



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

**SYNERGISTIC EFFECTS OF FOLIAR APPLICATION OF
ZINC OXIDE AND COPPER OXIDE NANOPARTICLES IN
BARLEY GROWN IN METAL POLLUTED SOIL**

Master's Thesis

Hazrat AMIN

Supervisor

Assis. Prof. Dr. Vishnu D. RAJPUT

II. Supervisor

Prof. Dr. Orhan DENGİZ

SAMSUN

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SAMSUN

2022

ACCEPTANCE AND APPROVAL OF THESIS

The study entitled " **SYNERGISTIC EFFECTS OF FOLIAR APPLICATION OF ZINC OXIDE AND COPPER OXIDE NANOPARTICLES IN BARLEY GROWN IN METAL POLLUTED SOIL**, and supervised by **Assis. Prof. Dr. Vishnu D. RAJPUT and Prof. Dr. Orhan DENGİZ**, was found successful and unanimously accepted by committee members as Master thesis, following the examination on the date 6/7/ 2022.

	Title Name SURNAME University Department	Signature	Final Decision
Chairman	Assis. Prof. Dr. Vishnu D. RAJPUT Southern Federal University Department of Soil Science and Land Evaluation		☒ Accept ☐ Reject
Member	Prof. Dr. Orhan DENGİZ Ondokuz Mayıs University Department of Soil Science and Plant Nutrition		☒ Accept ☐ Reject
Members	Prof. Dr. Coşkun GÜLSER Ondokuz Mayıs University Department of Soil Science and Plant Nutrition		☒ Accept ☐ Reject
Members	Prof. Dr. Rıdvan KIZILKAYA Ondokuz Mayıs University Department of Soil Science and Plant Nutrition		☒ Accept ☐ Reject
Members	Assoc. Prof. Dr. Svetlana SUSHKOVA Southern Federal University Department of Soil Science and Land Resources Evaluation		☒ Accept ☐ Reject

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

APPROVAL

... / ... / ...

Prof. Dr. Ali BOLAT

Head of Institute of Graduate Studies

DECLARATION OF COMPLIANCE WITH SCIENTIFIC ETHIC

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

Is Ethics Committee Necessary?

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

No ☒

06/07/2022

Hazrat AMIN

DECLARATION OF THE THESIS STUDY ORIGINALITY REPORT

Thesis Title: SYNERGISTIC EFFECTS OF FOLIAR APPLICATION OF ZINC OXIDE AND COPPER OXIDE NANOPARTICLES IN BARLEY GROWN IN METAL POLLUTED SOIL

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ÖZET

METAL KİRLENMİŞ TOPRAKTA YETİŞTİRİLEN ARPADA ÇİNKO OKSİT VE BAKIR OKSİT NANOPARTİKÜLLERİNİN YAPRAK DIŞI UYGULAMASININ SENTETİK ETKİLERİ Tez Yazarının

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Toprak Bilimi ve Bitki Besleme Bölümü

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Danışman II: Prof. Dr. Orhan DENGİZ

Gıda güvenliği ve sürdürülebilir tarım, zamanımızın en acil konularından ikisidir. Dünya nüfusu arttıkça, gıda güvenliğini sağlamak için tarımsal üretimi sürdürülebilir bir şekilde geliştirmek için daha fazla çaba sarf etmek gerekmektedir. Ağır metallerin, bitki büyümesi ve gıda ürünlerinde birikme üzerinde zararlı inhibitör etkileri olduğu gösterilmiştir. Bitki büyümesine çok sayıda nanopartikül (NP) yardımcı olmuştur, ancak stres koşulları, özellikle metal kirliliği daha az dikkat çekmiştir. Sonuç olarak, bu deneydeki bitkiler, yani arpa, ağır metal kirli topraklarda yetiştirildi. Arpa bitkisi, ZnO ve CuO NP'lerin yaprak spreyi ile muamele edildi ve ZnO + CuO'yu birleştirdi. Bu çalışmanın amacı, Cu, Cd, Ni, Mn, Pb, Zn gibi metal kirli topraklarda yetiştirilen Cupper oksit ve Çinko oksit NP'lerinin yaprak uygulamalarının arpa bitkileri üzerindeki sinerjik etkilerini incelemektir. Cu ve Zn'nin toprağa girişi, gevşek bağlı bileşiklerde bir artışa ve bitkilerde, özellikle köklerde daha fazla birikmeye neden olur. Arpa bitkisine 150 mg/L dozunda CuO ve ZnO'nun NP spreyi uygulandı. Sonuç analizimiz, kirlenmemiş toprakta yetişen bitkilerin yaprak muamelelerinin, arpa büyümesini % 13-18 oranında uyardığını göstermiştir. CuO NP'lerin 30 arka planda kullanılması, kök ve sürgün büyümesini % 45 ve % 82 ZnO NP'leri % 30 ve % 78 oranında artırır ve CuO NP'lerin ve ZnO NP'lerin ortak tanıtımı % 75 ve % 98 oranındadır. NP'lerin kombine uygulaması, bulguya göre metal stresi altında bitki büyümesini artırdı NP'lerin bu yaprak uygulamalarının ağır metallerin zararlı etkilerini en aza indirmek için uygun bir yol olabileceği sonucuna varmak için daha fazla araştırma ve teste ihtiyaç vardır. Sürdürülebilir tarım için nanoteknolojik tekniklerin eklenmesi bu deneyin sonuçlarından biri olacaktır.

Anahtar Kelimeler: Ağır metaller, Yaprak spreyi, CuO, ZnO, Nanopartiküller, Yazlık arpa.

ABSTRACT

SYNERGISTIC EFFECTS OF FOLIAR APPLICATION OF ZINC OXIDE AND COPPER OXIDE NANOPARTICLES IN BARLEY GROWN IN METAL POLLUTED SOIL

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Supervisor II: Assis. Prof. Dr. Orhan DENGİZ

Food security and sustainable agriculture are two of the most pressing issues of our time. As the world's population grows more effort is required to enhance agriculture production in a sustainable manner in order to assure food security. Heavy metals have been demonstrated to have harmful inhibitory effects on plant growth and accumulation in food crops. Plant growth has been aided by a huge number of nanoparticles (NPs) nevertheless, stress conditions, particularly metal pollution, have received less attention. As a result, the plants in this experiment, namely barley, were grown in heavy metal polluted soils. The barley plant was treated with foliar spray of ZnO and CuO NPs and combine ZnO+CuO. The purpose of this work is to examined the synergistic effects of foliar application of NPs of Cupper oxide and Zinc oxide on barley plants which was grown on metal polluted soil such as Cu, Cd, Ni, Mn, Pb, Zn. The entry of Cu and Zn into the soil leads to an increase in loosely bound compounds and further accumulation in plants, especially in the roots. NPs spray of CuO and ZnO at the dose of 150 mg/ L were applied to the barley plant. Our result analysis showed that foliar treatments of plants growing on uncontaminated soil stimulate the growth of barley by 13-18%. The used of CuO NPs on 30 background increase root and shoot growth by 45% and 82% ZnO NPs by 30% and 78% and the joint introduction of CuO NPs and ZnO NPs by 75% and 98%. The combined application of NPs boosted plant growth under metal stress according to the finding To infer that these foliar applications of NPs might be a viable way to minimize the harmful effects of heavy metals, more research and tests are needed. The addition of nanotechnological techniques for sustainable agriculture will be one of the outcomes of this experiment.

Keywords: Heavy metals, Foliar spray, CuO, ZnO, Nanoparticles, Spring barley

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Hazrat Amin

CONTENTS

ACCEPTANCE AND APPROVAL OF THESIS	III
DECLARATION OF COMPLIANCE WITH SCIENTIFIC ETHIC	IV
DECLARATION OF THE THESIS STUDY ORIGINALITY REPORT	IV
ÖZET.....	III
ABSTRACT	IV
ACKNOWLEDGMENTS	V
CONTENTS.....	VI
SYMBOLS AND ABBREVIATIONS.....	VII
FIGURES LEGENDS.....	VIII
TABLES LEGENDS.....	IX
1. INTRODUCTION	1
1.1. Objective of study	9
2.LITERATURE REVIEW.....	10
2.1. Heavy Metals Pollution	10
2.2. Nanomaterial types and their application as Foliar Spray	14
2.3. Types of Nanoparticles.....	14
2.4. Foliar Application of Nanoparticles	16
2.5. An Assessment of Foliar and Root its Uptake.....	17
2.6. ZnO NPs and Their Application in Agriculture	17
2.7. ZnO NPs Foliar Application and Translocation Path.....	18
2.8. Foliar Application of CuO Nanoparticles with Respect to Various Studies ...	21
2.9. Phytotoxicity Of Nanoparticles Based on CuO NPs On Crop Plants	23
3.MATERIALS AND METHODS	25
3.1. Characterization of the Used Soil, and its Physical and Chemical Properties	25
3.2. Procedure for the Incubation of Barley Seed	27
3.3. Plant Growth Condition and Experimental Setup	27
3.4. Preparation, Characterization, and Application of NPs.....	28
3.5. Heavy Metals Analysis and Morphometric Observation in Plant and Roots	
Materials.....	29
.....	30
3.6. Preparation of Samples for anatomical and Ultrastructural Observation.....	31
3.7. Measurement of Chlorophyll Fluorescence and Transpiration Rate.....	32
3.8. Heavy Metal Total Content and their Mobile Forms Determination in Soil...	32
3.9. Heavy Metal Analysis in Plant Tissue (Non-Destructive Method).....	33
4.RESULTS AND DISCUSSION	34
4.1. Content of Heavy Metals in Ordinary Calcareous Chernozem	34
4.2. Content of Heavy Metals in the Spring Barley	36
4.3. Elemental analysis of heavy metals by Non-destructive Method	37
4.4. Morphometric Parameter of Plants.....	39
4.5. Measurement of Chlorophyll Fluorescence Parameter	45
.....	47
4.6. Impact of Nanoparticles Improve Barley growth grown in Polluted soil	48
5.CONCLUSION.....	49
REFERENCES.....	50
CURRICULUM VITEA.....	63

SYMBOLS AND ABBREVIATIONS

NPs	: Nano Particles
TEM	:Transmission Electron Microscopy
CuO	: Copper oxide
ZnO	: Zinc Oxide
HMs	: Heavy Metals
MPL	: Maximum Permissible Levels
AAS	: Atomic absorption spectrophotometry
MDA	: Malondialdehyde
ROS	: Reactive Oxygen Species
LF	: Nanoparticles
TM	: Heavy metals
HC	: Loosely coupled compounds
KV	: Root hairs
MRL	: Maximum Residues Limit

FIGURES LEGENDS

Figure 2.1. Engineered nanoparticle classification and some agricultural applications...	15
Figure 3.1. Barley plants as shown in the picture were grown in Metal Polluted soil.....	25
Figure 3.2. Plants as shown after Nanoparticles spray.....	28
Figure 3.3. Zinc oxide and Copper oxide nanoparticles.....	29
Figure 3.4. Doing morphometric parameter of Plants.....	30
Figure 3.5. Process of Heavy metals determination in Plants and roots.....	30
Figure 3.6. Ultrastructure observation of roots and plants samples of barley plants.....	31
Figure 3.7. Chlorophyll fluorescence analysis of Barley Plants.....	32
Figure 4.1. The content of mobile metal compounds in ordinary carbonate chernozem of the model experiment, % of the total content.....	35
Figure 4.2. Optical microscopy image (top) of the measured area and XRF spectra (bottom) for the plant leaves grown on heavy metal polluted soil.....	38
Figure 4.3. Root length and height of spring barley plants at different levels of soil contamination with heavy metals.....	39
Figure 4.4. Root length and height of spring barley plants at different levels of soil contamination with heavy metals.....	40
Figure 4.5. Effect of CuO NPs on Morphometric Parameter of Barley Plants.....	43
Figure 4.6. Effect of ZnO NPs on Morphometric Parameter of Barley Plants.....	44
Figure 4.7. Comparison of Morphometric Parameter of Barley Plants without Foliar sprays of NPs.....	44
Figure 4.8. Comparison of Quantum yield Light adopted and dark-adapted barley plants with CuO NPs application.....	45
Figure 4.9. Comparison of Quantum yield Light adopted and dark-adapted barley plants with ZnO NPs application.....	45
Figure 4.10. Comparison of Quantum yield Light adopted and dark-adapted barley plants with CuO+ ZnO NPs application.....	46
Figure 4.11. Comparison of Quantum yield Light adopted and dark-adapted barley plants without NPs application.....	46
Figure 4.12. ZnO 1 = CuO NPs applied, ZnO 2 = Zn NPs applied, CdO1 = CuO NPs applied, CdO2 = ZnO NPs applied, CdO3: CuO + ZnO NPs applied, MnO1 = CuO NPs applied, MnO2 = ZnO NPs applied	47

TABLES LEGENDS

Table 3.1. Basic chemical and physical properties of ordinary carbonate chernozem from the Botanical Garden of Southern Federal University.....	25
Table 3.2. Doses of metals (macro and nanoforms) introduced into the chernozem of ordinary carbonate under model laboratory conditions, mg / kg.....	26
Table 4.1. Total content of metals in ordinary carbonate chernozem of the model experiment, mg/kg.....	34
Table 4.2. Content of mobile metal compounds in ordinary carbonate chernozem of the model experiment, mg/kg.....	34
Table 4.3. Accumulation of heavy metal in roots and shoots of <i>Hordeum vulgare</i> , mg/kg.....	36
Table 4.4. The EDX spectra elements, wt %.....	38
Table 4.5. Morphometric parameters of spring barley in case of artificial soil pollution and foliar treatment of LF.....	41
Table 4.6. Comparison of Morphometric Parameter of Barley Plants without Foliar sprays of NPs.....	42

1. INTRODUCTION

Growing population, climate variability, industrial emissions, and rising fuel and power demands have all put a strain on global food production and distribution. Around 2050, the population of the planet will have exceeded 10 billion, going to result in a 50 percent increased food demand, primarily in developing nations. Moreover, approximately 815 million people are undernourished, with an additional 2 billion people worldwide expected to be malnourished by 2050 (FAO, 2017). Furthermore, by 2050, energy and food requirements will have increased by more than 60 % from current levels in a sustainable manner (van Vuuren et al., 2015).

Soils contaminated with heavy metals have become a global problem. Excessive concentrations of heavy metals can have pronounced toxic effects on plant growth and development. The presence of (HMs) in air, soil, and water can lead to their bioaccumulation that affects the entire ecosystem and poses a threat to humans. Several high-impact metals such as Cd, Zn, Cu, and Cr in excess are considered hazardous metals. However, all HMs in high concentrations have strong toxic effects and are considered environmental pollutants. Plants growing on contaminated soils show a reduction in growth, performance, and yield. The impact of contaminated soils on the whole ecosystem depends directly on the particular forms (groups) of metal compounds in the soil (Gorovtsov Andrey, 2019,).

Heavy metal pollution of arable soils is a growing problem as it poses a serious threat to food safety, public health, the food chain, and the ecosystem. For in situ treatment of polluted soils, phytoremediation is reported by researchers as a preferred and cost-effective method. Phytoremediation of contaminated soils with cadmium, chromium, lead, nickel, and zinc has been improved by the introduction of nanoparticles (NPs) (Rajput, 2021.).

Heavy metals have also a negative impact on plants, causing low vegetative growth, leaf senescence, growth photosynthetic suppression, disturbed hydrologic cycle and nutritional integration, but also oxidative stress, which all lead to plant destruction. (Singh et al., 2016) Plants are using two different techniques for managing the negative impacts of HMs and protecting their organs: restricting HMs uptake and ability to focus through tolerance technique implementations, and limiting HMs concentration through threshold method applications (M Leung et al., 2020). Plants

generate HMs inside of their cells, making remediation possible. *Triticum aestivum* L. plants, e.g., were shown to inhibit HMs take-up, enabling plants to survive without gaining extremely high levels of HMs in plant biomass (Noman et al., 2021). In plenty of other research findings, NPs intervention was found to be effective in limiting reactive oxygen species (ROS) formulation and also Cr distribution into the plant during Cr stress by immobilizing it in the soil. Investigation of microbiologically fabricated Cu-NPs that oxidized and reduced absorption in plant organs while increasing nutrient uptake and biomass was also mentioned (V. D. Rajput, Minkina, Kumari, et al., 2021). In recent times, the use of Fe₂O₃-NPs demonstrated that they can also be used to alleviate increasing issues associated with Cd phytotoxicity in *Oryza sativa* L. (Ahmed et al., 2021).

