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EFFECT OF HEAVY METALS ON PHOTOSYNTHETIC PIGMENTS IN AGRICULTURAL PLANTS

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ABSTRACT

When an element is referred to as "toxic," it means that it is extremely dangerous, poisonous, undesirable, or present in abundance. Heavy metals such as mercury, cadmium, and lead cause toxic effects on plants. These heavy metals are typically found in industrial waste. can significantly affect soil quality, as well as its physical, chemical, and biological properties. Because industrial water is used on agricultural land, heavy metals are transferred and eventually concentrated in plant cells, rendering them poisonous. These have extremely negative effects on plant growth and development. These metals have higher atomic weights than water (over 4g/cm³). Heavy metal toxicity varies by species, depending on the metal's concentration, chemical form, pH, and other variables. These must be present in a small amount. This study looks at how different levels of heavy metals (Pb and Cd) affect the pigment content of specific agricultural plants (Triticum aestivum and Brassica juncea), with a focus on carotenoids, total chlorophyll, chlorophyll a, and chlorophyll b. Physiological measures such as the Membrane Injury Index (MII) and Relative Water Content (RWC) were also evaluated. ANOVA analysis revealed a significant decrease in pigment levels and water retention, as well as increased membrane damage, during heavy metal stress ($p < 0.05$). These findings highlight the importance of sustainable phytoremediation techniques and soil quality monitoring.

KEYWORD: *Heavy metal, plant growth, sustainable, photosynthetic*

INTRODUCTION

Global heavy metal poisoning of the environment from many sources, including industrial and agricultural activities, is a significant problem. While heavy metals are naturally present in soils at trace amounts, several human activities, including heightened environmental pollution from industry, agriculture, and mining, have led to detrimental toxic accumulations of heavy metals.

Phytoremediation is a remediation method for soils polluted with metals. Model phytoremediators include several tree species, including cottonwood, poplar, and willows. Trees are optimal for the cleanup of heavy metals since they can endure elevated concentrations of contaminants owing to their substantial biomass (Paz-Alberto and Sigua 2013). Due of their size and broad root systems, they may gather significant quantities of pollutants in their systems. Moreover, trees possess the capacity to sustain a region, mitigate erosion, and reduce the dissemination of toxins. They may be readily collected and extracted from the environment with no danger, facilitating the efficient removal of toxins (Paz-Alberto and Sigua 2013).

Certain heavy metals, including copper (Cu), zinc (Zn), cobalt (Co), and iron (Fe), are vital in trace quantities for many metabolic processes in plants. Excessive levels of any metal, whether necessary or non-essential, negatively impact plant metabolism (Hall 2002). In plants, metals mostly exert their harmful effects by destroying chloroplasts and disrupting photosynthesis. The inhibition of photosynthesis results from the disruption of photosynthetic enzymes and chloroplast membranes by metal ions (Aggarwal et al. 2012). In higher plants, heavy metal deposition in leaves indirectly diminishes photosynthesis by impairing stomatal function, hence affecting total photosynthesis and transpiration rates. The decrease of photosynthetic pigments by heavy metals indirectly impacts photosynthesis; thus, the use of non-destructive technologies and the simplicity of measurement facilitate the frequent use of photosynthetic pigments to assess stress for regulatory objectives (Aggarwal et al. 2012).

The susceptibility of plants to heavy metals is contingent upon a complex interplay of physiological and molecular mechanisms, including: (i) the uptake and accumulation of metals via the binding of extracellular exudates and cell wall components; (ii) the efflux of heavy metals from the cytoplasm to extracytoplasmic compartments such as vacuoles; (iii) the complexation of heavy metal ions within the cell with various substances, including organic acids, amino acids, phytochelatins, and metallothioneins; (iv) the accumulation of osmolytes and osmoproteins, along with the induction of antioxidative enzymes; and (v) the activation or modification of plant metabolism to facilitate the proper functioning of metabolic pathways and the rapid repair of damaged cellular structures (Cho et al. 2003).

