Faculty of Graduate Studies

Phytoremediation of Heavy Metal-Contaminated Soil Using Tobacco (*Nicotiana tabacum*) and Okra (*Hybiscus esculentus*)

المعالجة النباتية للتربة الملوثة بالمعادن الثقيلة باستخدام نباتي الدخان البامية

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By

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Table of Contents

DECLARATION

This is my original work and has never been submitted in part or whole for an award in any institution.

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DEDICATION

I would like to dedicate this hard work to my beloved mother for her emotional-spiritual support; I would not have arrived here without her prayers and encouragement

To my dearest father and dear brothers

To my beloved sister, and her daughter ' Eleen'

To my family, to my friends and everyone dear, those who gave me the support and encouragement

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As always Alhamdulillah, I am thankful to Allah for the patience and reconciliation that have gifted me to accomplish this long and difficult mission. Thank you God for giving me the ability to overcome these difficulties, and be able to arrive at this very moment.

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LIST OF TABLES

Tables in the appendix

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA: Analysis of Variance

ARIJ: Applied Research Institute – Jerusalem

BAF: Bioaccumulation Factor

BCF: Bioconcentration Factor

BOD: Biochemical Oxygen Demand

BTF: Biotranslocation Factor

Cd50: 50 mg/kg of Cd

Cd100: 100 mg/kg of Cd

Cd150: 150 mg/kg of Cd

Cd200: 200 mg/kg of Cd

CEC: Cation Exchange Capacity

Cu50: 50 mg/kg of Cu

Cu100: 100 mg/kg of Cu

Cu200: 200 100 mg/kg of Cu

Cu300: 300 100 mg/kg of Cu

DW: Dry weight

ICP- OES: Inductivity Coupled Plasma – Optical Emission Spectrometry

MIX: 300 mg/kg of Zn, 100 mg/kg of Cu, 100 mg/kg of Cd, and 100 mg/kg of Pb

NPK: Nitrogen, phosphorus, and potassium

Pb50: 50 mg/kg of Pb

Pb100: 100 mg/kg of Pb

Pb150: 150 mg/kg of Pb

Pb200: 200 mg/kg of Pb

TLC: Testing Laboratories Center (Birzeit University)

WHO: Word Health Organization

Zn300: 300 mg/kg of Zn

Zn500: 500 mg/kg of Zn

Zn800: 800 mg/kg of Zn

Zn1000: 1000 mg/kg of Zn

Phytoremediation of Heavy Metal-Contaminated Soil Using Tobacco (*Nicotiana tabacum*) and Okra (*Hybiscus esculentus*)

Abstract

Overall backdrop: Soil and water heavy metals pollution is considered one of the most serious problems encounter the environment, as a result of human activities. Heavy metals threaten the environment and human life that warranted finding appropriate solutions that are economically inexpensive as well as environmentally compatible. Phytoremediation is a bioremediation technique that takes the physiological capabilities advantages of plants to remove and reduce heavy metals from soil. Phytoremediation cleans up soil or water by plants that transform, absorb, and accumulate pollutants, thus reducing the heavy metals toxicity in the environment. Therefore, this study was concerned with selecting two plants that are abundant in Palestine and their cultivation is easy. The potential of tobacco and okra to remediate heavy metals from soil and the efficiency of the phytoremediation process were evaluated by measuring the translocation, bioconcentration, and bioaccumulation factors.

Main aims: present research aims at investigating the potential of tobacco (*Nicotiana tabacum*) and okra (*Hybiscus esculentus*) to phytoremediate heavy metal-contaminated soil, and investigating the ability of two plants to transfer heavy metals from soil to different plant parts, the study also investigates the effect of metals on different plant growth parameters .

Methodologies: Tobacco and okra plants were grown in pots containing soil contaminated with Zinc, Copper, Cadmium, and Lead. Four concentrations of each metal were prepared and mixed with the experimental soil to get the concentrations mg/kg soil of 300, 500, 800, and 1000 mg of Zn; 50, 100, 200, and 300 mg of Cu; 50, 100, 150, and 200 mg of Cd or Pb. After monitoring for

60 days, the experiment was terminated and the plants were harvested and partitioned into shoot and root. Then, the concentrations of the four heavy metals were measured in soil and plant parts using ICP- OES.

Noteworthy findings: results indicated that the Zn and Cu treatments did not show any significant impact in shoot length of tobacco plants. Whereas some high concentrations of Cd and Pb caused some reduction in tobacco shoot length. Similarly, okra plants recorded some decreases in shoot length due to high levels of Cd, Pb and Cu in soil. In addition, by the end of the experiment, there was no significant difference between the chlorophyll content in treatments of both plants and the control except for some Cu high concentrations. Furthermore, no significant reduction in okra fruit weight was observed due to exposure to metal-contaminated soil for 2 months.

For both tobacco and okra, all treatments showed significant increases in heavy metals concentration in plant tissues (root & shoot) compared to control. Tobacco and okra plants had the ability to translocate Cd from their roots to shoots. The bioaccumulation factor was observed in trend of Cd > Zn > Cu > Pb in tobacco plants, and Zn > Cd > Cu > Pb in okra. Concerning metal concentrations in soil, the highest average percentage of metal reduction by tobacco plants was 59.05% and 52.37% for Pb and Cd, respectively. In the case of okra plants, the highest average percentage of metal reduction was 57.65% and 51.72% for Pb and Cd, respectively.

General conclusion: the findings indicate that both, tobacco and okra, have the ability to grow, absorb, accumulate and translocate metals from soil. Both can be used effectively to phytoextract metals, especially Cd & Pb, from soil. Thus, the two can be considered for phytoremediation purposes of metal contaminated soil.

Key words: Phytoremediation. heavy metals. Zn. Cu. Cd. Pb. contaminated soil. accumulation. metal uptake. Tobacco. Okra.

المُلخص

المعالجة النباتية للتربة الملوثة بالمعادن الثقيلة باستخدام نباتي الدخان والبامية

خلفية عامة: نتيجة للنشاط البشري المتزايد، زادت مشكلة تلوث التربة والمياه بالمعادن الثقيلة والتي تعد من أخطر المشاكل التي تواجه البيئة وتهدد حياة الإنسان. يُظهر هذا التلوث الكبير في البيئة مدى أهمية إيجاد حلول معالجة غير مكلفة وصديقة للبيئة. المعالجة النباتية هي تقنية معالجة بيولوجية، تستفيد من القدرات الفسيولوجية الجوهرية للنباتات لمعالجة الوسائط الملوثة. إنّ آلية المعالجة النباتية تعتمد بالأساس على تنظيف التربة والمياه بواسطة النباتات التي تقوم بامتصاص الملوثات وتراكمها، وبالتالي تقليل سميتها في البيئة. لذلك اهتمت هذه الدراسة باختيار نباتات متوفرة بكثرة في فلسطين وكذلك سهولة زراعتها و العناية بها. تم تقييم قدرة كلاهما على معالجة المعادن الثقيلة من التربة وكذلك كفاءتهما في المعالجة النباتية عن طريق قياس عدة عوامل حيوية.

الأهداف الرئيسية: تهدف هذه الدراسة إلى تقييم إمكانية نبات الدخان ونبات البامية في معالجة التربة الملوثة بالمعادن الثقيلة وكذلك قدرتهما على نقل هذه المعادن الثقيلة من التربة إلى أجزاء النباتات، وكذلك قياس مؤشرات النمو في النباتات.

منهجيات الدراسة: تمت زراعة الدخان و البامية في قوارير تحتوي على تربة ملوثة بالزنك و النحاس و الكادميوم و الرصاص. تم تحضير أربعة تراكيز مختلفة من كل معدن و مزجها جيداً مع التربة المُعدّة للتجربة. التراكيز النهائية في التربة كانت 300 و 500 و 800 و 1000 ملغرام زنك لكل كيلوغرام تربة، بينما للنحاس كانت التراكيز 50، 100، 200، 300 ملغرام نحاس لكل كيلوغرام تربة. بينما لعنصري الرصاص و الكادميوم كانت التراكيز في التربة 50، 150،100، 200 ملغرام لكل كيلوغرام من التربة. تعرضت النباتات للمعادن الثقيلة المحددة لمدة 60 يوم من بداية زراعتها إلى حصادها. بعد حصاد النباتات وتقسيمها إلى جذوع وجذور تم تحضيرها لقياس تركيز المعادن الثقيلة فيها وكذلك تم قياس تركيز المعادن الثقيلة في التربة باستخدام جهاز **(ICP-OES)**

النتائج الرئيسية: أشارت النتائج إلى أن معاملات الزنك والنحاس لم يظهرا أي تأثير حقيقي على أطوال نباتات الدخان. في حين أظهرت بعض التراكيز العالية من الكادميوم والرصاص انخفاضا حقيقياً في طول نبتة الدخان. و كذلك بالنسبة لنبات البامية فقد كان هناك انخفاضا حقيقاً لأطوال النباتات نتيجة زيادة تراكيز الكادميوم والرصاص والنحاس في التربة. بالإضافة إلى ذلك في نهاية التجربة لم يكن هناك أي تأثير حقيقي على محتوى الكلوروفيل بين المعاملات و المعاملة المرجعية في كلا

النباتين باستثناء بعض التراكيز العالية من النحاس. علاوةً على ذلك، لم يلاحظ أي انخفاض معنوي في وزن ثمار البامية بسبب التعرض للتربة الملوثة بالمعادن لمدة شهرين.

بالنسبة لكل من التبغ والبامية ، فقد أظهرت جميع المعاملات زيادة معنوية في تركيز المعادن الثقيلة في أنسجة النبات (الجذور والجذوع) مقارنه بالمعاملة المرجعية. كان لدى نباتات الدخان و البامية القدرة على نقل الكادميوم من الجذور إلى الجذوع. بالنسبة لعامل التراكم الحيوي فقد كان الترتيب لنبات الدخان على النحو الآتي: أولاً الكادميوم ثم الزنك ثم النحاس وبعد ذلك الرصاص، بينما في نبات البامية كانت أكبر قيمة للزنك ثم الكادميوم وبعد ذلك النحاس ومن ثم الرصاص. أما فيما يتعلق بتركيز المعادن في التربة، فقد كان أعلى متوسط نسبة إزالة للمعادن في نباتات الدخان 59.05% و 52.37% للرصاص و الكادميوم على التوالي. بينما في نباتات البامية كانت أعلى نسبة 57.65% و 51.72% للرصاص و الكادميوم على التوالي .

الاستنتاج العام :تشير هذه النتائج إلى أنّ الدخان و البامية لهما القدرة على معالجة التربة الملوثة بالمعادن الثقيلة، ويمكن أن ينمو كلاهما في تربة ملوثة بتركيزات عالية جداً من المعادن الثقيلة، بالتالي يعتبر نباتي الدخان، البامية ك ‘مُستَخرَج نباتي‘

الكلمات المفتاحية: المعالجة النباتية. معادن ثقيلة. زنك. نحاس. كادميوم. رصاص. تربة ملوثة. تراكم.

Introduction

Pollution as an Environmental Problem

Pollution is the introduction of harmful material (pollutants) or energy into the environment making its components unsafe or inappropriate to use. Pollutants harm the environmental physical components and wildlife and negatively impact human health.

Pollution can be natural or manmade. Natural pollution originates from volcanoes, dust, forest fire, …. etc. Manmade pollution, on the other hand, is more diverse and serious and includes, emissions from fossil fuel, industrial pollution, oil refineries, municipal incinerators, smelters, cement factories and sulphuric and nitric acid manufacturing ….. etc. (Vaseashta et al., 2007).

The average pollution varies from site to site according to the sources and the rate of pollution in the site. Nowadays, the World is facing a serious and risky problem because of pollution, the negative effects of pollution are hazardous for people who live in polluted areas. The groundwater or surface water in the contaminated areas are threatened to be polluted too, which threatens drinking sources for people. For pollution to occur, the source of pollution must come in direct or indirect contact with the resource (Brasileira de Ciências Brasil Bidone et al., 2001).

Heavy metals and radionuclides are considered one of the most dangerous pollutants in the environment, which are carcinogenic, cytotoxic, and mutagenic. Globally, many elements of different environments (water, soil, and air) became contaminated by a diversity of metals, that could harm living organisms and hinder the normal biogeochemical cycles (Majeti, 2014).

Soil Pollution:

Soil pollution occurs when foreign matters enter the soil and affect its chemical and physical composition. Anthropogenic activities are the main factor that affects soil pollution, as there are a lot of activities that contribute to soil pollution like; industry solid waste disposal, intensively applying insecticide and fertilizers, discharging of raw domestic and industrial waste water, emissions from cars using leaded fuels, mining and smelting …. etc. These pollutants fall into two broad categories; organic and inorganic (Mirsal, 2004).

Regularly monitoring specific soil sites is an important way to control pollution; this monitoring occurs by determining the amount and concentration of pollution in a given location and period. Monitoring should provide data about the nature of the pollutants, sources, causes, concentrations, distribution, and the probability of treatment (remediation). The polluted soil can be treated immediately in its place, or it may be carried out in proper reactors or containers according to the pollutants' level, the level of risk, and the economic and time constraints. There are four remediation technologies to treat polluted soil: Physical method, Chemical method, Biological methods, Fixation methods, and Thermal destruction methods (Mirsal, 2004).

Heavy Metals

High dense metallic elements are called heavy metals; their densities are more than 4 g/cm^3 or greater than water by five-times or more (Length, 2007; Tchounwou et al., 2012). Heavy metals threaten the environment and human life. This is because they are very toxic, even at low concentration, and their tendency to accumulate in the environment as well as in living organisms. Therefore, they stay in the environment for years, even after removing their source of pollution (Gall et al., 2015).

Heavy metal pollution of the environment has speeded up since the beginning of the industrial revolution. Human activities are the main cause of entering heavy metals into the environment and affecting human life by accumulating in food, living organisms, and even the atmosphere (Oliveira Ribeiro et al., 2005). Sources of heavy metal pollution include traffic emissions, burning wastes, dumping wastes, atmospheric deposition, chemical fertilizers, urban effluents, and wastewater drainage into agricultural land for a long time (Ghiyasi et al., 2010).

Heavy Metals and Human Health

Heavy metals can cause various diseases like asthma, hypertension, poisoning, cancer, nervous system damage, reduced growth, organ damage, and death (Malassa et al., 2014; Rajeshwar & Sevarkodiyone, 2018). Heavy metals affect human and can enter to the human body through water and food, they can also be absorbed through the skin from air. (Malassa et al., 2014). Cadmium exposure affects human health by affecting vital organs such as kidney. Excess secretion of low molecular weight of proteins such as; α- and β -microglobulin lead to tubular dysfunction, thus kidney damage. Cadmium exposure can also affect the skeletal system and cause osteoporosis as a result of bone minerals deficiency (Järup, 2003). (Fu & Xi, 2020) reported that some heavy metals including mercury and cadmium could lead to cellular damage e.g. mitochondrial metabolism. Heavy metals can also disrupting the metabolic enzymes and causing hormonal disruption (Houston,2007).

Heavy Metals in Soil

Soil properties affect the behavior of the heavy metals in the soil, whereas their behavior depends on the pH, cation exchange capacity (CEC), texture, and redox potential. The fate of heavy metals depends on physicochemical processes like sorption, migration, dissolution, complexation, occlusion, precipitation, diffusion into minerals, absorption and sorption by

microbiota, binding by organic substances, and volatilization. Accumulated heavy metals in soil are subjected to various mechanisms which lead to metal level reduction, like plant uptake, deflation, leaching, or erosion. Complete removal for heavy metal from soil is almost impossible because the processes of depletion are very slow (Kabata-Pendias, 2010). As Balabanova et al., 2015 reported, the uptake of heavy metals from soil relies on the type of the soil, PH, soluble content, plant growth stages, types of species, and others.

Although some of the metals are essential for organisms like Fe, Co, Mn, Cr, Cu, Mo, Zn, V, Ni, and W, where they are needed as micronutrients and act as cofactors in biochemical reactions, they are toxic and risky when they present in high concentration. Also, there are other heavy metals non-essential like Cd, Hg, Ag, Pb, and Cr and very toxic even at low concentration, these non-essential metals can enter plants and replace their essential homolog and interposing with biological functions (Manara, 2012; DalCorso et al., 2019). High levels of heavy metals were also reported in liver, lungs, kidneys, and heart of some animals from the West Bank (Swaileh et al., 2009).

In order to clean up heavy metal pollution, scientists try many methods and technologies. Some of these were complicated technologies that are coasty, consumes a lot of energy and require highly skilled personnel. Other approaches were remediation technologies that are simple to apply, easy and cheap. Among the latest technologies are bioremediation technologies, which have gained wide acceptance due to their ability to remove heavy metals and pollutants from soil and water.

Bioremediation is a process based on the use of living organisms to remove or clean the pollutants and harmful substances from the environment (Mulligan et al., 2001). This process has become important for researchers during the last few years as it is natural, low-coast, low

technology and easy to apply (Korzeniowska & Stanislawska-Glubiak, 2015; Kumar et al., 2017). Some of the most common types of bioremediation technologies are microbial bioremediation, phytoremediation and mycoremediation.

Phytoremediation

Phytoremediation is a bioremediation technique that removes and recedes the pollutants from soil and water by using different species of plants and algae, plants can transfer, uptake, and stabilize contaminants, as well as destroy it. (Paz-Alberto & Sigua, 2013). This new technology is rapidly expanding as it has many advantages. It can be applied *in situ* without the need to excavate polluted soil or water; suitable to clean up large contaminated areas; cost-effective compared to other remediation technologies, low energy requirements, does not produce secondary pollution; aesthetically pleasing; high public acceptance (Odjegba & Fasidi, 2007; Yang & Shen, 2020).

Phytoremediation cleans up soil or water by enhancing microbial growth which thrives in the rooting zone of cultivated plants and by the plants themselves which transform, absorb, and accumulate pollutants (Korzeniowska & Stanislawska-Glubiak, 2015; Yang & Shen, 2020).

Phytoremediation represents a valuable way of reducing, immobilizing, detoxifying, and eliminating heavy metals from the contaminated soil, water, wastewater, sediments, and sludge. Hence, five types of phytoremediation techniques have been identified (Fig. 1): phytoextraction, phytodegradation, rhizofiltration, phytostabilisation, and phytovolatilization (Subhashini & Swamy, 2015).

Phytoextraction or phytomining:

Some plants are capable of accumulating the pollutants in their above ground parts (shoots and leaves). These plants (called hyperaccumulators) are planted to remove (extract) the contaminants from the soil or water of a site to the plant harvestable parts. Finally, the crop is harvested and accumulated metals are dealt with properly. This technique can be used to extract valuable metals from soil (i.e Gold).

Phytodegradation:

Phytodegradation is the phytoremediation technique that degrades organic pollutants either through the release of enzymes from roots (such as dehalogenase and oxygenase) that destroy pollutants or through metabolic activities within plant tissues that work to degrade pollutants.

Rhizofiltration:

Rhizofiltration is the process of removing contaminants or excess nutrients from water or wastewater through adsorption onto or absorption into plant roots. Usually, used to clean up aqueous sources using terrestrial or aquatic plant.

Phytostabilisation:

Is the process of remediating contaminants from soil, sediment, and sludge by restricting contaminant mobility and movement using the plant's roots which can absorb, accumulate, or precipitate the pollutants. This prevents pollutants from diffusing through the food chain or migration into the groundwater.

Phytovolatilization:

Some plants can absorb the contaminants, and pass it through their vascular system from the roots to the leaves and then release the pollutants to the atmosphere by evaporation. This process is considered as valuable remediation on commercial projects. It is worth noting that contaminants may be modified along the way through plants (Wani, et al., 2012).

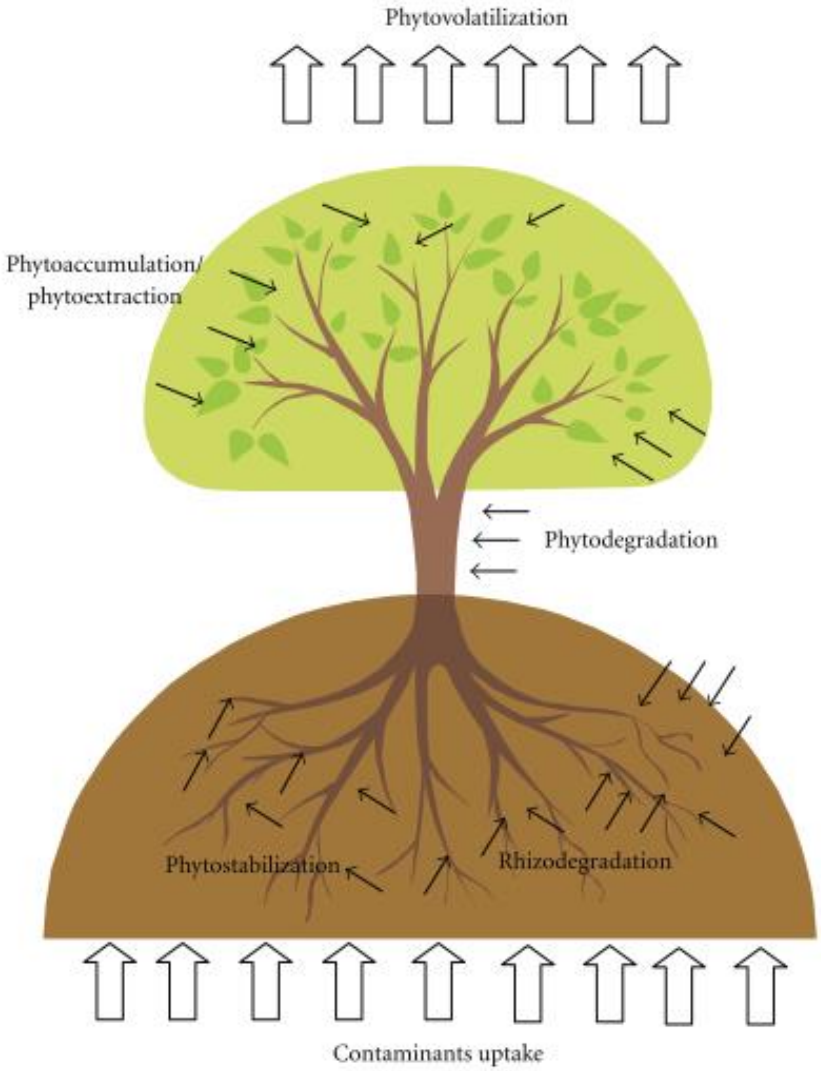

Figure 1: Phytoremediation strategies (Tangahu et al., 2011)

For phytoremediation, the plant will be selected in one of two ways; as a hyperaccumulator plant that has a potential to accumulate large amounts of metals and has a relatively low shoot biomass or a plant that produces a high shoot biomass with less ability to accumulate metals (Tlustoš et al., 2006).

Plants uptake the soil pollutants through the root, whereas the root system has mechanisms for preventing toxicity by a large surface area that absorbs and stores essential substance like nutrients and water for growth with other pollutants (Subhashini & Swamy, 2015).

Many plants have been used as phytoremediators, good pollutants accumulators (hyperaccumulators), and indicators of metal pollutants, most of them can accumulate only one toxic element (Khalid et al., 2018). Plants have improved themselves and developed various approaches to live and grow on soils contaminated by heavy metals (Balabanova et al., 2015).

A hyperaccumulator plant is defined as "A plant species whose shoots contain (in mg/kg^{-1}, dry weight) > 100 Cd, > 1000 Ni, Pb and Cu or > 10000 Zn and Mn when grown on metal rich soils" (Ali et al., 2012). So far, globally, four hundred plant types belonging to 45 families have been reported to be hyperaccumulators of metals. Brassicaceae is one of the most important groups of hyperaccumulators (Malik et al., 2010; Mudgal et al., 2010; Wu et al., 2010). Grasses showed higher ability to accumulate metals than shrubs and tree (Malik et al., 2010).

Plants parts collect and accumulate different concentrations of heavy metals, a high concentration of heavy metals was found to be accumulated in the edible as well as the inedible parts of vegetable species. (Overesch et al., 2007, Ismail et al., 2014).

Literature Review

Phytoremediation is cost-effective, aesthetically pleasing, consumes no energy, produces zero harmful emissions and easy to apply and manage. It searches and utilizes a group of plants known as “hyperaccumulators” to be used to absorb and accumulate toxic metals from soil or water.

The concept of removing and cleaning pollutants from the environment by plants is well known. In 1994, a study confirmed that *Thlaspi caerulescens* can be considered a successful hyperaccumulator due to its ability to accumulate very high concentrations of zinc from contaminated soil (Baker et al., 1994). This plant was the first one to be reported as a hyperaccumulator plant.

Phytoremediation can be applied to a wide range of pollutants, whether organic pollutants such as petroleum hydrocarbons or inorganic pollutants such as heavy metals (Cluis, 2004). The advantages of phytoremediation are the low cost, less destructive, improves ecosystem re-vegetation and restoration, environmentally friendly, aesthetic view of plant growing, as well as suitable to remediate a large area either in the terrestrial or aquatic environment (Majeti, 2014).

Okra plant (*Hybiscus esculentus L.)* is a seasonal edible herb planted throughout the World's tropical and subtropical areas. Okra could be planted as the sole crop or intercrop with yam and maize. It is rich in protein, carbohydrate, fats, minerals, and vitamins. So it very important in the human diet (Moyin-Jesu, 2007). Okra can easily absorb and fill its vacuoles with heavy metals (Ng, et al., 2016). Okra shoots were found to be filled with Pb and Zn, and the roots acted as a sink for Pb, Zn, and Cu, whereas its translocation factor and accumulation factor values are very high. Also, okra has a high tolerance for Pb accumulation in the roots and shoots (Ng, et al., 2016).

Tobacco plant (*Nicotiana tabacum L.*) is fast-growing and propagated (Stojanović et al., 2012). It is also known as hyperaccumulators for pollutants. The tobacco plant is used by plant physiologist and many examinations related to daylength, virus research, inanition, nitrogen metabolism, alkaloids, organic acids, and mineral deficiency inanition (Steinberg & Tso, 1958).

The tobacco plant is a good and promising plant for phytoremediation. Tobacco plants are very useful in absorbing pollutants, especially for sites contaminated with perchlorate. It also can accumulates Zn, Cu, Cd, Pb, and Mn in its leaves (Dguimi et al., 2009; Chitra et al., 2011).

Researchers work on changes in the selective gene of tobacco tolerance for heavy metals to enhance and improve the tobacco plants' ability of phytoremediation. To improve the accumulation, a combination of using natural chelators with tobacco was studied (Ellington et al., 2001; Sundberg et al., 2003; Boonyapookana et al., 2005; Pomponi et al., 2006).

Other studies were conducted to increase the phytoremediation efficiency of tobacco for methylmercury, Cd, Ni, and Zn by developing transgenic in tobacco plants (Daghan et al., 2010; Nagata et al., 2010; Chitra et al., 2011). A new strategy of transformation of the *N. glauca* of tobacco is considered promising phytoremediation (Pavlíková et al., 2004).

A study showed the ability of tobacco plants to phytoremediate uranium-contaminated mediums (uranium hyper-accumulator properties) (Stojanović et al., 2012). In addition, tobacco plant was found to be a very good accumulator for Cd (concentration in shoots reaching 100 mg kg^{-1}) (Bi, et al., 2011).

Toxicity of Heavy Metals to Plants

Heavy metals affect plant growth and biomass production and deactivates and damages plants' physiology and morphology. Plant growth can be affected by high concentrations of heavy metals in several ways; for instance, lead, nickel, and chromium affect chlorophyll production.

(Lamhamdi et al., 2013; Abou Auda et al., 2011); on the other hand, Cu, Cd, Ni, Pb, and Cr reduce the development of plants. (Zhi et al., 2015; Gardea-Torresdey et al., 2004). Additional studies were conducted on Ni, Cr, Pb, and Hg. it was reported that they affect the photosynthetic pigments and reduce the surface area of thylakoid as well as chlorophyll production. Also, they may affect electron transportation and enzymatic activity. (Gardea-Torresdey et al., 2005).

Though some plants find a way to adapt to contaminated media and grow in a polluted media with heavy metals, some of these plants can be considered metallophytes, properties of few of them depend on the presence of metals in the soil. *Thlaspi* plant is the most popular metallophytes in the World (Gardea-Torresdey et al., 2005).

Zinc is considered an essential element (needed by living organisms). It is common in alkaline soils. Zinc is essential for organisms; it works as a catalyst for the plants, also it has a role in the structural functions of plants. But, if it present in high concentration, it becomes toxic and lead to a decrease in plant growth and infertility (Ricachenevsky et al., 2015). Zinc in the soil can come from wastewater, urban fertilizers, emissions of waste incinerators, mining processing and the waste and residuals of these processes, and other anthropogenic activities (Zhao et al., 2003).

Although the elevated concentration harms the plants, some plants can accumulate high amounts of zinc and be beneficial for phytoremediation and remove the contaminants (Verkleij et al., 2009; Ricachenevsky et al., 2015).

Lugli & Mahler (2015); studied the behavior of cadmium, lead, and zinc contamination using simulations and numerical analysis, considering reactive transport and root process in phytoremediation. They found out numerical analysis is a useful experimental process for the phytoextraction process. Pedological and climatological data of a sub-tropical climate were taken to this simulation; accordingly, low water situations prefer stabilization for mobile contaminants,

but it restrains plant removal. On the other hand, the irrigation system that decreases crop water pressure had an adverse effect. For limited contaminant mobility, remediation wasn't useful or had significant advantages (Lugli & Mahler, 2015).

Ricachenevsky et al. (2015) found that trees tolerance mechanisms for toxic materials are higher than crops, as the concentration of heavy metals in trees is very high compared to agricultural crops. Therefore, they suggested focusing on zinc transporters' functional characterization in plants, all mechanisms related to uptake of zinc, and studying the relationship between homeostasis of zinc and physiological processes.

Copper is an essential micronutrient for living organisms. Natural Cu is the most plentiful element in mafic and intermediate rocks. The appropriate concentration of Cu in the soil is essential as a nutrient supply for humans, animals, and plants. Although Cu is classified with the most mobile heavy metals, Cu ions can precipitate with other ions like sulfide, carbonate, and hydroxide. So, Cu is considered an immobile element in the soil with a slight contrast in total content in soil characterization. Contaminated soil with Cu is created by fertilizers, agriculture waste, municipal waste, sprays, and emission from industrial facilities (Kabata-Pendias & Pendias, 2001).

Although some of the plants have a high tolerance to accumulate a high concentration of Cu in their tissues, Cu is considered a very toxic element (Kabata-Pendias & Pendias, 2001).

Korzeniowska & Stanislawska-Glubiak (2015) found out that *Spartina pectinata* is a suitable plant for Cu and Ni phytostabilisation. Sunflower plant was used in phytoremediation of Cu, as it is a fast-growing industrial oil crop with high biomass production (Jadia & Fulekar, 2008).

Cadmium Cd is the most toxic and dangerous non-essential element of the environment. It shows an adverse effect on the soil's biological activity, plant metabolism, and health of humans

and animals. Some plants can uptake Cd in an effective way; they have a high tolerance to accumulate Cd in their root and leaf systems. Cd is considered immobile in the alkaline media, but it has high mobility in acidic media, like soil with a pH that ranges between 4.5 to 5.5. It was found that Cd is more likely to accumulates in the surface soil (Kabata-Pendias & Pendias, 2001).

Several plants can accumulate Cd, like willow clones, Indian mustard, corn, alpine penny-cress, and sunflower. Cd is more likely to present in the contaminated soil with zinc; *Brassica juncea* could accumulate Cd from contaminated soil with a 200 mg Cd kg^{-1} dry weight (Jiang et al., 2003).

Wu et al, (2004) studied the ability of *Thlaspi caerulescens* to be phytoremediator in soil, and they found out it is a good Cd phytoremediator with 337 mg/kg^{-1} dry weight. They investigated *Thlaspi caerulescens* and they found that *T. caerulescens* could be a good phytoremediator in a soil. Kališová-Špirochová et al. (2003) they also reported that the *Z. mays* and *H. annuus* have a good ability to accumulate Cd (90 mg/kg dry weight) from Cd-contaminated soil.

Natural **Lead** is the least mobile non-essential element of heavy metal in soil; it originates from the bedrock. Contaminated soil with Pb obtained much attention as this element is very toxic for humans and animals. Pb can enter organisms through food, water, and soil dust inhalation. The concentration of lead in the surface soil is increasing in arable and uncultivated lands (Kabata-Pendias & Pendias, 2001).

Pb exist in the plants naturally, but there isn't any scientific basis that proves Pb is essential for any metabolic processes. The concentration of Pb within 2 to 6 ppb is helpful for the plant, but if this concentration increased, it will be considered toxic element to plant (Kabata-Pendias & Pendias, 2001).

Many plants can accumulate Pb effectively in high concentrations like *Brassica juncea*, which is used as a phytoremediator for Pb-contaminated soil at a concentration of 500 mg/kg Pb. In addition, *Zea mays* and *Helianthus annuus* can be used as Pb-phytoremediator for Pb-contaminated soil, which can accumulate up to 500 and 19 mg/kg dry weight of Pb respectively (Kališová-Špirochová et al., 2003).