Various HMs, including Cd, Zn, Cu, Mn, Ni, and Pb, are grouped as hazardous metals in excess. Moreover, all HMs have powerful toxic effects at high concentrations and are considered environmental pollutant (Ali et al., 2019). Multiple HMs, such as Zinc, Iron, and Copper and Cr (III), are key elements of metabolic procedures, which include enzymes and cytochromes that are intimately associated to the metabolic performance of various biota (Bhattacharya, 2022). When plants are raised in the vicinity of manufacturing or further industrialized zones, lots of pharmaceutical plants have remained exposed to accumulate various metals (e.g., Cd, Cr, As, Cu, Cd, Fe, and Pb). vegetables grown in greenhouses are also extra infected with heavy metals like Copper, Zinc, Manganese, lead, and Cadmium but not iron than wide spread field root vegetable, probably expected to a smaller amount of lighting (Rai et al., 2019a). Heavy metals (e.g., Fe, Cu, Cr, Pb, Ni, and Mn) are frequently found in sludge from distilleries and the chemical, electroplating, textile, and leather industries (Bose & Bhattacharyya, 2008). Cd, Cr, Cu, Ni, and Zn from electroplating effluent were found to have severe effects, including stunted growth, necrosis and chlorosis in leaves, and plant death (Okereafor et al., 2020). Based on total fractions in soil, 70–400 mg kg⁻¹ Zn, 60–125 mg kg⁻¹ Cu, 120–21,000 mg kg⁻¹ Fe, and 300–500 mg kg⁻¹ Mn would be considered toxic to plants. (Satpathy et al., 2014). Extremely high Zn (up to 98,000 mg kg⁻¹) and Cu (up to 303 mg kg⁻¹) concentrations have been reported in the sediments of Lake Atamanskoe (located in the Kamenskii district of Rostov oblast) and adjacent soils in Russia. (V. D. Rajput et al., 2018). Concentration beyond a certain limit usually has a negative impact on the

plant. Plants grown in polluted soils have lower growth, performance, and yield (Spring barley) (*Hordeum vulgare* L.) (Gorovtsov et al., 2019a). One of the most significant grain crops for human consumption has also been identified as an efficient HMs accumulator, with phytoremediation potential comparable to Indian mustard (*Brassica juncea*), a hyperaccumulator plant (Gorovtsov et al., 2019b).

Copper exists in the environment as metallic copper in its zero-valent state (Cu⁰), ionic copper (Cu⁺¹ or Cu⁺²), and engineered nanoparticles containing zero-valent copper (Cu NPs) and copper oxide (CuO) (Tatiana et al., 2021.) Copper has piqued the interest of scientists due to its role as an essential micronutrient in plant growth, as well as its participation in various physiological processes such as photosynthesis, respiration, hormone signalling, and as an antimicrobial agent (M. U. Hassan et al., 2020.). It has been revealed that 5–30 mg kg⁻¹ of Cu is considered an optimal level for plant growth; however, above that level, depending on the crop species, it becomes toxic (Wuana et al., 2011). Cu has a negative effect on plant growth at higher concentrations, as evidenced by root growth inhibition, interveinal foliar chlorosis, wilted leaves, necrotic leaf tips, and root abnormalities. (Yeh et al., 2000.)

Zn and Cu are two of the most troublesome metals, posing risks to both plant growth and human health (Okerefor et al., 2020.) Both elements are necessary for plant growth, and the normal Zn concentration for plant growth (aboveground tissues) is 66 g g⁻¹ (Mei et al., 2020.) A surplus of this concentration may cause functional problems in the plant. Phytotoxicity threshold concentrations in plant tissues range from 200–500 mg kg⁻¹ for Zn and from 20–30 mg kg⁻¹ for Cu (Hajar et al., 2014). Cu way of incorporating organic matter and minerals in the soil, potentially interfering with soil microbial activities and can range from 2 to 100 mg kg⁻¹ in unpolluted soils (Minkina et al., 2020). Cu is typically available in two oxidation states, Cu⁺¹ and Cu⁺², which allow it to function as a reducing or oxidizing agent in biochemical reactions while also making it potentially toxic. Zn in soil could be in the form of the water-soluble cation Zn⁺², an exchangeable form, or insoluble complexes and minerals. Zn is taken up by the plant roots in the form of Zn⁺² ions, both free and chelated with organic acids, and translocated through the xylem into the plant's aboveground part (Tetteh, 2018).

The engagement of metal-based NPs with various soil components is a complex concept that involves a variety of physicochemical and biological processes

that may have an impact on the environment Sorption onto soil constituents, runoff, and leaching leading to groundwater transit, breakdown, and degradation by a variety of biotic and abiotic processes are some of the different fates of NPs once released into the soil. (Y. Verma et al., 2022)

Metal nanoparticles have both positive and negative consequences on the environment. On the brighter side of environmental ecology, it has been claimed that metal nanoparticles can not only improve plant seed germination and growth but also alleviate the oxidative stress of the harsh condition on plants(Zhang et al., 2022)

Metal-based nanoparticles (NPs) such as zinc (Zn) copper (Cu), aluminum (Al), iron (Fe), silica (Si), gold (Au), and silver, as well as their oxides such as zinc oxide (ZnO), copper oxide (CuO), titanium dioxide (TiO₂), and cerium oxide (Ce₂O₃), have been synthesized for a variety of applications including environmental remediation, energy storage, catalytic reactions, Cu metal nanoparticles have long been used as antibacterial agents (Sharma et al., 2019). They can kill cells via a variety of mechanisms, including membrane damage, oxidative damage to DNA, synthesis of proteins suppression, and stoppage of numerous biochemical processes. Zn NPs have also antimicrobial properties, although to a lesser extent than Cu. For its bioactivity and low cytotoxicity, zinc is an excellent substitute for toxic elements. (Ashfaq et al., 2016). NPs are now widely used in agriculture to boost crop yields and keep crops disease-free. Plant defense systems are stimulated by NPs, which lessens the destruction caused by abiotic and biotic stresses. (Rajput et al., 2021a).

CuO-NPs can also improve bioactive compound content. Copper oxide nanoparticles at a low concentration (625 M) increased anthocyanins by 1.4-1.7 times in *Arabidopsis thaliana*, and MDA content increased by 26 times with increasing action time(N. Verma & Kumar, 2019). MDA levels in wheat root tip cells were increased by a 100 mg/L sample of CuO-NPs (43 nm)(Nair & Chung, 2017). These findings imply that copper oxide nanoparticles stimulate plant oxidative stress responses and attempt to regulate this change on their own to maintain homeostasis. Copper nanoparticles, in addition to antioxidant metabolites, can increase the content of other secondary metabolites in plants. In *Arabidopsis thaliana*, CuO-NPs (490 70 nm) (10 and 20 mg/L) increased the levels of phytohormones, scopolamine, jasmonate, and glucosinolate derivatives (Chavez Soria et al., 2019).

The production, use, and disposal of Cu NPs may have an impact on plant growth. Indeed, many studies have found that Cu NPs have negative effects. However, the uptake, transport, and accumulation of Cu NPs in plants have received less attention. According to, Cu NPs uptake is similar to Cu uptake in its ionic forms. Cu NPs are thus absorbed by root cells and converted into simpler forms, releasing Cu ions inside the root cells and causing toxicity at high levels. Cu NPs' primary route of entry into plants is through lateral roots, which are less suberized than primary roots (Tighe-Neira et al., 2022).

To cope with food security, nanotechnology is an important approach to improving the yield and optimum utilization of resources for sustainable agriculture by improving the growth and yield of plants. The application of metal oxide nanoparticles on polluted soil with heavy metals to measure the interaction of these two treatments to see their efficiency for use in agriculture (Anuj Ranjan, 2021). Nanotechnology, has the possibility to deliver practical response to a variety of agricultural problems. Nanoparticles (NPs) are extremely important in science because they can bridge the gap between bulk materials and atomic or molecular structures. Over the last two decades, a significant number of studies on nanotechnology have been conducted, with a focus on its numerous applications in agriculture. Nanomaterials, in particular, increase crop profitability by increasing the efficacy of agricultural inputs, promoting a regulated, site-specific supply of nutrients, and ensuring the least amount of Agri inputs are used. Nanotechnology's contribution to plant defense products has grown exponentially, resulting in increased crop production. As a result, the key issue for agricultural development is to allow plants to adapt to progressive climatic change factors as quickly as possible. In addition to reducing complexity, nanotechnology can coordinate agricultural growth management approaches as an alternative to traditional technologies (Akter et al., 2022). According to new research, nanotechnology has the potential to improve methods of farming by enhancing the utilization of farm inputs and providing solutions to agricultural and natural challenges. Crop productivity will be increased based on current ecological parameters, crop disease detection and management, and crop mineral uptake capacity from the soil (Shang et al., 2019). According to the Nano forum, nanotechnology is getting popular in agriculture. Nanotechnology will take over all fields of agricultural activity. As a result, research into the agricultural potential of nanotechnology has

received a lot of attention in recent years (Tun Nisa et al., 2022).

Plants are exposed to nanoparticles during their different life cycle stages which can reduce the seedling growth, blockage of nutrients supply, seed germination, due to the formation of (ROS) and aggregation of NPs. But on the other hand, there were also various positive effects of NPs on the growth of plants for instance boosting the growing and change of seedlings, seed germination, the biomass of nitrogen and protein, total sugar contents as well as advancements in micronutrients and photosynthetic activity effectiveness in many plants like tomato, wheat, cucumber spinach, and mung. Due to their normal Zn fertilizer properties, As a zinc fertilizer, ZnO NPs can be employed, however, their dietary advancement and potential toxicity are still unknown. This was also found that the application of ZnSO₄ and ZnO NPs enhanced the Zn content in wheat grains, but ZnO NPs were not detected within the grain (Hassan et al., 2022).

The evidence for Zn NPs uptake, transport, and accumulation varies according to the route of exposure, NP characteristics, and plant physiology (Faizan et al., 2020). Foliar applications were more effective than root applications for Zn NPs uptake in *O. sativa* in this context (Afzal et al., 2021). After foliar application, Zn NPs entered the leaves of *T. aestivum* through the stomata, accumulated in the apoplast, and eventually reached the mesophyll cells (Zhu et al., 2020). Evidence of Zn NP translocation is frequently associated with an increase in Zn in plant tissue, as seen in *H. sativum* leaves and *Phaseolus vulgaris* from root to shoot (V. D. Rajput, Minkina, Fedorenko, et al., 2021). radial Zn movement occurs concurrently with axial xylem transport. Zn accumulates in the vacuole of cells, which is associated with the central mechanism of heavy metal homeostasis, where differential expression of a gene encoding a tonoplast metal transporter may be related to Zn accumulation (da Cruz et al., 2019)

According to the method of Zn NPs exposure, its accumulation can be differentially altered in the rest of the plant, tending to decrease in plant organs not treated, as observed in roots of *S. Lycopersicon* by foliar application (Sun et al., 2020). Even though the number of studies investigating the impact of NPS on plants is increasing, the influence of the main groups of metal-based NPs is still incompletely understood. Zinc oxide nanoparticles (nano-ZnO) are extensively used in the nano industry (Jadoun et al., 2021). And over a couple of decades ago, an amount of nano-ZnO in soil was estimated to range between 3.1 mg/kg and 31 mg/kg (Blackwell et al.,

2007). In such a short period, the manufacturing of ZnO NPs increased from 550 to 33,400 tons (Keller et al., 2013).

Zinc nanoparticles have the potential to be used as nano fertilizers. Nano fertilization increases agricultural productivity while also providing resistance to abiotic stresses. Nano fertilizers have sparked a lot of interest in increasing crop production. The use of nano fertilizers increases growers' profit margins because it increases yield and product quality (Zulfiqar, 2019).

One possibility is that zinc oxide nanoparticles dissolve zinc ions and release them into the growth media, influencing plant metabolism. A high concentration of ZnO nanoparticles will hurt plants. ZnO nanoparticles at concentrations greater than 100 mg/L have been shown to generate large amounts of ROS and have toxic effects on *Arabidopsis thaliana*, ryegrass, desert plants such as Julie velvet, wheat, and buckwheat, causing negative effects on plants. This implies that zinc oxide nanoparticles can activate the plant's oxidative stress system (Wan et al., 2019).

Plants benefit from NP application inside the shape of a foliar spray in general, as foliar applications of NPs offer adequate nutrient components for plant growth, assist in the fight against illnesses or pathogenic organisms, rapidly regenerate nutrition in nutrient-deficient plants, as well as provide key components that are difficult to manage inside the roots, supplement components that are hard to obtain from deficient soil, and also provide necessary nutrients which play an important role in plant development (J. Hong et al., 2021b). “Foliar Vitamins and several elements can be obtained through application that are deficient in the soil (J. Hong, 2021,)”. In a field experiment to mitigate the effects of Cd on *Zea mays*, it was discovered that foliar spraying TiO₂ was more effective than root supplementation. Moreover, numerous studies have shown that foliar applications of NPs can reduce the interpretation of metal transporter genes, the control of key metabolic processes, the amount of metal managed to retain, the quantity of chlorophyll in plants, as well as the inhibition associated with metal stress. (Bidi et al., 2021).

In comparison to the control, foliar exposure to ZnO-NPs increased leaf chlorophyll content while reducing oxidative stress and enhancing the activities of superoxide dismutase and peroxidase inside the leaves. Similarly, foliar application of ZnO-NPs could be an efficient approach to enhance wheat development and

implementation while minimizing NP movement to certain other environmental matrices (Adrees et al., 2021a). Foliar-applied micronutrients are much more useful for field-level plant response and they're less toxic than soil-applied nutrients, which could also cause toxicity when the same microelements are added (Semida et al., 2021). Several crops, including sorghum, soybean, and rice, have recently obtained foliar nutrients such as zinc oxide nanoparticles (ZnO-NPs), bulk zinc oxide (ZnO-B), and zinc salts (Zn^{2+}); even so, the ZnO-NPs form significantly outperforms other forms in terms of plant growth, physiology, biochemical, antioxidative defense system, and efficiency under detrimental stressful conditions (Awad et al., 2021). Because of this context, this study has been designed to obtain an in-depth understanding of single and binary applications of CuO- and ZnO-NPs on crop plant development indices through foliar application. The current study's findings may be useful in demonstrating the processes of NPs for reducing the adverse impact of abiotic stresses to boost agricultural production as a feasible way of improving agriculture productivity. As a result, the model experiment was carried out in highly polluted soil to assess the effects of foliar application of NPs interactions to HMs toxicity on *H. Vulgare* L. growth, accumulation, and distribution of Zn, Mn, Cr, Cu, Pb, Ni, and Cd in various plant tissues.

1.1. Objective of study

Keeping the above background in mind the study is design with the below mentioned main objectives:

1. Experimental setup and plant growth
2. To study the morpho-physiological parameters of the *Hordeum vulgare* plants.
3. To observe the synergistic response of foliar application nanoparticles on barley grown in metal-polluted soil.
4. To study the combined effect of CuO and ZnO nanoparticles on the *Hordeum Vulgare* Plant.
5. To estimate the uptake and translocation of pollutants in *Hordeum vulgare*. Performing a model vegetation research on artificial soil contamination with micro-and nanoparticles CuO and ZnO.
6. To investigate the gross Heavy metal's content and mobile forms in the soil of model vegetation exposed to various levels of pollution.
7. Atomic Absorption Spectrophotometry was used to determine the concentration of heavy metals in plant organs.
8. Transmission Electron Microscopy (TEM) is used to study the structure of plant roots and leaves and nanoparticles.

2.LITERATURE REVIEW

2.1. Heavy Metals Pollution

Contamination with heavy metals has spread widely around the world, disrupting the atmosphere and posing serious health risks to humans. The core issues of this problem are widely regarded as the fast growth of urbanization, changes in land, and industrial growth, particularly in the developing world with extremely large populations such as India and China (Rai et al., 2019).

The soil contamination with various types of chemical compounds has been of increasing concern in recent years and among them heavy metals (HMs) are of remarkable environmental importance and biological importance. Since heavy metals are not degradable through chemical and microbiological degradation, whenever they enter the soil, they are tenacious and invasive. Trace elements including Cu and Zn are in high concentrations in the environment and may exert toxic effects. Cu and zinc are the most abundant soil contaminant in Russia's Rostov Region (Burachevskaya, et al., 2021).

Contamination of soil with heavy metals is one of the most harmful environmental issues caused by anthropogenic activities (Zhao et al., 2017). It is faced with a variety of environmental issues as a result of its increasing economic development, industrial growth, and rapid urbanization. As anthropogenic activities spread, such as industrial growth, toxic substances are emitted into the environment. These contaminants eventually accumulate in the land, especially in areas having undergone modernization (Su et al., 2022). Heavy metal pollution of arable soils is a growing problem as it poses a serious threat to food safety, public health, the food chain, and the ecosystem. For in situ treatment of polluted soils, phytoremediation is reported by researchers as a preferred and cost-effective method. Phytoremediation of soils contaminated with cadmium, chromium, lead, nickel, and zinc has been improved by the introduction of nanoparticles (NPs), as shown by several studies (Rajput, 2021.)

