098 mg/kg), and Zn (3133 mg/kg) were greatly increased in comparison to the global average concentrations of (15-40mg/kg) for Pb, and (55 mg/kg) for Zn (Table 5). The concentrations of Cr (34.1 mg/kg), Cu (55.7 mg/kg), and Ni (20 mg/kg), remained within acceptable natural values (Table 5). Concentrations of other elements followed their natural concentrations, valid for uncontaminated conditions, e.g. Al (32.8 g/kg), Ca (66.5 g/kg), and Fe (31.8 g/kg) (Table 5). The content of Mg (39.4 g/kg), Mn (823.1 mg/kg), P (2366.6 mg/kg), and S (1136.9 mg/kg) are heavily elevated between 9-47 times the global mean naturally occurring values (Table 5). Na and K values are highly elevated with Na (564.9 mg/kg), and K (5.9 g/kg)(Table 5). These heightened values make biochar types exceptionally important as they should not contribute more towards increasing those values.

Table 5. An average (n=3) concentration of elements in tested metal-contaminated soil

Cd	Co	Cr	Cu	Ni	Pb	Zn	Al	Ca
mg/kg							g/kg	
25	9.6	34	56	20	5098	3133	33	67
Fe	Mg	Mn	P	S	Mo	V	Na	K
g/kg		mg/kg						g/kg
32	39	823	2367	1137	14	72	565	6

Although there are heightened concentrations of many metals due to aforementioned factors, this soil analysis data provides an ideal study condition for the alteration of the variable metals of Cd, Pb, and Zn caused from the amendment of biochar along with the germination and growth of the test crop. Not only this, but as seen in the scanning electron microscopy (SEM) (Figure 3) and proven by (Table 5), a further understanding can be comprehended of how heavy metals are distributed, leached, and withheld in the tested soil type, as well as how to plant uptake and growth parameters are undergone.

As seen in (Table 5), the high density of larger soil particles along with the enabled porosity for water infiltration into this soil will allow multitudes of different plant species ample distributions of soil gas, and soil water to grow without restriction. This context will allow the furthered ability for the remediation of contaminated sites, along with a greater insight for strategy planning surrounding the management and conservation of natural resources, that lay within the vicinity of polluted regions. The next few paragraphs will further explain Cd, Zn, and Pb within soil systems for a greater understanding of how this study will utilize their functions towards a successful outcome.

The area is famously known for its mining and Pb-smelting plant, which the smelter started operating at the said location in 1896 (Nathanail, et al. 2004). However, it must be noted that the peak of Pb production occurred in the 1970s, which means the smelter emitted approximately 5000 kg of particles daily, containing a staggering 2000-2500 kg of Pb (Fugaš, et al.1984; Miler, et al. 2013). Although this specific plant had a filtering system installed in 1978 which helped to drastically reduce the daily emission of particles to 70 kg (Fugaš, M.; Hršak, J.; Souvent, P. 1984). By 1990, the smelting business was coming to a rapid slow down and so the smelter was converted into an industrial facility that was utilized for recycling Pb batteries (Vidic, et al. 2006). Although this process was less intrusive to the annual emissions, it was still responsible for emitting particles that ranged from 6500 to 1500 kg annually (Miler, et al. 2013). After careful analyses researchers have identified particles that they have classified as a combination of anthropogenic, geogenic–anthropogenic, and secondary products formed from the weathering process. The before mentioned geogenic–anthropogenic particles are recorded as ore minerals (Zn- and Pb-) which originated from waste deposits as a result of mining (Vidic, et al. 2006). After roughly a little over three hundred years of heavy pollution in the surrounding area, the region became a gradually eroding grassland, that boasts barren rocks and little plant life in comparison to what that landscape would under natural conditions without human interference (Kumar, et al. 2021). Due to the widespread contamination the region has been nicknamed Death Valley, making it the perfect starting point for a remediation project (Vidic, et al. 2006).

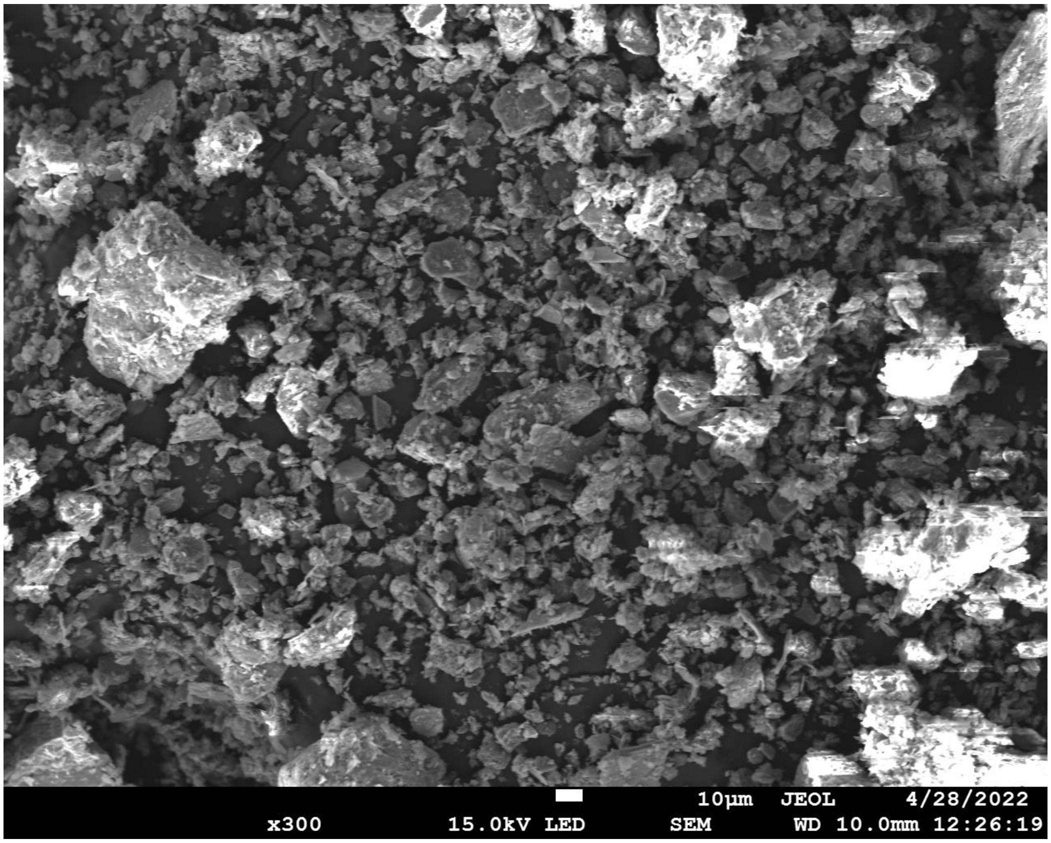


Figure 3. SEM micrograph of tested metal-contaminated soil

Cd loading in soils and the environment has been accelerated worldwide due to enhanced industrialization and intensified agricultural production, particularly in developing countries (Gardea-Torresdey, et al. 2004). Soil Cd pollution, resulting from both anthropogenic and geogenic sources, has posed an increasing challenge to soil quality and food security as well as to human health (Gao, et al. 2016; Filipović, et al. 2018). Cd demonstrates accelerated mobility along the food chain and within the environment, creating a vital need for its amelioration due to its harmful effects on human health and toxic nature towards biota at lower concentrations (Sanchez-Polo, et al. 2002). Due to Cd having no physiological function in plants, its extenuating presence in soils leads to various deleterious effects, ranging from causing physiological, chemical, and structural changes in some plants (Filipović, et al. 2016). There are species similar to industrial hemp that are able to mitigate the phytotoxic effects of Cd (and other toxic metals) to a varying potency dependent on the soil type (Gressel, et al. 2005). However, it should be noted that of all heavy metals, Cd is adjudged to be one of the most toxic to plants (Filipović, et al. 2018). Several exogenous substances are currently being utilized to mitigate the toxic effects of Cd in plants. Zn is one of the seven essential plant micronutrients that may be able to alleviate Cd toxicity due to their chemical similarity (Shen, et al. 2016; Rizwan, et al. 2019). Several studies have demonstrated that Zn can alleviate some of the toxic traits stemming from Cd in plants by stimulating multiple growth factors, which allow the regulation of Cd uptake while increasing the plant's photosynthetic rate, and reducing oxidative stress (Roy, et al. 2013; Rizwan, et al. 2017). It should be noted that the role of Zn on Cd accumulation within plants is still a highly debated topic and needs further research, however, several factors ranging from, genotypes, growth conditions, plant species, concentrations within the medium, and the exposure duration, are showing great promise in alleviating the very present issue that Cd is causing globally (Jones, et al. 2012).

