T.R.
ONDOKUZ MAYIS UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF SOIL SCIENCE AND PLANT NUTRITION

EVALUATION OF PHYTOREMEDIATION POTENTIAL OF SEDUM PLUMBIZINCICOLA

Master's Thesis

Onyinye Anthonia EZEIFEKA

Supervisor
Prof. Dr. Violina ANGELOVA
II. Supervisor
Prof. Dr. Coşkun GÜLSER

SAMSUN
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This thesis was supported by the European Union project of Erasmus Mundus Joint Master Degree in Soil Science (emiSS) with project number 610528-EPP-1-2019-1-TR-EPPKA1-JMD-M

SAMSUN
2023

ACCEPTANCE AND APPROVAL OF THE THESIS

The study entitled **"EVALUATION OF PHYTOREMEDIATION POTENTIAL OF SEDUM PLUMBIZINCICOLA"** prepared by **Onyinye Anthonia EZEIFEKA**, and supervised by **Prof. Dr. Violina ANGELOVA and Prof. Dr. Coşkun GÜLSER**, was found successful and unanimously accepted by committee members as Master thesis, following the examination on the date ....06.2023.

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This thesis has been approved by the committee members that already stated above and determined by the Institute Executive Board.

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Prof. Dr. Ahmet TABAK
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I hereby declare and undertake that I complied with scientific ethics and academic rules in all stages of my Master's Thesis, that I have referred to each quotation that I use directly or indirectly in the study and that the works I have used consist of those shown in the sources, that it was written in accordance with the institute writing guide and that the situations stated in the article 3, section 9 of the Regulation for TÜBİTAK Research and Publication Ethics Board were not violated.

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ÖZET

SEDUM PLUMBIZINCICOLA'NIN FİTOREMEDIASYON POTANSİYELİNİN DEĞERLENDİRİLMESİ

Onyinye Anthonia EZEIFEKA
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Toprak Bilimi ve Bitki Besleme Ana Bilim Dalı
Yüksek Lisans, Ağustos/2023
Danışman: Prof. Dr. Violina ANGELOVA
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Bu çalışma, gelecek vaat eden bir bitki türü olan *Sedum plumbizincola'nın* fitoremediasyon amaçlı potansiyelini araştırmaktadır. Araştırma, organik değişikliklerin ağır metallerin alımı ve Sedum plumbizincola tarafından mikro ve makro elementlerin birikimi üzerindeki etkisine odaklanmaktadır. Bulgaristan'ın Plovdiv kenti yakınlarındaki Demir Dışı Metaller Fabrikası civarından toplanan toprak örneklerinde kontrollü koşullarda denemeler yapılmıştır. Saksı denemesi, beş işlem ve üç tekrarlamalı (15 saksı) ile tam şansa bağlı blok deseni uygulanmıştır. Uygulamalar, herhangi bir organik değişiklik içermeyen bir kontrol grubunun yanı sıra, kuru toprağın ağırlığına bağlı olarak sırasıyla % 5 ve % 10 oranlarında solucan gübresi ve biyokömürü içermektedir. Organik maddelerinin uygulanması, toprağın fizikokimyasal özelliklerini önemli ölçüde etkilemiştir. Organik madde, elektriksel iletkenlik ve makro elementlerin (P, K, Ca ve Mg) ve mikro elementlerin (Fe, Mn, Zn) konsantrasyonlarının artmasına neden olmuştur. Sonuç olarak, organik madde uygulamaları Sedum plumbizincola'nın ağır metallerin ve mikro ve makro elementlerin alımını etkilemiştir. Çalışma ayrıca organik katkı maddesinin türüne ve miktarına bağlı olarak verim farklılıklarını gözlemlenmiştir. Spesifik olarak, % 10 solucan gübresi uygulamasına sahip varyant, kontrol numunesinden üç kat daha fazla Cd ve Zn alımına nened olmuştur. Buna karşılık, % 10 biyokömür uygulamasının mahsul verimi üzerinde ihmal edilebilir bir etkisi görülmüştür. Bu çalışmanın sonuçları, Sedum plumbizincola'nın fitoremediasyon potansiyelini en üst düzeye çıkarmak için organik madde uygulamalarının önemini vurgulamaktadır. Organik katkı maddeleri, toprak özelliklerini iyileştirerek ve besin mevcudiyetini etkileyerek ağır metallerin ve temel elementlerin bitkiler tarafından alımını kolaylaştırabilir. Sonuçlar, bitki performansını ve metal alım verimliliğini en üst düzeye çıkarmak için uygun organik düzenleyicilerin seçilmesinin ve optimal dozajlarının belirlenmesinin önemini vurgulayarak Sedum plumbizincola'yı fitoremediasyonda kullanmaya yönelik sürdürülebilir stratejileri anlamamıza katkıda bulunmaktadır.

Anahtar Sözcükler: Fitoekstraksiyon, Organik değişiklikler, Kirlenmiş topraklar, Sedum plumbizincicola.

ABSTRACT

EVALUATION OF PHYTOREMEDIATION POTENTIAL OF SEDUM PLUMBIZINCICOLA

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This study investigates the potential of *Sedum plumbizincola*, a promising plant specie, for phytoremediation purposes. The research focuses on the impact of organic amendments on the uptake of heavy metals and the accumulation of micro and macroelements by *Sedum plumbizincola*. Controlled experiments were conducted using soil samples collected from the vicinity of the Non-Ferrous Metals Work near Plovdiv, Bulgaria. The pot experiment followed a randomised complete block design, with five treatments and three replications (15 pots). The treatments included a control group without any organic amendments, as well as vermicompost and biochar amendments applied at rates of 5% and 10%, respectively, based on the weight of dry soil. The application of organic additives significantly influenced the soil's physicochemical characteristics. It led to increased concentrations of organic matter, electrical conductivity, and macroelements (P, K, Ca, and Mg) and microelements (Fe, Mn, Zn). Consequently, the presence of organic amendments affected Sedum plumbizincola's absorption of heavy metals and micro- and macroelements. The study also observed yield variations based on the type and quantity of the organic additive. Specifically, the variant with 10% vermicompost application produced Cd and Zn yields that were up to three times greater than the control sample. In contrast, the administration of 10% biochar had a negligible effect on crop yield. The results from this study highlight the significance of organic amendments for maximising the phytoremediation potential of Sedum plumbizincola. Organic additives can facilitate the uptake of heavy metals and essential elements by plants by enhancing soil properties and influencing nutrient availability. The results contribute to our understanding of sustainable strategies for employing Sedum plumbizincola in phytoremediation by emphasising the importance of selecting appropriate organic amendments and determining their optimal dosage in order to maximise plant performance and metal uptake efficiency.

Keywords: Phytoextraction, Organic amendments, Contaminated soils, Sedum plumbizincicola

ACKNOWLEDGEMENT

The success and completion of this research project required a great deal of inspiration, direction, help, and financial support. I would like to express my deepest appreciation to everyone who supported and helped me to complete my master's thesis.

Firstly, I want to sincerely thank my supervisor, Prof. Dr. Violina ANGELOVA for her unwavering support and kindness, and for the invaluable guidance she provided me with which positively influenced the success of this thesis. I enjoy the numerous lessons learned during my lab experiments, especially. Additionally, I sincerely appreciate my Co-supervisor, Prof. Dr. Coşkun GÜLSER. He stood in as a father and mentor throughout my program and I can never forget his impact on my professional growth.

Secondly, special thanks go to my parents, Mr. A.I Ezeifeka and Mrs. B.E Ezeifeka for their parental guidance and love towards making me a better person. I sincerely appreciate the efforts of my mentors, friends, and well-wishers. On a special note, I want to appreciate the following persons: Valentine Okonkwo, Emmanuel Uzochukwu, Marybeth Ugoh, Kelechukwu Onwukamike, Uche Ejidike and Enid Adiegwu, for their constant contribution, support, and encouragement which gave me the push I needed during my tough days.

Thirdly, I sincerely appreciate the opportunity and financial assistance given to me by the European Union project of Erasmus Mundus Joint Master Degree in Soil Science (emiSS) with project number 610528-EPP-1-2019-1-TR-EPPKA1-JMD-MOB.

Lastly, to the Library Department of the Agricultural University of Plovdiv, I am most grateful for the online access to paid databases and writing tools which helped me to get relevant resources for my thesis study.

I am grateful to you all!!!

Onyinye A. EZEIFEKA

CONTENTS

SYMBOLS AND ABBREVIATIONS

SYMBOLS

As	Arsenic
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Fe	Iron
Hg	Mercury
K	Potassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
Ni	Nickel
P	Phosphorus
Pb	Lead
Si	Silicon
Zn	Zinc

ABBREVIATIONS

ATSDR	Agency for toxic substances and disease registry
$BaCl_2$	Barium Chloride
$CaCO_3$	Calcium carbonate
EC	Electrical conductivity
EPA	European protection agency
HM	Heavy metals
HNO_3	Nitric acid
IICQ	Integrated index of contamination by heavy metals
K_2SO_4	Potassium sulphate
NaOH	Sodium hydroxide
OM	Organic matter
REE	Rare earth elements

FIGURES LEGENDS

TABLES LEGENDS

1. INTRODUCTION

We can't put enough emphasis on the problem of environmental contamination while discussing the effects of our daily actions. Environmental pollution is a major issue globally and comes in various forms such as soil, water, and air pollution. As a result of the consequences of pollution, some agricultural areas have become unsuitable for plants and animal production (Wang et al., 2020). The United Nations announced on November 16th, 2022, that the global population is over 8 billion. An increase in human and industrial activities that produce pollutants, notably heavy metals, is inevitable as a result of the need to enhance food production to feed the world's growing population.

Heavy metals (HMs) are referred to as non-degradable elements; thus, HMs linger in soils and pose a longstanding environmental concern (Wu et al., 2013; Ye et al., 2017; Suman et al., 2018). The chief sources of these hazardous contaminants within our environment include mining, ore smelting, sewage application to agricultural soils, and indiscriminate industrial and agricultural waste disposal (Li et al., 2019; Huang et al., 2020; Twarog et al., 2020). Heavy metal contamination has attracted the greatest attention due to its impacts on plant and animal life, plant death, groundwater contamination, animal toxicity, and impaired plant and animal metabolism. Soil heavy metal pollution has particularly garnered much public interest in the past few years, partly due to the growing concern for agricultural product safety and human welfare (Hu et al., 2014; Hu et al., 2017; Li et al., 2019). More than 500, 000, 000 hectares of land are contaminated by HMs at well above 5 million sites worldwide, with present soil HMs concentrations surpassing permissible or regulatory standards. (Liu et al., 2018).

Most remediation projects are becoming technically more challenging and less environmentally friendly, particularly chemical cleanup, which increases chemical hazards to people and in the environment (Khalid et al., 2017; Bhat et al., 2022; Xu et al., 2021). It is pivotal to reduce heavy metal pollution at its source and improve remediation solutions for polluted soils without damaging the ecosystem. Phytoremediation utilizes plants for cleanup, and it has a low environmental impact, is less expensive, and has lower complexity for decontaminating soils that have been contaminated (Saxena et al., 2020; Zou et al., 2021). Hyperaccumulator plant species have been identified as a group of plants having the physiological and morphological defense systems needed for effective countermeasures against heavy metal toxicity this implies that they can better withstand and thrive in metal-rich soils (Sidhu et al., 2017). *Sedum plumbizincicola*, which is an hyperaccumulator plant belonging to the Crassulaceae family, was first discovered in China and has greater potential to accumulate HMs in soils; especially Cadmium and, Zinc in its shoots (Wu et al., 2006; Wu et al., 2012).

This thesis therefore, aims to conduct a comparative study that will allow the determination of the influence of organic soil additives on the uptake of heavy metals, macro and trace elements by S. plumbizincicola, thus creating awareness about the phytoremediation potential and the possibilities of its use for phytoremediation of industrially contaminated soils in Bulgaria.

To achieve the objective thus formulated, it is necessary to solve the following specific tasks:

1. To determine the main soil characteristics and mineral composition of the soil used in the experiment.
2. To study the influence of organic amendments (vermicompost and biochar) on soil characteristics and the amount of phytoavailable forms of heavy metals, micro and macro elements.
3. To compare the effect of selected ameliorants on the accumulation of heavy metals, micro and macro elements by S. plumbizincicola
4. To evaluate the feasibility and effectiveness of the studied soil amendments for phytoremediation of Bulgarian industrially contaminated soils.

2. LITERATURE REVIEW

2.1 Heavy metals (HM)

Metallic elements with an atomic weight and density above 5g/cm^3 are called heavy metals. Lead, Cadmium, Mercury, Arsenic, and Chromium among others fall into this category. Heavy metals are naturally found within earth crust, but they can also find a way into the environment through anthropogenic activities like mining, smelting, and industrial processes. They stick around for long periods in the environment and can build to concentrations that are hazardous to animals and plants. Knowing the land use history of a tract of land can therefore give one an idea of the sources of HMs in the soil as well as their levels (Mahar et al., 2016; Sarwar et al., 2017). When they get to these toxic levels, they can cause damage to plant and animal health, pollution of our water and soil, and could also have negative impacts on our ecosystems and biodiversity. Heavy metal levels in the environment, food, and consumer products have been the subject of increased regulation and monitoring in response to these worries. Methods for detecting and monitoring heavy metals, as well as plans for limiting exposure and overseeing polluted areas are widely being developed (Mahar et al., 2016).

2.1.1 Sources of Heavy metals in the soil

Heavy metals source in soils can be categorized into two, namely natural and anthropogenic source. Natural source of HMs in soils are usually from the parent materials in which the soil has been formed from. The levels of HMs in natural soils will vary depending on the type of parent material and pre-existing minerals in them. Another natural source of HMs is volcanic eruption. On the other hand, the anthropogenic sources are usually from irrigation activities, sewage sludges, mining activities, oil spillage, agrochemical usage in the agricultural sector, smelting ores among other human activities. To better understand the extent of human contribution to the growing levels of environmental pollution, it is important to be aware of the natural levels of these HMs in the soil (Thornton, 1981). Of the list of human activities that add to the HMs concentration in the soil, industrial and agricultural activities top the list. There are reference standards to compare natural levels of HMs in the soil to the permissible or toxic limits that are dimmed unfit for plant and animal health. The most popular ones are the World Health Organization (WHO) and the Food and Agricultural Agency (FAO) reference standards.