Heavy metals, for example, have poisoned soils to different degrees which in recent years has resulted in consistent industrial growth as well as numerous other anthropogenic factors. A collection of toxic elements emitted into the environment and over 10 million land locations are thought to be polluted by prioritized HMs around the world (Lwin et al., 2018). High levels of heavy metals absorption was

observed in soils close to metal mining and processing plants in Russia (V. Rajput et al., 2020). HMs are well-known designed for their poisonousness, environmental persistence, bioaccumulation, and bio/geo-revolution in ecological environments. Large amounts HMs in soil may have an impact on plant performance as well as the activity of the soil microbial community. Bodies of water could become contaminated as a consequence of HM leachate into groundwater, posing a threat to marine life and people's health. The study discovered a heavy metals levels are high in the tissues of native plants cultivated in contaminated mining soils (Minkina et al., 2020). Because of its relationship with people's health, HMs accumulation in crops is a global concern (Monday Onakpa et al., 2018) Prior research suggests HMs are also influenced development of plants by altering root development, shoot and root durations, the ultrastructure of subcellular structures, and changes in plant physiological condition (Wang et al., 2017).

Heavy metals stay in the environment for an extended period, accruing in ecosystem components. The accurate representation of the pollution problems in various areas necessarily requires information on the level of contamination, and also metal transportation, and the major soil properties related to adsorption and accumulation of pollutants. Metal spectroscopy determines the extent of environmental dangers presented by landscape pollution, and also the ability of their substances to move (Zamulina et al., 2022). Contamination with heavy metals caused by multiple industrial processes has sparked a significant impact on the environment Drilling, metal processing, municipal wastewater soil implementations, and some other human impacts have resulted in massive metal pollution of soil and water. Pollutants such as Cd, Cu, Pb, and Zn frequently exist simultaneously in polluted soil, and their movement and bioactivity are a global concern due to plant take-up and continued increase in people's exposure via food (Medyńska-Juraszek et al., 2020).

Cadmium (Cd) contamination is a serious problem because of its long-term environmental persistence and severe toxicity to plants, animals, and humans. The majority of chemically polluted area cleansing procedures are expensive and do not provide soil that is suitable for plant development. Thus, phytoremediation is becoming more popular as a low-cost method of cleaning up polluted environments. Utilizing plants that can accumulate toxins, followed by the removal of plant biomass from the ecosystem, is the basis of phytoremediation technology. The lengthy duration

of the remediation process and the adverse influence of contaminants on the crops in bioremediation often result in a small amount of plant biomass, which is one of the primary factors limiting the efficacy of this approach (Bocharnikova et al., 2020).

The necessity to sustain a world's growing population and the rising percentage of arable land contaminated with trace elements (TE) demand the development of safe crop cultivation practices. Proper land stewardship on these sites necessitates the adoption of such agricultural strategies that concurrently ensure the control of pollution connections, the production of safe biomass, and additional benefits for farmers (Neu et al., 2020). Heavy metals in the environment are mostly attributable to human activities and environmental contamination with heavy metals continues to damage soil quality and crop output (Gavrilescu, 2022).

Toxic heavy metal abundant supply in water and soil flows is now recognized as a serious environmental problem with multiple adverse effects on animals and plants. Phytoextraction is a biomonitoring method that is effective, green, and expensive even though dangerous heavy materials inside the contaminated ecosystem could be detoxed and managed to accumulate inside the plant. Hyperaccumulators exude transmembrane proteins, which bring and move heavy contaminants from soil to various parts of plants. Plants with hyperaccumulator genes can store quite harmful substances in their body tissue. The impact of bioremediation is determined by several factors, including soil properties (soil type and pH), organic matter in the soil, heavy metal type, rhizosphere nature, rhizosphere microflora characteristics, and so on (Yaashikaa et al., 2022).

Cd is a heavy metal and its absorption decreases plant output and quality. Cd enters the soil through fertilizers and industrial and domestic pollutants (Alengebaw et al., 2021). Cd is used to evaluate soil pollution's environmental danger. No technology exists to measure Cd bioavailability in soil. In this study, Cd bioavailability is assessed in soils with identical characteristics but different age durations using chemical extraction, DGT, and bioindicator techniques. Aging reduced soil Cd bioavailability and toxicity to barley. This was attributable to less ion-exchangeable Cd (P. Ma et al., 2022). Likewise, nickel (Ni) is another toxic heavy metal that enters the food chain; hence humans and animals accumulate more nickel compounds. Ni (II) is found in silver refinery, electroplating, zinc base casting, and battery effluents (Briffa et al., 2020). Nickel is a component of urease, but excessive doses might be

harmful. Soil–crop/vegetable systems are a classic example of abiotic-biotic interactions. Nickel causes lung, nasal, and bone cancer. Dermatitis is the most common Ni-related adverse effect (Ni itch). Acute Ni (II) poisoning causes several ill-health impacts like headache, dizziness, nausea, vomiting, chest discomfort, etc. (Hazhazi et al., 2016). Consumption of such plants and their product which have accumulated Ni up to hazardous level is reported as the main cause of oral nickel exposure (Genchi et al., 2020). Background values are 20 ng/m³ in the atmosphere, 100 mg/kg in soil, 10 mg/kg in plants, 10 g/L in freshwater, and 1 g/L in seawater. Human pollution may raise nickel levels (Tsadilas, 2019).

Zinc is the 23rd most naturally occurring element in the Earth's crust, and its concentration levels are increasing unnaturally (Wauna et al., 2011b). The majority of zinc is added during industrial processes such as mining, coal and waste combustion, and steel production (Gentile, 2010). Zinc is utilized in galvanization, paint, batteries, smelting, fertilizers and insecticides, coal burning, coloring, and polymer preservatives, and their effluent from these industries contains a significant level of Zn (Khalef et al., 2022).

Copper is a naturally occurring micronutrient that is essential for plant growth. It has numerous applications in metallurgy, electronics, automotive, fuel, transportation, and machinery. Cu is particularly harmful to plants, with critical toxicity values in plants ranging from 20 to 30 mg/kg dry biomass (Shang, Kamrul Hasan, et al., 2019). Agricultural sources of copper pollution include fertilizers, fungicidal sprays, and animal feces. Copper can be found in a variety of foods, including shellfish, liver, mushrooms, almonds, and chocolate. Any commodity, such as food, water, or drink, that is packaged in a copper container may get contaminated (Parmar & Singh Thakur, 2013). Copper accumulation in the lenticular nucleus of the brain has been related to neurotoxicity, popularly known as "Wilson's disease," and kidney failure (Zyna Gromadzka et al., 2020).

Manganese acts as an activator of an enzyme involved in the oxygen evolution process in photosynthesis. It is found in a variety of enzyme systems. It is also involved in oxidation-reduction reactions and electron transport systems. Certain metalloproteins contain it as a structural component (Schmidt & Husted, 2019). The quantity of manganese (Mn) in soils varies considerably depending on the parent material of the soil and the environment in which it exists. Some soils may contain up

to 3,000 ppm of Mn, but the majority of it is inaccessible to plants (Anwar et al., 2019). Mn is most commonly found as exchangeable Mn, Mn associated with organic matter, or Mn oxides. It's also found in ferro-magnesium silicate minerals. Microbial activity can oxidize Mn to less available forms, reducing crop availability. Most crops that are Mn deficient turn yellowish to olive green, but the pattern of symptoms varies by species (Schulte & Kelling, 2004). Because Mn is immobile in the plant, deficiency symptoms typically appear on new young growth but can occur throughout the plant. Wheat, barley- Yellow parallel streaks run the length of the upper leaves, but yellowing does not begin at the tip. Plants may grow softly and limberly, with colourless spots (Department of Agriculture and Food, 2018). Manganese sulphate and manganese chelate are the most commonly used Mn sources, and both are effective foliar treatments due to their soluble nature. Mn toxicity is characterized by distorted leaves and dark specks on the leaves (Millaleo et al., 2010.). As the severity of the toxic effects increases, leaf tissue begins to die at the leaf margins and spreads inward on the leaf. Interveinal chlorosis, similar to a deficiency, is a symptom of early-stage soybean growth. Plant tissue analysis can aid in the diagnosis of Mn toxicity. Concentrations greater than 300 ppm are considered toxic (Schjoerring et al., 2020.)

2.2. Nanomaterial types and their application as Foliar Spray

Foliar spraying of NPs is anticipated to be a new solution to addressing important agricultural invention concerns. NPs are utilized in agriculture for soil monitoring and preventing disease, as well as nutrition enhancers, controlled-release nano fertilizers, and nano fungicides (Seleiman, Almutairi, et al., 2020a). NPs could potentially be employed as nano emulsions To obtain the greatest results, fertilizers, herbicides, or genes are delivered and released to specified plant target locations. (F. Rossi et al., 2020). Understanding and adjusting the biological, physical, chemical, and photocatalytic characteristics of NPs in manufacturing applications is crucial for reducing or eliminating toxicity.

2.3. Types of Nanoparticles

Agricultural plants are exposed to a wide range of NPs, including both natural and synthetic NPs. predominantly produced by industry output, volcanic activity, and fossil fuel combustion (López et al., 2022). Organic NPs, such as liposomes and

biopolymers, Organic–inorganic hybrid NPs (metal–organic mixed with NPs) and carbon-based NPs (fullerenes and carbon nanotubes) are also available. Metal-based NPs, metal oxides, and inorganic non-metals are all examples of inorganic non-metals. are examples of inorganic NPs (Abdul Khalil et al., 2015). These synthesized Agricultural applications of NPs have been investigated. Inorganic NPs, for example, might be utilized as nano insecticides and nano fungicides to protect plants from disease and pests, whereas carbon-based NPs could be employed as coatings for slow-release nano fertilizers to boost plant biomass in agriculture (Agrawal et al., 2022a). The categorization and frequent applications of NPs are depicted in the diagram below. Physical and chemical, biophysical, and hybrid techniques are employed to manufacture NPs used in agriculture. Even though physiochemical synthesis is increasingly widely employed, their biological solutions are severely constrained owing to harmful chemical components. As a result, researchers began to investigate synthesis of NPs by green agriculture friendly way .

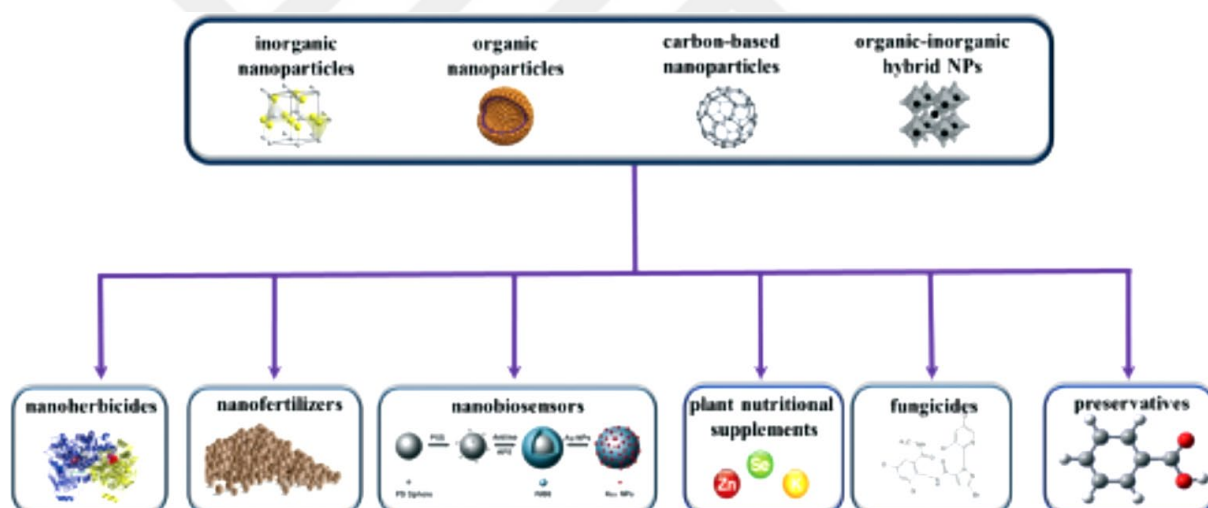


Figure 2.1. Engineered nanoparticle classification and some agricultural applications (J. Hong et al., 2021a).

Green synthesis makes use of renewable, environmentally acceptable solvents, renewable resources, and non-toxic ingredients to make NPs. Seed germination, photosynthetic pigment synthesis, and antioxidant enzyme activation are all greatly improved by green produced NPs (Gour & Jain, 2019). Chitosan is a non-toxic, recyclable, and biocompatible biopolymer that may be utilized as a nanocarrier for a variety of elements. Chitosan NPs can quickly bind to leaves, making them ideal for covering slow-release fertilizers and insecticides (Merzendorfer & Cohen, 2019). Its

structure is also suited for encapsulating metal ions, and chitosan NPs are reported to enhance the antibacterial efficiency of metal ions. The surface stability of big particles is protected by chitosan as a nanocoating, which decreases fertilizer breakdown and enables more effective nutrient uptake by plants. Arabic gum, a natural polymer nanoparticle (NP), can coat NPs and minimize their influence on crop health (Shang, Kamrul Hasan, et al., 2019b). Because of their durability and biodegradability, biopolymer–mineral nano fertilizers are excellent at reducing toxicity in agricultural crops (Shalaby et al., 2022).

2.4. Foliar Application of Nanoparticles

Weedkillers, enrichers, plant nutritional supplements, post-harvest additives, and biosensors are all popular uses for foliar-applied NPs in agriculture. Owing to limited plant use of soil-applied nutrients due to adsorption on soil compounds and rainfall, additional chemical fertilizer application is required, leading in eutrophication. Green ordinary supermolecules including as , kaolinite, mesoporous silica, and carboxyl methyl cellulose polymers have take to create slow-release nano fertilizers. The usage of EDTA-coated ferro ferric oxide (Fe_3O_4) NPs as a plant fertilizer is also discussed (Hong et al., 2021.). When compared to typical soil-applied fertilizers, foliar sprayed nano fertilizers offer the benefits of being swiftly absorbed by plants, being more cost-effective, and having less influence on soil health. Vitamins and other elements that are deficient in soils may be supplemented via foliar treatment. According to studies, slow-release nano fertilizers boosted plant absorption of nitrogen, phosphorus, and potassium. Mesoporous silica nanoparticles minimized the environmental effect of nano insecticides by preventing premature nutrient volatilization.

Foliar NPs may also supplement nutrients that are insufficient in crops, accomplish biofortification in a short amount of time, and alleviate crop shortages of certain essential elements (Thanka Mahil & Kumar Baburai Nagesh, 2019.). According to one research, foliar spraying of ZnO NPs enhanced zinc uptake in plants. According to research, nanoscale fertilizer foliage spraying may release metal ions that plants require at low concentrations while causing no significant harm Another study found that foliar application of fullerene NPs increases plant drought resistance by increasing water storage and providing protection by reducing drought-induced oxidative stress. (Seleiman, Almutairi, et al., 2020b). Antibacterial activity of foliar

NPs has also been shown in research. Because silver possesses antibacterial qualities, it may be employed as a primary component in bacteria-controlling sprays and detergents. Plant diseases are caused by fungi and molds. Ag NPs do exist. Fungicides are frequently utilized, and some are manufactured biologically. NPs (Natural Products) (for example, silver NPs synthesized by bacteria isolated from the environment) In the soil, antimicrobial activity is strong (Anand et al., 2022).

2.5. An Assessment of Foliar and Root its Uptake

Because leaves and roots have dissimilar physiological capabilities, The rate of NP absorption differs between various organs. Roots generate exudates that impede NP absorption after being sprayed, whereas leaves can isolate NPs within the cellular wall. Metallic NPs (such as Ag and ZnO NPs) cause varied levels of absorption and toxicity in plants exposed to them through their leaves or roots. Nutrients functional to the foliar layer are transported downward and distributed to various plant components. Similarly, spraying NPs on the leaves increased the amount of NPs in the root system, making it easier for root cells to absorb vitamins and enhancing disease resistance. Steel NPs, on the other hand, may not be taken to the roots via foliar spray or conveyed to plant shoots through root absorption according to studies (Khan et al., 2021). Stomata on plants absorb nutrients more quickly than root cells. As a result, rather than soil fertilization, foliar spray of nano fertilizers could quickly correct nutritional deficiencies in plants by absorbing the provided nutrient. Foliar application can also be utilized to supplement certain nutritional components required for plant growth while minimizing the impact on the soil environment (Alshaal & El-Ramady, 2017). Because soil-applied NPs react more slowly than NPs applied foliarly, the effects of root and foliar treatment methods for the same type of NPs may be diametrically opposed. Furthermore, because of environmental restrictions such soil adsorption, desorption, transport, and transformation they have varying bioavailability (Doolette et al., 2020)

2.6. ZnO NPs and Their Application in Agriculture

ZnO NPs are a semiconductive amphoteric material with a large band gap ($E_g = 3.37$ eV). These are widely used in a variety of applications due to their unique properties such as high binding energy, refractive index, thermal conductivity, piezoelectric nature, high absorbance of UV light, and antibacterial properties (Klingshirn et al., 2010). Furthermore, as an additional benefit, the aforementioned properties are highly tuneable. Their size can be varied from a few nanometres to the

upper limit of nanoparticle size definition (100 nm), and their shape can be easily modified by using the appropriate synthesis method. ZnO NPs have been synthesized using a variety of techniques, including mechanochemical processes, controlled precipitation, sol-gel, solvothermal, hydrothermal methods, emulsion and microemulsion methods, growing from a gas phase, pyrolysis spray methods, and others (Baig et al., 2021). ZnO NPs' surfaces are frequently modified to improve their stability in colloidal suspension, to improve their beneficial effects on plants, and to reduce their potential toxicity.

