

INTERACTIONS OF LEAD, ZINC AND NICKEL TOXICITY IN THEIR EFFECTS ON GROWTH RESPONSES OF *CICER ARIETINUM L.* AND *BRASSICA NIGRA L*

Abstract

Heavy metals are most available in the soil and aquatic ecosystems, with small amounts present in the atmosphere in the form of particles or vapours. Low doses of heavy metals can be attributed to a wide range of activities and the metals are non-degradable and persistent, thereby affecting the survival of plants and the level and pattern of biodiversity. The present study aimed to investigate the effects of potentially toxic elements of lead, zinc, and nickel on morphological and biochemical parameters in *Cicer arietinum* and *Brassica nigra*. A pot experiment was conducted to evaluate the sensitivity of chickpea and mustard seedlings to lead, zinc and nickel at 10, 20, 30, 40 and 50, ppm concentrations. It affected all growth parameters (germination, seedling growth, biomass, vigour index, biochemical constituents such as pigments, protein, carbohydrates and enzymes) studied in the test crop compared to the respective control. The lower concentrations of 10 ppm of heavy metals showed stimulatory effects on Chickpea and Mustard seedlings. The extract showed more inhibitory effect at 50ppm than at 10ppm concentration on germination, morphological and biochemical parameters of Chickpea and Mustard seeds. It may be due to the influence of different concentrations of heavy metals. The toxicity also slows down the speed of germination. This heavy metal also retards several developmental events in the crucial stages of test crops at the seedling stage. Lateral roots are essential for the successful establishment of seedlings and it is significant to note that the development of this root is affected by the heavy metallic ions. Similarly, toxicity

Authors

Leela P

Department of Botany
Rani Anna Government College for women
Tirunelveli, Tamil Nadu, India
leeladde72@gmail.com

Karthiga Meena P

Department of Botany
Rani Anna Government College for women
Tirunelveli, Tamil Nadu, India

increases with an increase in concentration. The germination percentage, protein, carbohydrate, shoot and root lengths, and photosynthetic pigments including carotenoids and chlorophyll a and b were all significantly decreased by seedlings. In Proline, a reversal of the trend was seen. From the experiment, it was noticed that lead is the least toxic of all the three heavy metallic ions studied, and it affects the germination of seeds and reduces root development. Heavy metals interfere with plant metabolism, resulting in the inhibition of plant growth and yield. The study suggests that the cultivation of crops like chickpeas and mustard in metal-polluted soils should be avoided or appropriate control measures be adopted to maintain the heavy metal content of the soil.

Keywords: Heavy metal, toxicity, cultivation, germination, photosynthetic, ecosystem: Heavy metal, toxicity, cultivation, germination, photosynthetic, ecosystem

I. INTRODUCTION

As immobile organisms, plants face various challenging environmental conditions throughout their life cycle that detrimentally impact their growth and developmental processes (Suleiman et al., 2020, 2021). The toxicity of heavy metals not only diminishes plant growth and productivity but also poses significant health risks to humans. Several metals and metalloids, including arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni), zinc (Zn), cobalt (Co), aluminum (Al), and chromium (Cr), induce severe toxicity upon entering the soil ecosystem, either through natural processes or anthropogenic activities (Ghorani-Azam et al., 2016; Luo L et al., 2020). The rapid industrialization and technological advancements have disrupted the natural geochemical cycle of these metal elements, leading to their increased accumulation in soil layers (Tak et al., 2013; Rai et al., 2021). This heightened bioaccumulation of heavy metals beyond safe thresholds has adversely affected both the natural food chain and microbial communities, posing an imminent threat to the ecosystem and the environment (Saha et al., 2017).

In plants, internal organelles such as mitochondria and chloroplasts, as well as root and shoot cells, sustain significant damage when heavy metals are absorbed by the roots and later transferred to the shoots. Plant morphology and survival rates are ultimately affected by this damage, which also causes oxidative stress and decreased energy production (Garg and Singla 2011). Many plant species are vulnerable to heavy metals, however some can even accumulate heavy metals in excess or show tolerance (Yan et al., 2020).

Elements that may be hazardous are frequently referred to as "heavy metals" or "trace metals." The term "trace metals" refers to elements found in soil in minute concentrations (usually expressed as 1 mol m^{-3}). With the exception of alkali metals, the elements that have metallic qualities with an atomic mass of more than 20 and a specific gravity greater than 5 are referred to as heavy metals, a loosely defined set of elements (Rascio and Navari-Izzo 2011). Some researchers define heavy metals as elements (apart from A, B, and Se) having an atomic density more than 6 gcm^{-3} (Park et al., 2011).

Transition metals, some metalloids, lanthanides, and actinides are the principal constituents of this group, and at low exposure levels, they may be hazardous to living things. Certain heavy metals, including Zn, Cu, Mn, Ni, Se, Co, Cr, and Mo, have beneficial biological effects on agricultural productivity because they are involved in vital biological processes (Kavamura and Esposito 2010; Rascio and Navari-Izzo 2011). Growing food crops on polluted soil has been linked to the buildup of heavy metals, according to numerous authors (Uzu et al. 2009, 2010, 2011a; Nabulo et al. 2010, 2011). By interfering with photosynthesis, water and mineral uptake, and nitrogen metabolism, excessive heavy metal uptake by plants can result in decreased crop productivity (Cambrolle et al. 2011; Buendia-Gonzalez et al. 2010; Dirilgen 2011; Shahid et al. 2011, 2012a, b, 2014b; Austruy et al. 2014; Hasanuzzaman et al. 2014). It is believed that the average lead content (Pb) in Earth's crust is 15 mg/kg . There are two recognized forms of lead in the terrestrial environment: primary and secondary. Primary Pb is of geogenic activates and integrated into minerals at the time of their formation, whereas secondary Vinod Kumar et al. (2019). Lead originates from radioactive decay of uranium and thorium. Lead is primarily used in the manufacture of lead acid batteries. Additionally, solders, alloys, wires, and chemicals all employ it. One of the most common harmful non-essential elements in soil is lead (Pb). According to Sharma and

Dubey (2005), soil and aerosol are the primary sources of the plant. Plant roots have a greater capacity to absorb lead, but there are very few opportunities for them to subsequently translocate to aerial portions. The amount of lead that is available in soil is mostly determined by factors like pH, particle size, and cation exchange capacity.

Zinc is an important plant micronutrient because, at a concentration, it is necessary for both good plant development and healthy cell metabolism (Dhankhar et al., 2012). It is an essential cofactor for many physiological activities, such as protein synthesis, enzyme activation, gene expression and control, metabolism of different macromolecules, and reproductive development. Nevertheless, a plant's physiology is altered and growth is inhibited when a larger quantity of zinc accumulates in it ($> 300\mu\text{g}^{-1}$ in dry weight) (Cakmak, 2000). Excessive zinc exposure in a growing medium inhibits a number of plant metabolic processes, stunts development, and induces senescence.

Nickel (Ni) plays a pivotal role as a constituent in various metalloenzymes, including superoxide dismutase, NiFe hydrogenases, M-reductase methyl coenzyme, urease, Co-A acetyl synthase, dehydrogenase carbon monoxide, hydrogenases, and RNAase. Furthermore, when Ni levels are elevated in the growth medium, it can have an impact on the activities of enzymes like amylases, proteases, and ribonucleases, subsequently influencing the processes related to the digestion and metabolism of food reserves during seed germination. Ni is recognized as an essential component for plants, albeit at lower concentrations ranging from 0.01 to 5 μg . The uptake of Ni from the growth medium primarily occurs through a combination of passive diffusion and active transport mechanisms.

1. **Heavy Metal Pollution in Soil:** The primary and most vital part of the ecological system—soil—is heavily contaminated with heavy metals all over the world (Shahid et al. 2013b; Austruy et al. 2014; Hakeem et al. 2014). The soil compartment receives a significant amount of pollutants annually from a variety of sources across the globe (Wannaz et al. 2012).
 - **Heavy Metal Uptake by Plants:** The portion of a metal's total content that is readily available to an organism in its immediate environment or that is dynamically made available over time is known as the metal's bioavailability in soil (Pauget et al., 2012). Most heavy metals are generally less soluble and available for plant absorption, with Pb and Cu being particularly less soluble (Punamiya et al. 2010; Sammut et al. 2010; Vega et al. 2010; Pourrut et al. 2011). Metals don't directly enter plant roots from the soil; instead, they undergo an initial adsorption onto plant roots. After that, they attach either directly to the mucilage polysaccharides on the surface of rhizodermal cells or to carboxyl groups in the uronic acid that surrounds the roots (Pourrut et al. 2011). According to Saifullah et al. (2009), there are multiple steps involved in the uptake of metals by plants: the release of metals from soil particles, the transportation of metals into the roots of the plant, the actual uptake of metals by the roots, and the translocation of metals toward the shoot.
 - **Heavy Metal Translocation to Shoots:** Verbruggen et al. (2009) state that transpiration is most likely the mechanism via which heavy metals are transported to aerial plant parts through the xylem (Liao et al. 2006). Most plants only translocate a very little amount of heavy metals to their shoot tissues. In some circumstances, 95

percent or more of the absorbed metal is sequestered in the roots, unless the plant is a hyperaccumulator or chelate-assisted (Małecka et al. 2008; Gupta et al. 2010; Jiang and Liu 2010; Yan et al. 2010; Duarte et al. 2007; Shahid et al. 2012c). When compared to other metals, Pb shows the most frequent metal translocation limitation phenomenon (Pourrut et al., 2011). The Casparian strip (Pourrut et al. 2011), accumulation in the plasma membrane (Jiang and Liu 2010), and precipitation in the intercellular space (Małecka et al. 2008; Pourrut et al. 2011) are the reasons for the restricted translocation of heavy metals to aerial plant parts. These metal transporter proteins are found in plasma or intracellular membranes and have cell-specific expression, suggesting that they may have a specific different function in different plant species. Research has indicated that in addition to the heavy metal transporter proteins that are naturally found in hyperaccumulators, the presence of organic ligands enhances the translocation of heavy metals from root to shoot (Shahid et al. 2012c, d, 2014d).

- **Heavy Metal Effects on Crop Productivity:** Within plants, heavy metals can trigger various morphological, physiological, and biochemical irregularities, ultimately leading to a reduction in crop yield. Nevertheless, the extent of these toxic effects induced by heavy metals depends on factors such as the duration of exposure, the developmental stage of the plant, the intensity of stress, the specific organ under examination, the variety of the species, and the concentration of the metal encountered by the organism in question.

2. **Effects of Heavy Metal on Germination:** Moosavi et al. (2012) found that seed germination is one of the physiological processes in the plant life cycle that is most susceptible to biotic and abiotic environmental impacts as well as hormonal interactions, including metals. Metals like copper and lead prevent seeds from germinating because they impede their ability to absorb water (Kranter and Colville 2011). The primary purpose of the seed germination test is to evaluate metal toxicity (Munzuroglu and Geckil 2002). Following seed coat penetration, germination is dependent on seed reserves to supply different metabolites for respiration; however, oxidative stress from metals impedes this process (Ko et al. 2012). According to Jozic et al. (2009), plants can absorb these metals through their roots, rain, and dust. Plants beside roadsides had four times higher amounts of Fe, Pb, Cu, and Mn than control plants, according to Celik et al. (2005).