(García et al. (2004) has reported that the *Piptatherum miliaceum* (Smilo grass) is a phytoremediator plant, whereas it can remediate soil contaminated with Pb at concentrations of 300 to 1,500 mg/kg Pb. Vogel-Mikuš et al. (2005) reported that a very high concentration of Pb can be accumulated in *T. praecox* that reaches 67,94 mg/kg DW. It is also proved that *Hemidesmus indicus* can act as Pb phytoremediation plant as it can eliminate more than 60% of Pb from Pb-contaminated soil at a concentration of 10,000 mg/kg (Chandra Sekhar et al., 2005).

Pollution in Palestine:

In Palestine, environmental issues, including pollution, are neglected due to mainly the complex situation caused by the Israeli occupation and settlements. Besides, environmental education and awareness campaigns are very limited and ineffective and environmental laws and regulations are rarely enforced.

In Palestine, two major sources of pollution can be recognized; solid wastes and wastewater. Other sources of pollution include; agricultural pesticides and fertilizers, quarries, water salinization … etc.

Wastewater networks cover about 50% of urban regions (Swaileh et al., 2001). In regions where sewer networks are lacking, wastewater from cesspits is transferred and emptied along roadsides of rural areas. Moreover, domestic and industrial wastewater from settlements containing high

concentrations of chemicals and heavy metals flows into agricultural lands contaminating soil and leaching hazardous material to groundwater.

Usually, the wastewater is treated by physical or chemical processes, both of which can be costly. However, chemical and physical processes are not effective in treating dye wastewater due to their complex molecular structure. There are also problems associated with disposing of concentrated sludge (Taştan et al., 2010; Abdel-Raouf et al., 2012). The key to treatment wastewater depends on removing biochemical oxygen demand (BOD), bacterial biofilm, toxicity, suspended solids, and nutrients (NO_3^-_N, NO_2^-_N, PO_4^{-3}_P, and NH_4^-_N). BOD is caused by the oxidation of organic material by microorganisms that use molecular oxygen to oxidize organic matter into CO2 and water. This can cause fish deaths and anaerobiosis through a reduction of dissolved oxygen in the water (Mantzavinos & Psillakis, 2004). Consequently, wastewater treatment focuses to reduce BOD levels. Further, removing nitrogen from wastewater is crucial; ion exchange, as well as nitrification and de-nitrification, are ways to address this problem (Abdel-Raouf et al., 2012; Wang et al., 2015). Over the past few years, biological methods have been more emphasized to remediate wastewater. The use of natural wastewater treatment methods has reentered; aquaculture systems are being used to treat and recycle domestic and industrial wastewater. They can be used to treat wastewater and address sanitary and environmental issues simultaneously while being economically feasible (Deng et al., 2007; Hussein et al., 2004). Aquatic macrophytes can remediate wastewater by accumulating heavy metals and toxic nutrients and by oxygen balance regulation through growing it in shallow ponds as an aquatic treatment system. Duckweed and water hyacinth are the most common of macrophytes that are used for bio-remediating wastewater, due to their immense biomass rate

(Sekomo et al., 2012). Cheng et al. (2002) studied the ability of duckweeds to grow at high level of nitrogen and phosphorus (240 mg/L of NH_4-N and 31.0 mg/L of PO_4-P), respectively.

The Applied Research Institute – Jerusalem (ARIJ, 2005) reported that services of collected solid waste in the West Bank and Gaza Strip cover almost 67% and 95% of the total population, respectively. Communities that are not covered with solid waste collection services dump and incinerate solid wastes openly and randomly. In any case, there are more than 180 open and uncontrolled dumpsites, which receive about 381,000 tons of solid waste every year in West Bank. 214 thousand tons of remaining wastes are burned each year on roadsides and empty lands (ARIJ, 2005) Solid wastes are burned in all open dumping sites, except in Abu-Dies and Nablus dumping sites where solid wastes are buried and covered with soil. Dura dumping site is the only dumping site that was reestablished and improved to be a sanitary and clean landfill (ARIJ, 2005). In addition, construction wastes are commonly dumped along roadsides of rural areas.

The disposal of hazardous solid wastes from Israeli settlements and industrial zones located in the West Bank causes serious environmental damage and results in grave harm to human health. In addition, large amounts of hazardous materials and wastes are illegally transferred from Israel and openly disposed in the West Bank causing soil and groundwater pollution. It was reported that more than 50 locations are used as dumping sites for settlements, which expose the Palestinian territories to the dangers of these wastes (ARIJ, 2015).

Hundreds of tons of e-waste are received every day to Palestinian townships from Israeli settlers. Ithna is one case of many communities; which receives 200-500 tons of e-waste and metal scrap every day (GIZ, 2014). According to ARIJ (2015) report, fertile lands are becoming contaminated as a result of illegal uncontrolled burning of e-wastes in order to obtain metals

from this e-waste. According to a study by Dabayneh et al. (2008), a natural alpha particle radioactivity was found in the soil in some parts of Hebron district.

All these practices result in damaging the water resources and soil, as well as polluting the air and affecting human health.

In an attempt to evaluate environmental pollution status in the West Bank, a few studies have been conducted. Malassa et al., (2013), studied several pollutants like pesticides, nitrates, chlorides, heavy metals, and analyzed their impact on the environment; Zn, Cu, Cd, Pb, Cr, TI, Co, Ni, Mo, Mn, and Ag metals are found out in analyzed groundwater samples from south of West Bank. The concentration of Cd, Al, and Pb of analyzed samples exceeded the limit of WHO level. 109.3 ppb of Pb was detected as the highest concentration of lead. Samhan & Ghanem (2009) studied the contamination of groundwater by nitrate and chloride in Tukaram area. They found that, in many samples, concentrations of nitrate were exceeding the WHO standards of 45 mg/L for drinking water.

Ghanem et al. (2011) also investigated the groundwater pollution with pesticides and heavy metals in the North West Bank. They detected Pb, Cd, and Cr compounds in the water with a Pb concentration higher than the WHO standards. According to a study by Swaileh et al. (2001) Nablus-Ramallah roadside soil and plants contained high concentration of metals, especially Pb. Therefore, awareness campaigns and environmental laws enforcement should go hand in hand with controlling, monitoring and remediating environmental pollutants in Palestine. (Swaileh et al., 2001 & 2009).

In Palestine, studies on hyper-accumulators and phytoremediation are almost lacking. Therefore, the present study aims at assessing the potential use of two plants to phytoremediate heavy metals (Cu, Cd, Pb & Zn) from artificially contaminated soil using phytoextraction technique.

Objectives

The main objective of this research is to investigate the potential use of two plants, tobacco (*Nicotiana tabacum*) and okra (*Hybiscus esculentus*) to phytoremediate heavy metal-contaminated soil through phytoextraction. Metals to be tested will include zinc, copper, lead, and cadmium.

This study will investigate the ability of two plants to transfer heavy metals from soil to different plant parts. The study will investigate different plant parameters like growth, chlorophyll concentration, root and shoot weights ….. etc.

Materials and Methods

In this study, two types of plants, *Nicotiana tabacum* and *Hybiscus esculentus,* were used to assess their ability to phytoremediate heavy metal-contaminated soil through phytoextraction. The plants were grown in pots containing soil contaminated with different concentrations of four heavy metals (Zinc, Copper, Cadmium, and Lead). After monitoring for 60 days, the experiment was terminated and the plants were harvested and partitioned into shoot and root. Finally, the concentrations of the four heavy metals were measured in soil and plant parts using ICP- OES.

Plant Material

Tobacco seedlings were purchased from a local plant nursery. Okra seeds were purchased from local market. Okra seeds were immersed in 4.5% $HCLO_3$ for three minutes and in distilled water for another three minutes. After that, the seeds were planted in a growing bed (Styrofoam seedling tray) with peat moss soil. Two to three seeds were planted in each well of the growing bed. After germination and growth, okra seedlings were transferred to mini-plastic disposable pots to reach the desired length of approximately 25 cm before transferring both types of seedlings into the metal contaminated soil.

Soil Preparation

Soil used in this experiment constituted of Terra rossa soil amended with Peatmoss and sand (Ratio by weight 1: 1:1) Terra rossa subsurface soil was obtained from Birzeit University campus garden (**Latitude:** 31° 57' 18.76" N; **Longitude:** 35° 10' 30.32" E) at a depth of 10 cm from the surface and transferred to the laboratory in plastic bags. In the laboratory, samples were sieved using an ecological sieve of 13.2 mm and 2.83 mm. Peatmoss and sand were purchased

from local market. From each one of the three soil types (Terra rossa, peatmoss and sand), 1kg was weighed and mixed well with the same weight from the other two types forming a final weight of 3 kgs of experimental soil in each pot.

Preparation of Metal Solutions

Glassware used was cleaned following the standard method of washing with soap, followed by acid wash using a mixture of hydrochloric and nitric acids (3:1). Finally, glassware was thoroughly rinsed with distilled water.

Four heavy metal salts were obtained from Biology Laboratories at Birzeit University to be used in this experiment. These salts were: zinc sulfate heptahydrate ($ZnSO_4.\ 7H_2O$; 287.5 g/mol, Sigma), copper (II) sulfate pentahydrate ($CuSO_4.\ 5H_2O$; 249.69 g/mol, Sigma), cadmium chloride anhydrous ($CdCl_2$; 183.32 g/mol, Sigma) and lead nitrate ($Pb\ (NO_3)_2$; 269.207 g/mol, Sigma). Stock solutions were prepared for each metal as 1000mg/L using analytical grade deionized water. From each stock solution, four concentrations of metals were prepared and mixed with the experimental soil to obtain the following nominal concentrations/kg soil: 300, 500, 800, and 1000 mg of Zn; 50, 100, 200, and 300 mg of Cu; 50, 100, 150, and 200 mg of Cd or Pb. Before being mixed with the experimental soil, the pH value of each solution was measured and adjusted to be between 5.13-5.25 using 1M HCl and 1M NaOH Lee & Chang (2011).

Experimental Design

After mixing with the corresponding heavy metal concentration, about 3kg of each experimental soils **(Table 1)** were kept in a 3L capacity black plastic pot (duplicate) at room temperature for 5 days to allow heavy metals to distribute evenly in the experimental soil.

Table 2: Weights of experimental soil used for each treatment.

Pot #	Tobacco		Pot #	Okra	
	Treatment	Soil Weight (kg)		Treatment	Soil Weight (kg)
1	Control-1	3.0059	1	Control-1	3.0001
2	Control-2	2.9930	2	Control-2	3.0193
3	Zn 300-1	3.0105	3	Zn 300-1	3.0297
4	Zn 300-2	2.9848	4	Zn 300-2	3.0562
5	Zn 500-1	2.9730	5	Zn 500-1	3.0223
6	Zn 500-2	3.0087	6	Zn 500-2	3.0148
7	Zn 800-1	3.0045	7	Zn 800-1	3.0193
8	Zn 800-2	3.0134	8	Zn 800-2	3.0113
9	Zn 1000-1	3.0181	9	Zn 1000-1	3.0124
10	Zn 1000-2	3.0159	10	Zn 1000-2	3.0061
11	Cu 50-1	2.9981	11	Cu 50-1	2.9915
12	Cu 50-2	3.0259	12	Cu 50-2	3.0107
13	Cu 100-1	3.0055	13	Cu 100-1	3.0244
14	Cu 100-2	3.0063	14	Cu 100-2	3.0439
15	Cu 200-1	3.0089	15	Cu 200-1	3.0022
16	Cu 200-2	3.0042	16	Cu 200-2	3.0169
17	Cu 300-1	3.0079	17	Cu 300-1	3.0333
18	Cu 300-2	3.0006	18	Cu 300-2	3.0163
19	Cd 50-1	2.9979	19	Cd 50-1	3.0323
20	Cd 50-2	3.0005	20	Cd 50-2	3.0149
21	Cd 100-1	3.0010	21	Cd 100-1	3.0309
22	Cd 100-2	3.0077	22	Cd 100-2	2.9993
23	Cd 150-1	3.0010	23	Cd 150-1	3.0114
24	Cd 150-2	3.0050	24	Cd 150-2	4.0148
25	Cd 200-1	3.0022	25	Cd 200-1	3.0633
26	Cd 200 2	3.0062	26	Cd 200-2	3.0330
27	Pb 50-1	2.9916	27	Pb 50-1	3.0160
28	Pb 50-2	3.0006	28	Pb 50-2	3.0434
29	Pb 100-1	3.0043	29	Pb 100-1	3.0159
30	Pb 100-2	3.0031	30	Pb 100-2	3.0284
31	Pb 150-1	2.9982	31	Pb 150-1	3.0612
32	Pb 150-2	3.0054	32	Pb 150-2	3.0329
33	Pb 200 1	3.0022	33	Pb 200-1	3.0120
34	Pb 200-2	3.0049	34	Pb 200-2	3.0268
35	Mix-1	3.0031	35	Mix-1	3.0634
36	Mix-2	3.9958	36	Mix-2	3.0354

The control experimental soil was not amended with any heavy metal solution. The "Mix treatments" were composed of experimental soil contaminated with the following concentrations of the four metals: Zn (300 mg/kg), Cu (100 mg/kg), Cd (100 mg/kg), and Pb (100 mg/kg).

After the plants reached a desirable length, they were arranged randomly into groups of four. Every group of four plants was planted with equal distances in one pot. After that, pots were placed in outdoor setting exposed to full sun light. Pots were randomly aligned into four columns to ensure the distribution of conditions to all pots and throughout the experiment the pots positions were randomly changed.

The study started in July and was concluded in September 2019. During this period, the plants were irrigated every two to three days and fertilized with NPK 22-8-11 fertilizer (1.75g/L) at a rate of 200 ml per pot every three weeks. The plants were also sprayed with 1.5 ml/L of the insecticide Dursban (Chlorpyrifos; $C_9H_{11}Cl_3NO_3PS$), three times during the experiment.

Plants Growth and Samples Processing:

The shoot length of each plant was measured at the beginning of the experiment and weekly thereafter. Similarly, chlorophyll content of each plant was determined by a chlorophyll meter (SPAD 502 Plus, Konica Minolta, Japan). In addition, plants were monitored and any signs of stress were recorded.

Finally, plants were harvested and washed well with tap water and rinsed with distilled water. Plant roots were cleaned thoroughly from adhering soil. For each plant, root length was measured from the point where the root was attached to the stem to the longest root tip. The shoot length was measured from the beginning of the stem to the apical meristem. After that, plants were divided into shoots, roots, and fruits (for okra plant). Roots, shoots, and okra fruits were separately weighed to record fresh weight.

Finally, all plant samples were oven dried at 60 °C to reach a constant weight. The dried samples were then weighed and recorded as dry weight for the plant. The samples were prepared for acid

digestion by grinding the plant samples well using a stainless-steel grinder, then storing the fine powder in clean 50 ml Falcon tubes.

Soil Samples Processing:

At the beginning and the end of the experiment, soil samples were randomly collected (from surface, middle and bottom) of each pot and stored in 50 ml falcon tubes. Later, samples were oven dried at 60 °C to reach constant weights. After that, soil samples were ground using pestle and mortar and then sieved through a 0.62 mm sieve, and stored in clean 50 ml Falcon tubes.

Samples Digestion and Metal Analysis:

The acid digestion of plant and soil samples was carried out using a microwave digestion system (MARS 6™, CEM®, USA). 0.5 grams of the plant samples and 0.2 grams of the soil samples were weighed using an analytical balance and placed into the MARSXpress Plus vessel. Next, 10 ml of concentrated suprapur nitric acid (69%) were added to the vessel, where the mixture was gently swirled and let to settle for 15 minutes as a pre-digestion step. After that, the torque was used to close the digestion vessels. Digestion vessels were aligned in the router of the device.

The programmed method was followed as recommended by the manufacturer. The digestion process went through three stages; the first stage was the ramping stage (15 min), the second as the holding stage (15 min), and the third was the cold stage (20 min). At the end of the digestion, volume of the digest was adjusted to a final volume of 10 ml with double distilled water. The digest was poured into a 25 ml clean glass vial, capped with Teflon and stored in the refrigerator (2-8 °C) for later heavy metal analysis. Blanks and two certified reference materials (V-10 hey and SL-1 Lake sediment; International Atomic Energy Agency) were run with the samples, in order to assess the accuracy of the analytical procedure. Finally, plant and soil digests were assayed for total content of Zn, Cu, Cd, and Pb using an Inductively Coupled Plasma Optical

Emission Spectrometer (Avio® 200 ICP-OES, USA) in the Testing Laboratories Center (TLC) at Birzeit University.

Calculations:

All concentrations of metals in plant and soil samples are expressed as mg/kg dry weight basis. Bioaccumulation factors (BF), bio-concentration factor (BCF) and translocation factor (TF) were calculated in order to compare the accumulation and translocation of heavy metals from root to shoot of the plants. These factors are expressed by the following formulas (Korzeniowska & Stanislawska-Glubiak, 2015):

$$\text{BAF} = \frac{Metal\ concentration\ in\ aboveground\ organ\ \left(\frac{mg}{Kg}\right)}{Metal\ concentration\ in\ soil\ \left(\frac{mg}{Kg}\right)}$$

$$\text{BCF} = \frac{Metal\ concentration\ in\ belowground\ organ\ \left(\frac{mg}{Kg}\right)}{Metal\ concentration\ in\ soil\ \left(\frac{mg}{Kg}\right)}$$

$$\text{TF} = \frac{Metal\ concentration\ in\ aboveground\ organ\ \left(\frac{mg}{Kg}\right)}{Metal\ concentration\ in\ belowground\ organ\ \left(\frac{mg}{Kg}\right)}$$

Statistical Analysis:

All data were expressed as Means ± Standard errors of the means. Data analyses were performed by IBM SPSS Statistics (version 26). One-way analysis of variance (ANOVA) was used to compare between means. Tukey Post-hoc test was used to determine which specific means are different. Pearson correlation and simple regression tests were performed when necessary.

Results and Discussion

A. Plant growth, chlorophyll content, and biomass

This experiment is a pot experiment, which was conducted in open air under the same conditions for both plants. Plants were monitored during the experiment and plant growth (shoot length) and chlorophyll content were recorded for all tobacco and okra plants at the beginning of the experiment (W0), after 1 week (W1), after 2 weeks (W2), after 4 weeks (W4) and at the end of the experiment (W8). Results are represented by figures below, while detailed descriptive statistics of the results can be found in the Appendix section.

1. Effect of Metals on Shoot Length:

I. Zn:

Shoot lengths of tobacco plants subjected to different concentrations of Zn for 2 months are shown in **Figures 2**. **Shoot lengths of tobacco plants** continued to increase normally and no significant difference in shoot length was observed between the control group and the treatments or between the treatments with each other. The average length of tobacco plant groups ranged between about 25 cm (Zn 800) and 39 cm (Zn 500). The average of the control group was about 30 cm. This indicates no significant adverse effects of Zn concentrations (up to 1000mg Zn/kg soil) on the growth of tobacco plants. No other abnormal growth symptoms were observed on any of the plants during the 2-month experiment.

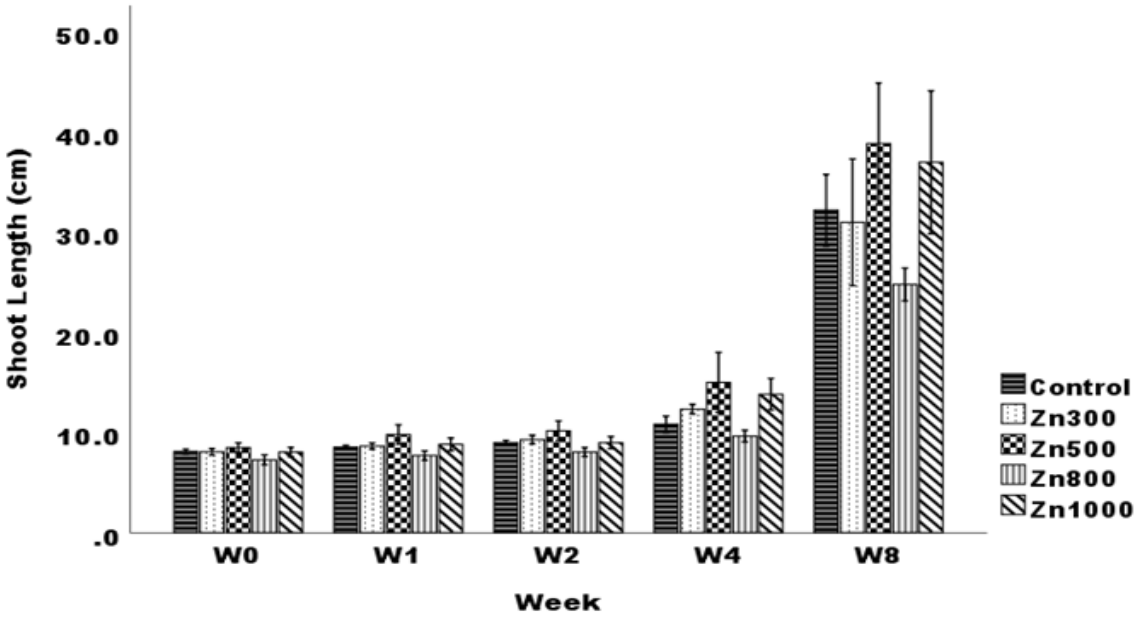

Figure 2: Shoot lengths of tobacco plant groups subjected to different Zn concentrations for two months. Values represent means±SE of 4 plants from 2 pots. No significant difference between the control and treatments at P<0.05.

Figure 3 shows **shoot lengths of okra plant** groups subjected to different concentrations of Zn. Average shoot lengths of okra plant groups at the end of the experiment ranged between about 43 cm (800 mg/kg soil) and 69 cm (1000 mg/kg soil). The average shoot length of the control group at the end of the experiment was 65 cm. On week 8, statistical analysis showed slight significant difference between the control group and Zn800 group. The average shoot length of Zn group was less than that of other groups from the beginning of the experiment. The highest shoot length among treatments was observed at Zn 1000 with no significant difference compared to the control. No other abnormal growth symptoms were observed on any of the plant groups subjected to Zn.

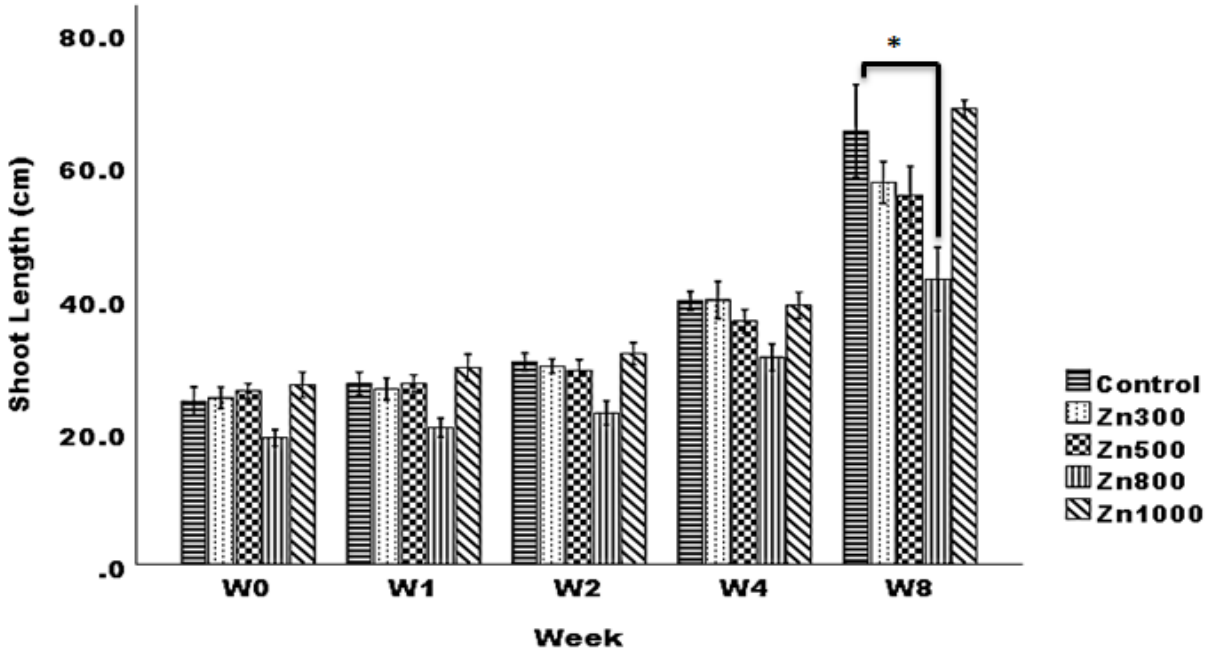

Figure 3: Shoot lengths of okra plant groups subjected to different Zn concentrations for two months. Values represent means±SE of 4 plants from 2 pots. *: Represents significant difference at P< 0.05.

As an essential metal, Zn can promote plant growth up to certain concentrations. Besides, plants can have mechanisms to deal with excess amounts of essential metals including Zn. The positive response of plants to the increasing Zn concentrations is due to the great role of Zn in physiological and biochemical processes in plants. Zn participates in metabolic reactions of carbohydrates, proteins, and auxins (Amooaghaie et al., 2017). Besides, several studies have shown that zinc has a significant role in growth and development of the reproductive parts of the plants, as well as it has a significant role in flowering and fertilization process (Pandey et al., 2006; Zhang et al., 2013; Davarpanah et al., 2016).

Zhi et al. (2015) found that the shoot length of *Eruca sativa* increased by 39.67% at a concentration of 5 mM Zn compared to 3 mM Zn. In addition, shoot length of *Eruca* significantly increased in all Zn concentrations (0.2Mm to 5mM Zn) compared to 0 mM Zn (Zhi

et al., 2015). Aydinalp & Marinova. (2009) reported that alfalfa plant had increased shoot growth by an average of 10 % more than the control at 40 ppm Zn. They concluded that the high concentrations of Zn increased the alfalfa's growth. These results are in agreement with the present study where at W8, Zn 1000 showed 20% & 2% higher growth than the control groups of tobacco and okra, respectively.

II. Cu

Figure 4 shows the relationship between Cu concentrations and **tobacco plant shoot lengths**. Results indicated no significant difference in shoot length between plant groups after being subjected to Cu for 2 months. The average shoot length of the control groups was about 32 cm, while the average of the treatments ranged between about 25cm (Cu 50) and 37 cm (Cu 100). The difference in percentage growth between the control and Cu300 was about 1% only.

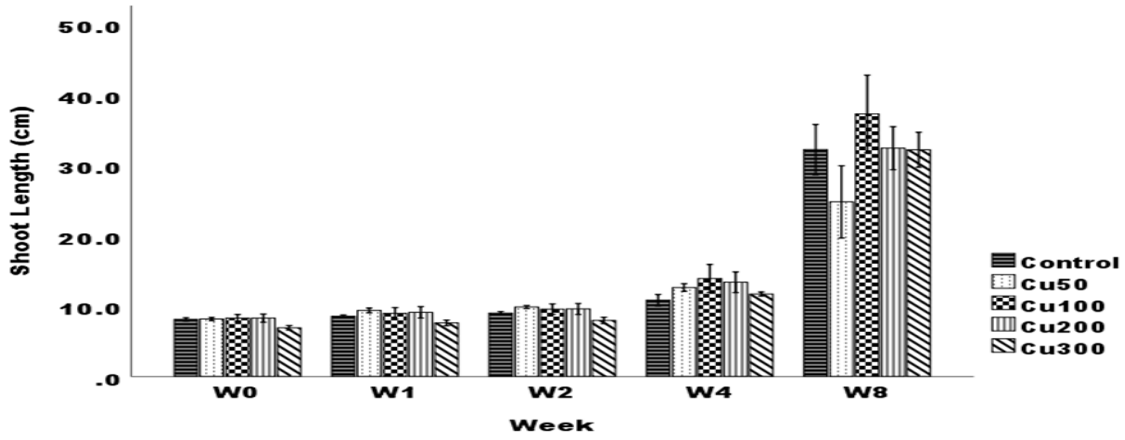

Figure 4: Shoot lengths of tobacco plant groups subjected to different Cu concentrations for two months. Values represent means±SE of 4 plants from 2 pots No significant difference between the control and treatments was observed at P<0.05.

In contrast to tobacco plants, **okra plants** were slightly affected by an increasing gradient of Cu concentration in soil. At the end of the experiment, mean shoot lengths of the groups ranged

between about 65 cm (Control) and 40 cm (Cu100). The decreasing trend in shoot length with increasing Cu concentration was clear (**Figure 5**). As Cu concentrations increased the shoot length of okra plants decreased. Statistical analysis showed a significant difference between the control group (65 cm) and Cu200 & Cu300 groups (39 cm & 40 cm, respectively). The highest shoot length of okra groups subjected to Cu was for Cu50 (64.00 cm). This indicates a negative impact of increasing Cu concentration in soil on okra plant growth.

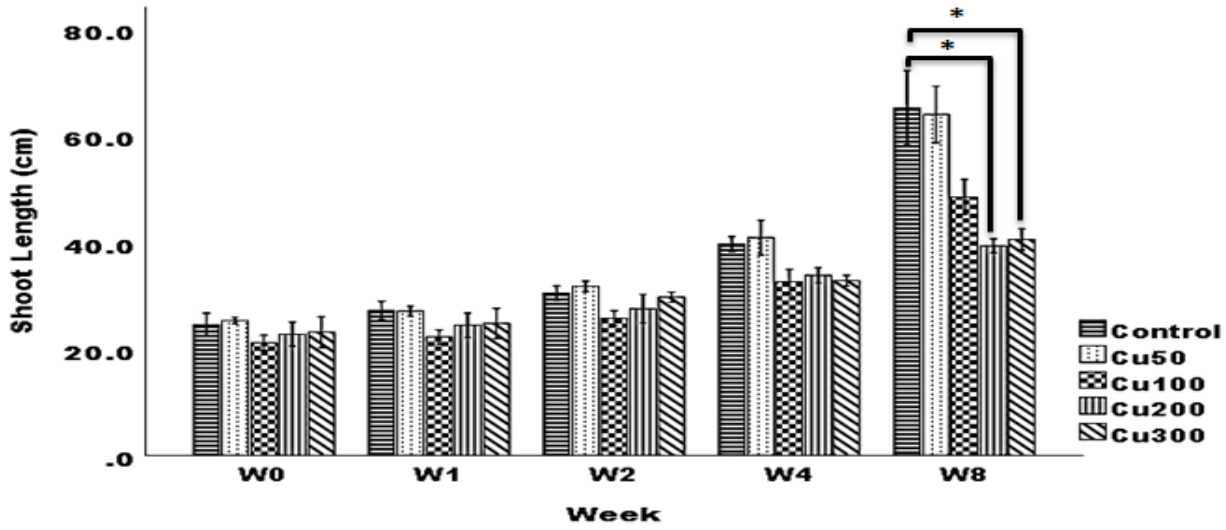

Figure 5: Shoot lengths of okra plant groups subjected to different Cu concentrations for two months. Values represent means±SE of 4 plants from 2 pots. *: Represents significant difference at P< 0.05.

Copper is an essential micronutrient, its deficiency causes a defect in plant growth through its role in photophosphorylation of the photosynthesis process, carbohydrate distribution, nitrogen fixation, and metabolism of the cell wall, in addition to oxidation-reduction processes in the electron transport chain. Cu also enters many enzymes as a structural-functional element (Jadia & Fulekar, 2008). Shoot growth of sunflower plant increased at 5, 10, and 20 ppm concentrations

of Cu (Jadia & Fulekar, 2008). However, they reported that the concentration of 50 ppm had significant decreases in shoot length compared to the control.

The same trend was observed in Ng et al. (2016). Cu treatments had the most effect on the growth parameters of okra plants compared to Zn and Pb treatments. They reported that the okra plants at Cu treatment had the lowest number of leaves compared to the control and other treatments. Gardea-Torresdey et al., (2004) reported a significant reduction of shoot length of *Convolvulus arvensis* after 15 days of growth in agar-based media containing 40 and 80 mg/l Cu compared to the control by an average of 43%, while 20 mg/l Cu increased shoot length with no significant differences compared to control.

III. Cd:

Figure 6 shows the effect of Cd soil concentrations on **shoot length of tobacco** plants. Results indicated that tobacco shoot lengths followed a clear decreasing trend with increasing Cd concentration. This became statistically significant by the end of the experiment where mean shoot lengths of all treatment were statistically less than that of the control group. Shoot length of the control reached 32.2 cm after 8 weeks, whereas mean shoot length of all treatments ranged between 18.45 cm and 15.4 cm. Shoot length in Cd exposed tobacco plants decreased by 52.4%, 65.6%, 68.6% & 70.0% for Cd50, Cd100, Cd150 & Cd200, respectively (**Figure 7**). Mean growth reduction of all treatments was 64.9% compared to the control.

