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

# COPING UP METAL STRESS (ZNO NPS) IN PLANTS BY APPLICATION OF VARIOUS FORMS OF BIOCHAR AMENDMENTS

Master's Thesis

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SAMSUN
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SAMSUN
2022

# ACCEPTANCE AND APPROVAL OF THE THESIS

The study entitled **"COPING UP METAL STRESS (ZNO NPS) IN PLANTS BY APPLICATION OF VARIOUS FORMS OF BIOCHAR AMENDMENTS "** prepared by **María Belén MOYA MEJIÍA**, and supervised by **Assist. Prof. Dr. Vishnu D. RAJPUT and Prof. Dr. Rıdvan KIZILKAYA**, was found successful and unanimously accepted by committee members as Master thesis, following the examination on the date 6.7.2022.

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| **Member** | Prof. Dr. Rıdvan KIZILKAYA<br>Ondokuz Mayis University<br>Department of Soil Science and Plant Nutrition | | ☒ Accept<br>☐ Reject |
| **Member** | Prof. Dr. Andon VASILEV<br>Agricultural University of Plovdiv<br>Department of plant physiology and biochemistry and genetics | | ☒ Accept<br>☐ Reject |
| **Member** | Prof. Dr. Coskun GÜLSER<br>Ondokuz Mayis University<br>Department of Soil Science and Plant Nutrition | | ☒ Accept<br>☐ Reject |
| **Member** | Prof. Dr. Orhan DENGIZ<br>Ondokuz Mayis University<br>Department of Soil Science and Plant Nutrition | | ☒ Accept<br>☐ Reject |

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

APPROVAL
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Prof. Dr. Ali BOLAT

Head of Institute of Graduate Studies

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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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Yes ☐ (If it necessary, please add appendices.)

No ☒

Maria Belén Moya M

6 /07 / 2022

María Belén MOYA MEJÍA

# DECLARATION OF THE THESIS STUDY ORIGINALITY REPORT

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# ÖZET

## BIOCHAR DEĞİŞİKLİKLERİNİN ÇEŞİTLİ ŞEKİLLERİNİN UYGULANMASIYLA TESİSLERDE METAL STRESİNİN (Zn O NPS) BAŞA ÇIKARILMASI

María Belén MOYA MEJÍA
Ondokuz Mayıs Üniversitesi
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Toprak Bilimi ve Bitki Besleme Bolumu
Yüksek Lisans, Haziran/2022
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Bu çalışmanın amacı, Güney Rusya'daki Atamanskoe Gölü bölgesindeki kirlenmiş topraklarda ZnO NP'lerinin etkisini azaltmak için farklı biyokömür dozlarının etkinliğini karşılaştırmaktı. ZnO NP'lerin bitkiler ve büyümeleri üzerindeki etkileri analiz edildi. Ayrıca, kirlenmiş topraktaki kirleticilerin türlenmesi ve hareketliliği ortaya çıkarılmış, bunun için Tessier sıralı ekstraksiyon yöntemi kullanılmış ve kullanılabilirliği tartışılmıştır. Toprakta ZnO NP'lerinin varlığı sonucu H. vulgare dokularında biyobirikim nedeniyle oluşan metal stresi ve toprak-bitki ilişkisindeki etkileri analiz edilerek biyokömürün etkilerini hafifletmedeki etkileri incelenmiştir. Mevcut çalışma, zayıf bağlı Zn bileşiklerinin (değiştirilebilir, kompleks haline getirilmiş ve spesifik olarak adsorbe edilmiş) içeriğini ve bunların fluvisol ve spolic tecnosol içinde *H. vulgare* için bulunabilirliğini araştırmaya işaret etti. Bu çalışmanın sonuçları, ZnO NP'lerin etkilerinin konsantrasyonu ile doğrudan ilişkili olduğu sonucuna varmıştır. Tohum çimlenmesini ve bitki biyokütlesini iyileştirebilir. Ancak mikrodispers formda 2200 mg/kg ZnO NP konsantrasyonu ile kök büyümesinde (%44) inhibitör görevi görürken, nanodispers formda %60'ını etkiler. En etkili biyokömür dozları, biyokömür değişiklikleri için 5 APC ve 10 APC Zn ile kirlenmiş topraktaki karbon sorbentlerinin %2.5 ve %5'iydi.

**Anahtar Sözcükler:** Anahtar Kelimeler: Biyokömür, Biyobirikim, *H. vulgare*, Metal stresi, Tessier sıralı yöntemi, ZnO NP'ler, fluvisol, spolik teknosol.

# ABSTRACT

## COPING UP METAL STRESS (Zn O NPS) IN PLANTS BY APPLICATION OF VARIOUS FORMS OF BIOCHAR AMENDMENTS

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The aim of this study was to compare the effectiveness of different doses of biochar to mitigate the impact of ZnO NPs in contaminated soils in the area of the Atamanskoe Lake region in Southern Russia. The effects of ZnO NPs on plants and their growth were analyzed. In addition, the speciation and mobility of the contaminants in the polluted soil were revealed, for this the Tessier sequential extraction method was used and its employability is discussed. Metal stress caused by bioaccumulation in the tissues of *H. vulgare* as a result of the presence of ZnO NPs in the soil and the implications in the soil-plant relationship are analyzed, thus the effects of biochar to aliaviate its effects. The present work pointed to investigate the content of weakly bound Zn compounds (exchangeable, complexed and specifically adsorbed) and their availability to *H. vulgare* in fluvisol and spolic tecnosol. The results of this study conclude that the effects of ZnO NPs are directly related to its concentration. It can improve seed germination and plant biomass. However, it acts as an inhibitor in root growth (44%) with a concentration of 2200 mg/kg of ZnO NPs in microdisperse form, while in nanodisperse form it affects 60%. The most effective doses of biochar were 2.5% and 5% of carbon sorbents in soil polluted with 5 APC and 10 APC of Zn for biochar amendments.

**Keywords:** Biochar, Bioaccumulation, *H. vulgare* , Metal stress ,Tessier sequential method, ZnO NPs, fluvisol, spolic tecnosol.

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Lastly, to my uncle Tom for always being there for me.

María Belén MOYA MEJÍA

# CONTENTS

# ABBREVIATION OF TERMS

| | |
|---|---|
| AAS | :Atomic Absorption Spectophotometer |
| BET | :Brunauer Emmet Teller |
| CEC | :Cation exchange capacity |
| FS | :Food security |
| HMs | : Heavy metals |
| HCl | :Hydrochloric acid |
| NPs | :Nano particles |
| NLDFT | :Density functional theory |
| OM | :Organic Matter |
| PBS | :Phosphate buffer solution |
| TEM | :Transmition electron microscopy |

# FIGURES LEGENDS

# TABLES LEGENDS

# 1 INTRODUCTION

## 1.1 Soil as the base of the soil food web

Soil is fundamental to life on Earth; it represents the very base of our food production, in fact, 95% of the food worldwide is being produced in soil. Nevertheless, soil is a vital living ecosystem that sustains plants and animals, thus soils have diverse functions that help to mitigate and adapt to climate change, filter water, improve resilience to floods and droughts (FAO, 2015b; UNEP & FAO, 2021; WSI, 2022). However, population growth, together with the overexploitation of natural resources, pollution and waste generated in the production of consumer goods, is causing significant negative environmental externalities. Furthermore, it seriously threatens the sustainability of these resources. In recent decades, there has been growing concern about continued population growth and the effects of soil change. According to (ONU, 2017) a study carried out showed that by the year 2030 the world population will reach a size of 8.6 billion people. In addition, it has been estimated that by 2050 the demand for food production will increase by 50% (FAO, 2015b). This will cause an expansion in the agricultural frontier and in could turn the intensification of the use of the territory to obtain food, since more and more food is needed (Lillo, 2019). Healthy soils are the key to food security and our own sustainable future (Rojas et al., 2016). Soil acts as a buffer against pollutants such as agricultural chemicals, organic wastes and industrial chemicals.

## 1.2 Role of soil in food security

Healthy soils are the foundation for healthy food production, the relationship between healthy soils and food security (FS) is of utmost importance. The soil is vitally important for the production of nutritious crops and filters and cleans tens of thousands of $km^3$ of water each year. As an important store of carbon, soils also help regulate emissions of carbon dioxide and other greenhouse gases, thus being essential for climate regulation (FAO, 2015a, 2015b; Rojas et al., 2016). A healthy edaphic ecosystem has high biodiversity, sufficient amounts of soil organic matter (OM), microorganisms, nematodes, arthropods and earthworms. The key microorganisms that are abundant in plant roots are bacteria, fungi, and protozoa. The OM is the basis of productive soils because it allows greater activity and diversity of the life present in the soil. As a result, it stimulates soil processes, which contributes to the health of crops. In addition, OM stimulates microorganisms in the soil, since it suppresses the presence of pathogenic microorganisms. It releases mineral nutrients and improves the stability of soil aggregates (Burbano-Orjuela, 2016; Rojas et al., 2016; WSI, 2022).

## 1.3 Causes of soil contamination

Nevertheless, soil contamination is the main global threat to soil resources. The soil is contaminated when the presence of certain chemical components from human activity alters its characteristics, and may present a risk to human health and the environment (FAO, 2015b; Rojas et al., 2016; UNEP & FAO, 2021). The levels of contamination of heavy metals (HMs) in the soil is increasing every year due to anthropogenic activities such as deposits in industrial areas, mining, disposal of waste with a high metal content, application of fertilizers, manure, sewage sludge, etc. pesticides, sewage irrigation, coal combustion residues and petrochemical spills (Alloway, 2013; Rodriguez-Eugenio et al., 2019; Ruvalcaba-Ruíz, D. et al., 2009). The effects of soil contamination have increased the concern in initiatives that allow remediation of soils that have been affected by heavy metals.

## 1.4 Heavy metals as pollutants

The excess concentration of HMs can produce pronounced toxic effects on plant growth and development. These elements occur naturally in low concentrations in soils. Many of them are essential micronutrients for plants, animals and humans, but in high concentrations they can cause phytotoxicity and damage human health due to their non-biodegradable nature, HMs tends to accumulate easily in living tissues and organisms (Minkina, Nevidomskaya, et al., 2018 a; Sharma et al., 2021). After HMs meet the ground, they can remain on the soil for a long period of time (Burachevskaya et al., 2021; S. Puga et al., 2006; V. D. Rajput, Gorovtsov, et al., 2021). Soil is the main sink for HMs, the health effects they produce are related on the forms in which they are added to soil and the prevailing environmental conditions. HMs reaching the soil go through numerous reactions with different soil components, which affect their solubility, mobility and availability in the environment (Ogundiran & Osibanjo, 2015). In Russia, extremely high concentrations of Zn (up to 98,000 mg kg-1) are reported in the sediments of Lake Atamanskoe (located in the Kamenskii district of Rostov oblast) and adjacent soils. The discharge of pollutants to the lake occurred during the decades between 1957 and 1987 due to waste from an industrial company located near the lake (Minkina, Nevidomskaya, et al., 2018).

## 1.5 Biochar as an alternative to mitigate the impacts of heavy metals

Bioremediation using biochar and nanoparticles are given as an alternative to recover the properties of the soil and in addition to mitigating the impact of heavy metals on the primary production levels of the food chain (Wuana et al., 2011). In recent decades, biosorption and bioaccumulation methods with plants have been used as alternatives to immobilize HM from contaminated soils. Studies conducted by (Misra & Ghosh Sachan, 2021) have given remarkable results related with the rol of NPs in the remediation of HMs in soils. The most common in situ remediation methods for contaminated soils are based on HMs immobilization. Soil cultivation by adding sorbents additives to it allows HMs stabilization, which leads to a decrease in their mobility and bioavailability, and has found widespread use in modern remediation technologies (Bezuglova et al., 2016). In addition, the choice of sorbents i.e., biochar should be based not only on their ability to firmly bind HMs, but also on creating optimal conditions for plant growth. The advantage of biochar is that it can be made from any type of agricultural waste, including a wide range of natural mineral and organic substances, industrial and agricultural wastes, and specially developed sorbents (Koptsik, 2014; Pukalchik et al., 2018), however, biochar is performed better in many cases such organic or inorganic pollutant remediation (Jatav et al., 2021; Mazarji et al., 2021; Qin et al., 2022; Rajput et al., 2022; Sabzehmeidani et al., 2021). The biochar is chemically inert and stable, demonstrates a high absorption capacity and huge porosity (Tomczyk et al., 2020).