2.1.2 Types of Heavy Metals

We can understand better, the adverse effects of HMs on the environment and helath of humans by classifying them into distinct groups, which can then be used to create risk-reduction plans that are both practical and efficient. Heavy metals have been classified into multiple groups according to their unique physical and chemical properties:

1. Essential and non-essential heavy metals: Essential HMs are needed in trace amounts, and they are very crucial for plant and animal development and metabolic processes. Essential HMs includes elements like Iron (Fe), Copper (Cu), and Zinc (Zn). Surprisingly, even the essential HMs in excess can be toxic when they exceed their normal levels. In contrast, non-essential HMs are group of metals which are not essential for plant or animal growth, and they include Cadmium (Cd), Lead (Pb), and Mercury (Hg). These group of HMs can have detrimental consequences at relatively low concentrations (Turkmen et al., 2009).

2. Metalloids: Arsenic and Selenium are examples of metalloids, which are elements with properties of both metals and nonmetals. When accumulated in living organisms, metalloids can cause toxicity even at low concentrations, making them a serious threat to both plant and human health.

3. Rare earth elements (REEs): The periodic table contains 17 elements known as rare earth elements (REEs), each of which has special chemical and physical properties with wide industrial applications. While most rare earth elements (REEs) are not classified as heavy metals, some REEs like gadolinium and europium can have harmful effects at extremely high concentrations. In the last couple of years, scientists have now taken a closer look at their toxicity and dangers associated with them (Kovarikova et al., 2019).

4. Redox-active heavy metals: Chromium, copper, and iron are all examples of redox-active heavy metals because they can partake in redox reactions which in

return produces reactive oxygen species (ROS), which can consequently damage cellular structures and lead to oxidative stress.

2.1.3 Heavy metal contamination versus Heavy metal pollution

Heavy metal contamination is described as the presence of HMs in an environment or substance in concentrations that exceed the background limit or what is normal. Heavy metal contamination is a prevalent issue that can be observed across various environments, encompassing soil, water, air, food, and living organisms. These contaminants pose a significant concern due to their potential adverse effects. On the other hand, the widespread availability of heavy metals within the environment at concentrations above levels deemed safe is called "heavy metal pollution." There are many different causes of HMs pollution, all of which can have serious effects on the ecosystem.

2.1.4 Impact of heavy metals on the ecosystem

Very high amounts of HMs have been found within the environment as a consequence of numerous anthropogenic activities that including mining, smelting, and industrialization. This acivities has contributed to negative consequences on people's health, farms, and water supplies. When released from both natural and manmade sources, HMs can travel great distances through media like soil, water, air, cans, and packaging materials before settling in the human organs and causing a wide range of problems (Gomaa, 2022).

Another major source of pollution in metropolitan areas is the inappropriate disposal of household garbage in landfills, which leads to the release of toxic chemicals into the groundwater and river systems there (UNECE, 2019). The amount of HMs in the environment has been considerably impacted by the usage of fertilizers and agrochemicals such as pesticides in agriculture and also through mining, chemical production, and other businesses that utilize and store a variety of hazardous compounds (Leng et al., 2021).

Heavy metals like Cd, Pb, As, and Hg can enter human bodies via the food-chain, leading to a variety of harmful consequencies such as damage to the liver, kidneys, and nervous system, as well as developmental disorders and even cancer. These metals can also cause damage to the soil and water bodies, leading to decreased agricultural productivity and the death of aquatic life. Therefore, it is vital to implement effective strategies to mitigate the negative effects of HM pollution on our environment and, human health. This can include proper waste management practices, reduction in the use of heavy metal-containing products, and the implementation of sustainable mining and industrial practices.

2.1.5 Impact of heavy metals on human health

Exposing human to high levels of heavy metals, can have significant adverse health effects. In order to protect humans from exposure to the most dangerous chemicals, the Agency for Toxic Substances and Disease Registry (ATSDR) compled a list of compounds, ranking them in order of their toxicity. Human health is most at risk from arsenic, lead, and mercury, the top three metals on this list (ATSDR, 2023).

Arsenic is the most dangerous substance since it can have toxicological consequences in almost all bodily systems (ATSDR, 2023). For instance, exposure to arsenic can cause skin lesions, cardiovascular disease, respiratory disease, neurological effects, and even cancer. In Brazil, there is a risk of high inorganic arsenic exposure from rice and infant cereals contaminated with heavy metals like arsenic, which can pose a public health concern (Nuryan 2021). The contamination often results from the use of pesticides containing heavy metals during crop cultivation.

Similarly, Lead, the second most toxic substance on the priority list, can cause brain damage, renal damage, anemia, and reproductive issues (ATSDR, 2023). Exposure to Lead can also lead to behavioral and developmental problems in children. Mercury, the third most toxic substance on the list, can cause neurological damage, skin rashes, and kidney damage. Cadmium exposure can cause lung cancer, and several heavy metals, such as arsenic and chromium, have been associated with skin and lung cancer, as well as respiratory and cardiovascular issues.

When untreated poultry dung containing heavy metals is dumped into waterways, it can endanger aquatic animals and cause an ecological imbalance, both of which have the potential to harm human health (Taiwo, 2022). It is, therefore, necessary to regulate the use of HMs and their release into our environment to reduce exposure to these toxic substances and prevent adverse health effects.

2.1.6 Impact of heavy metals on agriculture

Heavy metals can infiltrate the soil through diverse means such as chemical application, polluted water, and direct waste disposal on farmlands, which is a critical threat to the entire ecosystem (Rai et al., 2019). When these metals are absorbed by plants, they make their way into the foodchain and expose people to them through ingestation, breathing processes, or drinking contaminated water. Mengxue et al. (2021) conducted a research to ascertain the risk posed by heavy metals to enhance the quality of soils and to ensure agricultural product safety considering the impact of anthropogenic activities on different crop-growing soils. Soil types utilized for agricultural cultivation have been found to have wide variations in the Integrated Index of Contamination by Heavy Metals (IICQ). Major sources of these HMs come from air deposition and fertilization. These findings are in line with previous studies, including Muhammad et al. (2021), that have demonstrated the effects of anthropogenic activities on HM contamination in both soil and plants.

The increasing incidence of heavy metal pollution worldwide highlights the pressing need for effective environmental monitoring to mitigate the impact of these contaminants and establish sustainable strategies and treatments to ensure food safety. Poor agricultural practices have been shown to be a significant contributor to soil contamination with heavy metals. These harmful metals tend to accumulate in plant parts that can be consumed, posing a significant health hazard to consumers (Muhammad et al., 2021). Heavy metals could also have a negative effect on agriculture by contaminating soil and water, thereby affecting crop growth and quality as well as the development and health of animals.

To address this issue, it is essential to implement measures that enable us to do environmental surveillance and mitigate the threat of such contaminants. Collective action from governments, non-governmental organizations, farmers, and consumers is required. Promoting good agricultural practices that minimize soil contamination and encouraging the use of natural methods to detoxify contaminated soils is crucial.

Policies that regulate the use of heavy metals in industry and agriculture must be implemented globally to reduce the incidence of HMs pollution. For instance, the European Union (EU) has enacted legislation to place restriction on the use of certain heavy metals, such as cadmium, in fertilizers to prevent soil contamination (EU, 2019).

2.1.7 Impact of heavy metals on water bodies

Water is in an important natural resource that is useful for agriculture. Heavy metal pollution is a big concern for the health of water bodies and well-being of aquatic organisms. These metals are non-biodegradable and have the potential to accumulate in water, sediments, and aquatic organisms, leading to adverse effects on the ecosystem (Elkhatat et al., 2021). Heavy metals can infiltrate aquatic bodies from various sources such as industrial effluent, mine runoff, and sewage disposal (Elkhatat et al., 2021). The environment is negatively impacted by the buildup of HMs in water bodies. HMs like Cadmium (Cd), Lead (Pb), Zinc (Zn), and Copper (Cu) are typically present in waters at low concentrations (Kenan et al., 2021). However, they are considered major contaminants in water bodies and are primarily taken up by fish from the sediments and benthic invertebrates. These metals can cause bioaccumulation and biomagnification, which indirectly poses a risk to human health when exposed to contaminated fish (Majnoni et al., 2013).

Around 62% of the total heavy metal pollution (including lead, zinc, copper, nickel, cadmium) in the world's water systems comes from industrial waste discharge (Banerjee et al., 2015). Heavy metals can accumulate and have negative effects on aquatic creatures, thus it is crucial to keep an eye on the levels of these metals in water.

In Bangladesh's Rupsa River sediments, Ram et al. (2021) evaluated the pollution level as well as the distribution of heavy metals, as well as the ecological and health risks associated with those metals. They found that the sediments included high levels of copper (Cu), followed by nickel (Ni), lead (Pb), chromium (Cr), arsenic (As), and cadmium (Cd), in that order. After analyzing the environmental and physiological effects of these metals, scientists warned of a possible threat to human health.

Another study by Kenan et al. (2021) discovered that, the availability of HMs in water and soil caused seasonal variations in the haematological, biochemical, and histopathological characteristics of the pike fish (Esox lucius). Pike fish, which is a commonly preferred fish for human consumption, needs to be monitored for HMs such as Chromium (Cr), Copper (Cu), Lead (Pb), and Zinc (Zn) to avoid threats to human wellbeing, wildlife, and sustainable ecosystem (Kenan et al., 2021). Exposure to lead (Pb) from polluted water sources can cause neurological impairment in people and animals, while mercury poisoning may cause malformations in fish and other aquatic species (Kenan et al., 2021).

To sum this up, the ecosystem and public health are both at risk from heavy metal poisoning of water bodies. To stop their buildup and negative impacts, it is crucial to keep an eye on the amount of HMs present in water bodies, sediments, and, aquatic species. Additionally, strategies for reducing industrial waste discharge are critical in reducing the amounts of heavy metals present in water bodies.

2.2 Plant's heavy metal requirements

Even though heavy metals are typically thought of as being toxic, some of the plants we grow need traces of specific heavy metals for growth and development. These HMs are known as micronutrients, and they include iron, copper, zinc, manganese, molybdenum, and cobalt. These micronutrients are vital for several processes related to plant growth and development, including photosynthesis, respiration, and nitrogen fixation. Through their roots, plants take up these micronutrients from the soil through the plant-soil relationship. A number of factors (biotic and abiotic) can affect the availability of these micronutrients in the soil and these factors includes soil CEC, pH, temperature, organic matter content, and the presence of other nutrients. Additionally, specific transporters and chelating substances that prevent the buildup of toxic levels of HMs in plant tissues control the uptake of HMs by plants.

However, excessive levels of HMs in the soil can accumulate in the plant tissues, leading to toxicity and stunted growth. For instance, high amounts of Zn in soils hinders numerous plant metabolic functions, consequently causing conditions like stunted growth and senescence. Zinc toxicity in plants inhibits both root and shoot development. Toxicity caused by Zn also causes chlorosis in young leaves, which spreads to older leaves over time (Choi et al., 1996; Fontes and Coe, 1998).

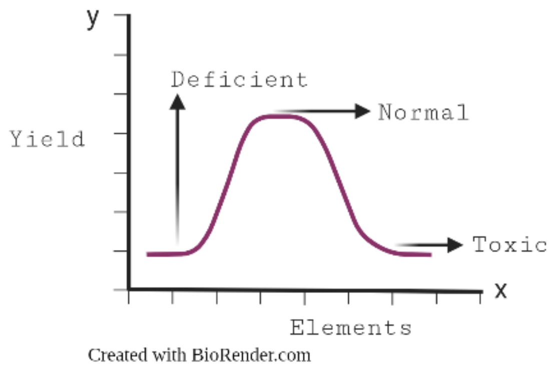


Fig.2.1. Heavy metal requirement for plants

2.3 Phytoremediation

The word *phyto* (which means "plant") originated from greek and the latin word *remedium* (meaning, to remove an evil) are the roots of the English word *phytoremediation*. Phytoremediation is a technique which employs the use of plants to eradicate, absorb, or decontaminate contaminants in soil, water, and air. It involves the absorption and modification of contaminants by plants through a variety of methods, including absorption, volatilization, and rhizodegradation. This approach has been utilized to a variety of contaminants, like HMs, organic pollutants, and radioactive elements, in ecosystems such as industrial sites, agricultural fields, and urban areas (Lin et al., 2021; Wang et al., 2019; Xing et al., 2022).

Several objectives could be achieved with phytoremediation. One reason could be the extraction of metals with a profit potential, such as nickel. Another goal can be a risk reduction through phytostabilization and sustainable soil management, where phytoremediation gradually enhances soil fertility and enables succeeding crop development with increased economic value (Vangronsveld et al., 2009; Van Aken, 2009). Multiple factors, including plant selection, soil quality, pollutant type and concentration, and environmental circumstances, have been proven to influence the efficacy of phytoremediation. In addition, researchers have devised other techniques to improve the efficacy of phytoremediation, including genetic engineering, bio-augmentation, and soil amendments (Xing et al., 2022).

In general, phytoremediation technology presents a viable and ecologically friendly method for tackling contamination challenges, and it is a field of research that is making rapid strides toward advancement (Singh et al., 2016).

2.3.1 Phytoremediation assisted processes

Phytoremediation can be facilitated by a variety of procedures that aim to improve the efficiency and efficacy of pollutant removal by plants. Among the most typical phytoremediation-assisted techniques are:

1. Rhizosphere biodegradation: this entails the utilization of plant roots and related microorganisms to breakdown soil pollutants into simpler absorbable compounds. Microorganisms in the rhizosphere (the region surrounding the roots) can degrade pollutants into less hazardous molecules that plants can absorb or release into the environment (Vishnoi and Srivastava, 2008).