Effects of Heavy Metals on Plant Growth: The most common physiological consequence of plant exposure to heavy metals is a decrease in growth (Hu et al. 2013). This exposure leads to alterations in leaf structure and physiology, resulting in diminished photosynthesis and respiration. Consequently, these changes disrupt metabolism and decrease energy production. Heavy metal exposure also affects transpiration and the transport of materials between different plant organs (Ying et al. 2010). Furthermore, under the stress of metal exposure, the roots' capacity to absorb nutrients and water is reduced (Poschenrieder and Barcelo 2004). Heavy metals exhibit substantial toxicity to plants, resulting in phytotoxicity, followed by symptoms such as chlorosis, reduced plant growth, decreased yield, impaired nutrient uptake, and a diminished capacity for nitrogen fixation in leguminous plants (Guala et al. 2010).

- 3. Effects of Heavy Metals on Photosynthesis:** The interaction between heavy metals and light-harvesting pigments, such as chlorophyll and carotenoids, leads to a reduction in their levels (Kuzminov et al. 2013). The energy transfer in light-harvesting antennae is interfered with by this contact. Furthermore, proteins involved in photosynthesis, such as Rubisco and proteins in the reaction centers, can be affected by heavy metals, changing their structure or composition (Franco et al. 1999). As a result, heavy metals obstruct both light- and dark-photosynthetic reactions from operating normally.

Because heavy metals are harmful to membrane lipids, high concentrations of heavy metals can also prevent electron transport on the acceptor side of PS II and between PSI and PSII (Rama Devi and Prasad 2004). Under Pb stress, many species have shown decreased chlorophyll content and photosynthetic activity (Pinchasov and Dubinsky 2006; Shahid et al. 2014). According to Soudek et al. (2014), shoots were less toxically affected by lower metal concentrations than roots were. Plants growing in polluted environments have lower photosynthetic efficiency, according to Padinha et al. (2000). According to Marques and Nascimento (2013), the negative effects of excess metals on photosynthetic electron transport may be the cause of the deterioration in photosynthetic functioning, which would result in decreased chlorophyll synthesis or increased chlorophyll breakdown. Cadmium in particular shows considerable toxicity to photosynthesis, especially in higher plants.

- 4. Effect of Heavy Metals on Nutrient Uptake:** Numerous investigations (Gopal and Rizvi 2008; Sharma and Dubey 2005; Chatterjee et al. 2004) have repeatedly shown that Pb exposure significantly affects how plants absorb nutrients. Moreover, it causes a decrease in the concentration of cations in *Oryza sativa* leaves, including Mg²⁺, Zn²⁺, Ca²⁺, Fe²⁺, and Mn²⁺ (Chatterjee et al. 2004).

Conversely, cadmium has been shown to impede stomatal openings (Hassan et al. 2011), which impacts the intake and movement of water (Vassilev et al. 1997). Cadmium inhibits photosynthesis in plants by interfering with the Calvin cycle enzymes (Nazar et al. 2012), which lowers the metabolism of carbohydrates (Khan et al. 2009)

- 5. Heavy-Metal–Induced Oxidative Stress:** In typical plant growth conditions, the levels of reactive oxygen species (ROS) within a plant are regulated by a defensive system that encompasses antioxidants, enzymes, and glutathione (GSH). Nevertheless, when ROS are not effectively eliminated, it can lead to oxidative stress. The excessive generation of ROS results in the oxidation and modification of cellular components such as amino acids, membrane lipids, DNA, and proteins (Yadav 2010; Rascio and NavariIzzo 2011). These interactions between ROS and cellular constituents give rise to disruptions in mitochondrial function, DNA integrity, cell membrane integrity, and eventually, cell death (Reddy et al. 2005; Clemens 2006; Yadav 2010; Shahid et al. 2011; Pourrut et al. 2011). This oxidative stress is closely linked to heavy metal-induced crop productivity issues.

Defense Mechanism of Plants against Heavy Metals Toxicity: Plants need to find constant ways to deal with stress in order to survive and continue producing crops. In order to combat metal toxicity and prevent tissue degeneration and cell damage, heavy metal hyperaccumulator plants have developed defense mechanisms (Shahid et al. 2012c;

Hakeem et al. 2014). These defense systems can function in tandem or independently. However, the type of plant and metal, as well as the extent and length of exposure, determine the effectiveness and activation of a particular defense system.

6. **Antioxidant Enzymes:** Many antioxidant enzymes are used in the secondary defense mechanism for heavy metal detoxification to regulate the excessive generation of reactive oxygen species (ROS). Ascorbate peroxidase (APX), peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) are some of these enzymes. Low molecular weight molecules involve in scavenging various forms of ROS include cysteine (Cys), proline (PRO), ascorbic acid (AsA), non-protein thiol (NPT), and glutathione (GSH) (Israr et al. 2011; Gao et al. 2010; Mou et al. 2011; Lomonte et al. 2010; Yasir et al. 2011; Ali et al. 2011; Pourrut et al. 2013; Shahid et al. 2012d). These molecules are in addition to these enzymes.
7. **Phytochelatin and Metallothioneins:** When a plant absorbs large amounts of harmful metals, it needs a safe sink to develop tolerance (Wojas et al. 2009; Hassan and Abbass 2011). The plants are able to avoid these metals' harmful impacts on many cellular functions in this way. Plant metal homeostasis depends critically on the vacuolar sequestration of heavy metals (Maestri et al. 2010; Xu et al. 2011). To lessen the otherwise detrimental impacts of heavy metals, hyper accumulator plants have the ability to sequester them into molecules. Certain natural chelators found in plants, such phytochelatin (PCs) and metallothioneins (MTs), are said to be the finest natural ligands for binding metals and transferring them to safe locations within plant cells called vacuoles (Israr et al. 2011; Xu et al. 2011). PCs and MTs are heavy metal-binding protein molecules that are rich in cysteines. By establishing mercaptide bonds with different metals, PCs and MTs play a crucial role in the detoxification of heavy metals in plants (Maestri et al. 2010). (Verbruggen e. 2009; Yadav 2010; Gupta et al. 2010; Jiang and Liu 2010).

In this context, a study was undertaken to elucidate the effects of three heavy metals interactions of lead, zinc and nickel toxicity in their effects on the growth and responses of *Cicer arietinum L.* and *Brassica nigra L.* by conducting a pot culture experiment with the following major objectives, to understand the Effect of heavy metals on seed germination and seedling growth, the protective role of proline against heavy metal stress and the photosynthetic pigments of seedling on lead, nickel and zinc stress.

II. MATERIALS AND METHODS

The present study was aimed to assess the effects of heavy metals on *Cicer arietinum L.* and *Brassica nigra L.* The plants *Cicer arietinum L.* and *Brassica nigra L.* was selected for this study. Heavy metals such as **Lead (Pb)**, **Zinc (Zn)**, and **Nickel (Ni)** have an impact on the growth and development of crops. The study was conducted in the Green House of Rani Anna Government College for Women, Tirunelveli. Sterilized and fertilized soil, Grow Bags(12×24 cm),

The seeds of *Cicer arietinum L.* and *Brassica nigra L.* were purchased from an authorised shop at Tirunelveli. Heavy metallic salts of Lead nitrate, Zinc sulphate and Nickel nitrate were used for studying metallic ions of lead, zinc and Nickel by dissolving 10mg,

20mg, 30mg, 40mg and 50 mg of respective salts in 1000ml distilled water, respectively. Different concentrations of Lead nitrate, Zinc sulphate and Nickel nitrate solutions such as 10ppm, 20ppm, 30ppm, 40ppm, and 50ppm were prepared from that stock solution. These salt solutions are used daily on the crops. Control seedlings were irrigated with water.

The grow bags are filled with fertile garden soil with silt, humus and sand. Healthy and uniform *C.arietinum L.* seeds and *B.nigra L.* seeds were chosen and pre-soaked in distilled water for three to four hours and The seeds were sanitized for 30 seconds using a 0.1% mercuric chloride solution, then carefully cleaned in distilled water many times to get rid of any remaining chemical, and then dried to prevent fungal growth.

Then, the crop seeds were sown in grow bags and uniformly irrigated with different concentrations of lead nitrate, zinc sulphate and nickel nitrate solution. They were maintained to each treatment, including control. The control grow bags were irrigated with distilled water. Each grow bag was irrigated with different concentrations of the heavy metal solutions daily for up to 15 days. Five days after sowing the seed, germination was noted. On the tenth day following germination, growth parameters including root and shoot lengths as well as the biochemical components of the test crop seedlings, including carbohydrates, proline chlorophyll a, chlorophyll b, and carotenoids, were measured.

III. GERMINATION PERCENTAGE

The percentage of germination was calculated by using the following formula:

$$\text{Germination Percentage} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

The seedlings of the test crop were selected randomly and uprooted from each treatment of the grow bags experiment; the shoot length, root length and total length were measured in centimetres. The seedlings of the test crop were selected randomly, uprooted and washed thoroughly with tap water. The seedlings were kept in a hot air oven at 80°C for 48 hours. The dry weights were recorded by using an electronic single-pan balance.

Seedling Vigour Index (SVI): The vigour index of seedling was calculated by following method suggested by Abdul-Baki and Anderson (1993).

$$\text{SVI} = \text{Germination (\%)} \times [\text{Shoot length(cm)} + \text{Root length(cm)}].$$

The test crops' biochemical constituents, such as pigments, carbohydrates, starch, protein and proline contents, were analysed.

IV. RESULTS

The most damaging element that significantly reduces agricultural productivity is heavy metal. The world's surface area affected by heavy metal pollution is growing every day. In irrigated croplands, the heavy metal effect is a more noticeable issue. The current study's findings and observations are listed below.

- 1. Seed germination (Table-1):** The seed germination of the test crops was reduced against the treatment of different concentrations of heavy metals such as Pb, Zn and Ni. At 50 ppm concentration of Pb the seed germination is (29.59%) in *C.arietinum* and (23.23%) in *B.nigra* and Zn treatment shows (45.91%) in *C.arietinum* and (47.47%) in *B.nigra* and then Ni treatment shows (47.95%) in *C.arietinum* and (50.50%) in *B.nigra* compared to control. The germination percentage decreased gradually as the concentration increased from 10 to 50 ppm.

