EFFECT OF HEAVY METAL STRESS IN *VIGNA RADIATA* L. (WILCZEK)

Project submitted

TO

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In partial fulfilment of the requirement in degree of

BACHELOR OF SCIENCE IN BOTANY

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CERTIFICATE

This is to certify that this project work entitled **“Effect of Heavy Metal Stress in *Vigna radiata* L. (Wilczek)”** is a bonafide piece of project work done by RIYA FATHIMA M.S **(**Reg.no**:** 210021022673**)** in the Department of Botany, Bharata Mata College, Thrikkakara under my guidance and supervision for the award of Degree of Bachelor of Science in Botany during the academic year 2021-2024. This work has not previously formed the basis for the award at any other similar title of any other university or board.

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DECLARATION

I hereby declare that this project entitled **"Effect of Heavy Metal Stress in *Vigna radiata* L. (Wilczek)"** is the result of work carried out by me under the guidance of Dr. Lins Simon, Department of Botany, Bharata Mata College, Thrikkakara. This work has not formed on the basis for the award at any other similar title of any other university of board.

RIYA FATHIMA M.S

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Chapter – 1

Introduction

Biosphere is severely contaminated by heavy metals and it has become a growing concern due to rapid urbanisation and industrialisation. Soil pollution can be very detrimental to the survival of all living beings, including plants, microbes and all the animals. Using sewage and wastewater that are not well treated have caused an intrusion of heavy metals like lead, arsenic, cadmium, and mercury in our agricultural lands and thus have been absorbed by the crops that tend to be eaten by humans themselves. Since heavy metals are non-degradable, they stay in the soil for a very long time.

Mung bean (*Vigna radiata* L.) is a Fabaceae plant that is one of the most important legume crops. It grows in tropical and sub-tropical regions around the world. It is an important pulse consumed all over the world, especially in Asian countries, and has a long history of usage as traditional medicine. It can be used as green manure and livestock feed. It has been known to be an excellent source of protein, dietary fiber, minerals, vitamins, and significant amounts of bioactive compounds, including polyphenols, polysaccharides, and peptides, therefore, becoming a popular functional food in promoting good health. Mung beans are also very cheap, reliable and easy to germinate, and offer a useful way to look at the germination process. In addition to being the prime source of human food and animal feed, it has short life cycle and plays an important role in in fixing atmospheric nitrogen and maintaining the soil fertility by enhancing the soil physical properties (Naik et al., 2020).

Lead is a chemical element. It has symbol Pb and atomic number 82. It is a heavy metal that is denser than most common materials. Lead is soft and also has a relatively low melting point. When freshly cut, lead is a shiny gray with a hint of blue. It tarnishes to a dull gray color when exposed to air. Lead is toxic, even in small amounts, especially to children. Lead is a devastating and persistent neurotoxin that accumulates in soft tissues and bones. It damages the nervous system and interferes with the function of biological enzymes, causing neurological disorders ranging from behavioural problems to brain damage, and also affects general health, cardiovascular, and renal systems.

Stress physiology in plants refers to the study of how plants respond, adapt, and cope with various environmental stresses. These stresses can include factors like extreme temperatures, drought, salinity, nutrient deficiencies, pollution, pathogens, and more. Stress in plants is managed through three primary strategies:

Stress Avoidance: Plants sidestep stress by adjusting their life cycles or growing in favorable spots within their habitat, avoiding the most challenging conditions. Adaptability: Plants change their structures, physiology, or genetics to better endure stress, adjusting root systems, metabolism, and other features to cope with environmental challenges. Tolerance: Some plants have inherent mechanisms to withstand stress, employing biochemical defenses, acclimation processes, or genetic adaptations to endure harsh conditions.

There are two main types of stress; Abiotic Stress and biotic Stress. Abiotic stress refers to environmental factors that negatively impact plant growth, development. Non-living elements like extreme temperatures, water availability, salinity, light, and atmospheric gases can significantly impact plant growth and productivity. Biotic stresses are adverse effects on plants caused by other living organism such as viruses, bacteria, fungi, parasites, insects, weeds, and competing plants. It is also caused due to the activity of man by cutting herbs and trees, twigs for fodder, fuels, and agricultural purposes.

The current concept of stress in plants has been well developed over the past 60 years. Any unfavorable condition or substance that affects or blocks a plant's metabolism, growth, or development is regarded as stress (Lichtenthaler, 1998). Vegetation stress can be induced by various natural and anthropogenic stress factors. Natural ecosystems are increasingly exposed to multiple anthropogenic stressors, including land-use change, deforestation, agricultural intensification, and urbanisation. Plants are bound to their habitat, they cannot run away many threatening environmental or anthropogenic stressors, and therefore need special mechanisms of stress avoidance and stress adaptation. One should not regard every little modification and change of a metabolic pathway, growth response, or development pattern of plants as a stress response.

The ecosystem is being ruined to the fact that the heavy metals are entering the food chain. These metals present in the soil absorbed by plants which can be hazardous both to the plant and also to the food chain that eats the plant, altering the properties of the soil thus impacting the quality of the soil, and also contaminating the water (Anil, 2023).

Industrial pollution is one of the major threats to the environment and human health. It affects the quality of air, water and soil. Among the sectors that generate the most pollution, industrial companies are responsible for a large share of emissions of greenhouse gases, particulate matter, heavy metals, toxic chemicals and waste. One of the areas that suffers the most from industrial pollution is agriculture. Agriculture depends on natural resources such as land, water and sunlight to produce food and other products. However, industrial pollution can degrade these resources and reduce agricultural productivity and profitability. Soil contamination, water pollution, air pollution, climate change are some of the impacts of industrial pollution on agriculture (Anil, 2023).