2. Phytoextraction: This is the process of pollutant uptake and accumulation in plant above-ground tissues including leaves and stems. Once (Zhu et al., 2020). Typically, the plants employed in this method have features such as a quick growth rate gathered, the contaminated plant material can either be burned or further processed to remove the contaminants, a large amount of biomass (above ground part) production, hyperaccumulation of heavy metal, HM translocation from root to shoot, tolerance to the detrimental effects of heavy metal, resilience for diseases and pests, etc. (Ali et al., 2013).

3. Phytostabilization: Phytostabilization strategies use plants to immobilize toxins in the soil thereby preventing them from migrating and being absorbed by other organisms. This can be achieved by modifying the chemistry of the soil and fostering conditions that encourage pollutants to bind to soil particles. These include the use of organic amendments to condition the soil physicochemical properties for optimal bioaccumulation process (Erakhrumen, 2007). The most successful

hyperaccumulator plants for phytostabilization are those that accumulate heavy metals in their belowground parts, thereby preventing their release into the food chain (Dickinson et al., 2009).

4. Phytodegradation/Phytotransformation: Leaves, stems, and roots can all participate in the phytodegradation process, which involves the direct breakdown of pollutants by plant enzymes. Organic pollutants, like those found in petroleum hydrocarbons, respond well to this method (Al-Baldawi et al., 2018).

5. Phytovolatilization: This process encompasses the uptake and transpiration of pollutants by plants, that are ultimately deposited into the atmosphere as volatile organic molecules (VOCs). This method is particularly useful for pollutants that are extremely volatile and quickly carried through the air (Ghosh and Singh, 2005). As a result of the transformation of mercuric ion into a significantly less dangerous elemental form, this method is predominantly utilized for mercury. This strategy is only a stopgap measure because the volatile form of Hg deposited into the atmosphere could be recycled back to the soil through precipitation (Sarma, 2011; Sarwar et al., 2017).

Overall, these phytoremediation-assisted techniques can be applied singly or in batches to address a variety of pollutant types (organic and inorganic) in various environments. Several criteria, including the kind and concentration of contaminants, soil characteristics, and plant species, influence the choice of the procedure to be employed.

2.3.2 Limitations of phytoremediation

Despite having numerous advantages over other remediation techniques, phytoremediation has some disadvantages. Firstly, for those who are impatient, phytoremediation may take several months to many years to provide the desired outcomes because it depends on the natural processes of plants (Khalid et al., 2017). Secondly, the hyperaccumulator plants utilized for this purpose are usually slow-growing and have little or no biomass. Furthermore, there are still issues with how to properly process and discard plant biomass that was gathered after phytoremediation. Although several methods have been proposed for the disposal of the biomasses from phytoremediation and these includes the compositing of the biomass (Garbisu and Alkorta, 2001), biomass pyrolysis into gas, liquid and solid (Al Chami et al., 2014; Stals et al., 2010, incineration i.e. burning of the dried biomass (Kovacs et al., 2013) etc. Also, in the tropics and subtropics, where climate change has had the greatest impact, pests and diseases have the potential to severely reduce the accumulation capacity of certain plants, rendering them useless (Cameselle and Gouveia, 2019). The mobility of pollutants may also be negatively impacted by agronomic techniques and soil amendments used during the remediation process.

2.3.3 Enhancing the effectiveness of phytoremediation using organic amendments

Plants in the hyperaccumulator group have the capacity to take up heavy metals in large amounts. In a study conducted by Wu et al. (2012), *Sedum plumbizincicola* had significantly greater concentration of Cd (by levels of 1.92 and 1.71 respectively) after being grown in soil that had been amended with ground rice straw or ground clove. Similarly, the use of chelating compounds like EDTA in the soil might enhance the dissolution and, uptake of heavy metals in plants, hence improving the efficacy of phytoremediation (Blaylock et al., 1997). Enhancing the efficiency of the phytoremediation process means the enhancement of plants growth and heavy metal bioavailability in soil. Metal bioavailability can be improved using organic materials with varied functional groups during phytoremediation. There are numerous amendments that can be used to enhance phytoremediation such as biochar, vermicompost, compost etc.

Biochar is one of the commonly used amendments for soils in remediation projects. It involves the pyrolysis of organic materials obtained from plants, industrial and agricultural activities, etc., in an anaerobic environment to form coal which are further processed into granules or powders for their intended use (Amusat et al., 2021). The product from this process has high specific surface area as well as a high carbon and hydrogen content plus sulphur. Biochar generally improves the physicochemical characteristics of the soil such that plan nutrients are easily made available for plant uptake. In agriculture, biochar is commonly applied to enhance the porosity of soil, increase soil water holding capacity and used for remediation studies. Biochar has been widely reported in recent studies to increase plant growth and plant biomass production by up to 10%, making it widely adopted alongside other phytoremediation techniques to increase HMs remediation procedures (Liu et al., 2013; Qiu et al., 2022).

Compost is also used as soil amendment to enhance phytoremediation due to its rich plant nutrient content (N, P, K, Fe, etc.) which is crucial in improving soil aeration and water retention. Compositing involves the degradation or humification of organic wastes into composts that are rich in nutrients and healthy microorganisms. Waste gathered from kitchens, agriculture, sludges are usually used for compositing (Lu et al. 2014). Adding compost to soil as an amendment can affect the bioavailability and the mobility of HMs, as well as their detrimental effects on plant and animal health (Walker et al., 2004). The reactions of various HMs to the introduction of compost can vary. As an illustration, organic matter has a lesser attraction for Arsenic than it does for cationic metals like Lead, Mercury and Cadmium (Fleming et al., 2013).

Furthermore, Vermicompost also is an organic amendment used in phytoremediation. Vermicomposting is basically a natural and biological process involving the use of earthworms to generate compost. Earthworms perform the duty of converting organic wastes into manure, which is nutrient-rich and used in bio-fertilizers, organic farming, and in some cases, sewage treatment. Vermicompost acts as a buffering agent by reducing the acidic phase and enhancing biostabilization of waste. It also plays the role of a bio-filter by the removal of heavy metals from leachate through adsorption (Kumar, 2005).

2.4 Hyperaccumulator plants

Hyperaccumulators plants are a group of rare plants that have the capacity to collect excessive concentrations of elements on their roots and shoots tissues above levels that are considered to be greater than normal without showing any signs of physiological stress (Bech et al., 2012; Edgar and Lojka, 2020; van der Ent et al., 2013).

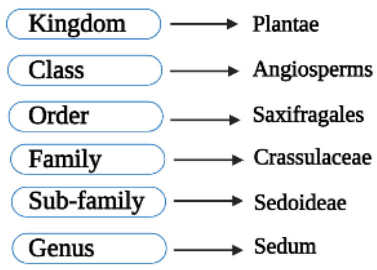
Plants categorized as hyperaccumulants are typically found on metalliferous soils where they always display hyperaccumulation of certain elements, plants found within these areas are referred to as "obligate" hyperaccumulators. On the other hand, some other hyperaccumulator plants that have a wider distribution, of which some of them are found on metalliferous soils exhibiting hyperaccumulation, while others seen on non-metalliferous soils without exhibiting extraordinary accumulation and plants within this category are called "facultative" hyperaccumulators (Pollard et al., 2002; Pollard et al., 2014).

While classifying hyperaccumulator plant species, trace element accumulation concentration in the plant's dry weight foliar tissue when growing naturally in their habitat is considered. These plants should contain >10,000µg g^{-1} manganese or >3000µg g^{-1} zinc, or , >1,000µg g^{-1} Nickel, Arsenic, Lead, or rare earth elements (REEs), >300µg g^{-1} Cobalt, Copper, or Chromium, >100µg g^{-1} Cadmium, Thallium, or Selenium, (Baker and Brooks, 1989; van der Ent et al., 2013).

2.5 Sedum plumbizincicola's taxonomy, characteristics and uses

X. H., Guo and S. B., Zhou ex L. H., Wu found plant *Sedum plumbizincicola* (Crassulaceae family) in 2005. The plant got its *plumbizincicola* name because it was discovered in western Zhejiang Province, China, near areas with plentiful lead and zinc deposits. Greater Cd and Zn hyperaccumulation have been found in S. *plumbizincicola* by a few studies (Wu et al., 2012; Violina, 2020; Wu et al., 2021).

Fig.2.2. Sedum plant



USDA, 2022

Fig.2.3. The Scientific Classifiaction of *Sedum plumbizincicola*

The succulent plant species *Sedum plumbizincicola* is indigenous to China. Just like many other succulent plants, it is a member of the Crassulaceae family. Sedum, also known as Lead (Pb) and Zinc (Zn) stonecrop, is a popular choice for phytoremediation projects because of the plant's tolerance for HMs and the accumulation of HMs like Lead (Pb) and Zinc (Zn) especially in mining- or industrial-contaminated environments. Heavy metals can bioaccumulate in the tissues of the plant, which can then be collected and removed from the site. This plant has been adopted for remediation purposes since 2006 after its discovery (Wu et al., 2006; Wu et al., 2018).

Sedum plumbizincicola is a low-growing plant with fleshy, yellow-green leaves that form rosettes. The waxy coating on the thick, cylindrical leaves helps to limit water loss. The plant normally attains a height of 10 to 20 centimeters and a width of 30 to 40 centimeters. This plant produces clusters of star-shaped, yellow flowers that attract pollinators such as bees and butterflies during the summer (Wu et al., 2018). While its appealing foliage and flowers make *Sedum plumbizincicola* useful in phytoremediation, the plant is also widely used as an ornamental. It is commonly used as a ground cover, in containers, or on rocks. The plant is suitable for gardens in arid regions and semiarid environments due to its tolerance of dryness and high temperatures. To cap it all, *Sedum plumbizincicola* is a tough and adaptable plant species that has several possible uses in environmental remediation and horticulture (Doyle, 2011; Wu et al., 2018).

2.5.1 Cultivation, propagation, climatic requirements of *Sedum plumbizincicola*

Soil requirement and climatic requirements:

Sedum plumbizincicola strives on soils with a pH of 6.0 to 7.5. Because of its resilience, *Sedum plumbizincicola* may thrive in a wide variety of environments and can survive in a array of soil conditions i.e., from drought affected soils to heavily contaminated soils. Overwatering the plant is not advisable in order to prevent root rot. Soil types like sand, loam, and rock are all suitable for *Sedum plumbizincicola*, though it does prefer excessive sunlight. Because of its resistance to dryness and high temperatures, *Sedum plumbizincicola* is an excellent choice for gardens in dry or semiarid climates (Wu et al., 2018).

2.5.2 Method of Propagation:

It is possible to propagate *Sedum plumbizincicola* by seeds, cuttings of the stems or by dividing established plants/tissue culture (Wu et al., 2010; Wu et al., 2018). Cuttings of healthy stems should be obtained in the spring or summer, dried for a few days, and then planted in soils with good drainage even though it can be propagated with fresh stems. Reproduction will be enhanced if a 3–5 cm stubble is maintained when cutting the stems (Li et al., 2009).

2.5.3 Features of *Sedum plumbizincicola* that makes it suitable for phytoremediation

HMs in the soil can be taken up by *Sedum plumbizincicola* and translocated into the plant's aboveground parts, making them more feasible for harvesting and removal.

Sedum plumbizincicola is an ideal phytoremediation plant due to its many beneficial traits, including the once below:

- Heavy metal tolerance: *Sedum plumbizincicola* may thrive in soil with high concentration of heavy metals without showing signs of toxicity or slowing growth. Consequently, the plant is able to flourish in soils that would damage other plants.
- *Sedum plumbizincicola* has a significant capacity for heavy metal accumulation in its tissues, especially its leaves and stems. Because of this, the plant is able to efficiently decontaminate the soil by eliminating heavy metals, hence lowering pollution levels and increasing soil quality.

- Fast growth and high biomass production: *Sedum plumbizincicola* is a fast-growing plant species that may produce large biomass in a short time.

(Wu et al., 2018).

3. MATERIAL AND METHODS

3.1. Selection of soils and organic additives for the experiment

3.1.1. Soils

Samples of soil were gathered from the uppermost soil layer, specifically the horizon situated at a depth of 0-20 cm, within a proximity of 0.5 km from the KCM-Plovdiv location. The sampling site has coordinates 42° 3' 25.3" N 24° 48' 55.1" E (350 m above sea level). The non-ferrous metals plant is located on the alluvial deposits of the Chepelaarska River. Within a radius of 5 km, the alluvial-fluvial and diluvial-fluvial soils are the most widespread and are the most affected by emissions from the smelter.

The soils are characterised by a neutral reaction (pH=7.2) and a high organic matter content (7.1%). In terms of mechanical composition, the soils are characterised as alluvial meadow soils (Table 3.1). Fig. 3.1 shows the diffractogram obtained from the X-ray phase analysis. The individual phases are identified by comparing the obtained intensities with the ICDD-PDF2 database of powder diffraction standards.

The secondary mineral kaolinite is mainly present in the soil sample in amounts of up to 53%. Quartz (28%) and muscovite (3%) are present in the sweat sample of the primary minerals.

Primary and secondary minerals were identified in the soil samples. Of the primary minerals, quartz (SiO_2)(28%) and the minerals albite $NaAlSi_3O_8$ (5%) and anorthoclase (Na, K)$AlSi_3O_8$ (8%), from the feldspar group, and of the secondary clay minerals kaolinite ($Al_2[Si_2O_5](OH)_4$) (53%) were identified. The carbonate minerals calcite $CaCO_3$ (4%) and dolomite $CaMg(CO_3)_2$ (1%, trace) were also identified.