Multiple poplar genotypes have been used to assess their physiological and molecular responses to various heavy metals. Sebastiani et al. (2004) examined the impact of organic waste enriched with heavy metals (Zn, Cu, Cr, and Cd) on biomass partitioning and heavy metal accumulation in the organs of two poplar clones (Populus deltoides x maximowiczii – clone Eridano and P. x euramericana – clone I-214). Gaudet et al. (2011) analyzed the physiological and molecular responses to cadmium stress in two genotypes of Populus nigra L. derived from disparate habitats. Robinson et al. (C2007) examined the efficacy of hybrid poplar for boron (B) phytomanagement using a lysimeter experiment and a field trial including B-contaminated wood waste. Zacchini et al. (011) investigated the impacts of cadmium accumulation and tolerance in Populus nigra and Salix alba. Despite extensive research, the precise processes behind heavy metal buildup and detoxification remain inadequately elucidated. The characterization of these pathways may enhance the phytoremediation capacity of Salicaceae plants. The results indicated a significant reduction in pigment concentrations and water retention, along with heightened membrane damage due to heavy metal stress, as verified by ANOVA ($p < 0.05$). These findings underscore the need for soil quality assessment and sustainable phytoremediation approaches.

REVIEW OF THE LITERATURE

Ghorbani et al. (2016,) A factorial pot experiment based on a full randomized block design was conducted in 2013 at Shahrood University of Technology's College of Agriculture to investigate the effects of salinity and lead (Pb) and cadmium (Cd) heavy metals on spinach plants. Three salinity levels (0, 4, and 8 dS/m) were used as factor A in the treatments, while four doses of HMs (control, Cd, Pb, and Cd+Pb) were used as factor B. The fresh and dry weight of spinach was shown to be unaffected by salinity, whereas both characteristics were significantly altered by HMs treatment. The Cd and Cd+Pb treatments yielded the lowest and greatest fresh and dry weights, respectively. Only chlorophyll a, chlorophyll b, and cartenoid levels were significantly impacted by salinity, which decreased them at the 8 dS/m level in addition to photosynthetic pigments of flavonoids and anthocyanin. Treatment with salinity increased the amount of salt and soluble carbohydrates in spinach leaves without changing the potassium content. Salinity and HMs only significantly interacted with carbohydrates, chlorophyll a, and chlorophyll b. The Cd+Pb and 8 dS/m treatment produced the most carbohydrates, whereas the no salinity (control) and Pb treatment produced the most chlorophyll a and chlorophyll b.

(Korsun and others, 2015) This research uses chlorophyll fluorescence to examine how heavy metal pollution affects maize photosynthesis. The findings indicate that polluted plants have altered Calvin cycle responses, reduced primary processes, and disruptions in the photosynthetic machinery. The kind of Kautsky curve changes when any link in photosynthesis changes. This makes it possible to determine how the photosynthetic machinery of plants is currently affected by heavy metals. Throughout the experiment, we observed variations in the fluorescence of maize chlorophyll leaves between control and heavy metal-contaminated ecotypes. Basic kinetic parameters, which are linked to the activities of the photosynthetic apparatus, govern the characteristic of curve formation. The photosynthetic machinery in maize leaves is disrupted during the early stages of growth (three to four leaves) and throughout the creation of the maize panicle when there is an excessive buildup of lead, zinc, and cadmium in the soil. Basic measurements including background fluorescence (Fo), maximum fluorescence (Fm), and stationary fluorescence (Fst) indicate a decline in photosynthesis's core processes as well as a violation of the Calvin cycle's coherence reactions. The version with a 100-fold extra background yielded a Kautsky curve with much smaller peak amplitudes than the others.

Sujatha and Priyadarshini (2014) With a lengthy biological half-life, cadmium (Cd) is a common heavy metal contaminant in the environment. Plants absorb it with ease. The current research employed varying doses of cadmium (cadmium chloride: $CdCl_2.H_2O$) representing 0.02, 0.04, and 0.06 mM to treat three pigeonpea (Cajanus cajan L.) cultivars LRG30, LRG41, and ICPL85063. Only 6–8 day old seedlings were used for the analysis of photosynthetic characteristics such as chlorophyll a, chlorophyll b, total chlorophyll, pheophytin a, pheophytin b, total pheophytin, and total carotenoids content. In the three pigeonpea cultivars, cadmium treatment at all investigated levels resulted in reduced chlorophyll and total carotenoid content and increased pheophytin content in the sequence of increasing externally supplied Cd concentrations. When pigeonpea cultivars LRG41 and ICPL85063 were exposed to Cd, their pigment content changed more than that of LRG30.