Due to enormous economic development and rapid growth in many fields, such as agriculture and industry, the environment is becoming more polluted. Certain environmental processes, such as synthetic industries, coal conversion, and waste burning, result in hazardous problems. Heavy metals are metals with a high atomic weight and density. Cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) cause an alarming combination of environmental and health problems (Alengebawy et al., 2021). Heavy metals arise from many sources, such as industry, mining, and agriculture. In terms of the sources in the agricultural sector, these can be categorized into fertilization, pesticides, livestock manure, and wastewater. Recently, the risk of heavy metals pollution in the environment has been increasing rapidly and creating turmoil, especially in the agricultural sector, by accumulating in the soil and in plant uptake. The heavy metals contamination problem has become urgent, and needs radical and practical solutions to reduce the hazards as much as possible. Even though heavy metals are needed for several organs of both plants and humans, they become toxic when their concentration exceeds the prescribed level. Many studies have been done in this area, reporting that the primary sources of heavy metals are agriculture, mining, agrochemicals, and industry. Agriculture and industry significantly influence heavy metals pollution in agricultural soil and plants. Plant roots are the essential point of contact for heavy metal ions transmitted from the soil (Alengebawy et al., 2021).

Due to the toxicant's longevity, penetration and accumulation in the food chain, it causes injurious health problems to the living organisms. Such contaminants can cause chronic diseases in the human body, such as lung cancer, renal dysfunction, osteoporosis, and cardiac failure. The accumulation of heavy metals in internal human tissues can affect the central nervous system, and act as a pseudo-co-factor or promotor of some health problems, such as seizures (epilepsy), headache, and coma. Heavy metal contamination is considered as a health threat to both adults and children. Pesticides are also hazardous to humans and other living organisms through contaminated food, water, or inhalation of contaminated air (Alengebawy et al., 2021).

This study is aimed to investigate the effect of lead stress on mung bean in several concentration of lead acetate along with a control solution. Distilled water is used as control treatment. In the present study, an attempt is made to access the effect of lead stress on chlorophyll content, membrane stability and protein test in *Vigna radiata*. The knowledge of physiological and biochemical basis of phytotoxicity thus gained helps in understanding the role of lead pollutants in the plant. This review also asses about the soil pollution caused by the heavy metals that adversely affects the agriculture field, and the associated risks of heavy metals in the soil of industrial and agricultural regions. And this study also explains how this lead metal can affect the growth of plants and thereby affecting the food which is taken up by living organisms.

1.2 Significance of the study

The study on the effects of heavy metal stress on *Vigna radiata* (mung bean) has significant implications for environmental science, agriculture, and public health. Heavy metals such as lead, cadmium, and mercury are notorious for their persistence in the environment and their ability to bioaccumulate, posing a threat to ecosystems and soil quality, especially in an industrial environment like Ernakulam. By examining the response of *Vigna radiata* to such pollutants, the research can deepen our understanding of plant resilience and the ecological impact of heavy metals, facilitating effective soil remediation and environmental management strategies.

In an agricultural context, *V. radiata* is a vital food crop, renowned for its nutritional benefits and versatility. Studying how heavy metals affect the growth and productivity of this plant species is crucial for safeguarding crop yields and food supply. The findings can help in devising agricultural practices that minimize crop exposure to these toxic substances, aid in breeding more resilient strains, and inform appropriate safety measures to ensure that heavy metals do not enter the food chain, mitigating health risks to consumers.

Moreover, the investigation may uncover the potential of *V. radiata* in phytoremediation efforts, an eco-friendly approach to cleansing contaminated soils. Understanding the plant's mechanisms of tolerance and accumulation could lead to innovative applications in environmental cleanup. Beyond its practical applications, the study also enriches fundamental scientific knowledge on plant biology and stress responses, potentially guiding future research and contributing to the development of environmental policies and safety regulations.

1.3 Objectives

- Collect and identify Vigna radiata from the local areas
- Develop a seed growing method for the treatment of the plantlets with heavy metal (Lead Nitrate) stress
- Estimate the level of Chlorophyll content under heavy metal (Lead Nitrate) stress
- Determination of Membrane damage caused by the heavy metal (Lead Nitrate) stress
- Quantification of Total protein at various concentrations of heavy metal (Lead Nitrate)

Chapter – 2

REVIEW OF LITERATURE

Modern concept of stress alleviation in plants were explained by Lichtenthaler (1998) and it explains the stress as unfavourable and environmental constraints in plants due to several factors. In the past 10 years an enormous increase has been occurred in the investigations and experiments conducted globally in concern with the stress tolerance mechanisms in plants and are recorded as numerous articles in the field of botany, plant physiology, ecophysiology, and plant biochemistry dealing with plant stress and plant stress detection. This process is yet continuing and may proceed in the future at an even more enhanced rate.

