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

COMPARATIVE STUDY OF ZnO NANOPARTICLES FOLIAR SPRAY AND BIOCHAR ON SPRING BARLEY GROWTH IN PAHs CONTAMINATED SOIL

Master's Thesis

Hadeer ELGENDY

I. Supervisor
Assoc. Prof. Dr. Svetlana SUSHKOVA
II. Supervisor
Prof. Dr. Rıdvan KIZILKAYA

SAMSUN
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2022

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ÖZET

ZnO NANOPARTİKLER YAPRAK SPREYİ VE BIOCHAR'IN PAH'LARLA KONTAMİNE TOPRAKLARDA BAHAR ARPA BÜYÜMESİ ÜZERİNE KARŞILAŞTIRMALI ÇALIŞMASI

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Toprak Bilimi ve Bitki Besleme Bölümü
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Polisiklik aromatik hidrokarbonlar (PAH'lar), tüm yaşam formları üzerinde büyük risk oluşturan kirleticiler olup hidrofobik ve termostabil yapıları bunları daha kalıcı ve bozulmaya karşı dirençli hale getirmektedir. Benzeri görülmemiş antropojenik faaliyetler nedeniyle çevrede yaygın olarak dağılım göstermektedirler. Biyokömür uygulamaları ve nanopartiküllerin yapraklara sprey şeklinde uygulanması sürdürülebilir ve yeni teknikler olarak giderek daha fazla teşvik edilmektedir. Bu çalışmanın amacı, uzun süreli kimyasal bulaşmaya uğramış Spolic Technosol ve yapay olarak PAH ile (2.5 MPC and 5 MPC) bulaşmış toprakta yetiştirilen yazlık arpa (*Hordeum staivum* L.) gelişimi, fizyolojik ve biyokimyasal karakteristikleri üzerine ayçiçeği kabuğu biyokömürünün (% 0.5,% 1 ve% 5), çinko oksit nanopartiküllerin (ZnO-NP'ler) yapraktan sprey şeklinde (<50 nm, 150 mg L-1 çözeltisi) uygulanması ile bunların kombine edilmiş uygulamalarının etkisinin belirlenmesidir. Elde edilen sonuçlar, ZnO-nanopartiküller ile ayrı ayrı ve % 0.5 biyokömür ile beraber uygulamasının, toprak ve bitkideki toplam PAH'ları ve BaP konsantrasyonunu azalttığını ve arpanın biyokimyasal ve fizyolojik özelliklerini geliştirdiğini göstermiştir. Kimyasal bulaşma altında ZnO-nanopartiküller ile %5 biyokömür, topraktaki PAH içeriğini %50'ye kadar azalttığı ve kimyasal kontaminasyon altında arpa büyümesini arttırdığı belirlenmiştir. Bunlara ilaveten, biyokömürün (% 1) ZnO-nanopartiküllerle beraberce uygulanması, hem yapay hem de kimyasal olarak kirlenmiş topraklarda yetişen bitkinin kök ve kök büyümesini iyileştirdiği saptanmıştır. Sonuçta, PSII'nin maksimum verimi (Fv / Fm) ve etkili verimi Y (II), PAH konsantrasyonuna ve ZnO-nanppartiküllere duyarlılık gösterdiği belirlenmiştir. ZnO-nanopartiküllerin yapraktan spreyi şeklinde uygulanmasının etkinliği üzerine biyokömürün etkili olduğu belirlenmiş ve PAH'lar üzerindeki kombine tedavi mekanizması hakkında daha fazla araştırma yapılması gerektiği önerilmektedir.

Anahtar Sözcükler: ZnO Nanopartiküller, Biochar, PAH'lar, Yaylı arpa, Antioksidatif enzimler, Klorofil floresansı.

ABSTRACT

COMPARATIVE STUDY OF ZnO NANOPARTICLES FOLIAR SPRAY AND BIOCHAR ON SPRING BARLEY GROWTH IN PAHs CONTAMINATED SOIL

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Polycyclic aromatic hydrocarbons (PAHs) are reluctant pollutants that impose a great risk on all life forms, their hydrophobic and thermostable structure makes them more persistent and resistant to degradation. They are widely distributed in the environment owing to unprecedented anthropogenic activities. Biochar and nanoparticles foliar spray amendment have been increasingly encouraged as sustainable and novel techniques. The objective of this study was to evaluate the impact of sunflower husk biochar (0.5%, 1%, and 5%), whether separate or combined with zinc oxide nanoparticles (ZnO-NPs) foliar spray (<50 nm, 150 mg L^{-1} solution) on spring barley (*Hordeum staivum* L.) growth, physiological and biochemical characteristics under artificial PAHs contamination (2.5 MPC and 5 MPC) in Haplic Chernozem and long-term chemical contamination of Spolic Technosol. The results showed that 0.5% biochar application, separate or combined with ZnO-NPs, reduced total PAHs and BaP concentration in soil and plant, and improved the biochemical and physiological characteristics of barley. While 5% biochar with ZnO-NPs under chemical contamination reduced PAHs content in soil up to 50% and enhanced barley growth under chemical contamination. Additionally, the combined treatment of biochar (1%) with ZnO-NPs improved stem and root growth in both artificially and chemically polluted soils. Finally, the maximum yield (Fv/Fm) and effective yield Y (II) of PSII showed sensitivity to PAHs concentration and ZnO-NPs. Broadly these results gave an evaluation of the biochar with ZnO-NPs foliar spray treatment efficiency. More investigation on the mechanism of combined treatment on PAHs is required to confidently suggest this course of amendment for toxicity mitigation of PAHs on plants.

Keywords: ZnO Nanoparticles, Biochar, PAHs, Spring barley, Antioxidative enzymes, Chlorophyll fluorescence.

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CONTENTS

ABBREVIATION OF TERMS

BAFv	: Translocation Factor in vegetation
BAFr	: Bioaccumulation Factor in root
BaP	: Benzopyrene
CAT	: Catalase
Fv/Fm	: Maximal Quantum Yield of PSII
HPLC	: High-Performance Liquid Chromatography
MDA	: Malondialdehyde
MPC	: Maximum Permissible Unit
NBT	: Nitroblue Tetrazolium
NPQ	: Non-photochemical Quenching
PAHs	: Polycyclic aromatic hydrocarbons
PAM	: Pulse-Amplitude-Modulation
PSII	: Photosystem II
PSI	: Photosystem I
Qp	: Photochemical Quenching Coefficient
SOD	: Superoxide Dismutase
SPAD	: Soil Plant Analysis Development
TBA	: Thiobarbituric Acid
TBARS	: Thiobarbituric Acid Reactive Substances
Y(II)	: Effective Quantum Yield of PSII
ZnO-NPs	: Zinc Oxide Nanoparticles

FIGURES LEGENDS

TABLES LEGENDS

1. INTRODUCTION

The advances in industry and agriculture increased the release of organic pollutants into the environment causing serious pollution issues (Dai et al., 2019). Soil pollution imposes a great risk on human health since the number of pollutants that enter the food chain is gradually becoming uncontrolled. These pollutants have a serious effect on the biodiversity of our ecosystems, the quality of water, air, and the food we consume (Zheng et al., 2020). Polycyclic aromatic hydrocarbons (PAHs) are highly toxic and persistent contaminants, widely emitted across the globe due to uncontrolled long-term anthropogenic activities. Their hydrophobic nature and thermostability make them a resilient contaminant in the environment. Several PAHs are found to be mutagenic, carcinogenic, teratogenic, and immune toxicogenic (Patel et al., 2020). Long-term exposure to PAHs could result in chronic diseases for humans such as cancer, kidney and liver damage, and skin irritation (Mojiri et al., 2019). The US Environmental Protection Agency (EPA) has categorized 16 of the PAHs as priority pollutants. Several PAH pollutants have been classified as probable human carcinogens by the International Agency for Research on Cancer (Agarwal et al., 2009). The listed 16 PAHs are acenaphthene, benzo[ghi]perylene, chrysene, acenaphthylene, benzo[a] anthracene, benzo[b]fluoranthene, anthracene, benzo[k]fluoranthene, benzo[a]pyrene, fluoranthene, Indeno[1,2,3- cd]pyrene, naphthalene, phenanthrene, dibenz[a,h]anthracene, fluorene, and pyrene (Mojiri et al., 2019). The incomplete combustion of biomass (such as wood) and fossil fuels (petroleum, coal) is the most prominent and ubiquitous source of PAHs in the environment (Agarwal et al., 2009). They are present in similar quantities in the aquatic and terrestrial ecosystems in addition to the atmosphere. However, their hydrophobic nature increases their deposition rates in soil/sediments. They are strongly adsorbed onto soil particles, and therefore, the soil ecosystem becomes an ultimate sink for PAHs (Patel et al., 2020). Soil PAH pollution can be classified into three categories, i.e., unpolluted ($\sum$ PAH < 200 μg kg^{-1}), weakly polluted (PAH 200–600 μg kg^{-1}), and heavily polluted (PAH > 1,000 μg kg^{-1}) (H. Wu et al., 2019). For more than 30 years, Lake Atamanskoe was used as a dump for industrial waste of the chemical plant «Kamenskvolokno» (formerly, ‘‘Himvolokno’’). It’s considered the oldest and the largest chemical fibers manufacturer in the south of Russia. of organic pollutants and heavy metals especially Zn in concentrations higher than the maximum permissible unit, making the plant the most hazardous textile enterprise in southern Russia (Linnik et al., 2021). The total reserves of Zn in the technogenic sediments as a result of $ZnSO_4$ discharge are estimated to be 30 kt (Bauer et al., 2018). However, in the past 20-30 years the lake water has evaporated forming active soil due to elongated dry periods in addition to the termination of industrial runoffs (Sushkova et al., 2019). Several studies have proved the presence of PAHs in soils not close to any urban and industrial activity. were found in high concentrations in areas with no industrial activity. Naphthalene, Phenanthrene, and Pyrene are the most abundant types of PAHs in tropical soil, on the other hand, temperate soils are dominated by heavier PAHs especially, benzo[a]fluoranthene (Kuppusamy et al., 2017). Remediation/reclamation of PAH-polluted soils is growing to be a matter of global concern. Soil remediation is performed with various techniques most of which involve the removal/isolation or alteration of the contaminant (Haritash, 2019). Initially, the adoption of risk-based remediation approaches for the removal of organic pollutants from soil had led to serious ecological consequences. Thereby, various physical, chemical, thermal, and biological techniques started to appear and the research on their applicability is still ongoing. Few of the established remediation techniques for PAHs polluted soil include incineration, thermal conduction, solvent extraction/soil washing, chemical oxidation, bioaugmentation, biostimulation, phytoremediation, composting/biopiles, and bioreactors. (Kuppusamy et al., 2017). Phytoremediation is an in-situ method that uses plants for PAH removal or their conversion into less toxic components (Patel et al., 2020). Organic pollutants are detoxified by plants by different mechanisms; phytoextraction (elimination of pollutants from soil and accumulation them in cell walls or vacuoles), phytovolatilization (release of volatile pollutants from soil to the atmosphere through plant organs), and phytodegradation (pollutants are broken down by enzymes released from the plant and/or plant-associated microbes). Phytoremediation is proposed to be an efficient technique for the remediation of contaminated soils. It does not interfere with the ecosystem, requires little manpower, and is also not very expensive when compared to traditional physicochemical methods (Cristaldi et al., 2017). The optimization and selection of the different plant species for the remediation processes are done by certain analytical and modern devices (O. V. Singh · R. K. Jain, 2003). Grasses are economically preferred for phytoremediation especially Spring Barley for their low nutrient needs, tolerance to acidity, cold condition, sought, fast growth, and dense fibrous root system (Patel et al., 2020). Hyperaccumulator plants are needed for heavily polluted areas, they can be adapted through genetic manipulation. Additionally, plants growing naturally in polluted sites can remediate polluted sites when given the proper nutritional requirements and environmental conditions (O. V. Singh · R. K. Jain, 2003). The Mechanism by which PAH becomes toxic to plants is minorly discussed and understood, in addition to the variation of phytotoxicity symptoms shown with different PAH and plant species (Alkio, Tabuchi, Wang, et al., 2005). PAHs induce a wide variety of responses in plants, leading to either tolerance or toxicity. Their impact on plant health depends on various environmental conditions, not only on the type and concentration of contaminant, temperature, or soil pH but also on the physiological or genetic status of the plant (Molina Lázaro & Segura Ana, 2021). The uptake with high concentration may result in oxidative stress and the formation of reactive oxygen species (ROS) (V. Rajput et al., 2021). ROS may lead to different outcomes varying from cell death to stress resistance. The understanding of plant responses to pollutants is key to enhancing phytoremediation technologies (Molina Lázaro & Segura Ana, 2021). Not enough is known about how plants take up, respond and break down PAHs on the cellular and molecular levels when compared to animals and microorganisms (Alkio, Tabuchi, Wang, et al., 2005). The extent of PAHs toxicity also depends on the PAHs degradation capacity of the natural microbiota around the root zone and plant's ability in the stimulation of soil microbes to degrade contaminants. However, plants stimulating capability is controlled by the environmental conditions, soil properties, root exudate composition and the chemical properties of the contaminants (Molina Lázaro & Segura Ana, 2021).