Table 1: Germination Percentage of *Cicer Arietinum L.* And *Brassica Ingra L.* Seeds Exposed To Various Concentrations Of Lead, Nickel And Zinc

Name of the Metal Ions	Lead		Zinc		Nickel	
	<i>Cicer arietinum</i>	<i>Brassica nigra</i>	<i>Cicer arietinum</i>	<i>Brassica nigra</i>	<i>Cicer arietinum</i>	<i>Brassica nigra</i>
Control	98	99	98	99	98	99
10	96 (-2.04)	97 (-2.02)	93 (-5.10)	95 (-4.04)	91 (-7.14)	92 (-7.07)
20	91 (-7.14)	94 (-5.05)	91 (-7.14)	81 (-18.18)	87 (-11.22)	79 (-20.20)
30	82 (-16.32)	93 (-6.06)	87 (-11.22)	72 (-27.27)	82 (-16.32)	71 (-28.28)
40	74 (-24.48)	84 (-15.15)	72 (-26.53)	64 (-35.35)	69 (-29.59)	60 (-39.39)
50	69 (-29.59)	76 (-23.23)	53 (-45.91)	52 (-47.47)	51 (-47.95)	49 (-50.50)

- 2. Seedling Length (Figures 1 & 2):** The root length of the test crops was reduced against the various concentrations of metallic ions. The reduction percentage of root length against Pb is (63.28%) in *C.arietinum* and in (66.25%) in *B.nigra* and against Zn (67.18%) in *C.arietinum* and (68.75%) in *B.nigra* and the root length against Ni is (67.18%) in *C.arietinum* and in (66.25%) in *B.nigra* at 15 days old seedling by 50ppm. But at 10 ppm, the heavy metal treatment observed a maximum percentage of root length over control, followed by 20, 30, 40 and 50 ppm.

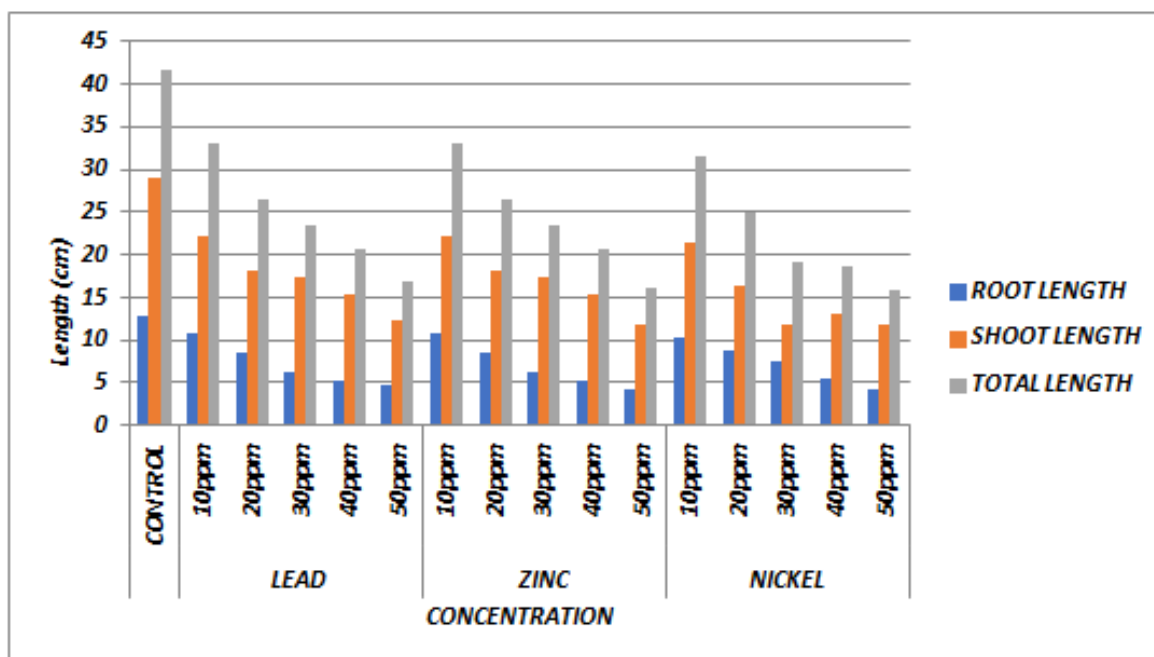


Figure 1: Effect of Lead, Zinc and Nickel Ions on Seedling Characteristics of *Cicer Arietinum*

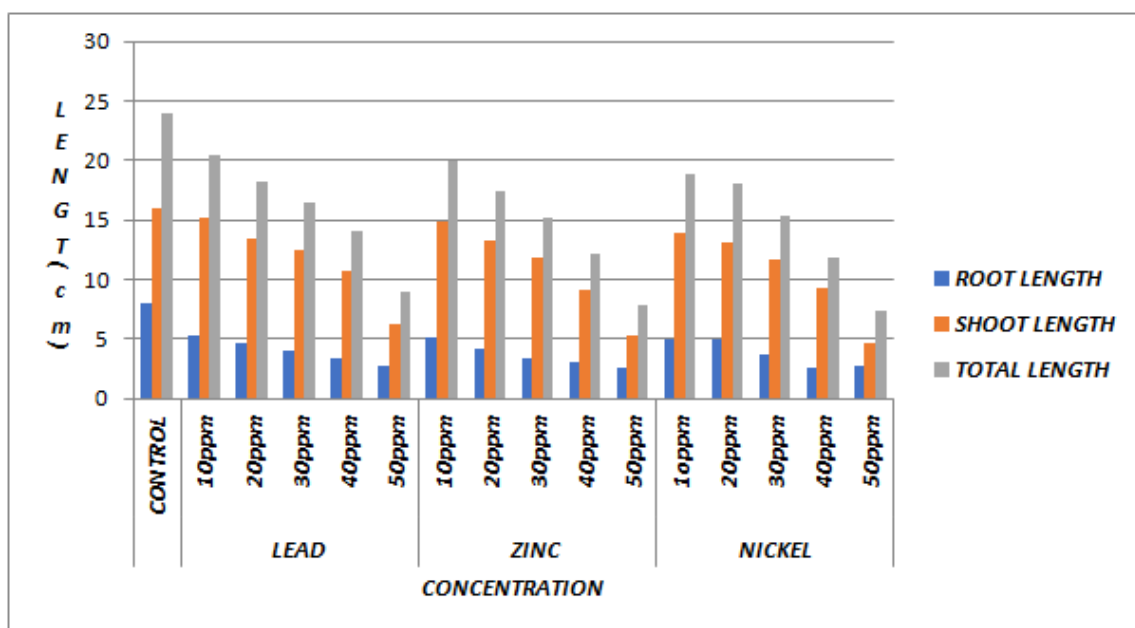


Figure 2: Effect of Heavy Metals on the Seedling Characteristics of *Brassica Nigra*

The values of shoot length were decreased in the test crop with increasing the concentration of the heavy metals. At 50 ppm concentration, the highest shoot length reduction percentage was recorded in the treatment of Pb on *C.arietinum* (57.43%) and in *B.nigra* (60.62%) and treatment of Zn on *C.arietinum* (58.82%) and in *B.nigra* (66.87%) and Ni in *C.arietinum* is (59.51%) and

in *B.nigra* (70.62%) compared to control. As the concentration increased from 10 to 50 ppm, the shoot length decreased concomitantly.

3. Fresh Weight and Dry Weight (Figures 3 &4): The fresh weight reduction percentage recorded in the treatment of Pb in *C.arietinum* is (61.23%) and in *B.nigra* (34.57%) and against Zn in *C.arietinum* is (63.76%) and in *B.nigra* (36.44%) and against Ni in *C.arietinum* is (71.37%) and in *B.nigra* (65.58%) compared to control. As the concentration increased from 10 to 50 ppm, the shoot length decreased concomitantly.

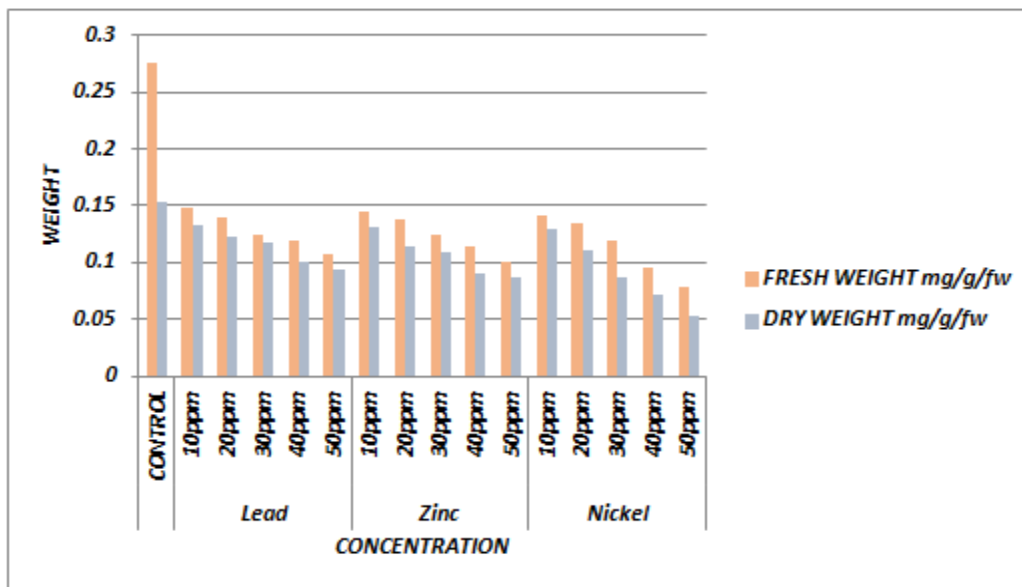


Figure 3: Effect of Lead,Zinc and Nickel Ions on Fresh Weight and Dry Weight of Cicer arietinum

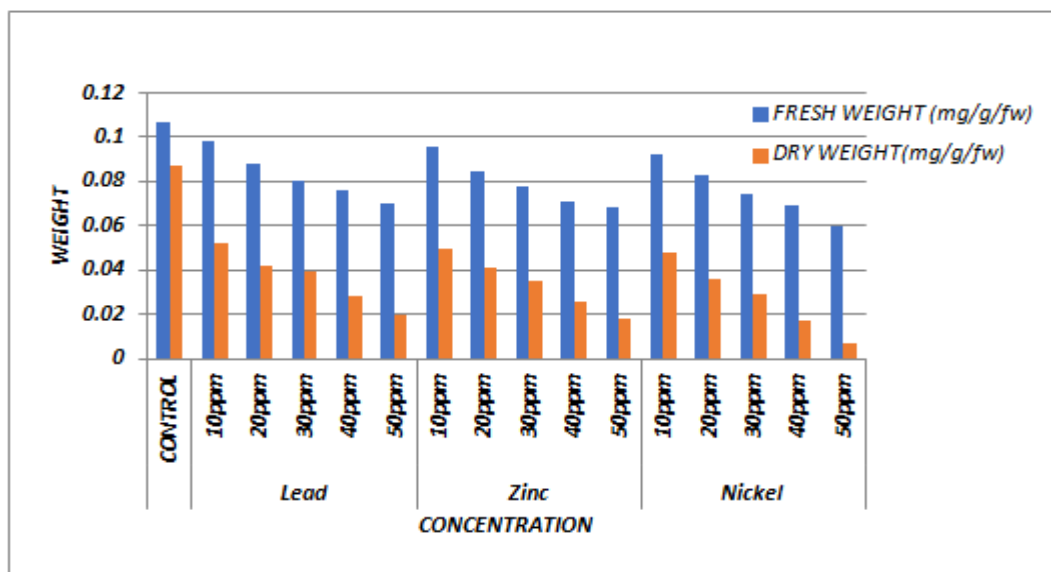


Figure 4: Effect of Lead,Zinc and Nickel Ions on Fresh Weight and Dry Weight Brassica nigra

The maximum value of dry weight was recorded in the control. The minimum dry weight value was observed in a 50 ppm concentration of heavy metals. As the concentration increased from 10 ppm to 50 ppm, the inhibitory effect also increased and the maximum inhibitory effect was caused by 50 ppm concentration.