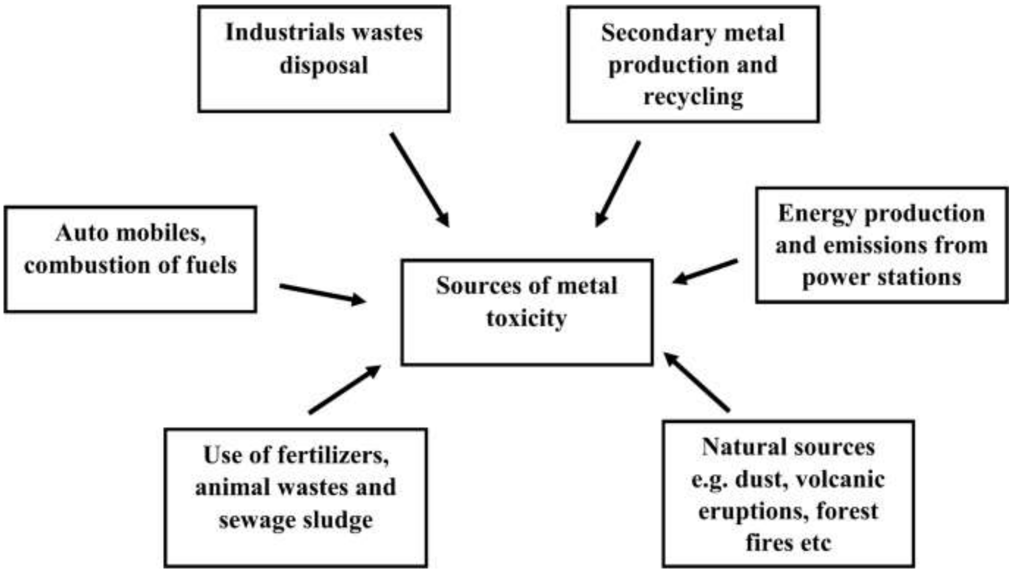

Fig. 2.1: Various sources of metal toxicity in soil

Angoorbala (2013) studied the effects of lead stress on different varieties (Virat, SML -668, A1 Gold and K-851) of mung bean (*Vigna radiata*) and observed that it occupies a very important position in Indian agriculture. Seeds of four different mung bean varieties were grown under laboratory conditions in dark at 0- 1500 µM concentrations of lead nitrate and the evaluation of morpho-physiological characters were done using standard markers.

Ahmed (2021) focused on the toxic effects of heavy metals like Cadmium (Cd), Lead (Pb), Copper (Cu) and Zinc (Zn), adversely influencing the agricultural ecosystem and human health. The information from the study highlighted various mechanisms plants undergo to mitigate the stress conditions and alterations. It also provides a deep insight into the understanding of environmental toxicants and their hazardous effects.

Varghese (2023) reviewed the impact of industrial pollution on agricultural fields and discussed the crop productivity. It also explains about the industrial pollution as one of the major threats to environment and human health and agriculture is tamed to be a major area that suffers the most from industrial pollution. Since it depends on various natural resources such as land, water and sunlight to produce food and other products, industrial pollution can degrade these resources and reduce agricultural productivity and profitability.

Hou et al, (2019) considered mung bean as an important pulse consumed all over the world and as a rich source of certain bioactive polyphenols, polysaccharides, peptides, and various health benefits. They gets into the blueprint for the better utilization of mung bean in food products to improve human nutrition and further encourage advancement in this field. In an experiment conducted by Muhammad Sajid and Aqeel Ahmad (2008), 30 days old plants of two mung bean were exposed to both lead and copper stress separately along with control variant. Application of copper and lead in both Mung bean cultivars caused a significant reduction in the CO_2 exchange and photosynthetic pigments. Higher concentration of lead particularly caused a significant inhibition of photosynthetic and transpiration rates and stomatal conductance compared to the same doses of copper.

Singh et al, (2003) investigated on the effect of lead on growth and nitrate assimilation of *Vigna radiata* (L.) seedlings in a salt affected environment and found out that inhibition of seedling growth and nitrate reductase activity in 5 days old *Vigna radiata* (L.) Wilczek cv. Pusa Baisakhi in the presence of lead acetate increased drastically, if NaCl was also present in the nutrient media along with the metal salt. The effect of two types of stresses, seriously tampered the plant, more drastically in the additive or even synergistic manner during the early growth phase of the seedlings.

Himani Singh (2017) demonstrated the effect of oxidative stress induced by lead in *Vigna radiata* L. seedling attenuated by exogenous nitric oxide and found out the effectiveness of nitric oxide (NO) on some biophysical and biochemical variables in *V. radiata* subjected to lead (Pb) toxicity. Pb adversely affected seedling growth and biochemical parameters of the test crop and the toxicity caused a significant decrease in growth. The effect of Pb on seedlings was mitigated by NO donor sodium nitroprusside (SNP) and Pb induced oxidative stress by enhancing the reactive oxygen species. SNP demonstrated a positive role against Pb toxicity which was evident from decreased activities of antioxidant enzymes. The SNP treatment enhanced plant tolerance against Pb toxicity.

Mitigation of lead toxicity in *Vigna radiata* genotypes by silver nanoparticles were studied and examined the application of silver nanoparticles (AgNPs) in two genotypes of Mung bean (*Vigna radiata)* for ameliorating the Pb toxicity (Fu Chen, 2022). The application of AgNPs substantially enhanced plant growth and helped them in surviving well in absence as well as presence of Pb. The results demonstrate that AgNPs mediate response(s) of plants under Pb stress and particularly contributed to heavy metal tolerance of plants and thus showing great promise for use in phytoremediation.