Table 3.1. Main soil physical characteristics of the tested soil

Sample	Particle size distribution, %				
	Coarse sand >2 mm	**Fine** sand 0.2-0.02 mm	**Silt** 0.02-0.002 mm	**Clay** <0.002 mm	**Silt +Clay** <0.02 mm
Soil KCM	34,95	31,92	13,74	19,39	33,14

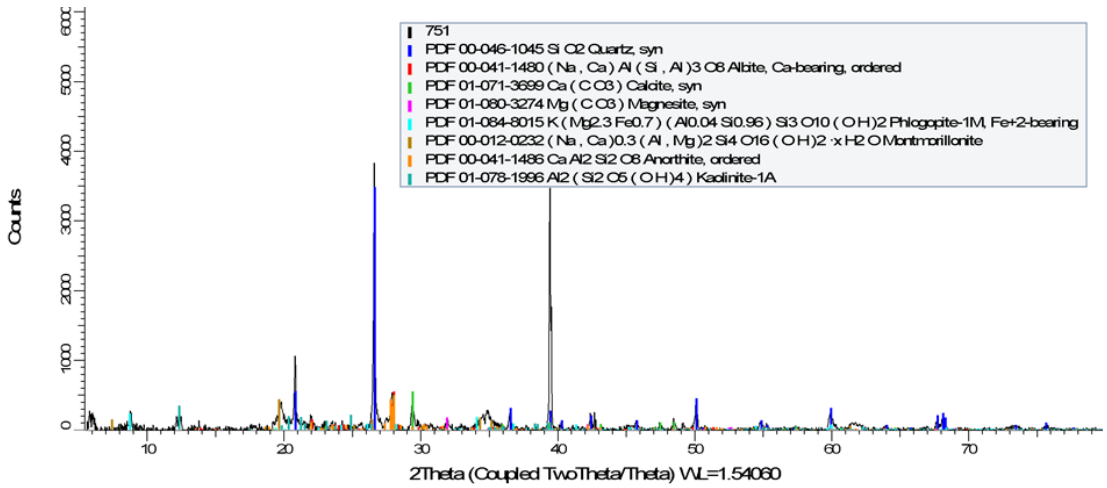


Fig.3.1: The diffractogram obtained from the X-ray phase analysis of the soil sample

3.1.2. Organic additives

Organic soil additives were tested within the framework of the study. Among the organic amendments, vermicompost obtained from the processing of compost (obtained by composting WWTP sludge with biodegradable materials (straw, wood chips, grass clippings and dry leaves) from California worms and biochar was selected.

Biochar is can be described as a carbon-rich solid material that is obtained via the pyrolysis of biomass (manure, sewage sludge, woody biomass and crop residues) in an environment with limited amount of oxygen. Biochar is added to soil to improve its physical properties, such as water holding capacity, nutrient retention, and plant growth. The effects of their addition to soils depend on many factors, including the properties of the raw material and processing conditions, soil type, environmental conditions and crop type. Research on the effects of biochar on plant yield and quality is often controversial. Positive or negative and sometimes neutral effects on crop yield and quality have been reported.

Table 3.2 presents the chemical properties of some of the organic additives used. Biochar has a neutral pH (6.7) and high EC values (88.4). The organic matter content (89.9%) was within the threshold (>60%) of the European Biochar Certification and according to IBI-Standard Class 1. The heavy metal content of biochar is low and below the maximum threshold values for Ordinary and Premium Quality (Pb 120 mg/kg, Cd 1 mg/kg, Cu 100 mg/kg, Hg 1 mg/kg, Zn 400 mg/kg).

Vermicompost has a slightly acid pH (6.4) with a medium to relatively high organic matter content (33.9%). The range of heavy metals in the vermicompost meets the requirements for organic soil conditioner according to the needs of the 2017 Regulation (Cd 3.0 mg/kg, Cu 400 mg/kg, Hg 2.0 mg/kg, Pb 250 mg/kg, Zn 1200 mg/kg).

Table 3.2. Chemical properties of the soil, biochar and vermicompost used in the study

Parameter	Soil	Biochar	Vermicompost
pH	7.2	6.7	6.4
EC, $\mu S.cm^{-1}$	165.6	88.4	3.5
Organic matter,%	7.1	89.8	33.9
P, $mg.kg^{-1}$	884.3	1653	1755
K, $mg.kg^{-1}$	4127.5	33125	6497.5
Ca, $mg.kg^{-1}$	30175	26250	19388
Mg, $mg.kg^{-1}$	11163	29610	910.3
Pb, $mg.kg^{-1}$	2314	2.9	32.5
Cu, $mg.kg^{-1}$	200.7	23	215.4
Zn, $mg.kg^{-1}$	3075.0	37.5	53.3
Cd, $mg.kg^{-1}$	53.8	0.54	0.96
Fe, $mg.kg^{-1}$	26475	336.1	5945
Mn, $mg.kg^{-1}$	818.5	54.2	423.3
Hg, $ng.g^{-1}$	327.9	24.9	721.1

MPCsoil (pH 6.0-7.4) - 320 $mg.kg^{-1}$ Zn, 100 $mg.kg^{-1}$ Pb, 2.0 $mg.kg^{-1}$ Cd, Hg-1.5 $mg.kg^{-1}$

3.2. Description of the experience

Vegetation pot experiments were conducted on heavy metal-contaminated soils obtained from the region of KCM- Plovdiv. This experiments were set in a 0.25 l containers with a dimension of 7x7x8 cm, in 5 variants with three replicates in this order: 1. Control - Soil (no additives). 2. Soil + vermicompost (5%). 3. Soil + vermicompost (10%). 4. Soil + biochar (5%). 5. Soil + biochar (10%).

The soils were afterwards sieved through a 2cm mesh sieve, and the tested organic soil amendments (vermicompost and biochar) were added at 5% and 100%

(recalculated based on the dry weight of the soil) and carefully harmonised with the soil manually using hand. The total amount of soil used in all the treatments was 150 g. In the treatments of the control experiment, the soil was placed directly into the containers, while in the treatments with the application of the organic amendments, the tested amendments were added to the soil in the amount of 5% and 10% (recalculated based on dry soil weight) and carefully mixed with the soil by hand. The soil moisture content was maintained at 75% of PPW. After an incubation period of 4-week, five seeds of the test plant *S. plumbizincicola* were sown in each pot. The containers bearing the plants were put in a growth chamber called Growarm 100 propagators, which provide a suitable constant temperature and humidity for seed germination and plant growth. The propagator has an 8W heated base that gently heats the seed containers, accelerating germination. The base is fitted with a capillary irrigation pad that retains water and ensures it is evenly distributed, preventing the seed containers from drying out or over-irrigating. The transparent lid is designed to handle any condensation and features two sliding vents to help control humidity and temperature. Maxibright LightWave T5 luminescent lamps were installed above the propagators to provide the necessary lighting. The blue-white colour spectrum of the lights is suitable for seedling development and the early growing season. The T5 tubes are mounted in a sturdy metal box with a reflector that ensures the light is directed from each line down to the plants where it is needed. Due to the reduced heat radiation, they were placed closer to the plants. After ten days, seed germination was recorded from the control and additive treatments. Plants were watered every other day, and weeds from the additive treatments were removed by hand. In some of the pots, the number of germinated seeds was more than one, and therefore thinning was done, and one plant per pot was left.

Harvesting took place 98 days after sowing. After being photographed, the harvested plants were cut in about an (1) inch above the soil, they were thoroughly washed with distilled water and placed in labelled paper bags. Fresh and dry biomass yield was recorded. Plants were oven dried at 80°C for 24 hours, and biomass was weighed. The plant roots were removed prior to air-drying and sieving the soils through a fine mesh sieve with a size of less than 2 mm. The sieved soils were then carefully stored in paper envelopes for future analysis.

3.3. Methods used in sample preparation for analysis

3.3.1. Soil samples

Determination of basic soil characteristics

For the purpose of determining the influence of the applied organic additives, soil samples collected were analysed one month after application to the soil. Pre-dried soils sieved using a 2 mm sieve were analysed for soil pH (ISO 10390), electrical conductivity (EC), and total organic matter (BDS EN 13039). The content of total (extracted with royal water, using the ISO 11466 analytical method) and DTPA-extracted mobile forms of heavy metals and micro and macro elements in the soils was determined before applying additives and four weeks after using the soil organic amendments.

The same procedures were applied to the organic amendments.

Utilising X-ray phase analysis, the mineral composition of the soils was determined. Powder X-ray diffraction patterns were recorded in the angular range from 5.3 to 80o2θ with a constant step of 0.02 o2θ on a Bruker D8 Advance diffractometer with Cu Kα radiation and a LynxEye detector. Phase identification was carried out using the Diffracplus EVA program, utilizing the ICDD-PDF2 database. The Rietveld method was used to perform phase quantification, and the Topas-4.2 software package was used to perform the analysis. The accuracy of the quantifications was 3-5%.

I. Soils - Total content

In preparing the samples for analysis for trace element and toxic metal content, the procedure described in the well-established international standard ISO 11466 was used:

- 3 g of air-dry soil was weighed to the nearest 0.001 g and moistened with 1 cm3 H2O. Add 28 cm3 of HCI:HNO3 mixture (3:1). The sample is left for 16 hours at room temperature. Place on a sand bath and heat for 2 hours at 180 to 200°C. Quantitatively transfer the residue to a volumetric flask by filtering through a blue band filter and bring the volume to 50 cm3. In addition to good reproducibility of the results, this method allows correct comparison of the results obtained with those of other authors.

II. Uptake (mobile) forms of heavy metals, micro and macro elements

The amount of nutrients and the mobile forms of heavy metals were determined by extraction with DTPA () solution. This method determined the mobile forms of PO_4-P, NO_3-N, K, Ca, Mg, Zn, Fe, Mn, Cu, and toxic metals (As, Se, B, Cd, Ni, Pb, Mo). The method's main advantage is the simultaneous determination of heavy metals, macro and trace elements in the extract by ICP-AES.

- Weigh 10 g of soil through a 2 mm mesh sieve into a 125 cm^3 Erlenmeyer flask and add 20 cm^3 of DTPA solution. Place the flask on a shaker, shake for 120 minutes, and then filter. The content of mobile forms of heavy metals, macro and trace elements is determined in the filtrate.

III. Mineralising and analysing plant samples for macro and trace element content.

The dry-ashing and microwave mineralisation method determined the heavy metal content in the above-ground mass of plant samples. EPA Method 3052: Suitable for sample preparation for atomic absorption determination of heavy metals. A Milestone 1200 MEGA microwave system with a 10 MRD 300 rotor (10 positions) was used.

- 0.5 g of the dried and well-homogenised sample was weighed to the fourth digit and transferred to Teflon dishes, after which 8 cm3 of hydrochloric and nitric acid were added to each in a 3:1 ratio. The mineralisation program includes three stages: (i) 5 minutes of 250 W non-pulsed irradiation; (ii) 5 minutes of 400 W pulsed irradiation; and (iii) 5 minutes of 600 W pulsed irradiation. After ventilation for 1 minute, cool the sample, and transfer it quantitatively to a 25 cm^3 volumetric flask.

A blank sample was also included in each series of mineralised samples by the described methods to assess the purity of the reagents and glassware used.

3.4 Apparatus used

i. Mineralisation of the samples

A Milestone 1200 MEGA microwave system with a 10 MRD 300 rotor (10 positions) was used to mineralise some soil and plant material samples. The maximum power is 1000 W, and the maximum pressure in the Teflon vessels is 30 bar.

ii. Quantifications

An atomic absorption spectrometer, "Analyst 200" by Perkin Elmer and an atomic emission spectrometer ICP-AES by Jobin Yvon Emission - JY 38 S, France were used in the determination of heavy metals, macro- and trace elements in soil and plant samples. The accuracy of the measurements was assessed by comparing the results obtained with the consensus values in the certificates of the reference materials used.

3.5. Statistical processing of the results

SPSS for Windows was used to perform statistical analysis on the data. The pH, organic matter content, and mobile forms of heavy metals in soil were correlated with the concentration of the elements in the tested plant using a correlation-regression analysis.

4. RESULTS AND DISCUSSION

4.1 Soil characteristics

The soil of the KCM area is defined as Alluvial-Fluvial (Mollic Fluvisol), and its main soil parameters are presented in Table 3.2. The virtually neutral pH of the soil correlates with its high carbonate and hydrogen carbonate content. The organic matter content is relatively high. Salinisation of the soils is not detectable.

The soils are characterised by a neutral reaction (pH =7.2), high organic matter content (7.1 %) and medium to high nutrient (P, K) availability. Total Zn, Pb, and Cd contents are high (3075.0 mg/kg Zn, 2314.0 mg/kg Pb and 53.8 mg/kg Cd, respectively) and many times exceed the maximum allowable concentrations (320 mg/kg Zn, 100 mg/kg Pb, 2.0 mg/kg Cd) (Table 3.2). The content of mobile forms of lead (extracted with DTPA) reached up to 962 (41.57%), cadmium - up to 28.64 (53.25%) and zinc - up to 282.9 (9.2%).

It is known that in soils closer to non-ferrous metal smelters, Zn and other metals levels can be relatively high (443 to 1,112 mg/kg) (ATSDR, 1994). In soils around a Zn smelter in Poland, this metal is present in 202-4832 mg/kg, averaging 1062 mg/kg (Diatta et al., 2008). According to Voegelin et al. (2008), the specific content of Zn in soils that are contaminated is primarily influenced by pH and Zn content. In acid soils, Zn was mainly found in mobile fractions (extraction: NH_4NO_3), while in neutral soils, it was mainly in mobilised fractions (extraction: NH_4-acetate). In highly Zn-contaminated soils, the formation of Zn-pyromorphite, $Zn_5(PO_4)_3OH$, was observed primarily on the root surface, suggesting the influence of the rhizosphere on this process.

Cd's content in the upper soil layers was found to be very high around the smelters.
Soil pH is directly related to the mobility and bioavailability of heavy metals. Most metals are available to plants, primarily in soils with pH <7. The mobile forms of Cd (Cd^{2+}) are formed primarily under acidic conditions due to their weaker binding interaction with the solid phase of the soil. However, $CaCO_3$ content significantly influences Cd mobility and affects the more accessible and mobile fractions n neutral and alkaline soils. A relationship between $CaCO_3$ content on the amount of

accessible forms of Cd and more significant accumulation in plants in carbonate environments has been found. Furthermore, it has been established that the amount of adsorbed Cd increases along with the amount of clay (Golia et al., 2007). Increasing clay contents in the soil can have dual implications on the behavior of metals, impacting their mobility and bioavailability. On the one hand, it can potentially impede the movement and accessibility of metals. Conversely, it can also yield beneficial effects by mitigating the toxic consequences of metal exposure on plant life.