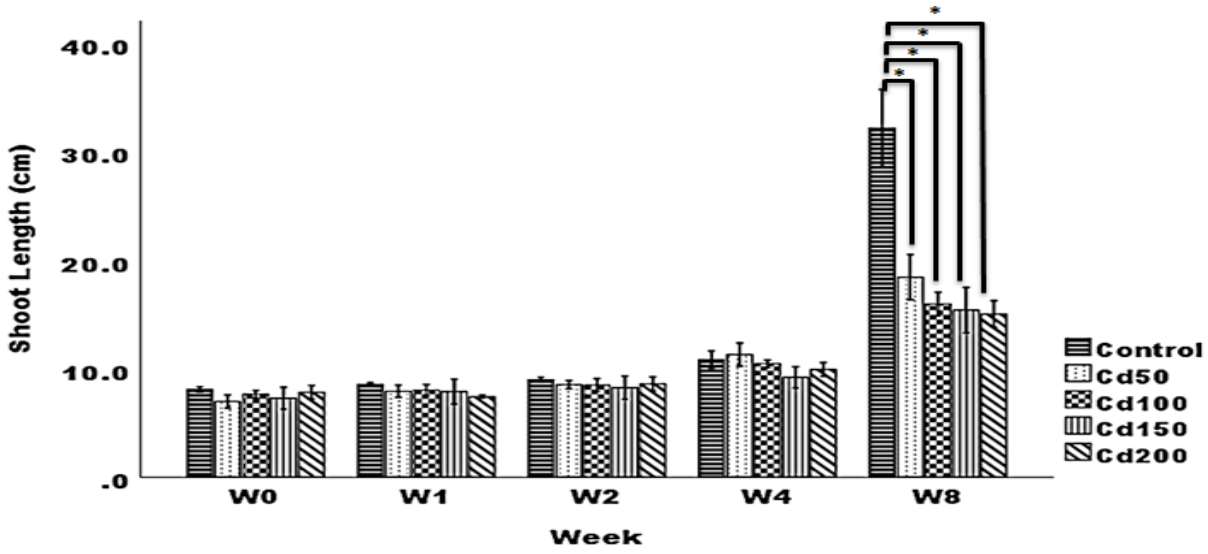

Figure 6: Shoot lengths of tobacco plant groups subjected to different Cd concentrations for two months. Values represent means±SE of 4 plants from 2 pots. *: Represents significant difference at P< 0.05.

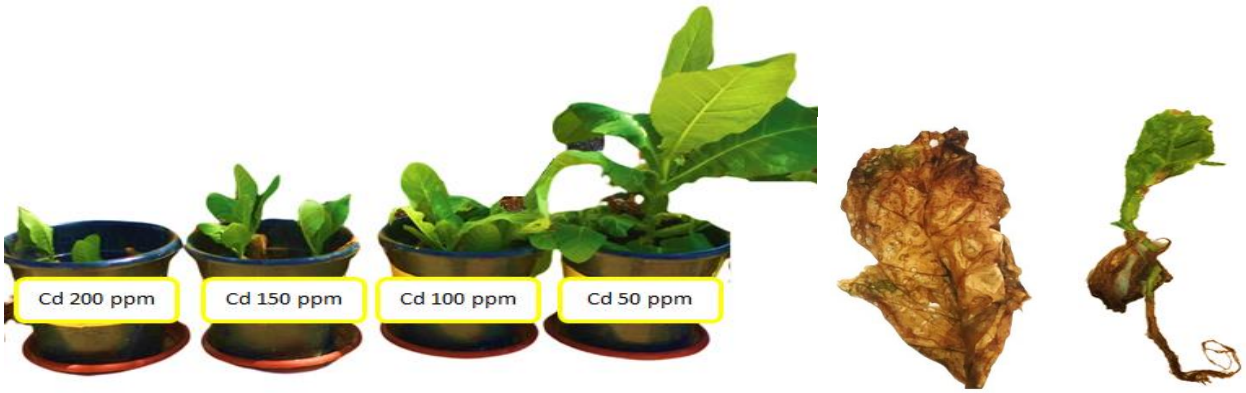

Figure 7: Effect of Cd contaminated soil on tobacco plant growth after two months of exposure.

Figure 8 shows the effect of different Cd soil concentrations on **okra plant growth**. Shoot lengths of plants subjected to Cd concentrations decreased significantly from the second week on (Cd200). By week 8, all mean shoot lengths were significantly less than that of the control group. At the end of the experiment, the average shoot length of the control reached 65.25 cm; while those of the treatments ranged between 38.2 cm (Cd200) and 43.2 cm (Cd 100). By the end of

the experiment, mean okra shoot lengths of the treatments were reduced by 61.6%, 57.5%, 54.2%, & 50.1% for Cd50, Cd100, Cd150, and Cd200, respectively. The average reduction in growth of all treatments was 55.85%.

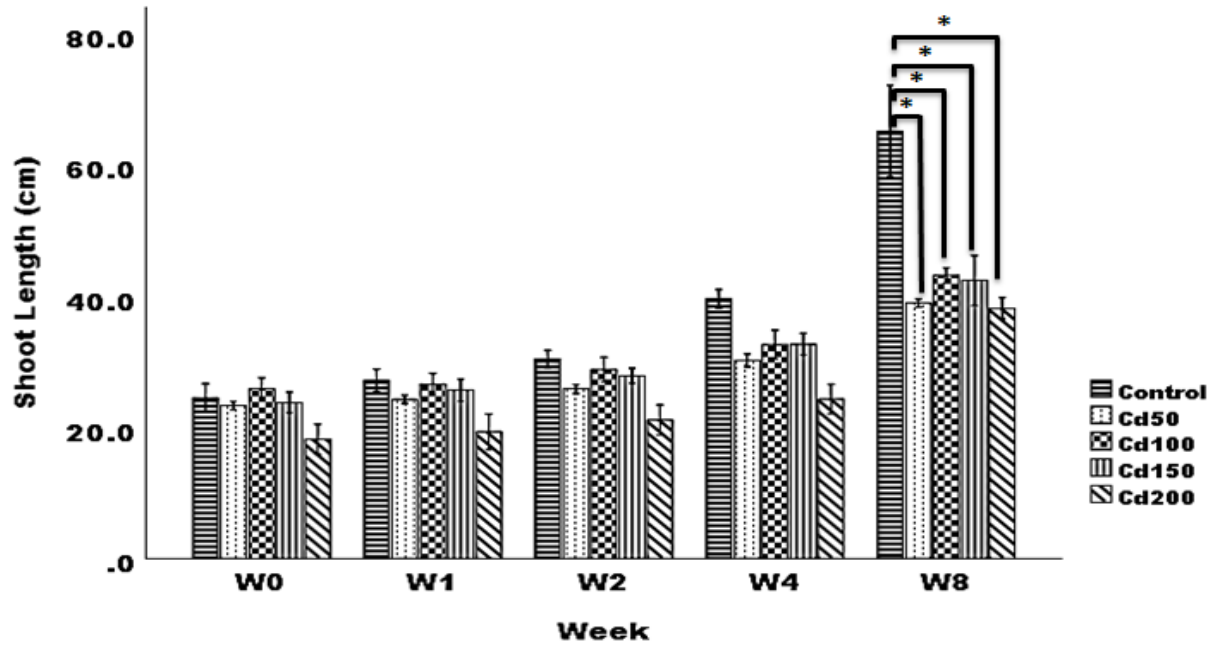

Figure 8: Shoot lengths of okra plant groups subjected to different Cd concentrations for two months. Values represent means±SE of 4 plants from 2 pots. *: Represents significant difference at P< 0.05.

Cd is a non-essential heavy metal that can accumulate in biological systems. Results of the present study are in agreement with the results of Chitra et al. (2011) on tobacco plants subjected to Cd contaminated soil using 10, 30, and 50 mg/kg of Cd. Zhi et al., (2015) reported that 5 mM Cd and 100 mM Cd decreased the shoot growth of *Arabidopsis halleri* by 45 % and 82%, respectively.

Cadmium can be toxic to plant in high concentrations (Abdulkhaliq et al., 2012). In the current study, some toxicity symptoms were observed on tobacco plants subjected to Cd (**Figure 7**). Cd

toxicity symptoms were growth inhibition, chlorosis, necrosis, and browning of root tips. Cd was reported to inhibit Fe (II) reductase in roots causing Fe (II) deficiency. Many symptoms of heavy metal toxicity are reported by Nagajyoti et al. (2010). Cd was reported to be absorbed enormously by *Brassica juncea*; this was combined with early development of oxidative stress confirming toxicity of this metal (Szollosi et al., 2009)

IV. Pb

Mean **shoot lengths of tobacco plants** subjected to different concentrations of Pb are shown in **Figure 9**. Statistically significant differences between control mean shoot length and treatments shoot lengths were observed only during the 8th week of the experiment. The mean shoot length of the control reached 32.2 cm while those of the treatments ranged between 14.35 cm (Pb200) and 32.6 cm (Pb 50). Compared to the control, growth of the plants subjected to Pb was reduced by 25%, 11%, 2%, & 65.5% for Pb50, Pb100, Pb150 & Pb200, respectively. The average reduction in growth of all treatments was 25.4%.

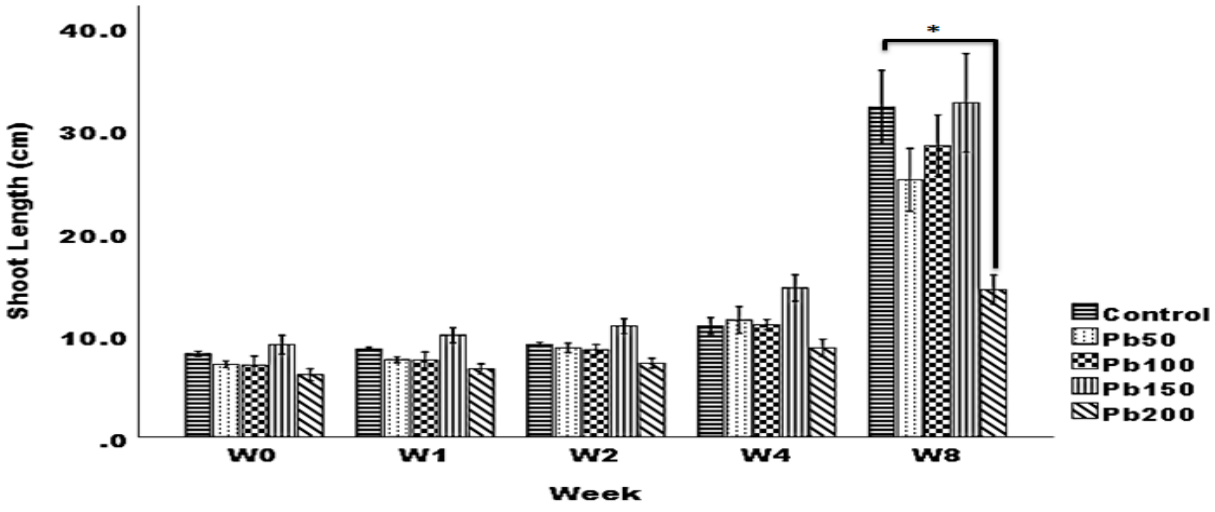

Figure 9: Shoot lengths of tobacco plant groups subjected to different Pb concentrations for two months. Values represent means±SE of 4 plants from 2 pots. *: Represents significant difference at P< 0.05.

Figure 10 shows the **mean shoot lengths of okra plants** during the two-months experiment. Mean shoot lengths of okra plants subjected to Pb had some significant differences from the control at the end of the experiment. Plants subjected to Pb50, Pb100, & Pb150 had significantly less shoot lengths than the control. Pb200, did not show such a difference by week 8 of the experiment. By the end of the experiment, the growth inhibition of the treatments compared to the control were 47.2%, 37.9%, 50.4% and +30.9% for Pb50, Pb100, Pb150, & Pb200, respectively. The average growth reduction for all treatments was 26.1%. The mean shoot length of the control group was 65.25cm, while those of the treatments were 42.25cm, 44.08 cm, 40.93 cm & 72.6 cm for Pb50, Pb100, Pb150 & Pb200, respectively.

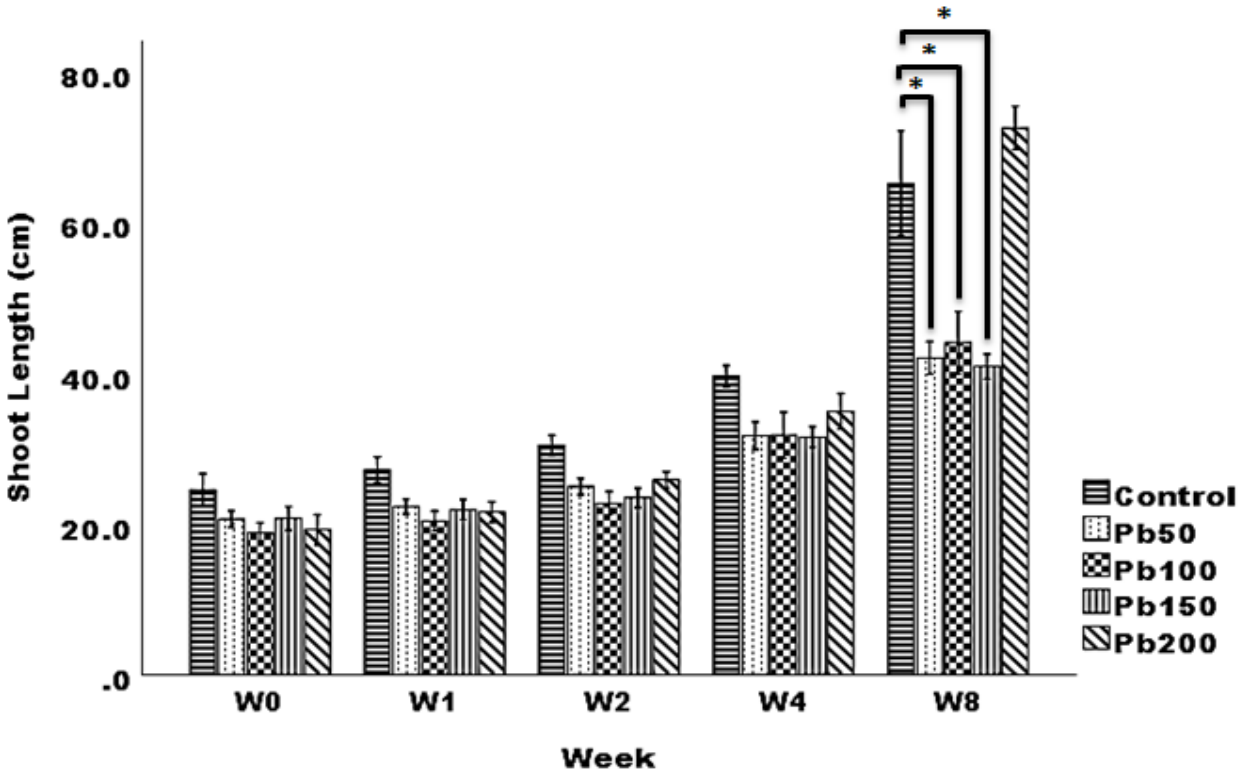

Figure 10: Shoot lengths of okra plant groups subjected to different Pb concentrations for two months. Values represent means±SE of 4 plants from 2 pots. *: Represents significant difference at P< 0.05.

Jadia & Fulekar (2008) showed that the shoot growth of Sunflower (*Helianthus annuus*) increased at level 5, 10, and 20 ppm of Pb. whereas, the 40 and 50 ppm Pb significantly reduced the shoot growth compared to the control plants. Alia et al. (2015) disclosed that the shoot length of Spinach (*Spinacia oleracea*) significantly decreased by 13 % compared to control at 500 mg Pb /kg soil.

Pb is a nonessential metal for any metabolic processes. The concentration of Pb within 2 to 6 ppb is helpful for the plant, but if this concentration increased, it will be considered toxic element to plant (Kabata-Pendias & Pendias, 2001). Houda et al., (2016) reported that some heavy metals as Pb limit the absorption of Ca, Fe, and Mg by disrupting the assimilation of these fertilization elements.

V. Mixture of Metals

Figure 11 shows results of **shoot length of tobacco plants** subjected to a mixture of metals. Statistical analysis revealed no significant difference between the control and the treatments during the whole experiment. Similar results were reported by Peralta-videa et al. (2002), when subjecting alfalfa plants to a mixture of Zn, Cu, Cd, and Ni.

Similarly, **Okra shoot length** did not show any significant difference from the control after two months of exposure to the metal mixture (MIX) (**Figure 12**). This indicates tolerance of the two plants to conditions where the environment is contaminated with a mixture of metals.

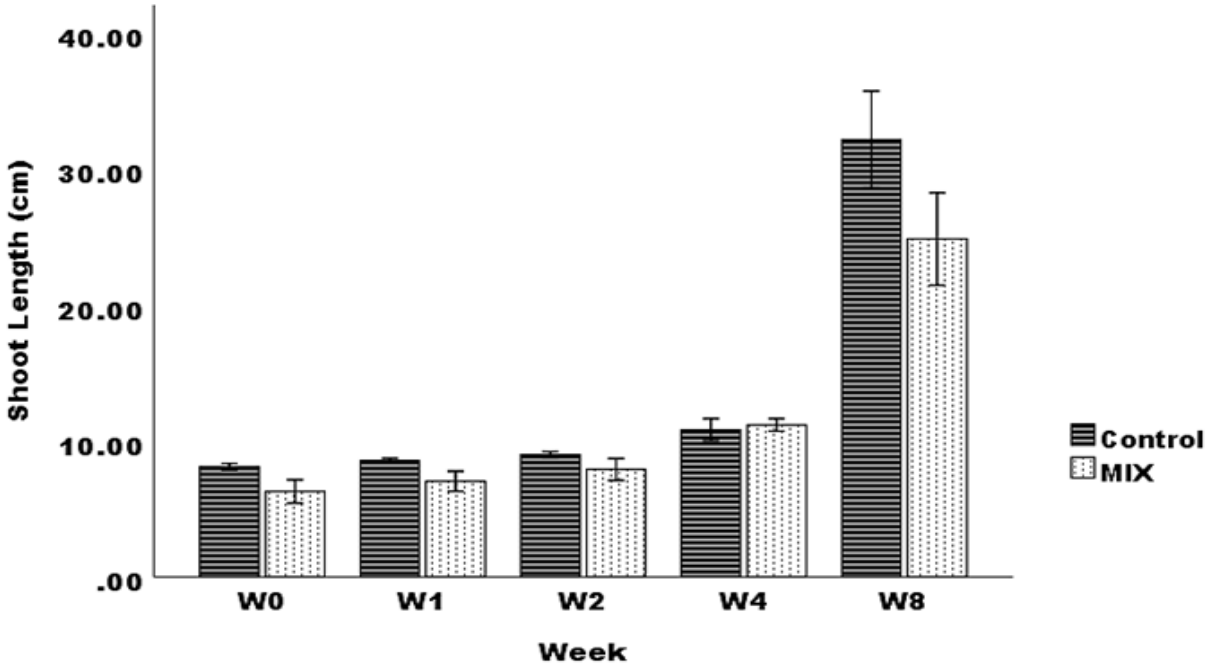

Figure 11: Shoot length of tobacco plants subjected to mix treatment for two months. Values represent means±SE of 4 plants from 2 pots. No significant difference was observed between the control and MIX at P<0.05.

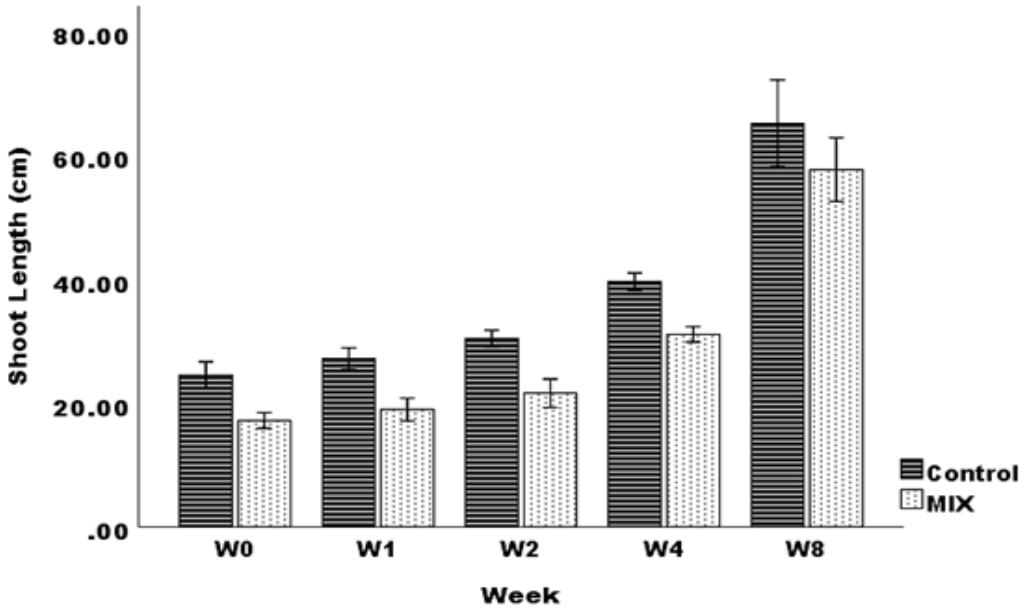

Figure 12: Shoot length of okra plants subjected to mix treatment for two months. Values represent means±SE of 4 plants from 2 pots. No significant difference was observed between the control and MIX at P<0.05.

2. Effect of Metals on Chlorophyll Content:

As an important photosynthetic pigment, chlorophyll is a major factor that determines the photosynthetic capacity and consequently, the growth of a plant. In normal response to growth and survival, plants alter the chlorophyll content to optimize light absorption and energy harvesting.

I) ZN

Figure 13 shows **chlorophyll content of tobacco** plants subjected to different Zn concentrations for 2 months compared to the control. Statistical analysis showed no significant difference in chlorophyll content between different groups. This indicates that Chlorophyll synthesis was not affected by different Zn concentrations. Although insignificant this may have been due to additional optimization measures taken by the plants to adapt to the harsh soil conditions imposed.

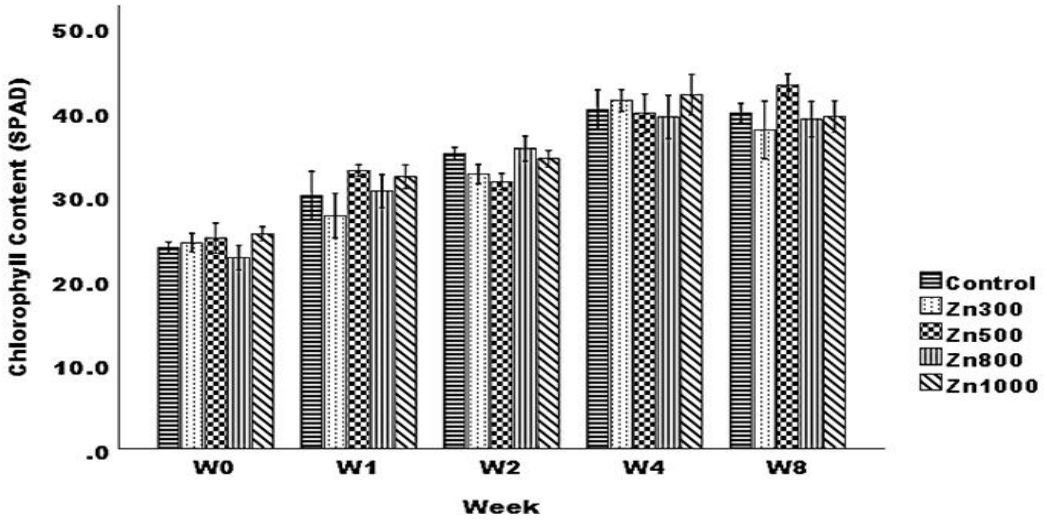

Figure 13: Chlorophyll content of tobacco plant groups subjected to different Zn concentrations for two months. Values represent means±SE of 4 plants from 2 pots. No significant difference between the control and treatments (P<0.05).

Similarly, **okra plants** subjected to different Zn concentrations did not show any significant difference in chlorophyll from the control **(Figure 14)**. This confirms that Zn concentrations,

under the current experimental conditions, do not affect chlorophyll synthesis in the plants studied.

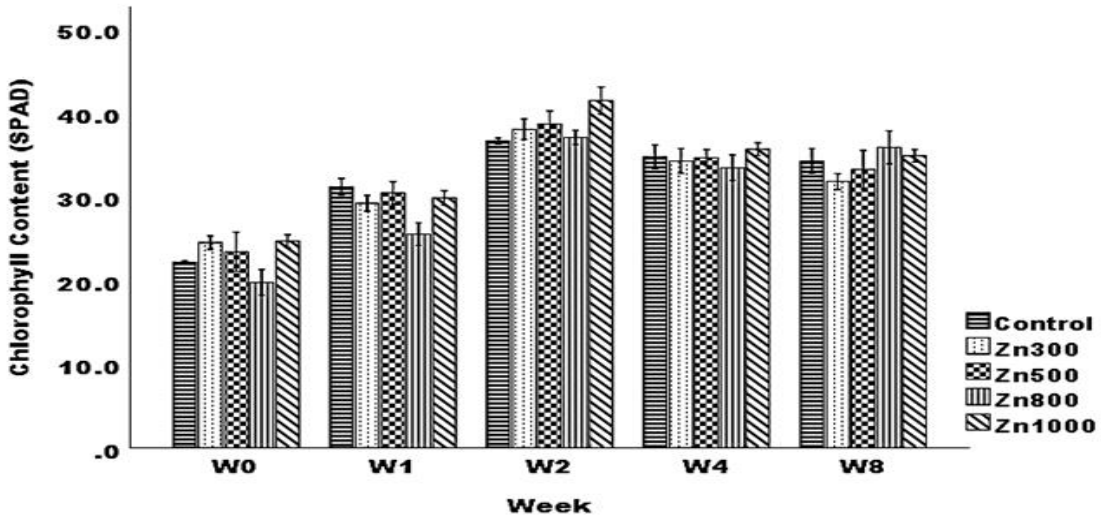

Figure 14: Chlorophyll content of okra plant groups subjected to different Zn concentrations for two months. Values represent means±SE of 4 plants from 2 pots. No significant difference between the control and treatments (P<0.05).

Some studies found that Zn significantly increased spinach chlorophyll a and b production (Abou Auda et al., 2011). However, there was no significant difference in total carotenoids between the control plants and treated Zn-plants. In contrary, Lefèvre et al. (2009) reported that the chlorophyll a and b and carotenoids of *Dorycnium pentaphyllum* were significantly decreased compared to the control when subjected to Zn at 100 µM.

II) Cu

Figure 15 shows **chlorophyll content of tobacco** plants subjected to Cu. After 8 weeks of exposure, no significant difference between the control and the treatments was observed. The chlorophyll content ranged between 34.68-43.43 chlorophyll units (SPAD).

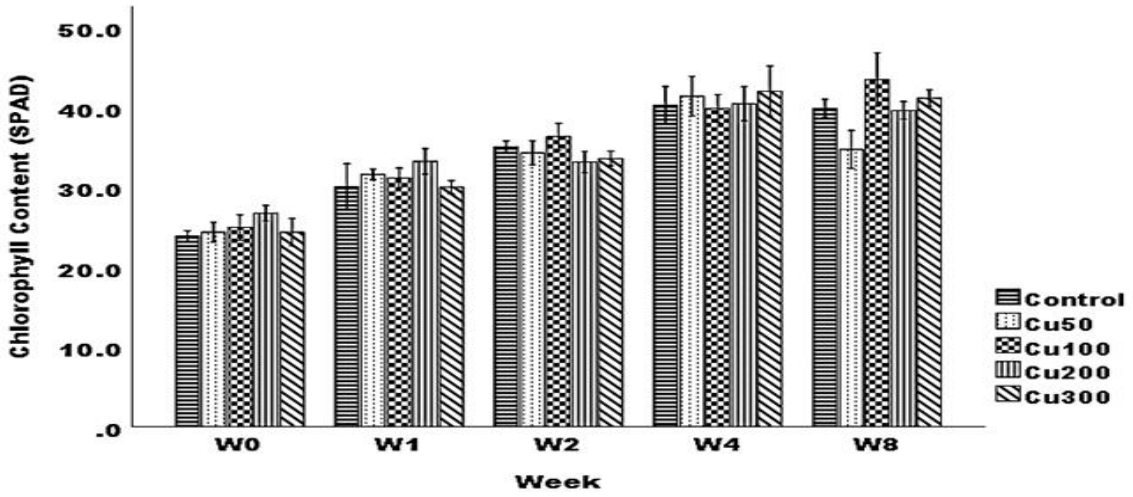

Figure 15: Chlorophyll content of tobacco plant groups subjected to different Cu concentrations for two months. Values represent means±SE of 4 plants from 2 pots. No significant difference between the control and treatments (P<0.05).

On contrary to tobacco, **okra plants** showed some statistically significant reduction in chlorophyll content after 8 weeks of exposure **(Figure 16).** Plants exposed to soil contaminated with Cu100 & Cu200 showed significantly less chlorophyll than the control. In addition, okra plants subjected to Cu showed some morphological signs of toxicity. These were mainly chlorosis and necrosis **(Figure 17).**

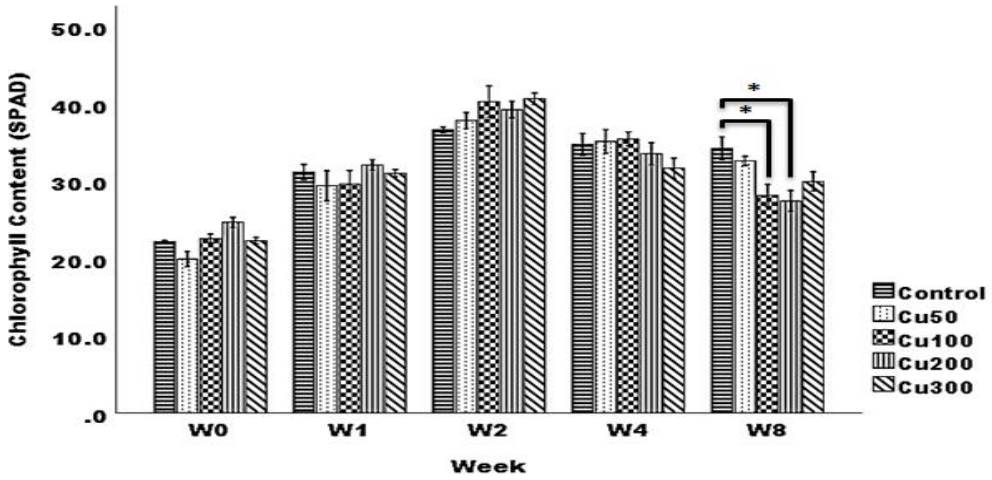

Figure 16: Chlorophyll content of okra plant groups subjected to different Cu concentrations for two months. Values represent means±SE of 4 plants from 2 pots. *: Represents significant difference at P<0.05.

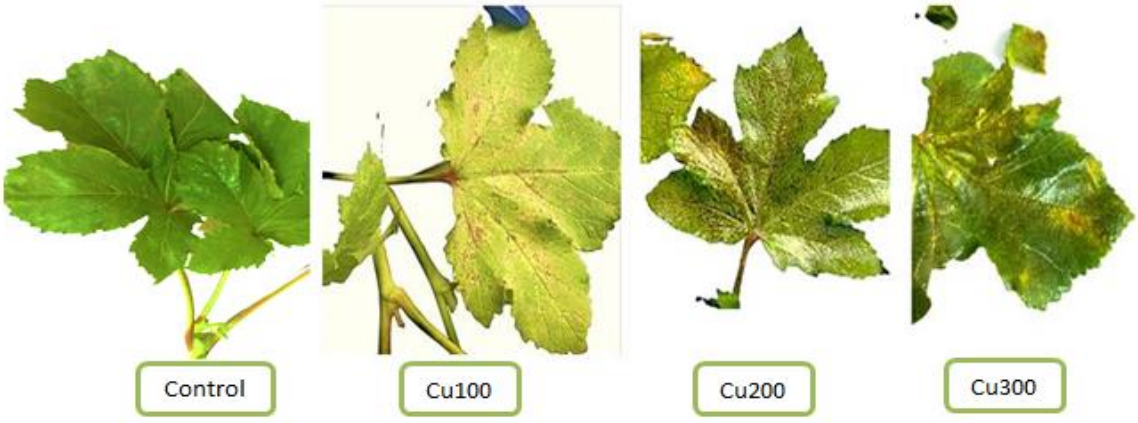

Figure 17: Cu toxicity symptoms on *Hybiscus esculentus* (okra) compared to the control.

Prasad et al. (2001) reported that the chlorophyll content and photosynthetic pigments in *Lemna trisulca* L. (duckweed) were significantly decreased in Cu at concentrations of 25 and 50 µM as well as the degradation of photosystem II of the plant. They also reported that the Cu treatment produced more morphological effects related to chlorophyll than Cd treatments. Cu could affect the membranes of chloroplasts by peroxidation; consequently, chlorophyll content was crucially decreased. Same results were reported in many studies, Shakya et al. (2008) study in *Thuidium delicatulum* (L.) and *Thuidium. sparsifolium*, Martins & Mourato (2006) in tomato plants.