Yasin Ashraf (2016) conducted an experiment with two mung bean cultivars, having three treatments, cadmium, lead, and cadmium + lead (Cd, Pb, and Cd + Pb) to growth medium leads to a drop in seed germination, length, fresh and dry biomass of shoots and roots, total chlorophyll, chlorophyll a, chlorophyll b, and soluble proteins in plants. Application of these metals in the growth medium evidently reduced the bio-absorption of Calcium (Ca), Magnesium (Mg), and Potassium (K). Overall, the metal-induced physio-biochemical changes resulted in reduced growth of both mung bean cultivars.

When the effect of Mo and Co on germination percentage, early seedling growth, leaf area, root development and pigment composition (chlorophyll and carotenoids) of mung bean were analysed, Reshma (2014) observed that Mo and Co treatment at 10, 50 and 100 μM L^{-1} affected germination percentage, early seedling growth, leaf area, root development and pigment composition (chlorophyll and carotenoids) of mung as compared to control.

Investigation on biochemical Changes under Chromium Stress on germinating seedlings of *V. radiata* were performed and observed that there is a gradual decrease in shoot and root length with respect to the increase in Cr concentration and the level of lipid peroxidation significantly increased along with the proline and antioxidant enzyme activity at higher Cr concentrations. Lipid peroxidation could be considered as an indication of membrane damage due to elevated production of reactive oxygen species (ROS), however, to combat oxidative damage by ROS antioxidant enzyme activity increased significantly, which indicates that antioxidant enzymes (SOD, CAT, APX and GR) play a crucial role during Cr stress during germination of *V. radiata* (Bhavin Suthar, 2014).

Saba Nazir (2021) studied the impact of industrial effluents and simulated acid rain on growth, productivity and metal contamination in mung bean and observed that environmental pollution poses a great health hazard to human beings, animals and plants. Industrial effluents and acid rain are the major pollutants that have a strong influence on the growth of crop plants. A pot trial was also conducted to examine the influence of industrial effluents and simulated acid rain (SAR) on the growth, productivity, biochemical and quality attributes of mung bean crop. They conclude that, simulated acid rain and pure form of industrial effluents have toxic effect on the growth, yield and quality of mung bean. The adequate dilution of effluents treatment is therefore needed before the disposal and reuse of wastewaters for irrigation purposes.

Importance of mung bean as an agricultural crop by reviewing relevant literature on the potential contribution of mung bean to food security and a balanced diet as well as the effect of mung bean cultivation on farm income and gender equality was observed as a result of a study conducted by Lisa Pataczek (2018) while measuring the role of mung beans in the altering environments. They also explained how the mung beans are gaining attention as a short-season crop that can tolerate dryland conditions, and fix atmospheric nitrogen, decreasing soil nutrient depletion.

Dongyan Tang (2014) reviewed the phytochemistry, metabolite changes, and medicinal uses of the common food in Mung Bean and its sprouts, and provided an insight into the nutritional value of mung beans and its sprouts, discussing chemical constituents that have been isolated in the past few decades, such as flavonoids, phenolic acids, organic acids, amino acids, carbohydrates, and lipids. Moreover, they also summarize dynamic changes in metabolites during the sprouting process and related biological activities, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antihypertensive, lipid metabolism accommodation, antihypertensive, and antitumor effects, etc., with the goal of providing scientific evidence for better application of this commonly used food as a medicine.

Abbas (2015) investigated the effects of industrial pollution on crop productivity and explained that the industrial effluents are a major health concern for all living matter on Earth. The components of these effluents are adversely affecting the environment, causing an imbalance in nature and as a result, in the natural processes going on in the ecosystems. As the title indicates, in this chapter they concerned with the types of substances industrial wastes can contain, their uptake by the plant influencing the uptake, transfer, and movement of other nutrients, and the effect they cause on the growth and biomass of crop plants.

Ranawake (2011) assessed the effect of water stress on growth and yield of Mung Bean and explained about the major abiotic stress, drought on Mung bean in the sub-humid, dry and intermediate zones of Sri Lanka. It revealed the response of Mung bean for the water stress at three different growth stages; 3 weeks after planting, 6 weeks after planting and 8 weeks after planting. Plant height, number of leaves, number of floral buds, dry matter weight of shoot system, number of lateral roots, length of tap root, number of root nodules, and dry matter weight of root system were also measured after one week of recovery period in water stressed plants at three different growth stages and along with control plants. Water stress significantly affected the measured parameters at 6 WAP. Only number of leaves was significantly affected when the drought applied at 8 WAP.

An investigation on the role of mung bean rhizosphere associated *Pseudomonas aeruginosa* GGRJ21 strain on drought stress alleviation in the host plant was carried out by Rupak K. Sarma (2013), apart from the conventional plant breeding and transgenic approaches, the application of plant growth-promoting rhizobacteria (PGPR) has under water deficit conditions has always been a promising approach to improve abiotic stress tolerance in crop plants. Fluorescent pseudomonads were isolated from mung bean rhizosphere by employing a culture-dependent approach. The field experimental data show an increase in biomass and better growth and development in inoculated and stressed plants when compared with untreated and stressed plants.

Study on the influence of drought stress on some traits in five mung bean genotypes including three cultivars, Taiwan (G1), Pakistan (G2) and India (G3) and two landraces Marvdasht (G4) and Arsenjan (G5) with diverse characters and origin was conducted in Iran. Results indicated that, all the studied traits except root length was influenced by drought stress conditions and based on the results of simple and combined ANOVA of studied traits, India cultivar was found to be with the highest grain yield under drought stress and non-stress conditions and Pakistan and Taiwan cultivars had the lowest grain yield in stress and non-stress conditions. They also suggested a strategy to reduce the effect of water stress on crop yield is only to use drought tolerant species and cultivars (Zare et al, 2012).