Calcite is the most abundant and relatively mobile form of Ca carbonate present in soils, which significantly influences soil pH and, therefore, trace element behaviour. Trace elements can co-precipitate with carbonates, be incorporated into their structure, or be sorbed by oxides (especially Fe and Mn) that have precipitated on carbonates or other soil particles. Metal ions can also influence precipitation processes on carbonates. The highest propensity to react with carbonates has been documented for Co, Cd, Cu, Fe, Mn, Ni, Pb, Sr, U and Zn. These processes are commonly observed in metal-contaminated soils. Carbonates can act as the dominant sink for trace elements in soil, but the most critical mechanisms regulating trace element behaviour by carbonates are related to soil pH variation. For example, $CdCO_3$, $Cu_2(OH)_2CO_3$ and $Zn_5(OH)_6(CO_3)_2$ are likely to occur in neutral or alkaline soils contaminated with these metals.

The Pb content of the soil is influenced by the presence of hydroxides of Fe and Mn, calcium carbonate and phosphate in the ground. At a higher pH (>6), Pb is taken up from the surface of the feldspar apparently by cation exchange processes. However, a discrete phase of hydricerusite is also observed at high pH (Chardon et al., 2008). In general, Pb accumulates near the soil’s surface, mainly due to its sorption by SOM. According to a study conducted by Sipos et al. in 2005, it was found that soil organic matter (SOM) plays an important role in the adsorption of lead (Pb). However, the fixation of Pb by clay minerals is significantly stronger. While the mobilization of Pb is generally slow, certain soil parameters like increased soil acidity and the formation of Pb-OM complexes can enhance its solubility.

4.2. Influence of organic amendments on basic soil agrochemical properties

Figure 4.1 illustrates the impact of both the type and quantity of soil amendments on physico-chemical properties and the influence of applied organic amendments on important soil characteristics. The results demonstrate the important link between soil amendments and variations in soil characteristics.

pH

Soil pH is a crucial soil characteristic that gives a general idea of the environment for plant optimal growth and development and the behaviour of macro- and trace elements.

The pH value changes the charge of iron oxides and sorption properties (e.g., clays). At low pH values, the sorption of metal cations and iron oxides is very low and increases rapidly at a pH of 6.5. The opposite effect is observed in soils with a lot of organic matter, as metal-humic complexes dissolve at higher pH and heavy metals become more soluble (Herms and Brümmer 1983).

From the results presented in Fig. 4.1, it can be seen that the pH values vary widely depending on the type of amount of ameliorant applied. The results show that the application of vermicompost and biochar increased soil pH relative to the control (Fig. 4.1). Adsorption of metals on biochar may be one of the mechanisms by which metals are immobilised (Beesley and Marmiroli, 2011). An increased soil pH may lead to adsorption and the precipitation of heavy metals (Cd as $CdCO_3$ (Mousavi et al, 2010), Cu as $Cu(OH)_2$ and Pb as $Pb_5(PO_4)_3OH$ (Cao et al., 2011), and to a decrease in their bioavailability (Zhang et al., 2013a). This may lead to a reduction in Cd, Cu, Pb and Zn content in the above-ground plant mass, as observed in the present study. The soil pH sensitivity to organic amendments is somehow attributed to their low buffering capacity (Neilsen et al., 1998).

Atiyeh et al. (2002) found that increased vermicompost applied to the soil led to a decreased in the soil pH. According to Albanell et al. (1998), the formation of NH_4^+, CO_2 and organic acids during microbial decomposition of vermicompost can cause soil pH to decrease.

Electrical conductivity (EC)

Electrical conductivity is a soil characteristics that indirectly indicates the total concentration of dissolved salts and is determined by directly measuring salts in the soil.

The electrical conductivity of the control soils (165.6 dS/m) for the soils of the KCM-Plovdiv area) increases after the application of the organic amendments and is also influenced by the amount of amendment applied. This increase became more pronounced obvious after the application of 5% and 10% vermicompost (468 and 891). Although the soil EC increased 3-4 times when vermicompost was applied, the EC was still low and did not lead to salinisation. Biochar application also led to an increase in soil electrical conductivity (EC). The results are consistent with the findings of Fellet et al. (2011), who found that the EC of mine tailings significantly increased with the addition of biochar.

The effect of increasing soil EC on plant uptake of heavy metals is dependent on the dominant ionic interactions in the soil solution.

Exchangeable cations and cation exchange capacity (CEC)

Applying organic additives leads to an increased amount of exchangeable cations Ca-$BaCl_2$, Mg-$BaCl_2$ and K-$BaCl_2$. The amounts of exchangeable cations varied significantly between the treatments; influenced by the type of additive and the dosage level. The highest concentrations of potassium, calcium and Mg were recorded with 10% vermicompost (Fig. 4.1). However, biochar application decreased the amount of exchangeable forms of K and did not significantly affect exchangeable Mg.

The application of vermicompost increased in soil CEC, with the varying dosages having an influence. As the amount of vermicompost applied increased, the CEC increased to 233.4 mmol/kg and 270.8 mmol/kg, respectively, relative to the control (211.5 mmol/kg). Using 10% biochar resulted in a marginal increase in soil CEC (217.1 mmol/kg). According to some authors, biochar application increased in soil

CEC, while according to others, biochar addition did not affect soil CEC. E.g. Xu et al. 2012 found an increase in soil CEC from 3.55 cmol/kg in the control to 4.90 cmol/kg and 6.12 cmol/kg when 3% and 5% biochar was applied. Similar results were also found by Ghorbani et al. (2019), according to which CEC of clayey sandy soil increased significantly by 20% and 30% due to adding biochar at 1% and 3%. In contrast, this increase was considerably lower (9% and 19%) in clay loam soil.

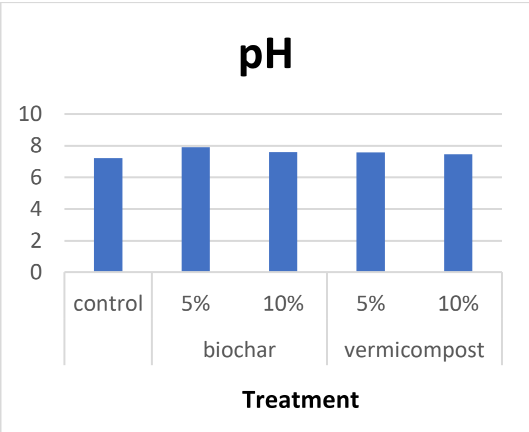
Soil organic matter (SOM)

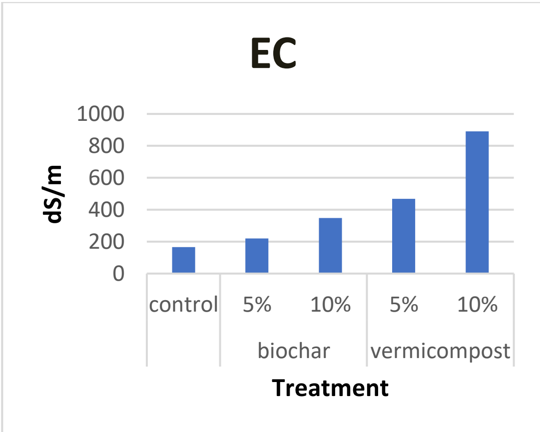
Soil organic matter is essential in soil due to its high cation exchange capacity and ability to form complex compounds. Soil organic matter is considered an additional source of nutrients (Sparks, 1995).

Soils treated with vermicompost and biochar have significantly higher organic matter content relative to the control, which increases proportionally with the amount of amendment applied.

Both organic amendments tested could increase soil organic matter (Figure 4.1), with no significant difference between them, despite biochar's higher organic matter content (88%).

Influence of organic amendments on mobile forms of heavy metals, macroelements (P, K, Ca and Mg) and trace elements (Cu, Fe and Mn) in soil.





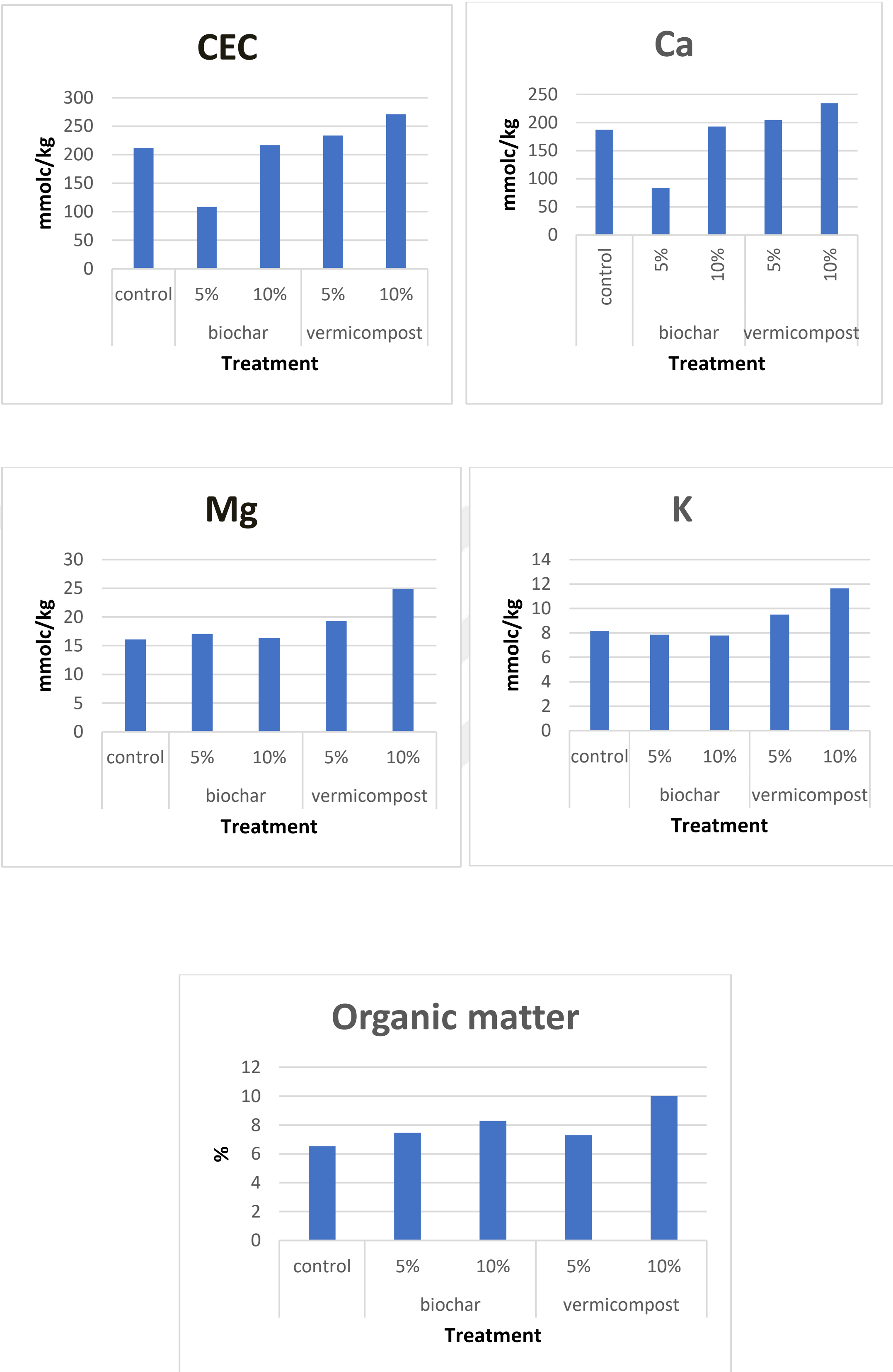


Fig.4.1. Influence of organic amendments on pH, EC, CEC, OM and concentrations of Ca, Mg and K

Heavy metals

The mobile forms of metals were measured using the DTPA extraction (diethylene triamine pentaacetic acid) method. Heavy metals extracted with DTPA are exchangeable or complexed with surfaces (e.g., clay minerals, organic matter) and, therefore, available for plant's uptake (Echevarria, 2018).

Soil amendments used in phytoremediation can significantly affect the mobile forms of Pb, Cd and Zn due to precipitation, complexation, sorption or changes in the degree of oxidation. However, this influence is often not unidirectional, necessitating case-by-case investigation.

Figure 4.2 presents the results obtained for the amounts of mobile forms of Pb, Cd and Zn extracted with DTPA from anthropogenically contaminated soil from the Plovdiv region used in the experiment and their variation after the addition of organic soil amendments. An important factor affecting the mobility of Pb, Cu, Cd and Zn is the amount of organic matter in the soil (McGrath et al., 2003). Many authors have found that Cd content decreases in soils with high organic carbon content and when organic fertilisers are applied. This effect can be explained by organic matter's high cation exchange capacity and its ability to form chelate complexes with Cd. Haghiri (1974), found that the decrease in the Cd content of plants with increasing amounts of applied organic amendments resulted from the soil's high cation exchange capacity.

The application of 5% vermicompost and 5% biochar resulted in a slight increase in the content of mobile forms of cadmium, while the higher dose of the additives had no effect. The decrease in mobile forms of Cd extracted with DTPA could be attributed to the high cationic capacity of organic matter and the ability to bind Cd from the soil. Korcak and Fanning (1985) found in their study, a positive correlation between DTPA-extracted forms of Cd and the amount of soil organic matter.
Results from this study, show a negative correlation between the amount of DTPA-extracted forms of Cd and soil organic matter.

A similar trend was observed for Zn. Applying 5% vermicompost and 5% biochar resulted in a slight increase in the content of the mobile forms of Zn to 297.3 and

305.1 mg/kg, respectively. In contrast, applying the higher dose of the additives had no effect (Fig. 4.2).

The tendency to decrease the amount of mobile forms with organic amendments was more pronounced for Pb. In most cases, its mobile forms fell.

According to Cao et al. (2011), the content but DTPA-extractable Cd, Cu, Pb and Zn in soil decreased significantly with the increasing amount of biochar applied, and the type of biochar had a significant effect, with fine biochar being more effective than coarse biochar. Cao et al. (2011) show that biochar can immobilise Pb in soil by adsorption and precipitation, which produces hydroxy pyromorphite. As a result, extractable Pb decreased, and this decrease was more significant with increasing amounts of biochar. Ok et al. (2010) suggest that the relatively high efficiency of biochar in immobilising Pb may result from the precipitation of Pb-hydroxide and Pb-phosphate.