Although Zn is perhaps responsible for sections of mediation, it can also be viewed as a toxic element to plants, at higher concentration levels. Zn performs imperative functions throughout numerous metabolic pathways within a plant, although it must be stated that as the ratio rises it becomes potentially noxious in soils resulting in various alterations within plants (Sun, et al. 2022). For example, reduced photosynthetic, growth, and respiratory rates, as well as enhanced generation of reactive oxygen species and imbalanced mineral nutrition (Gardea-Torresdey, et al. 2009). Zn can enter soils through various sources, ranging from volcanoes, the weathering of rocks, forest fires, mining and smelting activities as stated by (Moreno, et al. 1999; Moreno, et al. 2003), while sewage sludge, phosphatic fertilizers, and manure are other notable sources as confirmed by (Filipović, et al. 2020). As stated previously, this study will focus on soils that have been contaminated by mining activities, which showcase the differences between Zn essentiality and toxicity within plants (Kaur, et al. 2021). Although Cd is the most toxic heavy metal to plants, and Zn has its pros and cons, due to the anthropogenic activities stated previously, Pb has continued to be a growing issue due to the redistribution of Pb from the earth's crust to the soil and to the environment (Lu, et al. 2012).

Pb forms various complexes in conjunction with soil components, leading to only a small fraction of the Pb present as these complexes in the soil solution become phytoavailable (Inyang, et al. 2011). Despite its lack of essential functions within plants, Pb is absorbed within the rhizosphere via the apoplastic pathway or via Ca^{2+}-permeable channels (Pourrut, et al. 2011; Pourrut, et al. 2013). The behavior of Pb within soils, as well as the uptake by plants, is controlled by the soil pH, soil particle size, root exudation, speciation, cation-exchange capacity, root surface area, and the degree of mycorrhizal transpiration (if present)(Sun, et al. 2022). Following the uptake, Pb primarily accumulates in root cells, due to the metal being actively blocked by the *Casparian* strips found within the plant’s endodermis (Nanda, et al. 2011). Duly notable is that Pb is not just trapped by the Casparian strips, it is also held due to negative charges that exist from a root’s cell walls (Gerhardt, et al. 2009). The major issues that arise from the excessive accumulation of Pb within plant tissues are that the heavy metal will impair various morphological, physiological, and biochemical functions within plants, either directly or indirectly, inducing a wide range of deleterious effects (Inyang, et al. 2011). Thus, activating effective causation of phytotoxicity that occurs due to cellular membrane permeability alterations, due to the reaction Pb has with the active multitudes of groups of enzymes involved in the plant metabolism (Haque, et al. 2014). Continually noting that reactions within the phosphate groups of ADP or ATP are occurring, as well as the replacement of essential ions (Filipović, et al. 2020). Due to Pb's toxicity, plants will undergo the inhibition of lipid peroxidation, and ATP production, as well as undergo DNA damage due to the overproduction of reactive oxygen species (ROS) (Gao, et al. 2016). Other inhibiting factors of Pb are its effects on seed germination, seedling development, root elongation, transpiration, chlorophyll production, both protein, and water content, as well as plant growth (Filipović, et al. 2020). Plant vegetative growth is most notably negatively affected by; the inhibition of the Calvin cycle enzymes, which suffer from the impaired/distorted uptake of essential macro and micro elements, such as Mg and Fe, coupled with an induced deficiency of CO_2 which stems from the closure of stomata within the chloroplast ultrastructure, as well as the obstruction to the electron transport system (Dakora, et al. 2002). Plants are not defenseless under the stresses caused by Pb and have coping mechanisms built into their genetic structure that allow them to deal with Pb toxicity (Cao, et al. 2011). These mechanisms include a variety of components that work cross-functionally to reduce the uptake of Pb into cells, namely, the sequestration into vacuoles via the applied formation of complexes, binding Pb carried out by phytochelatins, amino acids, and glutathiones, as well as the synthesis of osmolytes (Haque, et al. 2014). Lastly, as a secondary type of defense mechanism, the activation of various antioxidants is utilized to combat the increase in production of Pb-induced ROS constitutes (Dakora, et al. 2002). With this being stated, the next section will take a deeper look into the biochar types and as aforementioned, how they can contribute to the amelioration processes with these metals already present in large quantities.

4.2. Physiochemical properties of tested biochar types

The biochar composed of wood chips (BC1) is of natural, sustainable wood products from wood chip production, in quality class A1, that is derived from mainly softwood (high proportion of spruce) as shown in (Figure 4). The main reason why this particular type of wood (spruce) is chosen for the creation of biochar is due to a few factors mainly being that its combustion point is very low, allowing the least amount of energy to be wasted on pyrolysis as well as that spruce grow easily in most zones throughout agroforestry designated areas, mainly due to their hardiness and ability to develop without the need of extra fertilizers, irrigation, or pesticides, according to the USDA.

