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Comparative Study of Heavy Metal Toxicity on Chlorophyll and Carotenoid Levels in *Vigna radiata L.* Seedlings

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Abstract

Rapid industrialization and urbanization have resulted in the contamination of natural resources, particularly water bodies and agricultural soils. This pollution has severely disrupted ecological balance and agricultural productivity, posing a serious threat to global food security. Among the various pollutants, heavy metals such as cadmium (Cd), lead (Pb), and nickel (Ni) are of particular concern due to their high toxicity, persistence in the environment, and tendency to accumulate in plant tissues. This study examines the effects of these heavy metals on the photosynthetic pigments of *Vigna radiata* L. (mung bean) seedlings, a nutritionally important legume crop. Seedlings were exposed to different concentrations of Cd, Pb, and Ni, and their total chlorophyll and carotenoid contents were measured. The results showed that all three heavy metals significantly reduced pigment levels, with the degree of reduction increasing alongside concentration. At 1000 ppm, cadmium caused the greatest decline in both chlorophyll and carotenoid levels, followed by lead and then nickel, establishing a toxicity order of Cd > Pb > Ni. The substantial decrease in photosynthetic pigments indicates impaired photosynthetic activity, leading to reduced plant growth and productivity. These findings underscore the urgent need for effective monitoring and management of heavy metal pollution to safeguard agricultural systems and promote sustainable food production.

Keywords*:* Heavy Metal, Toxicity, Chlorophyll Content, Carotenoid Levels, *Vigna radiata L.*, Seedling Growth.

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Introduction

The expansion of modern industries such as mining, chemical manufacturing and metal processing has led to the accumulation of increasing amounts of heavy metals in the soil. In addition to direct deposition, atmospheric pollution can result in acid rain, which alters soil chemistry and facilitates the release of heavy metals into the soil (Foy, 1984, 1988). Once present in the soil, these heavy metals can be absorbed and concentrated in plant tissues. While certain heavy metals are essential micronutrients for plant growth, excessive concentrations can be toxic and detrimental to plant health. Metals are an integral part of the Earth's composition and are naturally present in almost all rocks and soils. However, human activities such as the burning of fossil fuels, mining, smelting, and the disposal of industrial, agricultural, and domestic waste have significantly increased the release of metals into the environment. Metallic elements are commonly categorized into light metals and heavy metals. The term "heavy metals" generally refers to metals that are potentially toxic and have a density greater than 4.0 grams per cubic centimeter (Krishnamurthy, 1985). These metals can also be characterized by their ability to precipitate in acidic solutions when treated with hydrogen sulfide. Although heavy metals play important biological roles—as components of metallo enzymes and essential micronutrients for all living organisms (Wood, 1975) they can become hazardous when their concentrations exceed the trace levels required for normal physiological and nutritional functions. *Vigna rediata* L. (Moong) is a significant arid legume crop that is widely grown in arid and semi-arid regions of the nation. It contains minerals like calcium and phosphorus as well as amino acids like leucine. After chickpeas (49.66%) and pigeonpeas (15.67%), mungbean accounts for 11.38% of India's total pulse production, making it the third most important pulse crop (Directorate of Economics and Statistics, 2021-22). With a brief growing cycle of two to three months, mung beans (*Vigna radiata* L.), a pulse crop, are widely grown and consumed in Asia, India, and the warmer regions of Europe and America. The crop is farmed in various parts of the country during the spring/summer, kharif, and rabi seasons It is the best insurance for marginal land in the event of drought-related crop failure because it is a very drought-tolerant crop that requires few inputs to grow. In the nation's hot desert region, it is a crucial food legume for long-term economic significance.

In addition to being a fantastic source of protein, complex carbohydrates, essential amino acids, vitamins, and minerals, mung beans are also known for being easily digested. Carbohydrates, which comprise fiber, sugars, and carbohydrates, make up about 55% of mung bean seeds. For young toddlers whose digestive systems are still developing, mung bean starch is an ideal food ingredient due to its exceptional 99.8% digestibility rate. With a 77% digestion rate and making up 20–25% of the seed, protein is the second most abundant nutrient in mung beans. Mung beans include a range of vitamins and minerals, as well as 22.2 grams of protein, 345 kcal of energy, 1.2 grams of fat, and 62.9 grams of carbohydrates per serving Mung beans provide more health advantages than other kinds of legumes, like having a significantly lower trypsin inhibitor level, which improves protein absorption. Additionally, arginine, isoleucine, valine, and lysine are among the essential amino acids that are abundant in mung beans. Because of these characteristics, mung beans are ideal as a foundation or additional component in foods meant to enhance their nutritional value, especially for those who are malnourished. One practical way to address nutritional issues in pregnant women and toddlers is to create supplementary feeding programs (PMT) with locally available products, such as mung beans. Communities are expected to develop long-term food independence and enhance family nutrition by encouraging the use of locally obtained foods for PMT.