Phosphorus (P)

Soil P content is influenced by applied organic amendments (Figure 4.2). The lowest mobile P content was found in the control soil (2.32 mg/kg). Biochar application did not significantly affect the amount of mobile P. Soils treated with vermicompost contained significantly more P than the control (Figure 4.2). The application of 10% vermicompost resulted in an increase in the mobile P content to 5.0%. This is most likely due to its slow release from vermicompost under the action of soil microorganisms (Arancon et al., 2005). Similar results were found by Marinari et al. (2000), who found that soil phosphorus increased after the application of organic amendments.

It has been found that P added to soil with compost or other fertilisers can form complexes with Ca^{2+} ions (Korboulewsky et al. 2002), making them less available to plants. The adsorption of P introduced into the soil with compost is enhanced by the presence of Ca^{2+}, as a high P-fixing capacity characterises this element.

The availability of P in carbonate soils is affected by the presence of $CaCO_3$, where part of the available P becomes unavailable as $Ca_3(PO_4)_2$ at pH > 9 or 10, $Ca(H_2PO_4)_2$ at pH 7 and $CaHPO_4$ at pH 8. When organic fertiliser (organic meliorant) is applied, the bond of the phosphorus compounds to $CaCO_3$ is broken. As a result, much more significant amounts of P are available to the plant.

This study’s results agree with those obtained by other authors who found that application of vermicompost leads to an increase in soil P content (Padmavathiamma et al., 2008). Devliegher and Verstraete (1997) saw a significant increase in P content after vermicompost application, reaching values up to twice as high in some treatments and, in some cases, up to three times as high, which was attributed to the increased enzymatic activity of phosphatase by the worms. The increase in phosphorus extracted with DTPA following the application of vermicompost is attributable to the release of humic acids during the decomposition of organic matter. This process converts indigestible phosphorus forms into readily digestible forms. A significant positive correlation was found between assimilable P and soil organic matter C ($r = 0.91$), indicating that organic matter suppresses P fixation in soils (Afif et al. 1993), and the increase in soil pH is further consistent with organic matter. Due to oxidation and decomposition of soil organic matter, many nutrients, such as P, will become more mobile and available to plants (Gallardo-Lara and Nogales, 1987). The application of compost increases mobile P in the soil. Other authors have found similar results (Gallardo-Lara and Nogales, 1987).

There was a significant correlation between DTPA-extracted P and DTPA-extracted Ca ($r = 0.87$) and Mg ($r = 0.83$) (Table 3). The probable cause is the degraded sediment uptake products on the colloids' surface.

Potassium (K)

Potassium is an element that positively influences plant development by enhancing leaf growth, increasing total assimilation area, and increasing crop production yields and quality. It is involved in the regulation of carbohydrate metabolism and aids in the conversion of nitrogen to amino acids and proteins.

DTPA-derived potassium (K) increases significantly with the application of organic amendments, and this increase is more pronounced with applying 10% vermicompost (Figure 4.2). An increase in soil organic matter leads to a decrease in K fixation and hence an increase in its available forms (Olk et al., 1993). Due to the high K content of organic amendments, K increases, whereby the K content of the soil also increases. Similar to the results for P, there is a significant correlation between DTPA-derived K and organic matter (r=0.75).

Calcium and magnesium (Ca and Mg)

Applying vermicompost increased DTPA-Ca and DTPA-Mg content, which was more pronounced when 10% vermicompost was applied. The increase in soluble calcium and magnesium compounds by vermicompost application was attributed to their high levels (Table 3.2). In vermicompost, most nutrients (P, K, Ca) are present in plants' absorbable forms (Orozco et al., 1996).

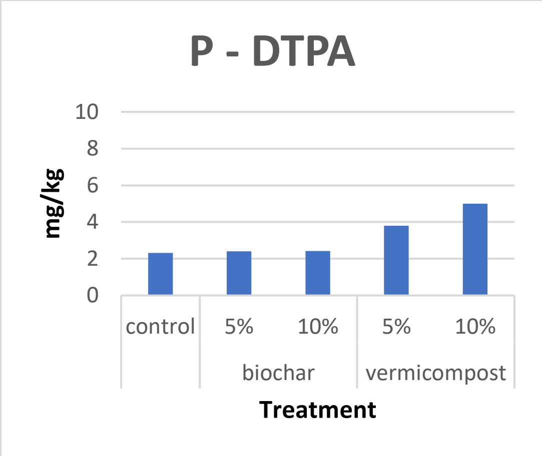
Biochar application resulted in a marginal increase in Ca and Mg content, with a more significant effect found when 5% biochar (81.3 mg/kg Mg) was applied.

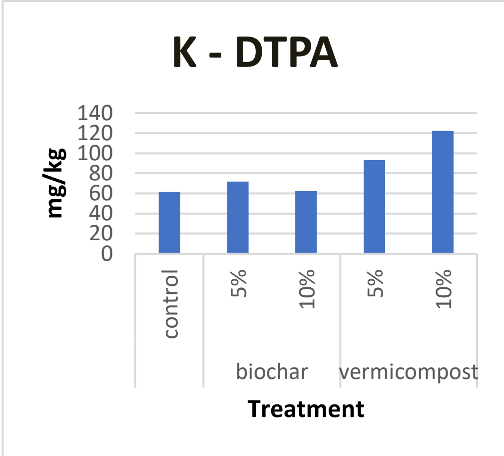
Copper, iron and manganese (Cu, Fe and Mn)

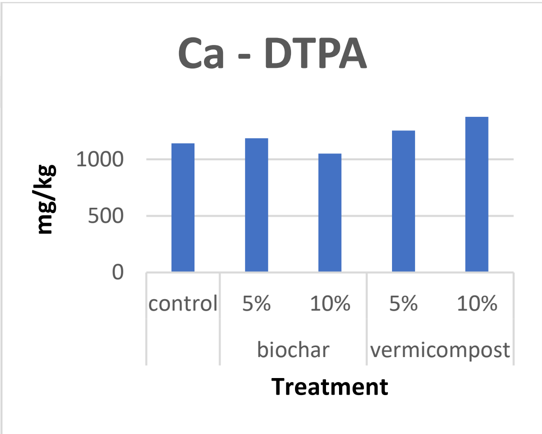
The Mn content increases with the application of organic additives. Application of vermicompost and biochar increased the amount of mobile Mn compared to the control. According to Jordao et al. (2006), the high content of Mn extracted with DTPA may be due to the dissolution of Mn precipitates (carbonates, hydroxides and phosphates) due to the activity of microorganisms, which changes the pH of the soil and the composition of the gas phase.

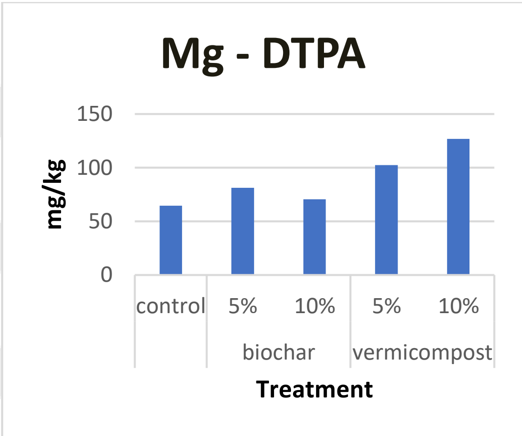
Fe mobility is significantly affected by the type and amount of additive applied. Adding 10% vermicompost increased the mobile Fe content to 8.4 mg/kg compared to the control (3.7 mg/kg).

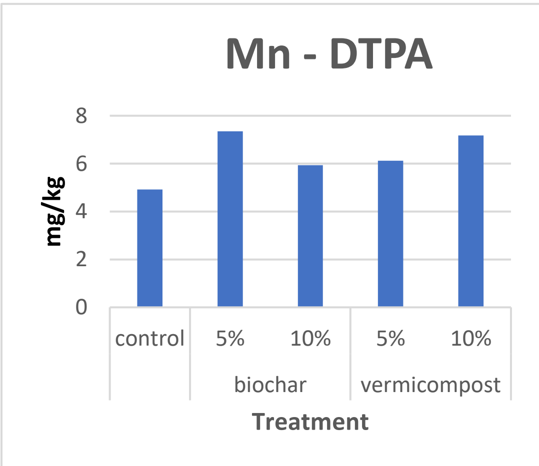
Organic amendments did not significantly affect the DTPA-extracted Cu content. Adding % biochar and vermicompost resulted in a marginal increase in the amount of mobile Cu, while a 10% addition had no effect. Temminghoff et al. (1998) found that pH and soil organic carbon content affects the amount of mobile Cu. Organic matter has been found to form much more stable complexes with Cu than with Zn (McBride, 1978). McGrath et al. (2003) found that total Cu content is strongly influenced by soil organic matter content as the amount of Cu present as Cu^{2+} increases and pH decreases. The authors suggest that the amount of mobile Cu will also be affected by the amount of organic matter in the soil and by the application of organic fertilisers.

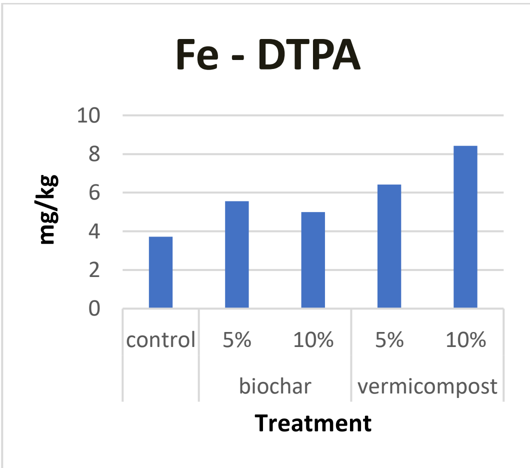

P - DTPA
mg/kg
10
8
6
4
2
0
control
5%
10%
5%
10%
biochar
vermicompost
Treatment


K - DTPA
mg/kg
140
120
100
80
60
40
20
0
control
5%
10%
5%
10%
biochar
vermicompost
Treatment


Ca - DTPA
mg/kg
1000
500
0
control
5%
10%
5%
10%
biochar
vermicompost
Treatment


Mg - DTPA
mg/kg
150
100
50
0
control
5%
10%
5%
10%
biochar
vermicompost
Treatment


Mn - DTPA
mg/kg
8
6
4
2
0
control
5%
10%
5%
10%
biochar
vermicompost
Treatment


Fe - DTPA
mg/kg
10
8
6
4
2
0
control
5%
10%
5%
10%
biochar
vermicompost
Treatment

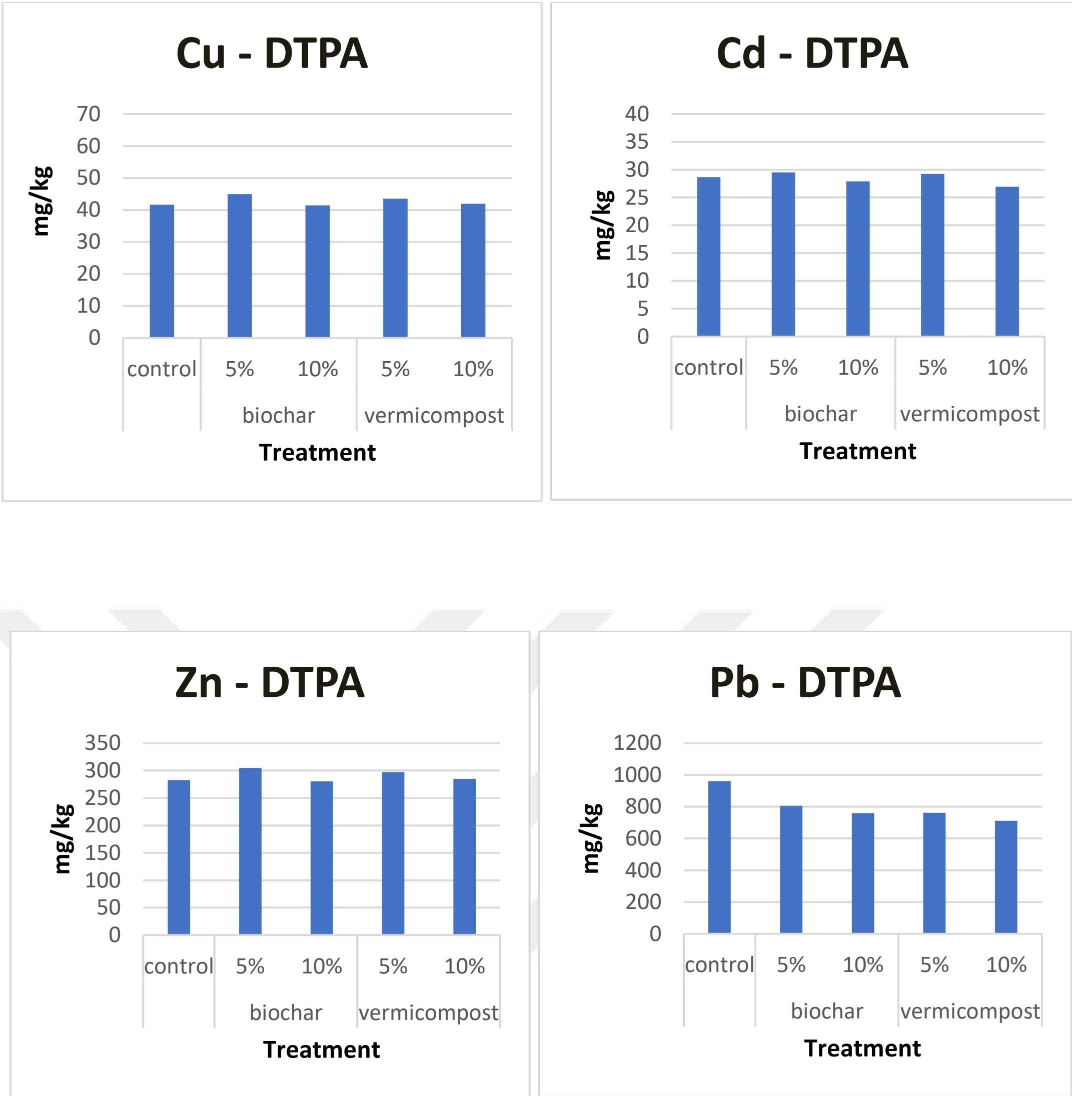


Fig.4.2. Influence of organic additives on mobile forms of elements in the soils

4.3. Evaluation of the Influence of organic amendments on plant development

4.3.1. Uptake of heavy metals, micro and macro elements by sedum (control)

Fig. 4.3 shows the results obtained for the heavy metal micro and macro element contents in the above-ground mass of sedum.