Biochar offers promising results for the benefit of agriculture and the reclamation of polluted arable lands (Kavitha et al., 2018). Commonly biochar is produced through the burning of organic matter at temperatures (highest treatment temperature, HTT) ranging from around 350 °C to over 750 °C in an oxygen-deprived condition. Additionally, a large spectrum of biochar is manufactured using different feedstock, for example, woody residues, crop straw, animal manures, sewage sludge, and food wastes (Joseph et al., 2021). Production of biochar can be done with different methodologies, such as flash carbonization, gasification, pyrolysis, and hydrothermal carbonization (Vijayaraghavan, 2019). Several studies reported biochar's efficiency in the adsorption of organic pollutants in water, but fewer studies discussed their application for soil remediation (Han et al., 2016). One of the best qualities of biochar is that it can be designed and tailored to address a specific land problem, from the feedstock type selection to the changes in pyrolysis conditions, also the pre-or post-production treatments, and finally their co-application with other materials such as mineral or organic fertilizers (Joseph et al., 2021). Several reports concluded biochar's ability to enhance soil physicochemical properties. Biochar can increase soil pH, strengthen soil water retaining capacity, raise soil fertility, reduce leaching of soluble macronutrients and heighten carbon sequestration (Han et al., 2016). It's composed of elements that can serve as macronutrients for plant growth, such as carbon, nitrogen, hydrogen, potassium, and magnesium (Kavitha et al., 2018). Biochar becomes incorporated with soil aggregates as it ages, to maintain its carbon content, which leads to stability in the microbial activity and plant root secretions (Joseph et al., 2021). Additionally, many studies reported the use of biochar to initiate and enhance organic contaminants' microbial degradation (Han et al., 2016). It was reported that biochar lowers the rate of PAHs biodegradation since the PAHs sorption to its surface reduced the rates of bioaugmentation and phytoremediation (Bianco et al., 2021). Unfortunately, there's a knowledge shortage on the effect of biochar on the phytoremediation of PAHs contaminated soils (Han et al., 2016).

Owing to the growing and broad use of nanotechnology in different fields like medicine, food industry, engineering, and pharmaceutics, their application in the agriculture sector was inevitable (Alabdallah & Alzahrani, 2020; Zulfiqar & Ashraf, 2021). Nanoparticles are utilized for purposes in agriculture for example the targeted delivery of active ingredients like fertilizers, herbicides, and pesticides, as nutritional sources specifically micronutrients, to alleviate the damaging effects caused by abiotic stresses (Imran et al., 2022; V. D. Rajput, Minkina, Fedorenko, et al., 2021; Yue Song, Meng Jiang, 2021; Zulfiqar & Ashraf, 2021). Nanomaterials are preferred over the bulk compounds due to their adjustable nanometric size (1-100nm) and large surface to volume area which contributes to their peculiar physicochemical characteristics (Kusiak et al., 2022; Zhao et al., 2020). Traditional methods of micronutrient application don't ensure the reach of nutrients to plants whereas a large portion of nutrients is lost due to hydrolyzation, leaching, or photodegradation (Farouk & Al-Amri, 2019). On the other hand, the Foliar application of nanoparticles takes an alternative pathway since the sprayed nutrients enter the plants directly through the cracks, wounds, or stomata of leaves or stem (Hong et al., 2021; Rossi et al., 2019). Several studies proved the efficiency of nano-fertilizer foliar spray, as an economic and sustainable approach because of the targeted application of the microelements in addition to the reduced toxicity when compared with soil application (Elsheery et al., 2020; Rossi et al., 2019). The purpose of nanomaterial addition during phytoremediation is the enhancement of plant biomass and elevation of their tolerance (B. Song et al., 2019). One of the most commonly studied, and inexpensive nanoparticles in the world is zinc oxide (ZnO NPs) (Farouk & Al-Amri, 2019; Tighe-Neira et al., 2018). Every year, 550–5550 tons of ZnO NPs are manufactured for different uses across the globe (Alabdallah & Alzahrani, 2020; V. D. Rajput, Minkina, Fedorenko, et al., 2021). ZnO NPs are non-enzymes, that modulate the up-regulation of stress-responsive genes to elevate plant stress tolerance (Kusiak et al., 2022). As an essential Micronutrient, they are involved in the biosynthesis of Auxins, carbohydrates, and proteins, cell wall integrity and the regulation of cellular proliferation, photosynthetic pigments, photosynthesis, and PSII activity (Farouk & Al-Amri, 2019; Imran et al., 2022). Foliar application of ZnO NPs was proved to succeed in tackling various abiotic stresses, for example, drought (El-zohri et al., 2021; Imran et al., 2022), chilling (Yue Song, Meng Jiang, 2021), Heavy metals (Venkatachalam et al., 2017), salinity (Alabdallah & Alzahrani, 2020; V. D. Rajput, Minkina, Kumari, et al., 2021), and finally organic pollutants (PAHs) (Kusiak et al., 2022). The combined treatment of biochar and ZnO NPs were applied in the field and studied under heavy metals stress showing positive plant tolerance to stress in addition to reduced pollutant availability (Ali et al., 2019; Bashir et al., 2021; Seleiman et al., 2020). There's a gap in knowledge on the combined application of biochar and ZnO NPs in PAHs polluted soils on the growth and phytoremediation efficiency of plants. Hence, this study will investigate the influence of soil-applied biochar and the foliar-applied ZnO NPs either alone or combined on PAHs accumulation and transportation by spring barley as a test plant.

Objectives of the Study

1-Test sunflower husk biochar ability to reduce PAH concentration in soil.

2- Evaluate the effect of PAHs toxicity on biomass growth of *Hordeum sativum* L. , Chlorophyll fluorescence kinetics, and the antioxidive response inside the tissues.

3- Study the effectiveness of the applied amendments on mitigating PAHs induced stress on *Hordeum sativum* L.

4-Compare the response of applied amendments in artificial and chemical pollution.

2. LITERATURE REVIEW

2.1. PAHs Effect on Soil Properties

Soil incubated with PAHs didn't have drastic changes in their physicochemical properties. Other studies confirmed that a low pollutant concentration won't alter soil's main properties (Wang et al., 2020; B. Wu et al., 2018). On the other hand, soil enzymatic activity showed significant changes, and low doses of Pyrene enhanced soil microbial activity (Wang et al., 2020). Hollender et al., reported an improvement in respiration rates in soil under stress since soil microorganisms can utilize pyrene as a substrate (Hollender et al., 2003; Lu et al., 2013). Similarly, microbial density is affected positively by PAHs addition at low concentrations as a form of adaptation to the change in the environment (Lu et al., 2014; Wang et al., 2020). On the contrary, the long-term soil contamination with high molecular weight PAHs clogs the pores between soil particles leading to decreased water infiltration rate. In addition to a drop-down in the number of microorganisms and reduced soil enzymatic activity (Haritash, 2019). Some soil properties regulate the degradation or retention of PAHs in soil, for example, organic carbon content, type of humus compounds, and clay or mineral content (Kuppusamy et al., 2017). It was reported in several studies, that PAH particles are more likely to adsorb to a finer-sized soil particle owing to the larger surface area (Haritash, 2019). Whereas, Svetlana et al., studied the physicochemical properties of a heavily polluted soil near an energy plant, and were found to be chernozems and meadow-chernozems with high organic matter content (2.2–2.9%), deep humus layer (70–100 cm) and physical clay content 33–67% and clay content 13–37% (Sushkova et al., 2019). A study by Gworek et al., reports that the variable concentration of organic matter in the soil plots may have led to the different PAH concentrations (Gworek et al., 2016; Sushkova et al., 2020). Additionally, Minkina et al., confirmed the spiking of BaP (Benzopyrene) in Haplic Chernozem soil and the synchronized increase in BaP with additional contamination of soil (Minkina et al., 2020). Agricultural lands close to industrial tend to suffer from high concentrations of PAHs, especially the more persistent ones like BaP. Li et al., reported the accumulation of total PAHs in the soil near the industrial park of Suzhou was found to be 796 µg kg $^{-1}$, 40% of this amount was overtaken by BaP (Y. Li et al., 2017). Furthermore, In the area near a domestic waste incineration plant, 300 µg kg $^{-1}$ of BaP were inspected in the soil (Jia et al., 2017). Also, Boente et al., comprehensive analysis of 150 soil samples in Langreo municipality, an area with high coal mining activity since 1850, indicated that in 79% of the samples the content of BaP was 0.24 µg kg $^{-1}$ which is 10 times higher than the dictated limit in Spain (Boente et al., 2020).

2.2 Bioaccumulation (BAF_r) and Translocation (BAF_v) of PAHs in Plant

There are two pathways for PAHs to enter plant tissues, either through the roots growing near PAHs polluted areas or by aerial tissues especially leaves when air is contaminated with volatile PAHs (Kumari et al., 2021). PAHs with low molecular are more readily translocated from roots to stem and leaves while high molecular weight PAHs are accumulated in roots (Kumari et al., 2021; Pullagurala et al., 2018). However, Zhan et al., reported the need for an energy-dependent carrier system in *Triticum aestivnm* L. (Kumari et al., 2021; Zhan et al., 2010). Svetlana et al., reported the phytoaccumulation of BaP in the tissues of spring barley grown on artificially polluted soil for 4 years with an annual uptake of BaP from 0.03 up to 0.06%. The results showed that the range of bioaccumulation coefficient by roots was 0.01-0.06 while in vegetative parts it ranged from 0.01-0.02. The data obtained from this study hypothesize that some of the degradation products of BaP can be used as an energy source in addition to plants having a vegetative biochemical barrier hindering BaP uptake (Sushkova et al., 2017). A total of 15 PAHs in winter wheat grown in different contaminated sites were measured. The results reported that the total PAH concentration was higher in roots (287–432 μg kg^{-1}) when compared to the upper parts (221–310 μg kg^{-1}). The study also showed that the dominant PAHs in plant tissues were 3 ring PAHs (acenaphthene, acenaphthylene, fluorene, phenanthrene, and anthracene), concentrated in the upper tissues with a percentage of (72.5– 82.7%), dramatically higher than roots (49.5–74.0%) and rhizosphere soil (36.3–65.7%) (Tian et al., 2018). *Zea mays* L. was assessed for PAHs reduction in a soil treated with degrading microorganism and organic fertilizer. The results propose the degradation of phenanthroline (PHN) into more soluble products and that rate of PAHs uptake increased with biomass growth. Bioaccumulation factor for total PAHs for roots, stems and leaves was 81.27, 34,87, 14.32 μg kg^{-1} FW respectively. Interestingly the data showed accumulation variability for similar ringed PAHs for example accumulation factor for phenanthrene (PHE) > anthracene (ANT) by 1.26 folds whereas fluorene (FLU) > Pyrene (PYR) by 3.68 folds. As for the translocation factor, in *Zea mays* L. there was a reported decrease in translocation from stem to leaves and from leaves to grains, with a BAFv value of 0.19 (shoot-root) and 0.12 (shoot-leaves) (Kumari et al., 2021). Gowerk et al., reported that plant types may contribute the uptake and translocation of PAHs, where the examined monocotyledons; couch grass (*Agropyron repens*), wood small-reed (*Calamagrostis epigejos*) showed a significant correlation between the concentration of 3-ring PAHs fluorene, phenanthrene, and anthracene in plant tissues with their content in the sewage sludge. However dicotyledons; wild buckwheat (*Polygonum convolvulus*), white goosefoot (*Chenopodium album*) didn't show the same correlation which predicted the uptake of PAHs through the leaves since dicotyledons have leaves with larger surface area, thereby are able to accept more PAHs from leaves compared with monocotyledons (Gworek et al., 2016; Pullagurala et al., 2018). The highest PAHs found in plant tissues was phenanthrene, where the collected monocotyledon (*Agropyron repens*) and dicotyledon (*Chenopodium album*) grown in a soil plot with phenanthrene concentration of 1.44 μg kg^{-1}, the amount of phenanthrene in plants was 0.348 and 0.185 μg kg^{-1}, respectively (Gworek et al., 2016).

2.3. PAHs Effect on Plant Morphological and Biochemical Characteristics

There's a shortage of information on the impact of PAHs on plant health and the mechanism of stress buildup when compared with other soil-borne contaminants (Elgendy et al., 2021). A comparative study by Panwar et al. proved the variation in root and shoot length in *Triticum aestivum* L. (wheat), *Brassica juncea* L. (mustard), *Helianthus annuus* L. (sunflower), and *Tagetes* L. (marigold) under PAHs stress. *Tagetes* L. at 100 μg kg^{-1} exhibited the highest decrease in shoot-root ratio. However, all plants studied, expressed a general growth reduction, necrotic and chlorotic lesions (Panwar & Mathur, 2019). Furthermore, Kumari et al., reported that *Zea mays* L. grown in PAHs polluted soil, expressed shoot length, root length, and fresh weight reduction by 11, 53, and 32% when compared to control (Kumari et al., 2021). Another study on *Arabidopsis thaliana* showed that phenanthrene exposure resulted in reduced growth of roots and stem, late flowering, and the appearance of white spots. In later growth stages, the white spots changed into necrosis, presumably due to the H_2O_2 causing cell mortality (Alkio, Tabuchi, Cruz, et al., 2005). A 4-year model experiment on BaP contamination indicated that the total length of spring barley was inversely proportional to the concentration of BaP. Hypothetically, because the ear length was sensitive to the increasing BaP in soil, thereby giving the whole plant a shorter overall outlook. Both the germination energy and plant weight were decreased, while the root length increased with BaP concentration (Sushkova et al., 2017). In rice, the plant biomass and chlorophyll content were reduced whereas the water content and chlorophyll a/b ratio were enhanced at 400 µg kg^{-1} of fluoranthene. Additionally the increase in fluoranthene content completely hindered the germination rate and energy in the plants (Hou et al., 2017). Also, Kummerova et al. (2012) observed the effect of fluoranthene on maize and pea plants, showing inhibition in the rate of seed germination and germination energy. This was probably connected with the endogenous hormonal levels changing, for example, cytokinin, ethylene, and Abscisic acid (Hasanuzzaman et al., 2019; Kummerová et al., 2012). The byproducts of PAHs resulting from aging, photodegradation, and weathering often give a more toxic contaminant due to increased polarity hence more solubility (Molina Lázaro & Segura Ana, 2021). Liu et al. (2021) reported the changes in the mitochondria and chloroplast structures as a consequence of the reduction in CO2 assimilation and phytohormones due to PAHs phytotoxicity (Hasanuzzaman et al., 2019; Liu et al., 2009). Changes in chlorophyll content translate the physiological balance and the stress extent in plants. *Zea mays* L. expressed a 26, 21, and 24% reduction in chlorophyll a, chlorophyll b, and total chlorophyll, respectively. Thereby, proving there’s a correlation between the plant’s reduced biomass and the photosynthetic pigment content (Kumari et al., 2021). Additionally, the total chlorophyll of *Tagetes* L. was reduced from 0.0049 to 0.0030 mg g^{-1} FW While in *Helianthus* it decreased from 0.010 to 0.008 mg g^{-1} U under PAHs stress (Panwar & Mathur, 2019). There is a significant elevation in the expression of enzymatic and non- enzymatic antioxidants under PAHs pollution. Examples are superoxide dismutase, catalases, peroxidase, and polyamines (Molina Lázaro & Segura Ana, 2021). In *Oryza sativa* L. grown under phenanthrene, and pyrene contamination expressed an increase in the antioxidative enzyme's activity. superoxide dismutase (SOD) content increased at 100, 200, and 400 μg kg^{-1} by 8, 46, and 56%, respectively when compared with control. The elevation in SOD and peroxidase (POD) content may translate into a protection mechanism for chloroplast or induce the increase of soluble protein in plant cells (J. H. Li et al., 2008). The comparative study by Panwar et al., showed that for *Helianthus annuus*, ascorbate peroxidase (APX), peroxidase (POD), and superoxide dismutase (SOD) were highly active at 100 mg. kg^{-1} PAHs with 20.37, 0.212, and 2.13 Unit g^{-1} FW, respectively. Whereas the polyphenol and proline concertation were enhanced to 0.909 and 0.732 Unit g^{-1} FW ,respectively, at the same PAHs concentration (Panwar & Mathur, 2019). *Amaranthus cruentus* growth near a thermal power unit resulted in the reduction of chlorophyll a content by 77%, and a 2 fold increase in the activity of SOD, Catalase (CAT), and POD (Tandey et al., 2020) . Moreover, the study of Tomar and Jajoo on wheat treatment with fluoranthene and photo modified fluoranthene showed a significant increase in POD and SOD by 67% and 19% for FLT and 91% and 21% for PFLT, respectively. While total proteins, growth regulators and CAT was found to be significantly reduced (Rupal Singh Tomar & Jajoo, 2015). Spinedi et al., noted the enhancement in POD and APX by 34.09% and 692% in *Marchanita polymorpha* L. under anthracene pollution (Spinedi et al., 2021).