Biochar can be obtained by heat treatment (pyrolysis) of plant materials and some industrial wastes, this process consists on a thermal decomposition of organic-matter-containing waste in the absence of an oxidizing agent, resulting in the formation of a solid charcoal-like residue and a pyrolysis gas containing highly boiling resin-like compounds (Devi et al., 2020; Mohan et al., 2006). Biochar is an effective sorbent for the remediation of contaminated soils. Studies carried out (Lu et al., 2017) )showed that the application of biochar from rice straw and bamboo wood to soils significantly reduced the concentration of mobile forms of HM, including Zn. The possibility of using sorbents to firmly bind pollutants in the soil is largely facilitated by their extremely high stability in the soil. Thus, the half-period of biochar mineralization reaches 100 years or more (Hazrat Ali et al., 2019; Andrey et al., 2019; Wuana et al., 2011; Zimmerman & Ouyang, 2019).

## 1.6 Biochar as sorbent for heavy metals

The specific pore surface area of sorbents is large and varies in the range of 400-2000 $m^2/g$, sorption capacity is 200-980 g/kg (Vasilyeva et al., 2007). The sorption of some pollutants by sorbent particle surface is thermodynamically more advantageous than their transition into solution (Mott & Weber, 1992). Adsorption, on the other hand, is a more accepted and widely used remediation technique for HM removal due to its economic feasibility and simplicity of operation in addition to its relative environmental friendliness (Grassi et al., 2012). In addition, carbon-based sorbents can provide nutrients for plants and allow them to retain more moisture (Kim et al., 2011; Kiran & Prasad, 2019). The use of biochar positively affects the pH, cation exchange capacity of soils, and content of nutrients in soils (Ding et al., 2016; Oni et al., 2019). Nanotechnology in agriculture has gained recognition in recent years (Rajput et al., 2022a). In soil, NPs may have a direct influence on soil microbial functionality, hence, it could enhance plant growth via improving soil physiochemical properties (Gorovtsov et al., 2020; Khan et al., 2021). Nanotechnology represents a potential method for polluted environments, such as soil (Kumari et al., 2021; Raffa et al., 2021). Soil remediation is among the most common applications of nanotechnologies. Adsorption, redox reactions, precipitation, and co-precipitation are just a few of the aspects NPs have been used to remove contaminants lately, all of which are aided by their enormous specific surface area (Raffa et al., 2021). The use of nanomaterials with bioremediation approaches has a beneficial effect as these particles are small (1-100 nm) with larger surface area and reactivity (Alazaiza et al., 2021).

## 1.7 ZnO Nanoparticles in the environment

Zinc based NPs have a great importance among the other metal-based NPs (Sturikova et al., 2018), and widely produced (550 - 5550 t $a^{-1}$) (Piccino et al., 2012; Rajput et al., 2018a; Tymoszuk & Wojnarowicz, 2020). It is extensively utilized in cosmetics industries , medicines (Anselmo et al., 2019), food and solar cells (Verma et al., 2019), and get released into the aquatic system (Nowack & Bucheli, 2007) , and accumulate in sediments (1300 t $a^{-1}$), in landfills (200 t $a^{-1}$), and even in urban soil (300 t $a^{-1}$) (Bundschuh et al., 2018). It can have a long lifetime and persistent in the environment depends on the size, shape, and surface of the particles (Mudunkotuwa et al., 2012; Vasileiadis et al., 2015; Velasco et al., 2020). ZnO NPs have both positive and negative effects on plant growth when used in agriculture (Duan et al., 2016; Faizan et al., 2021; Javed et al., 2017; Verma et al., 2019; Zoufan et al., 2020). According to a study conducted by (Singh & Kumar, 2018) in a experiment where ZnO NPs was implemented in *Raphanus sativus, i*t reduced root and shoot length of the plant, in another experiment conducted by (Priester et al., 2017) ZnO NPs caused leaf chlorosis and necrosis on *Glycine max.* Based on these studies the response of plants to ZnO NPs depends on the plant species, type, size, applied concentration, and media (Wang et al., 2015; Yusefi-Tanha et al., 2020; Zoufan et al., 2020). The application ZnO NPs as basal use could reduce soil pollutant toxicity and enhance plant growth at low concentration whereas excess application could project it as a emerging soil pollutant (Azarin et al., 2022; Faizan et al., 2018, 2021; Ghani et al., 2022; Jin et al., 2021; Rajput, et al., 2021b; Srivastav et al., 2021; Zoufan et al., 2020).

## 1.8 Heavy metal contamination in the study zone (Lake Atamanskoe)

Lake Atamanskoe and its surroundings have been polluted for more than 3 decades (Bauer et al., 2022; Gorovtsov et al., 2021), causing damage to the ecosystems in the area. Consequently, new deposits of mud and sediment have been created that are toxic to the environment and human health. This has caused infiltration of trapped water from sedimentation lakes, leading to the formation of a halo of highly contaminated groundwater, especially with zinc underneath. The importance of treating these soils lies in the fact that under Lake Atamanskoe there is an underground water reservoir that serves as a source of water for the city of Shakhtinsky (Burachevskaya et al., 2021; Minkina, Nevidomskaya, et al., 2018; Vodyanitskii et al., 2020). The speciation of heavy metals in soils that indicates the intensity of migration and accumulation of metals in the soil., the impact of soils contaminated by metals on the ecosystem depends directly on the metallic compounds (Minkina, Nevidomskaya, et al., 2018). Identifying the solid phases that control the mobility of metals is essential to understand and predict the behavior of metals in the environment (Ruvalcaba-Ruíz, D. et al., 2009). In the study area, the predominant soil is fluvisol. This soil predominates in the southern part of the country and is a soil with a high agricultural potential, hence the importance of this research (Burachevskaya et al., 2021).

## 1.9 Objective of present study

The considering effectiveness of biochar reducing soil pollutant toxicity and enhance plant growth; three different doses of biochars were applied in different level of ZnO micro and ZnO nano contaminated soil and following observations were performed;

a) Examine how biochar affects soil characteristics and how these changes affect plant development and growth.

b) To analyze the response of *Hordeum vulgare* plants to soils contaminated with different amounts of ZnO.

c) To compare how the plants cop up the metal stress with different biochar amendments.

d) The aim of this study was to analyze the accumulation and transformation of Zn in polluted soils and its effects on plants and soils through the Tessier method.

# 2 LITERATURE REVIEW

## 2.1 Heavy metals effects in the environment

HMs are harmful for human beings and toxic for the environment, in addition to its ability to interfere with the metabolic activities of living organisms. HMs are persistent, bioaccumulative, biotransforming and highly toxic, all of which means that can be found in ecosystems for long periods of time (Dunia & Heredia, 2017). Industrial, technological, agricultural, mining pollution and the indiscriminate use of various chemical fertilizers in the soil with HMs, which are finally incorporated into rivers, plants, animals and food, alter the sustainability of the food chain, causing potential risks in nature and society (Burbano-Orjuela, 2016). Soil contamination causes a chain reaction that has several effects, such as; alters soil biodiversity, reduces organic matter and affects its ability to act as a filter. In addition, the water stored in the soil and groundwater is contaminated, this causes an imbalance in the nutrient cycle (FAO, 2018; Rojas et al., 2016). The presence of HM in the air, soil and water can cause bioaccumulation that affects the entire ecosystem and represents a threat to humans. Various HMs like Cd, Zn, Cu and Cr in excess are considered as hazardous metals. Moreover, all HM in high concentrations have strong toxic effects and are considered environmental pollutants ( Ali et al., 2020a).

The adverse environmental and human effects that the HMs produced are related to their solubility and bioavailability in the soil. Excessive amounts of HMs available in the soil have been reported produces negative effects on the ecosystem (Ghosh et al., 2004). Some HMs affect the central nervous system and internal organs, in addition to having carcinogenic effects (Lee et al., 2007; Maas et al., 2010). In humans, after exposure to high levels, heavy metal toxicity has been associated with several physiological alterations. However, it is important to consider that living beings require small amounts of these metals, for example (Cu, Zn, Fe) for various biological functions. However, a low or excessive concentration of these can alter biochemical and/or physiological processes in the body(Hafeez et al., 2013; Londoño-Franco et al., 2016; Raffa et al., 2021).

## 2.2 Zinc oxide in soil as a pollutant

Zinc in oxide showed serious environmental problem due to their high levels of toxicity for the organisms with which it interacts once it enters in contact with the environment (Jimenez, 2017; Orozco Gutiérrez et al., 2021; Pabón et al., 2020; A. Singh et al., 2010). The accumulation of heavy metals in plants represents severe problems to the soil-plant systems, affecting the number, diversity, and activity of soil organisms, HMs inhibit the decomposition of soil organic matter (Burachevskaya et al., 2021). However, excess content of ZnO nano form also showed toxic effects of plants. Moreover, rarely studies are available on input of biochar in ZnO micro-nano polluted soils. In following sections, we have explained in-depth about role biochar as sorbent of pollutant and plant growth enhancer, forms of ZnO toxicity and Speciation of ZnO micro and ZnO nano in soil. When ZnO NPs are released into the soil, they can alter the soil's physical and chemical properties, interact with existing pollutants, produce new hazardous chemicals, and have an impact on soil functionality, microbial populations, plant growth, and yield. Due to the chemical composition of NPs, once they meet the environment, they can represent a risk to human health (Faizan, et al., 2021 a; Jatav et al., 2021; Jin et al., 2021).

According to studies carried out by (Wang et al., 2018) NPs carry out a variety of chemical and metabolic processes that could disrupt biological nitrogen fixation, damage plant cells and represent a significant risk to human health. For this reason, it is essential to develop safety and toxicological risk assessment standards, including the exposure route and safe exposure doses of ZnO NPs (Rajput *et al.*, 2018 c; García Montero *et al.*, 2021). ZnO NPs have been reported to be beneficial, when applied in small amounts to seeds in a pregerminative state. To have a better understanding of the effects of ZnO NPs, more experiments should be replicated in it, since the data known so far are from hydroponics. This will allow to deepen the interactions of NPs with plants in the soil, and in turn the effect of NPs on the physical-chemical properties in the soil and on human health (García Montero et al., 2021; Loureiro et al., 2018; Rajput et al., 2020a; Świątek et al., 2020).

## 2.3 Zinc oxide nanoparticles in soil: an emerging pollutant or soil health improver

ZnO NPs can be used as a replacement for traditional harmful chemical fertilizers exerts positive effect on the plant growth (Mazumder et al., 2020), ZnONPs have been employed as a significantly less expensive alternative to pesticides due to their antibacterial capabilities. According to (Hafeez et al., 2013; Zabrieski et al., 2015), the practice of ZnO NPS in agriculture has increased worldwide, as part of a strategy that promotes not only crop productivity but also contributes to plant development. The application of ZnO NPs allows control of pests and crop production, in the case of *Arabidopsis thaliana* strong inhibition of root growth, seed germination, and a decrease in the number of leaves was recorded (Woo Lee et al., 2010). Another study conducted by (Sharifan et al., 2020) showed that ZnO-NPs at a concentration of 100 µ/mL reduce the amount of cadmium and lead in *Petroselinum sativum* (parsley), *Spinaciae oleracea* (spinach), and *Coriandrum sativum* (cilantro) roots. In a rice plantation, on the coast of Ecuador, the reduction of cadmium and arsenide content in roots and shoots of *Oryza sativa L*. (rice) was recorded. This study shows that direct exposure to nanoparticles contributed significantly to phytotoxicity and underlines the need for eco-responsible disposal of waste and sludge containing metal oxide nanoparticles (Ochoa et al., 2020).

According (Yusefi-Tanha et al., 2020) to suggest that ZnO NPs fertilizers have great potential to improve yield and food quality, especially when grown in zinc-poor soils. An important advantage is that the presence of ZnO NPs can reduce the amount of heavy metals in edible plants, ZnO NPs have beneficial effects when low doses on plants are applied (Hayder Ali et al., 2021; Czyżowska & Barbasz, 2022). ZnO NPs can be considered as an emerging contaminant or driver of soil health, as long as soil properties are taken into account to avoid phytotoxicity while providing higher Zn accumulation in the plant (Czyżowska & Barbasz, 2022). ZnO NPs in small concentration can have positive affect to plants, but it poses a threat to more sensitive ones (Watson et al., 2015). ZnO NPs with diameters below 100 nm have superior catalytic properties, resulting from the large ratio of the nanoparticle surface to its volume (Yusefi-Tanha et al., 2020; Zabrieski et al., 2015).