III) Cd

Unlike shoot length, **chlorophyll content of tobacco** plants exposed to Cd did not show any significant difference from the control after 8 weeks of exposure **(Figure 18)**. The chlorophyll content ranged between 30.22 SPAD (Cd200) and 39.8 SPAD (Control).

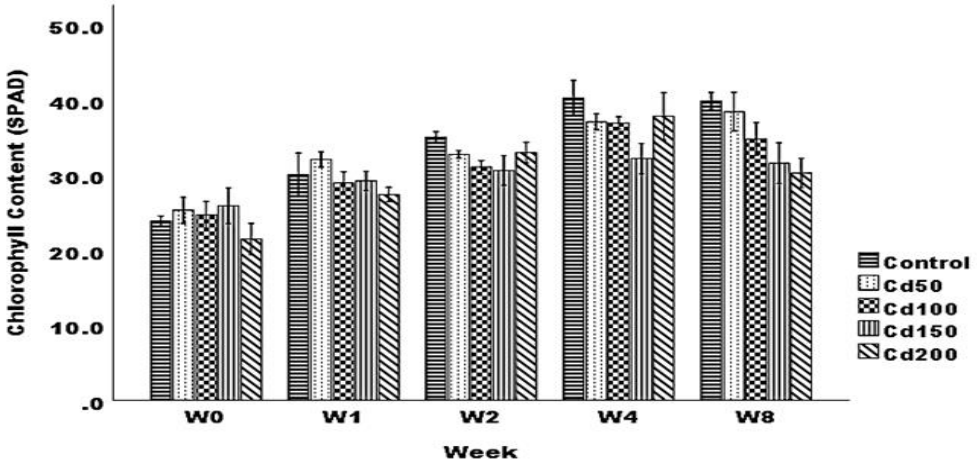

Figure 18: Chlorophyll content of tobacco plant groups subjected to different Cd concentrations for two months. Values represent means±SE of 4 plants from 2 pots. No significant difference between the control and treatments (P<0.05).

Similarly, **okra plants** subjected to Cd-contaminated soil did not show any significant effect on chlorophyll content of treatments and the control (**Figure 19).**

This indicates no observed toxicity of Cd to both plants when exposed to Cd under the current experimental conditions.

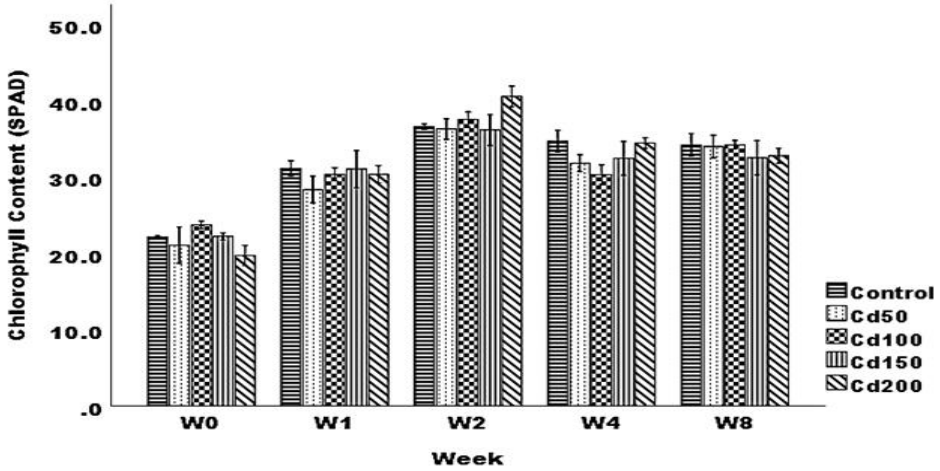

Figure 19: Chlorophyll content of okra plant groups subjected to different Cd concentrations for two months. Values represent means±SE of 4 plants from 2 pots. No significant difference between the control and treatments (P<0.05).

Abou Auda et al. (2011) found that increasing Cd concentrations decreased spinach chlorophyll a content. They also reported that chlorophyll b and total carotenoids were not affected. Hassanein et al. (2017) reported that wheat photosynthetic pigments decreased when grown in soil solution contaminated with 0.5- and 1.5-mM Cd. However, another study showed that chlorophyll b and carotenoids significantly increased at high dose of Cd (100 µM) (Lefèvre et al., 2009). Some other studies found that high Cd concentrations reduced the production and stability of chlorophyll by affecting Fe and Mg uptake, which are essential elements for chlorophyll production (Nagajyoti et al., 2010).

IV) Pb

Chlorophyll content of tobacco plants exposed to Pb treatments ranged from 34 to 41 (SPAD) slightly different compared to control plants (39.80 SPAD) **(Figure 20)**. **In okra**, the chlorophyll content in Pb treatments was ranged (30.98 to 35.98 SPAD) (**Figure 21)**.

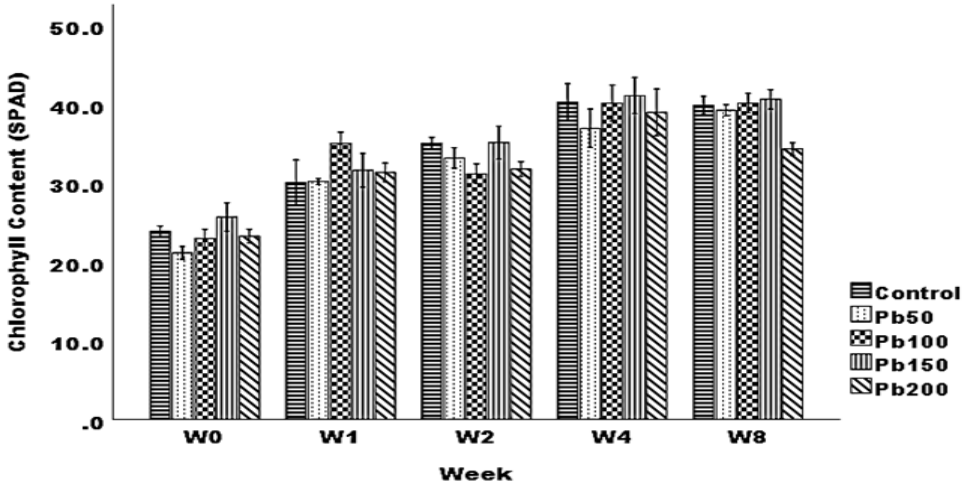

Figure 20: Chlorophyll content of tobacco plant groups subjected to different Pb concentrations for two months. Values represent means±SE of 4 plants from 2 pots. No significant difference between the control and treatments (P<0.05).

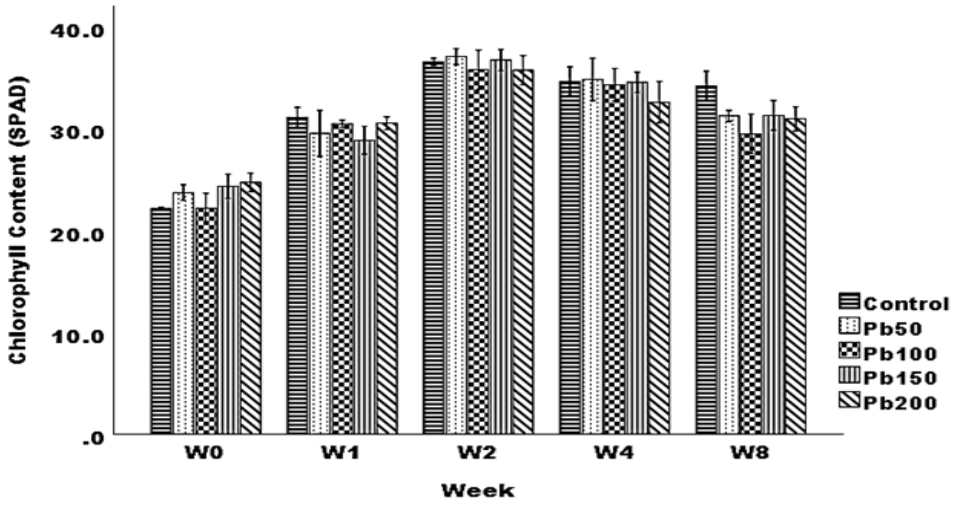

Figure 21: Chlorophyll content of okra plant groups subjected to different Pb concentrations for two months. Values represent means±SE of 4 plants from 2 pots. No significant difference between the control and treatments (P<0.05).

Heavy metal presence especially in high quantities leads to developing toxicity symptoms affecting the photosynthetic activities (Myśliwa-Kurdziel et al., 2004). Toxic heavy metal levels can leads to a reduction in photosynthetic pigments as well as the development of chlorosis caused by either reduce chlorophyll synthesis or increased chlorophyll destruction (Mobin & Khan, 2007, Nouairi et al., 2006). Heavy metals Toxicity doesn't only affect the photosynthetic pigments but also leads to inhibition of Calvin cycle, reduction in CO2 fixation and pigments aggregation as well as the formation of ROS which induces Chloroplast damage (B. Ali et al., 2015). Most authors concluded that the decrease in the pigments is caused by the inhibition of photosynthetic enzymes.

Faizan et al. (2014) conducted a pots experiment to assess the effect of treated wastewater (TW) and fertilizers in the production yield, growth, and heavy metal accumulation in okra plants. They reported that the concentration of heavy metals (Cd and Pb) is higher in soil irrigated with TW than in soil groundwater-irrigated (control). Okra growth was raised in plants treated with

TW compare to control. Besides that, the photosynthetic parameters and chlorophyll were high in plants treated with TW.

V) MIX

Figures 22 & 23 show **chlorophyll concentrations in both tobacco and okra plants**, respectively. No significant difference between treatments and the control was observed after 8 weeks of exposure of both plants to a mixture of metals. This indicates a good degree of tolerance of these plants to metals and their mixtures.

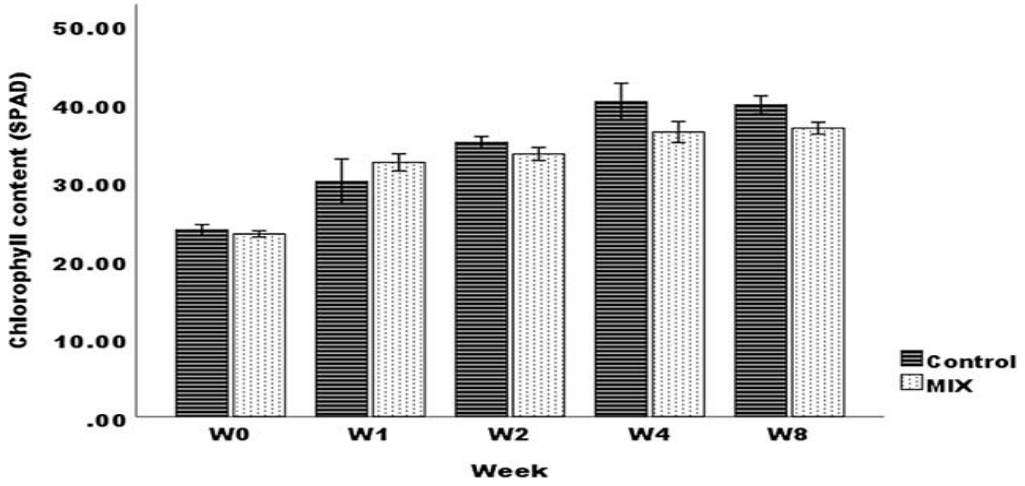

Figure 22: Chlorophyll content of tobacco plants subjected to MIX for two months. Values represent means±SE of 4 plants from 2 pots. No significant difference between the control and MIX (P<0.05).

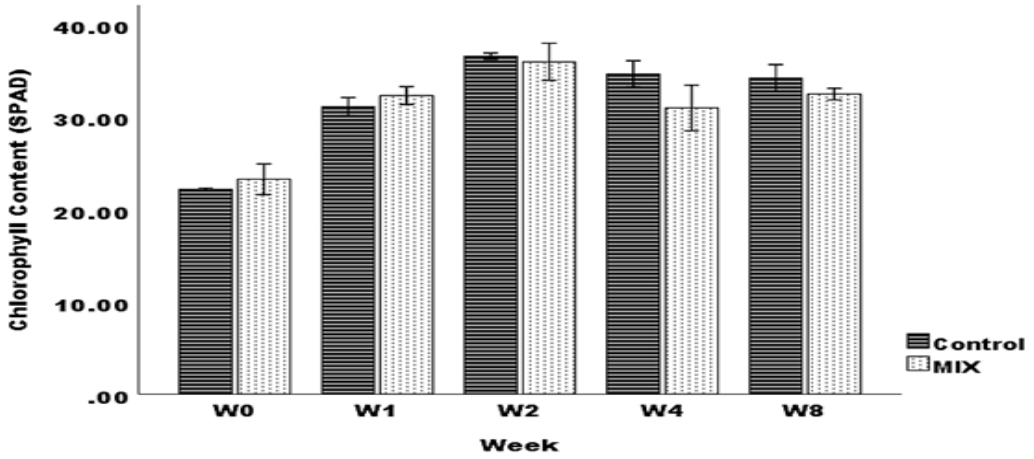

Figure 23: Chlorophyll content of okra plants subjected to MIX treatment for two months. Values represent means±SE of 4 plants from 2 pots. No significant difference between the control and MIX (P<0.05).

3. Effect of Metals on Shoot, Root and Fruit Biomass

In phytoremediation studies, plant biomass is very important as it may reflect the ability of plants to accumulate biomass and thus, heavy metals. Hyperaccumulator plants are characterized by having large biomass yields (Ali et al., 2013).

a. Effect of Metals on Plant Shoot Weight:

Figure 24 shows the **shoot dry weight of tobacco plants** subjected to different metal concentrations for 2 months. Statistical analysis revealed no significant difference between all treatments and the control. This indicates no significant effect of metals and concentrations studied on tobacco shoot dry weight. This is of great importance for possible use of tobacco in bioremediation of metal-contaminated soil.

On contrary to tobacco, **okra shoot weight** was affected by some metals and treatments after 2 months of the experiment **(Figure 25).** Zinc was the only metal that did not affect dry weight of the shoot of okra significantly. Okra planted in soil contaminated with Cu200

showed significantly less shoot dry weight than the control**.** Cd100, 150 & 200 significantly reduced the dry weights of okra compared to the control**.** Similarly, Pb100 & 150 reduced the dry weights of okra shoots significantly**.**

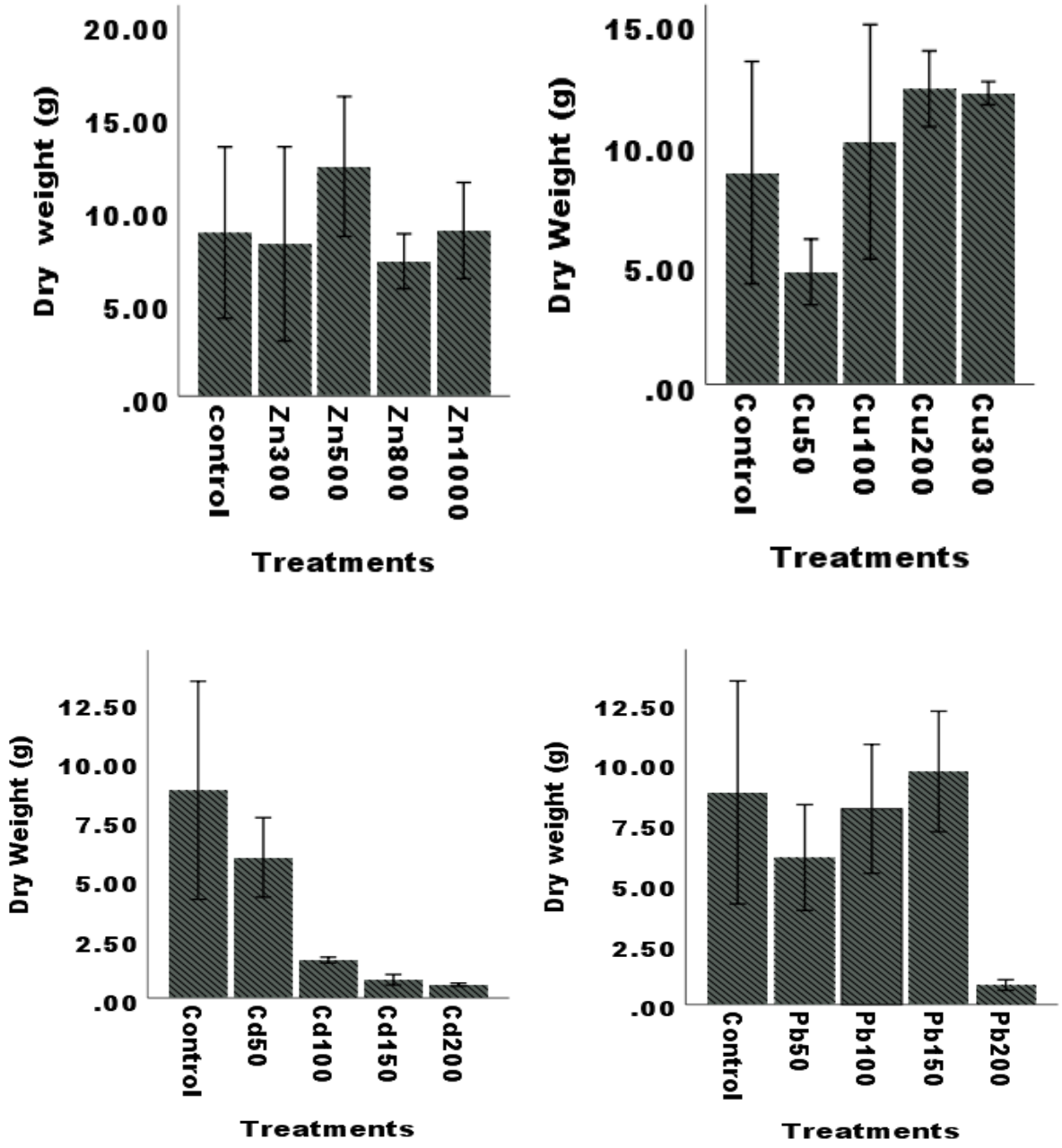

Figure 24: Effect of metals on tobacco shoot dry weight (mean ± SE, N=4). No significant difference between the control and treatments (P<0.05).

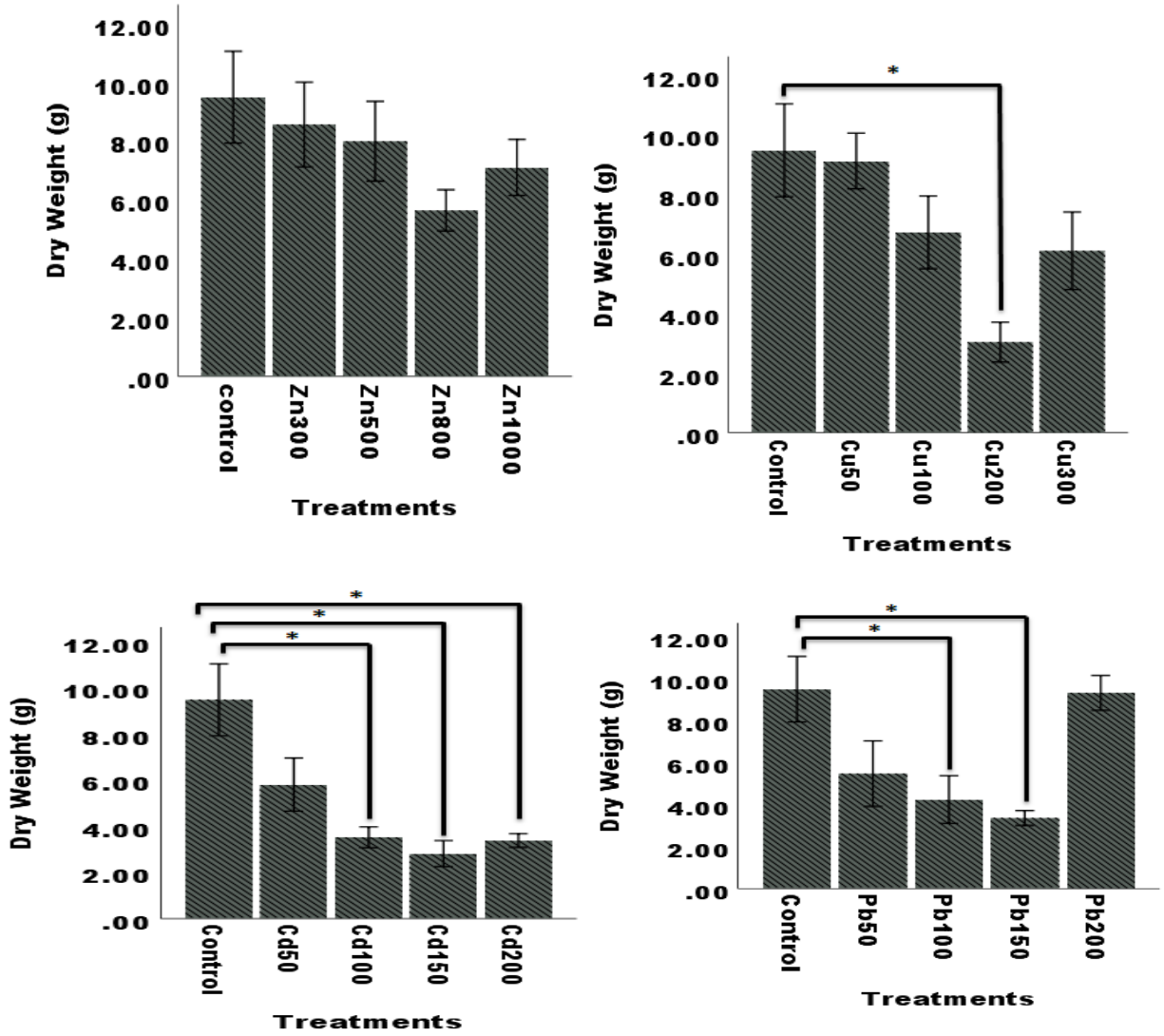

Figure 25: Effect of metals on okra shoot dry weight (mean ± SE, N=4). *: indicates significant difference between the control and treatments (P<0.05).

b. Effect of Metals on Plant Root Weight:

Tobacco dry root weights exposed to metal contaminated soil for 2 months did not show any significant difference from the control (**Figure 26**). These results, along with the previous results of shoot weight, confirm that tobacco plant is tolerant to the metals studied and can be considered for bioremediation purposes.

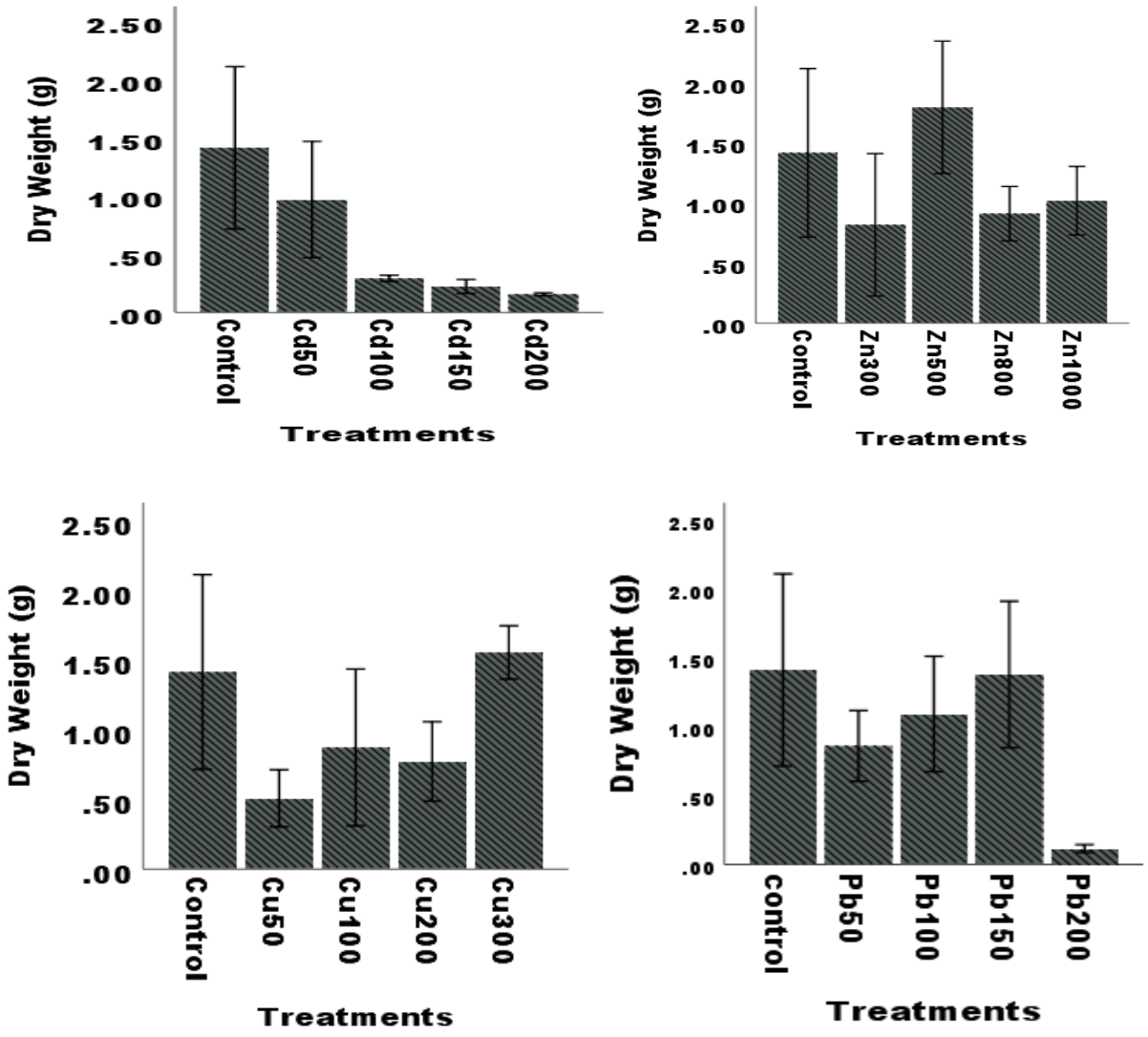

Figure 26: Effect of metals on tobacco root dry weight (mean ± SE, N=4). No significant difference between the control and treatments (P<0.05).

Okra root weights **(Figure 27)** were significantly affected only by Cd100, 150 & 200 compared to the control. Zinc, Cu and Pb did not cause any significant reduction in root weight of okra. This indicates sensitivity of okra roots to Cd contamination. This is in agreement with the sensitivity of the shoot of this plant to the same concentrations. In other words, okra weights of shoot and root were reduced due to cadmium contamination.

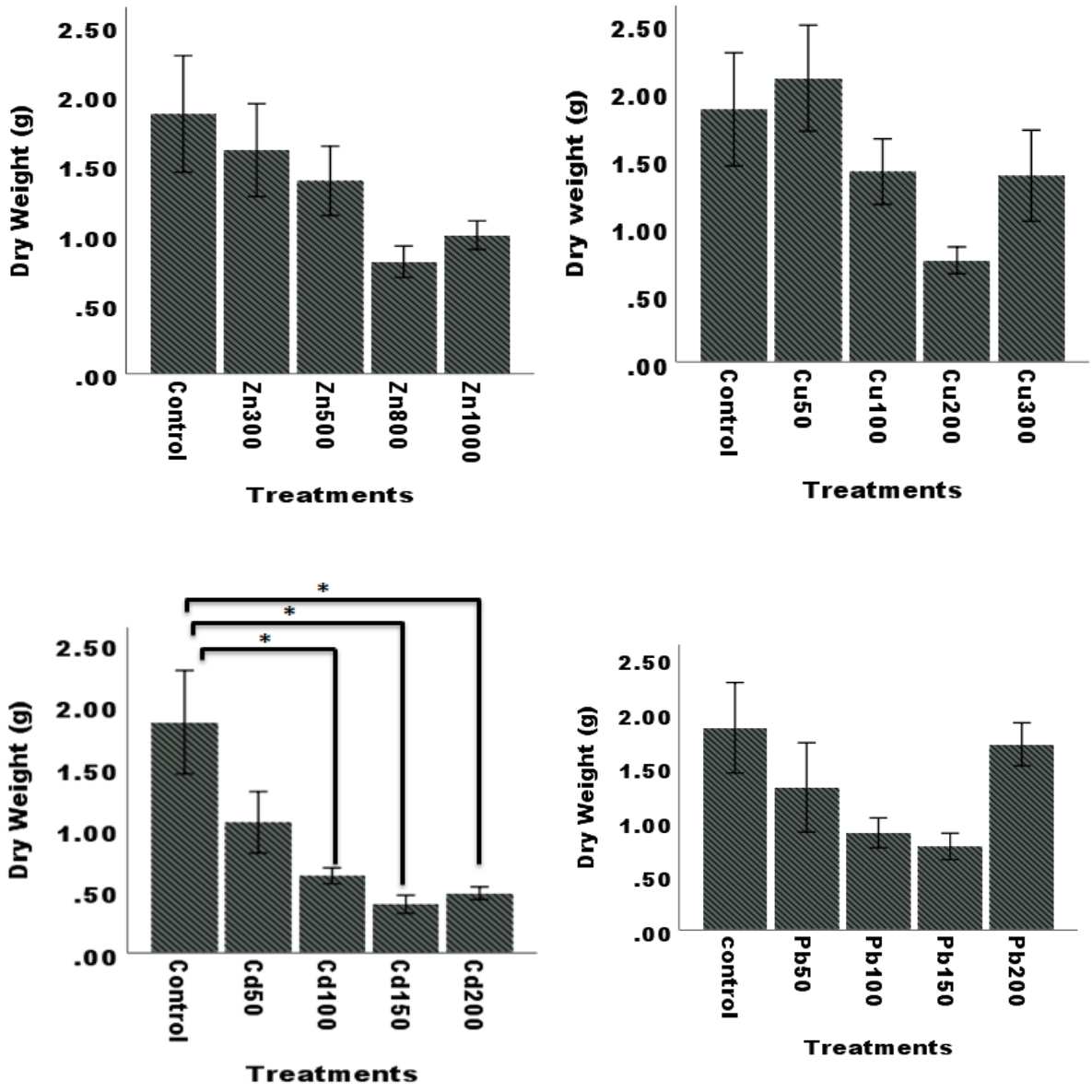

Figure 27: Effect of metals on okra root dry weight (mean ± SE, N=4). *: indicates significant difference between the control and treatments (P<0.05).

Lefèvre et al. (2009) reported that the shoot dry weight of *Dorycnium pentaphyllum* was significantly increased at 1 µM Cd compared to the control. Whereas the 10 µM Cd did not affect the shoot and root dry weight. The same study found that the 100 µM Cd significantly decreased the dry yield of both shoots and roots. Hassanein et al. (2017) study reported that the shoot dry weight of wheat (*Triticum aestivum*) was significantly reduced by 40% at (0.5, 1.0 and

1.5 mM Cd) compared to the control. As well as, the root dry weight was reduced by 33.3%. Tobacco plants did not show any significant difference in dry matter yield at all Cd concentrations for both wild type and transgenic plant (Daghan et al., 2008).

According to Lefèvre et al. (2009) a statistically significant increase in root dry weight at the lowest concentration of Zn (10 µM) was observed. While the shoot dry weight insignificantly increased compared to the control at the same concentration. Alia et al. (2015) reported that the highest dose of Zn (700 mg/kg) decreased the shoot and root dry mass by (5.7% and 14.5 %, respectively) compared to the control of spinach (*S. oleracea*).

Deepa et al., (2006) studied the potential of *Portulaca oleracea* to accumulate Cu in different types of soil with different Cu concentrations. They reported that the shoot and root dry weight decreased as the Cu concentration increases in all types of soil, i.e., the highest Cu concentration (2000 µg/g) had the lowest shoot and root dry weight (ranged from 0.1g - 0.35 g to all soil types). Dguimi et al. (2009) observed a reduction in both shoot and root of tobacco using the following Cd concentrations: 10, 20, 50,100 µM.

According to Grandgirard et al. (2002) the dry biomass yield of corn and sunflower plants was increased at 0.1 µM Pb and 0.5 Pb µM compared to the dry biomass yield of the control. Besides, a high dose of Pb concentration (200 mg/kg Pb) showed an adverse effect on shoot and root dry mass compared to the other concentrations. In present study, the dry mass of tobacco shoot and root at 200 mg Pb/kg were 0.81g and 0.12g insignificantly decreased from the control (8.8 g) and other concentrations**.**

According to Lamhamdi et al. (2013) the spinach dry weight at 15 mM Pb decreased by 29% compared to the control. Kosobrukhov et al. (2004) reported that the dry weight decreased in

Plantago major when it was exposed to Pb. Lead can compete with essential macro-elements e.g. (Mg, P, K, and Ca), and can reduce the uptake of these elements.

c. Effect of metals on Fruit Dry Weight

The effect of Zn, Cu, Cd, and Pb heavy metals on **fruit dry weight of okra** is shown in **Figure 28**. Although statistically insignificant, the lowest dry weights of okra fruit were observed in Cd treatments. Cadmium concentrations were adversely affected the dry weight of okra fruits. The fruit dry weight was reduced by 58.2 % compared to the control at Cd treatment-200 mg/kg. However, the trend in other metals was different. As the concentrations of Cu and Zn were increased the dry weight increased. While the highest dry weight was recorded at 50 mg/kg of Pb (6.13 g) compared to the control (3.18 g). In a comparison of fruit dry weight in control plants to that in treated plants on all metals, no significant difference was seen.