Sehrawat Nirmala (2015) noted the effect of salinity stress on mung bean during consecutive summer and spring seasons and the experiment was carried out at two different levels of salinity and observed significant variations and adaptability among stressed and non-stressed plants in both the varieties. The plant in early vegetative stage were found more resistant to salinity as compared to plants in late vegetative and reproductive stage. However, it underlined the need of screening of large mung bean germplasm for salt tolerance during spring season.

In a study conducted to evaluate the effects of high temperatures (>40/25 °C; day/night) during reproductive growth on performance of mung bean and to probe the mechanisms associated with reproductive failures, Ramanpreet Kaur (2015) recorded that a rise in temperatures as a result of climate change will have adverse effects on various crops. Since, Mung bean is grown as a summer-season crop in many parts of the world, the optimum temperature for growth of mung bean is 28–30 °C and an increase in temperatures above these limits, are likely to inhibit its potential performance.

Pooja Bangar (2019) in her study to assess the morphophysiological and biochemical response of mung bean at different developmental stages under drought stress, found out that, the vegetative stage was more sensitive to drought stress, which was further supported by correlation analysis. For the study, they performed a field experiment according to a completely randomized design on 25 mung bean varieties with 3 replicates per variety. Stress treatment was applied at 3 levels; control (no stress), vegetative stage (25 days after sowing), and reproductive stage (35 days after sowing).

Biochemical responses of Cr-tolerant and Cr-sensitive mung bean cultivars grown on varying levels of chromium was carried out by Samantary (2002), and observed at different concentrations of hexavalent chromium (as $K_2Cr_2O_7$) in hydroponics culture, seeds were germinated and grown in the presence or absence of chromium under controlled environmental conditions. Protein, pigment and enzyme analysis were conducted in both Cr-tolerant and Cr-sensitive cultivars of mung bean after 72 h of treatments. Chlorophyll and protein contents were reduced in Cr-sensitive cultivars more than those of the tolerant ones. The enzyme activity varied among the Cr-tolerant and Cr-sensitive ones. Activities of catalase, peroxidase, glucose-6-phosphate dehydrogenase and superoxide dismutase were greater in Cr-sensitive than tolerant cultivars.

Amit Kumar Mishra (2014) conducted a work on the biochemical and physiological characteristics of tropical mung bean cultivars against chronic ozone stress. The study thoroughly analysed the differences on the foliar injury, reactive oxygen species (ROS) generation, antioxidative defense system, physiology of two tropical mung bean cultivars exposed to elevated O_3 under near-natural conditions. Both cultivars were negatively affected by the pollutant, but the response was cultivar-specific. Photosynthetic pigments, photosynthetic rate, stomatal conductance, and photochemical efficiency reduced under elevated O_3 exposure and the extent of reduction was higher in one variety. Altogether, the results suggested that, the higher accumulation rate of ROS and limited induction of antioxidant defense system paved the way to more leaf injury and impairment of photosynthesis in HUM-2 than HUM-6 depicting its higher sensitivity towards elevated O_3.

Avnish Chauhan (2010) conducted a study on the effect of ambient air pollutants on Wheat and Mustard crops growing in the vicinity of urban and industrial areas and appraised the effect of different air pollutants like SO2, NOx, SPM and RSPM on bio physiological as well as yield characteristics of wheat and mustard plants grown at different sites in the urban and industrial areas. The wheat and mustard plants grown at polluted sites showed significant reduction in total chlorophyll, carotenoid, ascorbic acid, plant height, shoot fresh weight, root fresh weight and yield. The study indicated that parameters reductions in wheat and mustard grown at polluted sited correlated directly with the gaseous and particulate pollutants, which led to lower yield at polluted sites experiencing higher levels of pollutants.

When the effects of heavy metal pollution in soil and plant in the industrial area was investigated by Tahar (2011), the degree of contamination which soil and plants are burdened with some heavy metals was determined. Pb, Zn, Ni, Cu, Cd, Mn, Cr, Fe and As, then the accumulation of heavy metals in the soil and plant adjacent of area. This paper analyses the heavy metal contents within a 2-years period in the soil and plants at the beginning of the vegetation period. The presence of Pb, Zn, Ni, Cu, Cd, Mn, Cr, Fe and As, in the samples were analysed using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). Consequently, they revealed a health risk for human and livestock due to the spread of the metal pollution from waste dumpsites to agricultural areas.

Chapter- 3

Materials and Methods

To investigate the heavy metal tolerance potential of *Vigna radiata*, the following procedures were adopted.

3.1 Plant Material

Seedlings of Vigna radiata was selected for the study and the seeds were obtained from the local market and proper identification was done and stored for the study.

3.2 Experimental design

The experiments were set up in a completely randomized design in 7 containers filled with equal volume of water containing lead nitrate [$Pb(NO_3)_2$] concentrations of 0, 300, 600, 900, 1200, 1500 mM.

The seeds were germinated in seed beds. Twenty-one-day old seedlings were transferred to a container containing of equal amount of water. The lead nitrate treatment was started on the 21st day of seed germination by incrementing 300 mM per day to attain a final concentration of 1500 mM. One set of plantlets were kept as control without lead stress application and watered with tap water. Leaf and entire plant samples for the analysis were collected after 7 days of stress application and preserved for further analysis.