4. Photosynthetic Pigments (Figures 5&6)

Chlorophyll a, Chlorophyll b, Total Chlorophyll and Carotenoid: The contents of chlorophyll a, chlorophyll b, total Chlorophyll and carotenoid were reduced in *C.arietinum* and *B.nigra* at higher concentrations of the heavy metals. The inhibition rate was higher in Ni-treated test crops than in Pb and Zn. The minimum inhibition of pigments was recorded at 10 ppm. Among the three photosynthetic pigments, the maximum reduction was observed in Chlorophyll, followed by Carotene and Chlorophyll-b. The Chlorophyll a, Chlorophyll b, Total Chlorophyll and Carotenoid content of 50 ppm of Pb treatment in *C.arietinum* is (12.54%), (32.57%),(27.95%), (19.09%) and in *B.nigra* is (35.94%), (38.20%),(26.417%) (35.83%) decreases over control. The Chlorophyll a, Chlorophyll b, Total Chlorophyll and Carotenoid content of 50 ppm of Zn treatment in *C.arietinum* is (28.571%), (41.83%), (38.77%), (11.306%) and in *B.nigra* is (53.91%), (38.20%), (43.30%), (35.83%) when compared over control. The Chlorophyll a, Chlorophyll b, Total Chlorophyll and Carotenoid content of 50 ppm of Ni treatment in *C.arietinum* is (51.567%), (65.93%), (62.55%), (60.05%) and in *B.nigra* is (46.54%), (49.58%),(48.73%),(30.83%) compared over control.

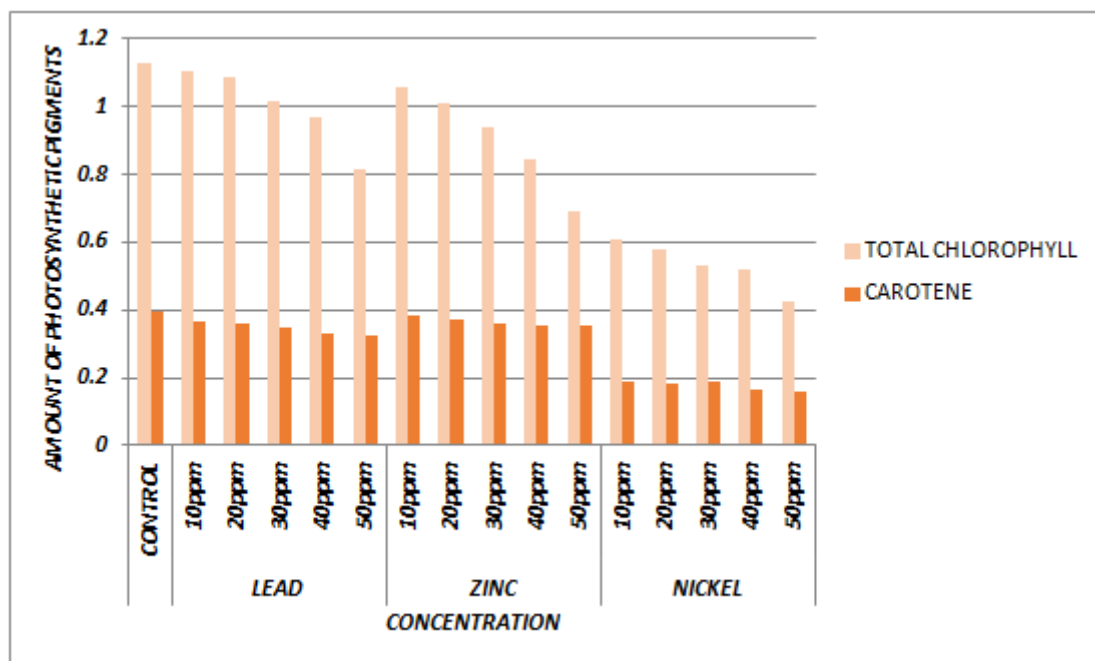


Figure 5: Effect of Lead,Zinc and Nickel Ions on Photosynthetic Pigments (Mg/Gfr.Wt of The Plant *Cicer Arietinum*

INTERACTIONS OF LEAD, ZINC AND NICKEL TOXICITY IN THEIR EFFECTS ON GROWTH RESPONSES OF *CICER ARIETINUM L.* AND *BRASSICA NIGRA L.*

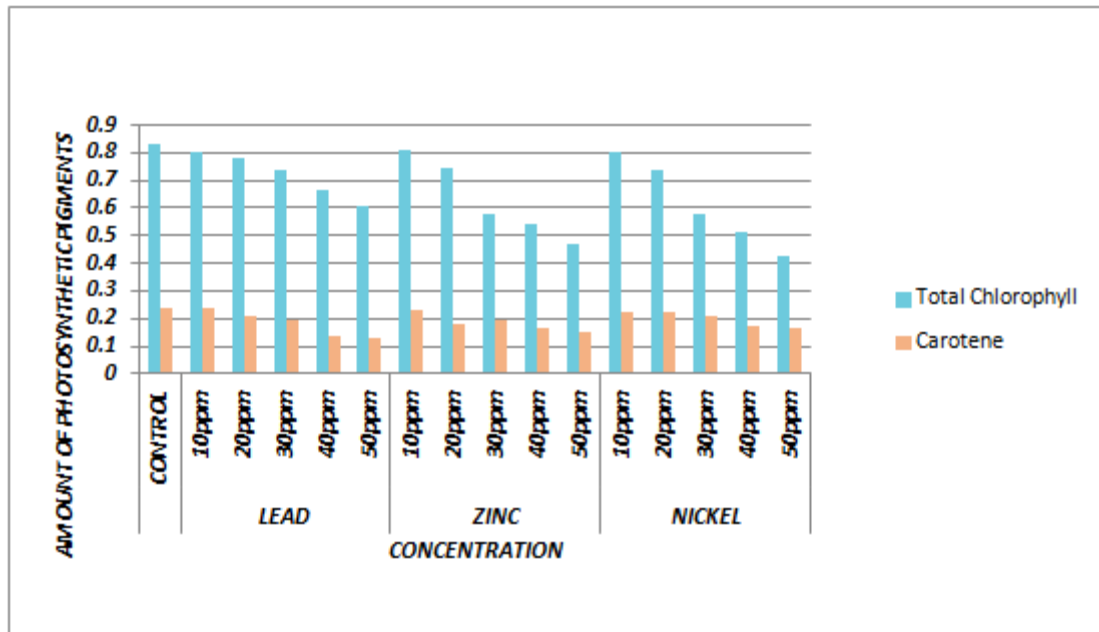


Figure 6: Effect of Lead,Zinc And Nickel Ions on Photosynthetic Pigments (Mg/Gfr.Wt) of the Plant *Brassica Nigra*

5. Protein (Protein 7&8): The Protein contents were progressively decreased in *C.arietinum* and *B.nigra* at higher concentrations, 50 ppm concentration of the heavy metals. The reduction rate was higher in Ni treated test crops than in Pb and Zn. The minimum inhibition was recorded at 10 ppm. At 50 ppm, the amount of Protein reduced against the treatment of Pb, Zn, and Ni is (36.52%), (56.52%), (66.08%) in *C. arietinum* and (41.39%), (58.06%), (70.96%) in *B.nigra*.

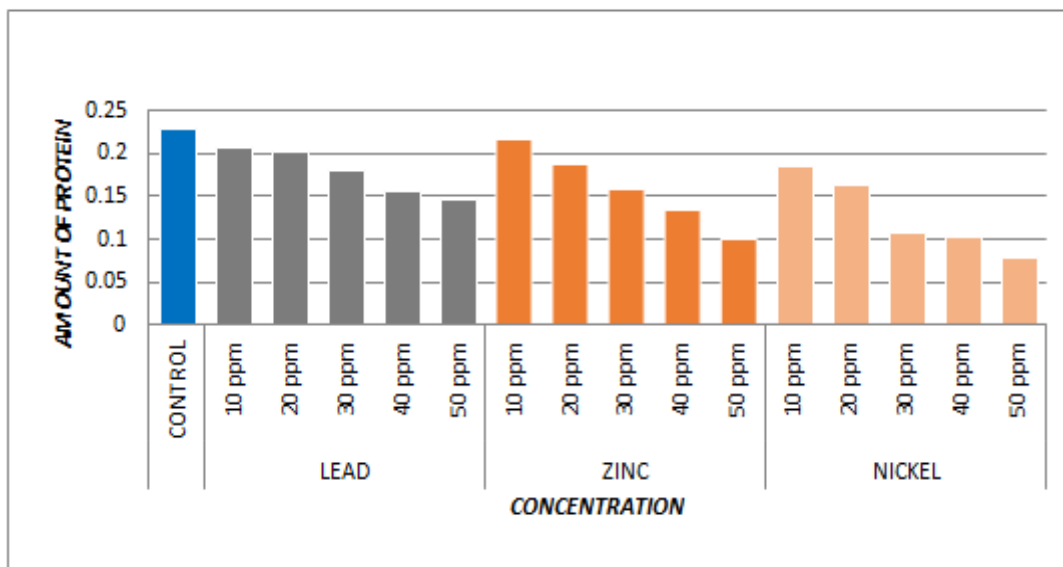


Figure 7: Effect of Lead, Zinc And Nickel Ions on Protein (Mg/Gfr.Wt) Content of *Cicer Arietinum*

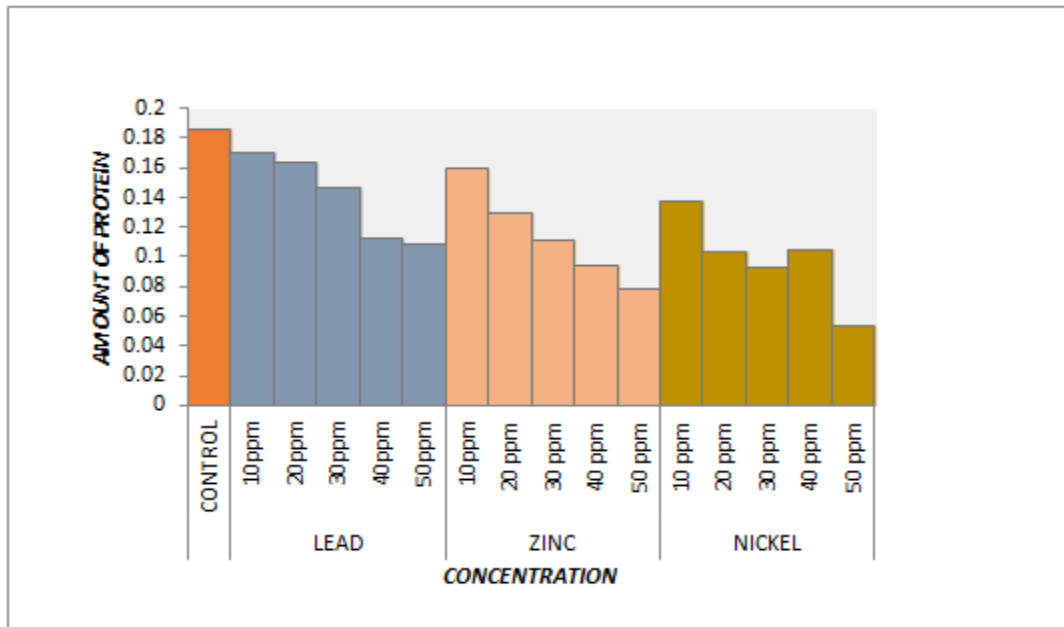


Figure 8: Effect of Lead,Zinc And Nickel Ions on Protein(Mg/Gfr.Wt) Content of *Brassica Nigra*

6. Carbohydrate (Figures 9 &10): The Carbohydrate contents were progressively decreased in *C.arietinum* and *B.nigra* at higher concentrations, 50 ppm concentration of the heavy metals. The reduction rate was higher in Ni treated test crops than in Pb and Zn. The minimum inhibition was recorded at 10 ppm. At 50 ppm, the amount of Carbohydrate reduced against the treatment of Pb, Zn, and Ni is (57.66%), (62.77%), (72.26%) in *C.arietinum* and (30.50%), (22.62%), (72.26%) in *B.nigra*.