## 2.4 Biochar as an efficient pollutant sorbent

The use of biochar as adsorbent materials and its use in problems related to environmental remediation have been notably increased in recent decades as they are materials that have a considerable adsorption capacity thanks to their highly developed porous structure that gives them a large specific surface (Hernández Morales & Piñeros Avendaño, 2017). Biochar is a carbon sorbent, result of a pyrolysis process of organic residues (El-Naggar et al., 2019). Biochar creates new structures for microorganisms that ensure soil aeration and the use of mineral nutrition elements (Burachevskaya et al., 2021; Orozco Gutiérrez et al., 2021). The capacity of biochar metal retention is based on the cation exchange capacity (CEC) found within the porous structure, moreover, having a high specific surface area and series of non-polar and polar substances, biochar has a strong affinity to heavy metals, nitrate, and phosphates that are inorganic ions in nature (Hernández Morales & Piñeros Avendaño, 2017; Kammann et al., 2015).

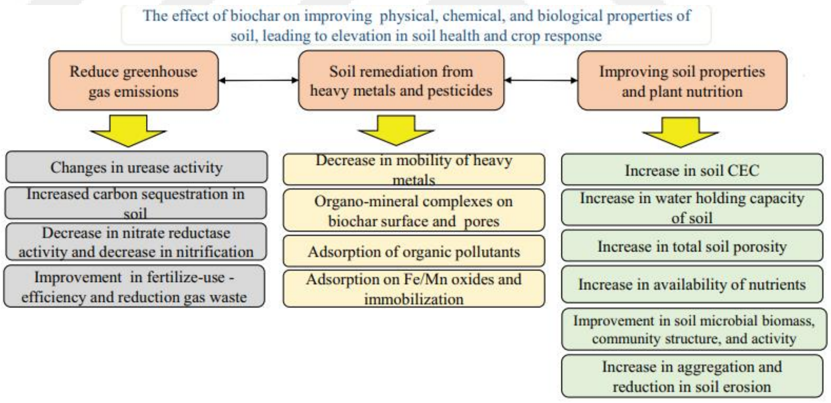

Figure 2.4. Effects of biochar in soil, crop and environment.

Application of biochar to agricultural soils has gained much attention in recent years, due to its potential for waste management, carbon sequestration, reduction of greenhouse gas emission, water and soil remediation, and enhancing soil fertility and crop production (Kuppusamy et al., 2016). Due to these characteristics, biochar is used in the application in the removal of heavy metal ions which have harmful effects for ecosystems when their concentration exceeds the limits indicated by regulatory entities (Anae et al., 2021; Han et al., 2016; Palansooriya et al., 2020; Rajput, et al., 2021b).

## 2.5 Biochar as a plant growth stimulant

The addition of biochar increases the specific length of the root and reduced its diameter and tissue density in plants (Orozco Gutiérrez et al., 2021). According to (El-Naggar et al., 2019), the addition of biochar had a positive impact on the water status of the plants and increased the biomass production of the aerial part, which promoted plant growth and production of the crop. The application of biochar in the soil can induce morphophysiological and metabolic changes in plants, improve the interactions they establish with soil microorganisms, including bacteria that promote plant growth, antagonistic fungi such as Trichoderma, additionally improves microbial population and arbuscular mycorrhizal fungi (Bezerra et al., 2019; Kocsis et al., 2022; Rajput, et al., 2021b). Biochar acts as a plant growth stimulant by significantly enhancing soil structure, reducing soil bulk density, and improving soil water retention, porosity, and aggregation (Arif et al., 2016; Beesley et al., 2015; Gorovtsov et al., 2019; Han et al., 2016; Orozco Gutiérrez et al., 2021). Studies shown that after applying biochar the maize yields increased by 20% after the first year and 12% the second year (Arif et al., 2016).

Soil application of biochars increased the bioavailability of nutrients to plants due to improvements in bio-physico-chemical properties of soil, organic carbon content, and pool of essential nutrients (Fru & Angwafo, 2018; Wrobel-Tobiszewska et al., 2016). An investigation carried out by (Shen et al., 2016) revealed that the application of biochar increased in yield and plant growth associated characteristics (increase spike length and 1000- grain weight, increase shoot and root weight, improve chlorophyll). Application of biochar comprising reduced nutrient leaching, increased nutrient bioavailability, and improved soil aeration, microbial environment, enzymatic activity, soil water retention, and water usability are widely reported, thereby facilitating an elevated crop performance (Burachevskaya et al., 2021; Gorovtsov et al., 2020; Haider et al., 2022; Kuppusamy et al., 2016; Rajput, et al., 2021b). The addition of biochar exerts a great influence on the availability of nutrients in the soil, increasing the availability of P, K, Ca, Mg and Cu, and reducing the availability of N (Abdelhafez et al., 2017; Escalante Reboledo et al., 2016; Han et al., 2016; Palansooriya et al., 2020). The characteristics of biochar give it the potential capacity to improve the physical-chemical properties of the soil and increase the productivity of crops, also contributing to the sequestration of carbon , which makes biochar a tool to fight against climate change (Kocsis et al., 2022; Lehmann & Joseph, 2009; Palansooriya et al., 2020). The effects of biochar on soil properties can vary depending on the characteristics of the biochar, which in turn depend on the properties of the material from which it is obtained and the pyrolysis conditions (Mukome et al., 2013). This explains why crop responses to biochar addition are highly variable (Biederman & Stanley Harpole, 2013; Liu et al., 2012).

The majority of research has focused on the outcomes of biochar on agricultural production, without exploring into the plant mechanisms that explain these responses. Thus, for example, very little research has been done on the responses of the root to the new conditions created in the soil after the addition of biochar. It is therefore necessary to study the responses of the different organs of the plant to the addition of biochar (Ruvalcaba-Ruíz, D. et al., 2009). The use of biochar establishes a positive and significant relationship between specific root length and plant production, suggesting that the increase in fine root proliferation after the addition of biochar increases crop yield. This fact is fundamentally related to greater access to available resources, favored both by a greater volume of exploration and a greater interaction of biochar-root particles (Ruvalcaba-Ruíz, D. et al., 2009).

## 2.6 Speciation of ZnO micro and ZnO nano

Engineered nanomaterials such as ZnO nanoparticles (NPs) will inevitably enter the environment because of the large quantities produced and their widespread application (Lv et al., 2015). Understanding the speciation process of ZnO allows us to understand the behavior of metals in plants, a study carried out by (Lv et al., 2015) showed that Zn 2+ ions were absorbed by the roots and also by the tissues of the corn.

This is the main pathway for Zn uptake by corn. Simultaneously, a small fraction of the ZnO NPs adsorbed on root surfaces enter the root cortex due to rapid cell division and elongation of root tips, some of which enter vascular systems via through the spaces of the Casparian fringe at primary root sites. Biotransformation of ZnO NPs to Zn phosphate within plants further limits their long-distance transport, resulting in negligible upward translocation of ZnO NPs in shoots ( Lv et al., 2015).

## 2.7 Sequential fractionation

In the study of the mobility of heavy metals, it is essential to determine the geochemical fractions of heavy metals in soils. Since the effect of HMs in the environment depends on their form of occurrence, studies of the accumulation and mobility of pollutants in soils are of current interest (Asmoay et al., 2019; Elmer & White, 2016; Houben et al., 2013; Minkina et al., 2018b). The most widely used methods are based on sequential extraction procedures whereby several reagents are used consecutively to extract operationally defined phases from the sediment in a sequence (Ghaderi et al., 2012).

There are several soil analysis techniques (Hortensius & Welling, 1996), in order to have a correct evaluation that the contaminated soil represents, it is necessary to establish the relationship between speciation and the mobility of contaminants ( Minkina et al., 2018a). The speciation of HMs is important to understand the roles that their different species play in the environment. Sequential fractionation extraction is the most widely used technique to determine the degree of association of HMs with other chemical species present in the soil (González Rodríguez et al., 2009; Martínez & Rivero, 2005). Sequential fractionation is a frequently used approach to evaluate metal distribution into different chemical forms present in a solid phase. Conceptually, sequential fractionation categorizes metals associated with chemically homogeneous fractions that, ultimately, affect metal availability (Beckett, 1989).Sequential fractionation can provide useful information to predict the future behavior of HMs in the environment (Silveira et al., 2006). However, fractionation schemes have not been standardized and the results of different procedures are not always comparable due to the lack of uniformity in the experiment conditions i.e (shaking time, reagents, number of extractions) (Silveira et al., 2006). From a chemical point of view standpoint, the poorly cristallized minerals are by far the most active component in the geochemical cycling of trace elements (Chao, 1984). Identifying the chemical solid phases or binding forms responsible for controlling the mobility of metals is essential to better understand the dynamics and fate of metals in the environment (Silveira et al., 2006). A significant issue in the study is the mobility of heavy metals in order to gain a better comprehension of how to determine their geochemical fractions in soils (Anae et al., 2021; Asmoay et al., 2019; Montanarella et al., 2016).

The potential toxicity of heavy metals in soil is a function of their mobility and bioavailability. Soil contamination has effects not only on human health, but also on the environment. The mobility of the metal depends on the phase in which the metal is produced, as well as physical and chemical processes that control the transformations between the phases (Asmoay et al., 2019). Due to the toxicity and the ability of heavy metals to accumulate not only in the initial area of affectation, but also to spread up the food chain and affect entire ecosystems, thus to their nonbiodegradable nature,  the contamination caused by these metals represents a problem (Dunia & Heredia, 2017; Ghaderi et al., 2012; Maas et al., 2010; Morillo et al., 2002; Yan et al., 2020).

It is fundamental to have a thorough understanding of the behavior of HMs in the soil, to assess the impact or behavior of HMs in the future. Information on total concentrations of metals alone is not sufficient to assess the environmental impact of polluted sediments because heavy metals are present in different chemical forms in sediments (easily exchangeable ions, metal carbonates, oxides, sulfides, organometallic compounds, ions in crystal lattices of minerals, etc.), which determine their mobilization capacity and bioavailability several methods for determining the different forms of metals in sediments are described in the scientific literature (Ghaderi et al., 2012; Konstantinova et al., 2020; Minkina et al., 2018a). The Tessier method is more suitable for the separation of the total technogenic component from contaminated soils (Burachevskaya et al., 2018).

# 3 MATERIALS AND METHODS

## 3.1 Study zone

The study site is located in the Kamenskii district of Rostov oblast, in the surrounding area of the Lake Atamanskoe (LA) at the Severskii Donets floodplain in the South of Russia. LA was used as a repository for industrial waste during the 1960s to 1990s ( Minkina et al., 2018b). Soil samples were collected from the soils formed at the bottom of a dried LA (Fig.1) where two types of soil predominate in the area; *Spolic technosols* and *Fluvisols* (Bauer et al., 2022). LA is among the most contaminated water habitats in Southern Russia, moreover it has been contaminated for a long time by outflow from industrial rayon factories near Kamensk-Shakhtinsky (Minkina, et al., 2018b).