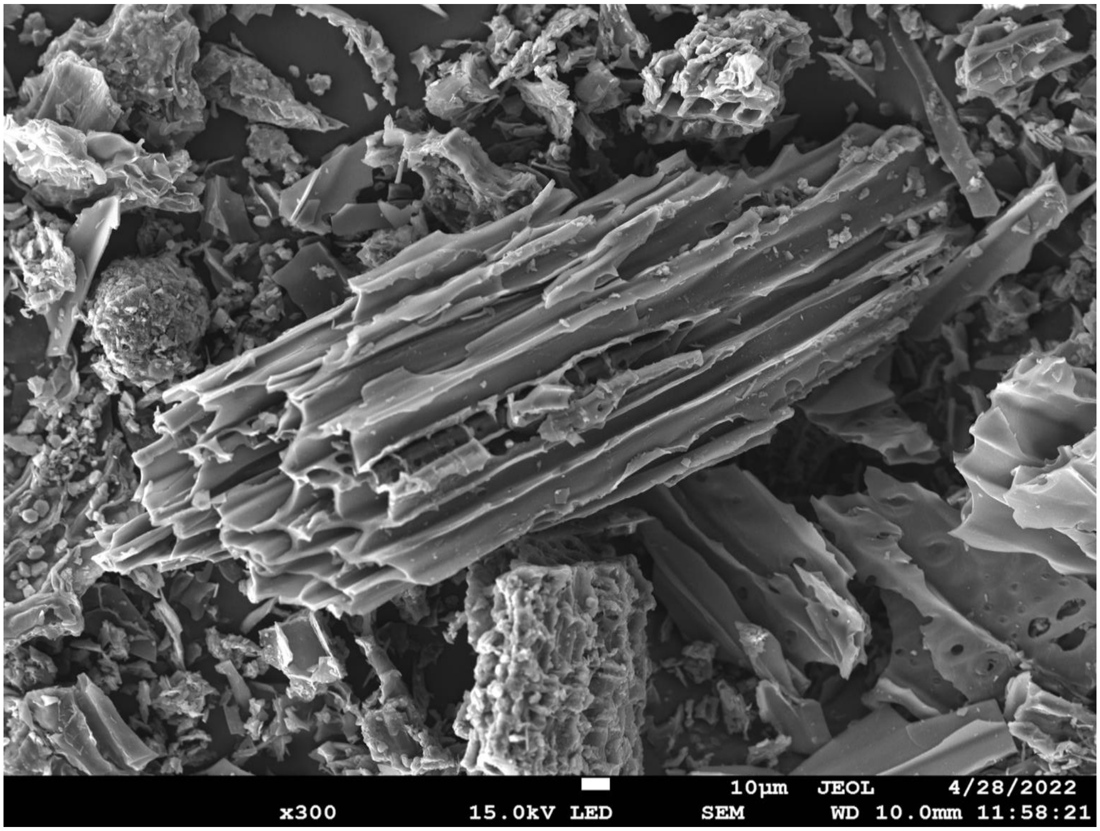


Figure 4. SEM micrograph of tested wood-derived biochar – BC1

As shown in (Table 7) the wood chip-derived biochar (BC1) has general parameters revealing the general bulk density to be (260 kg/m3) with a pH of 9.39 and an ash pH of 12.8. While its elementary analysis indicates that the total carbon percentage is 81% with the total organic carbon (TOC) that creates the C/N ratio of 67.9%, Nitrogen <0.08%, and the sulfur content being (<1000 mg/kg). As seen in (Table 6) there are only four types of polycyclic aromatic hydrocarbons, comprised of naphthalene, phenanthrene, anthracene, and fluoranthene, which compose a total value of (0.71 mg/kg). With this being said these values both individually and combined are far below the USA's Occupational Safety Health Agency guidelines for permissible exposure, allotting to the aforementioned statement regarding the extremely low-level threat to the environment that both processes of pyrolysis and the amendment of biochar incur to natural systems.

Table 6. Concentration of polycyclic aromatic hydrocarbons in tested biochar types

PAH (BC1)	mg/kg	PAH (BC2)	mg/kg
PAH total	.71 mg/kg	PAH total	4.20 mg/kg
Naphthalene	.55 mg/kg	Naphthalene	2.1 mg/kg
Phenanthrene	.12 mg/kg	Acenaphthene	<0.10 mg/kg

Anthracene	.002 mg/kg	Acenaphthylene	<0.10 mg/kg
Fluoranthene	.002 mg/kg	Fluorene	<0.10 mg/kg
		Phenanthrene	0.80 mg/kg
		Anthracene	0.30 mg/kg
		Fluoranthene	0.40 mg/kg
		Pyrene	0.50 mg/kg
		Benzo(a)anthracene	<0.10 mg/kg
		Chrysene	<0.10 mg/kg

Nutrient and grain analysis are necessary are exceptionally important in deterring the usefulness of biochar due to factors such as leachate potential, reaction complex within the soil, porosity, and distribution. As revealed in (Table 7), (BC1) is comprised of, (<0.08% mg/kg of elementary N), while other notable nutrient levels were K (7000 mg/kg), P (1600mg/kg), Mg (3000 mg/kg), Cu (28 mg/kg), Zn, (176 mg/kg), Mn (1520 mg/kg), and Fe (3800 mg/kg). Finally, the grain analysis reveals a favorable distribution within the three main size categories of, <0.1-1 mm containing 42.4%, 1-2 mm containing 25.8%, and 2- >6.3 mm containing 31.8% (Table 7).

Table 7. Nutrient analysis and grain size distribution of tested biochar types

Nutrient Analysis	BC1 Horse Manure	BC2 Wood Chip
N	<0.08% mg/kg	1.50% mg/kg
C	81%	64.30%
S	<1000 mg/kg	<0.20(+)% mg/kg
H	1.9%	1%
O	11.71%	13.10%
As	2.2 mg/kg	1.3 mg/kg
Pb	8 mg/kg	5 mg/kg
Cd	5.8 mg/kg	<0.4(+) mg/kg
Zn	176 mg/kg	118 mg/kg
Mn	1520 mg/kg	537 mg/kg
Fe	3800 mg/kg	1780 mg/kg
Cu	28 mg/kg	16.3 mg/kg
Grain Analysis		
<0.1 mm	0.1%	0.28%
0.1-0.63 mm	25.3%	34%
0.63-1 mm	17.%	11.1%
1-1.6 mm	4.9%	18.8%
1.6-2 mm	20.9%	17.4%
2-3.15 mm	7.1%	3.8
3.15-6.3 mm	24%	14%

>6.3 mm	0.5%	0.2%

The biochar derived from horse manure (BC2) as seen in (Figure 5) can be noted as highly porous with a tubular structure and high surface area. These qualities make it an extraordinary amendment to the soil in conjunction with the benefit of the added nutrients it is able to enhance as shown in (Table 7). This biochar type is composed of the following: Total dry substance 86.6%, ash 19.7%, carbon 64.3%, total organic carbon in ratio to nitrogen 57.7%, oxygen 13.1%, As (1.3 mg/kg), Pb (5 mg/kg), Cd (<0.4(+) mg/kg), and Zn (128 mg/kg), thus proving the ability for (BC2) to create a nutrient-rich space in order to generate higher growth rates that are linked to the coupled alteration of the soil in the form of added gas-water-exchange capacity of the due to the physical attributes that the biochar creates. These qualities reveal outstanding circumstances for this biochar type, however in comparison to that of the biochar derived from wood chips, there are higher amounts of Cd, Pb, and Zn, which is unfavorable in this research, due to the extensive contamination caused by the aforementioned three heavy metals.