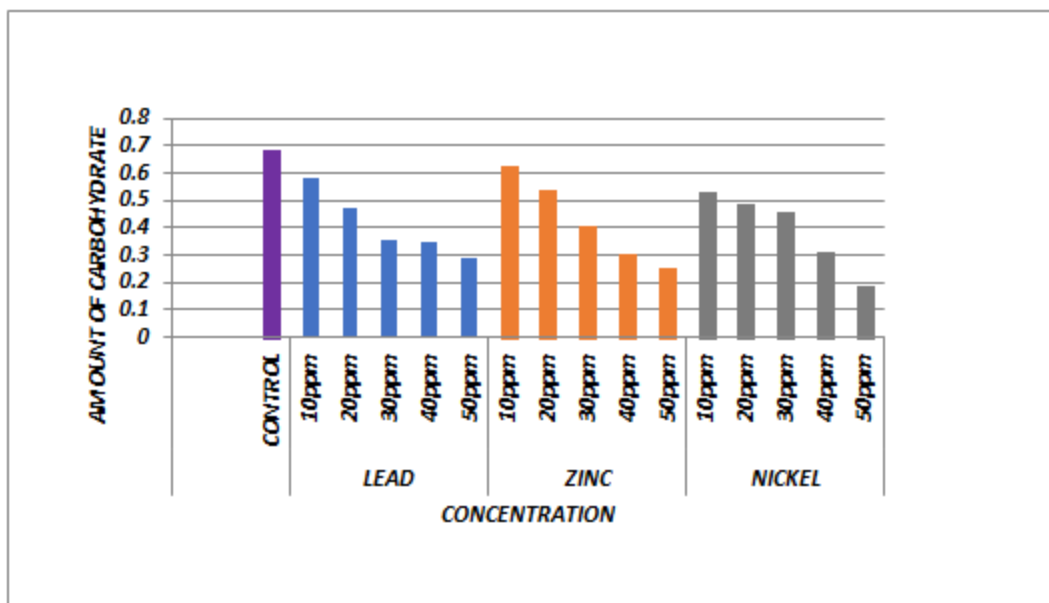


Figure 9: Effect of Lead,Zinc and Nickel Ions on Carbohydrate Content of *Cicer Arietinum*

INTERACTIONS OF LEAD, ZINC AND NICKEL TOXICITY IN THEIR EFFECTS ON GROWTH RESPONSES OF *CICER ARIETINUM L.* AND *BRASSICA NIGRA L.*

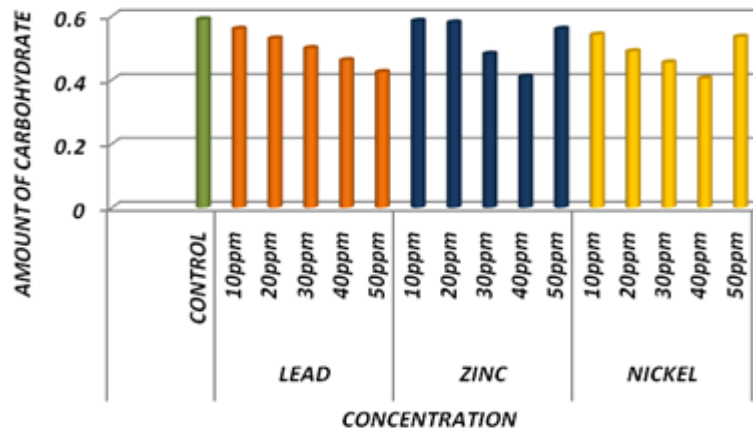


Figure 11: Effect of Lead, Zinc and Nickel Ions on carbohydrate content of Brassica nigra

7. **Proline Accumulation (Figures 11&12):** Proline is the amino acid formed in a high percentage of basic proteins. Free Proline plays an important role in plants under stress conditions. Though the Molecular mechanism has not been established for the increased level of Proline, one hypothesis refers to the breakdown of proteins to amino acids and conversion to Proline for storage. In such a way, Proline accumulated in the stressed plant leaves.

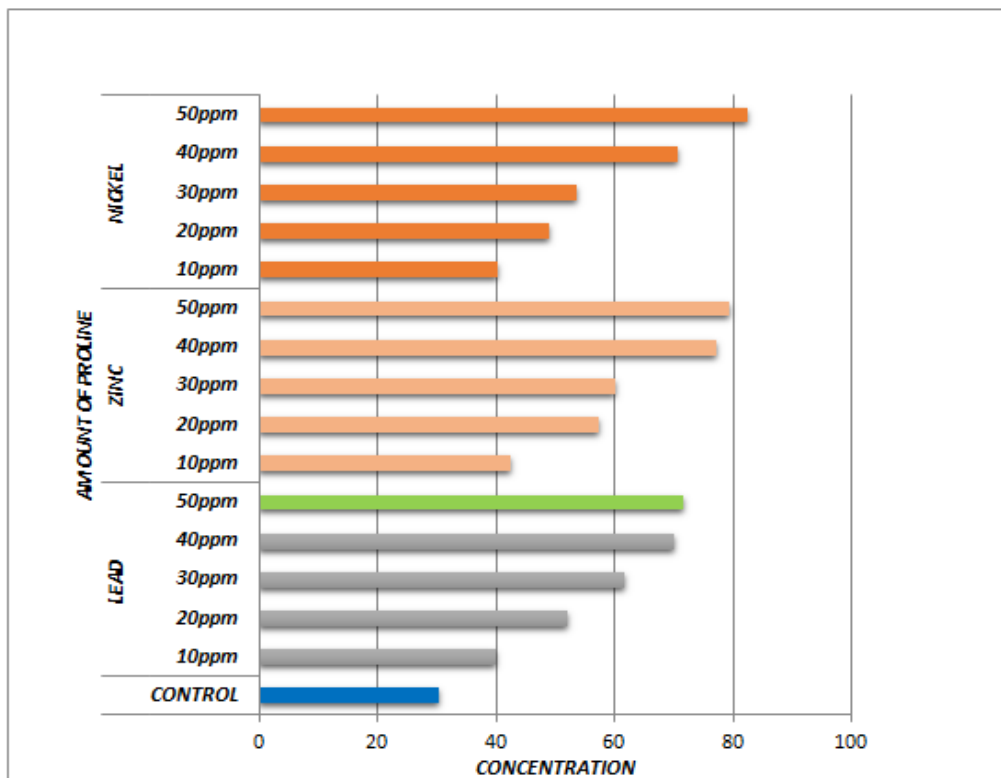


Figure 11: Effect of Lead, Zinc and Nickel Ions on Proline Content of Cicer Arietinum

The high amount of Proline accumulated in the Stressed Plants. In the following data, the amount of Proline accumulated in Nickel is high. The 50 ppm Concentration of heavy metals is most affected. The amount of Proline accumulation on the effect of Pb on *C.arietinum* is 71.42 $\mu\text{m/g/fr.wt}$ and on the effect of Zn is 79.22 $\mu\text{m/g/fr.wt}$ and on the effect of Ni is 82.25 $\mu\text{m/g/fr.wt}$. The amount of Proline accumulation on the effect of Pb on *B.nigra* is 74.45 $\mu\text{m/g/fr.wt}$ and on the effect of Zn is 80.08 $\mu\text{m/g/fr.wt}$ and on the effect of Ni is 101.29 $\mu\text{m/g/fr.wt}$.

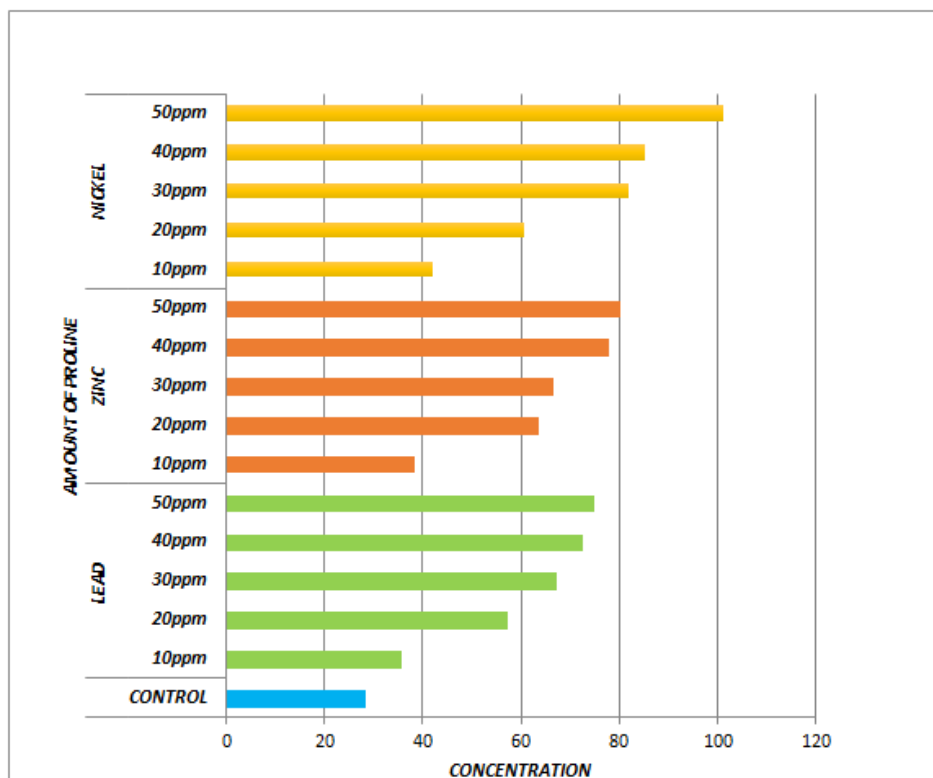


Figure 12: Amount of Lead,Zinc and Nickel Ions on Proline Content of *Brassica Nigra*

V. DISCUSSION

Natural elements like heavy metals cannot be organically broken down or eliminated. Without metal ions, life cannot grow or endure because it is primarily organic and inorganic. The elements that are found in trace amounts in natural biological systems are called trace elements. A trace element resulted from worries about the environment's declining quality. The fundamental constituents of plant, animal, and human life can be separated into two categories: major and trace elements. The latter category comprises essential and nonessential elements.