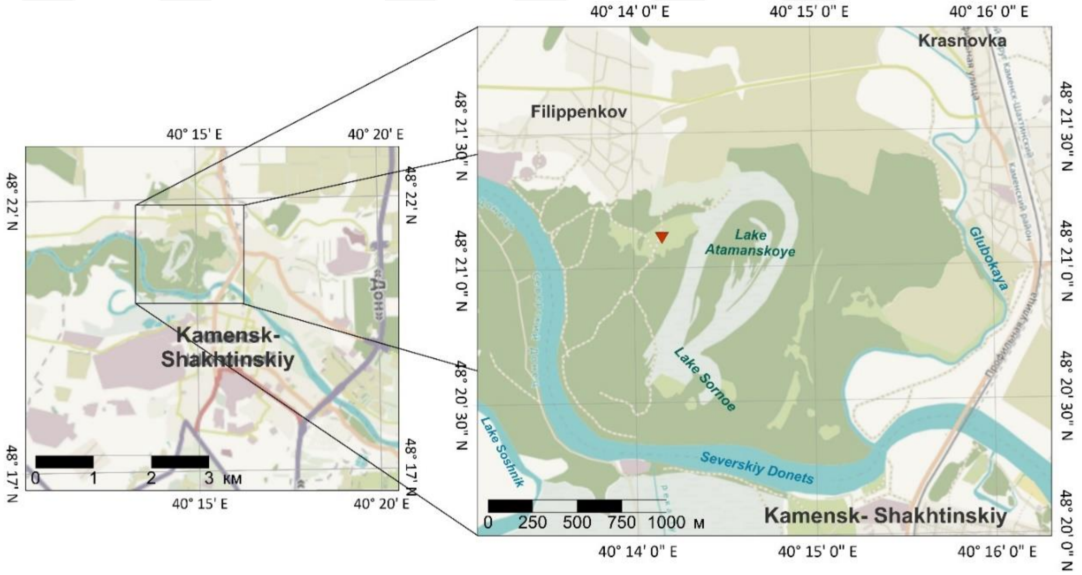

Figure 3.1.-Location of the study zone (Lake Atamanskoye)

In order to study the effect of biochar, a model experiment with artificial contaminated soil was carried out. The soil samples were collected from the Severskii Donets floodplain, in Southern Russia.

## 3.2 Soil sampling

Soil samples were collected from the soils formed at the bottom of a the dried lake Atamanskoe. Soil at the territory adjacent to the lake classified as Stagnic Phaeozem was taken as control. The polluted soil was classified as Spolic Technosol. The collected soil samples were immediately transported to the laboratory and mixed well. Then air-dried and used for the determination of soil physical and chemical parameters and HMs contents. The samples were taken from the indicated location, and selected soil was cleansed of plant residues and other impurities before being pulverized in a porcelain mortar and sieved with a 2-mm diameter hole. With 1 kg of soil, vegetable containers with a closed drainage system were filled with a volume of 2 liters. The amount of contaminants introduced was: 660 mg/kg; 1100 mg/kg (5 APC) and 2200 mg/kg (10 APC) Zn (GN 2.1.7.2511-09). The following scheme of pots with various pollutants with the combination of biochars and ZnO micro and nano was added (Table 1).

### 3.3 **Experimental set-up**

The experiment was laid according to the following scheme:

Table 3.1.- Variables of the experiment

| | |
|---|---|
| 1 | Control (Umbric fluvisols) |
| 2 | 5 APC (1100 mg/kg) ZnO macro |
| 3 | 5 APC (1100 mg/kg) ZnO nano |
| 4 | 5 APC ZnO macro + 1% biochar |
| 5 | 5 APC ZnO nano + 1% biochar |
| 6 | 5 APC ZnO macro + 2,5% biochar |
| 7 | 5 APC ZnO nano + 2,5% biochar |
| 8 | 5 APC ZnO macro + 5% biochar |
| 9 | 5 APC ZnO nano + 5% biochar |
| 10 | 10 APC ZnO nano + 5% biochar |
| 11 | 10 APC (2200 mg/kg) ZnO macro |
| 12 | 10 APC (2200 mg/kg) ZnO nano |
| 13 | 10 APC ZnO macro + 1% biochar |
| 14 | 10 APC ZnO nano + 1% biochar |
| 15 | 10 APC ZnO macro + 2,5% biochar |
| 16 | 10 APC ZnO nano + 2,5% biochar |

|  |  |
|---|---|
| 17 | 10 APC ZnO macro + 5% biochar |

Zn in alluvial and prairie soils was carried out both separately and jointly in macro and nanodisperse forms of ZnO. The incubation period of ZnO micro and nano in alluvial and prairie soils was one month, maintaining the humidity in the pots at 60% of the total humidity capacity of the field. Biochar were then introduced into the studied soils of the model vegetation experiment according to the scheme of the experiment in triplicate. In each pot containing 1 kg soil, twenty healthy barley seeds were sowed without any additional treatments. Controls included unpolluted soils. Before sowing *H. vulgare L.*, the pots were incubated (for about a week). The experiment employed 2.5 percent industrially manufactured wood biochar with surface areas of 500–800 m2/g, pore widths of 8–12 nm, and sorption capacities of 200–500 g/kg.

### 3.4 Characteristics of used ZnO nanoparticles

ZnO NPs powder was supplied by Alfa Aesar,USA. The particle size of the ZnO NPs powder was the 30-50 nm. There was no treatment given prior to the ZnO NPs. In order to prepare the required concentration, the ZnO NPs were put into double distilled water. The NPs solution was shaken and ultrasonicated (stabilization stage) to ensure well-mixed dispersion and reduce aggregation and agglomeration before being used in batch tests. Transmission electron microscopy (TEM) was used to analyze the characteristics of ZnO NPs, including particle shape (Fig.3).

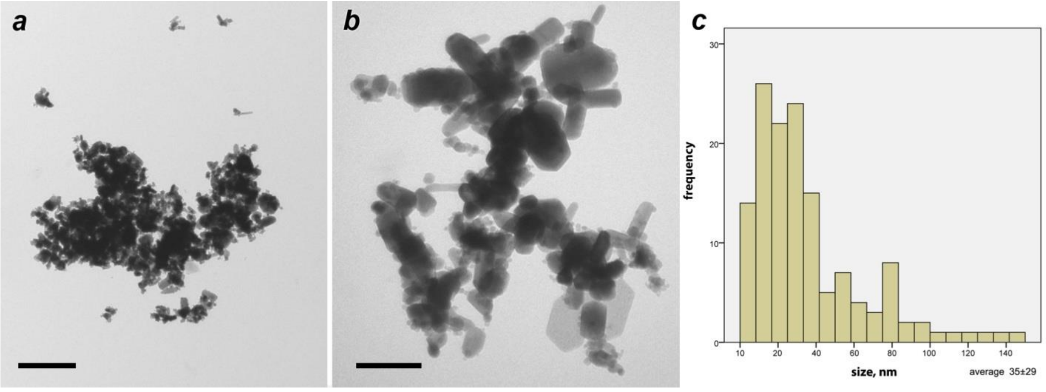

Figure 1.2- Zinc oxide nanoparticles. Scale bar (a - 400 nm, b - 100 nm).

Examination of ZnO NPs samples by TEM showed that it consists of single crystalline particles, predominantly elongated (Fig. 3.2 a, b). A small proportion of the particles are scattered, but most of the particles stick together into loose conglomerates with a size of 1000-2000 nm (Fig. 3.2 b). Particle sizes range from 10 to 150 nm. Occasionally, the relatively large particles are encountered with a size 100 nm and more and the highest frequency of the investigated particles sizes determines in the range from 15 to 40 nm (Fig. 3.2 c).

Table 3.2.- Characteristics of ZnO nanoparticles

| Parameters | ZnO |
|---|---|
| Initial particle size (nm) | < 50 |
| Hydrodynamic diameter (nm) | 39.6± 2.2 |
| Z-potential* (mV) | -33.3± 1.6 |
| Purity (%) | 97 |
| Shape, morphology | Irregular |
| Crystal structure | Spherical |
| Presence of other elements | Ba, Fe, Mg, Ca, K |

## 3.5 Heavy metals analysis in plant tissues and morphometrics parameters

The plant parts were separated into roots, leaves, and stems. Subsequently, they were washed with abundant distilled water and dried in an oven at 65°C. After that, they were ground and passed through a 0.25 mm sieve for HMs analysis. Regarding the preparation of plant tissues for the determination of HMs. 1 g of plant sample was used in the air dryer at 450 °C for 6 h. The ash was then dissolved in 5 mL of 20% HCl and filtered through 0.45 lm Whatman filter paper (Andrey et al., 2019). The analysis was performed using an atomic absorption spectrophotometer (AAS) (KVANT 2-AT, Kortec Ltd, Russia) at room temperature with a tuning fork wavelength: for Zn—213.9 nm, Mn—279.5 nm, Cr—357.9nm, Cu—327.4nm, Pb: 283.3nm, Ni: 352.1nm, Cd: 228.8nm.

## 3.6 Preparation of leaves samples according to Fedorenko method

The observation of structural and ultrastructural cells was carried out according to the method of (Fedorenko et al., 2018), and for this an optical microscope and TEM were used. This method uses a half 1 mm fresh leaf sample taken directly from the plants.

The collected samples were fixed using 2.5% glutaraldehyde/0.1M phosphate buffer solution (PBS) at room temperature for 2 hours. Fixed samples were washed with PBS. After washing, samples were incubated for 1 hour in 1% OsO4/0.2M PBS solution. Increasing concentration of ethanol and acetone (50%, 70%, 96% and 100% separately) were used by dehydration Dehydrated samples were embedded in Epon-812 embedding medium. Ultrathin sections (about 1 μm thick) were prepared with a microtome (Leica EM UC6, Germany), stained with 1% toluidine blue, and examined by light-optic microscopy (Mikmed-6 St. Petersburg, Russia) and TEM (Tecnai G2, Philips, The Netherlands).

## 3.7 Characterization of Wood Biochar

The sorbent morphology was studied using scanning electron microscopy (SEM Carl Zeiss EVO-40 XVP) and confocal microscopy (KM Keyence VK 9700). The imaging was carried out under standard conditions for non-conductive and low-contrast samples (low vacuum, 15 kV, increased emission), the conductive layer was not deposited (the study of morphology under inactive conditions). Determination of the elemental composition in powder samples of carbonaceous sorbents was carried out on a Max GV spectro-scan. For the case of porosity and specific surface area a low-temperature nitrogen adsorption approach on an ASAP 2020 volumetric analyzer was used.

The calculation of the porosity andsurface parameters was carried out by the Brunauer-Emmett-Teller (BET) method for N2 in the range of equilibrium values P/P0=0.05-0.33. The volumes of micro-meso- and macropores and the total limiting volume of the adsorption space were calculated. Determination of volumes of micro and mesopores of sorbents was performed using the comparative t-method using the Hurkins-Jura equation to calculate the thickness of the adsorbate statistical layer. The pore size distribution was calculated using the NLDFT (density functional theory) method.

In the study of the biochar composition, Fourier transform infrared spectroscopy (FTIR) was used. A DTGS detector was used to analyze IR spectra on an FSM-1202 spectrometer in transmission mode. The spectra was recorded in the range from 4000 to 400 cm–1 with a resolution of 4 cm–1 per 100 scans. A tablet of 200 mg KBr with a diameter of 13 mm was used as a reference sample. The test samples weighing 0.14 mg (wood biochar), 0.21 mg (sunflower husk biochar), and 0.18 mg (rice husk biochar) were crushed, mixed with KBr, and pressed into granules with a total weight of 200 mg.

In order to further study the composition, as well as the thermal and oxidative stability of materials, phase transitions, temperatures and kinetics of chemical reactions, Synchronous thermal analysis of biochar samples was used, including thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) (thermal analyzer STA 449 F5 Jupiter, Netzsch). For analysis, sorbent samples were placed in special corundum crucibles. The measurements were carried out in the temperature range of 25–800°C in an air flow (70 ml/min).

To determine the degree of crystallinity of the studied biochar, the method of X-ray phase structural analysis (XRD) (XRF) was used.

Each sample was pulverized first and then put as a dry powder to the measuring container. The measurements were carried out on a Brucker D2 Phaser diffractometer in the range from 20° to 90° with a step of 0.01°, the time for each step was 0.2 sec. A total of 5 passes were made in the intensity accumulation mode. The anode material was Cu, the detector used was LYNXEYE/SSD160.

### 3.8 Plant growth, morphological and anatomical observations

After one month of soil incubation, spring barley (*H. vulgare*) was sown (Fig. 4), the measurements of height of barley shoots when applying Zn to the soil in micro- and nanoform and different doses of biochar were collected.