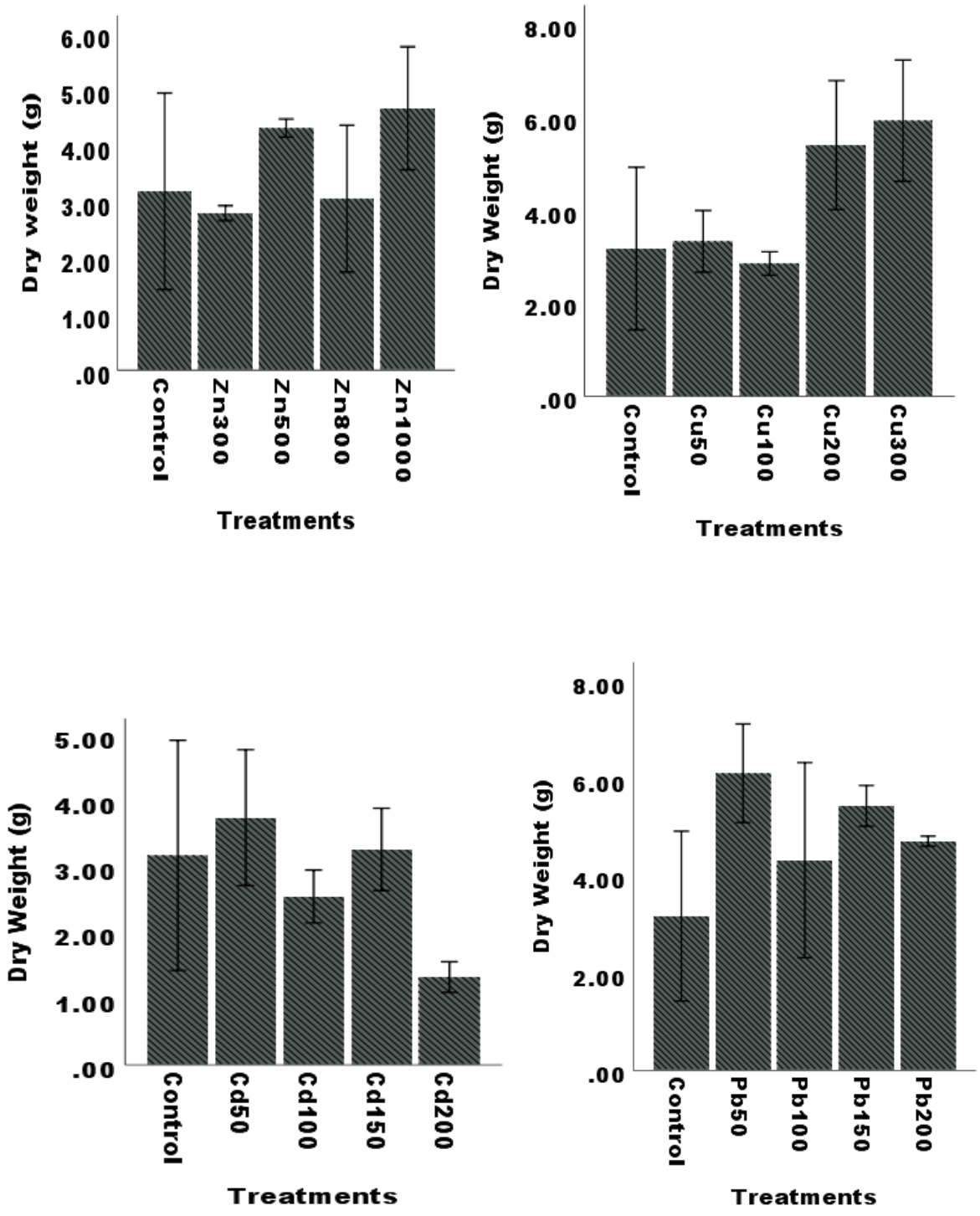

Figure 28: Effect of metals on okra fruit dry weight (mean ± SE, N=4). No significant difference between the control and treatments (P<0.05).

d. Effect of MIX on Weight of shoot, root and fruit of okra and tobacco

As shown in **Figures 29-30**, exposing **tobacco and okra** to a soil contaminated with a mixture of metals did not cause any statistically significant difference in shoot, root or fruit weight. Wang et al., (2016) studied the effect of mixed heavy metals stress on growth and accumulation of metals in *Salix* species. They reported no significant differences in root biomass of *Salix matsudana* and *Salix babylonica* between the control and mixed heavy metals (Zn, Cu, Cd, and Pb) at 5mM. Whereas, at the same concentration of treatments, the *Salix fragilis* had a significant decrease in dry root compared to the control. However, they observed that the shoot biomass of mixed heavy metal treatment significantly decreased compared to the control in three *Salix* species. Faizan et al.(2014) reported that the dry matter of okra plants was increased in plants treated with treated wastewater compared to the control.

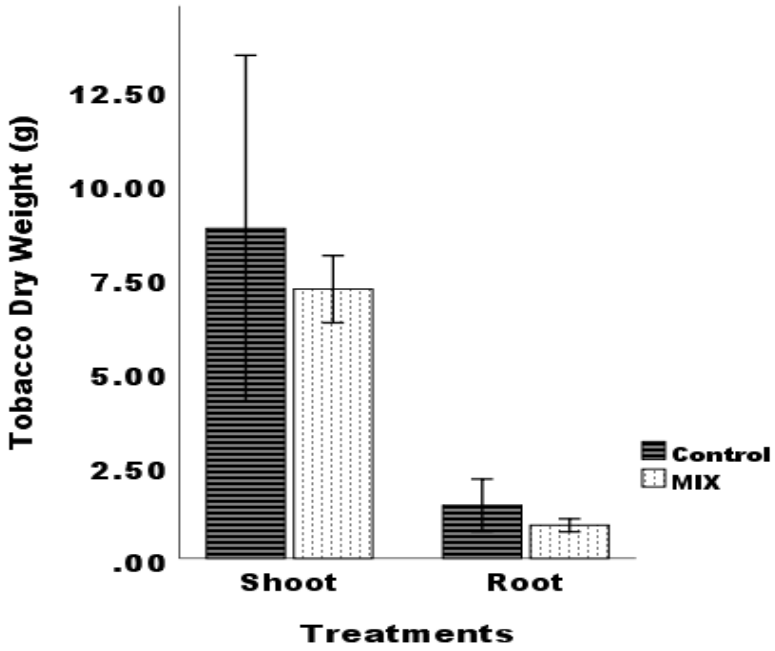

Figure 29: Effect of metal mixture (MIX) on tobacco shoot & root dry weight (mean ± SE, N=4). No significant difference between the control and MIX treatment (P<0.05).

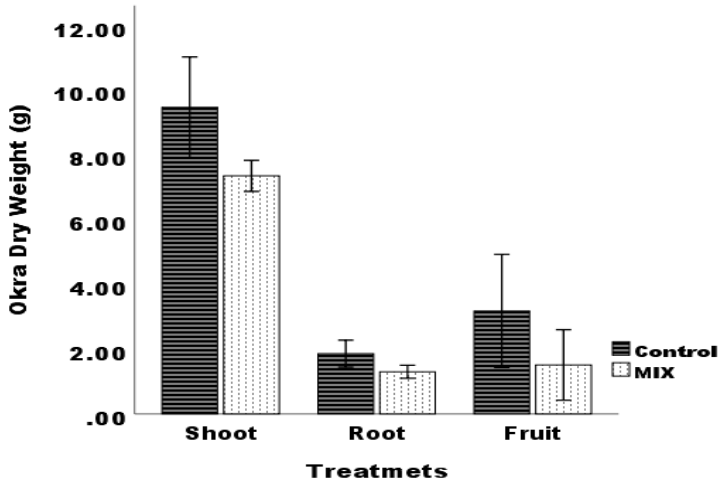

Figure 30: Effect of metal mixture (MIX) on okra shoot, root and fruit dry weight (mean ± SE, N=4). No significant difference between the control and MIX treatment (P<0.05).

B. Heavy Metal Analysis

Certified reference material of soil and plant material were run with all samples in order to assess the accuracy of the analytical procedure and for verification purposes. Results of the analyzed certified material are shown in **Table 2**.

Table 2: Metal concentrations (Mean±SE, mg/kg) of reference material to measured value and certified value.

		Plant Reference Material Values (Hay powder, IAEA-V-10) *			Soil Reference Material Values (Lake Sediment, IAEA-SL-1) *	
Metal	Measured Values N=14	Mean Value	95% Confidence Interval	Measured Values N=6	Mean Value	95% Confidence Interval [a]
Zn	23.06 ± 0.79	24	23 - 25	223.80 ± 28.26	223	213 - 233
Cu	9.04 ± 0.62	9.4	8.8 - 9.7	26.27 ± 2.93	30	24 – 36
Cd	0.36 ± 0.08	0.03	0.02 - 0.05	0.28 ± 0.05	0.26	0.21 – 0.31
Pb	0.90 ± 0.19	1.6	0.8 - 1.9	7.36 ± 4.48	37.7	30.3 – 45.1

*: Analytical Quality Control Services (AQCS) of the International Atomic Energy Agency.

1. Heavy Metal Phytoextraction by Plants

I. Heavy Metal Levels in Shoots, Roots and Fruits:

To evaluate the ability of the two plants to phytoextract and accumulate metals in their shoots, roots and fruits, four replicates of plant parts were prepared and analyzed to determine the heavy metal concentration in each part.

Shoots:

Figures 31 - 32 indicate the phytoextraction of the 4 heavy metals by shoots of both plants that were subjected to metals contaminated soil over 2 months. Heavy metal level in shoots of all tobacco treatments contained significantly higher levels of metals than the control **(Figure 31)**. Okra shoot metal levels followed the same trend except for Pb50 & Pb200 (**Figure 32).**

In shoots of both tobacco and okra, there were highly significant differences in Zn concentration between the control and Zn treatments. Tobacco plants grown in 800 ppm Zn phytoextracted 547.55 mg/kg of Zn in their shoots compared to the control (53.59 mg/kg DW) (10 folds). While in okra plants, the highest phytoextraction was observed at Zn1000 by an average of 514.8 mg/kg. There were also highly significant differences ($P \leq 0.003$) between Zn concentration of tobacco shoots grown in soil containing Zn300, 500, and 1000 ppm (199.45, 291.1, 233.65 mg/kg, respectively) compared to the control. Whereas, in shoots of okra plants, the treatments of Zn 300, Zn 500, and Zn 800 showed highly significant differences compared to the control demonstrating an increase of 665%, 1059%, and 1993% respectively.

Amer et al. (2013) conducted a hydroponic experiment in which *Atriplex halimus* was exposed to a metal solution. They found that Zn concentration in shoots was 4660 mg/kg DW at Zn level of 25 mg/L. Another study conducted on transgenic tobacco plants grown in sandy soil found that there was no significant difference in zinc concentrations in shoots between control and plant

lines of tested tobacco (Pavlíková et al., 2004). They reported that the highest concentration of Zn was found in one line of the transgenic plant (123.1 mg/kg DW) compared to the control (93.5 mg/kg DW).

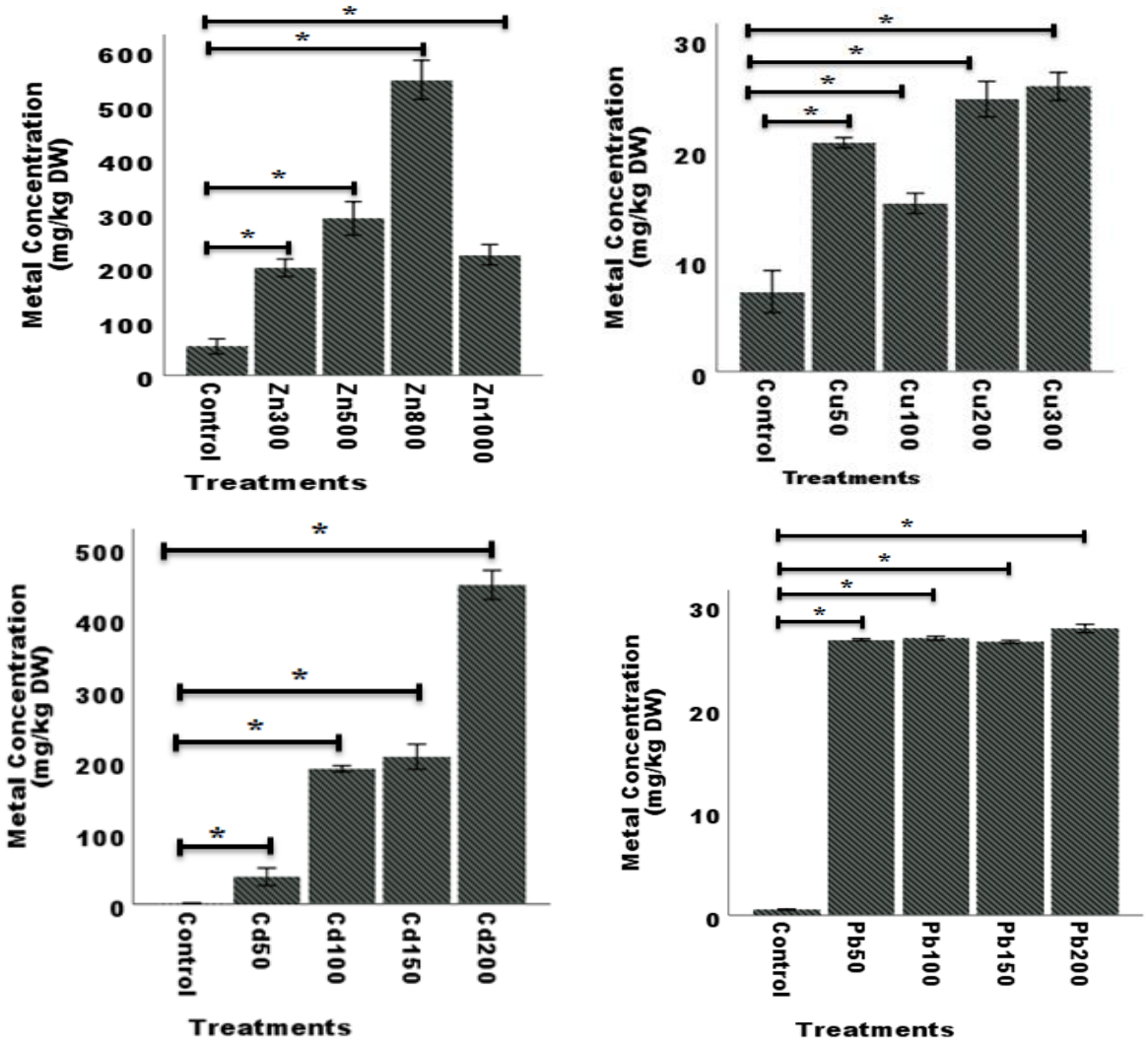

Figure 31: Phytoextraction of metals by tobacco shoots exposed to metal contaminated soil for 2 months. Values represent Mean± SE (mg/kg dry weight, N=4). *: indicates significant differences between the control and treatments (P< 0.05).

In a study on different weed species, heavy metals concentrations of plant and soils from polluted and unpolluted areas were measured, they found that the Zn concentration in shoots of *Polygonum lapathifolium* (L.), *Solanum nigrum* (L.), *Ambrosia trifida* (L.), *Chenopodium*

acuminatum (Wild), *Helianthus tuberosus* (L.), *Physalis angulata* (L.), *Abutilon theophrasti* (Medic.), and *Conyza canadensis* (L. Cronq) were (168.5, 94.7, 264.6, 154.9, 205.9, 180.8, 158.2, and 74.4 mg/kg DW, respectively) compared to concentration in unpolluted area (100 mg/kg DW) (Cui et al., 2007).

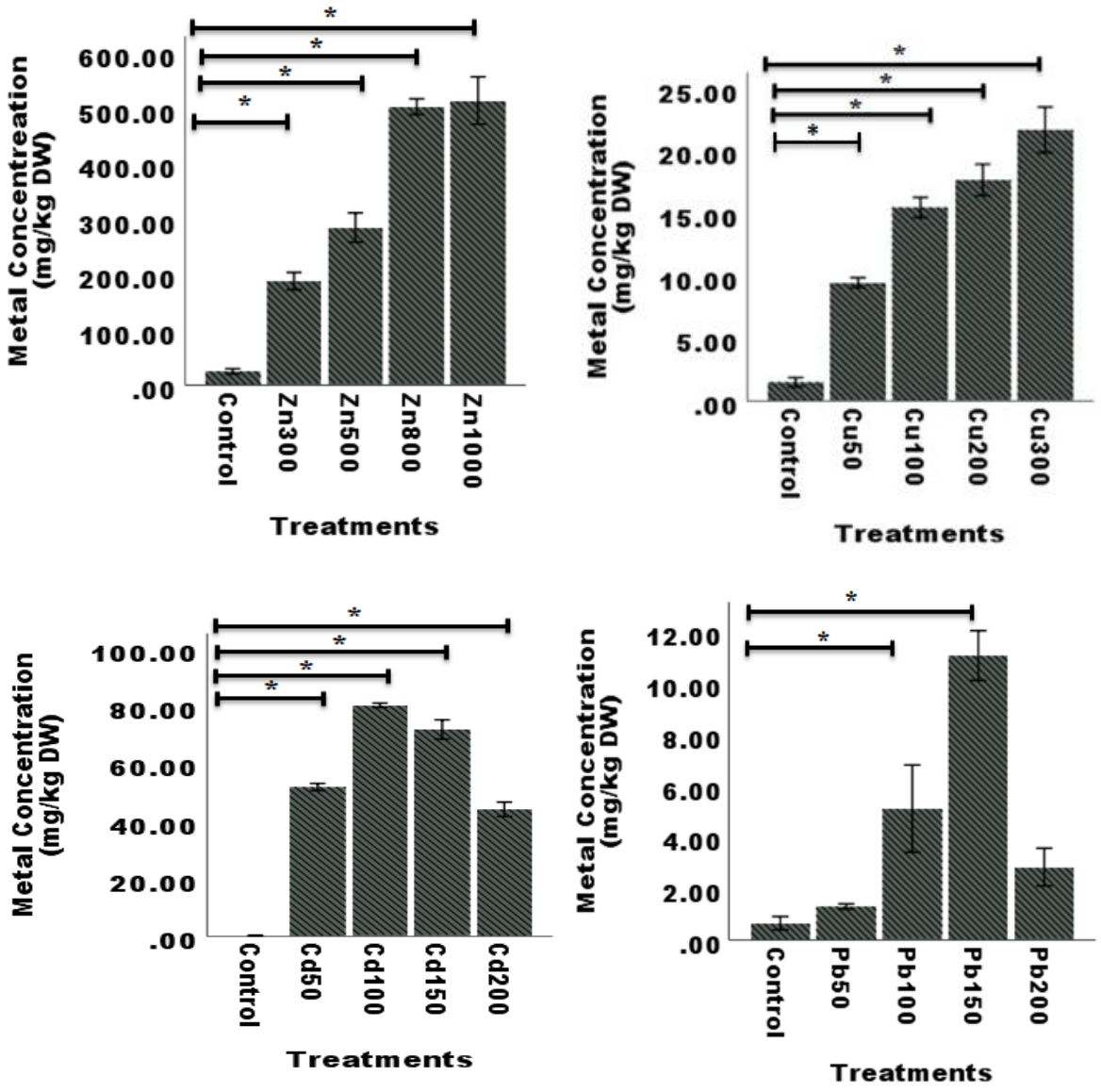

Figure 32: Phytoextraction of metals by okra shoots exposed to metal contaminated soil for 2 months. Values represent Mean± SE (mg/kg dry weight, N=4). *: indicates significant differences between the control and treatments (P< 0.05).

Cu concentrations in shoots of tobacco and okra treatments were significantly higher than those in the control plants. Cd content in the shoot of tobacco plant significantly increased as the Cd concentrations increased in Cd-contaminated soil. Cd concentrations in shoots and roots of tobacco and okra were significantly increased at all Cd treatments compared to the control. Shoots of tobacco at Cd200 had the highest Cd concentration (358.0.5 mg/kg) compared to the shoots of the control plant (0.95 mg/kg DW) with a highly significant difference.

In comparison, the shoots of okra plants showed the highest accumulation value in plants treated with Cd at a concentration of 100 mg/kg. Whereas, the lowest accumulation rate was observed in plants treated with Cd50.

Pb accumulation in shoots and roots exhibited the same manner at all treatments. As the results show, Pb concentrations in shoots and roots of tobacco were significantly higher, in all treatments, than the control. The Pb concentrations in shoots of Pb-subjected plants ranged between 26.50 to 27.85 mg/kg compared to the control (0.55 mg/kg DW). In shoots of okra plants, the highest accumulation value was recorded in plants treated with Pb150 (11.08 mg/kg DW). A significant difference was only observed in plants treated with Pb100 and 150 ppm.

Roots

Figure 33 & 34 show the levels of metals in roots of the two plants. Generally, the two plant were able to phytoextract significantly higher metals than the control. However, only tobacco plants subjected to the lowest level of Zn (Zn300) did not show any significant difference from the control. All other treatments were able to extract significantly higher levels of the four metals than the control **(Figure 33)**. This clearly indicates the high ability of tobacco to phytoextract the

four metals at the levels studied. On the other hand, all okra treatments significantly phytoextracted more Zn, Cd, & Pb and only Cu200 in their roots than the control.

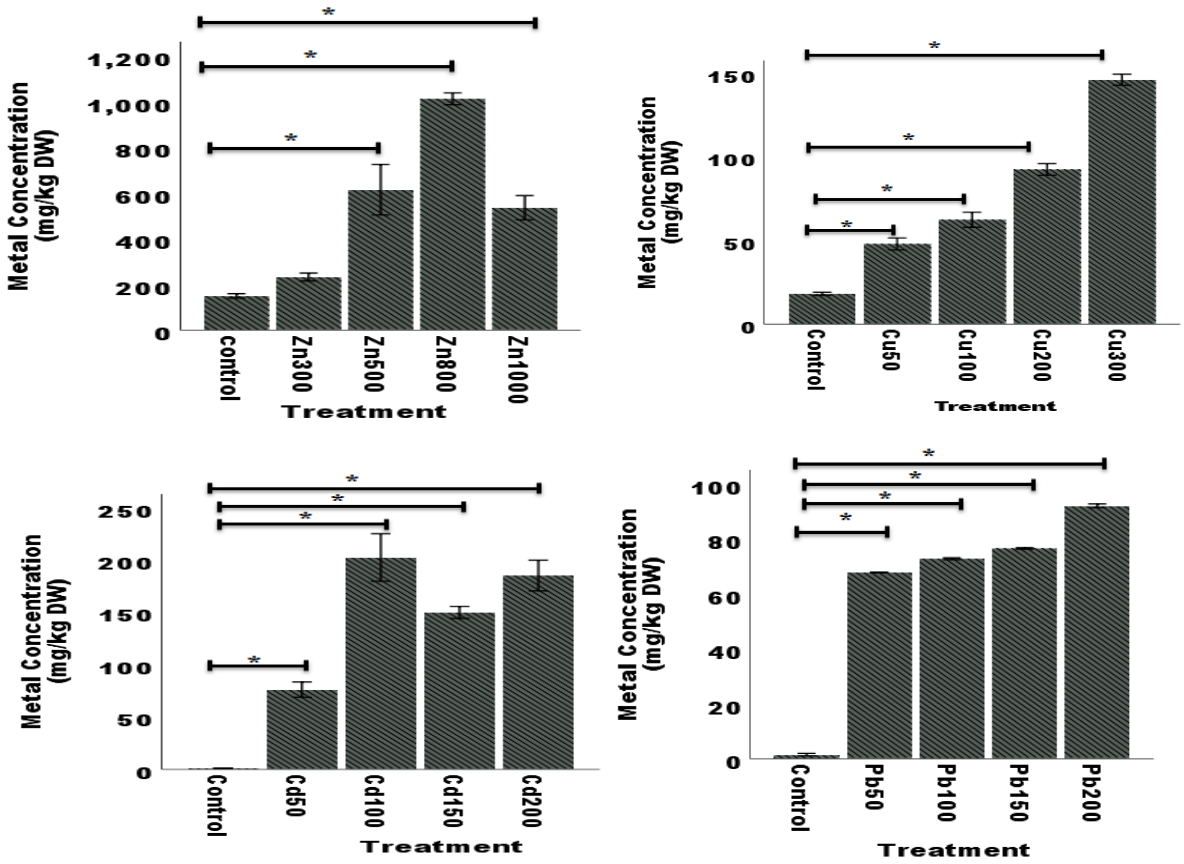

Figure 33: Phytoextraction of metals by tobacco roots exposed to metal contaminated soil for 2 months. Values represent Mean± SE (mg/kg dry weight, N=4). *: indicates significant differences between the control and treatments (P< 0.05).

Zn accumulation of some treatments was slightly higher in okra than in tobacco. For example, Zn accumulation in plants treated with Zn1000 was 536.25 mg/kg DW and 753.00 mg/kg DW in tobacco and okra, respectively. The same results had been recorded in different studies as well using different species. Korzeniowska & Stanislawska-Glubiak. (2015) concluded that the Zn concentration in roots was significantly higher than Zn in shoots for both studied species (*Spartina pectinata* and *Miscanthus giganteus)*. The same finding was reported in *Viola*

baoshanensis, *Rumex K-1*, *Vertiveria zizanioides*, *Rumex acetosa DSL*, and *Rumex acetosa JQW* according to Zhuang et al.(2007) study.

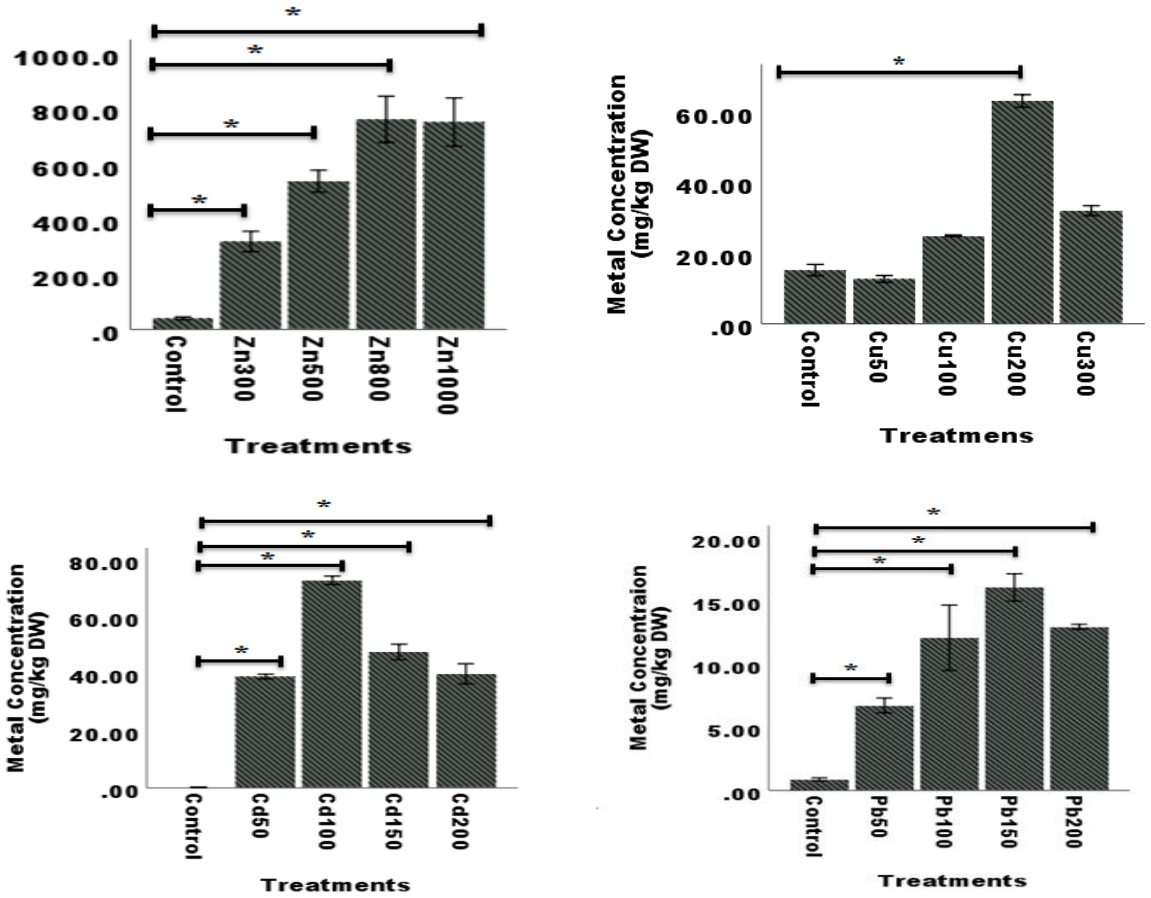

Figure 34: Phytoextraction of metals by okra roots exposed to metal contaminated soil for 2 months. Values represent Mean± SE (mg/kg dry weight, N=4). *: indicates significant differences between the control and treatments (P< 0.05).

However, Cu concentration in roots of tobacco increased as the Cu concentration in Cu-contaminated soil increased. As the highest concentration among Cu treatments was 300 ppm Cu, the Cu concentration in the root was highest at Cu 300 (148.36 mg/kg DW) significantly different compared to the control (18.13 mg/kg DW). There were highly significant differences in Cu concentrations of roots at all treatments compared to the control plant. On the other hand, the opposite trend was observed in the roots of okra plants. The highest accumulation value was recorded in plants treated with 200 mg/kg Cu (63.25mg/kg DW). Statistically, higher significant

differences were observed in plants treated with Cu 200. As shown above, the Cu concentrations in roots were higher than the concentrations in shoots for both tobacco and okra at all Cu treatments. The same results were reported in Marzilli et al. (2018). They found that the concentration of Cu in roots was significantly higher than the Cu concentration in shoots in *Populus alba* ($P<0.001$). They also reported that the highest concentrations of Cu (250 and 500 µM Cu) had the highest accumulation level in plants with significant differences compared to the control and low concentrations of Cu. Many studies also reported that Cu concentration in roots was higher than Cu concentration in shoots or (leaves and stem separately) in many plant species (Kacálková et al., 2009, Hajiboland, 2005, Ali et al., 2012).

According to statistical analysis, a significant increase was noted in all treatments compared to the control ($P=0.00$). The same results were obtained in okra roots. Cd accumulation was significantly increased in treated plants compared to the control plants ($p=0.00$). The roots in plants treated with 100 mg/kg of Cd had the highest accumulation value (72.80 mg/kg DW). In general, tobacco plants tend to accumulate more Cd than okra plants. In tobacco roots, however, the highest accumulation was observed at 100 ppm of Cd (202.50 mg/kg DW). Statistical analysis shows higher significant differences between all treatments compared to the control in Cd concentrations of tobacco's roots ($P\leq0.004$). Cadmium is considered as a mobile metal. The roots adsorbed Cd from soil solution then translocated to shoots via xylem. Rahmanian et al. (2011) reported that the concentration of Cd in shoots of millet, alfalfa, and couch grass were 15.1, 13.4, and 23.6 mg/kg DW, respectively. Alfalfa was also accumulated up to (202 mg/kg DW) of Cd in their shoots at Cd 50 ppm (Peralta-videa et al., 2002). In contrast, some studies observed that the root had more accumulation quantities of Cd compared to shoot parts. (Ali et al. (2012) study on *Trifolium alexandrinum*, reported that the roots had the highest accumulation

rate of Cd compared to the stem and leaves. Another study reported that the roots of *B. juncea* accumulate the highest concentration of Cd (81.9 mg/kg) (Ghosh & Singh, 2005).

In a comparison of Pb concentrations in roots, significant differences were observed in all treatments for both tobacco and okra. As the concentrations of treatments were increased the Pb concentrations in roots increased. In tobacco plants, the highest accumulation of Pb was observed in plants treated with Pb 200 ppm (92.13 mg/kg DW) compared to the control plants (1.50 mg/kg DW). In okra plants, the highest accumulation value was observed in plants treated with 150 ppm (16.13 mg/kg DW) compared to the control plants (0.88 mg/kg DW). Overall, okra plants have a limited ability to accumulate Pb. Okra plant was ineffective to take up and accumulate Pb from contaminated soil. These results were in agreement with the results in Hassan et al. (2018) study.

Unlike Cd, Pb accumulation was observed in roots more than Pb in shoots at all treatments for both plants. Hydroponically experiment contaminated with 300 mg/L Pb by Peralta-videa et al. (2002) found that the Kentucky bluegrass and Colonial bent grass accumulate more than 150 mg/kg dry weight in their roots. A significant difference was observed in Pb concentration in shoots of *T. praecox* up to 0.4% from the Pb concentration in soil (Vogel-Mikuš et al., 2005).