2.4. PAHs Effect on Chlorophyll-a Fluorescence

The lipophilic property of PAHs, favors their deposition in the thylakoid membranes of plant cells, leading to the modification within the photosystem II electron transport and eventually the blockage of the electron movement from PSII to PSI (Hasanuzzaman et al., 2019; Hou et al., 2017). Total chlorophyll is reduced in comparison with chlorophyll a/b ratio in C3 and C4 plants in presence of PAHs (Molina Lázaro & Segura Ana, 2021). It was found that Anthracene inhibits the photosynthetic activity of green algae. Through lowering the activity of oxygen electron complex (OEC) in anthracene-treated algal cells (Hou et al., 2017). In *Triticum aestivum* L., PSII quantum yield was negatively affected by fluoranthene (FLT) and photo modified fluoranthene (PFLT) treatment due to the increased non-photochemical quenching. It was noted that PFLT had more toxicity on PSII than FLT, where it caused a serious disturbance in the rate of electron transport by

PSII. Similar to PSII, PSI was increasingly inhibited at a high concentration of PFLT through the blockage of the oxidation-reduction kinetics of P700 (Rupal S Tomar & Jajoo, 2017). Furthermore, the treatment of wheat with Anthracene resulted in a reduction in the electron transport, trapping, and light absorption efficiency, thereby weekend the PSI and PSII quantum yield. Additionally, the measured Chlorophyll-a fluorescence induction curve was diminished in J–I and I–P phase (Jain & Jajoo, 2020). Tomar and Jajoo reported the impact of fluoranthene on the dark reaction in wheat. Their observations concluded a negative correlation between fluoranthene concentration and photosynthesis rate (Pn). Since there was a noticeable decrease in the Pn by 39, and 52 % at 5 µM and 25 µM, respectively. Consistently there was similar reduction in the stomatal conductance (Gs) and transpiration rate (Tr) but with no detected significant change in the internal concentration of CO2 (Ci) (Tomar et al. 2019; Tomar and Jajoo 2015). Oguntimehin et al., study in 2-year-old Japanese red pine seedlings fumigated with a solution of phenanthrene and fluoranthene and observed a reduction in the total photosynthesis rate, stomatal conductance, and Rubisco activity at 10 Mm concentration (Oguntimehin, Nakatani, & Sakugawa, 2008). Jia et al., highlighted the positive influence of phenanthrene and anthracene on the chlorophyll-a and chlorophyll-b in spinach, Chinese cabbage, shanghai green cabbage, and romaine (Jia et al., 2019). Ahmad et al., recorded a significant change in the chlorophyll-a in *Cucumis sativus* L.under 9 pesticide contamination. Since the photosynthetic parameters measured disclosed a decrease in the maximal quantum efficiency of PSII (Fv/Fm) by paraquat and in quantum efficiency of PSII (YII) by most of the pesticides because of photochemical quenching coefficient (qP) inhibition (Tomar et al. 2019). On the other hand, *Marchantia polymorpha* L. treated with anthracene displayed a decrease in the Chlorophyll-a and Chlorophyll-b by 31.6% and 38.4%, wasn't supported by significant change in maximum quantum efficiency (Fv/Fm) and photosynthesis rate (Pn) even though the value of photosynthesis was diminished to almost half of its value (Spinedi et al., 2021).

2.5. Role of Biochar on PAHs and Plant Growth

Biochar is gaining a lot of recognition as an environmentally safe and sustainable remediation approach for PAHs dissipation from aqueous solution and soils (Lamichhane et al., 2016; X. Li et al., 2020). The action of biochar on contaminants is either through adsorbing of contaminants onto its porous surface or altering the structure of the microbial community, hence, increasing their biodegradation (Lamichhane et al., 2016) The application of rice straw-derived biochar pyrolyzed at 600 °C (RS6) to a PAHs polluted soil, enhanced the percentage of biodegradation for individual PAHs from 40% to 58.84% and showed significant breakdown of individual PAHs in soil (Zhang et al., 2020). Also, biochar diminished the concentration of freely dissolved PAH content by 57% in a sewage sludge (Lamichhane et al., 2016; Oleszczuk et al., 2012). Biochar is reported to have a high activation temperature, thereby expected to show better adsorption capability towards HMW PAHs compared with LMW PAHs (Patel et al., 2020). Whereas biochar addition diminished the concentration of bioavailable HMW PAHs by more than 50% and LMW PAHs by 40% in a PAHs polluted soil (Lamichhane et al., 2016). Biochar can have a wide spectrum of properties, all depending on several control factors, such as feedstock type, time, and temperature of pyrolysis (Vijayaraghavan, 2019). The dissipation percentage of PYR and BaP peaked at a pyrolysis temperature of 500 °C. Where BaP and PYR adsorption by (*Enteromorpha prolifera*) EP-biochar reached 48.1% and 59.8%, respectively (Qiao et al., 2018). Additionally, wood-based biochar achieved a total PAH removal (pyrene, phenanthrene, and benzo(a)anthracene) by 60% (Lamichhane et al., 2016). Guo et al., noted a downgrade in the phytotoxicity and bioavailability of phenanthrene and pentachlorophenol with conifer biochar treatment (Guo et al., 2020). Furthermore, Svetlana et al., studied the impact of 1% and 5% sunflower husk biochar on the total PAHs and BaP content in both soil and spring barley. Where the results proved a successful mitigation percentage for 1% biochar at 400 µg kg^{-1} by 50 % and for 5% PAHs were reduced in plants by 40-60% at 800 µg $kg^{-1}$and 37-48% at 1200 µg kg^{-1} PAHs contamination (Sushkova et al. 2021). In addition to this, Kong et al., described the impact of different pyrolysis temperatures and feedstock on the efficiency of Biochar sorption to PAHs. Since it was revealed that, 500 ◦C pyrolyzed biochar was able to lessen PAHs content by more than 300 ◦C pyrolyzed biochar and that sawdust biochar was more effective in stimulating biodegradation when compared to wheat straw biochar (Kong et al., 2018). Biochar was proved to have plant stress mitigation potential in various studies. The application of 1% biochar to drought and salt-stressed quinoa enhanced their antioxidative response through the elevation of plant growth hormones. In addition to sorption of Na^+ and K^+ thereby reducing their uptake by plant (Kavitha et al., 2018). Also, Paneque et al., reported an increase in the water-use efficiency of sunflowers under drought stress at 15 t ha^{-1} dose of biochar (Kavitha et al., 2018; Paneque et al., 2016).

2.6. Nanoparticles Foliar Spray: Impact and Their Possible Effects on Plant Growth

Stomata can absorb nutrients at a rate faster than root cells. Since the soil-applied NPs may be less bioavailable because of their adsorption, transport, desorption, and transformation in soil (Hong et al., 2021).The spray of ZnO-NPs solution to *Lycopersicon esculentum* expressed a growth improvement after 45 days and 60 days of growth. Besides the optimum ZnO-NPs were found to be 50 mg L^{-1} giving an increase in the soil-plant SPAD chlorophyll, net photosynthetic rate, gaseous exchange, and internal CO2 by 32.1, 35.1, 29.1, and 31.2% respectively. Furtherly the antioxidant enzymes were presented in higher concentrations at the same ZnO-NPs treatment of 60 % for CAT, 74% for SOD, 55% for POD, and 54 % for proline. Also, the fruit number and fruit yield were elevated by 21 and 19.4% respectively, with higher lycopene and β- carotene content but lower ascorbic acid concentration (Faizan & Hayat, 2019). Another study demonstrated that 10 mg L^{-1} solutions of ZnO-NPs foliar supplication to 14- days old cluster bean plant upgraded shoot length, root length, and plant biomass by 31.5, 66.3, 27.1%, respectively (Ali et al., 2019). Rossi et al., showed that foliar spray of ZnO-NPs can efficiently increase the fresh and dry weight of *Coffea arabica* L. plants. Since the FW of roots and leaves increased by 37 and 95%, respectively, the DW of roots, stems, and leaves was enhanced by 28, 85, and 20%, respectively. Coffee plants also expressed an enhancement in the net photosynthetic rate by 55% and an improvement in Zn content in leaves by 1267.1 ± 367.2 mg kg^{-1} DW (Rossi et al., 2019). Besides this, another study on foliar ZnO-NPs application proved the influence of applied Zn on the concentration of other micronutrients in rice. Where the content of Mn, Fe, Cu in the shoot, Cu in grain, and Mn in root increased by the ZnO-NPs foliar application (Bala et al., 2019). The defense system in plants can be supported by the foliar application of NPs in coping with stress. By acting on various stress mitigating parameters in plants such as changes in hormonal levels, antioxidant enzyme activities, and other enzymatic activities (Hong et al., 2021). For example, *Leucaena leucocephala* seedlings treated with ZnO-NPs foliar spray exhibited high antioxidative activity through SOD, CAT, and POD elevated concentration under Cd and Pb contamination. Also, ZnO-NPs increased total soluble proteins and pigments concentration but lowered MDA activity in leaves (Venkatachalam et al., 2017). Zohri et al., evaluated green ZnO-NPs foliar application to tomatoes under drought stress. Where the results translated an increase in the shoot dry weight, and the activity of SOD, CAT, and APX by 2. 3.99. 3.23.and 2,82 folds, respectively at 25 mg L^{-1} concentration of ZnO-NPs. Similarly, at 50 mg L^{-1}, the enhancement was by 2.5, 4.5. 3.5, and 3.2-fold for the same parameters indicated. Furthermore, the concentration of ascorbic acid and free phenols were consistent while MDA and H_2O_2 content were reduced (El-zohri et al., 2021). In cucumber plant under drought stress, ZnO-NPs downsized the stressing effects of drought and increased the plant growth and biomass significantly. Since the foliar application of ZnO-NPs at 100 mg L^{-1} both photosynthesis and photosynthetic pigments in addition to PSII activity were all improved. The stress mitigation action of ZnO-NPs in cucumber was observed through the increased enzymatic and non-enzymatic antioxidant activity, especially for proline, glycine betaine, free amino acid, and sugars (Imran et al., 2022). Seleiman et al., studied the impact of heavy metals on sunflower growth with rice and cow manure biochar soil treatment and the foliar spray of ZnO-NPs. The results broadcasted a significant decrease in heavy metals availability in soil with the combination treatment of all three amendments. Especially for Pb, Cr, Cu, and Cd, their availability in soil was reduced by 78.6, 115.3, 153.3, and 178.5%, respectively. Additionally, heavy metals concentration was diminished from pant tissues by 1.13, 5.19, 3.88, and 0.26 mg kg^{-1} DM, respectively, for Pb, Cr, Cu, and Cd (Seleiman et al., 2020). Another combination of ZnO-NPs and biochar to hinder the bioavailability of cadmium on *Oryza sativa* L. showed a significantly low concentration of cd in roots at 100 mg kg^{-1} ZnO-NPs combined with biochar. Whereas Cd content in the above and belowground parts with combined treatments was lessened by 39% while for ZnO-NPs the reduction was 30% for aboveground tissues and 31% for belowground parts. Additionally, it had a positive effect on plant biomass, photosynthesis rate, and chlorophyll content (Ali et al., 2019). Likewise, in *Zea mays* L., the combined effect of ZnO-NPs with biochar or alone enhanced the plant biomass, gas exchange rate, and plant height, on the other hand, downsized the concentration of H_2O_2 and MDA. It was noted that ZnO-NPs with 100 mg L^{-1} showed the highest improvement in shoot and root growth by 68 and 55%, respectively when combined with biochar, whereas ZnO-NPs alone decreased Cd uptake in by 61 and 53%, respectively, by stem and roots of maize plant (Rizwan et al., 2019). However, it's noted that there's no recorded data on the effect of nanoparticles on plants growth under PAHs pollution or the combined application of any nanoparticle type with biochar (Elgendy et al., 2021).