3.3 Quantification of total protein

The leaf samples were homogenised in 1.5 ml 100mM phosphate buffer (pH 7.0) containing 1% (w/v) fresh PVP using a mortar and pestle. The samples were centrifuged at 10000 rpm for 15 min in refrigerated cooling centrifuge at 4^{o}C. The supernatant was collected and kept on ice and used for further assays.

Total protein content from plant tissues treated with different $Pb(NO_3)_2$ concentrations and control were determined using Lowry *et al.,* (1951) protocol.

- 50 µL of the protein extract of each sample was taken in a test tube and the volume was made upto 1mL with double distilled water.
- 5 mL of solution C was added to the mix and incubated at room temperature for 10 min.
- 0.5 mL of solution D was added and incubated in the dark for 30 min.

- The absorbance was measured using UV visible spectrophotometer (Shimadzu, Japan) at 660 nm.
- A standard curve was prepared using gradient concentrations of BSA (1 mg/mL) and the total protein content was measured.

Table 3.1: Reagents used for Lowry's method of protein measurement

Reagents	Preparation
Solution A	2.0 g of Na_2CO_3 dissolved in 0.1 N NaOH solution and final volume made up to 100 mL
Solution B	0.1 g Sodium potassium tartrate and 0.005 g $CuSO_4.5H_2O$ dissolved separately in distilled water. Both solutions were mixed together and final volume adjusted to 10 mL.
Solution C	100 mL solution A and 2 mL solution B were mixed well to obtain C.
Solution D (Folin's reagent)	Folin-Ciocalteau Phenol prepared by diluting with distilled with distilled water in the ratio 1:1 and kept at 4 ºC until use.

3.4 Quantification of Total chlorophyll content

Total chlorophyll content from the heavy metal (lead) stressed rice leaves was measured according to the Arnon (1949). The leaf (0.05g) from each treatment was homogenised in a pre-cooled mortar and pestle containing 10ml of 80% (v/v) pre-chilled acetone. The homogenate was centrifuged at 5000 rpm for 10 min at 4ºC in a refrigerated cooling centrifuge and the supernatant was collected. The procedure was repeated until the pellet became white in colour. The final volume was adjusted to 20 ml with 80% (v/v) acetone. The absorbance was read at 645 and 663nm in UV-Visible Spectrophotometer against the blank (80% v/v acetone). The quantity of total chlorophyll, chlorophyll *a* and chlorophyll *b* were calculated using the equations:

$$\text{Chl } a = 0.0127\ A_{663} - 0.00269\ A_{645}$$

$$\text{Chl } b = 0.0229\ A_{645} - 0.00468\ A_{663}$$

$$\text{Total chl} = 0.0229\ A_{645} + 0.00802\ A_{663}$$

Where, A_{663} = Absorbance of sample at 663 nm

A_{645} = Absorbance of sample at 645 nm

3.5 Membrane stability index (MSI)

Leaf membrane stability index (MSI) was calculated according to the protocol explained by Sairam (1994).

- Leaves were thoroughly washed in running tap water followed by washing with double distilled water. The leaves were heated in 10 mL double distilled water at 40˚C for 30 min. The electrical conductivity (C_1) was recorded using an electrical conductivity meter. Subsequently, the same samples were placed in a boiling water bath (100˚C) for 15 min and their electrical conductivity (C_2) was also recorded.
- MSI was calculated using the formula;

Membrane Stability Index (MSI) = $[1 - (C_1/C_2)] * 100$

Chapter – 4
Results & Discussion

4.1 Quantification of Chlorophyll content

The influence of $Pb(NO_3)_2$ on chlorophyll content in *Vigna radiata* was greatly pronounced when compared to the control plants. The total chlorophyll content of *V. radiata* used as the control (0 mM) was higher on the 7th day. In increasing concentrations of Lead Nitrate, the total chlorophyll content showed a decreasing pattern, indicating stress accumulation and the decreasing photosynthetic efficiency of the plant under heavy metal stress. The Chl. a was higher than Chl. b in the control treated varieties and in various concentrations (300, 600, 900, 1200 and 1500 µM) of lead stress. The increasing concentration of lead nitrate resulted in a steady decrease in the levels of Chl. b and Chl. a. At the highest concentration of stress (1500 µM), the total chlorophyll content was recorded to be 0.42 mg/g, which is a much lesser than that of chlorophyll levels of the plants treated with 300 µM levels of stress. The drastic drop in total chlorophyll content after the stress indicates the higher concentrations of lead nitrate increases the toxicity levels, which affects the chlorophyll content which is dependent on the exposure period.

Table 4.1: Chlorophyll content in *V. radiata* treated with different concentrations of $Pb(NO_3)_2$ ± SE, n=3

Concentration of $Pb(NO3)_2$	**Chl. A ± SE**	**Chl. B ± SE**	**Total Chlorophyll ± SE**
Control (0 mM)	0.80 ± 0.004	0.36 ± 0.003	1.16 ± 0.002
300 mM	0.71 ± 0.003	0.28 ± 0.004	0.99 ± 0.008
600 mM	0.66 ± 0.003	0.24 ± 0.006	0.90 ± 0.006
900 mM	0.58 ± 0.002	0.19 ± 0.003	0.77 ± 0.010
1200 mM	0.44 ± 0.003	0.15 ± 0.002	0.59 ± 0.005
1500 mM	0.31 ± 0.005	0.11 ± 0.003	0.42 ± 0.004