Many studies observed that the Pb concentration in roots was higher than in shoots on different plant species such as *Trifolium alexandrinum* (Ali et al., 2012), endurant weed plants (Cui et al., 2007), *Catharanthus roseus* (Subhashini & Swamy, 2015). The concentrations of Pb significantly increased as the level of Pb increased in all studied species; *Silene vulgaris*, *Noccaea caerulescens*, and *Matthiola flavida* (Mohtadi et al., 2012).

In general, plant shoots tended to accumulate more Cd than roots. These results were commonly observed in many studies regardless of plant species. In Houda et al. (2016) study that assessed

the effect of two poplar species (*Populus alba* and *Populus nigra*) to accumulate heavy metals from TWW for 90 days. They found that the two species accumulate Cd in their leaves more than the roots. Another study in Slovenia had studied the ability of *Thlaspi praecox* to hyper-accumulate of heavy metals from polluted areas, they found that a significant accumulation of Cd in shoots (5960 mg/kg) and shoot to root ratio was 5.6 for Cd (Vogel-Mikuš et al., 2005). Tobacco plants accumulated high concentration of Cd in their leaves with no any toxicity symptoms at 20 mg Cd/ kg soil (Tsadilas, 2000).

In several studies, *N. tabacum* has been proven to remediate and accumulate heavy metals as well as many pollutants from contaminated soils. A hydroponic study assessed the efficiency of *N. tabacum* to remove Pb in the presence and absence of chelating agent (EDTA), the study found that the Pb-removal was up to 30 % and 1.87 % at 2.5 µM Pb with and without EDTA, respectively (Boonyapookana et al., 2005). Talano et al. (2012) study served that the effectiveness of transgenic tobacco lines to remove and accumulate 2,4- dichlorophenol (2,4-DCP) from wastewater. Another study by Sundberg et al. (2003) reported that the ability of tobacco plants to uptake and accumulate perchlorate in plant tissues, which grown in hydroponic solutions with different concentrations of perchlorates. In Stojanović et al. (2012) study, two species of tobacco were used to remediate uranium-contaminated soil. They found the concentrations of uranium in tobacco leaves ranged from 3.50 to 4.18 mg/Kg DW in both types. However, Hassan et al. (2018) studied the potential of Rosselle (*Hibiscus sabdariffa* L), Amaranth (*Amaranthus Dubius*), and Okra (*Abelmoschus esculentus*) to accumulate heavy metals from contaminated soil, they reported that the highest concentration of Zn was recorded in okra plants at treatment treated with 50 mg/kg Zn, Ni, Pb, Cd, and Cr. In soil contaminated with 50 mg/kg of Zn, Ni, Pb, Cd, and Cr, a higher Cd concentration was found in okra plant

compared to other plants and respective control (Hassan et al,. 2018). The Cd accumulation in stems, roots, and fruits of okra was 0.80, 0.72, and 65 mg/kg DW respectively. Whereas, the Pb concentration in okra was 0.12 mg/kg DW compared to control (0.04 mg/kg). The Zn, Cu, and Pb concentrations were ranged from 8-20, 3.20- 8.40, and 3-9 mg/kg DW, respectively in fruits of okra that was irrigated by polluted water (Muazu et al., 2010). Okra plants tend to uptake essential metals and it has high capacity to retain them more than nonessential metals (Cd and Pb). Muazu et al. (2010) study is in agreement with this conclusion. The study in Wa Municipality (Ghana) reported that the maximum Pb content in tobacco and cigarettes was 8.3 mg/kg, which exceeds the allowed limit of the WHO/FAO (Sebiawu et al., 2014). Pal et al.(2013) study conducted on different crops collected from a contaminated site, they reported that, the Cd concentrations in shoots and roots of okra plant were 0.18 and 0.45 µg/g, respectively.

Figures 35-36 show heavy metal phytoextraction by tobacco and okra plants exposed to a mixture of the four metals. Compared to the control, tobacco shoots were found to significantly phytoextract and accumulate Cd & Pb. However, the roots of tobacco were able to significantly phytoextract and accumulate Cu, Cd & Pb. Whereas, Zn was not accumulated significantly in comparison to the control by both shoots and roots **(Figures 35)**. Okra shoots accumulated significantly only Cd but not Cu, Pb & Zn **(Fig. 36).** Roots of okra were able to accumulate metals the same way as roots of tobacco. Cd was the only metal to be phytoextracted and accumulated by shoots and roots of both plants and Zn was the only metal that was not accumulated by any of the two plants. Cu and Zn, both essential metals, were not accumulated significantly by shoots of both plants. This might indicate some regulatory mechanism as these metals are essential metals that can be regulated by certain metabolic pathways.

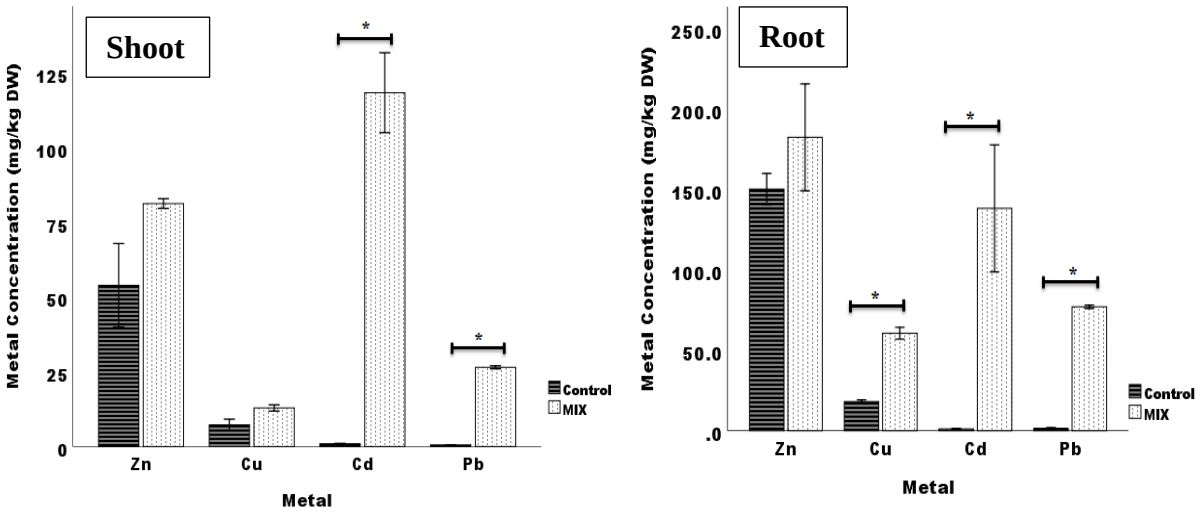

Figure 35: Phytoextraction of metals by tobacco plant shoots & roots planted in a soil contaminated with a mixture of metals for 2 months. Values represent Mean± SE (mg/kg dry weight, N=4). *: indicates significant differences between the control and treatments (P< 0.05).

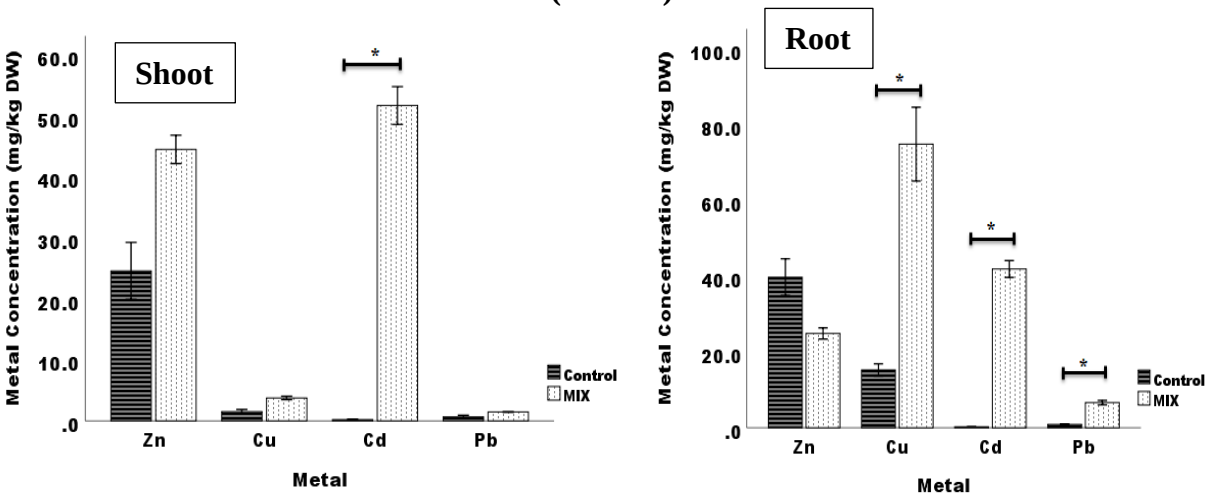

Figure 36: Phytoextraction of metals by okra plant shoots & roots planted in a soil contaminated with a mixture of metals for 2 months. Values represent Mean± SE (mg/kg dry weight, N=4). *: indicates significant differences between the control and treatments (P< 0.05).

Fruits

Okra fruits are considered as very important in the human diet (Moyin-Jesu, 2007). Metal accumulation in fruits was checked and results are shown in **Figure 37**. Okra fruits from all treatments contained significantly higher levels of Cd than the control. Pb, on the other hand, did

not accumulate significantly in fruits from any of the treatments. Fruits of okra from the 2 high treatments of Zn (Zn 800 & 1000) contained significantly higher levels than the control. Similarly, fruits of treatments Cu 100 & 300 showed significantly higher levels of Cu than the control. Results indicate that only Cd can be significantly accumulated in the fruits of all treatments compared to the control.

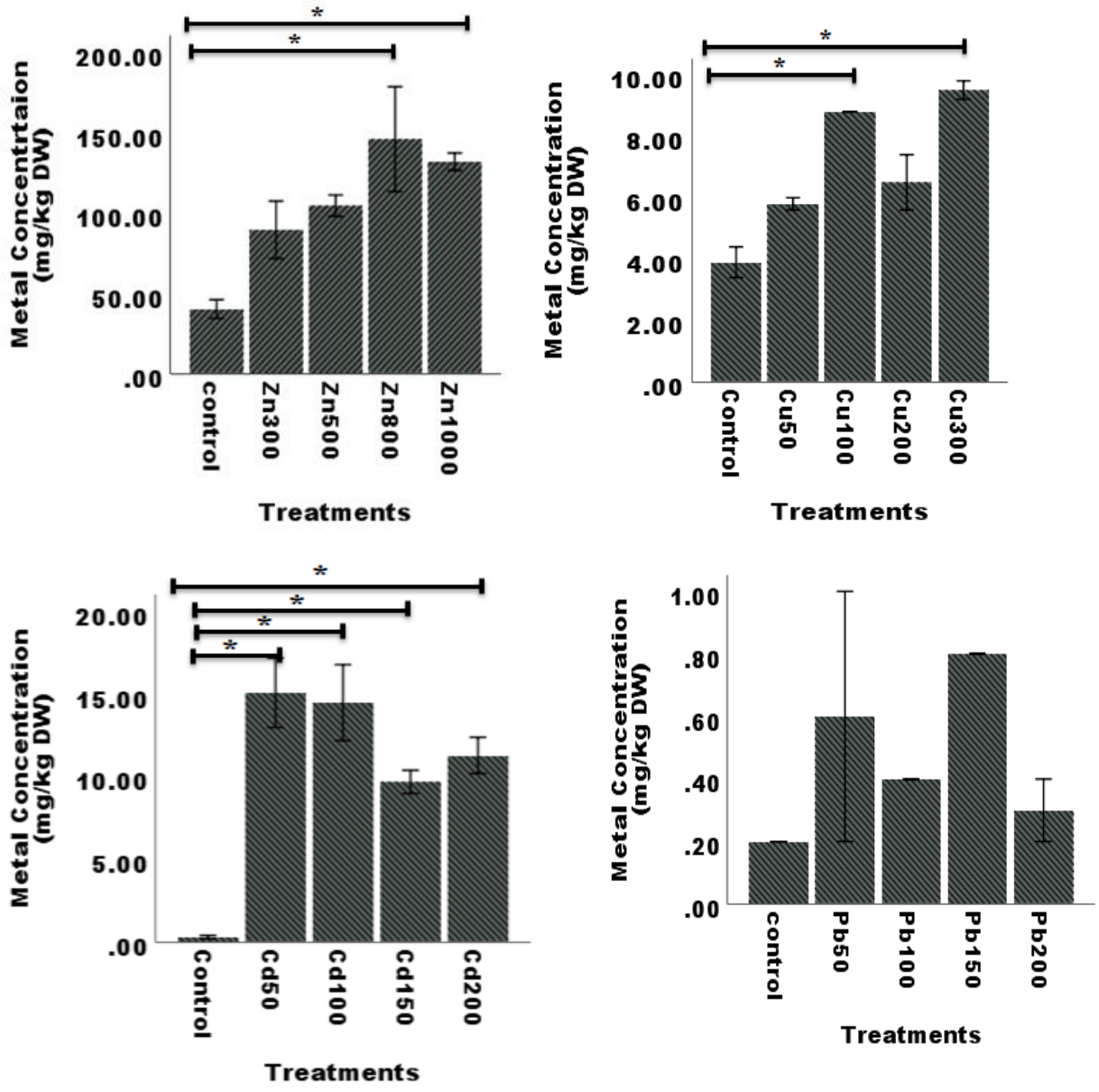

Figure 37: Accumulation of metals in fruits of okra plants subjected to metals for 2 months. Values represent Mean± SE (mg/kg dry weight, N=4). *: indicates significant differences between the control and treatments (P< 0.05).

Humans could be affected by heavy metals in contaminated fruits and vegetables. Heavy metals also alter the nutritional value of vegetables and fruits. Therefore, WHO and FAO limited the maximum permission levels of heavy metals in fruits and vegetables. **Table 3** shows the permission level of Zn, Cu, Cd, and Pb.

Table 3: The maximum concentration of heavy metals in vegetables permitted by FAO/WHO (Mensah et al., 2009)

Element	Max Allowable Concentration (mg/kg)
Zn	99.4
Cu	73.3
Cd	0.2
Pb	0.3

The results in present study show that the accumulation values of heavy metals in fruits were ranging between 61.5 to 104.6, 5.0 to 9.5, 7.9 to 15.1, and 0.3 to 0.8 mg/kg DW of Zn, Cu, Cd, and Pb, respectively. Some treatments of Zn, Cd, & Pb caused significantly higher levels than the permissible levels. Only levels of Cu in fruits from all treatments were below the permissible levels.

Zn concentrations in fruits of okra were significantly increased in plants treated with 800 and 1000 ppm of Zn (P=0.02 and 0.04, respectively) compared to the control demonstrating an increase of 263.2% and 228.4% than the control. While all other treatments didn't show significant differences compared to the control. The concentration of Zn at 500 ppm also was high in okra fruits. In comparison with the permissible level, okra fruit had a high level of Zn.

The concentrations of Cu in fruits were significantly higher in plants treated with 100 and 300 ppm of Cu (8.8 and 9.5 mg/kg DW, sequentially).

Okra plants could translocate Cd from roots to their leaves and fruits via xylem. Statistical analysis shows high significant differences between all treatments compared to the control in Cd concentrations of okra fruits ($P \leq 0.02$). The highest accumulation value was observed in plants treated with 50 and 100 ppm of Cd (15.1 and 14.5 mg/kg DW).

The Pb accumulation in okra fruit was shown in **Figure 39**. Statistically, no significant differences were observed in okra fruits at all treatments. The highest concentration of Pb was seen in plants treated with 150 ppm (0.8 mg/kg DW) which exceeded the permission level.

Bentum et al.(2017) studied the uptake and accumulation of heavy metals (Zn, Cu, and Pb) in okra fruits that were collected from two different farms in Cape Coast. Zn concentration in okra fruit was 2.45 and 1.54 mg/kg DW in both sites. Whereas, the Cu and Pb concentrations in okra fruit ranged between 1.94 -2.47 and 1.45- 1.91 mg/kg DW in both sites.

When planted in soil contaminated with a mixture of the four metals, okra fruits were found to accumulate only Cd to levels that were significantly higher (13.6 mg/kg DW) than the control (0.3 mg/kg DW) **(Figure 38).** This result of significant accumulation of Cd in both plants and their parts is consistent. This indicates an efficient phytoextraction of this metal by both plants.

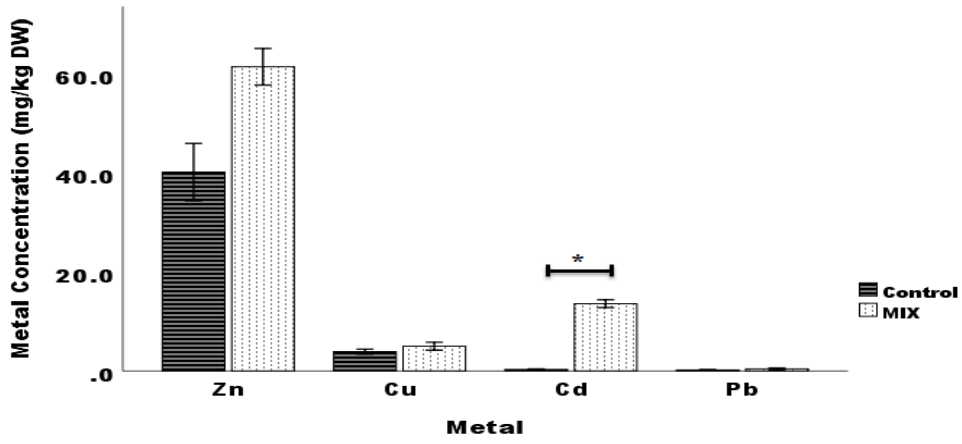

Figure 38: Accumulation of metals by fruits of okra plants exposed to soil contaminated with a mixture of metals for 2 months. Values represent Mean± SE (mg/kg dry weight, N=4). *: indicates significant differences between the control and treatments (P< 0.05).

1. Biotranslocation, Bioaccumulation and Bioconcentration Factors

Biotranslocation factor (BTF), bioaccumulation factor (BAF), and bioconcentration factor (BCF) are parameters calculated to evaluate the potential of a plant to remediate heavy metals and to evaluate the efficiency of the phytoremediation process (Usman et al., 2019).

BTF is referred to the ability of an accumulator plant to translocate heavy metals from belowground tissue (roots) to aboveground tissue (shoots). This is obtained through the ratio of heavy metal concentration in shoots to that in roots (Radziemska, 2018). Whereas the BAF and BCF refer to the ability of a hyperaccumulator plant to accumulate heavy metals, from soil or water, in their shoots and roots, respectively. BAF is calculated by the ratio of heavy metal concentration in shoots to that in soil (Balabanova et al., 2015), whereas, BCF is calculated by the ratio of heavy metal concentration in roots to that in soil (Boonyapookana et al., 2005).

If the BTF ratio is equal to or more than one, it indicates high effectiveness of a plant to transport heavy metals from roots to aerial parts. This is of great importance to phytoremediation as it enables easy harvesting of the shoot containing high levels of the pollutant at the end of the phytoremediation process.

Table 4 summarizes the BTF, BAF & BCF for tobacco plant. BTF for the treatments ranged between 0.44-0.86 for Zn, 0.18-0.44 for Cu, 0.67-2.03 for Cd & 0.3-0.4 for Pb. BTF for Zn, Cu & Pb decreased gradually with increasing metal concentration. Only BTF for Cd was found to increase with increasing concentration. The highest BTF (2.03) was observed for tobacco plants treated with Cd at a concentration of 200 mg/kg, with a significant difference compared to control. According to BTF, the 4 metals can be arranged as follows: Cd> Zn> Pb> Cu.

Compared to tobacco, for okra plant **(Table 5)**, the highest BTF (1.52) was observed in plants treated with Cd150 ppm, with a significant difference compared to control. BTF for the

treatments of okra ranged between 0.54-0.69 for Zn, o.28-0.75 for Cu, 1.11-1.52 for Cd & 0.2-0.7 for Pb. No clear increasing or decreasing trend was observed when metal concentration was increased. The BTF in tobacco plants was higher than that in okra plants. In general, higher BTF ratios were observed in plants treated with Cd compared to plants treated with other metals. According to BTF, the 4 metals can be arranged as follows: Cd> Zn> Cu> Pb.

Generally, BTF results indicates variability in the ability of tobacco and okra to translocate metals from the root to the shoot. For both plants, the highest BTF was observed for Cd followed by Zn. On average, BTF for both plants and all treatments was about 70% compared to the root. Both plants can be considered ideal for Cd phytoremediation. While Cd accumulates more in the shoot, Pb was found to accumulate more in the root. This is in agreement with the study of Wang et al. (2013) who found that Cd accumulated mainly in shoots of tobacco plant while Pb accumulated in roots.

Results of BAF and BCF for both plant types are summarized in **Tables 4 & 5.** BAF values for tobacco plant ranged between 0.29-0.74 for Zn, 0.07-0.4 for Cu, 0.92-1.97 for Cd and 0.14-0.6 for Pb. BCF values ranged between 0.7-1.52 for Zn, 0.42-0.93 for Cu, 0.88-2.11 for Cd and 0.48-1.53 for Pb **(Table 4)**. Generally, Tobacco accumulates higher levels of metals in their roots than in their shoots. However, Cd levels were high in both roots and shoots compared to the soil.

The results of this portion of the study demonstrates the ability of *N. tabacum* and *H. esculentus* to efficiently phytoextract Cd, baring a tolerance mechanism for Cd detoxification. These results are in agreement with the results obtained by Zinov'ev & Sole.(2004). They studied the potential of fifty-nine species of *Nicotiana* to take up Cd from Cd-contaminated and uncontaminated soil. They found that most species of *Nicotiana* including *Nicotiana tabacum* have the ability to translocate Cd and build up in their leaves.

Table 4: The Biotranslocation (BTF), Bioaccumulation (BAF), and bioconcentration (BCF) factors for tobacco plant.

Mean ± ER (N=4)			
Treatments	**BTF**	**BAF**	**BCF**
Control	0.38 ± 0.11	0.94 ± 0.24	2.60 ± 0.17
Zn300	0.86 ± 0.06*	0.74 ± 0.06	0.87 ± 0.06*
Zn500	0.65 ± 0.06	0.56 ± 0.07	0.86 ± 0.05*
Zn800	0.55 ± 0.05	0.82 ± 0.05	1.52 ± 0.04*
Zn1000	0.44 ± 0.07	0.29 ± 0.02*	0.70 ± 0.07*
MIX	0.49 ± 0.08	0.28 ± 0.01*	0.63 ± 0.12*
Control	0.39 ± 0.10	0.63 ± 0.16	1.57 ± 0.08
Cu50	0.44 ± 0.05	0.40 ± 0.01	0.93 ± 0.07*
Cu100	0.25 ± 0.00	0.16 ± 0.01*	0.65 ± 0.04*
Cu200	0.27 ± 0.01	0.12 ± 0.01*	0.43 ± 0.02*
Cu300	0.18 ± 0.01*	0.07 ± 0.00*	0.42 ± 0.01*
MIX	0.22 ± 0.01	0.13 ± 0.01*	0.58 ± 0.03*
Control	0.87 ± 0.14	0.60 ± 0.08	0.71 ± 0.08
Cd50	0.67 ± 0.10	0.92 ± 0.18	1.36 ± 0.12*
Cd100	1.04 ± 0.06	1.97 ± 0.05*	2.11 ± 0.21*
Cd150	1.44 ± 0.08	1.21 ± 0.04	0.88 ± 0.04
Cd200	2.03 ± 0.45	1.85 ± 0.28*	0.96 ± 0.08
MIX	1.56 ± 0.82	1.40 ± 0.16*	2.08 ± 0.11*
Control	0.54 ± 0.22	0.05 ± 0.01	0.12 ± 0.04
Pb50	0.40 ± 0.00	0.60 ± 0.01*	1.53 ± 0.02*
Pb100	0.37 ± 0.00	0.24 ± 0.00*	0.66 ± 0.01*
Pb150	0.35 ± 0.00	0.17 ± 0.00*	0.50 ± 0.00*
Pb200	0.30 ± 0.00	0.14 ± 0.00*	0.48 ± 0.00*
MIX	0.35 ± 0.01	0.31 ± 0.01*	0.88 ± 0.01*

*: represents statistically significant difference between the control and treatments ($P < 0.05$).

Table 5: The Biotranslocation (BTF), Bioaccumulation (BAF), and bioconcentration (BCF) factors for okra plant.

Mean ± ER (N=4)			
Treatments	**BTF**	**BAF**	**BCF**
Control	0.70 ± 0.20	0.77 ± 0.13	1.26 ± 0.18
Zn300	0.63 ± 0.13	0.65 ± 0.05	1.10 ± 0.13
Zn500	0.54 ± 0.08	0.56 ± 0.06	1.05 ± 0.07
Zn800	0.68 ± 0.08	0.72 ± 0.02	1.09 ± 0.11
Zn1000	0.69 ± 0.04	0.69 ± 0.05	1.01 ± 0.11
MIX	1.81 ± 0.13*	0.18 ± 0.01*	0.10 ± 0.00*
Control	0.11 ± 0.03	0.22 ± 0.06	2.13 ± 0.18
Cu50	0.75 ± 0.05 *	0.18 ± 0.01	0.24 ± 0.02*
Cu100	0.62 ± 0.04 *	0.19 ± 0.01	0.30 ± 0.00*
Cu200	0.28 ± 0.02	0.08 ± 0.01*	0.29 ± 0.01*
Cu300	0.68 ± 0.06*	0.06 ± 0.01*	0.10 ± 0.00*
MIX	0.06 ± 0.01	0.03 ± 0.00*	0.67 ± 0.01*
Control	0.92 ± 0.08	0.27 ± 0.04	0.29 ± 0.03
Cd50	1.33 ± 0.06*	0.88 ± 0.02*	0.66 ± 0.02*
Cd100	1.11 ± 0.01	0.83 ± 0.01*	0.75 ± 0.02*
Cd150	1.52 ± 0.09*	0.55 ± 0.02*	0.36 ± 0.02
Cd200	1.11 ± 0.04	0.24 ± 0.01	0.22 ± 0.02
MIX	1.24 ± 0.07*	0.56 ± 0.03*	0.45 ± 0.02*
Control	0.80 ± 0.29	0.10 ± 0.04	0.13 ± 0.02
Pb50	0.20 ± 0.03	0.03 ± 0.00	0.14 ± 0.01
Pb100	0.47 ± 0.21	0.05 ± 0.02	0.11 ± 0.02
Pb150	0.70 ± 0.09	0.08 ± 0.01	0.11 ± 0.01
Pb200	0.22 ± 0.06	0.02 ± 0.00*	0.06 ± 0.00*
MIX	0.23 ± 0.02	0.02 ± 0.00	0.08 ± 0.01

*: Represents significant differences between the control and treatments (P< 0.05).

BAF for the treatments of okra ranged between 0.56-0.72 for Zn, 0.08-0.19 for Cu, 0.24-0.88 for Cd & 0.02-0.08 for Pb. BCF values ranged between 1.01-1.10 for Zn, 0.1-0.30 for Cu, 0.22-0.75 for Cd & 0.06-0.14 for Pb. In general, okra accumulate more in roots than in shoot. Highest BAF & BCF values were for Zn in both plants. BAF & BCF can be arranged as: Zn>Cd>Cu>Pb. Daghan et al. (2013) reported similar results indicating inability of the plant to translocate the heavy metals Zn, Pb and Cu. Bentum et al. (2017) reported that the BCF values of Zn, Cu, and Pb in okra plants are less than one.

Tobacco plant has a phytostabilisation potential to Zn, Cu, and Pb. Balabanova et al. (2015) also reported that the BAF was lower than one in all studied species treated with Zn, Cu, Cd, and Pb. Based on the calculated BCF values, the Zn treated plants at 800 mg/kg, the Cd treated plants at 50 and 100 mg/kg including the MIX treatment, and the Pb treated plants at 50 mg/kg accumulated the heavy metals in the tobacco roots. There were significant differences in all treatments of Zn, Cu, and Pb compared to the control of each treatment.

The BTF values were found to be more than one in *M.jacquemontii*, *C.bijarensis*, *S.barbata*, and *C.juncea* and less than one in *C. botrys*, *C. virgata*, *A. verus*, *Z. clinopodioides*, *C. congestum*, *S. orientalis*, *Cousinia* sp, and *V. speciosum* with Zn-contaminated site. Whereas with Cu-contaminated site, the BTF values were found to be less than one in all studied species (Nouri et al., 2009). Tamaoki et al. (2016) studied the potential of sixteen plant species for phytoremediation of radiocesium-contaminated soil. They reported that the TF of radiocesium was 0.077 of the okra plant. In okra plants, the BTF and BAF values were found to be less than one at Cd treatments. Whereas, the highest BAF was observed in okra plant at 50 mg/kg of Zn (2.3). According to Hassan et al.(2018) study, okra plants have a good potential to translocate Zn from roots to shoot parts, as a BTF was more than one (1.5). This is in good agreement with our study which indicated high BAF and high (>1) BCF for Zn.

Tobacco plants subjected to a mixture of metals in soil had BTF values ranging between 0.22-1.56 with the following order: Cd>Zn>Pb>Cu. **(Table 4)**. BTF values were less than 1 except for Cd which equals (1.56). BAF and BCF values were also less than one except for Cd (BAF=1.4, BCF=2.08). BAF and BCF followed the same order: Cd>Pb>Zn>Cu. This indicates high ability of tobacco to absorb, accumulate and translocate Cd.

2. Total Metal Uptake by Tobacco and Okra Plants

Total metal uptake by roots and shoots were calculated by multiplying the metal concentration in shoot or root (mg/kg) by the shoot or root dry weight (kg). Total Metal uptake is considered an important factor to determine the phytoremediation efficiency of shoot and root.

The soil properties, plant species, and the properties of heavy metals are affecting the phytoavailability phenomenon. Phytoavailability phenomenon referred to the availability degree of contaminants in soil and the ability of plants to absorb and uptake heavy metals that are exposed to them (Laghlimi et al., 2015). When the exposure time and eruption rate of the solution increased the heavy metal uptake is increased.

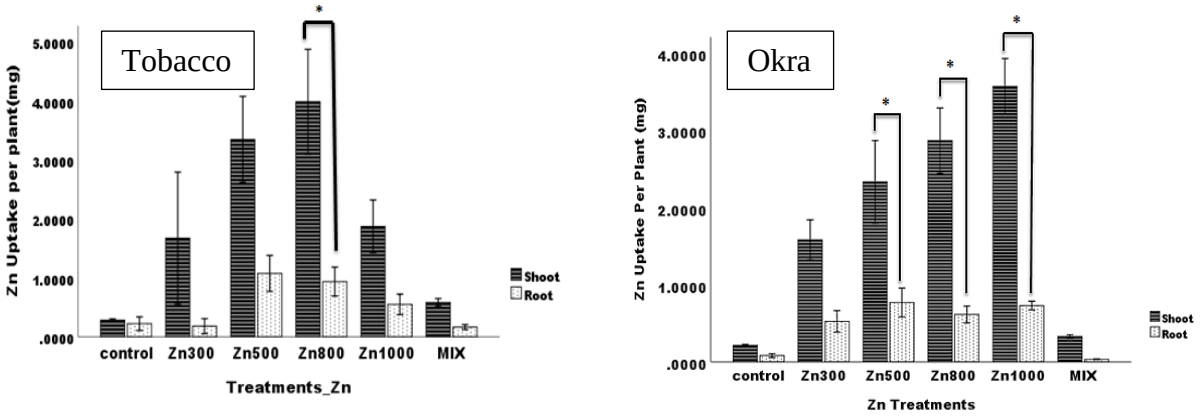

Figure 39: Zinc total uptake in shoots and roots of tobacco & okra plants. *: indicates significant differences between the control and treatments (P< 0.05). Values represent Mean±SE of 4 readings.

Figure 39 shows the content of Zn in shoots and roots of *N. tabacum*. The highest Zn content was observed in shoots at Zn 500 and Zn800 (3.34 and 3.97g per plant), which was statistically significant compared to control (0.28g) (P= 0.04 and 0.013, respectively). As well, a significant difference was observed between shoots and roots at Zn 800 ppm. Whereas in the roots, the highest content of Zn was observed at Zn500 (1.07g per plant) slightly significant difference

compared to the control (P=0.047). While the lowest shoot and root content of Zn was observed at MIX treatment.

Norouzi et al. (2014) studied the effect of different prior crops on uptake of Zinc by wheat. They found that the Zn uptake ranged from 19.2 to 51.8 % statistically significant compared to the control treatment.

