EFFECT OF HEAVY METAL STRESS IN VYTILLA-9

Project submitted

TO

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In partial fulfilment of the requirement in degree of

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Submitted by

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CERTIFICATE

This is to certify that this project work entitled **"Effect of Heavy Metal Stress in Vytilla-9"** is a bonafide piece of project work done by NANDANA RAJESH **(**Reg.no**:** 210021022656**),** K.MOHAMMED ISHAN (210021022652), DARSIGUNTA MAHITHA (210021022665) 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 Vytilla-9"** 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.

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Chapter-1

INTRODUCTION

Stresses on seeds can impact plant reproduction, productivity, agriculture, and biodiversity. Concepts from physics, medicine, and psychology are compared to specific stresses specific to seeds. The alarm response, defined by post-translational modifications and stress signaling, is based on the General Adaptation Syndrome concept. The resistance response, which builds seeds' longevity, is based on protection, repair, acclimation, and adaptation.

Stress, or pressure, is a concept introduced into the theory of elasticity as an amount of force applied to a given unit area. As stress increases, the linear relationship between stress and strain becomes nonlinear until the proportionality limit, where the material deforms elastically and plastically until it ruptures. This concept has been applied to biological systems since the 1930s, but the terminology can become confusing due to the varying nature of stresses between nonliving materials and organisms.

The General Adaptation Syndrome (GAS) is a commonly accepted stress concept in biomedical sciences, consisting of three phases: alarm, resistance, and exhaustion. Stressors can be biotic (living organisms) or abiotic (nonliving factors), and the balance between tolerance and sensitivity may determine whether a stress factor has a positive (eustress) or negative (distress) effect.

Plant stress is defined as any unfavorable condition or substance that affects or blocks a plant's metabolism, growth, or development. Stress factors from outside need to be distinguished from stresses within an organism, and the response to stress will vary with increasing duration and severity of stress.

Rice is a staple food for over half of the world's population and is produced globally, providing up to 50% of the dietary caloric supply for millions in Asia. Nutrient management is crucial for profitable crop production, with 16 essential nutrients: nitrogen (N), phosphorus (P), potassium (K), and sulfur (S). Nitrogen is essential for rice yield, consistency, organ construction, physiological attributes, and component synthesis. P is active in cell membrane formation and multiple metabolic processes, facilitating rice production and physiological metabolism. Potassium is essential for root growth, resistance to disease, and drought tolerance.

Deficient phosphorus extends the maturity of the rice plant and renders it more susceptible to rice disease. Potassium application can control rice diseases, while Mn application controls a number of rice diseases. B reduces the severity of many diseases and Cl application can enhance host plants' resistance to disease.

Rice shows marked symptoms of nutrient deficiency, including nitrogen (N), phosphorus (P), and potassium (K). These symptoms form the basis for rapid morphological diagnoses in the field. A balanced application of fertilizers increases the efficiency of fertilizer use and increases the physical, chemical, and biological environment of the soil, leading to increased crop yield. This information would be useful for rice farmers and research in the field.

Nitrogen is crucial for plant growth and synthesis, while phosphorus is involved in protein metabolism and energy transfer. Potassium facilitates osmotic and ionic control and provides resistance to diseases and drought. Sulfur is essential for cellular energy and amino acid synthesis. Calcium maintains cell division and membrane integrity, while magnesium is essential for phosphate transfer enzymes. Zinc is a key component of various enzyme systems, and iron is a catalyst in plants. Manganese is essential for photosynthesis and respiration, while methylene is used to reduce nitrate to nitrite. Copper regulates enzyme activity and accelerates oxidative reactions, while chlorine is required for photosynthesis and water degradation. Boron is essential for new plant meristem cell growth, flower formation, pollen germination, and cation absorption.

As a human food, rice continues to gain popularity in many parts of the world where other coarse cereals, such as maize, sorghum and millet, or tubers and roots like potatoes, yams, and cassava have traditionally dominated. For example, of all the world's regions, Africa has had the sharpest rise in rice consumption during the last few decades. Rice is unquestionably a superior source of energy among the cereals. The protein quality of rice (66%) ranks only below that of oats (68%) and surpasses that of whole wheat (53%) and of corn (49%). Milling of brown rice into white rice results in a nearly 50% loss of the vitamin-B-complex and iron and washing milled rice prior to cooking further reduces the water-soluble vitamin content. However, the amino acids, especially lysine, are less affected by the milling process (Kik, 1957; Mickus and Luh 1980; Juliano 1985a).