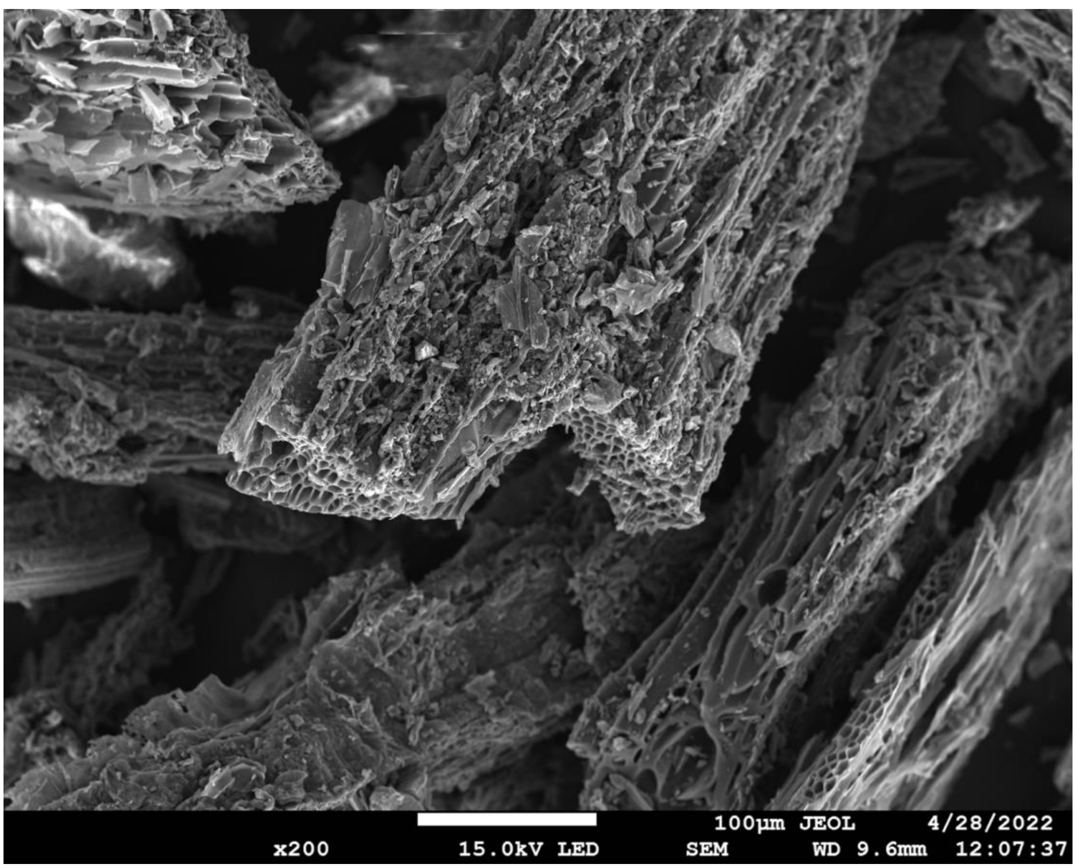


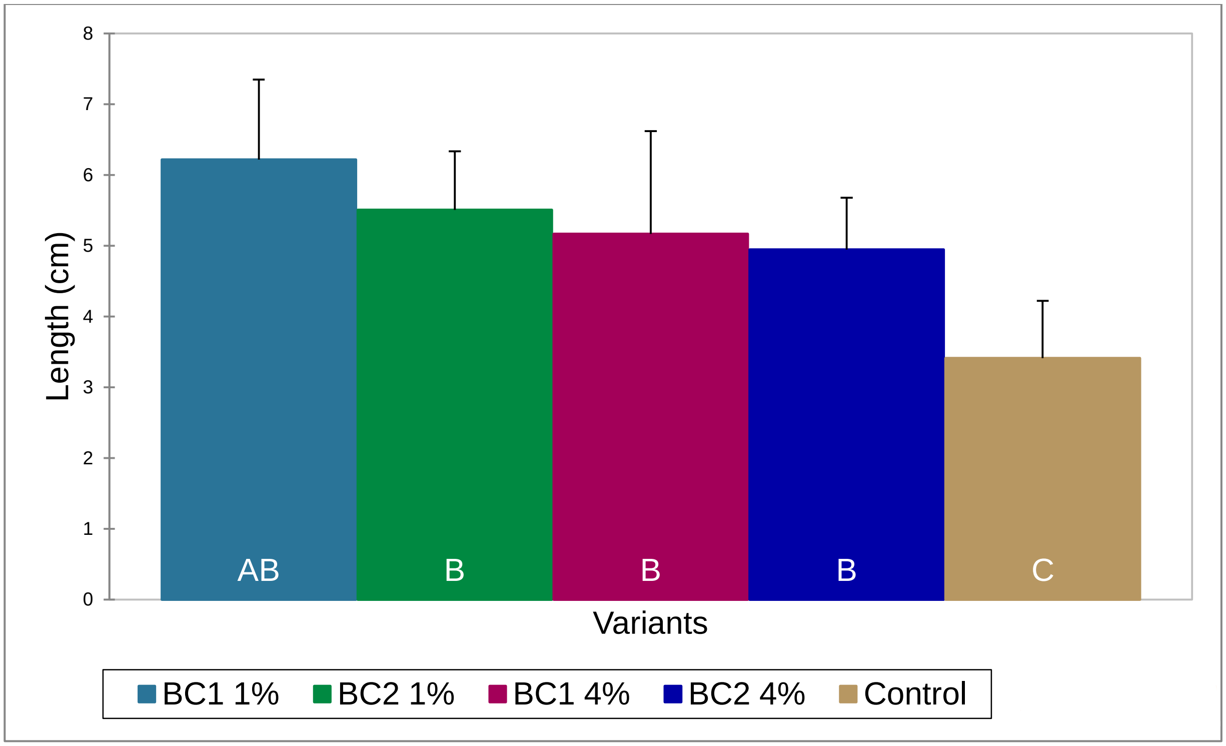
Figure 5. SEM micrograph of tested wood-derived biochar – BC2

Although the range of the different polycyclic aromatic hydrocarbons is almost identical to that of (BC1), the total amount of PAHs within (BC2) is about six times greater than that found within the wood chip composed biochar (Table 6). However, it must be noted that the total value being (4.20 mg/kg) is still below the USDA guidelines for said substances by quite far, therefore allowing this source to remain a valuable amendment to the soil. Shown in (Table 6) are the main values that are attributed to the above-stated total value, while the full list is comprised of sixteen PAHs instead of the four that were found within (BC1) (Table 6).

4.3. Impact of Biochar Amendment on Seed Germination of Test Crop

This portion of the experiment was proof of the understanding of the aforementioned influences that both chosen biochar types would have on seed germination if amended to contaminated soils. This process has been utilized as stated previously for almost two thousand years in principle due to people applying chard biomass onto areas where they attempted to produce agricultural products in hopes that it would help the soil help the plants. The results reveal that both biochar types, (BC1), and (BC2) were more effective in providing hospitable conditions for the test crop seeds to germinate at both low (1%) and high (4 %) percentages than that of no addition as revealed by the Control (Graph 1). These results also state that the lower (1%) amendments of both biochar types had a greater positive effect on the germination rates of the seeds than that of the higher (4%) biochar amendments. These results also state that in both amendment conditions of low (1%) and high (4%), biochar derived from wood chips (BC1) was more effective at inducing a higher rate of growth than that of biochar derived from horse manure (BC2), potentially due to the higher surface area, porosity, and nutrient combination found in the wood chips. Thus, allowing the statement to be made that biochar derived from wood chips (BC1) will provide a faster rate of germination of the test crop within this type of contaminated soil matrix than that of biochar derived from horse manure within the same amendment percentage or than that of no additional amendment as shown in the Control.

Based on (Graph 1) below, the above-stated results can be witnessed as a graphical and comparative summary revealing that all four biochar types had a positive impact on germination in comparison to that of the control group. This is able to be deduced because as seen in Graph 4.3.2 that although the control type has a statistically significant difference from the four biochar types, within the four types of biochar they are not statistically different enough to be granted their own letter group indicating overlapping areas of average values resulting from the mean. There as aforementioned, we can deduce that each biochar type will create a more hospital environment for Henola seed germination to occur, marking their effectiveness at the first vital stage of a remediation project, germination.



Graph 1. Average radical length of seed germination experiment

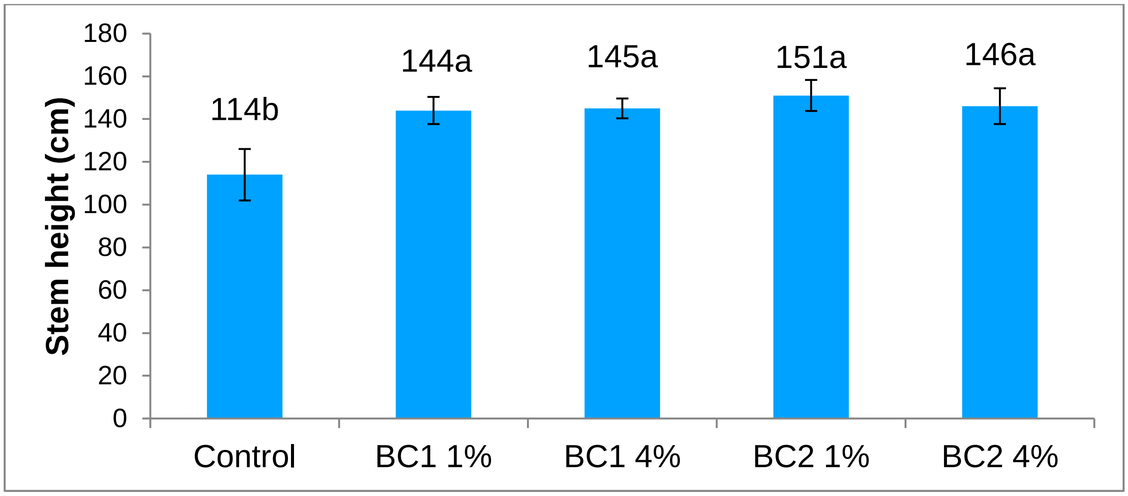
Whilst the results obtained have played a key role in understanding germination under stressed conditions, it should be considered that further experiments can be conducted due to the many properties that have a significant impact on biochar, such as the quality of feedstocks, as well as the type of organic material burned, which is heavily noted as having a direct impact on the quality of the final biochar product (Yao, et al. 2011). It should be noted that in the ideal situation, the utilization of clean and high-quality feedstocks will boast moisture content that is ranging between ten to twenty percent, as well as having high lignin amounts (Chen, et al. 2011). Moreover, the main functional organic groups, –OH, –O-, and –COO-, which are present within biochar are alkaline by nature, which means that they consequently contribute to an increased level of pH of the biochar (Chiou, et al. 2002; Yuan et al., 2011). It must be considered that these functional organic groups play a concise and considerable role in the influence of hydrophobicity, hydrophilicity, and adsorption of biochar, while they remain as a middle ground acting as a connection to form a buffer between both acids and bases (Chan, et al. 2007; Yuan, et al. 2019).