Chouhan and colleagues (2012) Finding out how heavy metals build up in plant leaves and how they affect the overall photosynthetic pigments of plants living amid industrial pollution was the goal of the current research. The accumulation of heavy metals such as Pb, Cd, Cu, and Ni in the leaves of naturally occurring plants found in Pithampur Industrial Area sectors 1, 2, and 3—namely, A. indica (Neem), C. gigantea (Aak), D. sissoo (Shishum), E. jambolana (Jamun), M. indica (Aam), and N. indicum (Kaner)—was assessed in the current study and contrasted with control plants. The current study's findings showed that, in comparison to the control, there was a notable buildup of heavy metals in the leaves of plants growing in Pithampur Industrial Area sectors 1, 2, and 3. A. indica showed the highest accumulation of heavy metals (59.11µg/gm of dry weight of leaves), whereas C. gigantea showed the lowest accumulation (14.68µg/gm of dry weight of leaves). Total photosynthetic pigment and heavy metal accumulation were negatively correlated. Sector -2 showed a significant negative correlation ($r = -0.61$), indicating that buildup was negatively impacting the overall amount of photosynthetic pigments.

(Pandey and others, 2013) Because they are sessile, plants are often subjected to a variety of severe environmental conditions that have a negative impact on their development, metabolism, and production. Drought, salt, gaseous pollution, and heavy metals are significant environmental stressors that have a significant impact on plant development among other abiotic stresses. In essence, the only way that life receives energy is via photosynthesis. In many plant species exposed to extreme climatic circumstances, a decrease in photosynthetic capability is often linked to a fall in production. Rapid urbanization, industrialization, and agricultural runoff have all contributed to an increase in heavy metals in the air, water, and soil. Numerous of these metals negatively impact plant development and metabolism, resulting in decreased photosynthesis, decreased chloroplast degeneration, decreased chlorophyll concentration, and suppression of enzyme activity.

OBJECTIVE OF THE STUDY

1. To examine the effects of varying concentrations of heavy metals (Pb and Cd) on pigment content in selected crop plants (Brassica juncea and Triticum aestivum),

2. To concentrate on chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids. Physiological parameters including Relative Water Content (RWC) and Membrane Injury Index (MII) were evaluated.

METHODOLOGY

The experiment focused on agricultural crop species like Brassica juncea and Triticum aestivum, chosen for their significant economic value and their responsiveness to environmental stressors. The plants were cultivated in pots filled with sterilized alluvial soil within a controlled greenhouse environment, with each treatment comprising three replicates to guarantee statistical reliability. Heavy metal stress was induced by irrigating the plants with salt solutions that contained varying concentrations (50, 100, and 150 ppm) of lead (Pb), cadmium (Cd), and zinc (Zn). The chemical salts utilized in this study included lead nitrate [$Pb(NO_3)_2$], cadmium chloride [$CdCl_2$], and zinc sulfate [$ZnSO_4 \cdot 7H_2O$]. A distinct control group was established, consisting of plants that received irrigation solely with distilled water.

Following a 30-day treatment period, mature leaves were collected from the plants for the purpose of pigment analysis. To quantify chlorophyll and carotenoids, 0.5 grams of fresh leaf tissue were homogenized in 80% acetone and then centrifuged at 5,000 rpm for 10 minutes to yield a clear supernatant. The extract's absorbance was quantified using spectrophotometry at wavelengths of 645 nm, 663 nm, and 470 nm. Pigment concentrations were determined using the equations established by Arnon in 1949: Chlorophyll a (mg/g FW) = $12.7 \times A663 - 2.69 \times A645$, Chlorophyll b (mg/g FW) = $22.9 \times A645 - 4.68 \times A663$, with Total Chlorophyll calculated as the sum of chlorophyll a and b. The estimation of carotenoid content was conducted using the following formula: Carotenoids (mg/g FW) = $(1000 \times A470 - 1.82 \times \text{Chl a} - 85.02 \times \text{Chl b}) / 198$. The calculations yielded accurate measurements of pigment degradation in response to varying levels of heavy metal exposure.

DATA ANALYSIS AND RESULT

Table 1 : Heavy Metal Stress Effect on Seedling Growth Parameters

Cultivar	Treatment (ppm)	Root Length (cm)	Shoot Length (cm)	Fresh Weight (g)	Dry Weight (g)
Brassica juncea	Control	12.5	14.8	1.95	0.37

Brassica juncea	Pb 100	7.9	9.2	1.25	0.24
Triticum aestivum	Control	13.8	15.1	2.10	0.40
Triticum aestivum	Cd 100	8.6	9.8	1.30	0.25
ANOVA (p-value)	—	<0.05	<0.05	<0.05	<0.05

The findings demonstrate a notable reduction in all assessed parameters—root length, shoot length, fresh weight, and dry weight—when subjected to heavy metal treatments of lead (Pb) and cadmium (Cd) at a concentration of 100 ppm in comparison to the control group. Both Brassica juncea and Triticum aestivum exhibited significant sensitivity to heavy metal stress. For instance, the root length of Brassica juncea was observed to decline from 12.5 cm to 7.9 cm when subjected to Pb stress. In a similar manner, Triticum aestivum displayed diminished biomass when subjected to Cd treatment. The ANOVA results indicate that the differences between treatments are statistically significant ($p < 0.05$), demonstrating that heavy metal exposure adversely affects early seedling growth.