Figure 3.3.- H.vulgare in different treatments

### 3.9 Methods to determine the speciation of ZnO micro and ZnO nano

To study the processes of transformation, identification and identification of HMs in the soil-plant structure, a multifactorial vegetative experiment was carried out on textured soils, in which several levels (5 and 10 APCs of Zn) and species were studied. (Mono and combined) were detected. Various types (carbon and mineral sorbents) and doses (2.5%, 5%) of sorbents were used. The total number of options for the experience is 48 samples to set up the model experiment, soils (0-20 cm) from the natural landscape, represented by intrazonal alluvial sandy soils and prairie heavy clay soils located in the floodplain of the Seversky Donets River were used as controls. According to (Tessier et al., 1979) the metals were fractionated into the following five fractions: exchangeable, bound to carbonates, bound to Fe and Mn oxides, bound to organic matter and residual fractions. The whole technogenic component of polluted soils is separated using this procedure (Burachevskaya et al., 2018). The most widely used methods are based on sequential extraction procedures whereby several reagents are used consecutively to extract operationally defined phases from the sediment in a sequence (Ghaderi et al., 2012). There are several soil analysis techniques (Hortensius & Welling, 1996), in order to have a correct evaluation that the contaminated soil represents, it is necessary to establish the relationship between speciation and the mobility of contaminants ( Minkina et al., 2018b).

### 3.10 Sequential extraction

The sequential fractionation method analyzes HMs realtive mobility and the forms of their bonds with soil components. This technique details the nature, modes of occurrence, biological and physical-chemical activity, mobility and transport of heavy metals. (Burachevskaya et al., 2018; Mohamed et al., 2019; Vilar et al., 2003). In order to study to study the mobility of HMs in the soil, a better and trough understanding of the determination of geochemical fractions is required (Minkina et al., 2018). According to several studies (Kumkrong et al., 2021; Morillo et al., 2002; V. D. Rajput et al., 2018; Tokalioglu et al., 2003; Vilar et al., 2003) the Tessier method is widely used for experiments in which it is desired to know the fractionation of heavy metals in different sediments, demonstrating objectivity in the results and validity in the scheme.

The composition of Zn compounds in soils was determined by the sequential fractionation method proposed by (Tessier et al., 1979). This method ensures the separation of five metal fractions: exchangeable (1 M MgCl2), carbonate-bound (1 M CH3COONa), Fe/Mn (hydr)oxide-bound (0.04 M NH2OH·HCl in 25% CH3COOH), organic matter-bound (0.02 M HNO3 + 30% H2O2 (pH 2), then 3.2 M CH3COONH4 in 20% HNO3), and residual (HF + HClO4, then HNO3conc.) fractions. The content of Zn in extracts was determined with an atomic absorption spectrophotometer (KVANT 2-AT, Kortec Ltd., Russia).

## 3.11 Statistical analysis

All laboratory studies were performed in triplicate. Statistical processing of the experimental data (means and standard deviations) was performed using the statistical functions of the STATISTICA 10.0 program. The results were considered statistically significant at $p \leq 0.05$.

# 4 RESULTS

## 4.1 Forms of Zn compounds in the Fluvisol and Spolic technosols

Based on the method of sequential fractionation of heavy metals (Tessier et al., 1979) studied the effectiveness of using biochar for the purpose of remediation of soil contaminated with ZnO macro- and NPs . An analysis of the change in the fractional composition of Zn compounds in soil under remediation conditions allows a deeper analysis of the mechanism of the effect of sorbents on the immobilization of metals, to study the redistribution of metal compounds and the direction of their transformation processes.

In uncontaminated Umbric fluvisols (control) observed following fractional distribution of Zn: residual > bound to Fe-Mn oxides > bound to organic matter > bound to carbonates > exchangeable (Table 3, Fig. 5). According to the model pollution, there is a change in the fractional composition of Zn, which is greatly influenced by the degree of metal dispersion (macro and nano). With an increase in the dose of Zn introduced into the soil , the share of mobile compounds ( the total content of the exchangeable and carbonate-bound fractions ) increases, the share of the metal associated with Fe- Mn (hydr) oxides, and the share of the residual fraction decreases. These changes are most pronounced in the variants of soil contamination with Zn in the form of nanoparticles.

In general, when ZnO is contaminated in the macrodispersed form, the trend of the fractional distribution of the metal in the meadow soil remains. On the option of applying to the soil 10 APC Zn in nanoform compared to the lower dose (5 APC ) and macroparticles , there are changes in the fractional distribution Zn compared to the control variant: residual > bound to Fe - Mn oxides > bound to carbonates > exchangeable_bound to organic matter (Fig. 5). The highest Zn mobility is observed: the sum of the exchangeable and carbonate-bound fractions reaches 25%. The dissolution of metal oxide nanoparticles depends on the surface area, which is larger for smaller particles (Borm et al., 2006) Various studies (Reed et al., 2012; Wong et al., 2009) show that nanoparticles dissolve faster than larger materials. Celebrated (Deng, 2016) that as a result of the transformation of Cu compounds in the (nano) form, some of the nanoparticles are oxidized (up to 20%) and dissolved to free $Cu^{2+}$ $^{ions}$ . Same as 10 APC soiling ZnO in the macroform, there is a noticeable increase in the metal in the fraction of Fe - Mn oxides and a decrease in the residual fraction, but more pronounced ( Fig . 5).

Introduction of biochar caused changes in the fractional composition of Zn in the soil. In all variants of the experiment with the introduction of a sorbent, a decrease in the mobility of the metal was noted. At lower doses of contamination (5 APC ), when biochar was introduced at a dose of 2.5%, the relative content of the exchangeable and carbonate-bound metal fractions decreased to the control level and lower at a dose of 5%. In all cases, a greater decrease was noted when the soil was contaminated with macroparticles. ZnO ( Fig . 5 ). With the introduction of 1, 2.5 and 5 % sorbent , the greatest changes were established for metal compounds associated with Fe and M n % when making a similar dose of metal nanoparticles. In variants with high soil contamination (10 APC ) Zn , these changes are less significant: there is an increase in the fraction by 3-7 % and by 3-6%, depending on the degree of metal dispersion and the dose of the sorbent ( Fig . 5).

The relative content of the Zn fraction associated with organic matter practically does not change at the studied doses of the introduction of the carbonaceous sorbent. The trend towards an increase in the content of this metal fraction after the introduction of biochar in variants with soil contamination with Zn nanoparticles is higher than in variants c metal macroparticles ( Fig . 5 ). Soil organic matter has a high binding capacity for metal oxides and influences the dissolution of nanoparticles (Waalewijn-Kool et al., 2013). One or two thirds of coal consists of amorphous carbon, part of which burns out during activation with the formation of pores of various sizes, which causes not only a large specific sorption surface, but also leads to an intensification in the content of organic matter in the soil (Lwin et al., 2018; O’Connor et al., 2018; Puga et al., 2015).

Table 4.1. .- Fractional composition of Zn in fluvisol and Spolic Technosols

| Variant | Total content | Exchangeable | Bound to carbonates | Bound to Fe-Mn oxides | Bound to organic matter | Residual | Sum of fractions |
|---|---|---|---|---|---|---|---|
| Control (Umbric fluvisols) | 110±1 | 1,0±0,1 | 4,7±0,2 | 9,2±0,6 | 8,2±0,5 | 77,5±1,4 | 100,6 |
| 5 APC (1100 mg/kg) ZnO macro | 1193±59 | 31,2±2,2$^{a}$ | 97,6±3,8$^{a}$ | 241,4±17,1$^{a}$ | 127,8±5,0$^{a}$ | 688,4±15,5$^{a}$ | 1186,3 |
| 5 APC ZnO macro + 1% biochar | 1185±71 | 21,5±0,5$^{b}$ | 58,9±2,7$^{b}$ | 329,6±12,5$^{b}$ | 117,7±7,6$^{a}$ | 649,4±2,9$^{b}$ | 1177,0 |
| 5 APC ZnO macro + 2,5% biochar | 1188±62 | 7,9±0,5$^{c}$ | 26,6±0,9$^{c}$ | 380,5±11,0$^{c}$ | 134,3±8,3$^{b}$ | 602,8±11,4$^{c}$ | 1152,1 |
| 5 APC ZnO macro + 5% biochar | 1189±76 | 7,0±0,5c | 23,8±1,2d | 405,6±20,2$^{c}$ | 119,0±3,1$^{a}$ | 607,7±15,7$^{c}$ | 1163,1 |
| 5 APC (1100 mg/kg) ZnO nano | 1194±65 | 67,3±1,0$^{a}$ | 72,4±2,5$^{a}$ | 332,5±16,5$^{a}$ | 124,6±8,0$^{a}$ | 573,2±14,1$^{a}$ | 1170,0 |
| 5 APC ZnO nano + 1% biochar | 1197±18 | 35,7±2,7$^{b}$ | 59,5±4,1$^{b}$ | 357,0±14,7$^{b}$ | 119,0±9,4$^{a}$ | 618,8±14,1$^{b}$ | 1190,0 |
| 5 APC ZnO nano + 2,5% biochar | 1191±63 | 20,2±1,5$^{c}$ | 45,0±1,9$^{c}$ | 418,6±14,1$^{c}$ | 143,6±1,9$^{b}$ | 539,7±21,4$^{c}$ | 1167,0 |
| 5 APC ZnO nano + 5% biochar | 1199±12 | 17,2±1,3$^{c}$ | 35,4±0,4$^{d}$ | 425,0±25,0$^{c}$ | 147,0±6,1$^{b}$ | 544,7±14,4$^{c}$ | 1170,0 |
| 10 APC (2200 mg/kg) ZnO macro | 2283±21 | 135±7,5$^{a}$ | 207,3±14,2$^{a}$ | 590,2±16,2$^{a}$ | 275,9±21,3$^{a}$ | 1099,6±12,1$^{a}$ | 2308,5 |
| 10 APC ZnO macro + 1% biochar | 2299±108 | 91,1±2,4$^{b}$ | 159,2±10,1$^{b}$ | 660,6±26,0$^{b}$ | 250,6±17,2$^{a}$ | 1116,3±66,1$^{ab}$ | 2278,0 |
| 10 APC ZnO macro + 2,5% biochar | 2302±48 | 85,6±3,1$^{b}$ | 130,2±7,4$^{c}$ | 690,0±32,4$^{b}$ | 225,1±5,9$^{b}$ | 1148,1±10.9 $^{bc}$ | 2279,0 |
| 10 APC ZnO macro + 5% biochar | 2295±175 | 45.4±1,5 $^{c}$ | 83.1±4,3 $^{d}$ | 756.8±20.4 $^{c}$ | 288.5±19.0 $^{a}$ | 1113.4±29,4 $^{a}$ | 2287,0 |
| 10 APC (2200 mg/kg) ZnO nano | 2301±182 | 251,0±18,9 $^{a}$ | 315.4±6,2 $^{a}$ | 673.1±18.5 $^{a}$ | 196.8±11,3 $^{a}$ | 838,4±57,3 $^{a}$ | 2275,0 |
| 10 APC ZnO nano + 1% biochar | 2291 ± 172 | 158.8 ± 10.4 $^{b}$ | 272.0 ± 13.9 $^{b}$ | 748.8 ± 51.6 $^{ab}$ | 226.9 ± 11.7 $^{b}$ | 862.2 ± 16.1 $^{a}$ | 2269,0 |
| 10 APC ZnO nano + 2.5% biochar | 2305 ± 117 | 113.0 ± 5.9 $^{c}$ | 162.6 ± 11.6 $^{c}$ | 787.7 ± 35.1 $^{bc}$ | 275.8 ± 8.8 $^{c}$ | 955.0 ± 27.6 $^{b}$ | 2294,0 |
| 10 APC ZnO nano + 5% biochar | 2309±27 | 76,5±5,8$^{d}$ | 115,0±6,1$^{d}$ | 827,1±63,7$^{bc}$ | 294,5±19,4$^{c}$ | 993,9±79,1$^{b}$ | 2307,0 |

Note: Letters indicate significant differences ($p<0.05$) between the soil with the same dose of pollutants and different doses of biochar, obtained as a result of calculating U-test.

The content of the residual fraction with the addition of biochar tends to increase at the maximum dose of contamination (10 APC ), which indicates a stronger fixation of Zn in the soil in the presence of biochar . On the variants with a lower level of pollution (5 APC ), the opposite trend is observed ( Fig . 5 ). In this way, on the basis of the obtained results of fractionation, regularities in the distribution of Zn according to the forms of compounds in Umbric were revealed fluvisols . It was found that the fractional distribution of Zn in Umbric fluvisols influences the level of soil contamination and particle size incoming pollutant (macro- and nanoform ). In uncontaminated soil, the predominant part of the metal is firmly fixed by soil components. With an increase in the dose and degree of dispersion of the introduced metal compounds, the mobility in the soil increases. The introduction of biochar into contaminated soil led to the immobilization of mobile Zn compounds . The sorbent showed high application efficiency on contaminated Umbric fluvisols . Fe and Mn (hydr)oxides play the greatest role in the strong fixation of Zn.