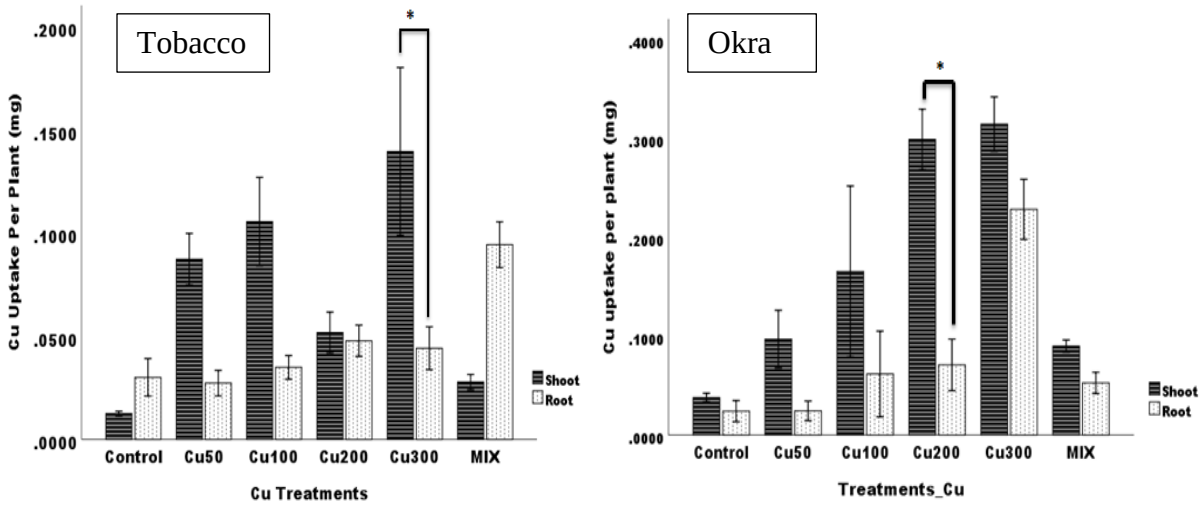

Figure 40: Cu total uptake in shoots and roots of tobacco & okra plants. *: indicates significant differences between the control and treatments (P< 0.05). Values represent Means±SE of 4 readings.

The content of Cu in shoots and roots of *N. tabacum* is shown in **Figure 40**. The Cu content in shoots at Cu 200 and Cu 300 was significantly higher than control and other treatments (P= 0.003 and 0.002, respectively) whereas in root a high significant difference was only observed at Cu300 (P= 0.00). As well as, there was a significant difference between shoots and roots at Cu200.

Total metal uptake in shoot and root of okra plants under Cu treatments is shown in **Figure 40**. Statistically significant increases were observed in Cu content at Cu100 and Cu300 compared to the control for shoots (P= 0.039 and 0.003), as well as the MIX treatment in roots (P=0.001). On

the other hand, A highly significant difference was observed between shoots and roots at the highest concentration of Cu treatments (P= 0.005).

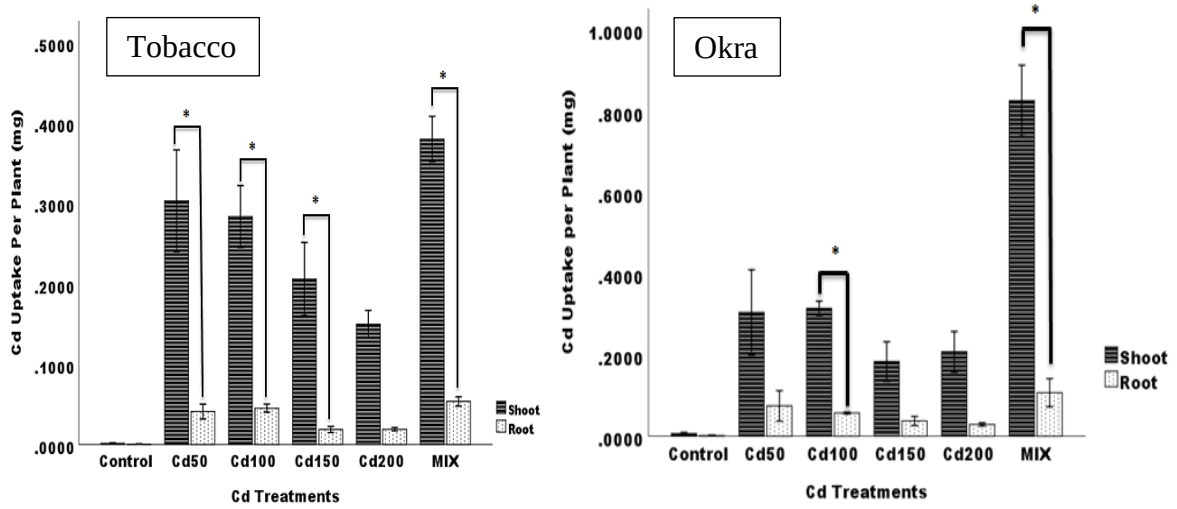

Figure 41: Cd total uptake in shoots and roots of tobacco & okra plants. *: indicates significant differences between the control and treatments (P< 0.05). Values represent Mean±SE of 4 readings.

The figures above show the metal uptake in shoots and roots per plant under Cd treatments (**Figure 41)** for both tobacco and okra. As the Cd concentration increased the content of Cd per plant decreased. Statistically, no significant differences were observed in Cd content at Cd100, Cd150, and Cd200 compared to the control for both shoots and roots of tobacco plants. Nevertheless, a significant difference was observed in the Cd content of shoots at Cd 50 and MIX treatments compared to the control (P = 0.049 and 0.00 respectively). In the case of Cd100, a significant difference was observed in Cd content between shoots and roots (**Figure 41)**. In general, the Cd content was observed higher in shoots than roots. Similar results were found by Daghan et al. (2008). They reported that the highest Cd content was observed in shoots of tobacco plants at 30 mg Cd/kg soil (790 µg per plant).

Whereby, okra plants had significant differences at Cd 50, 100,150 mg/kg, and MIX treatment for both shoots and roots with the exception in roots at 150 mg/kg of Cd. As well as, there were

significant increases between shoots and roots at the same treatments (P=< 0.001). In both plants, the highest significant difference was recorded between shoots and roots in MIX treatments.

The content of Pb in shoots and roots of tobacco and okra is shown in **Figure 42**. The Pb content in shoots of tobacco significantly increased at Pb100, Pb150, and MIX treatments compared to the control (P= 0.012, 0.002, and 0.049, respectively) whereas in root a significant difference was only observed at Pb150 (P= 0.048). While on the contrary, no significant differences were observed between shoots and roots at all Pb treatments.

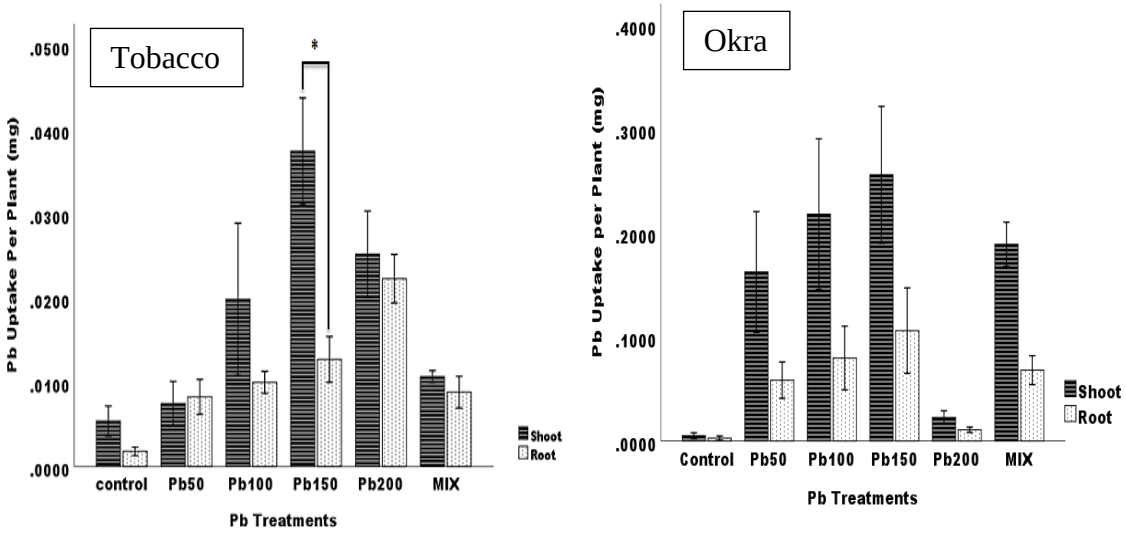

Figure 42: Pb total uptake in shoots and roots of tobacco & okra plants. Values represent Mean±SE of 4 readings.

Wang et al. (2013) reported that the uptake of Cd and Pb of tobacco tissues increased by increasing the concentration of each metal. A study conducted by (Mandakini et al., 2016) reported that the *Azolla pinnate* could remove the Cd and Pb in contaminated solution up to 88% and 86% at 0.5 Cd ppm and 8 Pb ppm, respectively. Pavlíková et al. (2004) study, they found that the Cd uptake was higher in leaves than roots and stems for both control and transgenic plant. Lugon-Moulin et al. (2006) also reported the same results; tobacco plants accumulate more

Cd in their leaves than other parts. In contrast, the Zn uptake by roots was higher than aboveground tissues in transgenic tobacco plants (Pavlíková et al., 2004).

The Pb content in shoots and roots of okra was shown in **Figure 42**. Regarding okra shoots, a significant difference was only observed in plants treated with 150 ppm of Pb compared to the control (P=0.004). However, in roots, significant increases were observed in plants treated with Pb at concentrations of 150 and 200 mg/kg. Plants treated with Pb 150 mg/kg showed a significant difference between shoot and roots of Pb content.

3. Heavy Metal Concentrations in Soil

Metals in soil can be translocated to plants and end up in the food chain reaching the human beings. Therefore, there are guidelines that regulate the permissible levels of metals in soil **(Table 6).**

Table 6: Concentrations of heavy metals in experimental soil permitted by FAO/WHO (Ismail et al., 2014)

Element	Concentration (mg/kg)
Zn	300
Cu	140
Cd	3
Pb	75

The initial and final concentrations of metals in soil were measured at the beginning and the end of the experiment. Initial concentration represents the concentration before planting, while final concentration is the concentration at the end of the experiment after plants harvesting. **Table 7** summarizes the initial, final and % reduction of heavy metals from soil by tobacco plants. Levels of all metals in experimental soil were found to decrease due to plant uptake. The percentages of

reduction of metals from soil ranged between 16.4-82. The highest average percentage of metal reduction was for Pb & Cd (59.05% & 52.37% for Pb and Cd, respectively). The essential heavy metals, Zn & Cu, were reduced at a much less percentages (Avg: 33.12% and 24.6% for Zn and Cu, respectively). These results indicate higher ability of tobacco to uptake more of the nonessential metals.

Table 7: Metal concentration (mg/kg) in experimental soil of treatments before tobacco plantation and at the end of the experiment. Values represent Mean±SE of 4 readings.

Metal	Treatments	Initial Concentration	Final Concentration	% Metal Reduction
Zn	Control	57.70 ± 0.10	29.00 ± 2.40	49.7
	Zn300	269.30 ± 1.10	193.80 ± 2.60	28.0
	Zn500	521.80 ± 23.80	380.90 ± 11.30	27.0
	Zn800	667.20 ± 3.80	528.10 ± 1.70	20.8
	Zn1000	772.30 ± 1.10	334.60 ± 36.40	56.7
				Avg=33.12
Cu	Control	11.60 ± 0.20	6.90 ± 0.90	40.5
	Cu50	51.70 ± 0.30	41.70 ± 0.30	19.3
	Cu100	95.60 ± 2.20	76.50 ± 6.30	20.0
	Cu200	215.50 ± 4.10	123.50 ± 13.90	42.7
	Cu300	349.40 ± 6.20	292.20 ± 9.00	16.4
				Avg=24.6
Cd	Control	1.60 ± 0.00	0.90 ± 0.10	43.8
	Cd50	56.20 ± 0.60	19.70 ± 0.30	64.9
	Cd100	95.60 ± 2.00	53.40 ± 5.20	44.1
	Cd150	151.70 ± 4.70	78.80 ± 8.40	48.1
	Cd200	193.50 ± 0.70	92.10 ± 1.50	52.4
				Avg=52.37
Pb	Control	12.00 ± 0.20	5.50 ± 1.30	54.2
	Pb50	44.50 ± 1.10	22.10 ± 0.30	50.3
	Pb100	111.20 ± 2.20	50.40 ± 19.40	54.7
	Pb150	153.50 ± 1.70	78.00 ± 15.60	49.2
	Pb200	194.50 ± 0.70	35.00 ± 0.80	82.0
				Avg=59.05

Metal concentrations in MIX soil planted with tobacco are shown in **Table 8**. Metal reduction from soil ranged between 36.9% and 63.9%. Highest removal percentages of metals were for Zn and Pb.

Table 8: Metal concentration (mg/kg) in experimental soil of MIX treatment before tobacco plantation and at the end of the experiment. Values represent Mean±SE of 4 readings.

MIX Treatment	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Metal Reduction
Zn	293.70 ± 4.70	106.10 ± 0.90	63.9
Cu	103.80 ± 0.40	64.90 ± 1.10	37.5
Cd	84.50 ± 3.90	53.30 ± 3.90	36.9
Pb	86.90 ± 0.10	42.80 ± 2.60	50.7

A comparison of the initial and final concentrations of metals in soil, higher significant differences was observed at all treatments to Zn, Cu, Cd, and Pb for both plant types, except the Cu50 and Cu100 and Pb 50 in tobacco planted experimental soil **(Figure 43).** These results indicate that the tobacco removes significant amounts of metals, especially Cd & Pb, from contaminated soil.

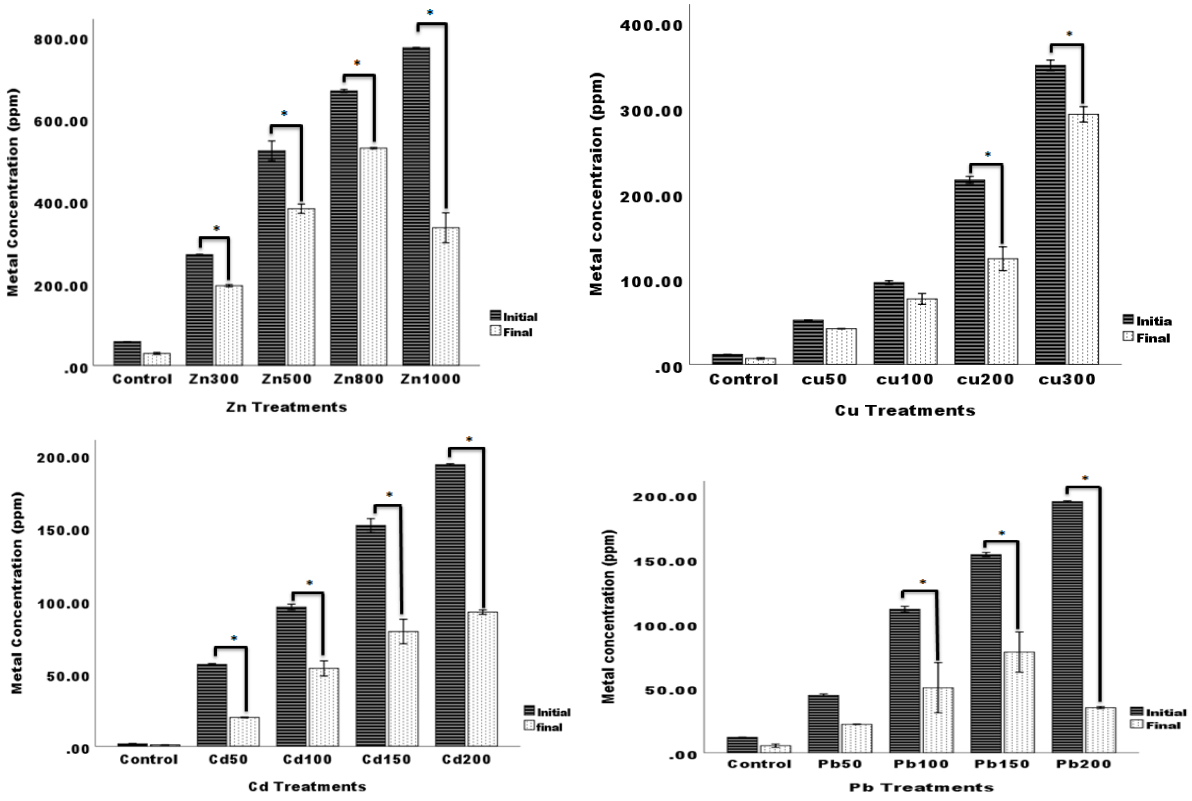

Figure 43: Metal concentration (mg/kg) in experimental soil of treatments before tobacco plantation and at the end of the experiment. Values represent Mean±SE of 4 readings.

Table 9 summarizes the initial, final and % reduction of heavy metals from soil by okra plants. Levels of all metals in experimental soil were found to decrease due to plant uptake of metals. The percentages of reduction of metals from soil ranged between 20.8-64.3. The highest average percentage of metal reduction was for the nonessential metals Pb & Cd (57.65% & 51.72% for Pb and Cd, respectively). The essential heavy metals, Zn & Cu, were reduced at a much less percentages (Avg: 41.05% and 36.25% for Zn and Cu, respectively). These results indicate higher ability of okra to uptake more of the nonessential metals.

Table 9: Metal concentration (mg/kg) in experimental soil of treatments before okra plantation and at the end of the experiment. Values represent Mean±SE of 4 readings.

Heavy Metals	Treatments	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Metal Reduction
Zn	Control	31.70 ± 1.10	18.63 ± 0.67	41.2
	Zn300	290.20 ± 3.00	198.02 ± 2.88	31.8
	Zn500	512.90 ± 7.50	286.26 ± 4.95	44.2
	Zn800	701.60 ± 8.80	415.16 ± 8.50	40.8
	Zn1000	742.10 ± 12.30	390.03 ± 2.60	47.4
				Avg=41.05
Cu	Control	7.10 ± 0.30	5.74 ± 0.31	19.2
	Cu50	53.80 ± 1.80	33.57 ± 0.73	37.6
	Cu100	82.50 ± 3.10	65.38 ± 0.67	20.8
	Cu200	215.50 ± 1.90	118.69 ± 2.42	44.9
	Cu300	332.50 ± 0.90	193.96 ±7.79	41.7
				Avg=36.25
Cd	Control	0.80 ± 0.20	0.40 ± 0.10	50.0
	Cd50	59.30 ± 0.30	21.98 ± 0.37	62.9
	Cd100	97.60 ± 0.80	46.73 ± 1.09	52.1
	Cd150	131.90 ± 3.10	75.46 ± 2.42	42.8
	Cd200	184.10 ± 0.50	93.75 ± 1.75	49.1
				Avg=51.72
Pb	Control	6.90 ± 0.30	4.83 ± 0.12	30.0
	Pb50	47.20 ± 2.40	16.84 ± 1.27	64.3
	Pb100	116.40 ± 0.80	52.52 ± 3.38	54.9
	Pb150	146.10 ± 1.10	68.88 ± 1.51	52.9
	Pb200	203.20 ± 2.00	84.39 ± 0.12	58.5
				Avg=57.65

All levels of metals in soil before the experiment and after the experiment were statistically significantly different from each other (**Figure 44).** This again emphasizes the ability of okra to uptake and accumulate heavy metals.

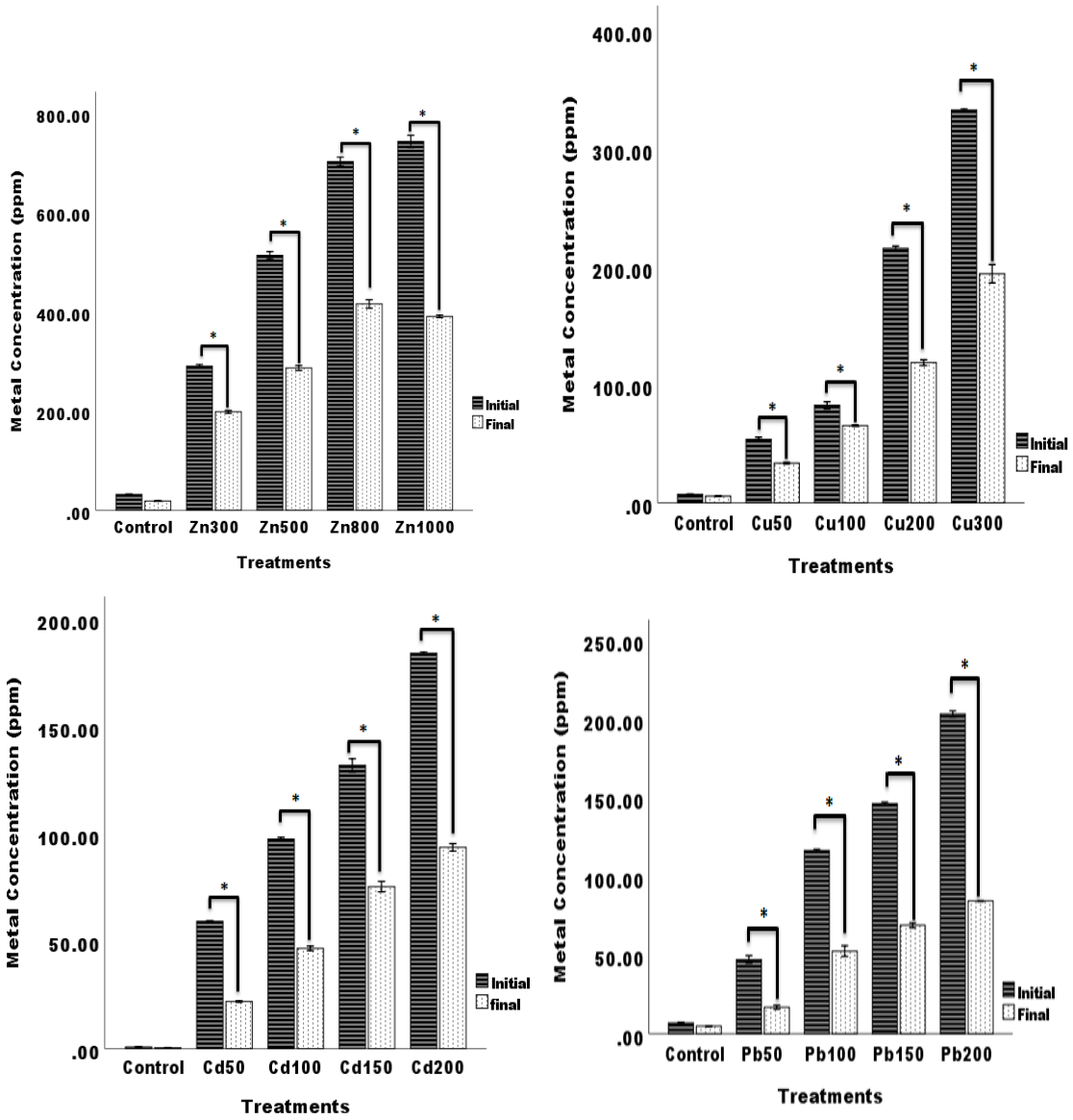

Figure 44: Metal concentration (mg/kg) in experimental soil of treatments before okra plantation and at the end of the experiment. Values represent Mean±SE of 4 readings.

The final metal concentration in soil contaminated with a mixture of metals statistically significantly decreased compared to the initial concentration for both plants **(Figure 45)**. The

highest difference between final and initial concentration was observed in zinc for tobacco plants.

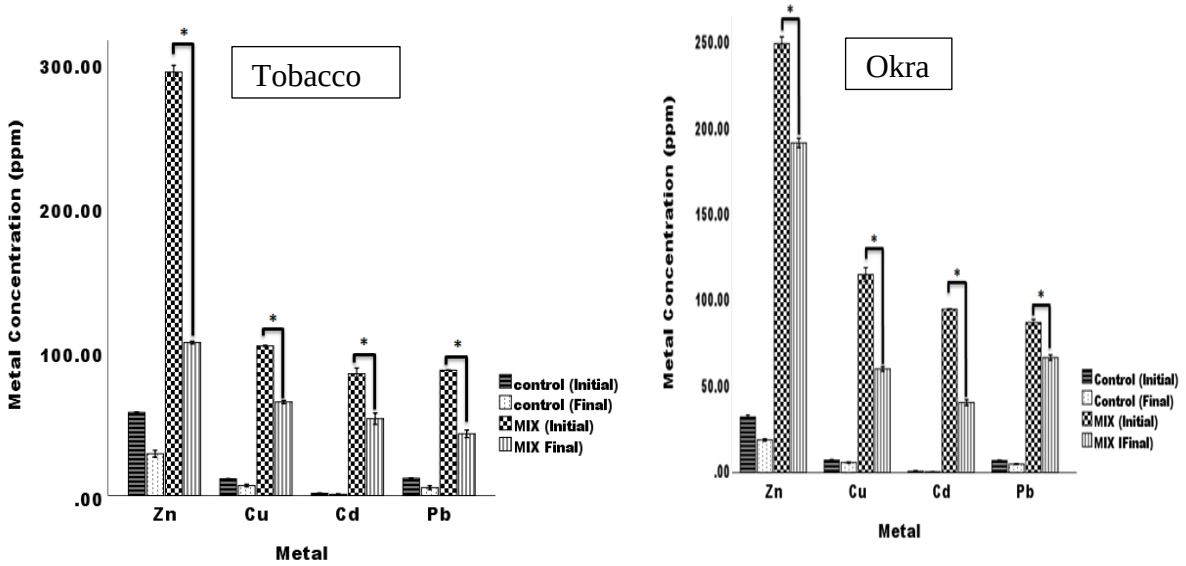

Figure 45: Metal concentration (mg/kg) in experimental soil MIX treatment before plantation and at the end of the experiment. Values represent Mean±SE of 4 readings.

Metal concentrations in MIX soil planted with okra are shown in **Table 10**. Metal reduction from soil ranged between 23.2% and 57.2%. Highest removal percentages of metals were for Cd and Cu.

Table 10: Metal concentration (mg/kg) in experimental soil of MIX treatment before okra plantation and at the end of the experiment. Values represent Mean±SE of 4 readings.

MIX Treatment	Initial Concentration (mg/Kg)	Final Concentration (mg/Kg)	% Reduction
Zn	245.00 ± 3.80	188.23 ± 2.66	23.2
Cu	113.10 ± 3.90	59.15 ± 1.34	47.7
Cd	93.30 ± 0.10	39.95 ± 1.71	57.2
Pb	85.70 ± 1.70	65.68 ± 1.57	23.4

Conclusions

From the results of the present study, the following conclusions can be made:

- The growth (shoot and root length & weight & chlorophyl content) of both tobacco and okra was generally not affected by metal treatments in the experimental soil (except for high concentrations of Pb & Cd).
- The growth of both plants was generally not affected by a mixture of metals in soil.
- Metal in contaminated soil did not affect okra fruit weight.
- With increasing metal concentration in soil, metal levels in plant tissues increased significantly.
- For both tobacco and okra plants, the accumulation of metals was found to be more in roots than in shoots for zinc, copper, and lead. While for cadmium, the accumulation was higher in shoots than roots.
- Cadmium can be significantly translocated from belowground parts to aerial parts.
- In two months, both plants were found to significantly reduce metal concentrations in soil by more than 50% especially for Cd & Pb.
- Both tobacco and okra plants have the ability to be used in phytoextraction of heavy metals from contaminated soil.

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Appendices

A. Plant growth, chlorophyll content, and Biomass

➢ Effect of Metals on Shoot Length

Table 1: Summary of shoot length measurements (cm) of tobacco plants subjected to different Zn concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	8.10±0.24 (7.60-8.60)	8.55±0.17 (8.20-9.00)	9.00±0.21 (8.60-9.60)	10.83±0.81 (9.50-13.20)	32.20±3.57 (27.10-42.60)
Zn300	8.07±0.34 (7.40-8.70)	8.65±0.31 (8.10-9.50)	9.30±0.42 (8.40-10.40)	12.33±0.48 (11.40-13.60)	30.98±6.32 (21.40-48.20)
Zn500	8.50±0.47 (7.40-9.70)	9.80±0.97 (7.60-12.00)	10.18±0.98 (8.10-12.50)	15.00±2.99 (10.50-23.20)	38.83±6.06 (30.10-56.00)
Zn800	7.28±0.50 (6.00-8.20)	7.70±0.47 (6.40-8.40)	8.05±0.45 (6.90-8.90)	9.65±0.58 (8.60-10.80)	24.78±1.63 (21.00-27.60)
Zn1000	8.05±0.47 (7.10-9.20)	8.82±0.63 (7.30-10.20)	9.00±0.61 (7.60-10.40)	13.83±1.58 (9.90-17.30)	36.98±7.13 (19.70-53.50)
Significance	NS	NS	NS	NS	NS

Table 2: Summary of shoot length measurements (cm) of okra plants subjected to different Zn concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	24.50±4.33 (20.30-30.50)	27.18±1.75 (22.90-31.30)	30.45±1.33 (27.90-34.10)	39.63±1.41 (35.50-41.80)	65.25±7.01 (51.90-81.00)
Zn300	25.00±3.21 (21.50-28.20)	26.40± 1.64 (23.00-29.50)	29.80±1.07 (27.60-32.60	39.78±2.79 (32.30-45.80)	57.48±3.71 (50.00-63.50)
Zn500	26.08±2.24 (23.10-28.20)	27.18±1.33 (23.80-29.50)	29.18±1.62 (25.30-31.90)	36.58±1.76 (31.50-39.50)	55.48±4.42 (46.00-63.50)
Zn800	19.00±2.51 (16.10-22.20)	20.55±1.42 (16.40-22.50)	22.78±1.81 (18.00-26.80)	31.15±2.01 (26.50-36.00)	42.93±4.79 (35.30-56.40)
Zn1000	27.00±3.83 (22.40-31.50)	29.60±2.00 (26.70-35.50)	31.70±1.56 (29.1036.50)	38.98±1.98 (35.50-43.70)	68.60±1.30 (66.60-72.30)
Significance	S	S	S	S	S

Table 3: Summary of shoot length measurements (cm) of tobacco plants subjected to different Cu concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	8.10±0.24 (7.60-8.60)	8.55±0.17 (8.20-9.00)	9.00±0.21 (8.60-9.60)	10.83±0.81 (9.50-13.20)	32.20±3.57 (27.10-42.60)
Cu50	8.15±0.23 (7.60-8.70)	9.38±0.34 (8.50-10.10)	9.85±0.22 (9.50-10.40)	12.63±0.54 (11.20-13.60)	24.78±5.12 (14.00-34.40)
Cu100	8.28±0.53 (7.30-9.50)	8.95±0.83 (7.50-10.80)	9.55±0.74 (8.20-11.40)	13.90±2.00 (11.40-19.80)	37.25±5.51 (28.30-53.30)
Cu200	8.28±0.58 (7.60-10.00)	9.10±0.81 (8.10-11.50)	9.58±0.78 (8.60-11.90)	13.38±1.47 (10.60-17.30)	32.40±3.05 (24.00-38.60)
Cu300	6.90±0.32 (6.20-7.50)	7.60±0.40 (6.50-8.30)	7.95±0.46 (6.60-8.60)	11.70±0.29 (11.00-12.40)	32.18±2.49 (27.20-39.00)
Significance	NS	NS	NS	NS	NS

Table 4: Summary of shoot length measurements (cm) of okra plants subjected to different Cu concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	24.50±2.16 (20.30-30.50)	27.18±1.75 (22.90-31.30)	30.45±1.33 (27.90-34.10)	39.63±1.41 (35.50-41.80)	65.25±7.01 (51.90-81.00)
Cu50	25.30±0.62 (23.80-26.80)	27.08±0.94 (25.00-29.00)	31.70±1.00 (29.80-34.20)	40.85±3.27 (35.10-47.20)	64.00±5.29 (49.20-73.10)
Cu100	21.10±1.43 (18.90-25.10)	22.20±1.36 (19.80-25.90)	25.73±1.42 (22.30-28.10)	32.53±2.39 26.60-37.50)	48.50±3.32 (41.50-55.80)
Cu200	22.75±2.25 (16.10-25.90)	24.40±2.29 (18.00-28.40)	27.53±2.68 (19.50-30.60)	33.78±1.42 (30.50-37.40)	39.33±1.31 (35.50-41.40)
Cu300	23.08±2.89 (15.40-28.80)	24.75±2.86 (16.80-29.70)	29.68±0.98 (28.00-32.00)	32.78±1.01 (30.90-35.30)	40.50±2.00 (35.20-44.70)
Significance	NS	NS	S	NS	S