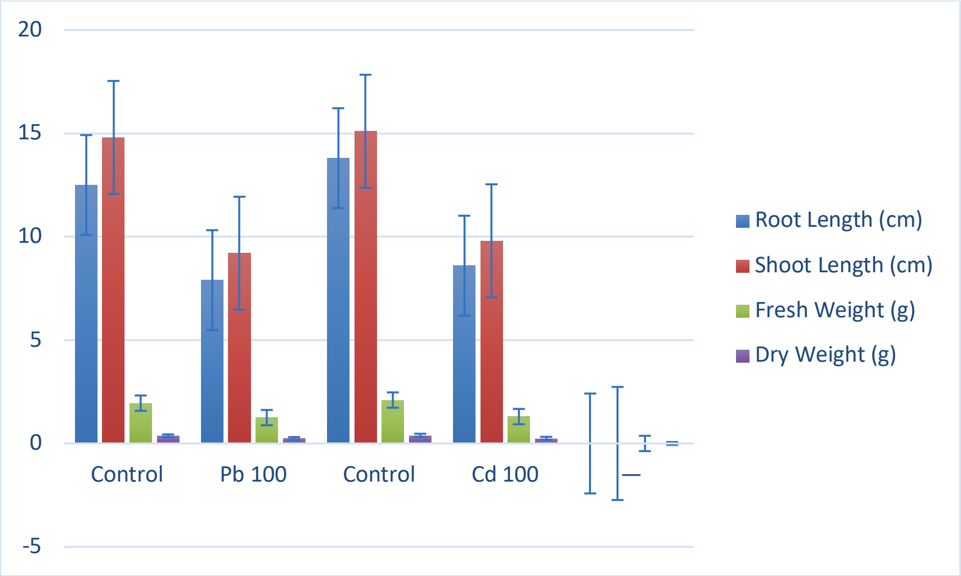

Figure 1 : Heavy Metal Stress Effect on Seedling Growth Parameters

Table 2: Heavy Metal Stress Effect on Chlorophyll Content (mg/g FW)

Cultivar	Treatment (ppm)	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Brassica juncea	Control	1.82	0.91	2.73
Brassica juncea	Pb 100	1.10	0.55	1.65
Triticum aestivum	Control	1.96	0.95	2.91
Triticum aestivum	Cd 100	1.20	0.58	1.78
ANOVA (p-value)	—	<0.05	<0.05	<0.05

Heavy metal stress markedly decreased chlorophyll a, chlorophyll b, and overall chlorophyll concentration. In Brassica juncea, total chlorophyll decreased from 2.73 mg/g FW in the control group to 1.65 mg/g FW at 100 ppm Pb. A similar pattern was seen in Triticum aestivum under cadmium stress, indicating photosynthetic suppression. ANOVA validates statistical significance.