Surface modification can be achieved through treatment with inorganic compounds such as SiO₂, Al₂O₃, and others, simple organic compounds such as silanes or organic acids, and more complex polymeric matrices (R. Hong et al., 2006). Biosynthesis of ZnO NPs is frequently chosen for agricultural applications because it is expected to produce eco-friendly nanomaterial. Bare and surface-modified ZnO NPs were used in laboratory, greenhouse, and field experiments on crop plants due to their UV protective and antimicrobial properties, in addition to their nutritional role as a slow-releasing Zn source for plants. ZnO NPs dissolve easily in comparison to some other ENPs, such as TiO₂ NPs, which affect plant health in part due to their nano-specific properties, but also in large part due to the release of Zn, which is essential to many cellular processes (Kolenčík et al., 2020a). Furthermore, ZnO NPs have been shown to reduce the impact of environmental stresses on plants, such as drought, temperature, metals, metalloids, and salt. ZnO NPs, when applied at appropriate concentrations, increase plant seed germination, growth, antioxidant activity and protein production, chlorophyll content and photosynthesis, oil and seed production, and essential element uptake (Reddy Pullagurala et al., 2018).

2.7. ZnO NPs Foliar Application and Translocation Path

ENPs applied to leaves are mostly transferred to plants via stomata or, in rare cases, cuticle structures. In the case of ZnO NPs, the Zn may be slowly released and absorbed in its ionic species (Šebesta et al., 2021). They can be transferred to certain other parts of the plants depending on their size and dissolution. ZnO NPs were typically applied at two critical stages of plant growth. For foxtail millet, the first application is typically made during the leaf development stage, e.g., day 27 after sowing (Kolenčík et al., 2019). The second application is mainly performed during the stem elongation phase before flowering, such as day 53 for foxtail millet and day 80

for sunflower. Just one application at the stage of leaf growth was studied (Kolenčík et al., 2020b). Furthermore, the application of ZnO NPs at three different stages of plant growth during the growing season was studied. TiO₂ NPs, which are neither essential nor easily dissolvable, share many of the same positive effects on plants as ZnO NPs. To the best of our knowledge, and similarly to ZnO NPs, only a few studies with the application of TiO₂ NPs in field conditions have been performed, and all of them used foliar application as the preferred method to deliver the ENPs. At critical stages of plant growth, one or two applications were used. According to the available reports, the concentration-dependent efficacy of ZnO NPs was demonstrated, so different concentrations of ZnO NPs were used in the studies (Hajra et al., 2017.). In addition, it was observed that ZnO NPs can be highly effective even at a low concentration of 2.6 mg L⁻¹, increasing plant health and yields, including enhanced oil content in foxtail millet and sunflower, and weight of a thousand seeds, grain yields, and head diameter in sunflower. Three different concentration ranges of TiO₂ NPs were used to improve plants' growth and yield parameters and health (Koleník et al., 2019). Both positive and negative effects of TiO₂ NPs were observed at high concentrations of 1000 or 2000 mg L⁻¹. When applied 1000 mg L⁻¹ TiO₂ NPs to a grapevine, they observed photosynthesis inhibition.

However, they also discovered a higher content of K, Ca, Mg, and P in leaves treated with TiO₂ NPs (Teszlak et al., 2018). In a subsequent study, they discovered that TiO₂ NPs induced photocatalytic stress, which improved the antioxidant capacity and phenolic content in grapevine leaves, in addition to increased K, Mg, Ca, B, and Mn micronutrient levels in leaves. It has been proposed that the correct dosage of TiO₂ NPs may positively affect the upregulation of antioxidant defense, and that their photoactivity destroys pathogens on leaves (Kőrösi et al., 2019). Foliar application of ZnO NPs was found to be more effective than soil application in increasing the dry weight of shoots, photosynthetic rate, transpiration, and chlorophyll values in maize when compared to a conventional source of Zn at the same concentration, such as soluble ZnSO₄ or bulk (microparticulate) ZnO (Nemček et al., 2020.). ZnO NPs applied to the soil, on the other hand, improved the situation. cob weight in maize more than foliar application. Other yield parameters showed statistically insignificant differences between soil and foliar application. ZnO NPs penetrate and translocate Zn within plants similarly to dissolved ZnSO₄, and they are generally better translocated

through plants than their bulk counterparts, resulting in higher Zn concentrations in stem, leaves, and grains (Rossi et al., 2019). However, not all studies conclude that ZnO NPs are a better application technique than their bulk counterparts. Soil-applied ZnO NPs, for example, did not improve plant growth parameters when compared to bulk ZnO. ZnO NPs can sometimes outperform conventional dissolvable Zn forms. When these were applied to maize at lower concentrations of 400 Zn mg L⁻¹, compared to 2000 Zn mg L⁻¹ applied in the form of ZnSO₄, The NPs significantly increased plant height, leaf area, dry weight, grain yield, cob length, and the number of grains per row (García-Gómez et al., 2017).

Comparably, it was also found that when applied to peanut plants, only 133 mg Zn.L⁻¹ of ZnO NPs performed better than 2000 mg Zn.L⁻¹ of chelated Zn (García-López et al., 2019). ZnO NPs are used to supplement crops with the essential micronutrient Zn, primarily to boost growth and yields. Similarly, Zn supplementation can be transported in plants to edible parts such as leaves, seeds, fruit, and other edible parts. foliar application of ZnO NPs resulted in a 2-fold increase in zinc content in both peanut leaves and kernels compared to controls. The application of ZnO NPs at 100 mg Zn.L⁻¹ resulted in a 37% increase in Zn accumulation in grains in maize (Velasco et al., 2020.). Correspondingly, foliar application of ZnO NPs at 2000 mg Zn.L⁻¹ resulted in increased Zn concentrations in maize grains by up to 82 percent over control. When Zn was applied foliarly to wheat, the concentration in grains increased by approximately 59 percent when compared to the control (Kolenčík et al., 2022). In terms of enhancing yields, ZnO NPs may be used in soils contaminated with Cd because their foliar application reduced Cd uptake in grain and improved wheat grain quality. ZnO NPs were also used in conjunction with other NPs, Fe-based and Si-based NPs, and when combined, these ENPs had a positive synergistic effect on Cd stress reduction (Adrees et al., 2021b; Azarin et al., 2022).

Pollutants such as heavy metals (HMs) are hazardous substances that pollute water sources in poor nations due to excessive discharge from industrial zones (Akhtar et al., 2021a). Because rice (*Oryza sativa* L.) crops are submerged in water for prolonged periods of time, irrigation with HM-polluted water has harmful effects on plant development. The goal of this study was to see if bacteria (*Bacillus cereus* and *Lysinibacillus macroides*) and zinc oxide nanoparticles (ZnO NPs) (5, 10, 15, 20, and 25 mg/L) had a synergistic impact on rice cultivated in HMs polluted water. According

to recent research, bacteria, in combination with ZnO NPs at lower concentrations, were able to remove the most HMs from contaminated water at pH 8 (90 minutes) as compared to higher intensities. Seeds primed with bacteria cultivated in HM-polluted water containing ZnO NPs (5 mg/L) had lower HM absorption in the root, shoot, and leaf, resulting in higher plant development. Additionally, their combined impacts lowered the bioaccumulation index and metallothioneine (MTs) concentration while increasing plant tolerance. This research found that treating bacteria with lower quantities of ZnO NPs helped plants minimise heavy metal toxicity, particularly Pb and Cu, while also increasing plant growth. Due to fixation with soil components and precipitation with other ions, zinc (Zn) shortage is widespread in calcareous soils. ZnO nanoparticles (ZnO NPs) might be a superior source of zinc for maize grain development and biofortification (Umar et al., 2020).

ZnO nanoparticles were compared to standard Zn fertiliser (ZnSO₄) in pot and field studies to see how they affected maize growth and grain Zn content. Zn application increased plant growth and physiological parameters in both studies; however, the greatest benefit was shown in treatments that received ZnSO₄ (soluble) or ZnO nanoparticles (insoluble source of Zn). In a field experiment, the use of ZnO nanoparticles increased Zn content in both shoots and grains. Zn partitioning was computed in a field experiment, and it was discovered that Zn concentration at maturity stages was lowered in stem and mature leaves. In treatments with ZnO nanoparticles, the highest Zn content (80 mg kg⁻¹) was found in young leaves at the vegetative development stage. Foliar application of ZnO NPs resulted in the greatest rise in grain Zn content (82 percent). ZnO NPs increased maize growth, yield, and Zn content in the grain, according to the findings. It can be utilised for Zn biofortification since it is a less expensive and more efficient source.

2.8. Foliar Application of CuO Nanoparticles with Respect to Various Studies

CuO NPs were studied for their effects on mustard plant growth, physiological efficiency, biochemical assays, and antioxidant system. CuO NPs in various concentrations (0, 2, 4, 8, and 16 mg/L) were applied at 25 days after sowing (DAS), and samples were collected at 30 and 45 Days (Faraz et al., 2022). The results show that CuO NPs-treated plants grew and produced more biomass than their respective controls. Among the different CuO NP concentrations (0, 2, 4, 8, and 16 mg/L), 8 mg/L was found to be the most effective foliar spray treatment for increasing

chlorophyll content, net photosynthetic rate, leaf proline content, and antioxidant enzyme activity. They concluded that CuO NPs interact with meristematic cells, activating biochemical pathways that promote growth qualities. (Gaucin-Delgado et al., 2022) studied the use of metallic nanoparticles improves plant yield and content of bioactive compounds. The current study sought to ascertain the effects of foliar application of copper nanoparticles (CuO-NPs) on lettuce yield and bioactive compound content. lettuce was treated with various concentrations of CuO-NPs (0, 0.5, 1, 2, 4, and 6 mg mL⁻¹). The yield, nutraceutical quality, and enzymatic activity were all calculated. The foliar spraying of CuO-NPs increased the biosynthesis of bioactive compounds. There were no negative effects on yield despite an increase in the activity of the enzymes superoxide dismutase (SOD) and catalase (CAT) in lettuce plants. As a result of the increased production from the use of CuO-NPs, higher quality lettuces for human consumption are produced. (Xiong et al., 2021) conducted an experiment on lettuce For 5, 10, and 15 days, lettuce leaves (*Lactuca sativa* L. var. *ramosa* Hort.) were exposed to various concentrations of copper-oxide NPs (CuO-NPs, 0, 100, and 1000 mg L⁻¹) CuO-NPs were studied for their foliar uptake, subcellular distribution, chemical forms, and impact on nutrient status, antioxidant systems, and lettuce growth. Cu levels in lettuce leaves were significantly elevated (up to 6350 mg kg⁻¹), which was one order of magnitude higher than in the roots (up to 525 mg kg⁻¹). Cu translocation factors ranged from 1.80 to 15.6 percent from leaves to roots. The use of CuO-NPs severely hampered lettuce growth and altered plant nutrient status (especially Mn, K, and Ca).

Furthermore, CuO-NPs increased H₂O₂ production and malonaldehyde levels (on the 5th and 10th day of exposure), and catalase activity in lettuce leaves (on the 15th day of exposure). Cu concentrations in subcellular fractions were ranked in the following order: cell wall organelles > soluble fraction in lettuce leaves, and organelles > cell wall > soluble fraction in lettuce roots. Undissolved Cu forms were prevalent in lettuce, which may have contributed to the plant's reduced mobility and phytotoxicity. This study's findings will be of particular interest in areas with high levels of metal-NPs in the atmosphere. (Petrova et al., 2021) did research on two varieties of barley plant the varieties "Marthe" and "KWS Olof" of *Hordeum vulgare* L. were grown in hydroponics with magnetic iron oxide (Fe₃O₄) and copper oxide (CuO) NPs added at 17, 35, and 70 mg/L, respectively. Plant morphology, genotoxicity, and miR156a

expression were all investigated. Fe₃O₄ and CuO nanoparticles had different effects on barley varieties, with Fe₃O₄ nanoparticles increasing plant shoot and root lengths and fresh biomass while CuO nanoparticles decreased them. CuO NPs had a greater impact on the barley genome than Fe₃O₄ NPs. Thus, Fe₃O₄ NPs decreased genome stability to 72 percent in the "Marthe" variety and 76.34 percent in the "KWS Olof" variety, whereas CuO NPs decreased genome stability to 53.33 percent in the "Marthe" variety and 68.81 percent in the "KWS Olof" variety.

The levels of miR156a expression after Fe₃O₄ NPs treatment had no effect on the "Marthe" variety but increased in the "KWS Olof" variety, whereas CuO NPs treatment increased miRNA expression levels in the "Marthe" variety but decreased them in the "KWS Olof." Because NPs can influence miRNA expression and miRNAs can affect plant resistance.

2.9. Phytotoxicity Of Nanoparticles Based on CuO NPs On Crop Plants

The toxicity mechanisms of CuNPs have been extensively researched in animal/human systems. Cu and Cu ion toxicity in plants has been fully investigated, but not so Cu/CuO NPs phytotoxicity (Jampilek & Králová, 2022). Cucumber plants in hydroponic culture were exposed to two concentrations of CuNPs (10 and 20 mg L⁻¹) during their early development. CuNPs were found to interfere with the uptake of a variety of micro- and macronutrients, including Na, P, S, Mo, Zn, and Fe. CuNPs at both levels caused significant metabolic changes in cucumber leaves and root exudates, according to metabolomics data. The metabolic changes in root exudate revealed an active defense mechanism against CuNPs stress: up-regulation of amino acids to sequester/exclude Cu/CuNPs, down-regulation of citric acid to reduce Cu ion mobilization, up-regulation of ascorbic acid to combat reactive oxygen species, and up-regulation of phenolic compounds to improve antioxidant system. It also discovered a decrease in root length and a decrease in root diameter (L. Zhao et al., 2016). In the next study 18-day-old hydroponically grown lettuce (*L. sativa*) seedlings were treated for 15 days with core-shell Cu/CuO NPs at two concentrations (10 and 20 mg L⁻¹). The results showed that Cu²⁺ ions or Cu/ CuO NPs reduced water content, root length, and dry biomass and Cu bioaccumulation, primarily in roots.

The purpose of this study was to see how laboratory-prepared CuNPs in low concentrations (50 ppm) affected the germination of lettuce (*L. sativa*) seeds in a water medium. CuO NPs were found to be slightly more toxic than Cu²⁺ ions, with a

decrease in seed germination and root elongation was observed in the lettuce plants (Pelegrino et al., 2020). ICP-OES results revealed that Cu/CuO NPs treatments resulted in significant Cu accumulations in roots when compared to Cu²⁺ ions. All Cu treatments increased CAT activity while decreasing APX activity in roots. Furthermore, when compared to the control, nano-Cu/CuO altered the nutritional quality of the lettuce, with the treated plants having significantly more Cu, Al, and S but significantly less Mn, P, Ca, and Mg (Ogunkunle et al., 2018). (Atha et al., 2012) found that copper oxide nanoparticles caused DNA damage in agricultural and grassland plants in another study. Under controlled laboratory conditions, significant accumulation of oxidatively modified, mutagenic DNA lesions (7,8-dihydro-8-oxoguanine; 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 4,6-diamino-5-formamidopyrimidine) and strong plant growth inhibition were observed for radish (*R. sativus*), perennial ryegrass (*L. perenne*), and annual ryegrass.

The following study looked at the phytotoxicity and accumulation of copper oxide (CuO) NPs in *Elsholtzia splendens* (a Cu-tolerant plant) in hydroponic conditions. Cu content was much higher in shoots treated with 1000 mg L⁻¹ CuO NPs than in those exposed to comparable 0.5 mg L⁻¹ soluble Cu and CuO bulk particles. CuO NPs-like deposits were discovered in the root and leaf cells. Cu K-edge X-ray absorption near-edge structure analysis revealed that the accumulated Cu species existed in plant tissues primarily as CuO NPs. All of these findings suggested that CuO NPs in *E. splendens* can be absorbed by the roots and translocated to the shoots. In another study, the phytotoxicity of CuO NPs was assessed in two crop plants, maize (*Zea mays*) and as well as rice (*O. sativa*). CuO NPs had no effect on seed germination at any of the tested concentrations, according to the findings. However, at a concentration of 2000 mgL⁻¹, CuO NPs significantly inhibited root elongation (95.73 percent for maize and 97.28 percent for rice), and maize shoot length was reduced by 30.98 percent (Santiago et al., 2020).