Another notable trait of the functional organic groups is that they actually hold negative charges within biochar and therefore assist in the enhancement of the cation exchange capacity. (Wang, et al., 2012). Some of these biochar types have been based on feedstocks that are derived from field/crop residues, woody biomass, and biosolids (Ahmad, et al. 2014). The issue that can arise is when contaminated feedstocks are put into use, which can range from feedstocks that have been sourced from railway embankments or some type of already contaminated land, which unfortunately can introduce a greater quantity and potentially a more toxic amendment into the soil, of which will generally either drastically increase soil pH and/or inhibit plants from absorbing the mineral compounds that were initially targeted (Burd, et al. 2000). The upside, however, is that because biochar can be manufactured through low-cost, and small-scale production methods of which are often utilizing modified stoves or kilns, whereas long as the quality of the initial organic matter is good enough the final product will be an acceptable amendment to the soil (Bian, et al. 2014). In large-scale operations, factors like the cost of the base organic material or the cost of running the factories or facilities to perform pyrolysis can greatly affect the demand for intensive production, which would ultimately need to utilize larger pyrolysis plants that in turn can process higher amounts of feedstocks, while maintaining a lower cost of the final product due to high output of biochar (Toková, et al. 2020). These lowered costs could potentially be mitigated by the added value that biochar can provide due to its enhancement of vegetative performances, which will be further explored in the next section.

4.4. Impact of Biochar Amendment on Vegetative Performances of Test crop

After understanding that all four biochar types create a statically more positive and hospital condition for the germination of Henola seeds, this section will continue to analyze and discuss the effects that the biochar types had on vegetative performances.

The stem diameter, and biomass are great indicators of the health of a plant and therefore it is vital to make a comparison of these parameters. As seen in (Table 8) the stem height measured in cm, coupled with the Fisher comparison t-test reveals that all four biochar types were statistically similar, however, they were also statistically different from the control, meaning that due to their height advantage the biochar types had a positive effect on this particular parameter. For plants, it is vital to compete for sunlight at the canopy layer which the treated soils helped to accomplish. Stem height as seen graphically in (Graph 2) presents a nice image of the above-mentioned results, as it allows one to picture what that canopy layer may look like, gaining a larger sense of insight into the positive effects of each biochar type. Steam diameter is another important factor within this parameter group, due to its vital role in distributing nutrients, and providing space for leaf, node, branch, and inflorescence growth, not to mention that it is also vital for a plant's ability to withstand harsh climatic conditions. The results as seen in (Table 8) reveal that there is not enough of a statical difference to be considered notable in favor or against the biochar types, however, it can be pointed out that the smallest diameter at the base of the plant was the control experiment. The second statical difference that must be noted in this situation occurs at the stem diameter in the middle or central region of the plants, when once again the four biochar types have outperformed the control, creating another mean average gap as pictured in (Table 8). Thus, revealing once again that the biochar types created another favorable condition for this growth parameter. It is interesting to compare all three of the stem diameters because, in the case of the base diameter, the average was lower in the control group, and once again it was lower in the middle or central region as well. However, as seen in (Table 8) the steam diameter on the top of the plants did not share the same characteristics as the other two diameter groups, with the control group being the second largest diameter on top. Although, this difference should not be viewed as a good sign for the control group, due to the plant becoming top-heavy in comparison to its smaller and potentially weaker central and bottom portion, creating a situation for the plant to be more prone to injury or death in the case of high winds or other negative climatic conditions.



Graph 2. Influence of applied treatments on stem height (cm). Means (+/- Stand. Error; n=3) with different letters are statistically different at $p<0.05$

Table 8. Influence of applied treatments on stem diameter (mm). Means (+/- Standard Errors; n=3) with different letters are statistically different at $p<0.05$

Treatment	Stem diameter at the base	Stem diameter in the middle	Stem diameter on the top
Control	6.3±.4a	4.6±.3b	2.7±.1a
BC1 1%	6.9±.5a	6.3±.3a	2.8±.6a
BC1 4%	7.3±.1a	6.7±.5a	2.7±.2a
BC2 1%	6.8±.5a	6.8±.4a	2.4±.3a
BC2 4%	7.3±.4a	6.2±.6a	2.4±.1a

Exploring the parameters of stem biomass both fresh and dry reveals a few interesting statical differences. Firstly, examining stem fresh biomass in (Table 9), it can be deduced that although there is not enough of a difference to determine all biochar types as superior for the biomass growing conditions it can be noted that (BC1 4%) wood chip, had a strong positive effect on the stem's biomass than that of the control group. Indicating that in fact the biochar derived from wood chips with an allotted higher dosage (4%) has indicative properties to cause a better growing environment for the plant to create significantly more stem biomass than that of the control group. This will be discussed in the next section and may signal a greater absorption of heavy metals from the soil matrix. Continuing the analysis over to the stem dry biomass it can be noted in a similar fashion to that of the fresh biomass with the exception that two groups of biochar created a significantly greater difference in total stem biomass than that of the control group. Thus, allotting to the fact that both (BC1 4%) and (BC2 1%) were able to grow at a stronger rate than that of the control group. From this data, the continued assumption that biochar does in fact create a more hospital soil environment for plant growth will be assessed by moving in leaves.

Table 9. Influence of applied treatments on stem fresh and dry biomass (g). Means (+/- Standard Errors; n=3) with different letters are statistically different at p<0.05

Treatment	Stem fresh biomass	Stem dry biomass
Control	20.9±3.5b	5.5±1b
BC1 1%	28.2±3.4ab	8.6±0.8ab
BC1 4%	36.5±2.1a	9.3±0.8a
BC2 1%	29.8±2.1ab	8.8±0.6a
BC2 4%	29.4±3.1ab	7.8±1.6ab

Leaf parameters were assessed based on two main groups, the first being the number of leaf tiers in height ascending the plant in conjunction with the total amount of leaves each plant grew. Secondly, the biomass of the leaves was also considered in both fresh and dry parameters. Examining (Table 10), the leaf tiers as well as the leaf total, one can tell that there is no statistical difference within these groups and that they are clearly able to produce and ascend approximately the same number of leaves. However, based on the biomass table as seen in (Table 10) there is a notable statical difference that shows a positive influence on fresh biomass between three of the biochar types, (BC1 1%), (BC1 4%), and (BC2 1%), in comparison to the control group. This suggests that although the number of leaves and tiers was statistically similar, the actual weight of the fresh leaves was quite different. This can be due to quite a few factors like water or possibly metal absorption and storage that will be further questioned and examined in section 4.5 as aforementioned.

Table 10. Influence of applied treatments on leaf parameters. Means (+/- Standard Errors; n=3) with different letters are statistically different at p<0.05

Treatment	Leaf tier	Leaf number	Leaf fresh biomass (g)	Leaf dry biomass (g)
Control	15±1.2a	30±2.3a	17±1.2b	4.9±0.1a
BC1 1%	15±1.7a	31±3.3a	24±3.5a	4.4±2a
BC1 4%	15±0.7a	29±1.3a	26±0.9a	5.8±1.1a
BC2 1%	13±2.7a	27±5.3a	24±0.8a	6.4±0.4a
BC2 4%	15±0.3a	31±0.7a	20±2.3ab	5.5±1.4a

The last portion of the above-ground plant that was required for a comprehensive understanding of the vegetative growth parameters is the biomass of the Inflorescence as seen in (Table 11). These results are quite interesting because they reveal that there is a statistical difference that has not been noted previously, where (BC1 1%), is the best performer in creating inflorescence-based fresh and dry biomass. Although it has been aforementioned that (BC1 1%) had the highest positive results in multiple different parameters, it is the first mentioned case of a statically more positive effect on the plant's growth. This can again be caused by a number of factors which will once again be further addressed in section 4.5.