For ecological, evolutionary, nutritional, and environmental reasons, heavy metal toxicity is becoming an increasingly important concern. Heavy metals are major environmental contaminants. According to Hawkes (1997), they are a class of metals and metalloids having atomic densities five times or higher than water, or more than 4 g/cm³. Heavy metal pollution in the biosphere is a result of growing urbanization and

industrialisation. It was most readily available in soil and aquatic environments, and it was only slightly more prevalent as vapor or particle matter in the sky. As many heavy metals are necessary for plant growth, their toxicity to plants varies depending on the type of plant, the particular metal, the concentration, the chemical form, the pH of the soil, and other factors. Certain heavy metals, such as Zn and Cu, either activate or cofactorize enzyme processes (Mildvan, 1970). Heavy metals like Co, Cu, Fe, Mn, Mo, Ni, V, and Zn are needed by organisms in very small amounts, but excessive concentrations of these elements can harm them and have no beneficial effect on them. They are therefore regarded as the "main threats" since they poison the water, soil, and air, damage plants and animals severely, and may be toxic or poisonous, all of which will kill living things.

The present study revealed the results of the activity of Lead, Zinc and Nickel on *C.arietinum* and *B.nigra*. These crops are economically important, commonly cultivated in Tirunelveli District of Tamil Nadu, India. The test crop was treated with different concentrations of (10, 20, 30, 40, and 50 ppm) of Lead, Nickel and Zinc. The germination of the test crop was observed on the fifth day with different concentrations of heavy metals. The inhibition of germination percentage ranged from 0 to 100 % (Table 1). A higher reduction degree of germination was observed in the 50ppm test crop. The inhibition depends on the concentration of the heavy metals, which may be due to the entry of water-soluble heavy metals into the seeds, inhibiting germination.

Zhang et al. (2002) discovered that the high As content affected the growth of seedlings and the germination of wheat seeds. Similarly, Helianthus annus plumule and radical length were observed to have decreased by Imran et al. (2013). L seedlings in the presence of As.

In Figures 1 and 2, it can be observed that the root length and shoot length of the test crop decreased when exposed to various concentrations of heavy metals. These findings align with the results of a study conducted by Ahmad et al. (2012), where they investigated the impact of cadmium on the germination of seeds and the growth of seedlings from four Wheat (*Triticum aestivum*) cultivars. Additionally, Kibra (2008) reported similar effects when studying the influence of mercury on various growth parameters of rice (*Oryza sativa*). Moreover, Keunen et al. (2011) noted that plants growing in environments rich in heavy metals exhibit reduced growth and yield, underscoring the role of heavy metal toxicity in impeding the overall growth performance of stressed plants.

The effects of heavy metals on the biomass of the test crop are shown in Figures 3 & 4. The dry weight was decreased over in respective control in chickpea and mustard seedlings. The biomass decreased steadily when the concentration of the heavy metals increased. The study of Nagajyoti *et al.* (2010) revealed that the heavy metals, once inside the cell, alter metabolism, which results in a reduction of growth and lower biomass accumulation. Hussain *et al.* (2013) reported a reduction in germination percentage, suppressed growth, and reduced biomass in maize (*Zea mays*) when treated with Lead (Pb). Figures 5 & 6 showed the reduction of photosynthetic pigments. Chlorophyll and carotenoid contents of the test crop were reduced significantly in all treatments of different concentrations of (10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm) heavy metals. Imran *et al.* reported a decrease in photosynthetic pigments when exposed to As. Dong *et al.* (2005) have also reported the harmful effects on various photosynthetic indices, such as

photosynthetic rate in tomato seedlings. Similarly, Li *et al.* (2012) reported that Cu, Zn, Pb and Cd depressed the Chlorophyll and Carotenoids level and quantum yield of PSII in *Thalassia hamprichii*, indicating that heavy metals have negative consequences of photosynthesis. As the Chlorophyll concentration decreased in all extract concentrations, the metabolite of carbohydrate-protein also decreased. In the heavy metal effects on the test crop, the contents of Chlorophyll a, Chlorophyll b, Total Chlorophyll, Carotenoid, Protein (Figure 7 and 8) and Carbohydrate (Figure 9 & 10) were reduced significantly. Mishra *et al.* (2006) reported that conversion of Chlorophyll a Chlorophyll b may occur during stress situations. In tolerant species Carotenoids serve as antioxidants against free radicals and photochemical damage.

Stress induced by heavy metals results in the excessive production of Proline content, as demonstrated in Figures 11 and 12, which depict the increased production of proline enzymes across various concentrations of heavy metals. According to earlier research by Israr *et al.* (2011), Gao *et al.* (2010), Mou *et al.* (2011), Lomonte *et al.* (2010), Yasir *et al.* (2011), Ali *et al.* (2011), Pourrut *et al.* (2013), and Shahid *et al.* (2012), different antioxidant enzymes that control ROS overproduction are part of the secondary defense mechanism against heavy metal detoxification. Catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and ascorbate peroxidase (APX) are among these enzymes. Moreover, low molecular weight compounds like cysteine (Cys), proline (PRO), ascorbic acid (AsA), non-protein thiol (NPT), and glutathione (GSH) contribute to the scavenging of different kinds of ROS in addition to these enzymes. Notably, plants subjected to irrigation with 50 ppm of Ni exhibited severe stunting and wilting by the end of the experimental period, displaying higher inhibition compared to the other two heavy metals, namely Pb and Zn, especially at higher concentrations.

VI. CONCLUSION

Based on the results, it can be concluded that the heavy metal induces toxicity to the plants. With the increased concentration of heavy metals, the test crop gradually affects it indetrimentally. It affected all growth parameters (germination, seedling growth, biomass, vigour index, biochemical constituents such as pigments, protein, carbohydrates and enzymes) studied in the test crop compared to the respective control. The lower concentrations of heavy metals showed promotory effects on Chickpea and Mustard seedlings. The extract showed more inhibitory effect at 50ppm than at 10ppm concentration on germination, morphological and biochemical parameters of Chickpea and Mustard seeds. It may be due to the influence of different concentrations of heavy metals. The investigation has shown that heavy metallic ions of Lead, Zinc and Nickel reduce seeds' germination percentage. The toxicity also slows down the speed of germination. This heavy metal also retards several developmental events in the crucial stages of test crops at the seedling stage. Lateral roots are essential for the successful establishment of seedlings and it is significant to note that the development of these root are affected by the heavy metallic ions. Similarly, toxicity increases with an increase in concentration. The effect of heavy metals such as Pb, Zn and Ni on morphological and biochemical parameters of the plant *C.arietinum* and *B.nigra* was carried out. Fifteen days following germination, morphological and biochemical analysis was conducted. The germination percentage, protein, carbohydrate, shoot and root lengths, and photosynthetic pigments including carotenoids and

chlorophyll a and b were all significantly decreased by seedlings. In Proline, a reversal of trend was seen.

Application of 10ppm heavy metal treatment resulted in only a small reduction in test seedlings of *C.arietinum* and *B.nigra* in which germination, seedling length, pigments, and bio-chemicals reduced significantly as compared to control. In contrast, both test seedlings' metrics were more negatively impacted by the 50 ppm heavy metal treatment. In this experiment, chickpea seedlings showed a greater inhibitory impact than mustard seedlings. According to the results, nickel is more poisonous and has an impact on the germination of seeds and the characteristics of seedlings in *C.arietinum* and *B.nigra* plants. Plants with an excess of Ni²⁺ experience a range of physiological changes, poisoning symptoms, and reduced growth. *C.arietinum* is the most affected than *B.nigra*. **NICKEL > ZINC > LEAD** The result observed that Zinc is less toxic than Nickel. Zn affects Plant growth and photosynthetic activity and reduces the seedling growth of *C.arietinum* and *B.nigra*. Excess of Zn²⁺ ions in plants causes Chlorosis in *C.arietinum* and *B.nigra*. From the experiment, it was noticed that lead is the least toxic of all the three heavy metallic ions studied, and it affects the germination of seeds and reduces root development. From the above result, we conclude that the effect of toxic heavy metals may be different for different species of plants and the concentration of the metallic ions is dependent.

REFERENCES

- [1] Ahmad.M.S.A.,Ashraf.M.,Hussain.M.,(2012) Phytotoxic effects of nickel on yield and concentration of macro-and micro-nutrients in sunflower (*HelianthusannuusL.*) achenes.J Hazard Mater 185:1295–1303
- [2] Ali.S.,Bai.P.,Zeng.F.,Cai.S.,Shamsi.I.H.,Qiu.B.,Wu.F.,Zhang.G.,(2011)Theecotoxicological and interactive effects of chromium and aluminum on growth , oxidative damage and antioxidant enzymes on two barley genotypes differing in Al tolerance.EnviroExpBot70:185–191
- [3] Austruy.A.,Shahid.M.,Xiong.T.,Castrec.M.,Payre.V.,Niazi.N.K.,Sabir.M.,Dumat.C.,(2014).Mechanisms of metalphosphates formation in the rhizosphere soils of pea and tomato:envi-ronmental and sanitary consequences.J Soils Sediments14:666–678
- [4] Austruy.A.,Shahid.M.,Xiong.T.,Castrec.M.,Payre.V.,Niazi.N.K.,Sabir.M.,Dumat.C.,(2014).Mechanisms of metalphosphates formation in the rhizosphere soils of pea and tomato: environmental and sanitary consequences.J Soils Sediments14:666–678
- [5] Buendia-Gonzalez.L.,Orozco-Villafuerte.J.,Cruz-Sosa.F.,Barrera-Diaz.C.E.,Vernon Carter.E.J.,(2010). Prosopis laevigataa potential chromium(VI) and cadmium(II) hyperaccumulatordes-ertplant.BioresourTechnol101:5862–5867
- [6] Cakmak.I., (2000). Possible roles of Zinc in protecting plant cells from damage by reactive oxygen species. New Phytologist, 146: 185-205
- [7] CambrolleJ.,Mateos-Naranjo.E.,Redondo-Gomez.S.,Luque.T.,Figueroa.M.E.,(2011).Growth, reproductive and photosynthetic responses to copper in the yellow-horned poppy,*Glaucium flavum Crantz*. Environ Exp Bot 71:57–64
- [8] Celik.A.,Kartal.A.A.,Akdogan.A.,Kaska.Y.,(2005).Determining the heavy metal pollution in Denizli (Turkey) by using Robinia pseudo-acaciaL. Environ Int31:105–112
- [9] Chatterjee.C.,Dube.B.K.,Sinha.P.,Srivastava.P.,(2004).Detrimental effects of lead phytotoxicityon growth, yield, and metabolism of rice. Commun Soil Sci Plant Anal 35(1–2):255–265 Chemosphere65:343–351
- [10] Clemens.S(2006).Evolution and function of phytochelatin synthases. J Plant Physiol 163:319–332
- [11] Deng.X.,Xia.Y.,Hu.W.,Zhang.H.,Shen.Z.,(2005).Cadmium-induced oxidative damage and protective effects of N-acetyl-L-cysteine against cadmium toxicity in *Solanum nigrumL*.J Hazard Mater 180:722–729
- [12] Dhankhar, R., Sainger, P.A. and Sainger, M. (2012). Phytoextraction of zinc: physiological and molecular mechanism. Soil and Sediment Contamination, 21: 115-133
- [13] Dirilgen.N.,(2011).Mercury and lead: assessing the toxic effects on growth and metal accumulation by *Lemnaminor*.Ecotoxicol Environ Saf 74:48–54