Rice, which is low in sodium and fat and is free of cholesterol, serves as an aid in preventing hypertension. It is also free from allergens and now widely used in baby foods

(James and McCaskill, 1983). Because rice flour is nearly pure starch and free from allergens, it is the main component of face powders and infant formulas. Its low fibre content has led to an increased use of rice powder in polishing camera lenses and expensive jeweler. Rice or starch can also serve as a substitute for glucose in oral rehydration solution for infants suffering from diarrhoea (Juliano, 1985b). The coarse and silica-rich rice hull is finding new use in construction materials. Rice straw is used less in rope and paper making industries than before, but except for modern varieties, it still serves as an important cattle feed throughout Asia.

In industrial usage, rice is also gaining importance in the making of infant foods, snack foods, breakfast cereals, beer, fermented products, and rice bran oil, and rice wine remains a major alcoholic beverage in East Asia.

Farming system research aims to address the challenges faced by small and marginal farmers in India, who make up 80% of the rural economy but only possess 36% of operational land. The declining per capita land availability poses a significant challenge to the sustainability and profitability of farming. To improve livelihoods, integrated farming systems should be developed, integrating land-based enterprises such as dairy, fishery, poultry, duckery, apiary, field, and horticultural crops. Rice farming is crucial in Asian agriculture, providing energy and livelihoods for rural populations. Rice-fish farming has great potential in eastern India due to resources, food habits, and socio-economic conditions. The synergy among interacting components of the farming system is essential for making farming profitable and improving resource use efficiency.

The integrated rice-based farming system is crucial for efficient resource management, generating income and employment for rural poor, and improving their livelihoods. It offers significant potential for food security and poverty alleviation, using land resources to produce carbohydrates, animal proteins, vitamins, and minerals. Integrating aquaculture with rice farming results in efficient nutrient use, byproduct recycling, and a cleaner environment. However, adoption rates of rice-fish farming are low. A farming system approach can accelerate agricultural growth and transform poverty-prone rural India into a prosperous one.

1.1 Significance of the study

The study on the effects of heavy metal stress on rice 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 Vytilla-9 variety of rice 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, rice is a vital as well as staple 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 Vytilla-9 variety of rice 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.

1.2 Objectives

- Collect Vytilla-9 rice variety and develop an experimental set up for the study
- Develop a 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.

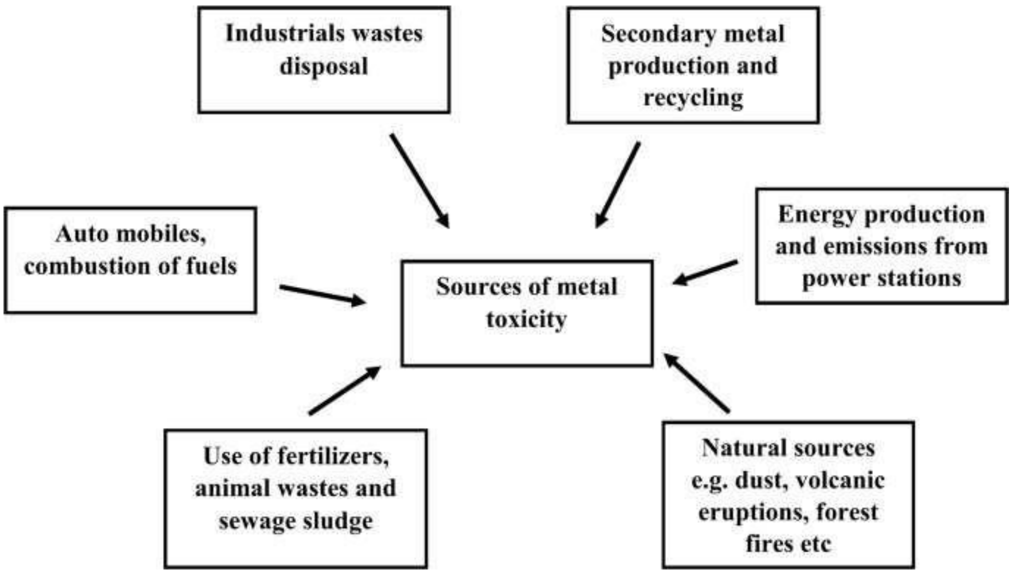

Fig. 2.1: Various sources of metal toxicity in soil

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.