Table 11. Influence of applied treatments on inflorescence fresh and dry biomass (g). Means (+/- Standard Errors; n=3) with different letters are statistically different at $p<0.05$

Treatment	Inflorescence fresh biomass	Inflorescence dry biomass
Control	6.0±1.2b	1.4±0.3b
BC1 1%	12.4±2.2a	2.8±0.5a
BC1 4%	3.7±1.7b	0.9±0.4b
BC2 1%	8.3±0.6ab	1.9±0.2ab
BC2 4%	4.7±2b	1.0±0.5b

Moving focus to the below-ground portion of the vegetative growth parameters means taking the plant's roots into account which had the fresh and dry biomasses observed. The interesting part of the root data is that only within the dry biomass was there a statistical difference, while there was not enough of a difference within the fresh biomass to be considered statistically different as shown in (Table 12). It should be noted, however, that in the fresh biomass results, the control's value did in fact show quite a low number (4.9 g) in comparison to those of the biochar types. The main statistical differences in the dry biomass were within (BC1 1%), (BC1 4%), and with the Control group. The dry biomass results could potentially be a component of chemical change within the roots after the drying process has ended, revealing more metals in the rootstock than in the fresh. This will be further investigated in section 4.5 with the mineral composition data.

Table 12. Influence of applied treatments on root fresh and dry biomass (g). Means (+/- Standard Errors; n=3) with different letters are statistically different at $p<0.05$

Treatment	Root fresh biomass	Root dry biomass
Control	4.9±0.8a	0.9±0.2b
BC1 1%	13.8±3.6a	1.9±0.2a
BC1 4%	14.9±3a	2.1±0.2a
BC2 1%	11.2±4.3a	1.7±0.4ab
BC2 4%	11.4±3.2a	1.5±0.4ab

The total plant biomass in both fresh and dry forms was analyzed to help in determining the overall vegetative growth of each group. As shown in (Table 13) the results turned out quite interesting once again, due to the fresh biomass consisting of (BC1 1%), (BC1 4%), and (BC2 1%), which were statistically different than that of the control while in the control only (BC2

1%) showed enough of a difference to be noted according to the fisher t-test. Due to the before-mentioned parameters and analyses, these results are quite expected and will be further investigated in the following section as the absorbed metal allotments come into play with not just their effects on growth, but also in how the addition of the biochar types may have enabled heightened absorption and phytostabilization.

Table 13. Influence of applied treatments on total plant fresh and dry biomass (g). Means (+/-Standard Errors; n=3) with different letters are statistically different at p<0.05

Treatment	Total plant fresh biomass	Total plant dry biomass
Control	48.8±4.8b	12.7±1.2b
BC1 1%	78.7±8a	17.7±1.5ab
BC1 4%	81.0±5.1a	18.1±1.4ab
BC2 1%	73.2±6.4a	18.7±0.7a
BC2 4%	65.9±9ab	15.7±3.1ab

Due to these aforementioned results, it must be noted that it is a well-known fact that heavy metals occur naturally within the soil environment due to the pedogenetic processes of weathering of parent materials, however, the levels occurring can be regarded as trace, because they are at quantities of (<1000 mg kg−1) and rarely toxic (Mehard, et al. 2010). Due to both the disturbance and acceleration of the earth's natural slowly occurring geochemical cycle of metals, the vast majority of soils ranging from rural and urban environments have accumulated one or more of the heavy metals above listed and defined, to values that are high enough to cause serious risks to human health, ecosystems, animals, plants, and other types of media (D'Amore, et al. 2005).

As these heavy metals build up in soil environments, they slowly become contaminants with an increasingly toxic nature (WHO, 2021). This is due to many factors including; (i) increased rates of generation onset from anthropogenic cycles being faster paced in comparison to natural cycles, and (ii) these metals are easily transferred from currently or formerly mined locations to random deposit sites based on the environmental surroundings, thus leading to the potential of direct exposure to take place due to people being unknowing of the surrounding toxins, (iii) heightened concentrations of the metals within discarded products, allowing leaching into the soils of which causes the receiving environment to build up intolerable levels of heave metals quickly,(iv) finally, the chemical speciation of which each individual metal is located at within the receiving environmental system generally renders it more bioavailable than its former natural biogeochemical makeup (WHO, 2021).

Projected models have shown that human-caused emissions into the atmosphere, of several heavy metals along with multitudes of other pollutants are on average three to seven times in magnitude higher than natural fluxes (WHO, 2007; WHO, 2021). It should also be noted that heavy metals that have built up within the soil from anthropogenic sources are much more mobile, therefore the heightened bioavailability compared to pedogenic, or lithogenic ones (WHO, 2021). Not only this but their bioavailability is influenced by a plethora of physical factors such as adsorption, sequestration, temperature, and phase association (Rajkovich, et al. 2012). They are also affected by a variety of chemical factors that influence their speciation within the thermodynamic equilibrium, along with complexation kinetics, lipid solubility and, water partition coefficients (WHO, 2021). Biological factors such as characteristics within each species, the trophic interactions, and the biochemical/physiological adaptations that occur naturally, play an important role in understanding how heavy metals are distributed (Mikanova, et al. 2006).

As mentioned previously, agriculture was the first noted method of metal contamination into the surrounding environment and continues to hold true to that claim due to the fact that in order to grow and complete their lifecycles, plants must acquire not only macronutrients (N, P, K, S, Ca, and Mg), but also essential micronutrients, (B, Zn, Mn, Fe, Cu, Mo, and Cl) (He, et al. 2011). Due to some soils being deficient in heavy metals such as, (Cu, Co, Fe, Mo, Ni, Mn, and Zn) which are essential for not only healthy plant growth but are also frequently utilized for crops as an addition to the soil or even as a foliar spray (Alengebawy, et al. 2021). This means that large quantities of fertilizers are regularly added to soils in almost all intensive farming systems to provide adequate N, P, and K for crop growth, however, the compounds that are used to supply these elements also contain trace amounts of heavy metals such as Cd and Pb, which are considered as impurities (Alengebawy, et al. 2021). The notable issue is that after continued fertilizer application, there is a significant increase in both Cd and Pb content within the soil, and because they have no known physiological activity, they can only cause greater issues to the global environment (WHO, 2021). The application of many brands of phosphatic fertilizers inadvertently adds not only both, Cd, and Pb but also other potentially toxic elements to the soil, such as F, and Hg which are slowly being more regulated, however in developing countries this is just not the case as of yet (WHO, 2021).

Fertilizers are not the only issue, as several common pesticidal products that are used quite extensively within both agriculture and horticulture contain substantial concentrations of metals. It should be noted that for these pesticides, most developed countries have placed heavy restrictions and regulations on the amendments to the plethora of products, generally forcing either the minimization or removal of certain additives (Diacono, et al. 2010). A great example of the continued allowance of heavy metals to be used in agricultural products can be that about 5% of the chemicals which are currently approved for use as both insecticides and fungicides within the United Kingdom are based on compounds containing metals such as Pb, Zn, Cu, Hg, and Mn (Wu, et al. 2017). A famous example of such a pesticide was the copper-containing fungicidal spray Bordeaux mixture (copper sulfate) that originated in the nineteenth century in France but has been banned in most of the EU and the United Kingdom due to its transgression into a pollutant over a few years of use (WHO, 2021). Compared to pesticides and fertilizers which are directed into a focused area of use and are heavily monitored, numerous biosolids, such as livestock manures, composts, and municipal sewage sludge have remained a consistent source of impartial accumulation of heavy metals such as Cd, Pb, Zn, Cr, Cu, Hg, Ni, Se, Mo, Tl, and Sb, into the soil (Plaza, et al. 2004; Wu, et al. 2017).