- [14] Duarte.B.,Delgado.M.,Caçador.I.,(2007).The role of citric acid in cadmium and nickel uptake and translocation,in *Halimioneportulacoides*.Chemosphere 69:836–840
- [15] Franco.E.,Aless and relli.S., Masojidek.J., MargonelliA, GiardiMT(1999). Modulation of D1 protein turn over under cadmium and heat stresses monitored by [35S]methioninein corporation.Plant Sci144:53–61
- [16] Gao.S.,Ouyang.C.,Tang.L.,Zhu.J.,Xu.Y.,Wang.S.,Chen.F.,(2010).Growth and anti oxidant responses in *Jatropha curcas* seedling exposed to mercury toxicity.J Hazard Mater 182:591–597
- [17] Gao.S.,Ouyang.C.,Tang.L.,Zhu.J.,Xu.Y.,Wang.S.,Chen.F.,(2010).Growth and anti oxidant responses in *Jatropha curcas* seedling exposed to mercury toxicity.J Hazard Mater 182:591–597
- [18] Garg,N.,Singla,P. Arsenic toxicity in crop plants: Physiological effects and tolerance mechanisms. *Environ. Chem. Lett.* **2011**, 9, 303–321.
- [19] Ghorani-Azam, A.,Riahi-Zanjani, B., Balali-Mood, M. Effects of air pollution on human health and practical measures for prevention in Iran. *J. Res. Med. Sci.* **2016**, 21, 65.
- [20] Gopal.R.,Rizvi.A.H.,(2008).Excess lead alters growth ,metabolism and translocation of certain nutrients in radish.Chemosphere 70(9):1539–1544
- [21] Guala.S.D.,Veg.F.A.,Covelo.E.F.,(2010).Thedynamics of heavy metals in plants oil interactions.Ecol Model 221:1148–1152
- [22] Gupta.D.K.,Huang.H.G.,Yang.X.E.,Razafindrabe.B.H.N.,Inouhe.M(2010).,The detoxification of lead in *Sedumalfredii*H. Isnot related to phytochelatin but the glutathione.J Hazard Mater 177:437–444
- [23] Gupta.D.K.,Huang.H.G.,Yang.X.E.,Razafindrabe.B.H.N.,Inouhe.M(2010).,The detoxification of lead in *Sedumalfredii*H. Isnot related to phytochelatin but the glutathione.J Hazard Mater 177:437–444
- [24] Hakeem.K.R.,Sabir.M.,Ozturk.M.,Mermut.A.,(2014).Soil remediation and plants:prospects and challenges.Elsevier,Waltham,pp1–724
- [25] Hasanuzzaman.M.,Alam.M.M.,Rahman.A.,*et.al*(2014).Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice(*OryzasativaL.*)varieties.Biomed Res Int 2014:1–17
- [26] Hassan.S.A.,Hayat.S.,Ahmad.A.,(2011).Brassinolide protects photosynthetic machinery against the cadmium induced oxidative stress in two tomato cultivars. Chemosphere 84:1446–1451
- [27] Hassan.S.A.,Hayat.S.,Ahmad.A.,(2011).Brassinolide protects photosynthetic machinery against the cadmium induced oxidative stress in two tomato cultivars. Chemosphere 84:1446–1451
- [28] Hossain.Z.,Komatsu.S(2013).Contribution of proteomic studies towards understanding plant heavy metal stress response.Front Plant Sci 3:310
- [29] Hu.Y.F.,Zhou.G.,NaXF.,Yang.L.,Nan.WBLX.,Zhang.Y.Q.,LiJ.L.,Bi.Y.R.,(2013).Cadmium interferes with maintenance of auxin homeostasis in Arabidopsis seedlings.J Plant Physiol 170:965–975
- [30] Israr.M.,Jewell.A.,Kumar.D.,Sahi.S.V(2011).Interactive effects of lead, copper, nickel and zinc on growth, metal uptake and antioxidative metabolism of *Sesbaniadrummondii*.JHazard Mater186:1520–1526
- [31] Israr.M.,Jewell.A.,Kumar.D.,Sahi.S.V(2011).Interactive effects of lead, copper, nickel and zinc on growth, metal uptake and antioxidative metabolism of *Sesbaniadrummondii*.JHazard Mater186:1520–1526
- [32] Israr.M.,Jewell.A.,Kumar.D.,Sahi.S.V(2011).Interactive effects of lead, copper, nickel and zinc on growth, metal uptake and antioxidative metabolism of *Sesbaniadrummondii*.JHazard Mater186:1520–1526
- [33] Jiang.W.,Liu,D.,(2010).Pb-induced cellular defense system in the root meristematic cells of *Allium sativum*L.BMC Plant Biol10:40
- [34] Jiang.W.,Liu,D.,(2010).Pb-induced cellular defense system in the root meristematic cells of *Allium sativum*L.BMC Plant Biol10:40
- [35] Jozic.M.,Peer.T.,Turk.R.,(2009).The impact of the tunnel exhausts interms of heavy metals to the surrounding ecosystem.EnvIRON Monit Assess 150:261–271
- [36] Kavamura.V.N.,Esposito.E.,(2010).Biotechnological strategies applied to the decontamination of soils polluted with heavy metals.BiotechnolAdv28:61–69
- [37] Khan.N.A.,Anjum.N.A.,Nazar.R.,Iqbal.N.,(2009).Increased activity of ATP-sulfurylase and increased contents of cysteine and glutathione reduce high cadmium induced oxidative stress in mustard cultivar with high photosynthetic potential.Russian JPlant Physiol 56:670–677
- [38] Ko.K.S.,Lee.P.K.,Kong.I.C.,(2012).Evaluation of the toxic effects of arsenite,chromate,cadmium,and copper using a battery of four bio assays.Appl Microbiol Biotechnol 95:1343–1350
- [39] Kranner.I.,Colville.L(2011).Metals and seeds: biochemical and molecular implications and their significance for seed germination.EnvIRON Exp Bot 72:93–105
- [40] Kuzminov.F.I.,Brown.C.M.,Fadeev.V.V.,Gorbunov.M.Y.,(2013).Effects of metal toxicity on photosynthetic processes in coral symbionts, Symbiodinium spp.JExp Marine Biol Eco 1446:216–227

- [41] Liao.Y.C.,Chien.S.W.C.,Wang.M.C.,Shen.Y.,Hung.P.L.,Das.B.,(2006).Effect of transpiration on Pb uptake by lettuce and on water soluble low molecular weight organic acids in rhizosphere.
- [42] Lomonte.C.,Doronila.A.I.,Gregory.D.,Baker.A.J.M.,Kolev.S.D.,(2010). Phytotoxicity of bio solids and screening of selected plant species with potential for mercury phytoextraction.J Hazard Mater173:494–501
- [43] Lomonte.C.,Doronila.A.I.,Gregory.D.,Baker.A.J.M.,Kolev.S.D.,(2010). Phytotoxicity of bio solids and screening of selected plant species with potential for mercury phytoextraction.J Hazard Mater173:494–501
- [44] Luo, L., Wang, B., Jiang, J., Huang, Q., Yu, Z., Li, H., Zhang, J., Wei, J., Yang, C., Zhang, H. Heavy metal contaminations in herbal medicines: Determination, comprehensive risk assessments. *Front. Pharmacol.* **2020**, *11*, 595335.
- [45] Małecka.A.,Piechalak.A.,Morkunas.I.,Tomaszewska.B.,(2008).Accumulation of lead in root cells of *Pisum sativum*.Act Physiol Plant 30:629–637
- [46] Marques.M.C.,doNascimentoCWA.,(2013).Analysis of chlorophyll fluorescence spectra for the monitoring of Cd toxicity in a bio energy crop (*Jatropha curcas*).J Photo chem Photobiol B127:88–93
- [47] Moosavi.S.E.,Gharineh.M.H.,Afshari.R.T.,Ebrahimi.A.,(2012).Effects of some heavy metals on seed germination characteristics of canola (*Barassicanapus*),wheat (*Triticumaestivum*) and saf-flower (*Carthamustinctoriosus*) to evaluate phytoremediation potential of these crops.JAgric Sci 4:1–19
- [48] Mou.D.,Yao.Y.,Yang.Y.,Zhang.Y.,Tian.C.,Achal.V.,(2011).Plant high tolerance to excess manganese related with root growth, manganese distribution and antioxidative enzyme activity in three grape cultivars.Eco toxicol Environ Saf 74:776–786
- [49] Mou.D.,Yao.Y.,Yang.Y.,Zhang.Y.,Tian.C.,Achal.V.,(2011).Plant high tolerance to excess manganese related with root growth, manganese distribution and antioxidative enzyme activity in three grape cultivars.Eco toxicol Environ Saf 74:776–786
- [50] Munzuroglu.O.,Geckil.H.,(2002).Effects of metals on seed germination,root elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*.Arch Environ ContamToxicol43:203–213
- [51] Nabulo.G.,Black.C.R.,Young.S.D.,(2010).Assessing risk to human health from tropical leafy vege-tables grown on contaminated urban soils.Sci Total Environ 408:5338–5351
- [52] Nabulo.G.,Black.C.R.,Young.S.D.,(2011).Trace metal uptake by tropical vegetables grown on soil amended with urban sewage sludge.Environ Pollut 159:368–376
- [53] Nazar.R.,Iqbal.N.,Masood.A.,Iqbal.M.,Khan.R.,Syeed.S.,Khan.N.A.,(2012).Cadmiumtoxicity in plants and role of mineral nutrients in its salleviation.AmJ Plant Sci3:1476–1489
- [54] Padinha.C.,Santos.R.,Brown.M.T.,(2000).Evaluating environmental contamination in Ria Formosa (Portugal) using stress indexes of *Spartin amaritima*.Mar Environ Res 49:67–78
- [55] Park.J.H.,Lamb.D.,Paneerselvam.P.,Choppala.G.,Bolan.N.,Chung.J-W., (2011) Role of organic amendments on enhanced bioremediation of heavymetal (loid) contaminated soils.J Hazard Mater185:549–574
- [56] Pauget.B,Gimbert.F.,Scheifler.R.,Coourdassier.M.,deVaufleury.A.,(2012).Soil parameters are key factors to predict metal bioavailability to snails based on chemical extractant data.Sci Total Environ 431:413–425
- [57] Pinchasov.Y.,Dubinsky.TBZ.,(2006).The effect of lead on photosynthesis, as determined by photo acustics in *Synechococcusleopoliensis* Cyanobacteria. Water Air Soil Pollut 175:117–125
- [58] Poschenrieder.C.,Barcelo.J.,(2004).Water relations in heavy metal stressed plants.In:Poschenrieder.C.,Barcelo.J(eds),Heavy metal stress in plants.Springer,Berlin,pp 207–299
- [59] Pourrut.B.,Jean.S.,Silvestre.J.,Pinelli.E.,(2011).Lead-induced DNA damage in *Vicia faba* root cells:potential involvement of oxidative stress.Mutat Res 726:123–128
- [60] Pourrut.B.,Shahid.M.,Douay.F.,Dumat.C.,Pinelli.E.,(2013).Molecular mechanisms involved in lead uptake, toxicity and detoxification in higher plants.In:Gupta.D.K., Corpas.F.J., Palma.J.M.,(eds). Heavy metal stress in plants.Springer,Berlin,pp121–147
- [61] Punamiya.P.,Datta.R.,Sarkar.D.,Barber.S.,Patel.M.,Das.P.,(2010).Symbiotic role of *Glomus mosseae* in phyto extraction of lead in vetiver grass[*Chrysopogonzizanioides*(L.)].J Hazard Mater177:465–474
- [62] Rai, K.K.; Pandey, N.; Meena, R.P.; Rai, S.P. Biotechnological strategies for enhancing heavy metal tolerance in neglected and underutilized legume crops: A comprehensive review. *Ecotoxicol. Environ. Saf.* **2021**, *208*, 111750.
- [63] RamaDevi.S.,Prasad.M.N.V.,(2004).Membrane lipid alterations in heavy metal exposed plants.
- [64] Rascio.N.,Navari-Izzo.F.,(2011). Heavy metal hyperaccumulating plants:how and why do they do it? And what makes them so interesting? Plant Sci (ShannonIreland) 180:169–181
- [65] Rascio.N.,Navari-Izzo.F.,(2011). Heavy metal hyperaccumulating plants:how and why do they do it? And what makes them so interesting? Plant Sci (ShannonIreland) 180:169–181