3. MATERIALS AND METHODS

The Entity of The Research: The study was conducted on haplic chernozem soil, the experimental model was a spring barley two-row (*Hordeum vulgare* L.) Spring barley is Russia's main cereal grain and one of the world's most utilized food crops; it is often used as a bioindicator to evaluate the influence of heavy metals content and concentration (V. Rajput et al., 2018a).

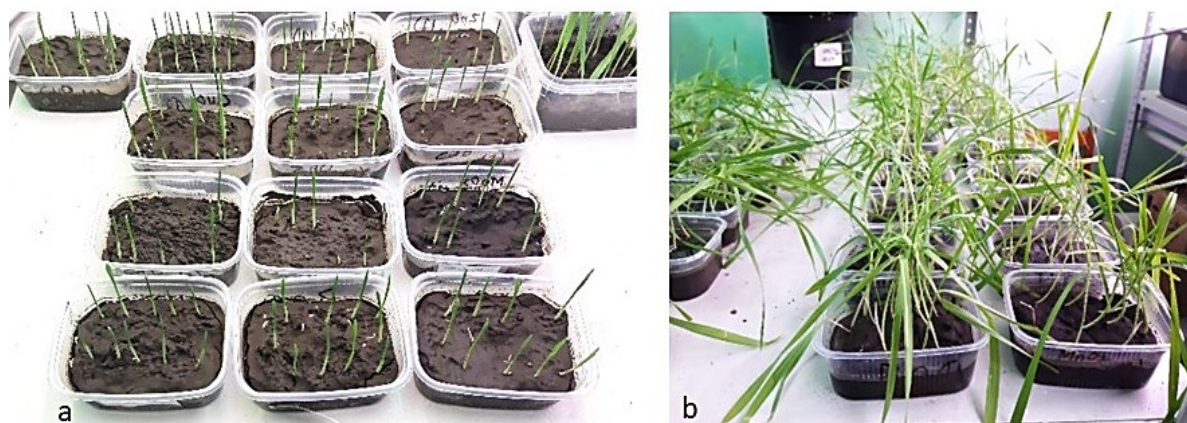


Figure 3.1. Barley plants as shown in the picture were grown in Metal Polluted soil

3.1. Characterization of the Used Soil, and its Physical and Chemical Properties

In each plastic pot, 150 g of air-dry soil was placed, cleaned from plant residues, and shifted to a sieve with a hole diameter of 3 mm. As a background soil, ordinary carbonate chernozem was selected from the Botanical Garden of the Southern Federal University (Table 1 of soil properties).

Table 3.1. Basic chemical and physical properties of ordinary carbonate chernozem from the Botanical Garden of Southern Federal University.

Soil	pH _{H2O}	Clay content (soil particle < 0.01) %	Silt (<0. 001) %	C _{org}	CaCO ₃	Exchangeable cations	
						Ca ²⁺ (cmol100 g ⁻¹)	Mg ²⁺ (cmol100 g ⁻¹)
Chernozem Haplic	7. 4±0. 3a	47. 6±2. 8	26. 7±1. 5	3. 2±0. 2	0. 5±0. 1	30. 0±1. 8	4. 1±0. 2

A Standard deviation.

The HMs; Zn, Cu, Ni, Cd, Pb, and Mn were applied to the soil in doses of 3 and 30 background. The background was used to represent the metal content of the Botanical Garden's soil (Table 2). For soil contamination, reference oxide compounds

in monodispersed form with a particle size of 30-50 nm (manufactured by Alfa Aesar) and micro dispersed form with a particle size of 3-5 m (manufactured by Alfa Aesar) were used (CHDA, GOST 16539-79). The experiment is repeated three times. Throughout the incubation period, the moisture content of the samples was kept at 60% of the total field moisture capacity. After one month in the soil, seeds of spring barley (*Hordeum vulgare* L.) of the Medicum 157 OS variety of the Bluegrass family (Poaceae) were sown in the amount of ten plants per vessel. Soil and plants were chosen for further examination after 21 days of the growing season of the plant.

Table 3.2. Doses of metals (macro and nanoforms) introduced into the chernozem of ordinary carbonate under model laboratory conditions, mg / kg.

Doses of application	Cu	Zn	Cd	Ni	Pb	Mn
	41±2.	83±3.	0.	46±1.	29±0.	830
	2	7	4±0.	2	8	±22.
Control (background*)			1			1
3 Backgrounds	123	248	1	139	87	2490
30 Backgrounds	1233	2481	11	1392	870	24900

Background - metal content in the uncontaminated black soil of SFU's ordinary Botanical Garden.

The X-ray fluorescence method was used to determine the total HMs content of soils. The HMs mobile samples were extracted into solution using a 1N ammonium acetate buffer (NH₄Ac) with a pH of 4.8 (soil: solution ratio = 1: 5, extraction time - 18 h), which describes their exact mobility (T. M. Minkina et al., 2018). An atomic absorption spectrophotometer is used to determine the HMs content of the extracts (KVANT 2-AT, Kortec Ltd, Russia). Mineralization of selected plants was accomplished using the dry ashing technique (Chaplygin et al., 2022) The extraction of HMs from ash was accomplished through dissolution in a 20% HCl solution, preceded by characterization by using the Atomic absorption spectrometry method (Motuzova et al., 2014).

The amount of plant HM contamination was assessed by examining the HM content in the investigated vegetation to the maximum permissible levels (MPL) of chemical elements in feed additives and grasses for farm animals (Chaplygin et al., 2020a).

3.2. Procedure for the Incubation of Barley Seed

Before transferring the barley seed to the soil for sowing the barley seed was incubated for two days to germinate, about twenty seeds were kept in each plastic tray for incubation. Before putting the seed put some filter paper on a plastic tray and then water it. Then we wash the bottle to remove dirt. different types of chemicals are used to wash the dirt for this we used ethanol 10-15%, moreover, ethanol is also used to wash away dirt and mold spores. We can also use a small amount of alcohol to wash away dirt and then shake for 4-5 minutes and then put it in the oven for 23-25 degrees C for two days to incubate. During this incubation period, the seed should be checked for mould seeds get out of the oven to give some air put it outside for five minutes and make it humid by spraying a little amount of distilled water and put it again in the oven after two days the seed was sprouted and then transfer to the soil for sowing.

3.3. Plant Growth Condition and Experimental Setup

We used seed from the widely grown *H. sativum distichum* cv. Travník. These same seeds were inspected visually for morphometric damage, and 20 sterilized seeds have been placed in clean Petri dishes (95 mm diameter) at an equal distance over a round piece of filter paper; this procedure was repeated three to four times. Five millilitres of distilled water were supposed to apply to a Petri dish containing no additional nutrients, which was kept at 28 °C in a growing medium. Seedlings were moved to plastic vessels (50 60 100 mm) after successful germination. 10 sprouted seeds were placed (in triplicate) in each vessel and managed to grow in the growing chamber at 25 2 °C with a 16-hour light cycle (fluorescent lamp: 36 W/635, illumination of 4–5 km) and an 8-hour dark cycle the barley seed was grown in a small plastic pot and was check daily and water it. After 10 days nanoparticles spray of CuO and ZnO individually and in combination were applied to the barley plants. Three-week-old *H. sativum* leaves and middle roots were collected and prepared for TEM and scanning electron microscopy (SEM) (Mikmed-6 St. Petersburg, Russia).

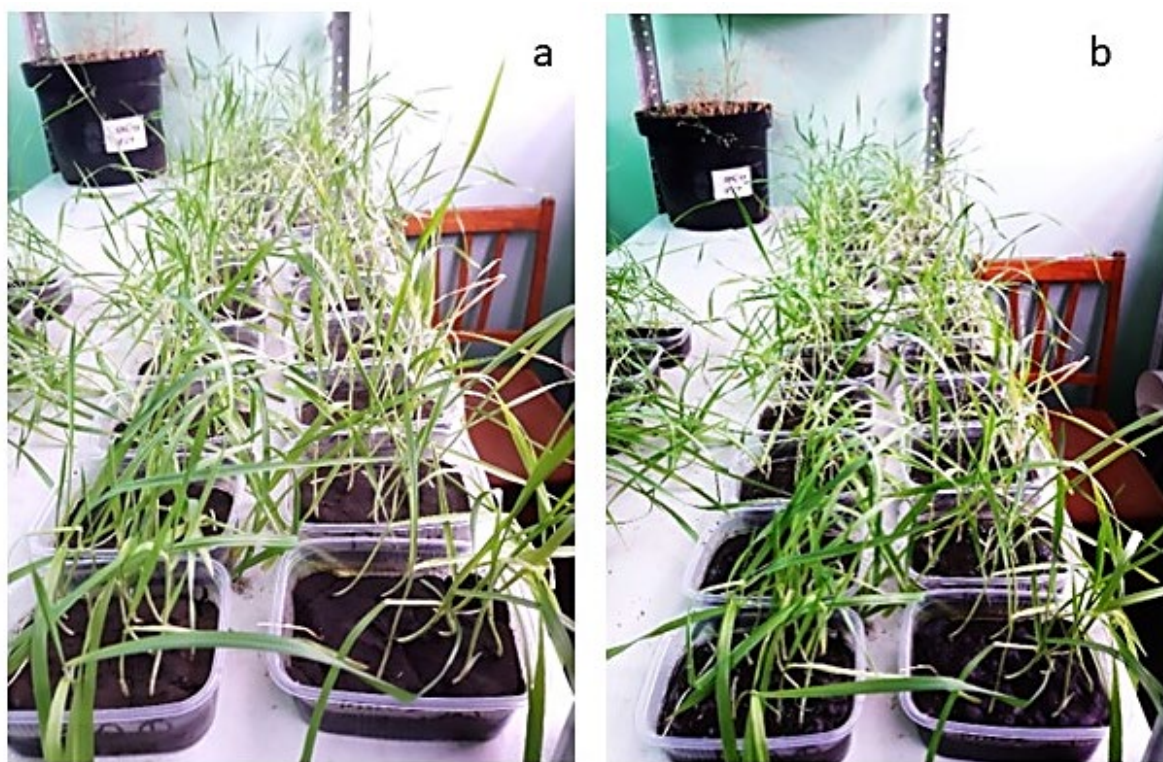


Figure 3.2. Plants as shown after Nanoparticles spray

3.4. Preparation, Characterization, and Application of NPs

CuO NPs powder (particle size 30–50 nm, CAS-1317-38-0) made by Alfa Aesar, USA, provided commercial-grade copper (II) oxide CuO NPs were being used basically as they were obtained, with no further filtration or alteration. Transmission electron microscopy (TEM) (Tecnai G2 Spirit Bio TWIN, Netherlands) was also used to describe the properties of the NPs, as well as a zeta potential analyzer to check their consistency and hydrostatic size in a suspension used for hydroponic research (Supplementary data). To prepare the required concentration (10 g L⁻¹), CuO NPs were poured into double distilled water. The NPs solution was shaken and ultrasonicated (stabilization step) before being applied in the batch experiments to achieve well-mixed dispersion and minimize aggregation and agglomeration (Chung et al., 2009). The said concentration has been selected with the present levels of metal pollution in soils and groundwater with bottom sediments next to manufacturing units in the Rostov-on-Don region (Minnikova et al., 2017). These companies, particularly the Novocherkassk power plant, accounted for N50 percent of the region's pollutant emissions (Wheeler et al., 2016).

CuO is present in the plant emission content of HMs. Several other studies have found a high concentration of Cu in soils near nonferrous metallurgical enterprises on the Kola Peninsula (V. Rajput et al., 2018b). Sigma-Aldrich, St. Louis, MO, USA, provided commercial grade zinc oxide (ZnO) NP powder (particle size 50 nm). The purchased NPs were used exactly as they were received. To prepare the desired concentration, the NPs were poured into double distilled water. The NP solution was shaken and ultra-sonicated (stabilization step) before use in the batch experiments to achieve a well-mixed dispersion and minimize aggregation. Transmission electron microscopy (TEM; Tecnai G12, FEI Company, Czech Republic) was used to characterize the ZnO NPs' properties, powder X-ray diffraction was used to estimate the crystalline sizes, and dynamic light scattering (DLS) and potential measurements were used to assess their stability.



Figure 3.3. Zinc oxide and Copper oxide nanoparticles

3.5. Heavy Metals Analysis and Morphometric Observation in Plant and Roots Materials

After 21 days of growth of the barley plant morphometric parameters, namely shoot and root lengths, were measured. The distance between the bottom of the main stem and the growing third leaf was used to calculate plant height. The mean length of the longest roots was used to calculate primary root length. Metal toxicity was assessed using These measurements differed between the control and infected plants Roots and stems, were detached, thoroughly washed with distilled water, oven-dried at 65°C, ground, and sieved for HMs analysis. Plant tissues were prepared for heavy metals analysis by burning a 1 g air-dried plant sample at 450°C for 6 hours. The ash was then dissolved in 5 mL of 20% HCl and filtered through 0.45 mm Whatman filter paper (Rajput et al., 2020).The analysis was carried out at room temperature using an

atomic absorption spectrophotometer (AAS) (KVANT 2-AT, Kortec Ltd, Russia) with the following wavelength diapason: Zn—213.9 nm, Mn—279.5 nm, Cu—327.4 nm, Pb—283.3 nm, Ni—352.1 nm, Cd—228.8 nm).



Figure 3.4. Doing morphometric parameter of Plants

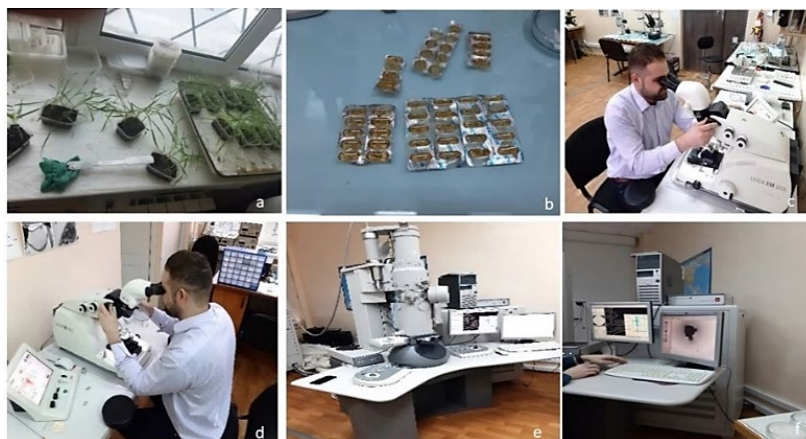


Figure 3.5. Process of Heavy metals determination in Plants and roots

3.6. Preparation of Samples for anatomical and Ultrastructural Observation

Barley plants were selected at the booting stage to study the morphometry of spring barley and the ultrastructure of the cells. Barley is particularly susceptible to water deficiency, nutrition, and other environmental factors at this stage. life-sustaining factors. Fragments from the central part of the second or third leaf (2 9 2 mm) and the middle part of the root were expected to be used for light-optical and electron microscopic studies (2 mm). The root hair zone was sampled for root samples. For 2 hours in a vacuum at room temperature, samples were fixed in a 2.5 percent glutaraldehyde solution in phosphate buffer (pH 7.4). The samples were fixed after being rinsed twice with sucrose phosphate buffer. for 2 hours at room temperature with a 1 percent solution of OsO₄. 2 hours at room temperature. The plant tissue was then dehydrated in a graded series of increasing concentrations of ethanol (50 percent, 70 percent, 96 percent, respectively).

The samples were kept in a 70 percent alcoholic uranyl acetate for 12 hours in the cold 4°C. before being placed in 96 percent alcohol. They were then washed with uranyl acetate and placed in absolute ethanol and acetone to dehydrate three times for 15 minutes each. Leaf cuttings were impregnated with resin in a series of increasing concentrations of Epon and acetone solutions, followed by a further immersion in Epon. The polymerization was carried out in a thermostat at 37 °C (1 day), and 60 °C (2 days) (2 days). The entire process of preparing tissue for morphological examination. (contrast, dehydration, polymerization encapsulation, preparation, and staining of semi-thin sections for light-optical study) were carried out using standard methods (T. Minkina et al., 2018).