A common practice that occurs globally is the amendment onto soils of certain animal wastes such as poultry, cattle, and pig manures, which are applied to cropland, pastures, and even urban areas as either solids or slurries for its favorable biochemical compositions (WHO, 2021). Although most manures are seen as valuable fertilizers, in both the poultry and swine industries, Zn, Cu, and As, are frequently added into the animals' diets as growth promoters, however, it is this addition that has been proven to cause a considerable buildup of metal contamination to the treated soils (McCutcheon, et al. 2003). Sewage sludge or more commonly referred to as biosolids is primarily composed of organic solid products, which are produced via the wastewater treatment process due to the ability to be beneficially recycled (Kucharski, et al. 2000). It is a common practice in many countries including developed countries to utilize biosolids for land applications that have been produced from urban waste treatment plants (Wang, et al. 2017). For example, it is estimated that in the United States alone, more than half of approximately 9.6 million dry tones of produced biosolids are utilized annually more land application methods, with a notable fact that agricultural utilization of biosolids currently occurs in every region of the country (Hossain, et al. 2011). In comparison, the Europe Union notes that over 42% of biosolids are utilized as fertilizer within agriculture practices (WHO, 2021). Due to the new building trend for composting biosolids with a mixture of other organic materials such as sawdust, straw, food scraps collected from urban areas, garden and cityscape waste, as well as plant biomass from agriculture, implications of metal contamination have begun to show some devastating measurements (Ye, et al. 2019).

These measurements are beginning to reveal that the heavy metals that are most commonly found in biosolids are now being found in the mixed compost which has reported high rates of Pb, Zn, Cd, Ni, Cr, and Cu (Schimmelpfennig, et al. 2012). These metal concentrations are governed not only by both the associated industrial activity that produced their initial compound but also by the type of process employed during the treatment of the initial biosolids (Ye, et al. 2019). In many countries metals that have been amended into soils via the application of biosolids have revealed a pattern of leaching downwards through the soil profile and into the groundwater of shallower soils (Razzaghi, et al. 2018). New Zealand has been leading the research on the contamination of groundwater within their soils that have been treated with biosolids over the last 20 years, revealing large increased concentrations of Cd, Zn, and Ni in the collected drainage leachates (Ye, et al. 2019). It must be noted, however, that further studies are being conducted in both New Zealand and throughout the developed world to prove that there are notably safe allotments of biosolids composted additions that have not contaminated the underlying groundwater (Salleh, et al. 2022). This is due to a variety of reasons such as the soil profile, type of organic products induced into the compost, level of the biosolids treatment plant, as well as soil depth, temperature, moisture content, and surrounding landscape (Salleh, et al. 2022).

4.5 Impact of Biochar Amendment on Mineral Composition of Test Crop

Taking the vegetative parameters into account, allows a deeper understanding of the effects that each heavy metal played within the aforementioned conditions of each group as they were analyzed statistically. The mineral composition of the test crops will also permit a full understanding of how the biochar amendments were able to assist in phytoaccumulation, phytostabilization, and bioaccumulation, and reveal the rate of hyperaccumulation by *Cannabis sativa* L. (Henola), which has not been studied in depth before. The parameters that were tested for each biochar type include Stem composition of (Cd, Pb, and Zn), Leaf - composition of (Cd, Pb, and Zn), Inflorescence - composition of (Cd, Pb, and Zn), and Root - composition of (Cd, Pb, and Zn). For each analysis, there are the captured values from the parameters listed above, as well as a standard error, and a fisher comparison test run, in order to accurately understand the data and determine if there are statistical differences within the observed data to support or reject the null hypothesis. This continued section will be broken down into paragraphs that will each go into depth surrounding each of the aforementioned vegetative performance parameters.

The composition of the stems for each group returned variables that highlight the statistical difference in multiple groups. As seen in (Table 14) all four biochar types were in a separate category showing lower Cd accumulation in the stem, while the control remained at the highest amount. As shown in (Table 14), Pb stands out as a varied case with the highest amount of accumulation presenting itself in the control, however, it remained in the same categorized group as that of (BC1 1%) and (BC2 1%), while both biochar types, (BC1 4%), and (BC2 4%) remained in their own separated category b, defining them as a lower accumulating unit. As shown in (Table 14) Zn was very similar to the case of Cd because the control remained in its own statistically separated group, once again accumulating the highest amount of the metal, while in this case, (BC1 1%), (BC2 1%), and (BC1 4%) were in their own statistically different group, of which, accumulated the least amount of Zn. (BC2 4%) was in the mixed category. In all stem experiments, the control group hyper-accumulated the highest amount of metal.

Table 14. Influence of applied treatments on mineral composition of stem (mg/kg). Means (+/- Standard Errors; n=3) with different letters are statistically different at $p<0.05$

Treatment	Cd stem	Pb stem	Zn stem
Control	1.5±0.1a	18.2±2.1a	29.2±2.5a
BC1 1%	0.9±0.2b	16.5±1.9a	19.4±0b
BC1 4%	0.6±0.2b	13.7±1.3b	17.4±2.2b
BC2 1%	0.7±0.1b	17.4±2.6a	19.4±2.5b
BC2 4%	0.5±0.1b	12.4±1.1b	22.1±3.2ab

The leaf accumulation of heavy metals was quite different as seen in (Table 15), due to the fact that Cd uptake and storage are the only metal group that showed a statistical difference within the fisher t-test. This analysis allows the understanding that (BC1 1%), (BC1 4%), (BC2 1%), and (BC2 4%), were grouped into a separate category than that of the control due to the control group storing larger amounts of Cd within its leaves as shown in (Table 16). Although it should be noted that the control group did not hyper-accumulate the most metals in every category, due to the data found within the Pb section (Table 15), that reveals wood chip-derived biochar types accumulated larger quantities (mg/kg) than the other groups, based on the numerical values portrayed. In the next section 4.6, the soil mineral composition will be analyzed, and doing so will allow greater insight into whether the plants or the biochar or both parties are responsible for the remediation of the carried metal groups.

Table 15. Influence of applied treatments on mineral composition of leaves (mg/kg). Means (+/- Standard Errors; n=3) with different letters are statistically different at $p<0.05$

Treatment	Cd leaf	Pb leaf	Zn leaf
Control	1.1±0.2a	16.2±1.8a	47.8±12.1a
BC1 1%	0.7±0.1b	17.0±1a	37.1±4.9a
BC1 4%	0.3±0.1b	17.1±0.9a	30.8±3.4a
BC2 1%	0.5±0b	15.9±0.2a	39.0±1.6a
BC2 4%	0.4±0.1b	13.5±3.3a	35.5±6.3a

The Inflorescence results showed similar findings to that of the leaves due to the same occurrence of the control being in its own categorized grouping according to the fisher t-test for Cd uptake, while the other biochar types were in the same category as each other. This can be seen in (Table 16) where the control had higher accumulated levels of Cd than the biochar types. In the case of Pb uptake, as shown in (Table 16) by the values listed in (mg/kg), (BC2 4%) had the highest weighted value. While in the other metal groups, the control as seen in (Table 15) and (Table 16) had the highest stored accumulation. The trend within the Cd group seems to be continuing and will be further discussed within the root analysis if it is found once again.

Table 16. Influence of applied treatments on mineral composition of inflorescence (mg/kg). Means (+/- Standard Errors; n=3) with different letters are statistically different at $p<0.05$

Treatment	Cd inflor.	Pb inflor.	Zn inflor.
Control	0.9±0.2a	2.8±0.7a	64.2±5.1a
BC1 1%	0.3±0b	2.3±0.2a	47.8±4.2b
BC1 4%	0.2±0b	2.3±0.1a	53.4±2.1ab
BC2 1%	0.2±0b	2.8±0.5a	51.4±6.1ab
BC2 4%	0.3±0.1b	3.0±0.9a	52.6±2.3ab

The root analysis as shown in both (Table 17) differs from the other mineral composition groups due to multiple variables in all metal groups accumulating at different statistical categories. As shown within (Table 17) the Cd group had the highest values revealed by category

(a) from the fisher t-test in both the Control and (BC1 1%), with the lowest accumulation occurring in (BC1 4%) and (BC2 1%). The accumulation of Pb, with the exception that the Control was grouped in the same category of (BC1 4%), (BC2 1%), and (BC2 4%), while (BC1 1%), had its storage at the largest value of Pb. Lastly, Zn like Pb, showed that (BC1 1%) accumulated the largest amount of Zn and was categorized by itself, while (BC1 4%), and (BC2 1%), stored the least amount, being categorized in their own group (c) as seen in (Table 17).