3. MATERIALS AND METHODS

3.1. Sample Collection and Characterization

The experiment design was built with two soil types to research the features of the Haplic Chernozem properties during PAHs contamination and combine the application of the biochar and special amendment with zinc oxide nanoparticle foliar spray. The approbation of the developing bioremediation method will be tested on the Technosols chemically contaminated with PAHs at the ultrahigh level which adjoined to the chemical enterprise working from 1964 at the territory of Atamanskoye lake, Rostov Region. The reflection of the treatments applied on the plant's physiological parameters especially oxidative enzymes activity, the morphometric characteristics of the plant, photosynthesis parameters along with the soil amendment efficiency will be measured to evaluate whether the separate application of biochar into the soil or the combined application with zinc oxide nanoparticle foliar spray had the highest positive impact on plant growth and development.

3.1.1. Carbonate Haplic Chernozem

Haplic Chernozem soils are present in large quantities in the large agro-industrial regions of southern Russia, such as the Rostov Region and Krasnodar Krai territory (Minkina et al., 2020). The samples were taken from the upper (0–20 cm) layer of the soil in the State Soil Preserve "Persianovsky preserved steppe" of Rostov region (south of the Russian Federation) located far from contaminated sources. The physical and chemical characteristics of this soil were analyzed and represented in (Table 1) (Sushkova et al., 2017).

Table 3.0.1.: The physical and chemical properties of Haplic Chernozem

Silt particles (<0.02 mm) (%)	**Clay particles (<0.002 mm) (%)**	**$C_{org.}$ (%)**	**pH**	**CaCO3 (%)**	**Ca^{2+}+$Mg^{2+,}$ Mmol (+) $100g^{-1}$**	**CEC, Mmol (+) $100g^{-1}$**
48.1±1.4*	28.6±1.2	3.7±0.3	7.3±0.1	0.3±0.1	35.0±3.0	37.1±2.9

3.1.2. Spolic Technosol

The mentioned soil is positioned on the area of Lake Atamanskoe in the Seversky Donets River floodplain, southern Russia, Rostov Region. The lake was used as a wastewater disposal basin for a chemical factory after the 1950s and dried up in 1990. Soil monitoring plot number 57 grid was selected as the most contaminated with PAHs regarding previous research (Figure 1) (Sushkova et al., 2019).

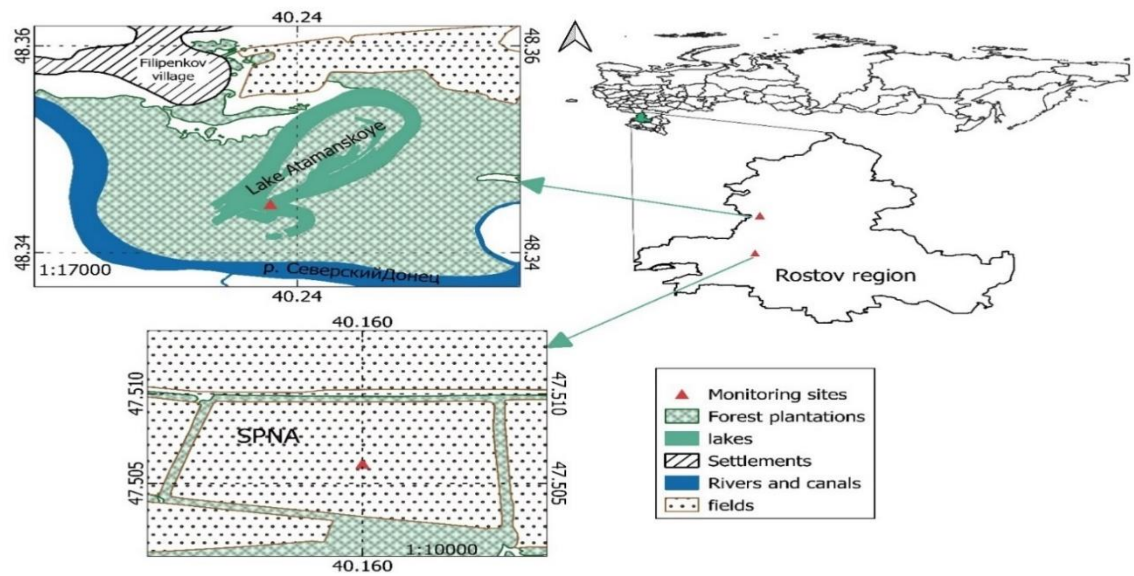

Figure 3.1.: Soil sampling map (Haplic Chernozem and Atamanskoye spolic technosol)

The sampling was taken also at 0 to 20 cm depth, where the samples were selected according to ISO 10381-1 (2002). They were stored in polyethylene bags and taken to the laboratory. Soil samples of the same area plot were mixed, homogenized, air-dried, and sieved through a 2 mm sieve. The main physical and chemical characteristics of the soil were determined at the certified analytical laboratory (Certificate No. POCC RU 0001.511127) following ISO Guide 34 (2009) methods. Furthermore, PAHs were extracted from the soils by the standardized method to remove the coextracted compounds from the soil with saponification forwarded by HPLC (High-performance liquid chromatography) analysis for identification and quantification of PAHs in soil (Figure 2) (Sushkova et al., 2017).

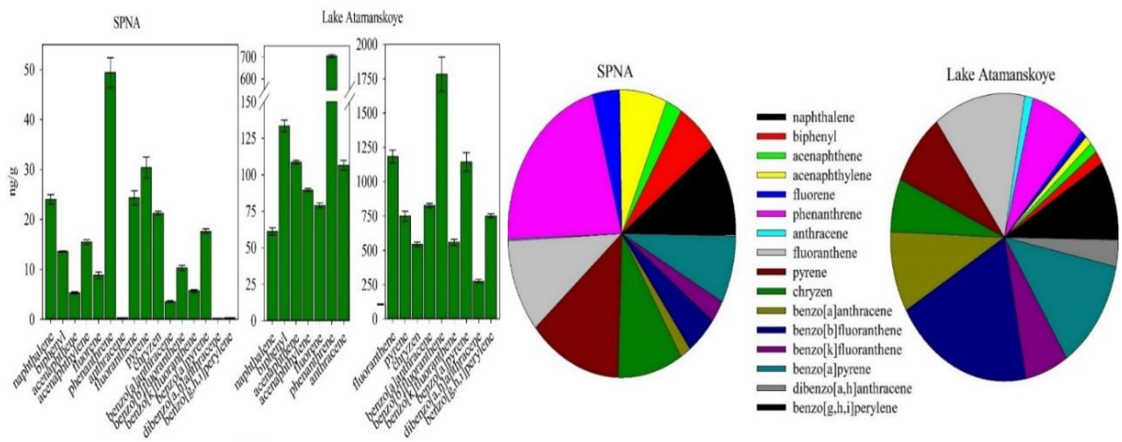

Figure 3.2.: Diagrammatic representation for PAH levels in Atamanskoye lake and the specially protected natural area (Gorovtsov et al., 2021).

3.2. Experiment Design

The selected soils were cleaned of plant residues and other inclusions, grounded in a porcelain mortar, and passed through a sieve with a hole diameter of 3 mm. 200 g of air-dried soil was placed in the vegetative plastic pots. The uncontaminated soil (Haplic Chernozem) was divided into Control, biochar and/or foliar spray of ZnO NPs treated soil, and PAHs artificially contaminated samples (Table 2). A similar design was applied for the chemically polluted Spolic Technosols apart from adding an external PAHs source. For soil contamination, an aqueous solution of benzo[a]pyrene (BaP) in acetonitrile (50 and 100 μg kg^{-1}), which corresponds to 2.5 and 5 MPC according to the concentration referenced by Kasimov et al., was added to the soil surface of the selected samples

(Kasimov et al., 2016). The above-mentioned concentrations were selected to equalize the pollution content in both soil types. During the incubation period, moisture content was maintained at 60% of the total field moisture capacity in the phytotoxicity model laboratory experiment (GOST RISO 22030-2009) (Sushkova et al. 2021). After one month of PAHs spiking with soil particles, biochar was added, and 10 seeds of Spring barely were sown in each sample pot. Zinc oxide nanoparticle ZnO-NPs foliar spray was applied to 18-day-old plants.

Table 3.0.2. Experiment Scheme

Soil type	**Samples no**	**Sample type**	**PAHs**	**Biochar**	**Nanoparticles**
Haplic Chernozem	1	**Control**			
	2	**0.5% Biochar**		0.5%	
	3	**0.5% Biochar+150ppm ZnO-NPs**		0.5%	150 mg L^{-1}
	5	**1% Biochar**		1%	
	6	**1% Biochar +150 ppm ZnO NPs**		1%	150 mg L^{-1}
	7	**2.5 MPC PAHs**	2.5 MPC		
	8	**2.5 MPC PAHs+0.5% Biochar**	2.5 MPC	0.5%	
	9	**2.5 MPC PAHs+0.5% Biochar+150 ppm ZnO NPs**	2.5 MPC	0.5%	150 mg L^{-1}
	10	**2.5 MPC PAHs+150ppm ZnO NPs**	2.5 MPC		150 mg L^{-1}
	11	**5 MPC PAHs**	5MPC		
	12	**5 MPC PAHs+ 1% Biochar**	5MPC	1%	
	13	**5 MPC PAHs +1 % Biochar+ 150 ppm ZnO NPs**	5MPC	1%	150 mg L^{-1}
	14	**5 MPC PAHs +150 ppm ZnO NPs**	5MPC		150 mg L^{-1}
Technosol	15	**Control**			
	16	**5% Biochar**		5%	
	17	**5% Biochar+ 150 ppm ZnO NPs**		5%	150 mg L^{-1}
	18	**150 ppm ZnO NPs**			150 L^{-1}

3.3. PAHs Extraction and Quantification in Soils and Plant tissues

PAHs were extracted from the soils using the standardized method to remove the co-extracted compounds from the soil extracts with saponification (Minkina et al., 2020). The soil moisture level was recorded before PAH extraction. Briefly, 1 g of the air-dry soil was subjected to a rotary evaporator. Afterward, 20 ml of 2% solution KOH diluted with ethanol was added to the soil sample, then the mixture was refluxed in the water bath. The saponification of soil's lipophilic compounds would increase PAHs recovery and reduce the content of coextracted compounds. The supernatant of the resulting mixture was separated in an Erlenmeyer flask: n-hexane (15 mL) and distilled water (5 mL) were added for the better separation of the layers. The mixture was shaken for 10 min and then transferred to a dividing funnel. Similarly, this step was repeated twice, the n-hexane is washed with distilled water until a neutral pH is reached. The resulting n-hexane layer in the diving funnel was carefully moved into a separate vessel and placed in a dark space. subsequently, 5 g anhydrous Na_2SO_4 was added to eliminate any water mistakenly passed through the analyzing solution. After 8 hours, the resulting extract was evaporated until dry by using a bath of the rotary evaporator under a temperature of 40 ^oC. Finally, the residue was redissolved in 1 mL acetonitrile for further analysis.

PAH concentration in the extracts was measured by HPLC (Model 1260, Agilent Technologies, USA, 2014) with fluorescence detection following the ISO 13859:2014 requirements. The chromatographic peaks of each PAH were identified by comparing the retention times to that of the analytical standard samples. Sigma- Aldrich (Merch) was used as the standard solution. The certified reference materials and calibration curves were used for the calculation of the limits of detection (LODs) and limits of quantification (LOQs) according to Minkina et al., (Minkina et al., 2019). A calibration standard of PAH mixture was injected after every six samples to correct for drift in retention time within a run. Quality control of every HPLC detection was performed according to Agilent Application Solution. For the developed methods of extracting the target PAH in the soil, a random component of the measurement error was estimated which for the concentration range of 2–200 μg kg^{-1} was 3.5–14%.

The following equation was used to estimate the PAH's content:

$$Cs = k\,Si \times Cst \times \frac{V}{Sst \times m}. \quad (1)$$

where Sst and Si – respective areas of target PAH peaks in chromatograms of standard and sample solutions; Cst – target PAH concentration in standard solution (μg mL-1); k – coefficient of target PAH recovery from a sample; V – volume of acetonitrile extract used for HPLC (mL); and m – the mass of the sample (kg).

The statistical analysis of the data was carried out using Statistica 11.0 software, where the mean, median, minimum, and maximum values, and coefficient of variation (CV) were calculated. Statistical significance of the differences among means was determined by the least significant difference (LSD) test. Differences were considered not significant at values of $p > 0.05$. For the data obtained, the levels of difference were statistically significant. The effect of soil properties on PAH accumulation was assessed using Spearman rank-order correlation.

Bioaccumulation for PAHs in roots and vegetative part of barley plants (BAFr and BAFv respectively) were calculated as a ratio between its concentrations in the specified plant part (Cr and Cv respectively) to its PAHs concentration in soil (Cs) according to the next equations (Sushkova et al., 2018b).

$$BAFr = \frac{Cr}{Cs};\ \text{or}\ BAFv = \frac{Cv}{Cs}. \quad (2)$$

3.4. Biochar Application

The used biochar for the model experiment was prepared from sunflower husks at a pyrolysis temperature of 700°C. The structure of the used biochar was studied by scanning electron microscopy (SEM) (Figure 3), (microscope Carl Zeiss EVO-40 XVP) and confocal microscopy using a 3D laser scanning microscope (KEYENCE VK-9700. Generation II, Japan), both the topography (surface) and morphology (microgeometry) of bio sorbents fragments were studied. Non-conductive and low-contrast samples were recorded under normal settings (low vacuum, 15 kV, increased emission) (SUSHKOVA et al., 2021). Additionally, the elemental composition of biochar was measured and represented (Figure 4). Biochar was added to the selected soil samples in the experiment design after 30 days of PAH spiking. For soils with 0.5,1, and 5% biochar amendment 1, 2, and 10 g, respectively, of the prepared sunflower husk biochar, were weighed and mixed with the soil in zip-locks plastic bags to make sure the dose is homogenized in the soil.

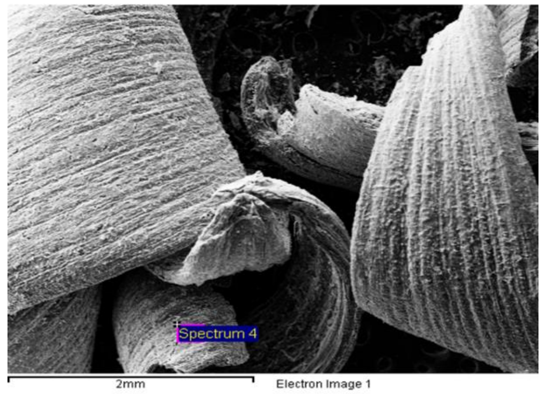

Figure 3.3.: SEM imaging of sunflower husks biochar produced at pyrolysis temperature 700°C.