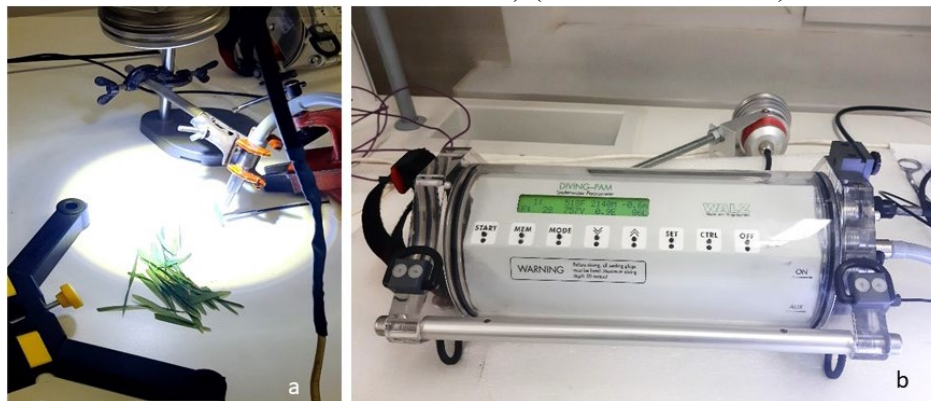


3.7. Measurement of Chlorophyll Fluorescence and Transpiration Rate

Plants remained dark-adapted in a laboratory room. For 20-30 minutes before chlorophyll fluorescence measurements, with dark curtains and no artificial light source. The room temperature was the same as the plant's growth environment during the measurement. One of the most important parameters of chlorophyll fluorescence, the maximum quantum yield of photosystem II (F_v/F_m), has been used as a non-destructive and accurate tool to precisely determine the physiological state of plants.

Figure 3.6. Ultrastructure observation of roots and plants samples of barley plants.

Chlorophyll fluorescence has enormous potential as a tool for monitoring the interaction of NPs with plants (Malea et al., 2022). A pulse amplitude modulated (PAM) fluorometer was used to measure the maximum quantum yield of photosystem II (F_v/F_m) at room temperature (Diving PAM, Waltz, Germany). Photosynthetic photon flux density (PPFD) The white measuring light had a PPFD of 1800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and the red saturating flash had a PPFD of 0.15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Before measurements, all samples were dark-adapted for 20-30 minutes. Transpiration was measured using the PC-gravimetric method (computer connected to lab balance, Adventurer TM Pro, Ohaus) (time resolution 3 s) and calculated as $\Delta F/\Delta t$



where F denotes the difference between initial and final weight and t denotes time.

3.8. Heavy Metal Total Content and their Mobile Forms Determination in Soil

The ground, an air-dried mixed soil sample was sieved through a 1-mm sieve (Medvedeva et al., 2022). To determine the content of Heavy Metals in the soil, a parallel extraction scheme was used (Semenkov et al., 2022). The prepared soil sample

Figure 3.7. Chlorophyll fluorescence analysis of Barley

(5.0 g) was treated with the following solutions (50 mL each): (1) an ammonium acetate buffer solution (1 M CH₃COONH₄) with a pH of 4.8, (2) a 1 percent solution of EDTA in 1 M CH₃COONH₄, and (3) 1 M HCl. The extraction took 18 hours. The extraction was carried out at room temperature, and the extracts were filtered after 3 minutes of initial agitation. The metal compounds extracted with 1 M CH₃-COONH₄ are exchangeable. The 1 percent EDTA in 1 M CH₃COONH₄ extracts exchangeable metal compounds as well as those bound in organometallic complexes; thus, the difference in metal content between the 1 percent EDTA in 1 M CH₃COONH₄ and 1 M CH₃COONH₄ extracts should characterize the metal content in complexes with organic matter in the soil. The 1 M HCl extracts both adsorbed metals and exchangeable metal compounds. A significant portion of the specifically adsorbed metals is associated with loosely bound compounds that are bound with iron, aluminum, manganese oxides and hydroxides, and carbonates. The contents of the adsorbed metal compounds are calculated by subtracting the amounts extracted with 1 M HCl from the amounts extracted with 1 M CH₃-COONH₄. These compounds are recognized as a transitional group between metals that are firmly and loosely bound (Mazarji et al., 2022). The mobile form of Heavy metals content in soil extracts was determined using an atomic absorption spectrophotometry analyzer (AAS) (KVANT 2-AT, Kortec Ltd.). X-ray fluorescence on a SPECTROSCAN MAKC-GV was used to determine the total mobile form of heavy metals content in the soils studied in the model experiment (Russia).

3.9. Heavy Metal Analysis in Plant Tissue (Non-Destructive Method)

Non-destructive X-ray fluorescence (XRF) spectroscopy was used to analyze the elements. The middle leaves of 2-week-old *H. sativum* seedlings were collected and processed for XRF (leaves were dried at room temperature and the surface was flattened with coverslips). An optical microscope (Mikmed-6, JSC "Lomo", St. Petersburg, Russia) was used to collect images of the areas for XRF analysis. A microfocus spectrometer M4 Tornado was used to measure the XRF spectra (Bruker Corp., Billerica, MA, USA). To have better statistics for light elements, the X-ray tube was operated at 50 kV and 600 μ A, and the sample analyses were performed in a vacuum (Si, P, S, Cl). For each sample, data were collected from different areas (for the control sample, XRF spectra were collected). Physiological Indices from three different areas) and averaged 200 points in each.

4.RESULTS AND DISCUSSION

4.1. Content of Heavy Metals in Ordinary Calcareous Chernozem

The gross content of heavy metals in the ordinary calcareous chernozem in the control variant corresponds to the background level (Mandzhieva et al., 2017) and does not exceed the approximate permissible concentration (APC) (Butko et al., 2021). When pollutants are introduced into the soil at a dose of 3 background, the content of metals increases by 4 times for Pb, Mn, Cu, Ni, Zn and by 8 times for Cd compared to the background, while compared to the APC, the increase occurs by 1 (Cd, Cu, Zn) – 3 (Pb, Ni) times (Table 4.1). On variants of 30 backgrounds, the total content of Mn increases 30 times compared to the control and 18 times compared to the MPC, Cu and Ni - 31 times compared to the control, 10 and 18 times, respectively, compared to the OPC, Zn - 32 and 12 times, Pb - 36 and 29 times, and Cd - 66 and 6 times. When plants are sprayed with a solution of Cu and Zn nanoparticles, their total content in the soil does not change.

Table 4.1. Total content of metals in ordinary carbonate chernozem of the model experiment, mg/kg

Options/ metals	Pb	CD	Mn	Cu	Ni	Zn
Control	25±4	0.2±0.01	876±53	43±2	48±3	81±5
3 backgrounds	107±6	1.5±0.1	3433±206	159±10	202±10	323±19
30 backgrounds	913±64	11.8±0.8	26291±1577	1301±65	1468±88	2639±132
UEC*	130	2	1500**	132	80	220

* UEC - SanPiN 1.2.3685–21, 2021

** MPC - SanPiN 1.2.3685–21, 2021

In uncontaminated carbonate ordinary chernozem, there is a low content of mobile forms Cu, Zn, Ni, Pb and Mn, not exceeding 2% of the total content, which is a regional feature of ordinary carbonate chernozem (T. M. Minkina et al., 2011, 2013, 2014), which is characterized by a high content of organic matter, physical clay and silt and has in its composition highly dispersed forms of carbonates (Table 4.1). The exception is Cd, the content of mobile compounds of which corresponds to 6% of its total content.

Table 4.2. Content of mobile metal compounds in ordinary carbonate chernozem of the model experiment, mg/kg

Options/ metals	Pb	CD	Mn	Cu	Ni	Zn
Control	0.4±0.02	0.01±0.001	21±2	0.3±0.01	0.9±0.1	1.8±0.1
3 backgrounds	7±0.3	0.2±0.1	154±9	6±0.3	11±1	25±1
30 backgrounds	85±5	1.8±0.1	1349±94	89±6	136±8	269±16
MPC*	6	-	140	3	four	23

* MPC - SanPiN 1.2.3685–21, 2021

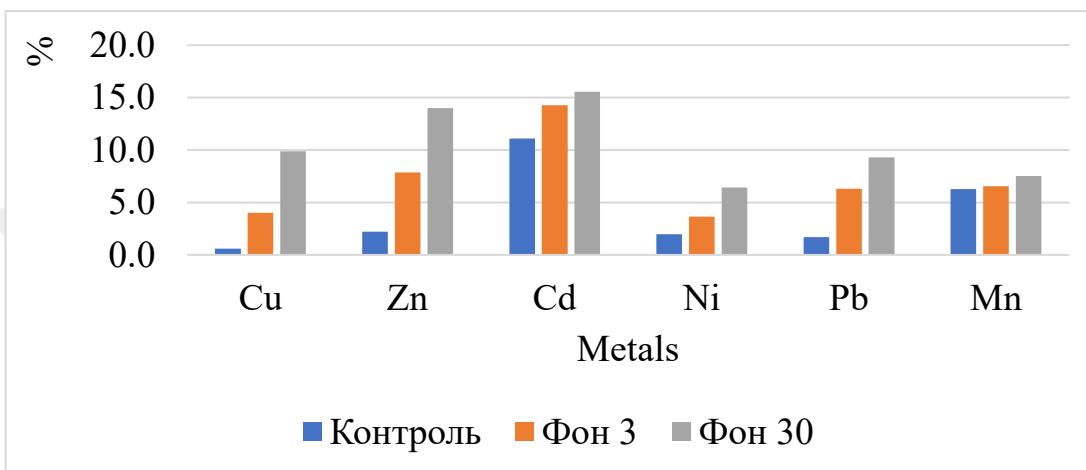


Figure 4.1. The content of mobile metal compounds in ordinary carbonate chernozem of the model experiment, % of the total content.

The introduction of metals into ordinary calcareous chernozem leads to an increase in both the total content and mobile compounds in the soil (Table 4.2). It has been established that the introduction of metals at a dose of 3 background contributes to an increase in the content of mobile compounds Cu and Mn in ordinary carbonate chernozem up to 4% of the total content, Zn - 8%, Cd - 14%; Ni - 5%; Pb - 6% (Fig. 2). In the variants with the introduction of 30 backgrounds of metals, an increase in the mobility of the elements under consideration is also noted, however, to a greater extent than in the variants with the introduction of 3 backgrounds: Cu up to 7% of the total content, Zn up to 10%, Cd up to 16%, Ni up to 9%, Pb up to 9% and Mn up to 5%.

The mobility of metals in soil depends on the chemical compound (Table 4.2, Figure 4.1). According to the degree of increase in mobility at a dose of 3 backgrounds, metals are distributed in the following sequence, % of the total content: Zn by 6% > Pb by 5% > Cd and Cu by 3% > Ni by 2%; at a dose of 30 backgrounds - Zn by 12% > Cu by 9% > Pb by 8% > Cd and Ni by 4% > Mn by 1%. The unequal mobility of

heavy metals is due to differences in the solubility of the compounds introduced into the soil and the strength of the metal bond with the solid phases of the soil (Y. V. T. Minkina & Vodyanitskii, 2022; Pinskii et al., 2014). The higher the dose of metals, the more their mobility increases. It was found that Cd is the most mobile metal, Ni is the least mobile (Table 4.2, Figure 4.1).

4.2. Content of Heavy Metals in the Spring Barley

There is an excess of the MRL Cu in the stems of spring barley on the variant of 30 backgrounds up to 2 times (Table 3) The greatest excess of MRL is noted for Cd and is 12 times when 30 backgrounds are added. A concentration of 6 MRLs is observed for 30 Pb backgrounds, 5 MRLs for Ni, and 1 MRL for Zn. Thus, it was found that the mobility of heavy metals in the soil and their accumulation in spring barley depend on the level of soil contamination. With an increase in the dose of applied metal compounds, their bioavailability and accumulation in the roots and aerial parts of barley increase.

Table 4.3. Accumulation of heavy metal in roots and shoots of *Hordeum vulgare*, mg/kg

Experimental variants	Treatment, 150 ppm	Root	Shoot	Experimental variants	Treatment, 150 ppm	Root	Shoot
Pb				Cu			
Control		1.3±0.1	2.1±0.1	Control		13.3±0.9	12.4±0.7
Control -1	CuO NPs	2.0±0.1	2.2±0.1	Control -1	CuO NPs	15.2±1.0	21.8±1.2
Control-2	ZnO NPs	1.6±0.1	1.6±0.1	Control-2	ZnO NPs	11.8±0.8	10.1±0.6
Control-3	CuO+ZnO	1.4±0.1	1.9±0.1	Control-3	CuO+ZnO	14.7±1.0	19.3±1.1
PbO 3		9.0±0.6	2.4±0.1	CuO 3		19.3±1.3	17.2±1.0
PbO 30		106.4±7.1	29.7±1.8	CuO 30		119.6±8.1	54.7±3.1
PbO 3-1	CuO NPs	10.1±0.7	2.3±0.1	CuO 3-1	CuO NPs	17.5±1.2	15.6±0.9
PbO 3-2	ZnO NPs	9.5±0.6	2.2±0.1	CuO 3-2	ZnO NPs	13.9±0.9	12.4±0.7
PbO 3-3	CuO+ZnO	8.6±0.6	2.5±0.1	CuO 3-3	CuO+ZnO	14.7±1.0	18.3±1.0
PbO 30-1	CuO NPs	61.4±4.1	9.7±0.5	CuO 30 -1	CuO NPs	86.5±2.7	39.0±2.3
PbO 30-2	ZnO NPs	63.0±4.2	8.1±0.5	CuO 30 -2	ZnO NPs	70.1±4.8	27.9±1.5
PbO 30-3	CuO+ZnO	50.2±3.4	5.6±0.3	CuO 30 -3	CuO+ZnO	75.5±5.2	29.4±1.6
MPL for grasses (Provisional...,1987)			5.0	MPL for grasses (Provisional ..., 1987)			30
Cd				Ni			
Control		0.2±0.01	0.1±0.01	Control		0.8±0.1	0.2±0.01
Control -1	CuO NPs	0.1±0.01	0.1±0.01	Control -1	CuO NPs	1.0±0.1	0.4±0.02
Control-2	ZnO NPs	0.2±0.01	0.1±0.01	Control-2	ZnO NPs	1.1±0.1	0.2±0.01
Control-3	CuO+ZnO	0.1±0.01	0.1±0.01	Control-3	CuO+ZnO	0.9±0.1	0.1±0.01
CdO 3		2.7±0.2	1.0±0.1	NiO 3		10.0±0.7	1.9±0.1
CdO 30		19.5±1.3	3.7±0.2	NiO 30		87.9±6.0	12.5±0.7
CdO 3-1	CuO NPs	2.3±0.3	0.6±0.04	NiO 3-1	CuO NPs	7.6±0.5	1.3±0.1
CdO 3-2	ZnO NPs	2.4±0.2	0.5±0.03	NiO 3-2	ZnO NPs	5.3±0.4	1.2±0.1

CdO 3-3	CuO+ZnO	1.6±0.1	0.2±0.01	NiO 3-3	CuO+ZnO	3.1±0.2	0.9±0.1
CdO 30-1	CuO NPs	14.1±1.0	2.2±0.1	NiO 30-1	CuO NPs	61.7±4.1	8.3±0.5
CdO 30-2	ZnO NPs	15.0±1.1	1.8±0.1	NiO 30-2	ZnO NPs	65.9±4.3	6.2±0.4
CdO 30-3	CuO+ZnO	9.8±0.7	1.1±0.1	NiO 30-3	CuO+ZnO	41.9±2.8	2.2±0.1
MPL for grasses (Provisional ..., 1987)			0.3	MPL for grasses (Provisional ..., 1987)			3
Mn				Zn			
Control		33.8±2.1	28.3±1.6	Control		16.1±1.0	14.2±0.9
Control -1	CuO NPs	31.0±1.9	26.7±1.5	Control -1	CuO NPs	17.8±1.2	13.5±0.8
Control-2	ZnO NPs	33.1±2.1	22.5±1.3	Control-2	ZnO NPs	20.4±1.3	19.6±1.2
Control-3	CuO+ZnO	38.7±2.4	29.0±1.6	Control-3	CuO+ZnO	19.7±1.4	18.2±1.1
MnO2 3		76.8±4.8	57.1±3.2	ZnO 3		46.6±3.0	24.3±1.5
MnO2 30		231.5±14.4	125.0±7.1	ZnO 30		139.4±9.1	56.4±3.5
MnO2 3-1	CuO NPs	46.0±2.9	32.8±1.8	ZnO 3-1	CuO NPs	29.5±1.8	23.8±1.4
MnO2 3-2	ZnO NPs	41.4±2.6	30.3±1.7	ZnO 3-2	ZnO NPs	32.7±2.2	27.5±1.6
MnO2 3-3	CuO+ZnO	36.9±2.3	29.7±1.6	ZnO 3-3	CuO+ZnO	31.9±2.1	25.2±1.5
MnO2 30-1	CuO NPs	156.1±9.7	59.1±3.3	ZnO 30-1	CuO NPs	84.2±5.4	42.9±2.7
MnO2 30-2	ZnO NPs	144.0±8.9	76.2±4.2	ZnO 30-2	ZnO NPs	95.6±6.3	46.4±2.9
MnO2 30-3	CuO+ZnO	102.6±6.4	46.9±2.6	ZnO 30-3	CuO+ZnO	79.8±5.2	48.7±3.0
				MPL for grasses (Provisional ..., 1987)			50

± – the standard deviation (SD)

* Exceedance of MPL for grasses is typed in bold

Provisional maximum permissible levels (MPL) for some chemical elements and gossypol in forage for farm animals and feed additives (1987). Moscow (in Russian).