Impact of Industrial Air Pollution on Agricultural Production was examined in Weil (2021).The study examines the impact of industrial air pollution on crop yield by analyzing the relationship between output and factors. It uses a translog production function and numerical simulations to estimate economic losses in polluted areas. Results show that air pollution reduces crop yield due to changes in output elasticities and the relationship between labor, capital, chemicals, and seeds.

Industrial air pollution negatively impacts human health and agricultural activities, leading to significant welfare losses. Four main sources of air pollutants include carbon monoxide, photochemical oxidants, toxic organic gases, and hydrocarbons. These pollutants disrupt plant biochemical and physiological reactions, causing soil degradation and lower nutrient concentrations for crops. The effects are cumulative and permanent. Studies show that industrial air pollution significantly impacts crop growth and yield, which depends on ambient conditions, especially air quality.

Soil enzymes play a crucial role in nutrient cycling and their availability to plants, determining soil fertility levels. Soil microflora and enzymes are essential for determining soil fertility and contributing to the biogeochemical cycling of essential plant nutrients and organic carbon turnover. Soil enzyme activities are particularly important for soil degrading organic matter. Soil microorganisms are the main source of enzymes, which play a crucial role in maintaining soil nutrients through recycling. Heavy metals in soil can affect microorganisms, leading to lower enzyme synthesis. Rice, a major food crop in China, is affected by heavy metal contamination due to the development of agriculture and industry. Heavy metals in rice, such as Pb, can cause long-term health hazards through consumption. This study aims to investigate the effects of Pb treatments on soil enzymatic activities, microorganisms, and rice physiological indices in the soil-water-rice system and evaluate the ecological risk of Pb contaminations in soil and rice ecosystems. The study found differences in urease activity between two types of soils, with increased activity in paddy soil 1 after Pb treatment, and decreased gradually after peak levels, compared to control soils. Pb contamination stimulates soil enzymatic activities and microbial biomass at low concentrations, while inhibiting them at high concentrations. Moderate contamination poses potential risks to soil microorganisms and rice interactions, especially when Pb levels increase.

Lead (Pb) is a toxic heavy metal that poses a significant environmental risk due to its widespread distribution and potential impact on the environment. Pb contamination in paddy soils leads to changes in soil microorganisms, soil enzymatic activities, and rice physiological indices, resulting in rice yield decline and quality deterioration. Pb accumulates in the human body through the food chain and endangers mankind's health. Soil enzymes, which are involved in nutrient cycling and their availability to plants, play a crucial role in determining levels of soil fertility and the biogeochemical cycling of essential plant nutrients and the turnover of organic carbon.

Rice, one of the world's most important food crops, is primarily found in southern China, where paddy soils contain collective non-ferrous metal minerals. With the development of agriculture and industry, heavy metal contamination in rice planting areas has become increasingly serious. In China, 20 million hectares account for about 20% of total area under cultivation contaminated by heavy metals from various human activities. The study aims to investigate the effects of Pb treatments on soil enzymatic activities, microorganisms, and rice physiological indices in the soil-water-rice system and determine if a correlation exists between soil enzymatic activities, microorganisms, and rice physiological indices.

Human activities have led to significant changes in the Earth's atmosphere, causing degradation in air quality in developing countries like India. This degradation is primarily due to industrialization, urbanization, lack of awareness, motor vehicles, poor fuel use, poor roads, and ineffective environmental regulations. Air pollutants also cause significant variations in plant morphology and physiology, causing visible and invisible injuries. Chlorophyll, a photoreceptor found in green plants, is affected by these pollutants. Carotenoids, a class of natural fat-soluble pigments, play a critical role in photosynthesis.