Table 17. Influence of applied treatments on mineral composition of roots (mg/kg). Means (+/- Standard Errors; n=3) with different letters are statistically different at $p<0.05$.

Treatment	Cd root	Pb root	Zn root
Control	39.2±9.6a	189±9b	374±54ab
BC1 1%	41.9±4.6a	298±39a	431±28a
BC1 4%	18.7±1b	133±12b	215±21c
BC2 1%	20.1±2.3b	157±32b	246±42c
BC2 4%	33.3±4.6ab	157±30b	296±27bc

4.6 Impact of Biochar Amendment on Mineral Composition of Soil

As shown in (Table 18) the soil matrix varied very heavily on the composition of metals left remaining in the soils after the phytoremediation project had taken place. As seen in (Table 18), Cd was left over at the highest rate in the Control group, being labeled an (a) category by the fisher t-test while (BC1 1%) as categorized (b) had the lowest remains values of Cd. Within (Table 18) the metal groups were not able to be broken down into their own categories due to values ranging within each other's mean group range. These results coupled with the seed germination data (section 4.3), vegetative parameter results (section 4.4), and metal mineral composition of plants (section 4.5), to help paint a picture of the total outcome that biochar has on both the soil matrix and plant growth parameters. Overall, these results will be determined in the conclusion section based on the three stated hypotheses as aforementioned.

Table 18. Influence of applied treatments on mineral composition of soil (mg/kg). Means (+/- Standard Errors; n=3) with different letters are statistically different at $p<0.05$.

Treatment	Cd soil	Pb soil	Zn soil
Control	23.1±0.7a	4.8±0.2a	3.0±0.1a
BC1 1%	22.5±0.3ab	4.7±0a	2.9±0.1a
BC1 4%	21.4±0.2b	4.5±0.1a	2.8±0.1a
BC2 1%	22.7±0.3ab	4.7±0.1a	3.0±0.1a
BC2 4%	22.2±0.6ab	4.5±0.1a	2.9±0.1a

There has been a considerable number of studies aimed at the modification of biochar that can be considered an extremely viable method for the improvement of its stabilization efficiency for heavy metals in soils (Rajapaksha, et al. 2016). These modifications are being greatly explored with types of improvements ranging from the utilizing hydroxides, iron compounds, organic solvents, and acids, which have all been undergoing extensive research by (Lu, et al. 2017) since approximately 2013. Expanding the current knowledge within these modification test groups can potentially create a viable solution that will have the ability to alleviate the need for high application rates due to the increase in the number of surface functional groups as well as enhance the cation exchange capacity (Anawar, et al. 2015). This would also mean that changes can be optimized toward a specific surface area of biochar, which will undoubtedly need the enhancement of the metal sorption capacity of the biochar (Cui, et al. 2011).

However, it must be noted that the majority of modified biochar has been accomplished with a single modifying compound or solution, such as iron compounds, HNO3, and H2SO4, etc., with very few studies utilizing multiple modeling agents simultaneously (Cantrell, et al. 2012). Thus, it can be argued that there is a great potential benefit within, (i) multiple-modifying agents in order to improve properties of biochar, (ii) after the biochar has been multiple-modified it may have a higher stabilization efficiency towards heavy metals depending on the different soils it is being employed within, (iii)and lastly that the application of multiple-modified biochar may have the ability to improve a plethora of soil components (Bridgwater, et al. 2012; Ondrasek, et al. 2021). Unfortunately, it is still extremely difficult to exactly clarify the stabilization mechanism of biochar mainly due to the complexity of each individual soil environment in conjunction with the limitations of currently available analytical techniques (Wang, et al., 2020). There have been a few promising potential mechanisms that have been proposed including but not limited to, electrostatic adsorption, co-precipitation, ion exchange, complexation with oxygen-containing functional groups, and an electron that resides within the pi bond(s) of either a double bond or a triple bond, or in some cases the conjugated p orbital (Bolan, et al. 2014). Currently, investigations surrounding the application of biochar which only slightly altered the soil pH and cation exchange capacity have been revealed, as well as the fact that many of the alkaline components within the tested biochar may have been removed during the modification process (Yuan, et al. 2011). Thus, it can be induced that the ion exchange and co-precipitation which are controlled by both alkaline groups and pH may in fact not be the main underlying mechanisms behind the shift within stabilizing heavy metals in contaminated sites (Beesley, et al. 2011). The chemical speciation calculations that were performed with Visual Minteq while utilizing the NICA-Donnan model indicated that Cu and Pb were present in all percolates, including the Control, exclusively as organo-complexes with dissolved organic carbon fractions (humates and fulvates). However, within the chemical pool of Cd and Zn, their free cationic forms (Cd^{2+}, Zn^{2+}) and chloro-complexes were confirmed in addition to organo-complexes (Ondrasek, et al. 2022). Proving that biochar derived from wood chips and from horse manure can create a positive statistically significant difference in a metal-contaminated soil.

5. CONCLUSIONS

An in-depth analysis of tested metal-contaminated soil confirmed that examined metals exceeded the global average values for metal in soil by multifold; Pb 127-fold, Zn 56-fold and Cd 25-fold. The addition of tested biochar types, derived from wood chips and horse manure, decreased the levels of high metal concentrations within the contaminated soil solution matrix by approximately 3% for Cd, 34% for Zn, and 93% for Pb. SEM analysis confirmed that micro to nano pore structure, surface area, and viable space for gas exchange in the amended soil was positively altered from the addition of the biochar types.

The mineral composition of the test crop showed lower heavy metal concentrations of Cd, Zn, and Pb in the examined tissues under applied biochar types. However, further long-term studies are required to validate the claim concerning full remediation of the metal-contaminated soil. Based on the results gathered from this research, it can be stated that out of the four types and levels of biochar tested, the biochar derived from wood chips with a 1% rate, was the strongest amendment in altering the mobility and chemistry of metals in tested soil. This biochar type encouraged higher germination rates, allowed for greater phytostabilization of Cd, Zn, and Pb, within the soil matrix as well as induced a higher growth performance of all tested vegetative parameters than of the control and horse manure-based biochar type.

The results presented in this research could serve as a foundation for future scientific studies that may further develop new biochar-based polymers. Some of which can be utilized as strategies toward the chemical amelioration, and bioremediation of natural or artificial ecosystems that have been contaminated by heavy metals in the soil.

6. References

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Curriculum Vitae

Jonti Evan Shepherd was born on the 17th of August 1992 in Johannesburg, South Africa. Finishing high school at Torrey Pines in San Diego, California, United States, he enrolled at San Francisco State University, where he finished a bachelor's of science degree in Environmental Studies (natural resource management and conservation). Due to working full time from the age of 15, upon completion of his degree he decided to move into a successful professional career path, leading to multiple experiences in all three major sectors of government, non-profit, and private industry as well as establishing his own company in 2018 called Whatwastes. In 2020, he enrolled in the master study program called INTER-EnAgro (Environment, Agriculture and Resource Management) at the Faculty of Agriculture at the University of Zagreb, where he has completed his master’s of science degree. As a passionate researcher, environmentalist, change-maker, operational leader; dreamer and storyteller, Jonti is pushing forward to accomplishing his Ph.D. in the interactions between soils-plants-microorganisms and their roles in global systems cycles. Where he has dedicated himself to research, development, innovation, design, and planetary exploration. While remaining an advocate for conservation, community science education, preservation of natural habitats, bioremediation, and green redevelopment, he will continue on his journey to re-shape the world into a more regenerative circular system of cycles.