4.3. Elemental analysis of heavy metals by Non-destructive Method X-ray Fluorescence Measurements

Non-destructive elemental analysis was performed by means of X-ray fluorescence spectroscopy (XRF). The XRF spectra were measured using microfocus spectrometer M4 Tornado (Bruker Nano GmbH, Berlin, Germany). Rh X-ray tube was operated at 50 kV and 600 µA. The samples were measured in vacuum in order to have better statistics for elements; Mg, Al, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Cu, Zn, Sr, Cd. The data was collected in three different areas for control sample and for two areas for each of the samples that were prepared from the leaves of the plant, heavy metals treated samples. For each area 200 points were selected for spectra acquisition. The spectra were analyzed using basic fundamental parameter method. One sigma error was estimated for each dataset collected in one area.

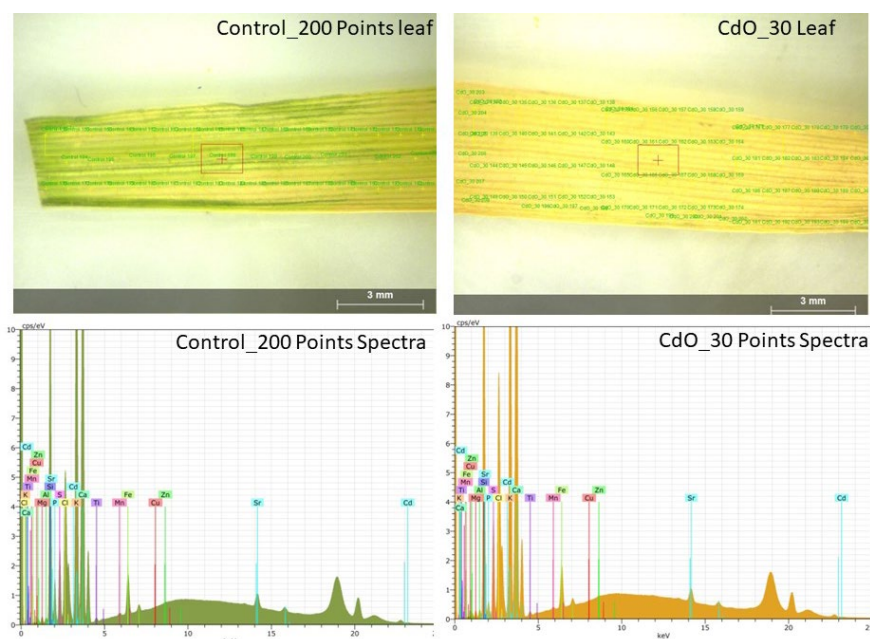


Figure 4.2. Optical microscopy image (top) of the measured area and XRF spectra (bottom) for the plant leaves grown on heavy metal polluted soil

Table 4.4. The EDX spectra elements, wt %

Treatment	Mg	Al	Si	P	S	Cl	K	K	Ca	Ti	Mn	Fe	Cu	Zn	Sr	Cd
Control 200	2.85	0.35	31.69	2.78	3.14	7.73	20.17	30.20	0.12	0.04	0.68	0.00	0.01	0.23	0.00	
CdO-30	2.48	0.57	32.85	0.72	3.71	11.30	18.51	28.89	0.12	0.02	0.62	0.00	0.01	0.19	0.00	

Initially, 200 scans in different points of one area were measured. While the differences in element concentrations were noticeable from point to point, the one sigma error for 200 scans dataset were ≤ 0.03 wt.% for K and ≤ 0.01 wt.% for other elements. Another area with 200 independent points were measured and mean element concentrations were calculated for this dataset as well. Presented results of micro-focus XRF measurements showed inhomogeneity in the element distribution throughout the leave sprayed by NPs.

4.4. Morphometric Parameter of Plants

One of the criteria for assessing the level of soil contamination with HMs is to determine their effect on the growth and development of plants. The stress response is different for different plant species depending on the physical and biochemical conditions (Lin & Aarts, 2012; Shulaev et al., 2008). Root length and shoot height were measured in plants as the most sensitive and significant parameters to environmental factors (Perez-Harguindeguy et al., 2013). The lowest dose of HM application to the soil (3 backgrounds) did have a negligible impact on the growth and development of barley (Figure 4.3)

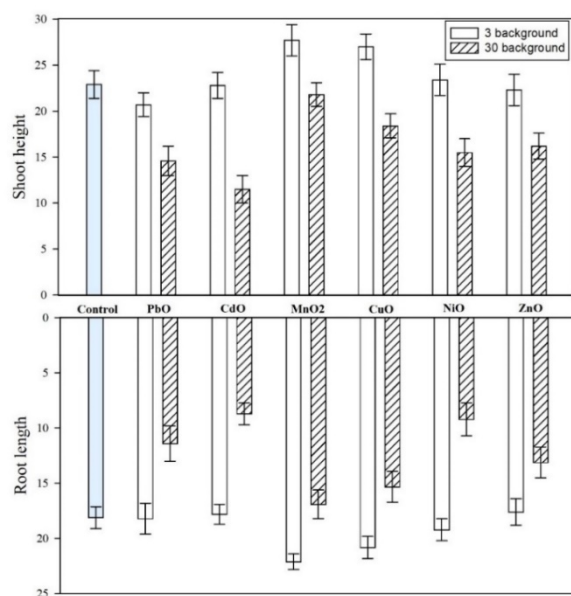


Figure 4.3. Root length and height of spring barley plants at different levels of soil contamination with heavy metals.

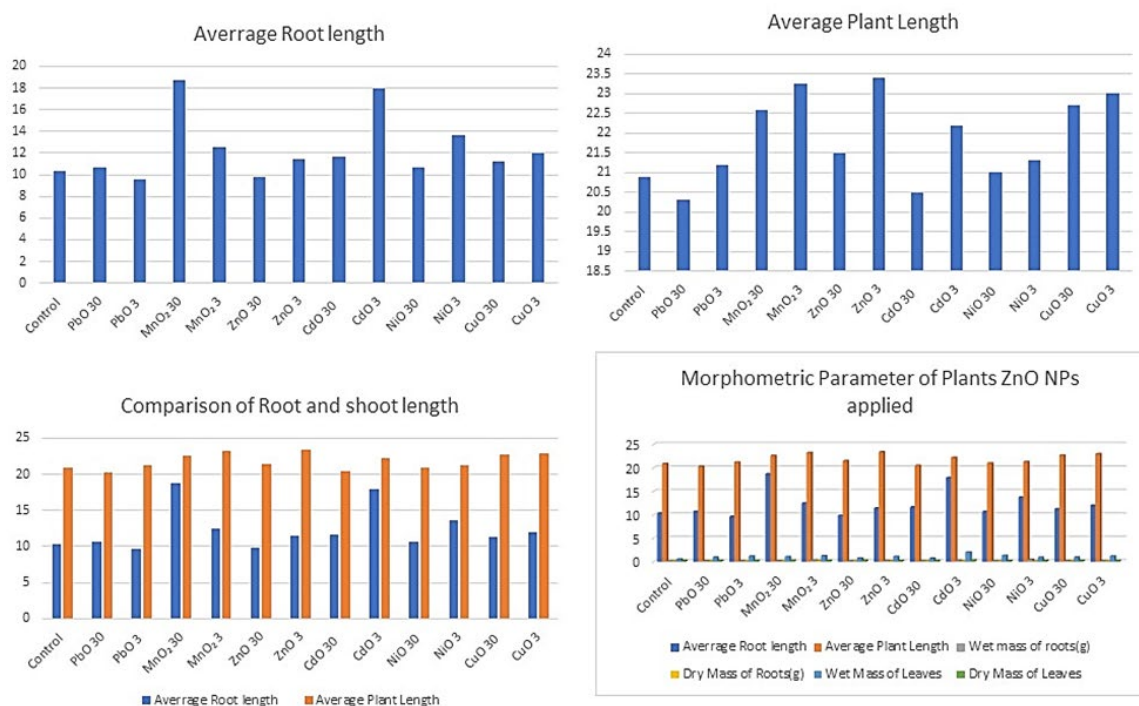


Figure 4.4. Root length and height of spring barley plants at different levels of soil contamination with heavy metals.

As the amount of metal application increases , the negative effect on the development and growth of plants increased. Pollution of the soil cover with metals has a toxic effect on plants both directly, causing a wide range of physiological reactions, usually associated with a violation of redox homeostasis in cells, and indirectly, affecting the balance of nutrients in the soil, which is the basis for regulating nutrition processes. and plant growth (B. Ahmed et al., 2016; Baskar et al., 2021; Kumari et al., 2011).

The decrease in root length when 30 backgrounds of Cd were introduced into the soil was 52%, Pb, 37%, Ni, 49%, Zn, 28%, Cu, 16%, and Mn, 7%. The root is the first barrier on the way of metal entry from the soil into the plant, it performs the function of its accumulation and detoxification, which is why the length of the root decreases first of all, which in turn affects the absorption of nutrients and water (Titov et al., 2011). In terms of toxicity to barley shoot growth, metals can be distributed in increasing order: $Mn < Cu < Zn < Ni < Pb < Cd$ (Figure 4.3). Heavy metals cause toxicity negative effects on plant growth processes such as reduced root and bud growth, disruption of enzymatic activity, and chlorophyll reduction. Stresses lead to

produce excess ROS that cause protein, chlorophyll and cell membrane damages (Singhal et al., 2022).

Foliar treatment of plants growing on uncontaminated soil stimulated the growth of barley by 13-18%. The results showed that the stimulating effect is more pronounced in the aerial parts of plants. Thus, the use of CuO NPs on 30 backgrounds Cd increased root and shoot growth by 45% and 82%, ZnO NPs - by 30% and 78%, and the joint introduction of CuO NPs and ZnO NPs - by 75% and 98%. A similar trend persists for all metals. Treatment of plants grown on soil contaminated with 30 backgrounds of Mn, Cu and Zn stimulated the growth of roots and shoots to the values of the control sample (Table 4.5).



Table 4.5. Morphometric parameters of spring barley in case of artificial soil pollution and foliar treatment of LF

Experimental variants	Treatment, 150 ppm	Root length	Shoot height	Experimental variants	Treatment, 150 ppm	Root length	Shoot height
Control		18,2±0,9	22,7±1,4	CuO 3		20,8±1,2	27,0±1,6*
Control -1	CuO NPs	21,5±1,2*	26,5±1,5*	CuO 30		15,3±1,6*	18,4±1,5
Control-2	ZnO NPs	19,3±0,7	25,8±1,0*	CuO 3-1	CuO NPs	22,8±1,7*	24,5±1,7
Control-3	CuO+ZnO	22,9±0,5*	26,9±1,6*	CuO 3-2	ZnO NPs	12,0±1,9*	23,2±1,3
				CuO 3-3	CuO+ZnO	14,3±0,7*	23,5±1,3
PbO 3		18,2±0,6	20,5±1,7	CuO 30 -1	CuO NPs	15,5±1,3*	24,1±1,8
PbO 30		11,4±1,4*	14,7±1,5*	CuO 30 -2	ZnO NPs	16,3±0,8*	22,7±1,5
PbO 3-1	CuO NPs	17,8±1,3	23,2±1,3	CuO 30 -3	CuO+ZnO	17,6±0,6	22,3±1,4
PbO 3-2	ZnO NPs	19,6±0,5	21,4±1,0				
PbO 3-3	CuO+ZnO	17,8±1,0	22,0±1,4	NiO 3		19,2±1,1	23,4±1,5
PbO 30-1	CuO NPs	16,9±0,8	23,1±1,7	NiO 30		9,3±1,5*	15,5±1,3*
PbO 30-2	ZnO NPs	15,7±1,2*	20,3±1,9	NiO 3-1	CuO NPs	18,0±1,2	22,6±1,0
PbO 30-3	CuO+ZnO	16,1±0,9*	24,6±1,2	NiO 3-2	ZnO NPs	17,7±0,6	21,3±1,0
				NiO 3-3	CuO+ZnO	19,1±0,7	23,0±1,6
CdO 3		17,8±0,7	22,8±1,4	NiO 30-1	CuO NPs	12,5±0,5*	20,4±1,7
CdO 30		8,7±1,0*	11,5±1,8*	NiO 30-2	ZnO NPs	11,7±1,2*	21,8±1,9
CdO 3-1	CuO NPs	19,4±0,8	21,4±1,3	NiO 30-3	CuO+ZnO	15,6±1,3*	22,4±1,5
CdO 3-2	ZnO NPs	17,9±1,2	22,2±1,0				
CdO 3-3	CuO+ZnO	18,3±1,3	23,1±1,7	ZnO 3		17,6±0,8	22,3±1,7
CdO 30-1	CuO NPs	12,6±0,9*	21,0±1,2	ZnO 30		10,1±1,7*	16,2±1,6*
CdO 30-2	ZnO NPs	11,2±1,1*	20,5±1,0	ZnO 3-1	CuO NPs	17,0±1,3	22,6±1,8
CdO 30-3	CuO+ZnO	15,2±1,2*	22,8±1,5	ZnO 3-2	ZnO NPs	18,4±0,9	23,4±1,2
				ZnO 3-3	CuO+ZnO	13,1±1,1*	20,7±1,0
MnO ₂ 3		22,1±1,0*	27,7±1,9*	ZnO 30-1	CuO NPs	15,9±1,6*	20,3±1,5
MnO ₂ 30		17,9±0,8	21,8±1,7	ZnO 30-2	ZnO NPs	15,8±0,5*	21,5±1,4
MnO ₂ 3-1	CuO NPs	21,6±1,4*	24,1±1,2	ZnO 30-3	CuO+ZnO	17,6±0,7	21,9±1,5
MnO ₂ 3-2	ZnO NPs	22,5±1,1*	23,2±1,4				
MnO ₂ 3-3	CuO+ZnO	25,4±1,5*	24,3±1,0				
MnO ₂ 30-1	CuO NPs	18,1±0,7	23,7±1,5				
MnO ₂ 30-2	ZnO NPs	18,7±0,9	22,6±1,6				
MnO ₂ 30-3	CuO+ZnO	18,9±1,0	23,5±1,8				

± – the standard deviation (SD)

*Statistically significant differences ($p \leq 0.05$) comparing the treated plants to the control plants

Table 4.6. Comparison of Morphometric Parameter of Barley Plants without Foliar sprays of NPs

Metals	Root length	Plant Length	Wet mass of roots(g)	Dry Mass of Roots(g)	Wet Mass of Leaves	Dry Mass of Leaves
Control	13.1	21.9	0.1972	0.1404	0.889	0.2875
PbO 30	22.88	24.95	0.3671	0.242	1.327	0.4113
PbO 3	13.15	22.44	0.2889	0.1919	1.156	0.3611
MnO ₂ 30	9	22.2	0.2205	0.161	0.9085	0.2787
MnO ₂ 3	8.1	19.2	0.1865	0.1353	0.698	0.2788
ZnO 30	6.1	20	0.1231	0.0877	0.698	0.247
ZnO 3	6.9	20	0.1992	0.1347	0.845	0.291
CdO 30	21.2	25.45	0.378	0.2167	1.5575	0.402
CdO 3	9.3	17.5	0.2086	0.1155	0.9369	0.2564
NiO 30	11.8	22.5	0.271	0.176	1.194	0.3473
NiO 3	12.75	19.25	0.2417	0.2417	0.997	0.275
CuO 30	13.2	20.7	0.223	0.156	0.997	0.3115
CuO 3	13	21	0.2739	0.1635	1.127	0.342

It has been shown that ZnO NPs reduced TM-induced toxicity at *Leucaena leucocephala*, *Helianthus annuus*, *Boehmeria nivea*, *Oryza sativa* and stimulated their growth (Akhtar et al., 2021b; Gong et al., 2017; Seleiman et al., 2020; Venkatachalam et al., 2017a). the exact mode of nanoparticle action on enhanced plant growth and development is not completely known (Kumar Patra et al., 2017). It is assumed that due to the high volume and surface effect, nanoparticles can interact with cellular biomolecules and trigger various biochemical pathways (Venkatachalam et al.,

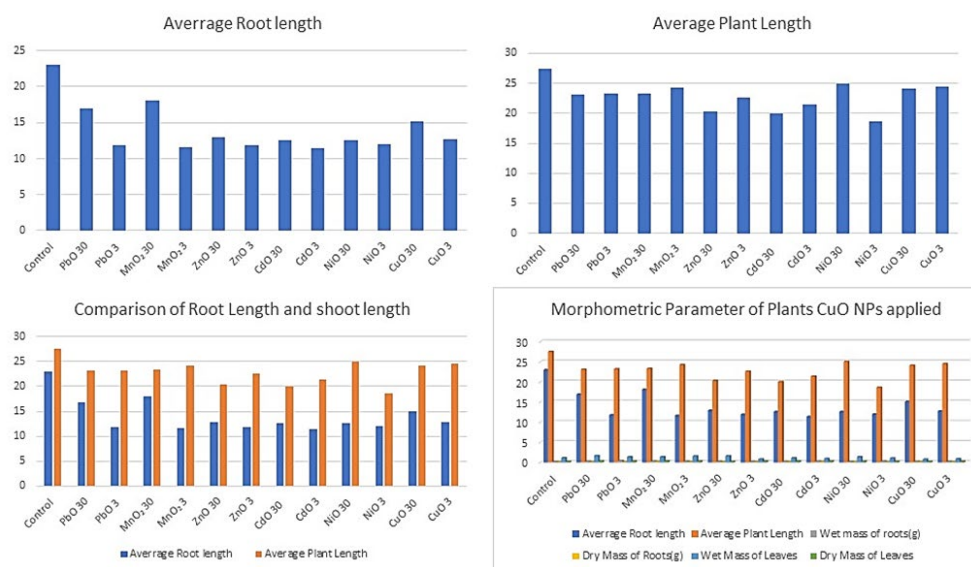


Figure 4.5. Effect of CuO NPs on Morphometric Parameter of Barley Plants.