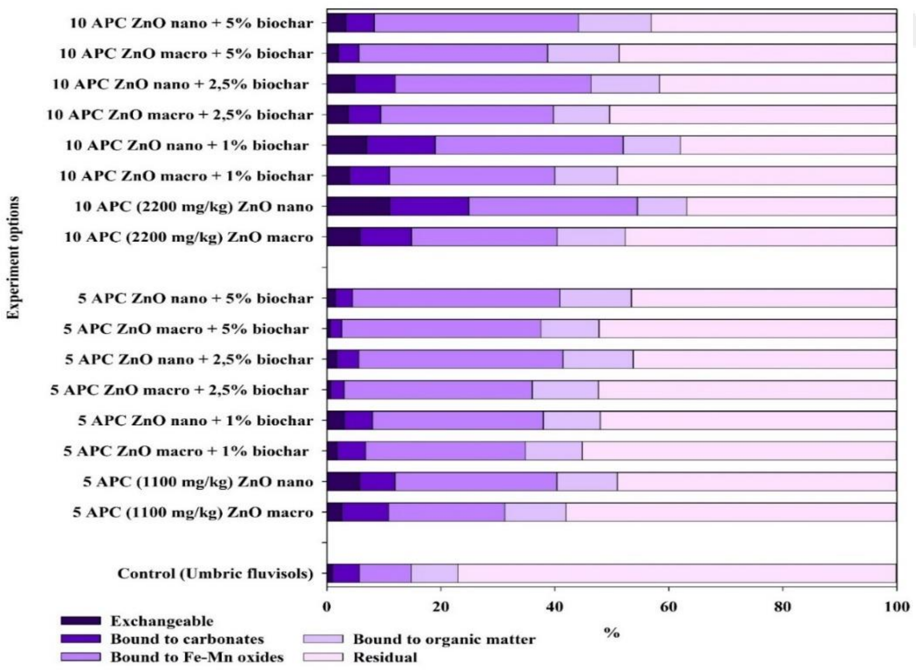

Figure 4.1.- Fractional distribution of Zn determined by the Tessier method.

## 4.2 Structure and chemical composition of biochar

Table 4.2.- Average elemental composition of the studied biochars.

| *Element and ash content, %* | | | | | *Atomic ratio* | | | |
|---|---|---|---|---|---|---|---|---|
| *C* | N | H | O | Ash | H/C | O/C | C/N | (N + O)/C |
| *77.3* | 2.4 | 4.8 | 7.3 | 8.2 | 0.75 | 0.07 | 37.9 | 0.10 |

As indicated in Table 4, the content ranges for C, N, H, and O are 77.3%, 2.4%, 4.8%, and 11.8%, respectively. Based on the atomic ratio of the elements, we can assume that the system of conjugated aromatic bonds is well developed in biochars (Table 4). The degree of aromaticity indicates that aromatic compounds prevail in the studied sorbents, mainly in the wood biochar. According to other studies (Xu et al., 2021), biochar with a high C:N ratio promotes the immobilization of microbial nitrogen (Table 4). This will lead to a decrease in the flow of greenhouse gases and an increase in organic carbon content in the soil.

The results of XRF are given in table, when determining the elemental composition of biochar in the samples, the maximum allowable concentrations of trace amounts of HMs were not exceeded (even for food raw materials and food products). Woody biochar has an increased content of calcium (26.30% CaO).

## 4.3 Biochar morphology analysis

Table 4.3.-XRF analysis of different biochar samples.

| *Element* | *Wood biochar* |
|---|---|
| $TiO_2$, % | 0.26 |
| *CaO, %* | 26.30 |
| $Al_2O_3$, % | 7.20 |
| $SiO_2$, % | 8.80 |
| $Fe_2O_3$, % | 4.17 |
| $P_2O_5$, % | 9.40 |
| $K_2O$, % | 3.94 |
| *MgO, %* | 3.47 |
| *V, mg/kg* | 0.00 |
| *Cr, mg/kg* | 18.04±0.92 |
| *Mn, mg/kg* | 407.66±10.18 |
| *Co, mg/kg* | 6.52±0.29 |
| *Ni, mg/kg* | 20.10±0.92 |

| | |
|---|---|
| *Cu, mg/kg* | 23.47±0.99 |
| *Zn, mg/kg* | 27.70±1.30 |
| *Sr, mg/kg* | 31.07±1.28 |
| *Pb, mg/kg* | 0.00 |

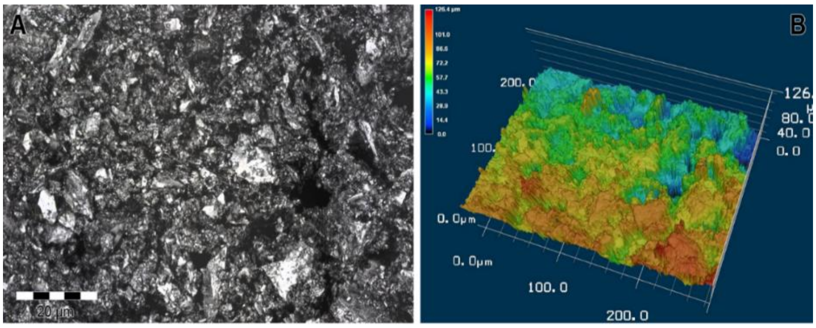

Figure 4.2.- Surface of woody biochar: A) SEM photo; B) 3D model

The SEM measurements, supplemented by 3D modelling, showed that the sorbents have a biogenic structure inherited from the raw starting materials (Fig. 4.2). The microscopy images showed that the surface morphology of wood biochar (Fig. 4.2A, B) was heterogeneous with a compact block. Moreover, the pores were irregular in shape (Fig.4.2A). The size of the individual particles ranges from 1 to 10 µm. There are monolithic inclusions up to 50 µm in size, which are the remains of the vascular system of wood. The surface relief of the particles was not uniform.

Table 4.4.- Physical characteristics of the biochar samples

| *Mean particle size (mm)* | *Specific surface area (m²/g)* | *Pore volume, V (cm³/g)* | | | |
|---|---|---|---|---|---|
| | | ΣV | $V_{macro}$ >50 nm | $V_{meso}$ 2-50 nm | $V_{micro}$ <2 nm |
| *0.5-4* | 612±25 | 2.73 | 1.31 | 1.14 | 0.28 |

Wood biochar has a surface area of 612 m$^2$/g (Table 4.4). Such a high value of the specific surface area is explained by the characteristics of the raw material. The total pore volume is 2.73 cm$^3$/g. At the same time, macro and mesopores predominate in the structure of the pore space.

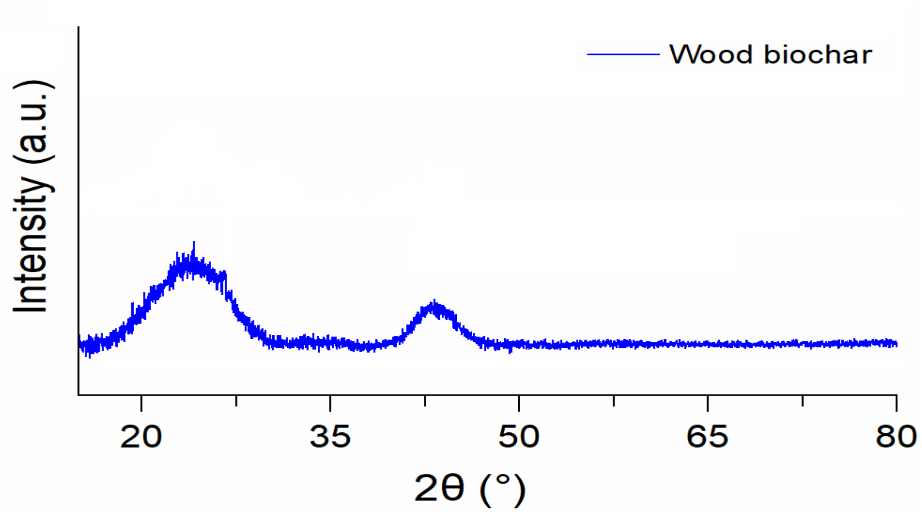

Figure 4.3.- Difraction pattern of woody biochar

The peaks of the diffraction pattern of wood biochar are expressive, but smooth. This shape of the diffraction pattern indicates the general amorphous structure of the studied sorbent. Two phases are detected on the graph: 2θ = 22° and 41°. These peaks belong to the quartz phase, and the other two protrusions, to the calcite phase (El-Naggar et al., 2019; Kim et al., 2011; Yang et al., 2015).

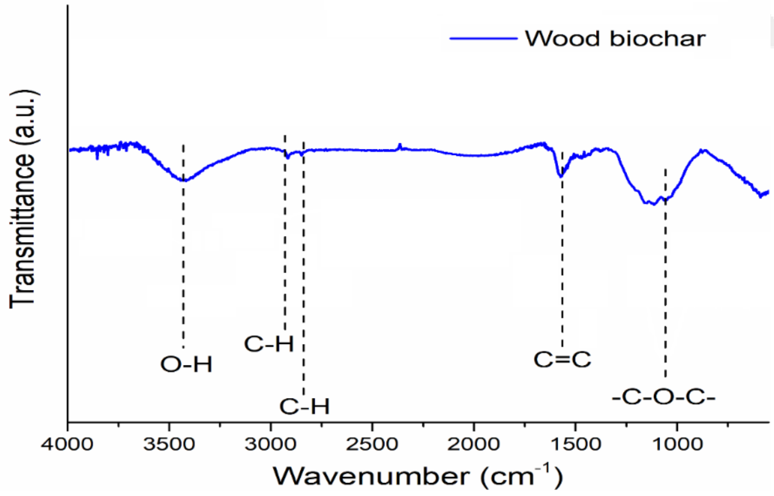

Figure 4.4.- FTIR spectra of biochar

The FTIR spectra of wood biochar in Fig. 4.4 showed a strong peak at approximately 3100-3600 cm$^{-1}$, corresponding to OH stretching vibrations from the hydroxyl functional groups or adsorbed water. The narrow peaks at 2918 and 2846 cm$^{-1}$ are probably associated with the stretching vibrations, both asymmetric and symmetric of the aliphatic C-H groups of cellulose, respectively. The bands at approximately 1570 and 1476 cm$^{-1}$ can be attributed to the stretching vibrations of C=C and bending vibrations of C–H, respectively. The strong and broad peak at 900–1250 cm$^{-1}$ was attributed to the symmetric valence groups -C-O-C- or phenolic functional groups and ethers.

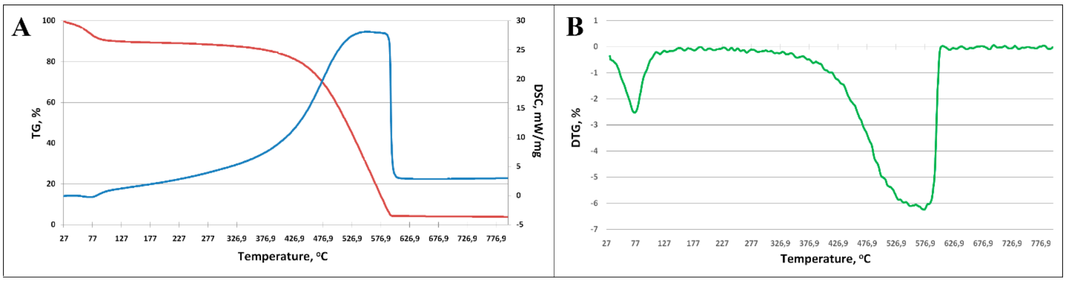

Figure 4.5.- Results of simultaneous thermal analysis of wood biochars:

A) - graph of synchronous thermal analysis of wood biochar; B) - curve of the derivative of thermogravimetric analysis of wood biochar.