Table 5: Summary of shoot length measurements (cm) of tobacco plants subjected to different Cd concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	8.10±0.24 (7.60-8.60)	8.55±0.17 (8.20-9.00)	9.00±0.21 (8.60-9.60)	10.83±0.81 (9.50-13.20)	32.20±3.57 (27.10-42.60)
Cd50	6.98±0.61 (6.20-8.80)	7.93±0.56 (6.90-9.50)	8.53±0.38 (7.80-9.60)	11.30±1.07 (9.50-14.20)	18.45±2.08 (14.30-23.50)
Cd100	7.65±0.34 (7.00-8.60)	8.03±0.52 (7.10-9.50)	8.50±0.59 (7.40-10.10)	10.45±0.35 (9.50-11.10)	15.95±1.10 (13.40-18.50)
Cd150	7.28±1.01 (5.50-10.10)	7.88±1.14 (5.90-11.00)	8.25±1.07 (6.40-11.20)	9.20±0.98 (6.90-11.60)	15.40±2.09 (10.50-19.40)
Cd200	7.83±0.63 (7.00-9.70)	7.43±0.15 (7.10-7.80)	8.60±0.65 (7.60-10.50)	9.93±0.64 (8.20-11.00)	15.05±1.22 (12.80-18.30)
Significance	NS	NS	NS	NS	S

Table 6: Summary of shoot length measurements (cm) of okra plants subjected to different Cd concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	24.50±2.16 (20.30-30.50)	27.18±1.75 (22.90-31.30)	30.45±1.33 (27.90-34.10)	39.63±1.41 (35.50-41.80)	65.25±7.01 (51.90-18.00)
Cd50	23.33±0.64 (21.90-24.60)	24.30±0.61 (23.10-25.50)	25.88±0.68 (24.50-27.40)	30.25±0.97 (28.50-33.00)	38.98±0.61 (37.20-40.00)
Cd100	25.88±1.71 (22.40-29.60)	26.63±1.65 (23.30-30.20)	28.83±1.88 (25.50-32.70)	32.68±2.17 (28.40-37.90)	43.20±1.18 (40.50-46.00)
Cd150	23.80±1.56 (20.50-26.60)	25.70±1.69 (22.50-30.00)	27.88±1.14 (52.00-30.50)	32.70±1.65 (29.70-36.90)	42.45±3.81 (33.00-50.00)
Cd200	18.20±2.30 (12.30-23.50)	19.35±2.72 12.50-25.80)	21.13±2.30 (16.30-27.40)	24.33±2.26 (19.00-30.00)	38.20±1.64 (33.60-41.00)
Significance	NS	NS	S	S	S

Table 7: Summary of shoot length measurements (cm) of tobacco plants subjected to different Pb concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	8.10±0.24 (7.60-8.60)	8.55±0.17 (8.20-9.00)	9.00±0.21 (8.60-9.60)	10.83±0.81 (9.50-13.20)	32.20±3.57 (27.10-42.60)
Pb50	7.08±0.32 (6.40-7.90)	7.53±0.29 (7.00-8.30)	8.68±0.45 (7.60-9.80)	11.40±1.31 (9.60-15.30)	25.08±3.07 (20.40-33.40)
Pb100	7.00±0.87 (5.10-9.20)	7.48±0.80 (5.50-9.40)	8.50±0.51 (7.50-9.90)	10.95±0.50 (9.50-11.80)	28.43±2.99 (24.00-37.20)
Pb150	8.98±0.91 (7.30-11.50)	9.90±0.73 (8.40-11.90)	10.80±0.73 (9.80-12.90)	14.53±1.29 (21.20-17.00)	32.60±4.81 (18.60-40.40)
Pb200	6.05±0.59 (5.00-7.50)	6.63±0.48 (5.60-7.50)	7.18±0.48 (6.30.8.10)	8.68±0.84 (7.20-11.00)	14.35±1.42 (11.00-17.00)
Significance	NS	NS	NS	NS	S

Table 8: Summary of shoot length measurements (cm) of okra plants subjected to different Pb concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	24.50±2.16 (20.30-30.50)	27.18±1.75 (22.90-32.30)	30.45±1.33 (27.90-34.10)	39.63±1.41 (35.50-41.80)	65.25±7.01 (15.90-81.00)
Pb50	20.65±1.11 (18.80-23.60)	22.33±0.98 (20.10-24.80)	24.98±1.10 (22.40-27.00)	31.73±1.83 (27.50-35.80)	42.05±2.16 (35.60-44.80)
Pb100	18.80±1.35 (25.50-21.50)	20.40±1.30 (16.90-22.60)	22.75±1.56 (18.30-25.50)	31.80±3.05 (25.80-38.60)	44.08±4.15 (36.70-52.00)
Pb150	20.73±1.57 (16.10-23.10)	21.90±1.31 (18.20-24.30)	23.48±1.30 (19.70-25.60)	31.55±1.38 (28.50-35.20)	40.93±1.64 (38.00-45.50)
Pb200	19.23±2.00 (13.50-22.50)	21.58±1.37 (18.00-24.00)	25.85±1.09 (23.00-28.10)	34.98±2.35 (29.00-40.50)	72.60±2.89 (64.00-76.40)
Significance	NS	S	S	NS	S

Table 9: Summary of shoot length of tobacco plants subjected to mix treatment for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	8.10±0.24 (7.60-8.60)	8.55±0.17 (8.20-9.00)	9.00±0.21 (8.60-9.60)	10.83±0.81 (9.50-13.20)	32.20±3.57 (27.10-42.60)
MIX	6.28±0.86 (4.00-8.00)	7.03±0.74 (5.10-8.70)	7.90±0.81 (5.50-9.00)	11.18±0.46 (10.50-12.50)	24.88±3.41 (18.00-32.70)
Significance	NS	NS	NS	NS	NS

Table 10: Summary of shoot length of okra plants subjected to mix treatment for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	24.50±4.33 (20.30-30.50)	27.18±1.75 (22.90-31.30)	30.45±1.33 (27.90-34.10)	39.63±1.41 (35.50-41.80)	65.25±7.01 (51.90-81.00)
MIX	17.18±2.53 (15.40-20.80)	18.95±1.83 (15.70-23.00	21.58±2.31 (17.50-26.10)	31.10±1.24 (29.20-34.50)	57.75±5.15 (47.70-69.60)
Significance	NS	NS	NS	NS	NS

- **Effect of Metals on Chlorophyll Content**

Table 11: Summary of chlorophyll content measurements (SPAD unit) of tobacco plants subjected to different Zn concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	23.83±0.68 (22.5-25.4)	30.00±2.88 (23.7-36.00)	35.03±0.73 (33.5-36.6)	37.00±3.04 (31.8-43.9)	39.80±1.15 (37.80-43.00)
Zn300	24.43±1.1 (21.2-26.1)	27.63±2.66 (20.00-32.3)	32.55±1.14 (30.7-35.6)	41.33±1.29 (37.8-43.3)	37.83±3.41 (28.0-43.60)
Zn500	24.98±1.78 (21.3-29.1)	33±0.67 (31.8-34.6)	31.68±0.97 (29.00-33.2)	39.78±2.3 (34.1-44.5)	43.10±1.37 (39.70-46.40)
Zn800	22.65±1.45 (18.9-25)	30.55±1.97 (27.0-34.2)	35.6±1.5 (33.00-39.9)	39.35±2.57 (35.1-46.0)	39.08±2.11 (34.1-44.40)
Zn1000	25.48±0.83 (23.2-27.1)	32.25±1.42 (28.2-34.8)	34.4±0.98 (31.8-36.5)	42.00±2.40 (36.1-47.3)	39.40±1.86 (35.40-40.10)
Significance	NS	NS	NS	NS	NS

Table 12: Summary of chlorophyll content measurements (SPAD unit) of okra plants subjected to different Zn concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	22.15±0.14 (21.90-22.40)	31.13±0.99 (28.90-33.70)	36.58±0.36 (35.60-37.30)	34.68±1.41 (32.30-38.20)	34.20±1.47 (31.10-37.70)
Zn300	24.45±0.79 (22.70-26.10)	29.15±0.94 (27.50-13.10)	37.98±1.22 (35.80-41.10)	34.20±1.47 (30.70-37.80)	31.75±0.95 (30.70-34.60)
Zn500	23.38±2.34 (17.10-27.20)	30.38±1.36 (26.80-33.40)	38.60±1.57 (35.10-42.20)	34.58±0.98 (31.80-36.00)	33.18±2.28 (29.40-39.80)
Zn800	19.73±1.53 (15.80-23.30)	25.48±1.34 (21.50-27.30)	37.00±0.83 (35.10-38.40)	33.40±1.53 (30.00-37.30)	35.83±1.97 (30.60-40.10)
Zn1000	24.63±0.78 (23.40-26.90)	29.80±0.87 (27.40-31.10)	41.40±1.62 (37.40-45.30)	35.60±0.75 (33.80-37.40)	34.80±0.76 (32.70-36.30)
Significance	NS	S	NS	NS	NS

Table 13: Summary of chlorophyll content measurements (SPAD unit) of tobacco plants subjected to different Cu concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	23.83±0.68 (22.5-25.4)	30.00±2.88 (23.7-36.00)	35.03±0.73 (33.5-36.6)	37.00±3.04 (31.8-43.9)	39.80±1.15 (37.80-43.00)
Cu50	24.33±1.24 (221.-27.2)	31.55±0.65 (30.8-33.5)	34.25±1.52 (32.1-38.7)	41.35±2.49 (35.7-47.6)	34.68±2.37 (28.7-40.2)
Cu100	24.93±1.58 (20.7-28.0)	31.13±1.23 (27.7-33.3)	36.28±1.67 (33.0-39.3)	39.8±1.75 (35.0-43.1)	43.43±3.35 (34.2-49.5)
Cu200	26.7±0.95 (24.0-28.5)	33.17±1.59 (30.4-37.4)	33.1±1.34 (30.1-36.3)	40.4±2.13 (34.5-44.3)	39.58±1.1 (36.8-42.1)
Cu300	24.33±1.71 (20.8-29.0)	29.95±0.84 (27.5-31.2)	33.55±0.92 (30.9-35.0)	41.98±3.15 (37.3-51.1)	41.1±1.03 (39.4-44.1)
Significance	NS	NS	NS	NS	NS

Table 14: Summary of chlorophyll content measurements (SPAD unit) of okra plants subjected to different Cu concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	22.15±0.14 (21.90-22.40)	31.13±0.99 (28.90-33.70)	36.58±0.36 (35.60-37.30)	34.68±1.41 (32.30-38.20)	34.20±1.47 (31.10-37.70)
Cu50	19.88±0.99 (17.30-21.80)	29.35±1.93 (26.00-33.90)	37.78±1.04 (35.60-40.60)	35.08±1.53 (31.50-38.00)	32.58±0.59 (31.10-33.70)
Cu100	22.55±0.55 (21.60-24.10)	29.55±1.80 (25.20-33.90)	40.23±2.03 (37.40-46.10)	35.43±0.90 (33.50-37.00)	28.08±1.44 (25.50-32.00)
Cu200	24.63±0.65 (22.80-25.80)	32.05±0.67 (30.70-33.40)	39.20±1.05 (37.30-42.00)	33.48±1.42 (30.50-36.10)	27.40±1.33 (24.20-30.50)
Cu300	22.25±0.43 (21.30-23.30)	30.98±0.48 (29.70-31.90)	40.60±0.74 (39.20-42.50)	31.63±1.30 (29.50-35.40)	29.90±1.25 (26.40-32.20)
Significance	NS	NS	NS	NS	S

Table 15: Summary of chlorophyll content measurements (SPAD unit) of tobacco plants subjected to different Cd concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	23.83±0.68 (22.5-25.4)	30.00±2.88 (23.7-36.00)	35.03±0.73 (33.5-36.6)	37.00±3.04 (31.8-43.9)	39.80±1.15 (37.80-43.00)
Cd50	25.28±1.74 (21.8-29.8)	32.03±1.00 (30.5-34.8)	32.73±0.52 (31.70-33.8)	37.38±2.84 (30.50-43.00)	38.40±2.58 (31.50-43.30)
Cd100	24.68±1.76 (19.6-27.3)	28.9±1.51 (25.6-32.9)	31.03±0.87 (28.7-32.6)	35.85±1.69 (32.40-40.50)	34.78±2.18 (31.30-41.10)
Cd150	25.88±2.37 (19.2-30.3)	29.17±1.27 (25.4-30.8)	30.58±1.99 (24.9-33.6)	32.48±1.72 (29.70-37.50)	31.55±2.70 (26.80-39.00)
Cd200	21.45±2.07 (17.2-25.2)	27.38±0.94 (25.4-29.6)	32.95±1.37 (29.5-35.7)	36.13±1.30 (32.30-37.90)	30.23±1.96 (26.70-34.90)
Significance	NS	NS	NS	NS	NS

Table 16: Summary of chlorophyll content measurements (SPAD unit) of okra plants subjected to different Cd concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	22.15±0.14 (21.90-22.40)	31.13±0.99 (28.90-33.70)	36.58±0.36 (35.60-37.30)	34.68±1.41 (32.30-38.20)	34.20±1.47 (31.10-37.70)
Cd50	21.05±2.38 (18.60-28.20)	28.33±1.74 (23.30-31.00)	36.28±1.35 (34.50-40.30)	31.80±1.12 (28.80-33.90)	33.98±1.48 (31.70-38.10)
Cd100	23.73±0.48 (22.80-25.00)	30.35±0.86 (29.20-32.90)	37.53±1.00 (36.00-40.30)	30.30±1.29 (27.70-33.50	34.23±0.56 (33.00-35.70)
Cd150	22.20±0.46 (21.40-23.50)	31.03±2.44 (25.70-36.90)	36.15±2.03 (31.50-39.80)	32.43±2.21 (27.00-37.60)	32.50±2.25 (28.20-37.60)
Cd200	19.70±1.33 (17.40-23.20)	30.35±1.14 (28.40-33.20)	40.55±1.35 (37.60-44.00)	34.43±0.70 (32.80-36.20)	32.78±0.97 (31.00-34.70)
Significance	NS	NS	NS	NS	NS

Table 17: Summary of chlorophyll content measurements (SPAD unit) of tobacco plants subjected to different Pb concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	23.83±0.68 (22.5-25.4)	30.00±2.88 (23.7-36.00)	35.03±0.73 (33.5-36.6)	37.00±3.04 (31.8-43.9)	39.80±1.15 (37.80-43.00)
Pb50	21.1±0.83 (19.3-23.2)	30.15±0.39 (29.2-31.0)	33.15±1.29 (30.6-36.2)	36.58±1.05 (34.2-39.0)	39.2±0.70 (37.5-40.9)
Pb100	22.95±1.14 (21.1-26.2)	35.00±1.41 (32.7-38.7)	31.13±1.26 (29.2-34.7)	38.35±0.30 (37.7-39.0)	40.1±1.23 (37.1-42.8)
Pb150	25.65±1.80 (20.4-28.00)	31.58±2.13 (28.4-37.6)	35.08±2.08 (31.7-41.1)	37.83±1.2 (35.6-40.1)	40.58±1.23 (37.4-43.2)
Pb200	23.23±0.87 (22.125.8)	31.3±1.19 (28.8-33.8)	31.73±0.94 (29.2-33.7)	36.6±0.64 (35.3-38.2)	34.28±0.80 (32.8-36.4)
Significance	NS	NS	NS	NS	S

Table 18: Summary of chlorophyll content measurements (SPAD unit) of okra plants subjected to different Pb concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	22.15±0.14 (21.90-22.40)	31.13±0.99 (28.90-33.70)	36.58±0.36 (35.60-37.30)	34.68±1.41 (32.30-38.20)	34.20±1.47 (31.10-37.70)
Pb50	23.73±0.77 (22.20-25.60)	29.55±2.26 (23.10-32.70)	37.10±0.78 (35.50-39.20)	34.85±2.07 (31.20-40.80)	31.28±0.53 (29.70-32.00)
Pb100	22.23±1.44 (19.20-25.50)	30.50±0.35 (29.90-31.50)	35.80±1.95 (30.50-39.60)	34.33±1.61 (31.20-38.70)	29.53±1.91 (24.00-32.70)
Pb150	24.35±1.18 (21.40-26.70)	28.88±1.37 (26.50-32.80)	36.75±1.02 (34.50-39.10)	34.58±1.00 (31.60-35.90)	31.33±1.47 (27.50-34.50)
Pb200	24.73±0.91 (22.50-26.80)	30.55±0.64 (29.00-31.70)	35.78±1.44 (31.50-37.50)	32.63±2.03 (27.20-36.90)	30.98±1.19 (27.70-32.80)
Significance	NS	NS	NS	NS	NS

Table 19: Summary of chlorophyll content measurements (SPAD unit) of tobacco plants subjected to mix treatment for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	23.83±0.68 (22.5-25.4)	30.00±2.88 (23.7-36.00)	35.03±0.73 (33.5-36.6)	37.00±3.04 (31.8-43.9)	39.80±1.15 (37.80-43.00)
MIX	23.33±0.39 (22.2-24.00)	32.45±1.09 (30.0-35.2)	33.53±0.83 (31.4-34.9)	35.8±1.4 (32.3-38.1)	36.83±0.76 (34.80-38.30)
Significance	NS	NS	NS	NS	NS

Table 20: Summary of chlorophyll content measurements (SPAD unit) of okra plants subjected to mix treatment for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatment	W0	W1	W2	W4	W8
Control	22.15±0.14 (21.90-22.40)	31.13±0.99 (28.90-33.70)	36.58±0.36 (35.60-37.30)	34.68±1.41 (32.30-38.20)	34.20±1.47 (31.10-37.70)
MIX	23.25±1.66 (19.00-26.60)	32.33±0.96 (29.90-34.00)	35.98±2.00 (31.90-40.50)	30.98±2.46 (23.90-35.00)	32.48±0.63 (31.20-33.70)
Significance	NS	S	NS	NS	NS

➢ Effect of Metals on Shoot, Root, and Fruit Biomass

Table 21: Summary of shoots and roots dry weight (g) of tobacco plants subjected to different Zn concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots
Control	8.8±4.62 (3.6-22.62)	1.42±0.7 (0.51-3.5)
Zn300	8.2±5.23 (0.96-23.22)	0.82±0.59 (0.21-2.59)
Zn500	12.37±3.77 (6.07-22.53)	1.79±0.55 (0.9-3.28)
Zn800	7.26±1.48 (4.66-11.51)	0.91±0.23 (0.46-1.51)
Zn10000	8.91±2.59 (2.63-13.28)	1.02±0.28 (0.43-1.54)

Table 22: Summary of shoots, roots and fruit dry weight (g) of okra plants subjected to different Zn concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots	Fruit
Control	9.48±1.56 (6.11-12.57	1.87±0.42 (0.91-2.78)	3.18±1.75 (1.43-4.93)
Zn300	8.55±1.44 (4.66-11.45)	1.61±0.33 (0.76-2.36)	2.80±0.13 (2.66-2.93)
Zn500	7.99±1.35 (4.95-11.52)	1.39±0.25 (0.96-1.91)	4.31±0.16 (4.15-4.47)
Zn800	5.64±0.70 (3.76-6.80)	0.81±0.11 (0.53-1.05)	3.05±1.31 (1.75-4.36)
Zn10000	7.10±0.95 (5.54-9.52)	1.00±0.10 (0.76-1.26)	4.66±1.10 (3.57-5.76)

Table 23: Summary of shoots and roots dry weight (g) of tobacco plants subjected to different Cu concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots
Control	8.8±4.62 (3.6-22.62	1.42±0.70 (0.51-3.50
Cu50	4.67±1.37 (2.25-8.22)	0.51±0.21 (0.27-1.12
Cu100	10.08±4.87 (1.21-22.15)	0.87±0.56 (0.15-2.55
Cu200	12.29±1.57 (9.77-16.73)	0.77±0.29 (0.12-1.42
Cu300	12.1±0.48 (11.06-13.09)	1.56±0.19 (1.01-1.83

Table 24: Summary of shoots, roots and fruit dry weight (g) of okra plants subjected to different Cu concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots	Fruit
Control	9.48±1.56 (6.11-12.57)	1.87±0.42 (0.91-2.78)	3.18±1.75 (1.43-4.93)
Cu50	9.12±0.93 (7.22-11.10)	2.10±0.39 (1.41-2.91)	3.34±0.66 (2.68-4.00)
Cu100	6.73±1.22 (4.16-9.98)	1.41±0.24 (0.87-2.03)	2.86±0.25 (2.61-3.12)
Cu200	***** 3.04±0.67 (1.42-4.23)	0.75±0.10 (0.54-1.01)	5.41±1.39 (4.02-6.80)
Cu300	6.11±1.30 (3.09-9.43)	1.38±0.34 (0.66-2.26)	5.94±1.30 (4.63-7.24)

'*' represent significant differences between the control and treatments ($P < 0.05$)

Table 25: Summary of shoots and roots dry weight (g) of tobacco plants subjected to different Cd concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots
Control	8.8±4.62 (3.6-22.62)	1.42±0.7 (0.51-3.5
Cd50	5.96±1.68 (1.3-9.2)	0.97±0.5 (0.23-2.36
Cd100	1.61±0.12 (1.37-1.87)	0.29±0.03 (0.22-0.35
Cd150	0.77±0.22 (0.39-1.29)	0.22±0.06 (0.11-0.35
Cd200	0.56±0.06 (0.46-0.75)	0.16±0.01 (0.12-0.18

Table 26: Summary of shoots, roots and fruit dry weight (g) of okra plants subjected to different Cd concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots	Fruit
Control	9.48±1.56 6.11-12.57	1.87±0.42 0.91-2.78	3.18±1.75 (1.43-4.93)
Cd50	5.81±1.15 2.65-7.76	1.06±0.25 0.40-1.59	3.76±1.03 (2.72-4.79)
Cd100	***** 3.52±0.45 2.74-4.83	***** 0.62±0.06 0.51-0.77	2.56±0.40 (2.16-2.96)
Cd150	***** 2.82±0.57 1.56-4.29	***** 0.39±0.07 0.22-0.54	3.27±0.63 (2.65-3.90)
Cd200	***** 3.39±0.30 2.57-3.98	***** 0.48±0.05 0.36-0.59	1.33±0.23 (1.10-1.57)

'*' represent significant differences between the control and treatments ($P < 0.05$).

Table 27: Summary of shoots and roots dry weight (g) of tobacco plants subjected to different Pb concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots
Control	8.8±4.62 (3.6-22.62)	1.42±0.7 (0.51-3.5
Pb50	6.1±2.19 (1.83-12.15)	0.86±0.26 (0.25-1.51
Pb100	8.12±2.67 (1.89-14.7)	1.1±0.42 (0.37-2.03
Pb150	9.67±2.5 (3.86-15.97)	1.38±0.53 (0.42-2.73
Pb200	0.81±0.22 (0.23-1.3)	0.12±0.03 (0.06-0.19

Table 28: Summary of shoots, roots and fruit dry weight (g) of okra plants subjected to different Pb concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots	Fruit
Control	9.48±1.56 6.11-12.57	1.87±0.42 0.91-2.78	3.18±1.75 (1.43-4.93)
Pb50	5.47±1.56 1.47-9.09	1.32±0.41 0.44-2.43	6.13±1.02 (5.11-7.15)
Pb100	***** 4.24±1.13 2.28-6.58	0.90±0.14 0.62-1.26	4.34±2.01 (2.33-6.35)
Pb150	***** 3.36±0.36 2.70-4.11	0.77±0.12 0.52-1.06	5.46±0.42 (5.04-5.88)
Pb200	9.31±0.83 7.15-11.18	1.71±0.20 1.33-2.27	4.73±0.10 (4.62-4.83)

'*' represent significant differences between the control and treatments ($P < 0.05$).

Table 29: Summary of shoots and roots dry weight (g) of tobacco plants subjected to mix treatment for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots
Control	8.8±4.62 (3.6-22.62)	1.42±0.7 (0.51-3.5)
MIX	7.18±0.9 (5.06-9.12)	0.88±0.17 (0.41-1.23)

Table 30: Summary of shoots, roots and fruits dry weight (g) of okra plants subjected to mix treatment for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots	Fruit
Control	9.48±1.56 (6.11-12.57	1.87±0.42 (0.91-2.78)	3.18±1.75 (1.43-4.93)
MIX	7.36±0.48 (6.13-8.40)	1.31±0.20 (1.02-1.90)	1.52±1.09 (0.43-2.61)

B. Heavy Metal analysis

Table 31: Summary of shoots and roots metal concentration (mg/kg DW) of tobacco plants subjected to different Zn concentrations. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots
Control	53.95±13.97 (12.2-70.4)	150.00±9.68 (121.00-160.50)
Zn300	***** 199.45±16.19 (171-239.2)	232.75±17.15 (195.00-273.00)
Zn500	***** 291.1±31.02 (206.4-343.8)	* 447.63±16.16 (404.00-482.00)
Zn800	***** 547.55±36.23 (439.8-591)	* 1,013.25±25.87 (969.00-1,074.00)
Zn10000	***** 223.65±18.88 (182-263.2)	* 536.25±53.08 (428.50-635.00)

'*' represent significant differences between the control and treatments ($P < 0.05$)

Table 32: Summary of shoots and roots metal concentration (mg/kg DW) of <u>tobacco plants</u> subjected to different Cu concentrations. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots
Control	7.25±1.91 (1.60-9.60)	18.13±0.94 (16.00-20.50)
Cu50	* 20.75±0.46 (19.60-21.60)	* 48.00±3.59 (38.00-54.50)
Cu100	* 15.25±0.92 (13.40-17.80)	* 62.50±4.51 (53.50-75.00)
Cu200	*24.70±1.61 (22.20-29.40)	* 92.63±3.48 (85.50-100.00)
Cu300	* 25.85±1.27 (22.80-28.40)	* 146.38±3.29 (141.50-156.00)

'*' represent significant differences between the control and treatments (P< 0.05)

Table 33: Summary of shoots and roots metal concentration (mg/kg DW) of <u>tobacco plants</u> subjected to different Cd concentrations. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots
Control	0.95±0.13 (0.60-1.20)	1.13±0.13 1.00-1.50
Cd50	* 52.05±10.47 (26.20-77.40)	* 76.25±7.32 56.50-90.00
Cd100	* 187.07±3.77 (181.40-194.20)	* 202.50±22.67 165.50-266.50
Cd150	* 211.00±10.90 (189.40-224.40)	* 150.00±5.77 140.00-160.00
Cd200	* 358.05±53.88 (238.40-468.20)	* 185.25±14.66 145.00-210.50

'*' represent significant differences between the control and treatments (P< 0.05)

Table 34: Summary of shoots and roots metal concentration (mg/kg DW) of tobacco plants subjected to different Pb concentrations. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots
Control	0.55±0.05 (0.40-0.60)	1.50±0.46 (0.50-2.50)
Pb50	* 26.75±0.10 (26.60-27.00)	* 67.88±0.13 (67.50-68.00)
Pb100	* 26.90±0.19 (26.40-27.20)	* 72.88±0.31 (72.00-73.50)
Pb150	* 26.55±0.15 (26.20-26.80)	* 76.63±0.24 (76.00-77.00)
Pb200	* 27.85±0.40 (27.20-29.00)	* 92.13±0.69 (90.50-93.50)

'*' represent significant differences between the control and treatments ($P < 0.05$)

Table 35: Summary of shoots, roots and fruit metal concentration (mg/kg DW) of okra plants subjected to different Zn concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots	Fruit
Control	24.60±4.68 (16.20-33.00)	39.75±4.84 (29.50-48.50)	40.2±5.8 (34.4-46)
Zn300	* 188.10±15.70 (149.80-224.40)	* 319.13±36.92 (234.00-389.50)	89.5±17.9 (71.6-107.4)
Zn500	* 285.00±26.55 (232.40-336.60)	* 538.25±39.91 (478.50-656.00)	104.6±6.6 (98-111.2)
Zn800	* 503.60±14.30 (465.60-529.20)	* 762.88±84.16 (592.00-985.00)	* 146.0±32.6 (113.4-178.6)
Zn10000	* 514.80±42.85 (392.80-587.00)	* 753.00±87.82 (566.00-919.50)	* 132.0±5.4 (126.6-137.4)

Table 36: Summary of shoots, roots and fruit metal concentration (mg/kg DW) of okra plants subjected to different Cu concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots	Fruit
Control	1.50±0.37 (0.80-2.40)	15.25±1.61 (12.00-18.50)	3.9±0.5 (3.4-4.4)
Cu50	* 9.47±0.40 (8.85-10.51)	12.75±0.97 (10.00-14.50)	5.8±0.2 (5.6-6.0)
Cu100	* 15.48±0.81 (13.27-17.14)	25.00±0.20 (24.50-25.50)	* 8.8±0.0 (8.8-8.8)
Cu200	* 17.70±1.26 (14.93-21.01)	* 63.25±1.79 (61.00-68.50)	6.5±0.9 (5.6-7.4)
Cu300	* 21.71±1.83 (17.70-26.54)	32.13±1.40 (29.00-34.50)	* 9.5±0.3 (9.2-9.8)

'*' represent significant differences between the control and treatments (P< 0.05)

Table 37: Summary of shoots, roots and fruit metal concentration (mg/kg DW) of okra plants subjected to different Cd concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots	Fruit
Control	0.20±0.00 (0.20-0.20)	0.23±0.03 (0.20-0.30)	0.3±0.1 (0.2-0.4)
Cd50	* 51.88±1.25 (50.00-55.50)	* 39.05±0.83 (37.00-40.60)	* 15.1±2.1 (13-17.2)
Cd100	* 80.25±0.78 (78.50-82.00)	* 72.80±1.48 (70.00-76.00)	* 14.5±2.3 (12.2-16.8)
Cd150	* 71.88±3.36 (62.00-77.00)	* 47.65±2.76 (44.00-55.80)	* 9.7±0.7 (9.0-10.4)
Cd200	*44.13±2.51 (37.00-48.50)	* 40.00±3.55 (32.20-49.40)	* 11.3±1.1 (10.2-12.4)

'*' represent significant differences between the control and treatments (P< 0.05).

Table 38: Summary of shoots, roots and fruit metal concentration (mg/kg DW) of okra plants subjected to different Pb concentrations for two months. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Treatments	Shoots	Roots	Fruit
Control	0.65±0.26 (0.20-1.40)	0.88±0.13 (0.50-1.00)	0.2±0.0 (0.2-0.2)
Pb50	1.30±0.10 (1.20-1.60)	* 6.75±0.60 (5.50-8.00)	0.6±0.4 (0.2-1.0)
Pb100	* 5.11±1.70 (1.14-7.95)	* 12.13±2.60 (7.50-18.00)	0.4±0.0 (0.4-0.4)
Pb150	* 11.08±0.97 (9.09-13.63)	* 16.13±1.09 (14.00-19.00)	0.8±0.0 (0.8-0.8)
Pb200	2.84±0.73 (1.14-4.54)	* 13.00±0.20 (12.50-13.50)	0.3±0.1 (0.2-0.4)

'*' represent significant differences between the control and treatments (P< 0.05).

Table 39: Summary of shoots and roots metal concentration (mg/kg DW) of <u>tobacco plants</u> subjected to mix treatment. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Metal	Treatments	Shoots	Roots
Zn	Control	53.95±13.97 (12.2-70.4)	150.00±9.68 (121.00-160.50)
	MIX	81.3±1.62 (77-83.8)	182.13±33.17 (122.50-243.00)
Cu	Control	7.25±1.91 (1.60-9.60)	18.13±0.94 (16.00-20.50)
	MIX	12.90±1.04 (10.00-14.60)	* 60.50±3.67 (49.50-64.50)
Cd	Control	0.95±0.13 (0.60-1.20)	1.13±0.13 1.00-1.50
	MIX	*118.40±13.36 (80.00-142.00)	* 177.33±3.93 172.00-185.00
Pb	Control	0.55±0.05 (0.40-0.60)	1.50±0.46 (0.50-2.50)
	MIX	* 26.50±0.50 (26.00-28.00)	* 76.88±1.03 (75.00-79.50)

'*' represent significant differences between the control and treatments (P< 0.05).

Table 40: Summary of shoots, roots and fruits metal concentration (mg/kg DW) of okra plants subjected to mix treatment. Values represent means±SE of 4 plants from 2 pots. Minimum and maximum values are between brackets.

Metal	Treatments	Shoots	Roots	Fruits
Zn	Control	24.60±4.68 (16.20-33.00)	39.75±4.84 (29.50-48.50)	40.2±5.8 (34.4-46)
	MIX	44.55±2.33 (38.80-50.20)	24.88±1.48 (21.00-28.00)	61.5±3.7 (57.8-65.2)
Cu	Control	1.50±0.37 (0.80-2.40)	15.25±1.61 (12.00-18.50)	3.9±0.5 (3.4-4.4)
	MIX	* 3.75±0.29 (3.40-4.60)	74.88±9.76 (57.00-92.50)	5.0±0.8 (4.2-5.8)
Cd	Control	0.20±0.00 (0.20-0.20)	0.23±0.03 (0.20-0.30)	0.3±0.1 (0.2-0.4)
	MIX	* 51.75±3.10 (43.50-58.00)	* 41.90±2.22 (36.80-47.60)	* 13.6±0.8 (12.8-14.4)
Pb	Control	0.65±0.26 (0.20-1.40)	0.88±0.13 (0.50-1.00)	0.2±0.0 (0.2-0.2)
	MIX	1.45±0.05 (1.40-1.60)	* 6.63±0.59 (5.00-7.50)	0.4±0.2 (0.2-0.6)