- [66] Rascio.N.,Navari-Izzo.F.,(2011). Heavy metal hyperaccumulating plants:how and why do they do it? And what makes them so interesting? *Plant Sci (ShannonIreland)* 180:169–181
- [67] Reddy.A.M.,Kumar.S.G.,Jyothsnakumari.G.,Thimmanaik.S.,Sudhakar.C.,(2005).Lead induced changes in antioxidant metabolism of horse gram (*Macrotylomauniflorum*(Lam.)Verdc.) and bengal gram(*Cicerarietinum*L). *Chemosphere*60:97–104
- [68] Saha. S., Saha. B.N., Pati. S., Pal. B., Hazra. G.C., Agricultural use of sewage sludge in India: Benefits and potential risk of heavy metals contamination and possible remediation options—a review. *Int. J. Environ. Technol. Manag.* **2017**, 20, 183–199.
- [69] Saifullah.M.E.,Qadir.M.,deCaritat.P.,Tack.F.M.G.,DuLaing.G,Zia.M.H.,(2009). EDTA-assisted Pb phyto extraction.*Chemosphere* 74:1279–1291
- [70] Sammut.M.L.,Noack.Y.,Rose.J.,Hazemann.J.L.,Proux.O.,Depoux.M.,Ziebel.A.,Fiani.E.,(2010).Speciation of Cd and Pb indust emitted froms interplant.*Chemosphere*78:445–450
- [71] Seleiman, M.F., Al-Suhaibani, N., El-Hendawy, S., Abdella, K., Alotaibi, M., Alderfasi, A. Impacts of long-and short-term of irrigation with treated wastewater and synthetic fertilizers on the growth, biomass, heavy metal content, and energy traits of three potential bioenergy crops in arid regions. *Energies* (2021), 14, 3037.
- [72] Seleiman, M.F., Santanen, A., Makela, P.S. Recycling sludge on cropland as fertilizer—Advantages and risks. *Resour. Conserv. Recycl.* **2020**, 155, 104647.
- [73] Shahid.M.,Pinelli.E.,Pourrut.B.,Silvestre.J.,Dumat.C.,(2011).Lead-induced geno toxicity to *Vicia faba*L.roots in relation with metal cell uptake and initial speciation.*Eco toxicol Environ Saf*74:78–84
- [74] Shahid.M.,Pinelli.E.,Pourrut.B.,Silvestre.J.,Dumat.C.,(2011).Lead-induced geno toxicity to *Vicia faba*L.roots in relation with metal cell uptake and initial speciation.*Eco toxicol Environ Saf*74:78–84
- [75] Shahid.M.,Arshad.M.,Kaemmerer.M.,Pinelli.E.,Probst.A.,Baque.D.,Pradere.P,Dumat.C., (2012a) Long term field metal extraction by pelargonium : phytoextraction efficiency in relation with plant maturity.*Int J Phytorem*14:493–505
- [76] Shahid.M.,Dumat.C.,Aslam.M.,Pinelli.E.,(2012b).Assessment of lead speciation by organic ligands using speciation models.*Chem Spec Bioavailab* 24:248–252
- [77] Shahid.M.,Dumat.C.,Pourrut.B.,Sabir.M.,Pinelli.E.,(2014b).Assessing the effect of metal speciation on lead toxicity to *Vicia faba* pigment contents.*J Geochem Explor* 144:290–297
- [78] Shahid.M.,Dumat.C.,Silvestre.J.,Pinelli.E.,(2012c).Effect of fulvic acids onlead-induced oxidative stress to metal sensitive *Vicia faba*L.plant.*Biol Fertil Soils* 48:689–697
- [79] Shahid.M.,Pinelli.E.,Dumat.C.,(2012d).Review of Pb availability and toxicity to plants in relation with metal speciation; role of synthetic and natural organic ligands.*J Hazard Mater* 219–220:1–12
- [80] Shahid.M.,Pinelli.E.,Dumat.C.,(2012d).Review of Pb availability and toxicity to plants in relation with metal speciation; role of synthetic and natural organic ligands.*J Hazard Mater* 219–220:1–12
- [81] Shahid.M.,Pinelli.E.,Pourrut.B.,Dumat.C.,(2014d).Effect of organic ligands on lead-induced oxidative damage and enhanced antioxidant defense in the leaves of *Vicia faba* plants.*J Geo chem Explor*144:282–289
- [82] Sharma.P.,Dubey.R.S.,(2005).Lead toxicity in plants.*Braz J Plant Physiol* 17(1):35–52
- [83] Uzu.G.,Sauvain.J-J.,Baeza-Squiban.A.,Riediker.M., Hohl.M.S.S., Val.S.,Tack.K.,Denys.S.,Pradere.P.,Dumat.C.,(2011a).In vitro assessment of the pulmonary toxicity and gastric availability of lead-rich particles from a lead recycling plant.*Environ Sci Technol* 45:7888–7895
- [84] Uzu.G.,Sobanska.S.,Aliouane.Y.,Pradere.P.,Dumat.C.,(2009).Study of lead phytoavailability for atmospheric industrial micronic and sub-micronic particles in relation with lead speciation.*Environ Pollut* 157:1178–1185
- [85] Uzu.G.,Sobanska.S.,Sarret.G.,Munoz.M.,Dumat.C.,(2010).Foliar lead uptake by lettuce exposed to atmospheric fall outs.*Environ Sci Technol* 44:1036–1042
- [86] Vassilev.A.,Yordanov.I.,Tsonev.T.,(1997).Effects of Cd²⁺ on the physiological state and photosynthetic activity of young barley plants.*Photosynthetica* 34(2):293–302
- [87] Vega.F.A.,Andrade.M.L.,Covelo.E.F.,(2010).Influence of soil properties on the absorption and retention of cadmium, copper and lead, separately and together,by 20 soil horizons: Comparison of line are regression and tree regression analyses.*JHazardMater*174:522–533
- [88] Verbruggen.N.,Hermans.C.,Schat.H.,(2009).Molecular mechanisms of metal hyperaccumulation in plants.*New Phytol* 181:759–776
- [89] Verbruggen.N.,Hermans.C.,Schat.H.,(2009).Molecular mechanisms of metal hyperaccumulation in plants.*New Phytol* 181:759–776

- [90] Wannaz.E.D.,Carreras.H.A.,Rodriguez.J.H.,Pignata.M.L.,(2012).Use of bio monitors for the identification of heavy metals emission sources.Ecol Indic20:163–169
- [91] Wojas.S.,Hennig.J.,Plaza.S.,Geisler.M.,Siemianowski.O.,Skłodowska.A.,Ruszczyiska.A.,Bulska.E.,Antosiewicz.D.M.,(2009).Ectopic expression of Arabidopsis ABC transporter MRP7 modifies cadmium root-to-shoot transport and accumulation.Environ Pollut 157:2781–2789
- [92] Xu.H.,Song.P.,Gu.W.,Yang.Z.,(2011).Effects of heavy metals on production of thiolcompounds and antioxidant enzymes in *Agaricus bisporus*.EcotoxicolEnvironSaf74:1685–1692
- [93] Yadav.S.K.,(2010).Heavy metals toxicity in plants:an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants.SAfrJMarSci76:167–179
- [94] Yadav.S.K.,(2010).Heavy metals toxicity in plants:an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants.SAfrJMarSci76:167–179
- [95] Yan, A., Wang, Y., Tan, S.N., Mohd Yusof, M.L., Ghosh, S., Chen, Z. Phytoremediation: A promising approach for revegetation of heavy metal-polluted land. *Front. Plant Sci.* **2020**, *11*, 359.
- [96] Yan.Z.Z.,Ke.L.,Tam.N.F.Y.,(2010).Lead stress in seedlings of *Avicennia marina*,acommonman-grove species in South China,with and without cotyledons.Aquatic Bot 92:112–118
- [97] Yasir.P.Y.,Hakeem.K.R.,Chandna.R.,Ahmad.P.,(2011).Role of Glutathione reductase in abiotic stress. In:Parvaiz.A., Prasad.M.N.V.(eds),Abiotic stress responses in plants.Springer,NewYork,pp149–158
- [98] Yasir.P.Y.,Hakeem.K.R.,Chandna.R.,Ahmad.P.,(2011).Role of Glutathione reductase in abiotic stress. In:Parvaiz.A., Prasad.M.N.V.(eds),Abiotic stress responses in plants.Springer,NewYork,pp149–158
- [99] Ying.R-R.,QiuR-L.,Tang.Y-T.,Hu.P-J.,Qiu.H.,ChenH-R,ShiTH,MorelJL(2010)Cadmium tolerance of carbon assimilation enzymes and chloroplast in Zn/Cd hyperaccumulator *Picrisdivaricata*. JPlantPhysiol167:81–87
- [100]Zhuang.P.,McBride.M.B.,Xia.H.,Li.N.,Li.Z.,(2009).Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshanmine.South China Sci Total Environ 407:1551–1561