4.2 Membrane stability index

The membrane stability index of *V. radiata* showed a downregulation proportionate to the levels of lead nitrate. In the non-stressed condition, *V. radiata* showed a MSI of 60.45 and it started a declining trend along with the application of lead nitrate, reaching upto 33.96 in 1500 mM concentration of lead nitrate. There was a sudden decline in the level of membrane stability index soon after the application of stress and the decrease was very steady corresponding to the increasing levels of lead nitrate. In the stressed condition, the highest MSI was observed in 300 mM concentration, however the lowest was recorded at the highest concentration of stress (1500 mM). The results implicated that metal stress has a greater impact on membrane stability of the plant under heavy metal stress. The decline in the membrane stability of the crop indicates the increased membrane permeability and damage, as lead nitrate interacts with membrane lipids and proteins, disrupting their structure and function. It also suggests that lead nitrate exerts a considerable destabilizing effect on cell membranes, with higher concentrations correlating with greater impairment of membrane stability.

Table 4.2: Membrane stability index in *V. radiata* treated with different concentrations of $Pb(NO_3)_2$ ± SE, n=3

Concentration of $Pb(NO_3)_2$	**MSI ± SE**
Control (0mM)	60.45 ± 0.54
300 mM	53.34 ± 0.72
600 mM	48.93 ± 0.64
900 mM	44.35 ± 0.41
1200 mM	38.28 ± 0.38
1500 mM	33.96 ± 0.63

4.3 Determination of Protein content

Quantitative assessment of protein content in the presence of increasing concentrations of lead nitrate indicate a clear trend of altered response in *V. radiata* correlating with the levels of lead stress. At low concentrations of lead nitrate (300 mM, 600 mM) there was a negligible impact on the protein quantitation. However, as the lead concentration increased, there was a noticeable decrease in the protein content, suggesting that lead nitrate stress induced protein dysregulation. When exposed to moderate levels of lead nitrate, a more significant decrease in protein content was observed. This indicates that lead nitrate -induced proteotoxic stress causes alterations in protein degradation rates. In the highest concentration of lead nitrate applied, *V. radiata* showed a pronounced reduction in total protein content (0.164 mg/g), whereas in the non-stressed condition it was 0.392 mg/g. this underscores the toxic impact of heavy metal stress on cellular protein machinery, potentially leading to disrupted protein synthesis and enhanced protein degradation processes. The recorded observations suggest a dose dependant relationship between lead nitrate stress and protein content. As metal stress increased, it highlights the negative impact of heavy metal contamination of cellular protein dynamics.

Table 4.3: Total protein content in *V. radiata* treated with different concentrations of $Pb(NO_3)_2$ ± SE, n=3

Concentration of $Pb(NO_3)_2$	**Total Protein ± SE**
Control (0 mM)	0.392 ± 0.008
300 mM	0.344 ± 0.009
600 mM	0.273 ± 0.004
900 mM	0.200 ± 0.010
1200 mM	0.176 ± 0.005
1500 mM	0.164 ± 0.006

4.4. Discussion

The investigation on the impact of heavy metal stress on the physiological and biochemical processes of *V. radiata* has revealed significant alterations in protein levels, chlorophyll content and membrane stability, indicative of the plants' stress responses.

The observations of the present study correlates well with established patterns seen in other leguminous and non-leguminous plants. Plant affected with heavy metal stress demonstrate responses; causing ionic imbalance and inducing oxidative stress (Hasegawa et al, 2000). In response to heavy metal stress, several toxic intermediates are produced in plants depending on varietal plasticity and stress intensity, whereas; metal induced H_2O_2 accumulation is non-localized and it sternly affects the overall plant growth and productivity (Jiang, 2002). The acclimation of plants to stress conditions is often associated with upregulation of reactive oxygen species (ROS) such as superoxide (O_2^-), hydroxyl radical (HO) and hydrogen peroxide (H_2O_2), that are toxic to cells, produced during the aerobic metabolism. The generation of ROS production upsurges during stress because of the disruption of electron transport system and also due to the metabolic activities that occurs in chloroplasts, mitochondria and various cell organelles (Ahmad *et al.,* 2008). The non-enzymatic and enzymatic antioxidants also play a vital role by interacting with each other in order to scavenge the reactive oxygen species mediated by the over expression of different genes and thus helping the tolerant plants to acclimatise to the adverse environmental conditions (Orabi *et al.,* 2015; Sofo *et al.,* 2015).

The examined crop plant in the present study demonstrated a decreasing trend in the chlorophyll content corresponding to the increasing metal stress, which can be attributed to the decrease in the photosynthetic efficiency of the plant. Chandra and Kang (2016) in their study the physiological effects of mixed heavy metals, Cadmium (Cd), Copper (Cu), Chromium (Cr) and Zinc (Zn) in four popular hybrids, they also observed a decrease in chlorophyll content in three of the hybrids, which thereby showed a significant decline in the photosynthetic efficiency of the plant. When the assessment of heavy metal stress was done in soybean (Glycine max) and mung bean (Vigan radiate) plants, Mao et al, (2018) observed that there was a significant alteration in the morphology and physiological characteristics of the plant. The biochemical and physiological characters were examined using different parameters and the results was in accordance to the results obtained in the present study. With the rise of heavy metals, they observed a decrease in chlorophyll content and leaves were noted with chlorosis symptoms at high metal concentrations. Furthermore, the effect of heavy metals in V. radiata was evidently noted by the reduction in chlorophyll content and efficiency of PSI and PSII in grapevines treated with various levels of heavy metals (Cramer, 2010) also aligns with the current study. This implies that heavy metals may disrupt the chlorophyll synthesis or accelerate the pigment degradation, impairs the photosynthesis, hinders the metabolism and lower the energy production as documented by Fu and Xi (2020). Hence the degradation of chlorophyll can be a direct effect of heavy metal interference with the chlorophyll synthesis pathway or an indirect result of oxidative damage to the structure.