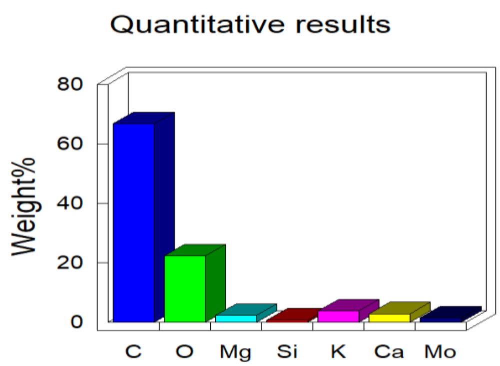

Figure 3.4.: Elemental Percentage in Sunflower husk biochar

3.5. Barely Growth and Performance

A variety of a two-rowed Ratnik *Hordeum sativum* of the family *Poaceae* was used as a test culture crop. Spring barley was used as a test culture crop. Since this variety of Spring barely is the main grain variety grown in the Rostov region. In each pot, 20 spring barley seeds were sown and incubated to grow in growth chambers for 14 days before they were taken for hyperspectral imaging and chlorophyll-a florescence measurement in the botanical garden. The samples were returned to the growth chambers and allowed to grow further. The 24 days old plants were transferred once more to the botanical garden the hyperspectral imaging and chlorophyll-a florescence measurement. Eventually, the morpho-biometric readings of the plants were recorded, and the plants were divided into roots and shoots for the biochemical assays for anti-oxidative enzymes and PAHs quantification.

3.6. Foliar Application of ZnO-NPs

Afterward, the 18 days old spring barley plants (10 selected plants) were foliar sprayed with 150 mg L^{-1}solution of ZnO-NPs. Prepared by dissolving 0.15 g of ZnO-NPs (< 50 nm particle size) in 100 ml of distilled water and a plastic spray bottle was used for the even distribution of ZnO on the leaves of barley.

3.7. Biochemical Analysis

3.7.1. Malondialdehyde (MDA) Activity

Malondialdehyde (MDA) is one of the end products formed via the decomposition of specific primary and secondary lipid peroxidation products. Its reaction with 2-thiobarbituric acid (TBA) works on the nucleophilic addition reaction producing a pink-colored adduct at high temperature and low pH. (Janero, 1990). The end product of MDA reaction with thiobarbituric acid (TBA) gives (TBARS), in which its absorbance is measured at 532–535 nm. (G. N. M. Kumar & Knowles, 1993; S. Kumar et al., 2017).

500 mg of plant sample were ground with 1ml of 20% TCA (Thiobarbituric acid) solution in a mortar. The resulting mixture was centrifuged at 12,000 rpm for 5 min and the supernatant was used transferred to 3 test tubes. Each tube contained 300 µl of supernatant with 1.2 ml of 0.5% TBA (50 mg of TBA dissolved in 10 ml of 20% TCA).

The resulting samples were incubated in the water bath at 100 oC for 20 min. Afterward, the samples were cooled down to room temperature and centrifuged at 3000 rpm for 15 min. Finally, the sample absorbance was measured at 532 nm and 600 for nonspecific absorption correction.

Calculations

To calculate the content of MDA, coefficient of extinction e = 155 $mM^{-1}cm^{-1}$ is used,

According to the formula:

$$C = \frac{\left(\frac{\Delta D}{155}\right).X.V}{(m.\Delta m).1} \tag{3}$$

Where C is MDA concertation, mmol/g dry weight, ΔD is the optical density difference for the sample at 532 nm and 600 nm, 155 is the coefficient of MDA extinction at 532 nm, mM^{-1} cm^{-1}, X is dilution (the total volume of the reaction mixture is divided by the amount of the extracted sample introduced), V is the volume of extract, ml, m is the weight of raw material, g, Δm is the ratio of dry weight to raw, 1 is the length of the optical path, cm.

3.7.2. Superoxide Dismutase (SOD) Activity

These enzymes are considered metalloproteins meaning they are activated by the metallic cofactors Cu, Zn, Mn, or Fe. They are found in the chloroplasts, mitochondria, cytosol, peroxisomes, and the apoplast. Their role is to catalyze the enzymatic transformation from O_2•− to H_2O_2. (Szollosi, 2014). The activity of SOD is quantified by inhibiting the generation of superoxide anion radical adrenaline in adrenochrome by nitroblue tetrazolium (NBT), under an alkaline environment.

A plant tissue of 100 mg was weighed and ground in a chilled mortar with 1.5 ml of extraction buffer, then the mixture was centrifuged at 10,000 rpm for 20 min. there were 2 mixtures prepared for analysis.

Reaction mixture:

3.5 ml Na-carbonate buffer

100 µl extract

0.15 ml NBT

Control mixture

3.5 ml Na-carbonate buffer

0.15 ml NBT

The reaction was initiated in a water bath preheated at 37 ºC with 20 µl of 0.1% adrenaline addition, 3 minutes later the reaction was inhibited by 100 µl of 3% HCl. Sample absorbance was measured at 540 nm.

Calculations

$$T(\%) = \frac{E\text{cont} - E\text{op}}{E\text{cont}} \quad (4)$$

Econt is the control optical density, Eop is the sample optical density, whereas, T is the inhibition percentage of the recovery reaction of NTS. As a conditional unit of enzyme activity, 50% inhibition of NBT is considered.

The activity of SOD activity was calculated using this formula and is expressed in c.e/ min×mg:

$$\text{SOD}\,\frac{T\%}{50\% \times V \times t \times C} \quad (5)$$

V – extract volume (ml), t – incubation time (min), C – concentration (mg/ml)

3.7.3. Catalase (CAT) Activity

Catalase activity is measured by the rate of hydrogen peroxide decomposition into water and oxygen. The determination of this activity is done using the spectrophotometer using Aeby's methodology (Aeby, 1984).

The buffer solution was prepared as follows: 1.36 g KN_2RO_4 and 400 mg NaOH were each dissolved and brought to 50 ml in separate flasks. The resulted solutions 0.2 M KH_2RO_4 and 0.2 M NaOH were mixed to prepare 50 mM K, Na-phosphate buffer, pH 7.8 and 7. where 25 ml of 0.2 M KH_2RO_4 and 22.25 ml of 0.2 M NaOH were incorporated and brought to 100 ml volume with distilled water. pH was adjusted to 7.8 with either concentrated H_3PO_4 or 5% NaOH. While the pH=7 buffer solution was prepared with 25 ml of 0.2 M KH_2RO_4 and 14.55 ml of 0.2 M NaOH was mixed with distilled water to give a total volume of 100 ml. As for the extraction buffer, 2 ml of 50 Mm K, Na- phosphate buffer (pH=7.8) was mixed with 20 µl of 100 Mm phenylmethylsulphonyl fluoride (FMSF) solution. Lastly, 3 ml of 3% hydrogen peroxide is dissolved with 4.5 ml of distilled water to prepare a 0.6 M hydrogen peroxide solution.

2.95 ml of 50 Mm K, Na phosphate buffer (pH=7) is mixed with 30 ml extraction buffer. The reaction starts once a 20 µl of 0.6 M H_2O_2 is added. For the control sample, all the reagents were added except for the substrate (0.6 M H_2O_2). The change in optical density was measured at 240 nm every second for 100 seconds.

Calculations

Calculation of catalase activity in relational units per gram of dry weight is carried out according to the formula:

$$A = (\Delta D.V.X)/(T.L.m.\Delta m) \quad (6)$$

Where is:

A – enzyme activity, ΔD – change in optical density (difference between the optical density at the beginning of the reaction and the optical density at a finite point in time), V – the total volume of the resulting extract (ml), X – final dilution of the extract in the cell (the volume of the reaction mixture is divided by the amount of the extract introduced), T – reaction time (s), L – layer thickness (cm), m – the weight of the attachment (g), Δm is the ratio of dry weight to fresh weight.

3.8. Chlorophyll-a Fluorescence (OJIP)

The Maximal quantum yield of photosystem II (Fv/Fm) is a nondestructive and precise method used to attain information on a plant's physiological state (V. Rajput et al., 2018). Chlorophyll-a fluorescence (OJIP) was measured twice after 14 days and 24 days of sowing the seeds in the polluted soil. Photosynthetic efficiency was evaluated after 20 min of incubation in a room with dark curtains to ensure reaction centers of PSII were photosynthesized and electron transfer was induced (Agnieszka et al. 2021; Rajput et al. 2019). The pulse modulated (PAM) fluorometer (Diving PAM, Waltz, Germany) was used to assay the maximal quantum yield of photosystem II (Fv/Fm) of plants at room temperature. The device was set up to red measuring light of 0.15 mmol photon m^{-2} s^{-1} and white saturating flash was 1800 mmol photons m^{-2} s^{-1} for Photosynthetic photon flux density (PPFD) estimation. Twenty readings were recorded by selecting fresh leaves and all the leaves were measured under the same condition.

3.9. Statistical Analysis

Statistical processing of the obtained results was carried out using STATISTICA 7 and Sigmaplot 12.5 software. The normality of the distribution in the samples was calculated using the Shapiro–Wilk test. To analyze the differences between the mean lengths of roots and stems of barley, one-way ANOVA was used, followed by the determination of differences between pairs of Tukey's post hoc test—Honestly

Significant Difference (HSD). For other data. The significance of differences between the mean values of enzymatic activity was assessed using Student's t-test at p-level < 0.05.

3. RESULTS AND DISCUSSION

4.1. PAHs Concentration in The Tested Soils

In control soil, total PAHs and BaP content ranged from 227.9 to 215.7µg kg $^{-1}$ and 18.1to 18.3µg kg $^{-1}$, respectively, which is less than the Russian federation state accepted standards (GOST 17.4.1.02.-83, 2004) (Sushkova et al., 2016) for BaP content in soils. Whereas Minkina et al., confirmed the spiking of benzopyrene (BaP) in chernozem soil (Minkina et al., 2020). The addition of biochar reduced the initial concentrations of both total PAHs and BaP in soil. Furthermore, 1% biochar and its combined treatment proved to be more efficient in reducing total PAHs than BaP content in soil compared with 0.5% biochar. A similar reduction in total PAHs and BaP concentration with 1% biochar and 5% biochar was reported by Svetlana et al., (SUSHKOVA et al., 2021). However, separate application of ZnO-NPs to leaves had less effect on PAHs content in the soil. where it's proposed that ZnO-NPs reduced PAHs uptake, thereby no significant reduction in PAHs was observed. Similarly the combined addition of biochar and ZnO-NPs on to rice, reduced Cd concentration in soil more than ZnO-NPs alone (Ali et al., 2019). Furthermore, Yue et al., reported the decrease in PAHs uptake in *Amaranthus tricolor* L. treated with foliar spray of ZnO-NPs (Cai, et al., 2022) . The effect of applied treatment on the concentration of PAHs in unpolluted Haplic Chernozem is represented (figure 5).

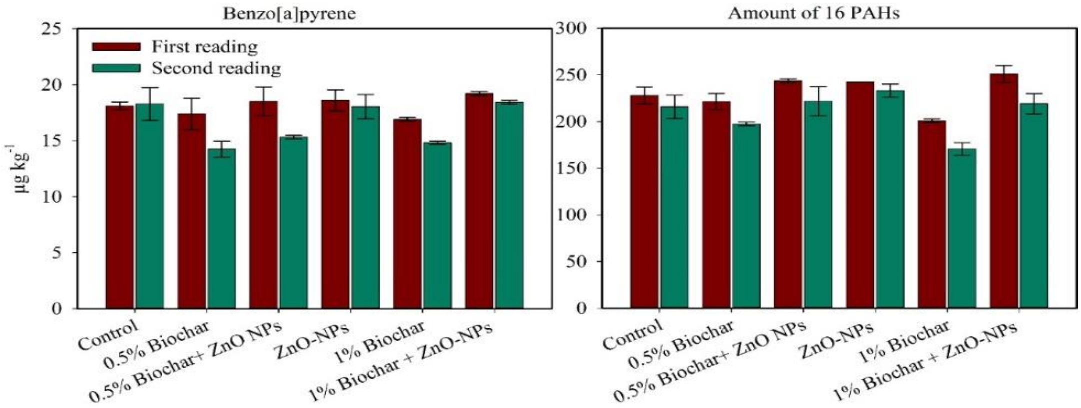

Figure 4.5.: PAHs concentration in unpolluted haplic chernozem

In soils with artificial contamination, the initial concentration of BaP in 2.5 MPC soil was 61-66.5 µg kg $^{-1}$ and in 5 MPC was 101.3-105.4 µg kg $^{-1}$. Both concentrations were higher than the added dose of BaP and the accepted range for BaP in the soil in reference to (GOST 17.4.1.02.-83, 2004). This proposes the presence of PAHs in the used Haplic Chernozem originally or that the PAHs spiking to soil wasn't complete.