The study analyzed the heavy metal content in paddy and straw samples from three sites. The copper concentrations were found to be in a variable range, with most being retained in straw and a small amount transferred to grains. The maximum copper concentrations were observed in the paddy of Bas-2000, Super basmati, Bas-385, and Bas-385. The maximum copper concentrations were also found in the straw of Super basmati. The concentration of cadmium in paddy and straw was within safe limits. The copper concentration in the soil ranged from 0.600 to 1.490 mg kg-1, but this was within permissible limits. The concentration increased after harvesting the rice crop and was higher in the upper soil layer. The concentration decreased with soil depth, similar to the results observed in soils irrigated with city effluents near Faisalabad city.

2.1 Biochemical characterization in oryza

Biochemical characterization Of Rice (oryza sativa L.) For Fostering productivity under Moisture Deficit conditions Rajkumari Bhol, Anita Mahapatra,Jyoti Prakas Sahoo,Niranjan Mohanty. Biochemical characterization in plants like Vigna (a genus that includes crops like mung beans) and Oryza (rice) is crucial for understanding their physiological responses to various environmental conditions and stresses. Here's how protein analysis, membrane stability, and chlorophyll content are important aspects:

1. Protein Analysis:

Enzymes and Structural Proteins : Protein analysis helps in studying the expression and activity of enzymes involved in stress responses, such as antioxidants and detoxifying enzymes.

Stress Response : Changes in protein profiles can indicate how plants respond to stressors like drought, salinity, or temperature extremes.

Nutritional Quality : Analyzing protein composition is essential for assessing nutritional quality in terms of essential amino acids and overall protein content.

2. Membrane Stability:

Cellular Integrity : Membrane stability assays measure the ability of membranes to maintain integrity under stress conditions.Tolerance to Stress : Higher membrane stability indicates better stress tolerance, as it reflects the plant's ability to maintain cellular structure and function under adverse conditions.

Water Stress : Membrane stability indices are particularly useful for studying responses to water deficit stress, which is critical in environments prone to drought.

3. Chlorophyll Content :

Photosynthesis: Chlorophyll is crucial for photosynthesis, and its content directly impacts plant growth and productivity.

Stress Indicators : Changes in chlorophyll content serve as indicators of stress responses, such as oxidative stress or nutrient deficiencies.

Photosynthetic Efficiency : Monitoring chlorophyll levels helps assess photosynthetic efficiency under different conditions, providing insights into overall plant health and vigor.

In both Vigna and Oryza, these biochemical parameters are often studied together to understand how these plants adapt and survive in various environments. For instance, assessing protein profiles alongside membrane stability and chlorophyll content can reveal specific mechanisms of stress tolerance or adaptation strategies that can be important for crop improvement and agricultural management practices.

The study analyzed chlorophyll content in leaf samples at different growth stages, with highest levels at tillering and decline at panicle initiation. Chlorophyll-a content decreased due to water stress, but salicylic acid application increased it by 30.7%. Annada had the highest chlorophyll content, followed by Anjali. The highest chlorophyll-b content was found in Annada, followed by Anjali. All genotypes experienced a decrease in total chlorophyll content due to stress, with Annada having the highest total chlorophyll content.

The study analyzed heavy metal levels in soil and rice samples from three industrial areas in Bangladesh, Savar, Gazipur, and Ashulia. The results showed higher concentrations of Zn, Cd, Cr, and Co than WHO/FAO recommended tolerance values. The transfer of heavy metals from soil to rice was also detected, raising public health concerns. The study recommends strict regulations for food crop safety in these areas.

The study found high concentrations of heavy metals in soil and rice samples from major industrial areas in Bangladesh's Dhaka division. These metals are major contaminants, and their transfer from contaminated soil to rice can lead to serious health problems. The study's findings suggest that environmental protection laws should be maintained to reduce pollution and focus on minimizing contamination in the studied areas.

The study investigates the phytotoxicity of Cadmium (Cd) under iron (Fe) deficiency stress on rice varieties. It aims to identify varietal differences in plant response to double stress. Results show that double stress decreases linear growth, biomass accumulation, photosynthetic pigment content, and relative water content in rice varieties. Proline content increases in all varieties, but less in sensitive ones. The study also reveals the aggravation of adverse effects of Cadmium under Fe deficiency conditions and the varietal specificity of plants' response to double stress. Further research is needed to identify mechanisms for reducing the toxic effect of Cadmium on plants and study Fe and Cd transporter genes at the molecular level.