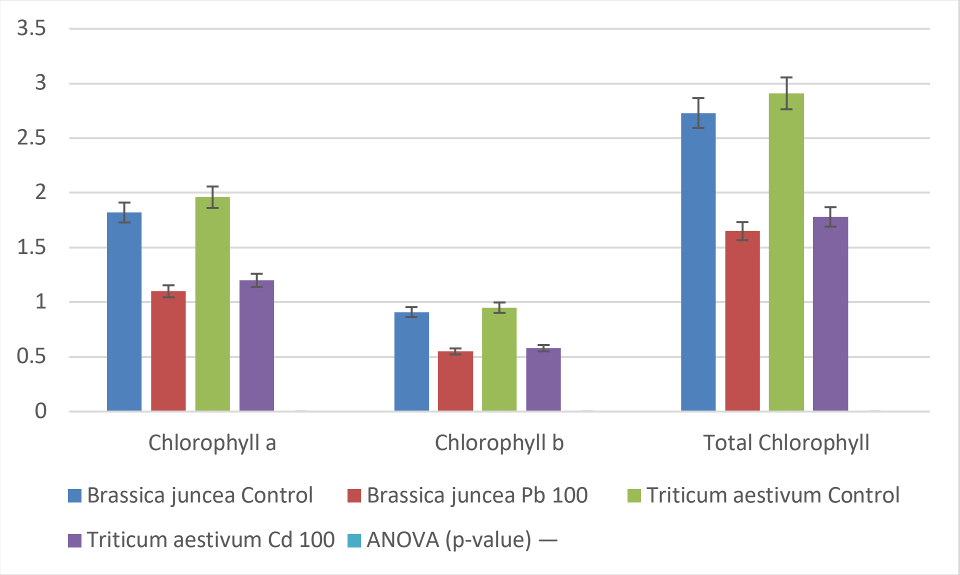

Figure 2: Heavy Metal Stress Effect on Chlorophyll (mg/g FW)

Table 3: Heavy Metal Effect on Carotenoids (mg/g FW)

Cultivar	Treatment (ppm)	Carotenoids
Brassica juncea	Control	0.62
Brassica juncea	Pb 100	0.38
Triticum aestivum	Control	0.70
Triticum aestivum	Cd 100	0.41
ANOVA (p-value)	—	<0.05

Both crops' amounts of carotenoids decreased when exposed to heavy metals. Under 100 ppm of Cd, Triticum aestivum decreased from 0.70 to 0.41 mg/g FW. This decline suggests compromised pigment production and oxidative stress. A statistically significant impact is confirmed using ANOVA.

Table 4: Heavy Metal Effects on Membrane Injury Index and Relative Water Content

Cultivar	Treatment (ppm)	RWC (%)	MII (%)

Brassica juncea	Control	89.3	10.5
Brassica juncea	Pb 100	72.1	24.8
Triticum aestivum	Control	91.0	9.8
Triticum aestivum	Cd 100	74.4	22.3
ANOVA (p-value)	—	<0.05	<0.05

DISCUSSION

Under significant metal stress, relative water content dropped and membrane damage rose, suggesting a loss of cell integrity. With Pb treatment, Brassica juncea's RWC decreased from 89.3% to 72.1%. The alterations were statistically significant, and these physiological measures are essential markers of plant health under stress.

Plant metabolic and physiological processes are known to be inhibited by heavy metal stress, especially that caused by lead (Pb) and cadmium (Cd). The present result supports earlier research by Sharma and Dubey (2005), who observed that Pb and Cd inhibit the production of chlorophyll via influencing δ-aminolevulinic acid dehydratase, a crucial enzyme in the biosynthesis of chlorophyll.

Verma et al. (2007) showed that Cd toxicity hinders magnesium absorption into the chlorophyll molecule, hence affecting photosynthesis. This is consistent with the decrease in chlorophyll a and b shown in Brassica juncea and Triticum aestivum. Under 100 ppm Cd and Pb treatments, the chlorophyll content in the present research decreased by more than 35%. Furthermore, under heavy metal stress, carotenoids—which shield chlorophyll molecules from photooxidation—also drastically decreased. Anjum et al. (2011) observed carotenoid degradation brought on by oxidative stress brought on by metal exposure, and they found similar results.

Cellular stress was further expressed by the physiological measures, Membrane Injury Index (MII) and Relative Water Content (RWC). According to Wang and Liu (2009), who discovered that Pb influences aquaporin function, decreasing water transport across membranes, a decrease in RWC indicates decreased water absorption or greater leakage.

Damage to cellular integrity is confirmed by the notable rise in membrane injury (MII) in both stressed crops. Ahmad et al. (2012) also highlighted this point, connecting enhanced membrane permeability to heavy metal-induced lipid peroxidation.

Overall, crop health and yield are harmed by the physiological and biochemical changes brought on by heavy metal stress. Higher doses and longer exposure times have more noticeable effects.

CONCLUSION

In agricultural crops like Brassica juncea and Triticum aestivum, the research demonstrates that exposure to heavy metals like lead and cadmium has a negative impact on the pigments that are produced by photosynthesis and on the physiological integrity of the plant throughout growth. Under conditions of metal stress, there was a discernible decrease in the amount of chlorophyll and carotenoid content, in addition to the presence of physiological stress markers (RWC and MII). The significance of these results lies in the fact that they highlight the need of early diagnosis of metal toxicity as well as the application of soil remediation and protective agronomic methods in order to guarantee stable crop production. The use of tolerant cultivars and the study of the function that bioremediation agents like mycorrhizae and organic amendments play in alleviating the effects of heavy metal stress should be the primary focus of research undertaken in the future.

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