"Life is a physico-chemical phenomenon." Photosynthesis, which transforms light energy into chemical energy, is one of the most crucial processes that sustain life. Plant cells' chloroplasts contain pigments that play an essential part in this process. The main pigment in charge of absorbing light energy during photosynthesis is chlorophyll, the green pigment present in plants. Both chloroplasts and chromoplasts contain carotenoids, a group of yellow to orange pigments that include yellow, orange, brown, and red chemicals, in addition to chlorophyll. By transferring absorbed light energy to chlorophyll a, carotenoids help encourage photosynthesis and retain chlorophyll from photooxidation. Effective photosynthetic activity depends on their precise spatial arrangement in relation to chlorophyll inside the chloroplast's lamellar system. One helpful measure of a plant's photosynthetic ability and general production is the amount of chlorophyll it contains. The rate of photosynthesis and chlorophyll content are closely related, and they can change in response to a number of environmental conditions, such as the presence of contaminants, heavy metals, and mineral fertilizers. substances in *Vigna radiata* L. seedlings, with an emphasis on determining limits of hazardous and critical concentrations. . Given this, the current study is to evaluate the effects of nickel (Ni), cadmium (Cd), and lead (Pb) on the amounts of carotenoid and chlorophyll in *Vigna radiata* L. seedlings, with an emphasis on determining toxic and critical concentration levels

Material and Method

Vigna radiata L. certified seeds have been obtained from Jaipur, Rajasthan's Durgapura Agriculture Research Station. Glass-stoppered bottles were used to store the seeds. The seeds were surface sterilized using a 0.1% $HgCl_2$ solution for two minutes after being initially chosen based on size and color homogeneity (Mishra, 1968). They were then rinsed three times with distilled water. Following sterilization, the seeds were immersed in solutions with different concentrations of nickel sulfate, lead sulfate, and cadmium sulfate (10, 50, 100, 200, 500, and 1000 ppm) for two hours. The control group consisted of seeds that were steeped in distilled water for two hours. Following treatment, the seedlings were moved to filter paper-lined petri dishes that had been steeped in the appropriate metallic solutions. Five replicates, each containing 10 seeds, were made for every heavy metal concentration.Throughout the experiment; the matching metallic solutions were used to periodically wet the filter sheets. Ten days of laboratory conditions were used for the studies, which included exposure to diffuse light and an average temperature of 25±2°C. The number of seeds that germinated was noted on the tenth day. The methods of Kirk and Allen (1965) and Arnon (1949) were used to determine the total amounts of carotenoid and chlorophyll, respectively.

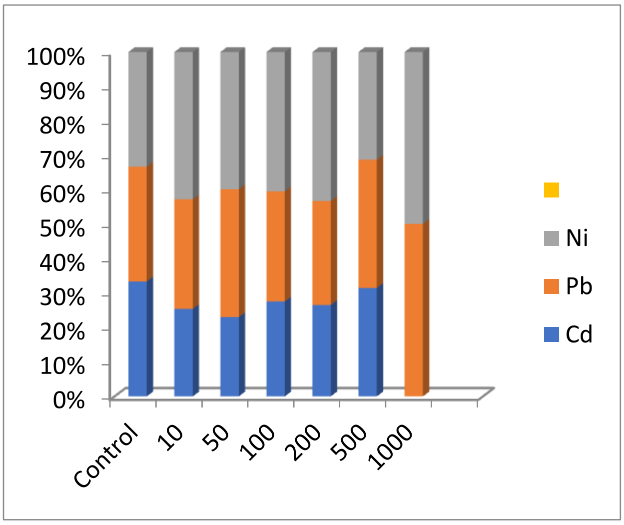
Results and Discussion

Tables 1 and 2 exhibit the data showing how heavy metals affect the overall amounts of carotenoid and chlorophyll.

Table1. Effect of heavy metals on chlorophyll contents (mg/g fresh weight, mean ±SD) in Vigna radiate L. Seedling

Heavy Metals		Concentrations (ppm)					
	Control	10	50	100	200	500	1000
Cd	2.65±0.012	2.71±0.21	2.432±0.016	2.542±0.0084	2.022±0.063	1.86±0.041	0.000±0
Pb	2.65±0.012	3.40±0.059	3.93±0.081	2.952±0.071	2.30±0.091	2.20±0.013	1.76±0.051
Ni	2.65±0.012	4.580±0.29	4.22±0.023	3.74±0.0073	3.306±0.0094	1.852±0.0015	1.758±0.0013

SE = Standard Error



Comparative Effect of heavy metals on chlorophyll contents in *Vigna radiate L*. Seedling

Table 2.Effect of heavy metals on carotenoid contents (mg/g fresh weight, mean ±SD) in *Vigna radiate L*. Seedling:

Heavy Metals		Concentrations (ppm)					
	Control	10	50	100	200	500	1000
Cd	0.517±0.0067	0.0.475±0.0015	0.323±0.0017	0.305±0.0015	0.255±0012	0.245±0.0010	0.000±0.000
Pb	0.517±0.0067	0.515±0.000748	0.565±0.001581	0.5846±0.000529	0.49875±0.000944	0.460±0.001781	0.4632±0.000427
Ni	0.517±0.0067	0.526±00.00015	0.545±0.0018	0.517±0.0017	0.454±0.0010	0.448±00.10	0.435±0.0014

(i) Heavy Metals' Impact on Total Chlorophyll:

The results indicate that lead (Pb) and nickel (Ni) were less inhibitory to total chlorophyll content compared to cadmium (Cd) (Table 1). An increase in chlorophyll content was observed at lower concentrations of Pb and Ni. However, in the case of Cd, a reduction in chlorophyll content was evident even after exposure to 10 ppm. At a concentration of 1000 ppm, Cd had a severe toxic effect, resulting in the death of the seedlings and the greatest reduction in chlorophyll content. For Pb and Ni, a noticeable decline in chlorophyll levels began at 200 ppm.

(ii)Effect of Heavy metals on Carotenoids:

At lower concentrations, heavy metals generally increased carotenoid content, except for cadmium (Cd), where it was lower than in the control (Table 2). However, carotenoid content significantly decreased at higher concentrations. At 1000 ppm, carotenoid content was 0.43 mg/g in nickel (Ni) and 0.46 mg/g in lead (Pb), while the control had a carotenoid content of 0.49 mg/g fresh weight.

An analysis of the pigment contents revealed significant changes in both chlorophyll and carotenoid concentrations in *Vigna radiata* L. seedlings treated with heavy metals (Tables 1 and 2). At lower concentrations, the levels of these pigments increased; however, they decreased at higher concentrations, with the exception of cadmium (Cd), which caused a drastic reduction even at lower concentrations (50 ppm and 100 ppm). Among all the heavy metals tested, Cd was found to be the most toxic to pigment content.

In angiosperms treated with heavy metals such as Cd, Cu, Hg, Pb, Mn, and Ni, Ning, Wang *et al*. (2024), Siedlecka *et al*. (2001), Divyashree and Lavaniya (2023), and Jain *et al*. (2011) noted a decrease in chlorophyll accumulation. According to Kupper *et al*. (1996), several heavy metals have been demonstrated to substitute magnesium (Mg) in the porphyrin ring of chlorophyll and bacteriochlorophyll. Gautam *et al*. (2008) and Shouric (2022) discovered that the addition of cadmium (Cd) dramatically reduced the net photosynthetic rate. Lower Pb concentrations, however, had little effect on the amount of carotenoid and chlorophyll in *Vigna radiata* L. The results of the present investigation are consistent with those of Prasad *et al*. (2004), who documented comparable alterations in photosynthetic pigments and electron transport activity in the liverwort Riccia spp.

In *Holcus L*., Symeonidis and Karataglis (2008) found a negative relationship between rising heavy metal concentrations and chlorophyll content. They also out that tolerant genotypes exposed to different Pb and Zn concentrations may be distinguished from non-tolerant genotypes by their greater chlorophyll levels as compared to controls. Likewise, Jain and Bhansali (2008a, 2008b) examined how heavy metals affected the pigment content of Cyamopsis tetragonoloba seedlings of cultivars RGC 936 and RGC 1002. Their research showed that, in comparison to zinc (Zn), copper (Cu), and nickel (Ni), cadmium (Cd) and lead (Pb) significantly decreased the total chlorophyll concentration at 1000 ppm. In a different study, Sagardoy *et al*. (2008) found that sugar beet plants subjected to zinc sulfate showed a significant decrease in carotenoid and chlorophyll concentrations, as well as an increase in the chlorophyll a/b ratio and improved de-epoxidation of violaxanthin cycle pigments. Reduced Photosystem II efficiency was also linked to higher zinc concentrations. In their study of the combined effects of zinc and cadmium on Ceratophyllum demersum's photosynthetic machinery, Aravind and Prasad (2004) showed that cadmium (Cd) not only significantly reduced the amount of carotenoid, chlorophyll-a, and chlorophyll-b, but also exhibited a marked toxicity to the entire photosynthetic system.

As Sinha *et al*. (2002) examined how manganese toxicity affected *Vigna radiata*'s pigment levels and photosynthetic rate, they found that the total carotenoid content significantly decreased at all manganese sulfate doses evaluated as compared to the control. Furthermore, when manganese concentrations increased, chloroplast activity and the photosynthetic rate as determined by CO_2 uptake progressively decreased. Similar to this, Kumar (1999) found that Catharanthus roseus's leaves senesced more quickly when exposed to high concentrations of heavy metals, with cadmium showing the most noticeable harmful effects. Cadmium was found to be the heavy metal that had the greatest negative impact on *Vigna radiata* L.'s pigment content. The heavy metals' relative toxicity was arranged as follows: Cd > Pb > Ni.

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