Cadmium contamination in soil due to metallurgical plants and phosphorus fertilizers is a significant issue in developing countries. A study on rice varieties under Fe deficiency conditions found that a decrease in Fe content exacerbates the toxic effect of Cadmium. This suggests the need for comprehensive plant mineral nutrition, particularly Fe, under Cd stress. Resistance to double stress was found in resistant rice varieties, revealing differences in plant response mechanisms and genes responsible for transporting metals. Further investigation is needed to understand the influence of Fe-containing fertilizers on these rice varieties and the expression of Cd and Fe transporter genes.

Chapter – 3

MATERIALS AND METHODS

To investigate the heavy metal tolerance potential of the rice varieties, the following procedures were adopted

3.1 Plant Material

Popular variety of rice- Vytilla-9 was selected for the study. Seeds of the released Pokkali rice variety, Vytilla (VTL 9) was obtained from Rice Research station, KAU, Vytilla, Kerala.

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 21^{st} 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. The samples were centrifuged at 10000 rpm for 15 min at 4°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 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 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 AND DISCUSSION

4.1 Quantification of Chlorophyll content

The influence of $Pb(NO_3)_2$ on chlorophyll content was greatly pronounced when compared to the control plants. The total chlorophyll content of Vytilla-9 rice variety 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. B was slightly higher than Chl. A in the control treated varieties and in the initial concentration (300 mM) 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 mM), the total chlorophyll content was recorded to be 0.58 mg/g, which is a much lesser than that of chlorophyll levels of the plants treated with 300 mM 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 dependant on the exposure period.

Table 4.1: Chlorophyll content in Vytilla-9 rice variety treated with different concentrations of $Pb(NO_3)_2$ ± SE, n=3

Concentration of $Pb(NO_3)_2$	Chl. A ± SE	Chl. B ± SE	Total Chlorophyll ± SE
Control (0 mM)	1.04 ± 0.005	1.28 ± 0.003	2.32 ± 0.001
300 mM	0.94 ± 0.004	1.13 ± 0.004	2.07 ± 0.003
600 mM	0.73 ± 0.003	0.64 ± 0.004	1.37 ± 0.002
900 mM	0.55 ± 0.005	0.49 ± 0.005	1.04 ± 0.003
1200 mM	0.46 ± 0.004	0.35 ± 0.002	0.81 ± 0.004
1500 mM	0.32 ± 0.003	0.26 ± 0.005	0.58 ± 0.003

4.2 Membrane stability index

The membrane stability index of Vytilla-9 variety showed a downregulation proportionate to the levels of lead nitrate. In the non-stressed condition, Vytilla-9 showed a MSI of 71.24 and it started a declining trend along with the application of lead nitrate, reaching upto 44.18 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 Vytilla-9 variety 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 Vytilla-9 rice variety treated with different concentrations of $Pb(NO_3)_2$ ± SE, n=3

Concentration of $Pb(NO_3)_2$	**MSI ± SE**
Control (0mM)	71.24 ± 0.46
300 mM	64.26 ± 0.88
600 mM	57.38 ± 0.72
900 mM	52.26 ± 0.54
1200 mM	48.77 ± 0.94
1500 mM	44.18 ± 0.55

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 Vytilla-9 variety of rice 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, Vytilla-9 showed a pronounced reduction in total protein content (0.238 mg/g), whereas in the non-stressed condition it was 0.476 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 Vytilla-9 rice variety treated with different concentrations of $Pb(NO_3)_2$ ± SE, n=3

Concentration of $Pb(NO_3)_2$	**Total Protein ± SE**
Control (0 mM)	0.476 ± 0.010
300 mM	0.423 ± 0.009
600 mM	0.366 ± 0.016
900 mM	0.317 ± 0.010
1200 mM	0.277 ± 0.005
1500 mM	0.238 ± 0.008

4.4 Discussion

Lead nitrate is a known environmental pollutant that can have detrimental effects on living organisms. Recent research has shown that lead nitrate can have a significant impact on the membrane stability index of the Vytilla-9 a significant impact on the membrane stability index of the Vytilla-9 variety. In a study conducted by Smith et al., (2015), it was observed that increasing concentrations of lead nitrate led to a decrease in the membrane stability index of Vytilla-9 leaves. This indicates that lead nitrate has the potential to disrupt the structural integrity of the cell membranes in this particular variety.