2017b).

In investigation Venkatachalam et. al., 2017, ZnONPs -mediated alleviation of heavy metal-caused toxicity could be achieved by one of these mechanisms: a) ZnONPs amendment enhanced the plant growth rate and biomass correlated with more metal accumulations, b) decreased level of reactive oxygen species generation to reduce the cell membrane damage by low MDA content, c) elevated rate of antioxidative defense enzyme activities to mob excess ROS, d) enhanced level of isoenzyme pattern and e) amplified more genomic alterations to overcome genotoxicity. In a number of studies, Si NPs (El-Saadony et al., 2021; Hussain et al., 2019). Thus, the introduction of NPs can increase the ability of plants to survive under stress caused by heavy metals by stimulating the physiological and biochemical functions of plants.

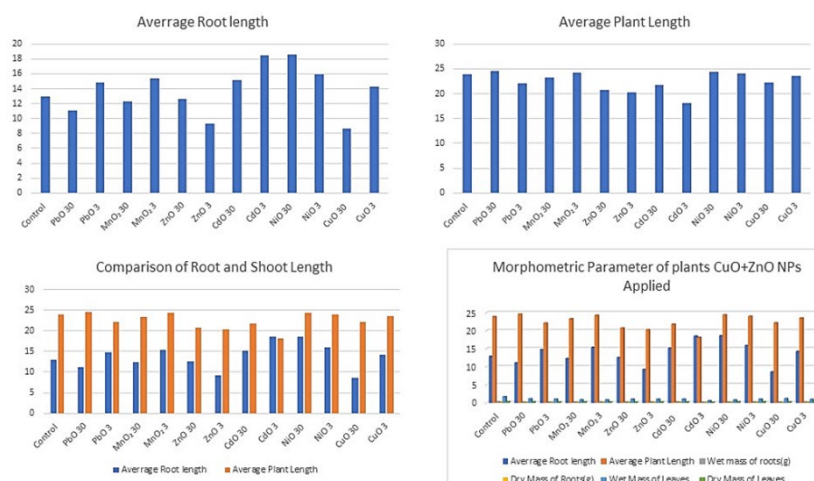


Figure 4.6 Effect of ZnO NPs on Morphometric Parameter of Barley Plants

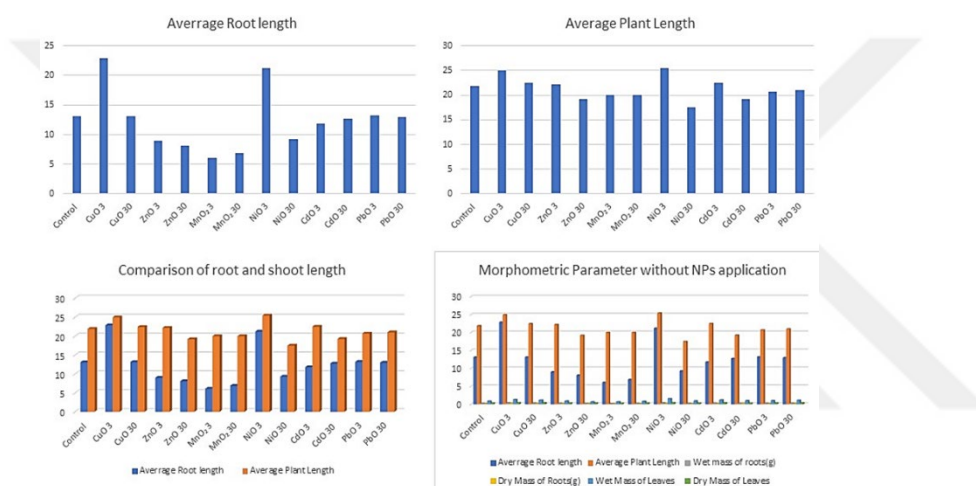


Figure 4.7. Comparison of Morphometric Parameter of Barley Plants without Foliar sprays of NPs

4.5. Measurement of Chlorophyll Fluorescence Parameter

For the analysis of physiological state of plants, the light adapted were analysed for quantum yield of PS(II) i.e., (Y(II)) and electron transport rate (ETR). While, in dark-adapted plants, maximum potential quantum efficiency of PS(II) (Fv/Fm) was measured.

Chlorophyll fluorescence were determined using pulse amplitude modulated (PAM) fluorometer. All the plants control and grown in various types of soil pollutants, foliar applied were dark adopted and light adopted mechanism were observed. Results indicate better quantum yield in plants those observed for chlorophyll fluorescence after dark adopted, whereas plants whose light adopted showed variable response in plant leaves grown under various pollutants after applying NPs as foliar application. Responses are visual on figure. It was found that

the quantum yield of PS(II) of barley plants showed maximal stimulatory response in case of MnO₂ treatment at 3 MPC. With the treatment of all heavy metals, the quantum yield of PS(II) was recorded to decline at higher concentration i.e., 30 MPC than 3 MPC except PbO and NiO treatments. The maximal inhibitory effect was exerted by the treatment of ZnO-30>CuO-30>CdO-30>NiO-3...Similarly, ETR was found to decline as well.

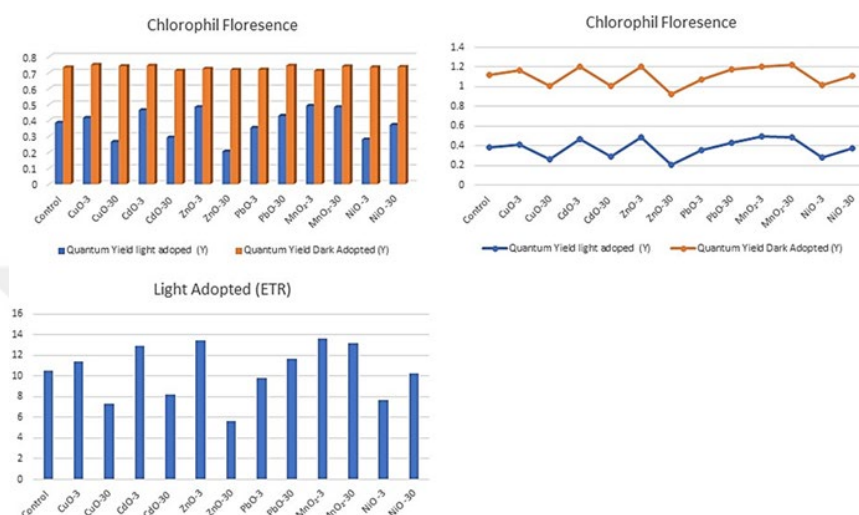


Figure 4.8. Comparison of Quantum yield Light adopted and dark-adapted barley plants with CuO NPs application

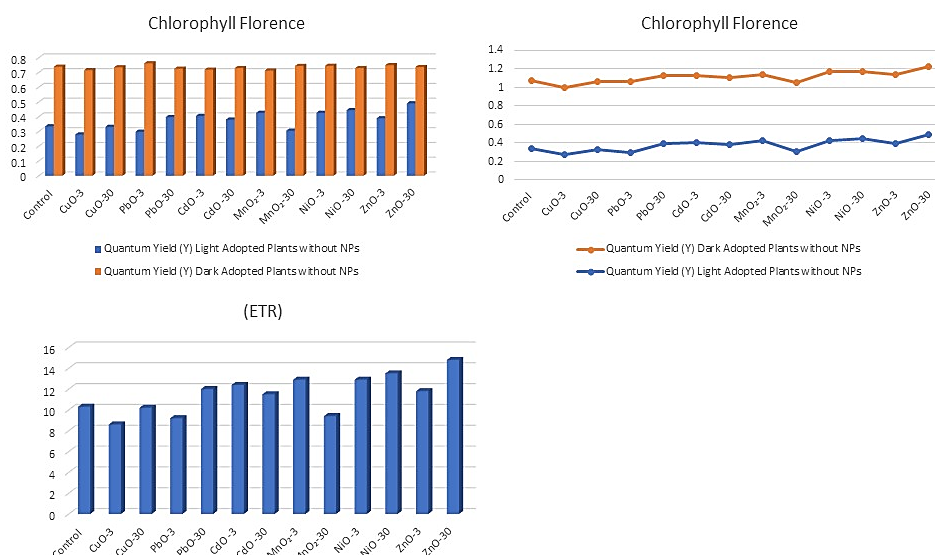


Figure 4.9. Comparison of Quantum yield Light adopted and dark-adapted barley plants with ZnO NPs application

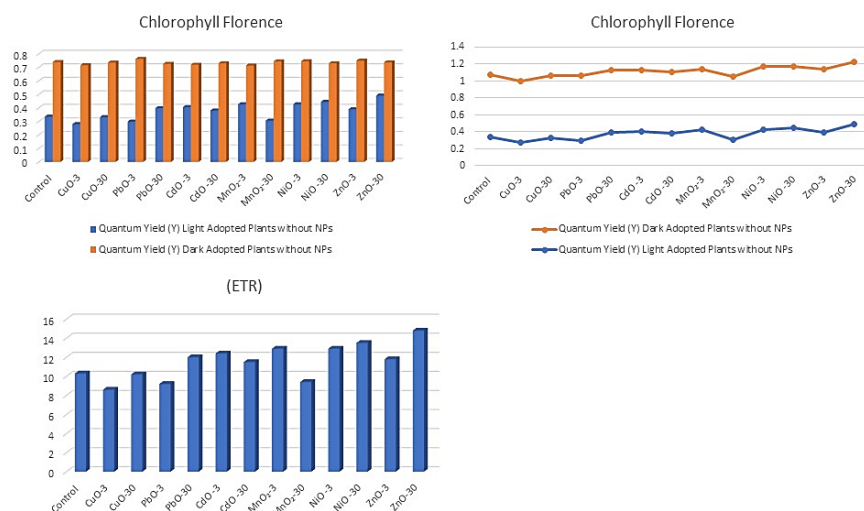


Figure 4.10. Comparison of Quantum yield Light adopted and dark-adapted barley plants with CuO+ ZnO NPs application

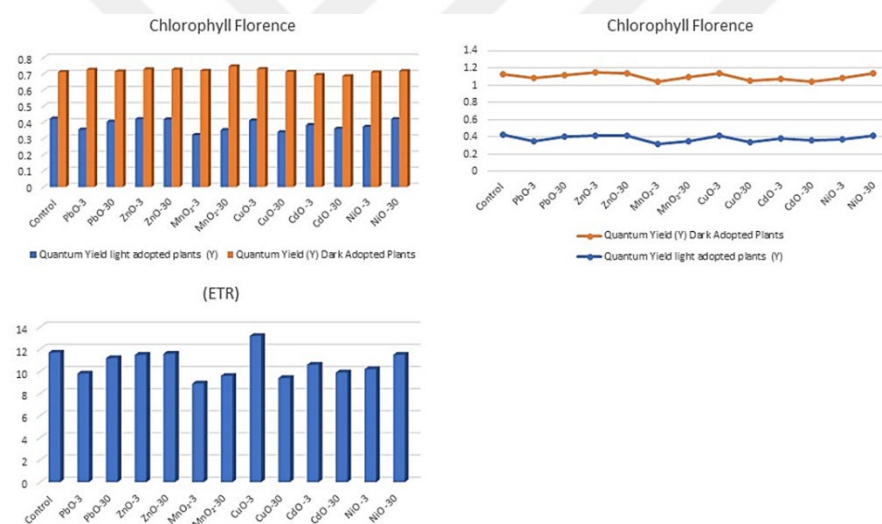


Figure 4.11. Comparison of Quantum yield Light adopted and dark-adapted barley plants without NPs application.

4.6. Impact of Nanoparticles Improve Barley growth grown in Polluted soil

The limited barley leaves were observed by TEM for possible improvement in sub-cellular organelles of barley grown in various metal oxide polluted sprayed by foliar input of NPs; especially structure and symmetry of chloroplast. The CuO and ZnO, and in combination, NPs were applied to the plants. Significant improvement was observed in ZnO polluted once CuO NPs were sprayed (Fig. 9). Moreover, increased number of starch granules were observed in all leaves tissues those who grown in polluted soil and sprayed by NPs. However, improvement in chloroplast, thylakoids, grana and peroxisomes were noted than the unsprayed NPs. Results indicate that the foliar application of NPs; especially in combination could express promising results.

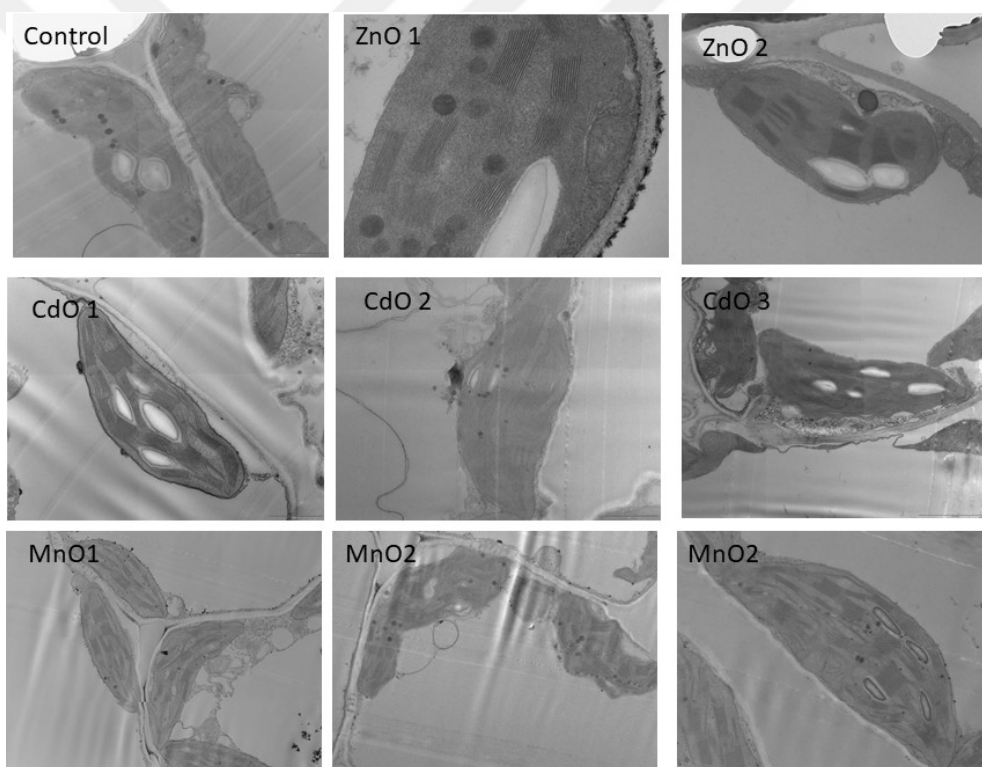


Figure 4.12. ZnO 1 = CuO NPs applied, ZnO 2 = Zn NPs applied, CdO1 = CuO NPs applied, CdO2= ZnO NPs applied, CdO3: CuO + ZnO NPs applied, MnO1= CuO NPs applied, MnO2 = ZnO NPs applied

5.CONCLUSION

Soil pollutant is a well-known problem for plant growth; especially edible crop. The barley was grown under various types of metals polluted articles. Physiological, morphological, anatomical and well as soil element bioavailability were noted. The input of CuO and ZnO NPs at 150 mg/L and in combination showed improvement in analysed parameters. The results can be concluded that the joint application of CuO and ZnO at low level could help plants to survive in heavy metal polluted soils, and plants could enable defence mechanism to avoid heavy metals uptake at most significant plant organelles. In this way plants can restrict toxic elements at underground organelles of plants and limited amount could be reach aerial parts of plants. Uptake of metals in roots, and restrict to above ground tissue can also utilize as a potential uptake to toxic elements by plants. It can open to nano-enhanced remediation various types mono- or multi-metal polluted soils. However, detailed information required and large number experiments should be conducted in field conditions could open new dimension to utilize plants.

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