Additionally, the highest reduction in BaP and total PAHs percentage was found in the treatment with 2.5 MPC pollution with 0.5 % biochar and ZnO-NPs foliar spray separately or combined. Where 0.5% biochar decreased total PAHs and BaP by 17.50 and 19.70 %, respectively. And studies showed that biochar was reported to have a higher tendency toward HMW PAHs than LMW PAHs owing to its high activation energy (Patel et al., 2020). In 5 MPC BaP Polluted soil, 1% biochar alone or combined with ZnO-NPs had no effect on PAHs and in some cases was an added source of PAHs to soil. Studies indicate the presence of PAHs in fast pyrolyzed biochar ranging from 0.3 to 45 µg kg^{-1} which is higher than several quality standards (Hale et al., 2012).Also, Janu et al., indicated that pyrolysis temperature had 38.7% control over the variance in infrared sorption behavior of biochar (Janu et al., 2021). Moreover, the Total PAHs and BaP range found in long-term polluted technosol were between 7538.3- 7522.4 µg kg^{-1} and 728 -779 µg kg^{-1}, respectively. Furthermore, the results showed a significant decrease of 50% in BaP and total PAHs expressed in combined treatment of 5% biochar with nanoparticles, afterward comes only 5% biochar treatment. There were no studies found on combined biochar and ZnO-NPs effect on PAHs concentration. But one of the explanations is that the enhanced growth of barley plants with biochar and ZnO-NPs could indirectly increase the uptake of PAHs from the soil. similarly, the combined application was reported to decrease soluble Cd in the soil while ZnO-NPs increased Zn concentration in plants (Rizwan et al., 2019). However, ZnO-NPs foliar spray in chemically polluted soil didn’t have a significant reduction in both total PAHs and BaP concentration. The impact of applied treatments on total PAHs and BaP concentration in artificially polluted Haplic Chernozem and chemically polluted Technosol is diagramed in figures (6,7).

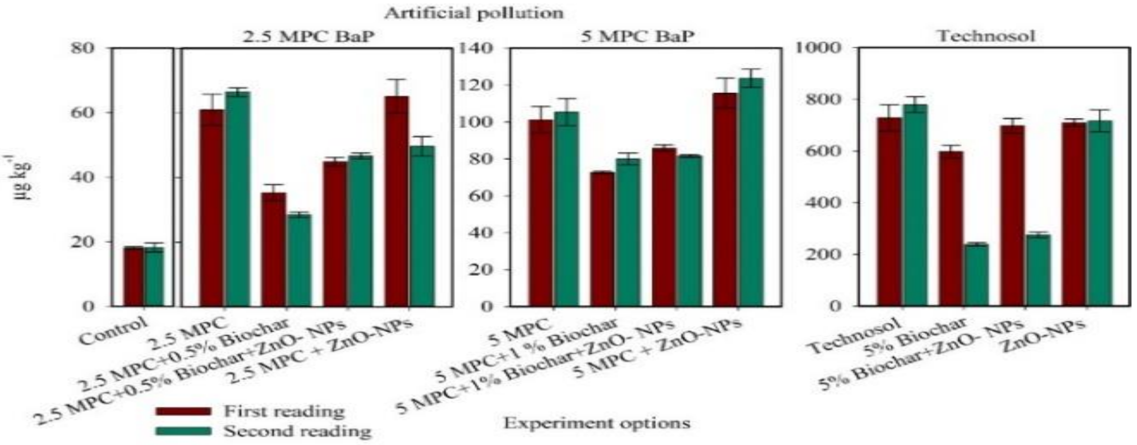

Figure 4.6.: BaP concentration in artificially polluted haplic chernozem and chemically polluted technosol in response to biochar and ZnO-NPs amendments.

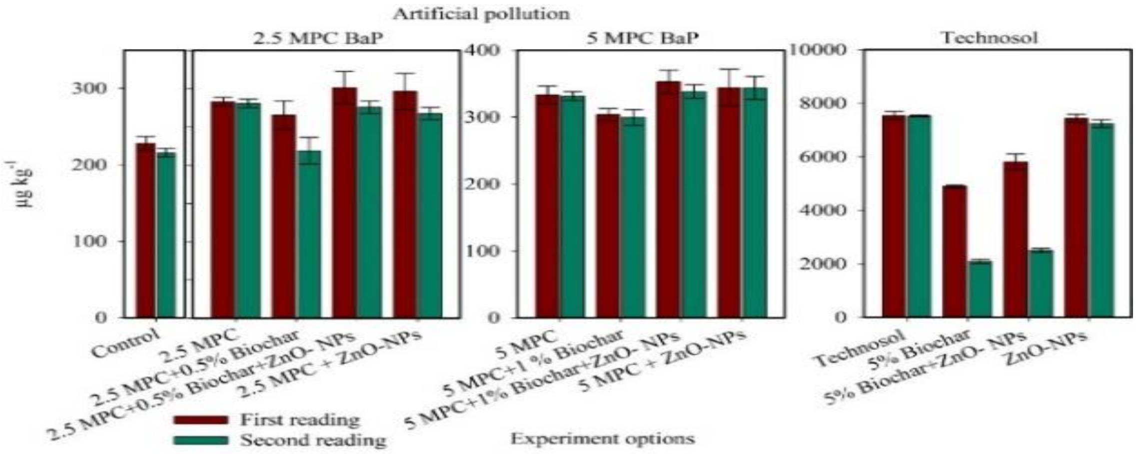

Figure 4.7.: Total PAHs concentration in artificially polluted haplic chernozem and chemically polluted technosol in response to biochar and ZnO-NPs amendments.

4.2. Effect of Biochar and ZnO-NPs on PAHs Accumulation in Roots and Shoots of Spring Barley

Concentrations of total PAHs and BaP in stem and roots of spring barley in the experiment variants are represented in figure (7). The results showed that PAHs content in the roots ranged from 72.5 to 67.8 μg kg^{-1} while 21.7 to 20.5 μg kg^{-1} in the stem of control plants. And that 1% biochar was the most efficient in reducing the bioavailable PAHs followed by combined 0.5% biochar with ZnO-NPs. However, ZnO-NPs and 0.5% biochar separate application significantly increased both total PAHs and BaP content in stem and root tissues compared with control plants. The explanation for this can be that the added biochar was an added source of PAHs to soil (Quilliam et al., 2013; SUSHKOVA et al., 2021), due to a sudden change in the pH led to desorption of PAHs from biochar surface (Bianco et al., 2021). While the improved biomass growth with ZnO-NPs may contribute to the increased PAHs concentration inside the plants. Similar outcomes were expressed by Rizwan et al., and Seleiman et al., (Seleiman et al., 2020), (Ali et al., 2019). On the other hand, in the artificial pollution with 2.5 MPC BaP, the treatment with 0.5% biochar reduced total PAHs content in root and stem. Furthermore, the combined application of ZnO-NPs and biochar significantly reduced BaP in the stem. Moreover, barley grown in the chemically polluted technosol, exhibited an excess of PAHs concentration in plant tissues, where the average concentration of total PAHs and BaP was 986 and 34.8 µg kg^{-1}, respectively in roots and 506 and 22 µg kg^{-1}, respectively in the stem. Out of the applied amendments, 5% biochar significantly diminished total PAHs concentration in stem and root by 47 and 59%, respectively. Proving the success of 5% biochar in reduction of bioavailable PAHs to plants in heavily PAHs polluted soil. Also in another study, 5% wood efficiently reduced PAHs in contaminated sediments, making it close to control (Bianco et al., 2021). Furthermore, the combined treatment reduced PAHs in stem and root by 45 and 35%, and ZnO-NPs foliar spray reduction was by 32.7 and 3.01%, respectively.

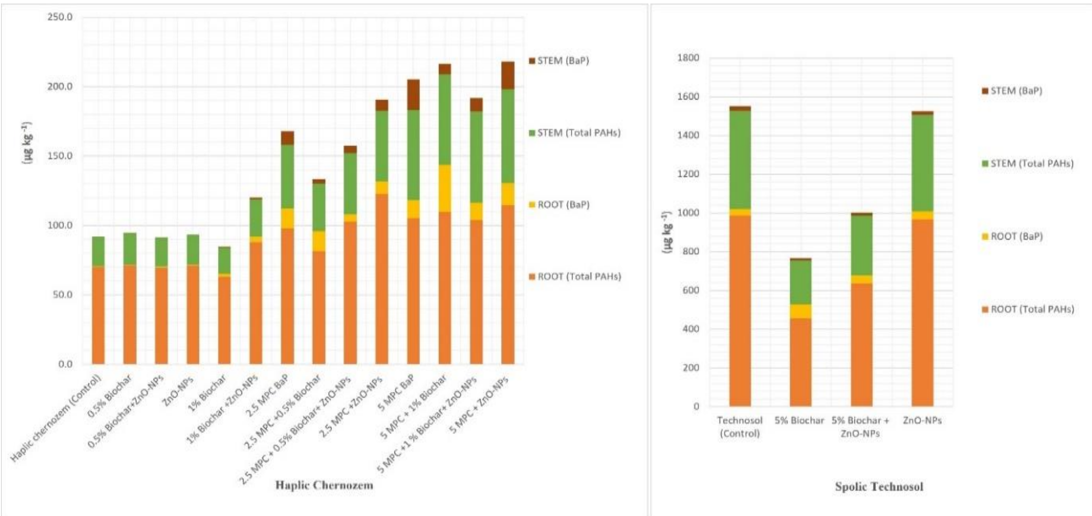

Figure 4.8.: Effect of biochar and ZnO-NPs application to PAHs concentration in root and stem of Spring barley.

4.3. Effect of Biochar and ZnO-NPs on the Bioaccumulation (BAFr) and Translocation (BAFr) of PAHs in Plants

A large sum of studies was dedicated to the explanation of PAH accumulation in plants through air-leaves systems, while very few thoroughly discussed the uptake from soil and their root-shoot transfer (soil-root-shoot system) (Tao et al., 2009). The bioaccumulation coefficient in control plants was 0.4 and 0.02 for total PAHs and BaP, respectively. Several reports indicated that spring barley is capable of accumulating considerable amounts of BaP in roots (Sushkova et al., 2018a). Also studies showed that as the concentration of PAHs in soil increases, it's followed by an increase in accumulated PAHs inside roots (Sushkova et al., 2020). In unpolluted chernozem, BAFr of total PAHs in barley tissues generally increased with the applied treatments. Whereas ZnO-NPs decreased BAFr of PAHs more effectively than biochar and their combined treatment. Yue et al., reported that, foliar spray of ZnO-NPs and SiO2 NPs reduced atmospheric deposition of PAHs in *Amanthrus tricolor* L (Cai et al., 2022). However, there were no found studies that explain the effect of ZnO-NPs on PAHs accumulation by roots. On the other hand, the artificial pollution with 2.5 and 5 MPC BaP elevated BAFr of BaP in barley roots, indicating phytoaccumulation of BaP in barley tissues with added BaP pollution to soil (Sushkova et al., 2017). But 1% biochar treatment lowered BAFr of total PAHs and BaP in barley tissues, whether single or combined with nanoparticles. Owing to their reduced bioavailability and removal from soil by biochar and the enhanced plant barrier mechanism to BaP and total PAHs with 0.5 and 1% biochar (SUSHKOVA et al., 2021). Total PAHs and BaP bioaccumulation in chemically polluted technosol were 1.13 and 0.1, respectively which is exceedingly high compared with unpolluted and artificially polluted Haplic chernozem. Where the high capacity of PAHs accumulation in spring barley shows the efficiency of their remediation capabilities. Similar PAHs bioaccumulation results were obtained in *Phragmites australis* found near the heavily polluted Atamanskoye lake (Southern Russia, Rostov Region) (Sushkova et al., 2020). Whereas 5 % biochar and combined treatment effectively reduced BAFr of total PAHs to 0.65 and 0.73, respectively. Similarly, 5% biochar was effective ($p<0.05$) in reducing the bioavailable PAHs for uptake in *Brassica rapa* L. (Khan et al., 2015). The representation of BAFr and BAFv values for total PAHs and BaP in spring barley are represented in figures (9,10).

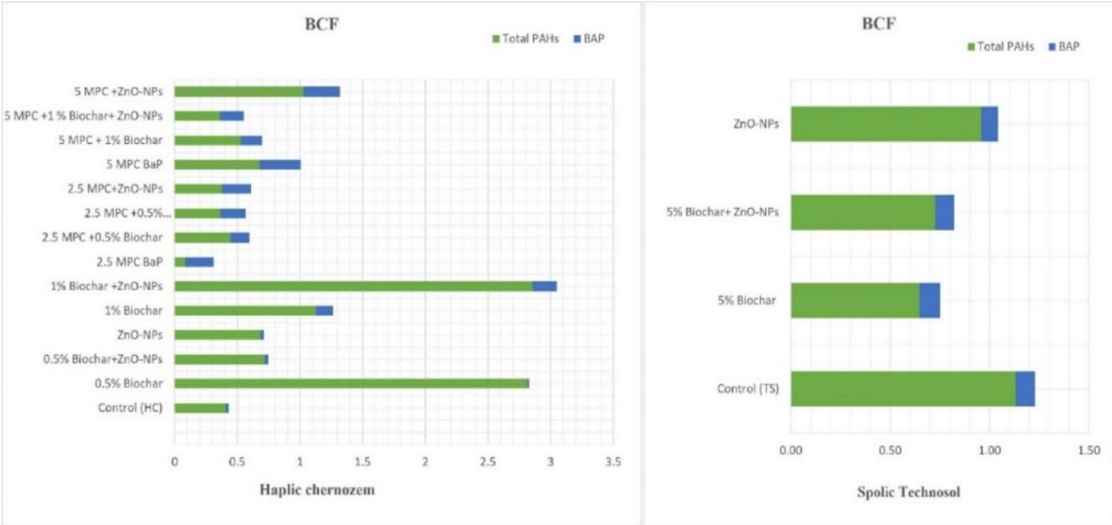

Figure 4.9: Effect of biochar and ZnO-NPs application on BAFr of total PAHs and BaP in barley tissues.

Additionally, the translocation coefficient of total PAHs and BaP for control plants in unpolluted haplic chernozem was 0.27 and 0.31, respectively. This indicates that PAHs concentration in shoots is correlated with their concentration in soil and roots (Tao et al., 2009). BAFv for total PAHs when compared with BAFv of BaP was elevated with biochar and ZnO-NPs treatment. Also, 0.5% biochar significantly increased the BAFv of 3-ringed PAH naphthalene to 0.67 when compared with control. Where it’s confirmed in various studies that LMW PAHs are more likely to be transported from root to stem compared with HMW PAHs (Kumari et al., 2021; Pullagurala et al., 2018). In artificial pollution of 2.5 MPC, BAFv of total PAHs and BaP was 0.51 and 0.68, respectively, while in 5 MPC was 0.57 and 0.65, respectively. This concludes that out of total PAHs in soil BaP concentration was the most dominant since it reported that BaP concentration in plants is correlated with its concentration in soil (Sushkova et al., 2018a). Moreover, the application of combined treatment of ZnO-NPs with biochar increased BAFv of total PAHs and BaP compared with other treatments. The exact reason for the enhanced BaP and total PAHs BAFv values with combined biochar and nanoparticles application isn’t previously reported. However, their combined application with other stressors confirmed a significant growth increase (Ali et al., 2019), so the increased plant biomass may attribute to more uptake of PAHs.