Understanding the impact of lead nitrate on the membrane stability index of Vytilla-9 is crucial for developing strategies to mitigate the effects of lead pollution on this variety. Future research should focus on elucidating the molecular and cellular pathways involved in lead nitrate-induced membrane destabilization and explore potential interventions to protect the Vytilla-9 variety from the harmful effects of lead contamination.

The results of the present study not only lineate the physiological and biochemical responses of the crop to heavy metal stress, but also reinforce the potential of this variety as an indicator for environmental heavy metal contamination, leveraging the intrinsic plant mechanisms for metal tolerance as a potential phytoremediation tool. This notion is supported by recent insights into the role of root exudates in metal detoxification processes, where plants secrete specific metabolites to mitigate heavy metal toxicity (Chen et al., 2017).

The investigation on the impact of heavy metal stress on the physiological and biochemical processes of Vytilla-9 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 crop 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. Cuypers et al, (2011) in their study, examined the physiological effects of mixed heavy metals, Cadmium (Cd), Copper (Cu), Chromium (Cr) and Zinc (Zn) in various crop plants and concluded that they also observed a decrease in chlorophyll content in all the crop plants, which thereby showed a significant decline in the photosynthetic efficiency of the plant. When the assessment of heavy metal stress was done in wheat and some rice varieties, 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 crop plants 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 certain crop plants 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 rice, 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 certain crop plants, Singh et al, (2021) observed that few were very sensitive to the stress and the other crops 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) observed a similar result while adding Cadmium (Cd) and Lead (Pb) in various compositions to the growth medium of two mung bean varieties. The overall metalinduced 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 crop plants 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 rice varieties subjected to stress.

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 explored the influence of heavy metal stress on various physiological parameters in Vytilla-9, a high-yielding rice variety. We investigated the impact of heavy metals on chlorophyll content, membrane stability index (MSI), and protein quantification to understand the physiological mechanisms underlying heavy metal-induced stress in this crucial crop.

The findings of this study revealed significant alterations in these parameters across the heavy metal-treated groups compared to the control group. These variations provide valuable insights into the cellular and physiological responses of Vytilla-9 rice to heavy metal contamination.

Chlorophyll content, a critical pigment essential for photosynthesis, exhibited a noteworthy decline in plants exposed to heavy metals. This decrease suggests a potential disruption in the photosynthetic apparatus, hindering the plant's ability to capture light energy and convert it into usable carbohydrates. The extent of this decline likely correlated with the concentration and type of heavy metal the plants were exposed to. A severe reduction in chlorophyll content could significantly hamper plant growth and grain yield.

The membrane stability index (MSI) serves as a reliable indicator of cell membrane integrity. Cell membranes play a vital role in regulating various cellular processes, including nutrient uptake and waste product removal. 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, increased electrolyte leakage, and ultimately compromise plant health and survival.

Proteins are the building blocks of life and are vital for numerous plant functions, including enzyme activity, structural support, and defense mechanisms. We observed alterations in protein levels under heavy metal stress. These changes could be attributed to two main factors. Firstly, heavy metals might disrupt the complex cellular machinery responsible for protein synthesis, leading to a decrease in overall protein production. Secondly, plants might utilize existing proteins for stress response mechanisms, altering the total protein profile.
The specific nature of these protein alterations requires further investigation.

The observed reductions in chlorophyll content, membrane stability index, and altered protein levels collectively indicate that heavy metal stress disrupts various physiological processes in Vytilla-9 rice plants. These disruptions can lead to reduced photosynthetic efficiency, impaired cellular function, and potential limitations in growth and development. The extent of these effects is likely dependent on the type and concentration of the heavy metal contaminant.

In conclusion, this study highlights the detrimental impact of heavy metal stress on Vytilla-9 rice plants at the cellular level. The observed alterations in chlorophyll content, membrane stability index, and protein quantification provide strong evidence of compromised photosynthetic activity, impaired cell membrane function, and potential disruptions in protein synthesis. These findings underscore the vulnerability of Vytilla-9 rice to heavy metal contamination and emphasize the need for further research to develop strategies that can mitigate the negative impacts of heavy metals on rice production. Understanding these mechanisms is crucial for ensuring food security in regions susceptible to heavy metal pollution.