The alteration in the membrane stability index in the current study suggests that heavy metal stress leads to lipid peroxidation and membrane damage, reinforcing the findings of Srivastava et al., (2017). The integrity of cellular membranes is crucial for cell survival, and its disruption under metal stress which signifies a critical threshold in the plant's tolerance capacity. They observed a significant alteration in the membrane integrity when the concentration of metal was increased in the plant. Singh et al, (2017) evaluated the effectiveness of NO on some biophysical and biochemical variables in *V. radiata* subjected to lead (Pb) toxicity. They concluded that Pb toxicity marked a significant increase in the rate of lipid peroxidation, thereby severely affecting the integrity of the membrane. Similar results were also obtained in various studies in Vigna varieties, which were subjected to Chromium (Cr), Copper (Cu) and Lead (Ld) stress and observed that the rate of lipid peroxidation significantly increased, contributing to the poor membrane integrity (Atta et al., 2023; Rath and Das, 2021).

When the germination, seedling growth, biomass production, antioxidant enzymes, electrolytic leakage, oxidative stress and protein content were evaluated in V. radiata, Singh et al, (2021) observed that few plants were very sensitive to the stress and both the cultivars examined exhibited a negative corelation in morpho-physiological characteristics under high concentrations of Cr and suggested that plants subjected to high concentrations of stress were noted with a sudden decline in protein content and it negatively affected the crop productivity. Ashraf et al, (2016) added Cadmium (Cd) and Lead (Pb) in various compositions to the growth medium of two mung bean varieties and observed a similar result. The overall metal-induced physio biochemical characters showed a negative effect corresponding to the intensity of the stress and the alteration in the protein content was very significant hindering into the reduction in the plant growth and productivity. Production of photosynthetic pigments and seed germination was found to be seriously affected in V. mungo variety subjected to various concentrations of heavy metal stress (Rath et al, 2021). Highest intensity of metal stress led to the production of more ROS and causing more adverse effects in the crop. Results regarding the protein content in the present study is also in agreement to the previous works in the evaluation of heavy metal stress in Vigna plants.

Varying abiotic environmental conditions result in causing environmental pressure to morpho-physiological functions of a plant. When plants are affected with high concentrations of heavy metals, tolerant varieties may mitigate the stress by upregulating the antioxidant system and effectively scavenging out the stress, while the sensitive varieties undergo with tampering in the morphological as well as physiological conditions. Studies suggest that desks to be cleared for engineering heavy metal stress tolerance in plants for developing improved heavy metal tolerance crop plants and challenging the heavy-metal induced threats to sustainable agricultural system and for qualitative and quantitative improvements in economic yield of crop plants (Dey and Mondal, 2016).

Chapter – 5

Summary and Conclusion

This study investigated the impact of heavy metal stress on various physiological parameters in Vigna radiata (mung bean). The experiment assessed the chlorophyll content, membrane stability index (MSI), and protein quantification in plants exposed to different concentrations of heavy metals.

Our findings revealed significant alterations in these parameters compared to the control group, indicating cellular damage and physiological stress in response to heavy metal exposure. The degree of these changes varied depending on the specific heavy metal and its concentration.

Chlorophyll content, a crucial indicator of photosynthetic activity, exhibited a noticeable decline in heavy metal-stressed plants. This suggests a potential disruption in the photosynthetic machinery, leading to reduced energy production and hampered growth. The extent of this decline likely correlated with the severity of the heavy metal stress.

The membrane stability index (MSI) serves as an indicator of cell membrane integrity. Our results demonstrated a decrease in MSI in heavy metal-treated plants, suggesting damage to the cell membrane structure. This damage can lead to impaired cellular function and electrolyte leakage, ultimately compromising plant health. Further investigation into the specific mechanisms by which lead nitrate affects the membrane stability index is warranted. Studies have suggested that lead nitrate may induce oxidative stress and lipid peroxidation, which could contribute to membrane damage. Additionally, the inhibition of antioxidant enzymes and alteration of membrane lipid composition may also play a role in the observed decrease in membrane stability index.

Protein quantification plays a vital role in plant growth and development. We observed alterations in protein levels under heavy metal stress. These changes could be attributed to either the disruption of protein synthesis processes or the utilization of existing proteins for stress response mechanisms.

In conclusion, the observed alterations in *V. radiata* physiology and biochemistry suggest that heavy metal stress imposes a multifaceted challenge to the plant, leading to a cascade of changes across various levels of biological organization. Future studies may focus on elucidating precise molecular pathways and the potential utilization of Vigna. This study also aims that heavy metal stress has a detrimental effect on *V. radiata* plants at the cellular level. The observed reductions in chlorophyll content, membrane stability index, and altered protein levels indicate compromised photosynthetic activity, impaired cell membrane function, and potential disruptions in protein synthesis. These findings highlight the vulnerability of *V. radiata* to heavy metal contamination and emphasize the need for strategies to mitigate the negative impacts of heavy metals on agricultural production.