The cadmium contents in the aerial mass of the plants reached 912.5 mg/kg, significantly above the threshold value set for cadmium hyperaccumulation (100 mg/kg). The results obtained indicate the ability of *S. plumbizincicola* to accumulate zinc in the above-ground mass (5440 mg/kg), but the values are lower than the threshold value set for zinc hyperaccumulation (10000 mg/kg). *S. plumbizincicola* has shown promising potential in extracting Cd and Zn from contaminated soils, as evidenced by successful greenhouse and field experiments (Wu et al. 2008, 2012, 2013a) from mine tailings and wastewater. The Cd and Zn contents were as high as 1,470 mg/kg and 14,600 mg/kg, respectively. A probable reason for the lower Cd and Zn accumulation in this study is due to the different soil characteristics under *S. plumbizincicola* cultivation. In Zhejiang Province (China), the soils are sandy, acidic, and highly leached, whereas the soils of the CCM area are alluvial grassland with high carbonate content. The results obtained, confirm that the metal uptake efficiency of *S. plumbizincicola* is significantly higher in acidic soils than in alkaline soils (Li et al. 2014).

The uptake efficiency was calculated by enrichment factor (EF=Cshoots/Csoils) The BAF (BAF=Cshoots/Me soils) values reach up to 17 for cadmium and up to 2 for zinc. These values indicate once again the ability of sedum to accumulate zinc and cadmium in its above-ground mass. Results from this study confirm that the tested plant is a hyperaccumulator and can be used for cadmium and zinc phytoextraction.

Hyperaccumulators are tolerant to soil contamination with heavy megals. A likely reason for this is protective mechanisms involving metal exclusion, active excretion, limited distribution of toxic metals in sensitive tissues, binding of metals to cell walls, chelation by organic molecules, and compartmentalisation in vacuoles (Vo¨geli-Lange and Wagner 1990). Vacuolar sequestration and binding to cell walls are thought to play a critical role in metal hyperaccumulation, and these mechanisms are involved in metal tolerance, transport, and storage in plants (Frey et al. 2000). Cao et al. (2014) found preferential localisation of Zn in the epidermis of young and mature leaves of *S. plumbizincicola*, as well as higher Zn concentrations in the mesophyll of young leaves, which plays an important role in Zn tolerance and hyperaccumulation. With respect to Cd, it was found to be localised in the cell walls and organelle fraction for both young and mature leaves of *S. plumbizincicola*.

The lead content reaches 194.3 mg/kg in the leaves.

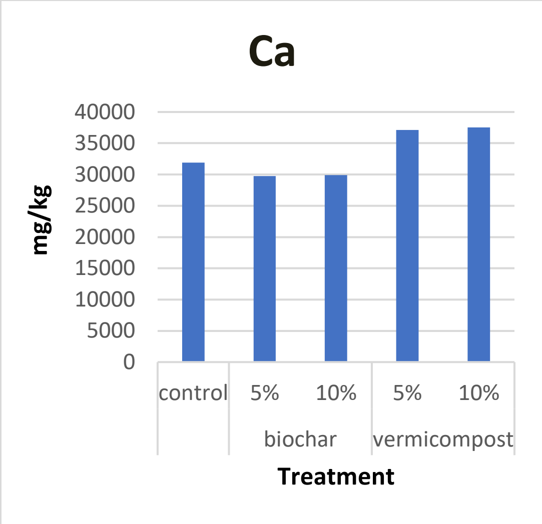
The upward movement of lead (Pb) from the roots to the above-ground parts of plants is typically limited. Once Pb enters the plant's roots, it promptly interacts with phosphates, carbonates, and bicarbonates present in high concentrations within the intercellular spaces. This interaction causes Pb to precipitate as phosphates or carbonates, preventing its transportation through the xylem. Our findings align with Wu et al.'s discovery that *S. plumbizincicola* exhibits a low ability to accumulate Pb, despite the soil containing a higher amount of mobile Pb.

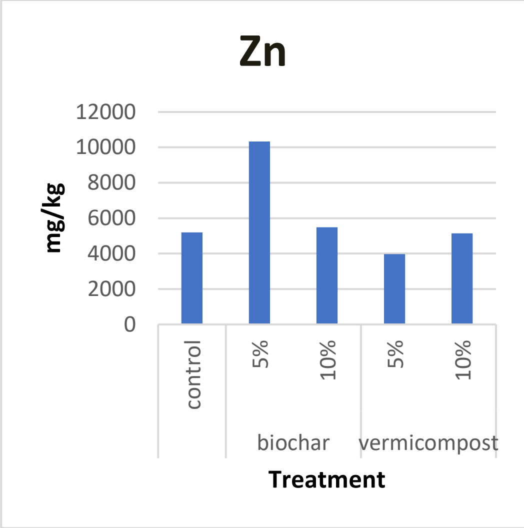
The Cu and Mn contents in the above-ground mass of sedum reached 6.8 mg/kg and 22.6 mg/kg, respectively, values lower than the critical values for plants (20-100 mg/kg Cu, and 400-2000 mg/kg Mn) (Chaney, 1989).

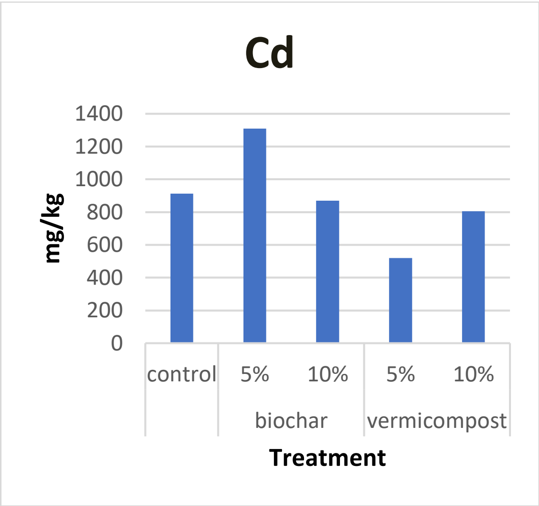
The Fe content reaches 248.8 mg/kg and is within the normal range (50-250 mg/kg).

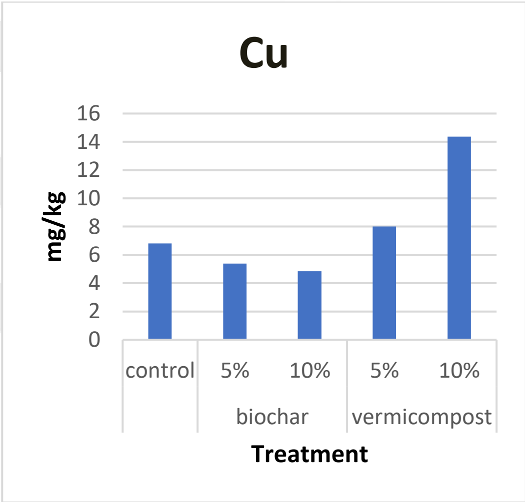
The Ca content in the above-ground mass of sedum reaches 31918.8 mg/kg. In the study conducted by Proctor (1971), it was established that calcium (Ca) plays a crucial role in reducing the toxicity of heavy metals. It appears that plants growing in contaminated soil possess a mechanism that enables them to absorb higher amounts of calcium. This increased calcium absorption serves as a compensatory mechanism to counteract the toxic effects of various heavy metals. Further research conducted by Broadhurst et al. (2013) revealed the remarkable ability of certain hyperaccumulator plant species to uptake calcium in their leaves. Additionally, plants belonging to the Crassulaceae family, as noted by Rössner and Popp (1986), are recognized as calcitrophic species, meaning they naturally possess a significant amount of water-soluble calcium (White 2003).

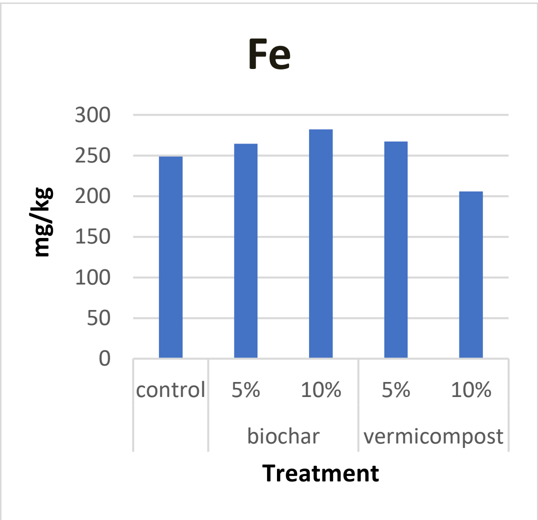
The K, P and Mg contents reach up to 8150 mg/kg, 981.3 mg/kg and 3148.8 mg/kg, respectively, in the above-ground mass of *S. plumbizincicola*.

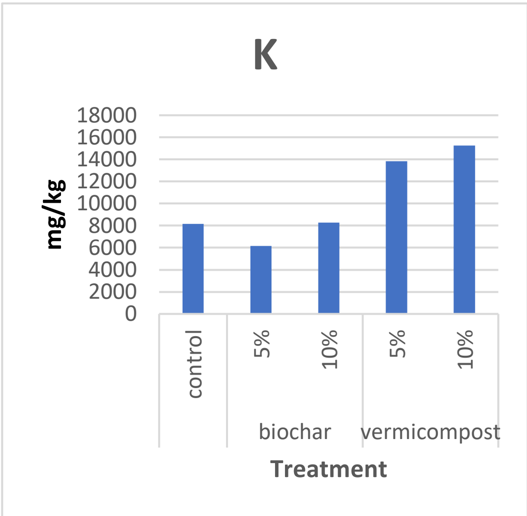

Ca
mg/kg
40000
35000
30000
25000
20000
15000
10000
5000
0
control
5%
10%
5%
10%
biochar
vermicompost
Treatment


Zn
mg/kg
12000
10000
8000
6000
4000
2000
0
control
5%
10%
5%
10%
biochar
vermicompost
Treatment


Cd
mg/kg
1400
1200
1000
800
600
400
200
0
control
5%
10%
5%
10%
biochar
vermicompost
Treatment


Cu
mg/kg
16
14
12
10
8
6
4
2
0
control
5%
10%
5%
10%
biochar
vermicompost
Treatment


Fe
mg/kg
300
250
200
150
100
50
0
control
5%
10%
5%
10%
biochar
vermicompost
Treatment


K
mg/kg
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
control
5%
10%
5%
10%
biochar
vermicompost
Treatment

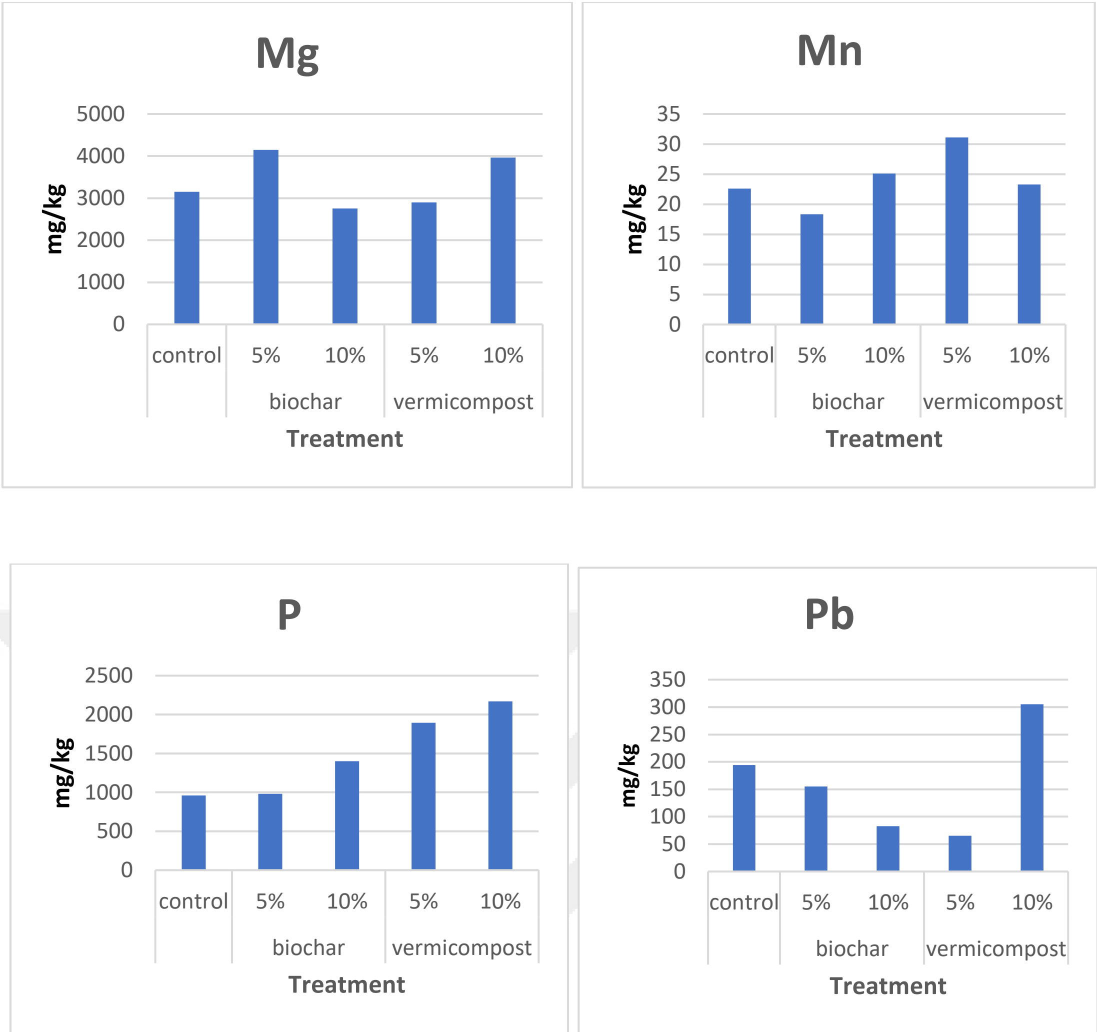


Fig.4.3. Influence of organic ameliorants on the accumulation of heavy metals in the above-ground mass of *S. plumbizincicola*

4.3.2. Influence of organic amendments on accumulation of heavy metals, micro and macro elements by *S. plumbizincicola*

- Influence of organic ameliorants on the accumulation of heavy metals in the above-ground mass

Fig. 4.3 presents the results obtained on the effect of the applied additives on the heavy metal, micro and macro element contents in the above ground mass of sedum. The application of 5% vermicompost resulted in the decrease of Pb content from 194.3 mg/kg in control to 65.5 mg/kg, Zn from 5191.9 mg/kg to 3967.1 mg/kg and Cd from 912.5 mg/kg to 519.0 mg/kg (Figure 4.3). Application of 10% vermicompost had no significant effect on zinc and cadmium contents compared to control, while an increase was observed for lead.