In chemically polluted technosol, there was no significant effect of added treatment on BAFv of PAHs. However, the results showed that BAFv of BaP is higher than Total PAHs in all barley plants growing under chemical pollution. And that 5% biochar showed a slight decrease in BAFv of Total PAHs compared with control. Studies on plants' uptake of BaP, reported that BaP concentration in roots is often less compared to soil (Sushkova et al., 2022) and that its translocation is controlled by transpiration rate (Pullagurala et al., 2018). However, the exact mechanism of uptake and translocation of PAHs and particularly BaP is still not clear and requires further investigation.

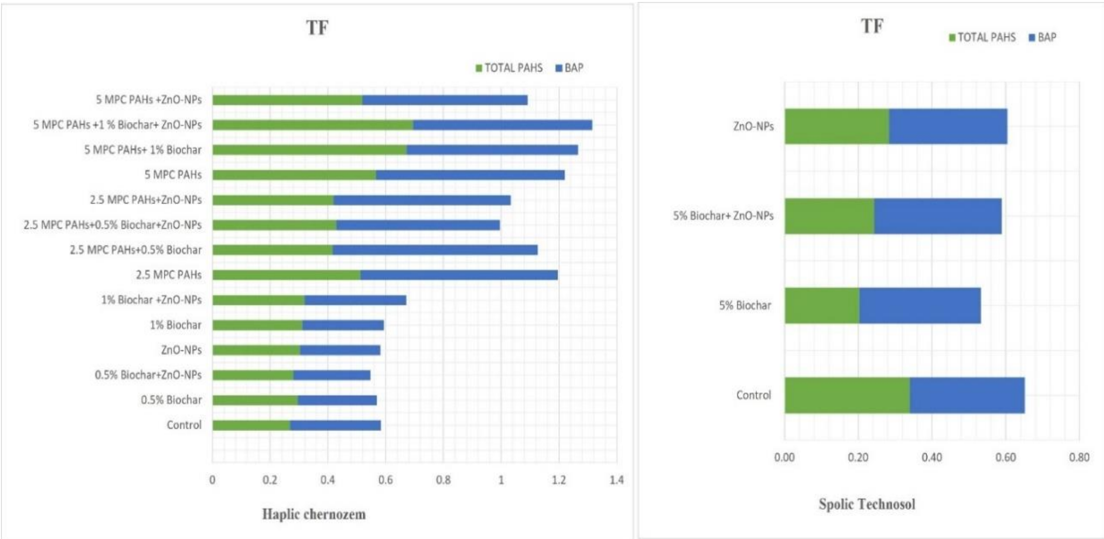

Figure 4.10.: Effect of biochar and ZnO-NPs application on BAFv of total PAHs and BaP in barley tissues.

4.4. Morphological Changes in Spring Barley

The effect of applied treatments and the level of pollution on the morphometric parameters of Spring barley according to the factor analysis of variance is represented in table (3).

Table 3.1. Effect of applied treatments and the level of pollution on the morphometric parameters of Spring barley according to the factor analysis of variance

Calculation block	Experiment options	Roots		Stems	
		F	p	F	p
1	Control	1.61	0.16	6.63	<0.00002
	0.5% Biochar				
	0.5% Biochar+100 NPs				
	NPS				
	1% Biochar				
	1% Biochar + NPs				
2	2.5 MPC PAHs	23.6	<0.000001	23.31	<0.000001
	2.5 MPC PAHs+0.5% Biochar				
	2.5 MPC PAHs+0.5% Biochar+ NPs				
	2.5 MPC PAHs+ NPs				
3	5 MPC PAHs	11.31	<0.000001	20.02	<0.000001
	5 MPC PAHs+ 1% Biochar				
	5 MPC PAHs +1 % Biochar+ NPs				
	5 MPC PAHs +200 NPs				
4	Control	12.09	<0.000001	21.14	<0.000001
	5% Biochar				
	5% Biochar+ NPs				
	NPs				

And the effect of applied treatments and pollution levels on the stem and root growth of barley are represented in figures (11,12). In the control variant, it was found that the median value of the length of the roots and stems of barley was 14.1 cm and 28.5 cm, respectively. However, the used biochar doses didn’t have a significant effect on the stem and root growth in unpolluted soil. Similar results were obtained by Nelissen et al. (2015), in a 2-year field experiment, the applied woody biochar didn’t affect spring barley yield (Nelissen et al., 2015). Furthermore, Kloss et al., reported that biochar application to mustard, barley, and red clover showed no variation in crop growth, except with biochars prepared at high pyrolysis temperature (Kloss et al., 2014). While the combined treatment of biochar with ZnO-NPs gave the highest root length when compared with other treatments in both unpolluted and polluted soils. Various studies furtherly confirmed the positive effect of combined biochar and ZnO-NPs on alleviating stress and increasing plant biomass (Guo et al., 2020),(Zhang et al., 2020),(Bashir et al., 2021). Additionally, with 0.5% biochar and ZnO-NPs treatment, root length ranged from 15-24 cm, and with 1% biochar and ZnO-NPs ranged from 17- 28 cm. And separate ZnO-NPs foliar spray increased stem, and root length to 36±0.5 and 27±0.3 cm, respectively. Moreover, 1% biochar addition to unpolluted soils enhanced stem and root growth more effectively than 0.5% biochar. The adsorptive properties of the sunflower husk used in this study align with the results obtained by Svetlana et al (SUSHKOVA et al., 2021). Five MPC BaP was more toxic to stem and root growth than 2.5 MPC even with the added treatment. These results were in line with induced BaP toxicity on barley growth by Aleksei et al. (2021) (Fedorenko et al., 2021). Although the combined treatment successfully mitigated the toxicity effect. Where 0.5% biochar and ZnO-NPs increased root and stem length by 35 and 27% and by 50 and 24 % with 1% biochar and ZnO-NPs. Since the foliar application supports the antioxidative response in plants in order to reduce stress growth reduction. Through the activation of various stress mitigating parameters inside the plants such as elevation of growth promoters, in addition to enzymatic and non-enzymatic antioxidative response antioxidant enzyme activities, and other enzymatic activities (Hong et al., 2021). In maize, the combined application of ZnO-NPs with biochar enhanced plant biomass, gas exchange rate, and plant height, while downsizing the concentration of H_2O_2 and MDA content in plant tissues under stress. Additionally, in rice growing under Cd stress, the combined treatment significantly reduced the contaminant concentration in plant tissue and enhanced on plant biomass, photosynthesis rate, and chlorophyll content (Ali et al., 2019). Moving on to long-term technogenic pollution (Technosol), the maximum stem and root length was 22.7 and 16.4 cm, respectively. The applied amendment to spolic technosol showed positive increase in stem and root growth. Where 5% biochar application enhanced root growth and the combined treatment of biochar with ZnO-NPs increased stem length significantly. Since the high levels of co-contamination by heavy metals in this soil forced roots to grow further into soil in order to escape the pollution on the surface

(Franco et al., 2011). However, ZnO NPs foliar application had less enhancement on stem and root growth, possibly due to induced toxicity on the plants by the dose of applied nanoparticles spray and the PAHs concentration in the soil.

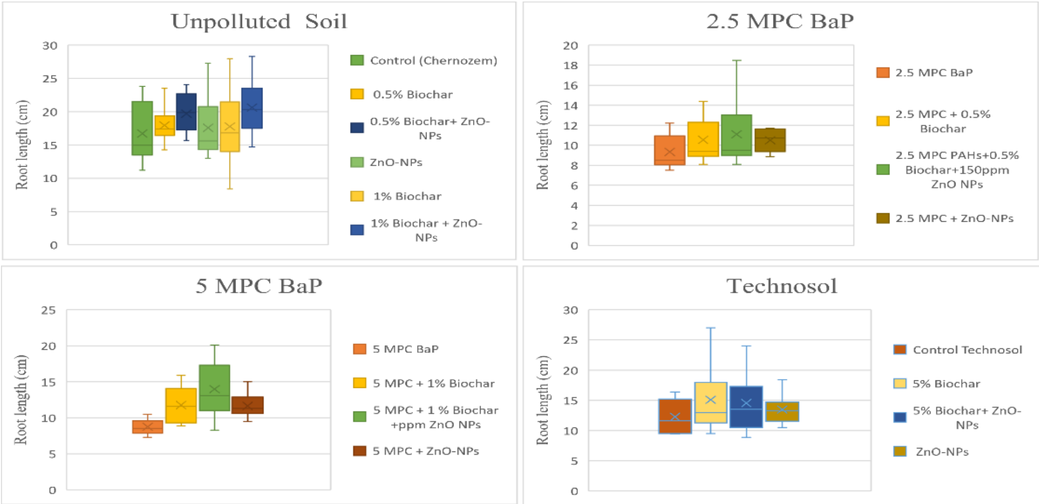

Figure 4.11.: Length of barley roots in different variants of the experiment

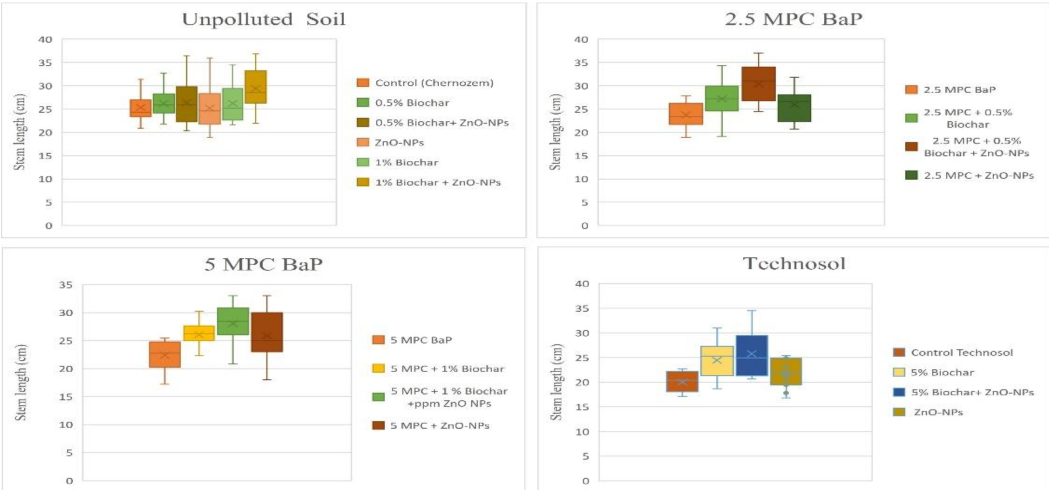

Figure 4.12.: Length of barley stem in different variants of the experiment.

4.5. Antioxidative Enzymes activity

SOD (Superoxide dismutase) and CAT (Catalase) concentration in different experiment variants (Table 4). The results showed that SOD and CAT concentration was 3.4, and 1.12 µmol mg^{-1} min^{-1} FW, respectively, in control plants of haplic chernozem. All applied amendments to unpolluted haplic chernozem increased SOD and CAT concentrations in plant tissues compared with control. On the other hand, 1% biochar had the lowest SOD and CAT increase in plant tissues. This proposes that biochar addition increased PAHs in soil and induced an antioxidant response in barley.

Similarly, SOD activity was increased up to 2 times compared with other enzymes in *Hordeum vulgare* L. with biochar amendment to soil (Krzyszczak et al., 2022). However, ZnO-NPs and their combination with biochar gave the highest SOD and CAT in the plant. Also, Dogaroglu et al., reported that ZnO-NPs application with 80 mg kg^{-1} concentration increased SOD and CAT activity in *Hordeum vulgare* L. and SOD concentration was more sensitive to ZnO nanoparticles concentration than CAT (Doğaroğlu & Köleli, 2017). Furthermore, Tahir et al., confirmed the increase in SOD and CAT activity with biochar treatment in plants under stress (Abbas et al., 2018).

Table 3.2. SOD (Superoxide dismutase) and CAT (Catalase) concentration in different experiment variants.

Experiment Variants	**SOD (μmol mg^{-1} min^{-1} FW)**	**CAT (μmol mg^{-1} min^{-1} FW)**
Control	3.36	1.12
0.5% Biochar	5.75	1.66
0.5% Biochar+ZnO-NPs	8.85	1.67
ZnO-NPs	5.38	2.65
1% Biochar	4.92	1.24
1% Biochar +ZnO-NPs	9.29	2.79
2.5 MPC PAHs	11.36	2.07
2.5 MPC PAHs+0.5% Biochar	4.21	1.81
2.5 MPC PAHs+0.5% Biochar+ZnO-NPs	3.45	2.15
2.5 MPC PAHs+ZnO-NPs	4.06	2.73
5 MPC PAHs	6.21	4.77
5 MPC PAHs+ 1% Biochar	6.65	3.92
5 MPC PAHs +1 % Biochar+ ZnO-NPs	6.38	1.37
5 MPC PAHs +ZnO-NPs	5.39	1.78
Control	5.07	3.19
5% Biochar	1.61	1.18
5% Biochar+ ZnO-NPs	3.40	0.43
ZnO-NPs	3.58	0.03

Furthermore, 2.5 MPC BaP had a more significant effect on SOD activity than on CAT however the opposite happened under 5 MPC. This indicates that SOD is more sensitive to PAHs pollution than CAT. These results were supported by the previous study on *Bruguiera gymnorrhiza*, which reported the positive correlation of SOD content with the level of stress (H. Song et al., 2012). Also, the combined treatment of biochar with ZnO-NPs to artificially polluted soil effectively alleviated stress symptoms in plants. In chemically polluted technosol, SOD and CAT concentration in untreated variants was 5.1 and 3.2 µmol mg^{-1} min^{-1} FW, respectively. Whereas 5% biochar treatment significantly reduced SOD and CAT to 1.6 and 1.2 µmol mg^{-1} min^{-1} FW, respectively. Similar results were indicated by the addition of biochar in bean seedlings under salinity stress (Farhangi-Abriz & Torabian, 2017). Diagrammatic representation of Superoxide dismutase (SOD), Catalase (CAT), and Malondialdehyde (MDA) in barley in the experiment variants (Figure 13,14). As for MDA, its concentration in the stem and root of control plants was 0.0388 and 0.0165 µmol g^{-1} FW, respectively, However, these concentrations are found to be higher than the normal range of MDA concentration in barley plants (Dawood et al., 2012). Out of the applied amendments to unpolluted chernozem, combined biochar (0.5%) with nanoparticles had the most significant effect on MDA concentration in both stem and root. The increase in MDA associated with ZnO-NPs application was previously reported by Abdel Latef AAH et al. (Abdel Latef et al., 2017). While in artificially polluted 2.5 MPC, 0.5% biochar addition significantly reduced MDA content in the roots from 0.0187 to 0.0058 µmol g^{-1} FW. Moreover, plants grown under chemical pollution didn't show a variation in MDA concentration when compared to control plants of unpolluted soil. However, ZnO-NPs application diminished MDA content in roots to 0.0004 µmol g^{-1} FW.