The results of STA wood biochar showed that heating the sample at 95–100 °C removed hygroscopic water and sorbed gases, and various volatile low-molecular-weight organic compounds from the sorbent surface (TGA curve). This is displayed on the DSC graph as an endothermic process (Fig. 4.5A) and the DTG curve (Fig. 4.5B) for a given temperature range. The weight loss of the sample at this stage was 9.55%. When biochar was heated to 250-300 °C, gradual combustion of residual fragments of lignin and cellulose began, which intensified at 390-400 °C owing to the amorphous carbon oxidation of the main component of biochar. This exothermic process was observed in the DSC curve (Fig. 4.5 A). This was accompanied by a significant and most intense weight loss (Fig. 4.5 A, B), which, upon reaching 600 °C, amounted to 83.55%. The total weight loss of the sample was 96.12%.

## 4.4 Plants morphometrics

The study of the distribution of Zn in barley plants revealed a tendency of predominant accumulation of pollutants in the root system, which is typical for all plants of the Bluegrass family (Chaplygin et al., 2020). This nature of the distribution is observed in all variants of the model experiment and allows us to attribute barley to the plants-excludors of HMs.

The results showed that with increasing levels of soil pollution, the metal content in plant increases. For example, when 110 mg/kg Zn in microfrom is applied, the content in the roots and above-ground part of plants was 8 and 5 times higher than the plants used as a control. Even when applying 2200 mg/kg, it was 17 and 10 times higher (table 4.5).

Table 4.5 shows the Zn content in *H. vulgare* when the metal is applied to the soil in micro and nanoform and different doses of biochar.

Table 4.5.- Zn content in barley (*H. vulgare*).

| Rate of HM application, (mg/kg) | Subsurface part | aboveground part |
|---|---|---|
| Control (Umbric fluvisols) | 21,9±0,9 | 19,5±1,0 |
| 5 APC (1100 mg/kg) ZnO macro | 174,8±7,1 | 96,4±4,8 |
| 5 APC (1100 mg/kg) ZnO nano | 282,5±17,0 | 167,1±10,0 |
| 5 APC ZnO macro + 1% biochar | 150,6±9,6 | 43,6±2,5 |
| 5 APC ZnO nano + 1% biochar | 247,3±15,8 | 71,2±4,1 |
| 5 APC ZnO macro + 2,5% biochar | 95,2±6,1 | 35,5±2,0 |
| 5 APC ZnO nano + 2,5% biochar | 126,7±8,0 | 45,1±2,6 |
| 5 APC ZnO macro + 5% biochar | 46,9±3,0 | 22,7±1,3 |
| 5 APC ZnO nano + 5% biochar | 58,0±3,7 | 34,8±2,0 |
| 10 APC (2200 mg/kg) ZnO macro | 363,4±14,5 | 190,2±7,6 |
| 10 APC (2200 mg/kg) ZnO nano | 527,3±31,6 | 301,5±18,1 |
| 10 APC ZnO macro + 1% biochar | 305,6±20,5 | 138,6±7,6 |
| 10 APC ZnO nano + 1% biochar | 459,3±30,8 | 253,0±13,9 |

|  |  |  |
|---|---|---|
| 10 APC ZnO macro + 2,5% biochar | 190,2±12,7 | 64,9±3,5 |
| 10 APC ZnO nano + 2,5% biochar | 258,5±17,3 | 105,2±5,8 |
| 10 APC ZnO macro + 5% biochar | 106,7±7,1 | 32,7±1,7 |
| 10 APC ZnO nano + 5% biochar | 142,1±9,5 | 46,1±2,4 |

± – the standard deviation (SD)

Due to the smaller particle size, the dissolution of the nanodisperse form of ZnO occurs faster than in microdisperse form. Perhaps this is due to the higher accumulation of Zn in barley plants introduced in the form of nanooxide. Thus, on variants with soil contamination of 2200 mg / kg Zn with nanoform, the content of HMs in the roots and above-ground part increased by 24 and 15 times, and with microform by 17 and 10 times (Table 6). It should be noted that significant differences in metal accumulation depending on the form of application were observed in the roots, and smaller differences in the above-ground parts of barley. A higher content of Zn at the root may also indicate significant adhesion of ZnO at the surface of the roots, with the ability to adhesion clearly higher in ZnO. A decrease in the mobility of metal in the soil when using a carbon sorbent leads to its less accumulation in plants. With an increase in the amount of sorbent applied, the Zn content in barley decreases. On variants with soil contamination of 2200 mg / kg Zn in nanoform, the metal content in the above-ground part of plants decreases by 16% when 1% biochar is applied, by 65% at 2.5% biochar and 85% at 5% biochar. A number of works have also revealed a decrease in the content of heavy metals when applying biochar to contaminated soil (Al-Wabel et al., 2015; Chen et al., 2018; Mohamed et al., 2017).

The study of the effect of the HMs present in the soil can be used to explain the dimension of soil contamination through the impact that plants have received in their development and growth. The introduction of ZnO in micro- and nanodisperseed forms has affected the growth and development of spring barley. Large changes were observed when the metal was applied in the form of nanoparticles and were observed only when a high dose (2200 mg/kg) of Zn was applied. The height of the plants varied insignificantly relative to the control on variants with soil contamination of 1100 mg/kg ZnO, however, there was a decrease (21%) in the length of the roots when applying NPs (Figure 4.6-4.7).

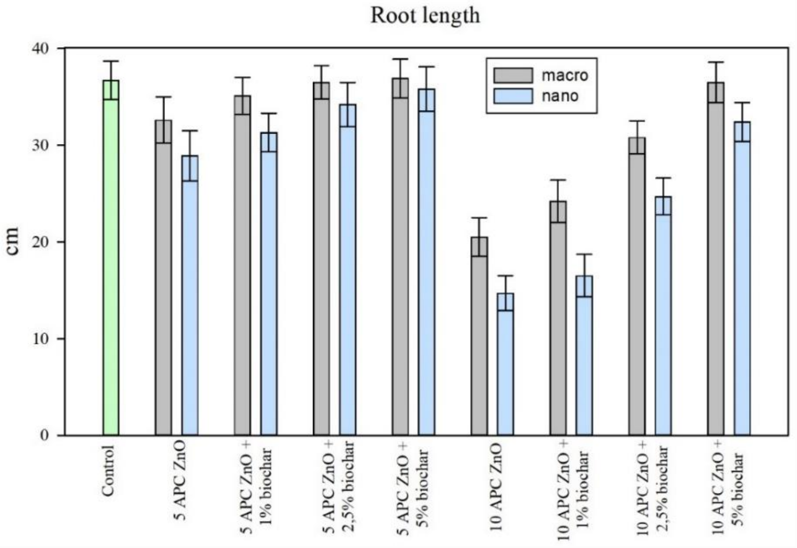

Figure 4.6.- Length of barley root when applying Zn to the soil in micro- and nanoform and different doses of biochar.

It is assumed that at an optimal concentration, nano ZnO can be used as a plant growth stimulant or fertilizer. In (Awasthi et al., 2017), the treatment of wheat seeds (*Triticum aestivum*) with 50 mg/l-1 LF ZnO improved seed germination and plant biomass. In the works of (Bandyopadhyay et al., 2015; Patra et al., 2013), it was found that at a dose of nano-ZnO 250 and 500 mg / kg, the germination energy and germination of alfalfa seeds and beans relative to control increased, but the length of the roots and the height of the shoots decreased. The phytotoxicity of Zn lies primarily in oxidative stress accompanied by lipid peroxidation, which ultimately leads to membrane damage at the cellular level (Ajey Singh et al., 2017).

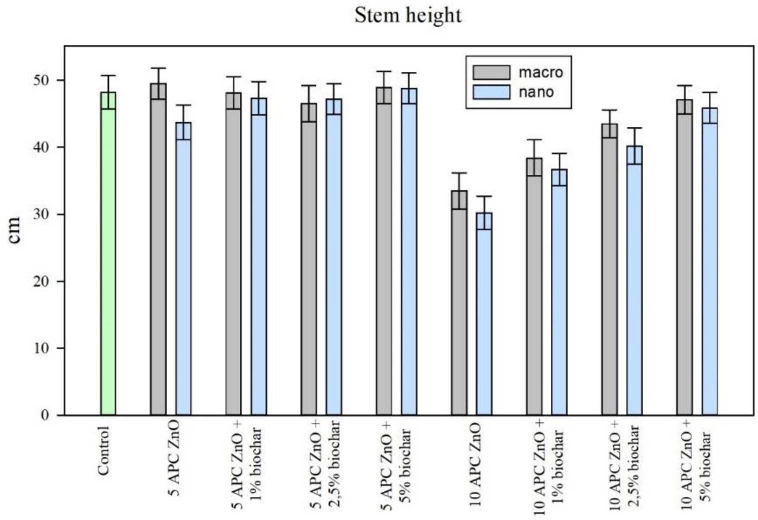

Figure 4.72.-Height of barley shoots when applying Zn to the soil in micro- and nanoform and different doses of biochar.

According to the study, the greatest inhibition of barley growth is characteristic of the roots. Thus, the length of the roots decreases by 44% when applying 2200 mg / kg ZnO in microdisperse form and with the nanodisperse form by 60% (Fig. 10). The decrease in the length of the roots and the height of plant shoots when exposed to ZnO of different dispersions is well consistent with the data of other researchers (Dimkpa et al., 2012; Lin & Xing, 2007; Priester et al., 2012; X. Wang et al., 2018).

Biochar has been shown to be effective in reducing the phytotoxicity of heavy metals in contaminated soils through the immobilization of bioavailable toxic metals (Almaroai et al., 2014; Herath et al., 2015). The use of biochar as a sorbent is a cost-effective strategy for the remediation of heavy metal-contaminated soils and crop growth (Beesley et al., 2013; Houben et al., 2013; Teodoro et al., 2020).The application of 5% biochar with soil contamination of 2200 mg / kg ZnO most effectively affects the growth of vegetative organs of barley. So, the length of the roots and the height of the shoots increases by 1.5-2 times, reaching the control values.

Visible symptoms of stress caused by metal toxicity in plants are expressed in previous metal-induced changes at the structural and ultrastructural level. These changes at the cellular, tissue and organ level are, in turn, either the result of the direct interaction of heavy metals with structural components or a more indirect consequence of changes in metabolism (Barceló & Poschenrieder, 2004).

Microscopic examination of sections of the roots of spring barley grown on the control version showed the anatomical structure of the root of a monocotyledonous plant (V. Rajput et al., 2018). Outside the root is a single-layered epiblema, some cells of which are elongated into root hairs (Figure 4.8). Root hairs have a length of 150 - 200 µm. Under the layer of the epiblema lie several layers of parenchymal cells. Most of them are corked and has a larger size than the cells of the epiblema. Next are the endoderm cells. Their size is smaller, and the radial and transverse walls have thickenings. The central part of the root section is occupied by the pericycle and conductive tissues. Pericycle cells are larger, slightly elongated in radius and have thin cell membranes. The large vessels of xylem are surrounded by living cells of the xylem parenchyma. Large pores in the cell walls connect them to the vessels. The phloem consists of sieve tubes, companion cells, and the phloem parenchyma.

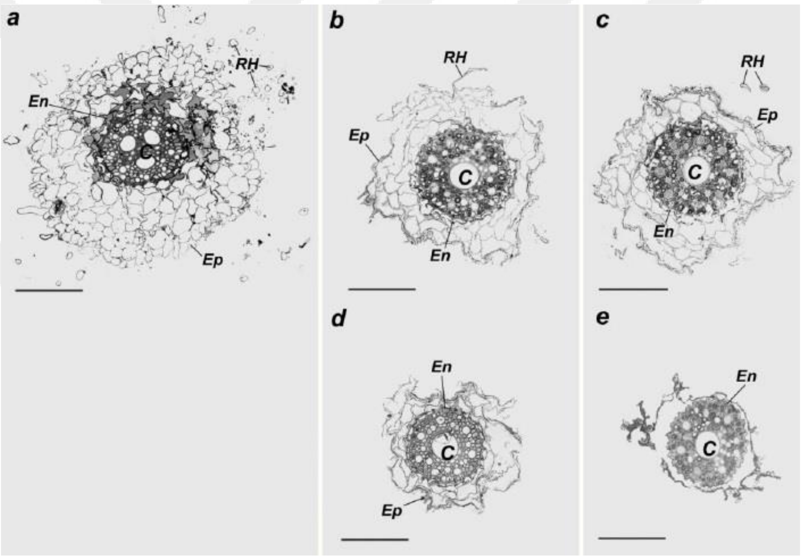

Figure 4.8.- Cross sections of barley roots.  Central cylinder. Scale segment -100 µm

Where a is control, b corresponds to 2200 mg/kg macro ZnO + 5% biochar,

c corresponds to 2200 mg/kg nano ZnO + 5% biochara, d has 2200 mg/kg macro ZnO, and e corresponds to 2200 mg/kg nano ZnO. For the case of the abreaviations, root hairs (RH), epiblemma (Ep) and endoderm (En).