Application of 5% biochar led to an increament in Zn content up to 10325.8 mg/kg and Cd to 1309.6 mg/kg while decrease was observed in lead to 155.3 mg/kg. Application of higher dose of biochar did not significantly affect the Cd and Zn contents of plants. The results obtained can be explained by the amount of mobile forms of heavy metals. The heavy metal content was also influenced by the dilution effect resulting from the increase in plant biomass.

In studies by Österås and Greger (2006), it was found that calcium can reduce the uptake, translocation and the accumulation of Cd in plants. It has been suggested that Cd^{2+} (and other non-essential metal ions) enter plant cells through systems designed for uptake of essential cations. Ca is believed to reduce the harmful effects of Cd toxicity in plants through three mechanisms. Firstly, Ca helps regulate plant growth, thereby reducing the accumulation of Cd in plants. Secondly, Ca boosts the activity of antioxidant enzymes in plants, which helps counteract the oxidative stress caused by Cd. Thirdly, Ca enhances various activities such as photosynthesis, physiology, and metabolism in plants, which in turn regulates signaling pathways dependent on calcium transport (Guo et al., 2018). Moreso, some studies have shown that calcium can also increase Cd absorption by Sesbania sesban and *Brassica juncea* (Franziska and Hans 2015,).

Biochar application resulted in a decrease in Cu uptake by *S. plumbizincicola*, and this decrease was more pronounced when 10% biochar was applied (4.84 mg/kg). Vermicompost application resulted in an increase in Cu content from 6.8 mg/kg in control to 14.4 mg/kg.
Applying biochar increased the Fe content of the above-ground tissues of *S. plumbizincicola*. As the amount of supplement used increased, Fe rose from 248.8 mg/kg in control to 264.6 mg/kg and 282.2 mg/kg. The application of 5% vermicompost also increased content to 267.4 mg/kg, while a decrease was observed in the 10% vermicompost variant (205.7 mg/kg).

An increase in the manganese content of the above-ground mass was found only in the variants with 10% biochar (25.1 mg/kg) and 5% vermicompost (31.1 mg/kg) compared to the control (22.6 mg/kg).

The application of vermicompost resulted in a significant increase in the P and K content of the above-ground mass of *S. plumbizincicola* compared to the control. Application of a higher dose (10% vermicompost) resulted in a significant increase in leaf P and K contents (P from 959.4 mg/kg in control to 2168.1 mg/kg in 10% vermicompost, K from 8150 mg/kg to 15255.2 mg/kg and 5964 mg/kg, respectively). The increase in P and K may be due to the effect of the additive used on the uptake and/or movement of these elements by the plant. Vermicompost contains significant amounts of P and K, resulting in an increase in their content in the soil and their uptake by plants.

However, applying 5% biochar decreases the potassium and phosphorus content of the above-ground plant mass (Fig. 4.4).

Applying organic additives affects the P content of the above-ground plant mass. The P content increased, with the highest content found when 10% biochar was applied. The higher P content in biochar (Table. 3.2) was responsible for the increased P uptake by leaves in biochar treatment.

The results of Lu et al. (2014) showed that biochar treatment significantly affected the P concentration in the above-ground plant mass, where the total P uptake by the plant mass was increased considerably.

Regression analysis showed a relationship between the yield from an above-ground mass of S. plumbizincicola and P uptake (R = 0.91), consistent with the results of Lu et al. (2014). Similar results were obtained by Huang et al. (2012), who found that P fertiliser treatment resulted in a greater growth rate and Zn extraction from the hyperaccumulator *Sedum alfredii*.

Biochar has the ability to bind metal ions on surfaces, resulting in a decrease in the availability of metals. However, it is important to note that this process can also immobilize certain essential plant nutrients, leading to a reduction in plant growth (Beesley et al., 2011). In the study of Lu et al. (2014) biochar from bamboo and rice straw significantly increased the dry weight of *S. plumbizincicola* shoots, possibly due to reduced metal toxicity by improving soil pH and increased availability of P.

The application of biochar resulted in a decrease in Ca content from 31918.8 mg/kg in control to 29770.71 mg/kg in the 5% biochar treatment, while an increase was observed with the application of vermicompost at both rates (Fig. 3.2)

Mg is an essential nutrient required for plant growth. Ca, K, and Na are known to inhibit Cd uptake by plants. Mg competes with heavy metal ions for uptake by roots and affects Cd accumulation and transport in the plant. The effect of organic additives on Mg uptake by *S. plumbizincicola* leaves is not unidirectional. Applying 5% biochar and 10% vermicompost increased leaf Mg content, while using 5% vermicompost and 10% biochar resulted in a decrease in Mg content. An increase in Mg content was found only in the treatments with the application of 5% biochar 4148.2 mg/kg and 10% vermicompost 3965.4 mg/kg compared to the control (3148.8 mg/kg).

Notably, the effect of ameliorants on Ca and Mg uptake by sedum leaves was significantly less pronounced than that of K and P. Similar results were obtained by Gallardo-Lara and Nogales (1987), who reported that no significant changes in Ca and Mg contents were observed after the application of organic meliorates. Applying biochar and vermicompost to the soil increased the mobile forms of Ca and Mg (Fig. 3.2), and this increase did not correspond to the Ca and Mg content of sedum leaves compared to the control. Likely, the results obtainedand this increase do not reach the Ca and Mg content of *S. plumbizincicola* leaves compared to the control. The increased plant biomass can explain the results obtained due to the application of organic amendments to the soil. The formation of more significant biomass decreases the micro and macro elements taken up by some plants (e.g. willow) (a dilution effect in the organic matrix occurs).

4.3.3. Influence of soil amendments on plant biomass

Biometric studies were carried out to study the biological response of the test plants to the soil-applied organic amendments. Plant biomass was determined by weight at harvest.

Figure 4.4 shows the effect of organic amendments on plant growth and their development. The biomass produced was significantly influenced by the additives used. The application of vermicompost has pronounced a beneficial impact on plant development and increases above-ground mass. Increasing the vermicompost dose from 5 % to 10 % increased plant biomass from 25.0 to 51.7 g fresh weight.

Adding 10% biochar increased the biomass to 19.0 g, while a significant decrease (11.4 g) was recorded with a 5% addition.

Cadmium and zinc yields of the plants were also calculated. Applying organic additives significantly aff *S. plumbizincicola* ects plants' cadmium and zinc yield, as the additive type does have an influence. In the 10% vermicompost application variant, the cadmium and zinc yield was 2 to 3 times higher than the control. Applying 5%, vermicompost resulted in a decrease in cadmium and zinc yield. Using 10% biochar resulted in a marginal increase in cadmium and zinc yield (Fig 4.4).

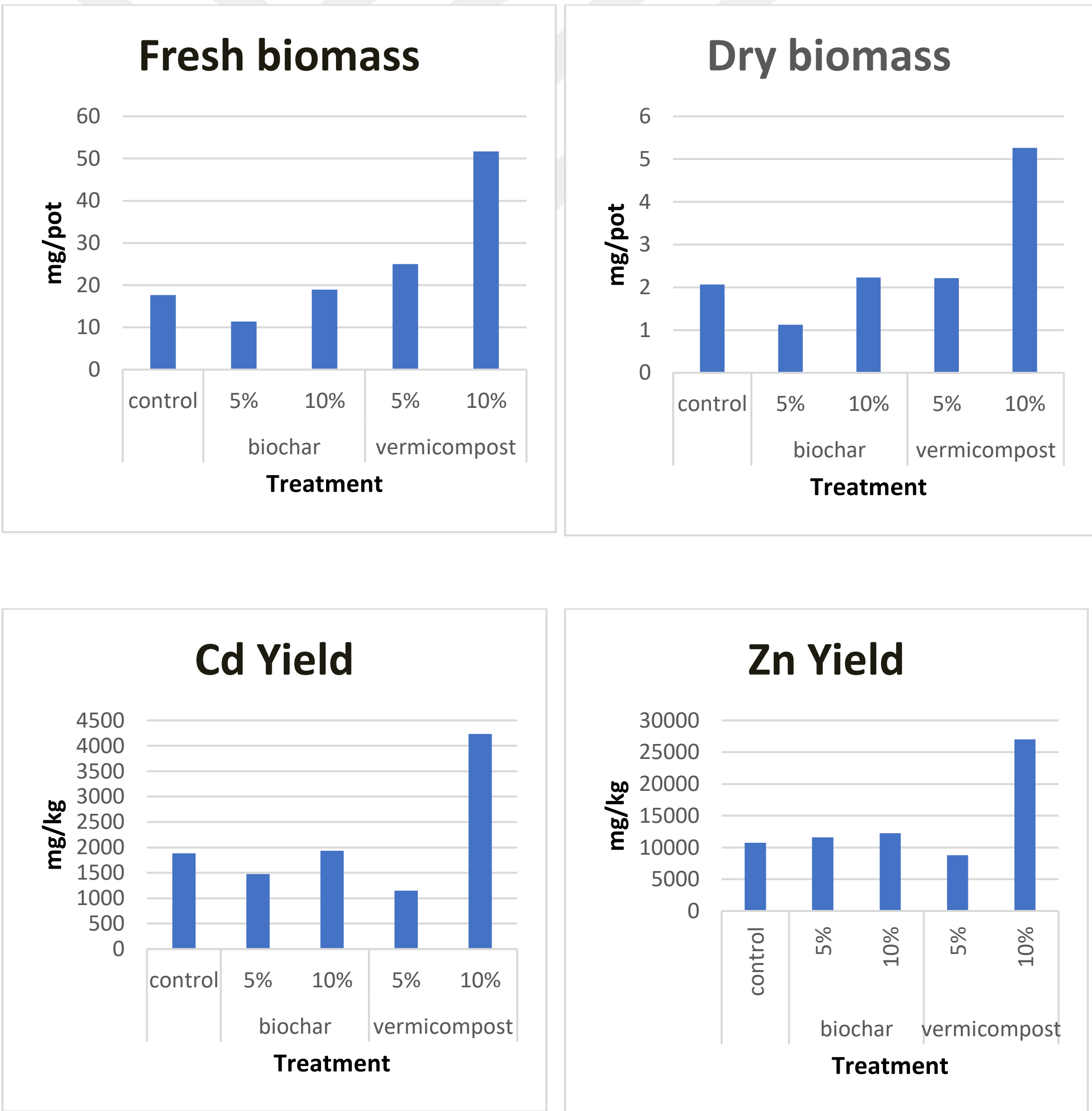


Fig.4.4. Influence of soil amendments on the biomass of *S. plumbizincicola* and yield of Cd and Zn

5. CONCLUSIONS

Based on the obtained results, the following more essential conclusions can be drawn:

1. *Sedum plumbizincicola* can be called cadmium hyperaccumulator and zinc accumulator. The high concentration of Cd and Zn in leaves and high bioconcentration factor (BCF Cd - 12, BCF Zn - 2) indicate the possibility of *S. plumbizincicola* being used in phytoextraction of heavy metal-contaminated soils in Bulgaria.

2. The incorporation of organic additives into soil significantly alters its physical and chemical properties. It increases the soil's organic matter, electrical conductivity, and essential macroelements (such as phosphorus, potassium, calcium, and magnesium) and trace elements (including iron, manganese, and zinc). The extent of this increase depends on the type and composition of the organic additive used.

3. The effects of organic additives on the mobile forms of cadmium and zinc is not unidirectional. Applying 5% vermicompost and 5% biochar resulted in a slight increase in the content of cadmium and zinc mobile forms. In contrast, the higher dose of the additives resulted in a slight decrease in cadmium and had no significant effect on zinc.

4. The application of vermicompost and biochar increased mobile Mn, Fe, Ca, Mg, P, K, to a decrease in mobile Pb content but did not significantly affect the content of DTPA extracted Cu.

5. Both the type and amount of organic additives used affect the amount of biomass. Vermicompost has a significant beneficial effect on plant growth, resulting in an increase in above-ground biomass when compared with biochar. However, the application of 10% biochar yielded a more favourable results.

6. Organic additives positively affect yield, influenced by the type of additive and dose. Cadmium and zinc yields were up to 3 times higher in the variant with a 10% vermicompost application. Application of 10% biochar results in a significant increase.

7. It is necessary to optimise the number of additives to obtain an optimal balance between the growth of plants and the accumulation of cadmium and zinc in the above-ground mass for optimal phytoextraction. Field studies must also be

conducted to evaluate the effectiveness and potential benefits of vermicompost application to the soil for cadmium and zinc phytoextraction.

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Fully Funded Erasmus Mundus Master's degree (EMJMD) Scholarship **2021**