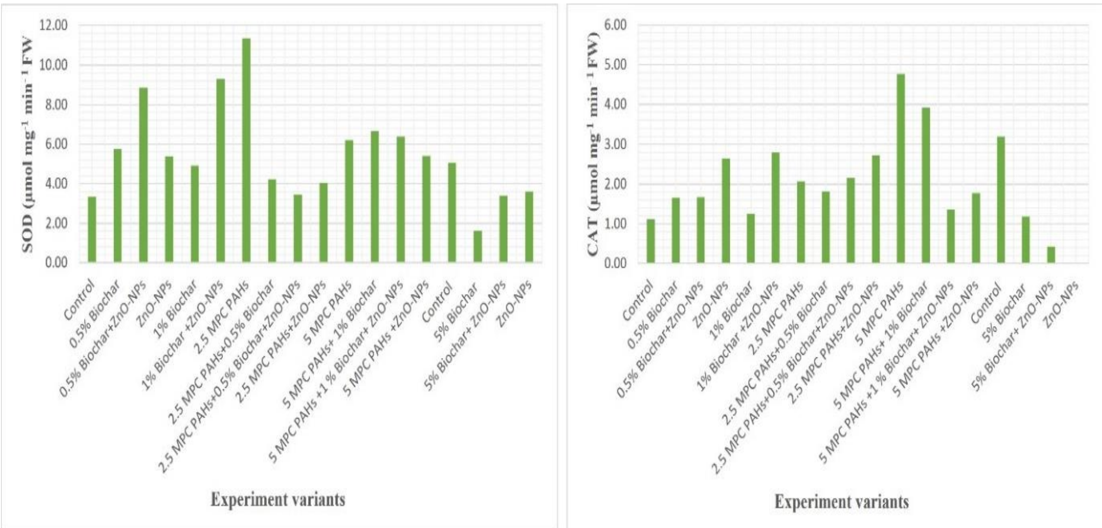

Figure 4.13.: Superoxide dismutase (SOD) and Catalase (CAT) concentration in barley in response to experiment variants.

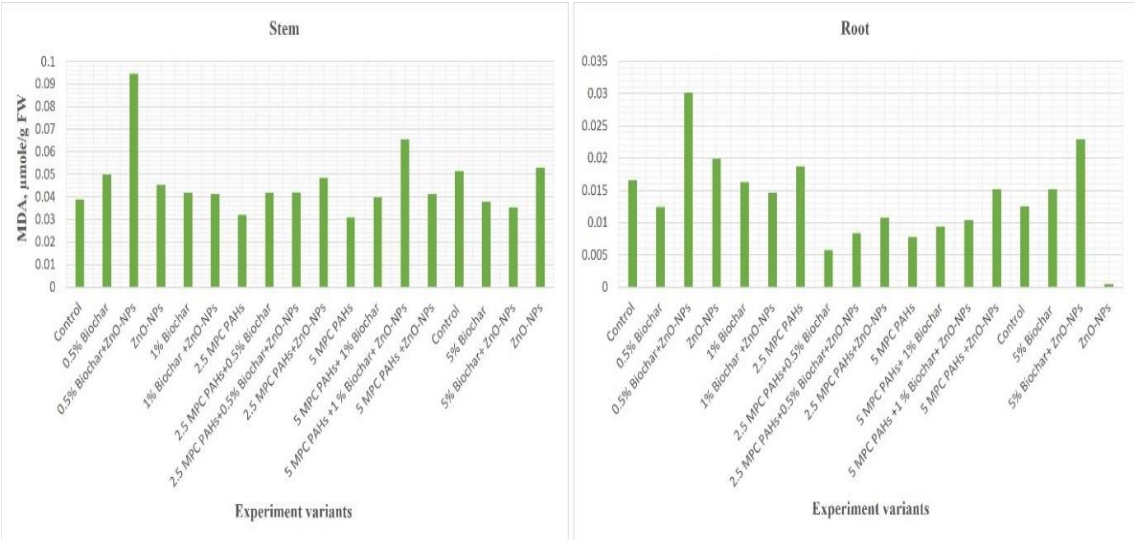

Figure 4.14.: Malondialdehyde (MDA) concentration in stem and root of barley in response to experiment variants.

4.6. Chlorophyll- a Fluorescence Response

Generally, changes in the maximum quantum yield Fv/Fm and quantum yield efficiency of photosystem II (YII) in response to PAHs pollution and applied treatments varied significantly. In the first survey of photosynthesis response, in 14 days old spring barley plants, the maximum quantum yield (Fv / Fm) of control plants was 0.87, within the normal range for higher plants (Maxwell & Johnson, 2000). Moreover, the results showed that, in unpolluted soil 0.5% biochar application didn't have a significant effect on Fv/Fm values. While 1% biochar revitalized the maximum quantum yield (Fv / Fm) to be almost similar to control plants. However, ZnO-NPs and their combination with biochar exhibited a sharp decrease in Fv/Fm to 0.72 and 0.73. Additionally, the artificial contamination with BaP significantly decreased Fv/ Fm in barley when compared to control plants. As a result of the blockage of the reaction centers of photosystem II by PAHs and inhibition of electron movement from PSII to PSI (Hasanuzzaman et al., 2019; Hou et al., 2017). This alteration in photosystem kinetics was furtherly increased with ZnO-NPs treatment. Hence ZnO-NPs were proposed to have a toxic effect on the maximum quantum yield of PSII in concentrations above 100 mg L^{-1} as previously reported by Singh et al (Singh et al., 2018). Further along, their systemic reduction for Fv/Fm in *Vicia faba* was reported at 200 and 300 mg L^{-1} ZnO-NPs concentrations as a function of time after they enter the leaves (Pedruzzi et al., 2020). Also, spraying with 400 mg L^{-1} ZnO-NPs solution increased the non-photochemical quenching (NPQ) and stimulated cyclic electron flow around PSI in *Pisum sativum* L. (Elshoky et al., 2021). These results propose the possibility of shading effect by ZnO-NPs to the available light for photosynthesis (Aruoja et al., 2009). Moreover, biochar treatment of artificially polluted soils contributed to the maximum quantum yield only in variants without nanoparticle treatment to values of 0.85 and 0.83 at 2.5 MPC and 5 MPC BaP in the soil. Haider et al. reported that biochar addition maintained the osmotic and water potential in maize under abiotic stress as well as increased the electron transport chain (ETR) and Y(II) (Haider et al., 2015). A similar trend was observed for plants grown in Spolic Technosol, however, there has been no significant increase in Fv/Fm in spring barley with biochar application to the soil. while in the second survey, there was a notable decrease in Fv/Fm. With the lowest value of Fv/Fm (0.52) in plants treated with ZnO-NPs. Possibly due to magnified stress on plants with PAHs in soil and the high concentration of ZnO-NPs in leaves. The maximum and effective quantum yield of photosystem II in spring barley of various two measurement periods in different experiment variants (Figure 15,16).

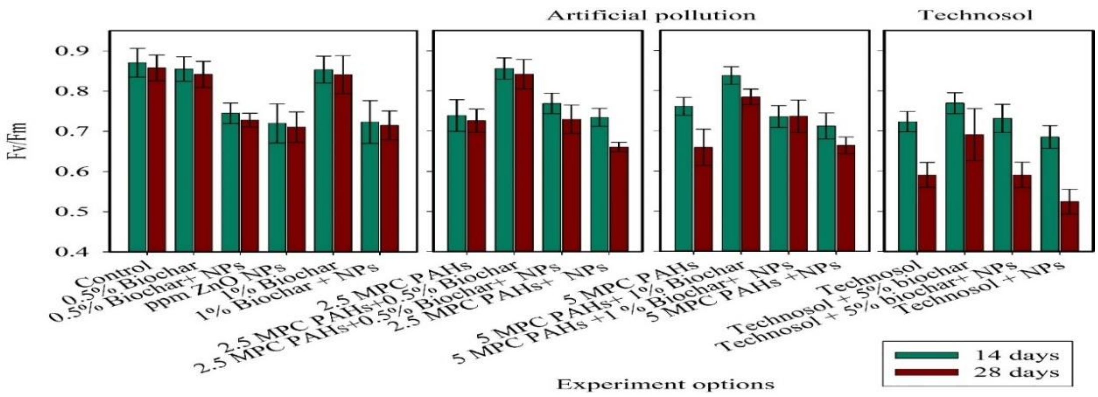

Figure 4.15.: Maximum quantum yield of photosystem II (Fv/Fm) in spring barley over two measurement periods in different experiment variants.

It's proposed that the reduction in Y(II) values was due to either the photosynthetic pigments degradation or inhibition of enzymes involved in their biosynthesis (NADPH-protochlorophyllide-oxidoreductase) by PAHs pollution (Oguntimehin et al., 2008). However, the quantum yield efficiency of photosystem II (YII) was less sensitive in assessing the stress-caused contamination in barley. Moreover, biochar was successful in mitigation of PAHs induced toxicity on photosynthesis and increased Y(II) values in addition to reducing toxicity effects of ZnO-NPs on Y(II). Noticeable differences in the light (Fv/Fm) and dark (YII) adaptations of barley were observed in the second measurement period (28 days). Where the results indicated an increase in Y(II) in the second measurement period compared with Fv/Fm, except in plants growing on chemically polluted technosol and treated with ZnO-NPs. Similarly, biochar addition to contaminated soil rejuvenated Y (II) close to that of control plants.

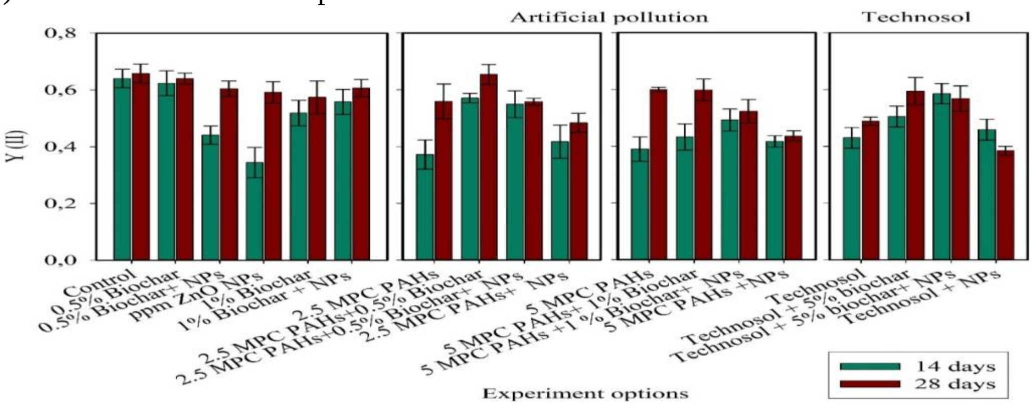

Figure 4.16.: Effective quantum yield of photosystem II (Y II) in spring barley over two measurement periods in different experiment variant

5. CONCLUSION

The results showed that biochar application (0.5%) reduced the concentration and bioaccumulation (BAFr) and translocation (BAFv) factor of total PAHs and BaP in unpolluted and artificially polluted haplic chernozem. While the separate application of biochar (5%) or combined with ZnO-NPs reduced 50% of total PAHs and BaP in chemically polluted Technosol. Moreover, in unpolluted soil, 1% biochar was more efficient in reducing PAHs concentration in the stem and roots of barley while 0.5 % biochar was more effective in artificially polluted haplic chernozem. Additionally, in all the experiment variants, BAFr of total PAHs was higher than BaP while vice versa for BAFv. Furthermore, 1% biochar with ZnO- NPs increased the growth of both roots and stem of barley, in unpolluted and artificially polluted Haplic Chernozem more significantly than in other treatments. While 5% biochar with ZnO was most effective in enhancing barely growth in chemically polluted Technosol. Furthermore, the quantification of antioxidative enzymes indicated that, superoxide dismutase (SOD) was more sensitive to PAHs contamination than catalase (CAT). Also, application of ZnO-NPs either separate or combined with biochar (0.5 %) reduced both SOD and CAT in plant tissues under 2.5 BaP pollution. Additionally, the high levels of MDA found in unpolluted Haplic chernozem were accompanied by a similar increase in SOD and CAT in plant tissues. Additionally, Maximum yield (Fv/Fm) and effective yield Y (II) of PSII were sensitive to PAHs increase as well as the treatments applied. Since all applied biochar doses enhanced the yield, while ZnO-NPs induced a significant reduction in both values. Finally, it was noted that there are no studies on the combined effect of biochar and foliar spray of ZnO-NPs on plants under PAHs pollution. Hence, this is the first study to combine and evaluate their role in mitigating PAH toxicity on *Hordeum sativum* L. under artificial and chemical pollution.

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3. The technogenic factor of PAH accumulation in floodplain soils of the Don River Delta
4. Impact of soil-applied biochar and foliar application of ZnO NPs on plant growth in PAHs contaminated soils

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