In the variants with ZnO contamination, the anatomical structure of the roots has a amount of significant differences from the control samples. The cells of the epidermis are highly differentiated, predominantly do not have a cytoplasm and a nucleus, and consist only of cell walls. The epidermis has sparse and shorter than in the control root hairs in the microdispersed form of metal introduction, and is absent in the nanoform (Figure 4.8 d, f). Parenchymal cells of the cortex in the nanodispheric form of ZnO introduction are strongly reduced and consist of one layer of cells, and in some areas they are completely absent. Endoderm cells are clearly visible and arranged in 1 layer. Their cell walls have an uneven thickness, increasing towards the center. The central cylinder, occupying on average a significantly larger share of the total root cross section than in the control sample, consists of the pericycle and conductive tissues. At the same time, the absolute total area of conductive tissues in all variants is approximately the same as in the control. The cells of the pericycle lie in one layer, slightly elongated along the radius and have thin membranes. Behind the pericycle are the cells that make up the conducting tissues. The vascular bundles, as in the control, are built according to the radial type and have one large vascular bundle in the central part of the central cylinder, from which smaller bundles of phloem and xylem diverge radially. Similar results were obtained in earlier studies on the effects of ZnO on other plant species (Azarin et al., 2022; Radi et al., 2018). The revealed structural changes in the roots can affect the absorption of water and nutrients, which negatively affects the development of the whole plant (Adams et al., 2017).

On the variants using 5% biochar, the outer layer of the epiblem of the barley root, as in the control, has living cells with root hairs (Figure 4.8 b, c). However, the root hairs are much shorter and rarer than in the control. The parenchymal layer is more developed than the contamination variants. At the same time, the cells of this layer are more deformed and disorganized than in the control and have a smaller size. They, for the most part, do not have cytoplasm and organelles and consist only of cell walls that carry a mechanical function. There are areas where the integrity of the cell walls is impaired and several cells are combined into one cavity. The structure of the endoderm cells and the central cylinder is not disturbed.

The structure of the tissues of the leaf plate of the control variant is characterized by an orderly organization and uniform localization of cells in the leaf chlorenchyme (Figure 4.8 a). The division of mesophyll cells into palisade and spongy parenchyma is weakly traced. The cells of the palisade parenchyma are arranged in one row under the upper epidermal layer and occupy about a quarter of the thickness of the leaf plate section. They have a rounded shape, fit snugly to each other. The cells of the spongy parenchyma are located in the central and lower parts of the leaf and are adjacent to the lower epidermis. The shape of the cells is more elongated. Their spatial organization is characterized by a small proportion of tightly contacting cells and the presence of an extensive intercellular space.

## 4.5 Anatomical and ultrastructural parameters of *H. vulgare*

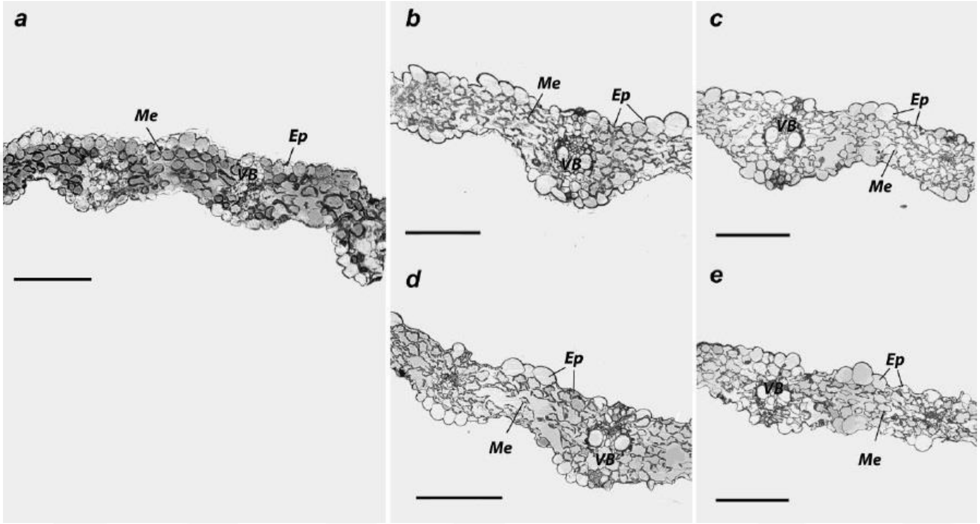

Figure 4.9.- Cross sections of barley leaf plates. Scale segment 100 µm

In the case, a is control, b corresponds to 2200 mg/kg macro ZnO + 5%biochar, c is 200 mg/kg nano ZnO + 5% biochar, d corresponds to 2200 mg/kg macro ZnO , and lastly 2200 mg/kg nano ZnO. Where Ep means epidermis, Me means mesophyll, VB means conducitve beam, In the variants with ZnO contamination, the organization of cells in leaf chlorenchyma is less ordered than in the control variant, regardless of the degree of metal dispersion. The division of the mesophyll into columnar and spongy parenchyma, as in the control, is weakly expressed (Figure 4.9 d). The cells of the palisade parenchyma are located in 1 row under the upper epidermal layer and occupy about a quarter of the cut area of the leaf blade. Unlike the control, they are slightly elongated in width and do not fit tightly to each other, along the long axis of the leaf plate the spongy parenchyma cells are flattened.

Compared to the cell organization of the walls of plants subjected to control treatment, there is less order in the cell organization. An insignificant proportion of cells maintaining close contact and recording a wide intercellular space was recorded.There is a tendency to reduce the number of cells per unit area and plastids in the parenchyma cell. HMs at the cellular level in plants generate a decrease in the intensity of photosynthesis, since they alter the ultrastructure of the chloroplasts, which in turn causes a decrease in the content of pigments in plants (Molas, 2002; Yüzbaşioğlu et al., 2017). Since chloroplasts and mitochondria are the main organelles necessary for photosynthesis (Yoshida et al., 2011) the changes recorded in these organelles are related to a decrease in metabolic processes, which are responsible for plant growth.

# 5 CONCLUSION AND RECOMMENDATIONS

Sequential fractionation helped to determined the presence of Zn in the studied soils. Speciation forms of Zn in the studied soil samples were identified. It was found tha metal mobility increases in technologically contaminated soils. Neverthless, the highest Zn content is associated with the least available soil fractions. The experimental results indicated that the addition of biochar amendments is an effective alternative for the bioremediation of Zn contaminated fluvisols and spolic technosols. Biochar has proven to be useful in decreasing Zn uptake by *H. vulgare*. For all 3 of the concentrations of biochar registered (1%,2.5%, 5%), the application of carbon sorbents into the contaminated soil resulted in the immobilization of loosely bound metal compounds (exchangeable, complexed, and specifically sorbed forms) due to their highly porous structure. The increase in the dose of the sorbents, showed two effects, first, the inactivating effect of the toxicity of Zn was increased and second, biochar showed a higher level of efficiency. The results showed that with increasing levels of soil pollution, the metal content in plant increases. For example, when 110 mg/kg Zn in microfrom is applied, the content in the roots and above-ground part of plants was 8 and 5 times higher than the plants used as a control. Even when applying 2200 mg/kg, it was 17 and 10 times higher. The most effective doses of biochar were 2.5% and 5% of carbon sorbents in soil polluted with 5 APC and 10 APC of Zn for biochar amendments.

# 6 DISCUSSION

The use of biochar establishes a positive and significant relationship between specific root length and plant production, suggesting that the increase in fine root proliferation after the addition of biochar increases crop yield. This fact is fundamentally related to greater access to available resources, favored both by a greater volume of exploration and a greater interaction of biochar-root particles (Ruvalcaba-Ruíz, D. et al., 2009).

Biochar promotes HMs immobilization causing a decrease in greenhouse gas flux and an increase in soil organic carbon content. Biochar reduces heavy metal phytotoxicity through immobilization of heavy metal bioavailability. By acting as a sorbent, the alternative of using biochar as a cost-effective tool for the remediation of contaminated soils is highly recommended. The more heterogenous the surface of the biochar is, the more ZnO NPs will be able to absorb. In addition, a high level of CaO (26.3) was found, which agrees with the high level of atomic ratio of Carbon and Nitrogen recorded (37.9). Biochar with a high C:N ratio promores the immobilization of microbial nitrogen, which helps decrease the flow of greenhouse gases and an increase in the quantity of organic carbon content in the soil.

Application of biochar ammendments decreased HMs accumulation levels of Zn. Biochar increases water retention capacity of the soil and this contributes to the improvement growth of *Hordeum vulgare*, however more techniques are required to provide a more suitable habitat for soil microorganisms and to help with the uptake of heavy metals from the contaminated soil (Rajput et al., 2021b). In the case of absortion of HMs it is important for the sorvent to have a high value of macro and meso pores with a wide absortion center, which in this case (612 $m^2/g$) contributes to capt HMs.

The structure of the biochar is heterogenous which means that it has more possible absortion center. We can use the colors as a guide for the size of the absortion centers, the blue color indicates a greater range of the absorption center than the green color.

The application of biochar in soil, helps plants to counteract the stress caused by the bioaccumulation of ZnO NPs, however studies have registered damage at the cellular level that affects plant growth (Andrey et al., 2019; Priester et al., 2017). The results of this study conclude that the effects of ZnO NPs are directly related to its concentration, it will act as a plant growth precursor or growth inhibitor for plants. It can improve seed germination and plant biomass. However, it acts as an inhibitor in root growth (44%) with a concentration of 2200 mg/kg of ZnO NPs in microdisperse form, while in nanodisperse form it affects 60%. In addition, there was a decrease in the length of the roots and the height of the shoots of the plants. The growth of *H. vulgare* in contaminated soils causes accumulation of heavy metals both in the root, as well as in the tissues of the plants with the leaves or the stem.

It should be noted that large differences in metal accumulation depending on the form of application were observed in the roots, and smaller differences in the above-ground parts of barley. A higher content of Zn at the root may also indicate significant adhesion of ZnO at the surface of the roots, with the ability to adhesion clearly higher in ZnO.

The application of 5% biochar with soil contamination of 2200 mg / kg ZnO most effectively affects the growth of vegetative organs of barley. Consequently, the length of the roots and the height of the shoots increases by 1.5-2 times, reaching the control values.In the variants with ZnO contamination, the anatomical structure of the roots has a number of significant differences from the control samples. The cells of the epidermis are highly differentiated, predominantly do not have a cytoplasm and a nucleus, and consist only of cell walls. The revealed structural changes in the roots can affect the absorption of water and nutrients, which negatively affects the development of the whole plant (Adams et al., 2017).

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## CURRICULUM VITAE

In 2012, I graduated from high school after studying 3 years in the specialization of chemical biological sciences. Later on I studied Biology with a major in Ecology and Management at the Universidad del Azuay, Cuenca, Ecuador, from where I studied with a full scholarship and I graduated in 2017. While working on my graduation thesis I worked with rural communities and the organic waste disposal in their farms which allowed me to later on get a job in a project at Universidad del Azuay. I worked there until 2020, when I started the Erasmus Mundus Master in Soil Science and studied in 3 different Universities; Turkey, Ondokuz Mayıs University, Bulgaria University of Agriculture in Plovdiv and the Southern Federal University in Russia. At this university my graduation thesis project was related with bioremediation and biochar. In 2021, my internship was done at the University of Nova Lisboa, Department of Biology.

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**Publications :**

**1.** Moya, B. Rajput, Vishnu, Sushkova, S**.** Coping up metal stress (ZnO NPS) in plants by application of various forms of biochar amendments ( A review).

**2.** López, J., Moya, B., Sánchez, C. (2018). Patterns of use of dry toilets in nine rural schools in the provinces of Azuay and Morona Santiago, South of Ecuador. Universidad Verdad.

**Won Awards, Incentives and Scholarships**

**1.** Erasmus Mundus Scholarship EMISS 2020-2022

**2.** UDA scholarship 2012-2017

**3.** Honor mention for thesis at the annual Biology Conference of the University of Azuay.