EFFECT OF LEAD TOXICITY ON GROWTH, DEVELOPMENT AND YIELD OF RICE

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This is to certify that the thesis enlightens, **"EFFECT OF LEAD TOXICITY ON GROWTH, DEVELOPMENT AND YIELD OF RICE"** submitted to the faculty of agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in AGRICULTURAL BOTANY** embodies the result of a piece of bona fide research work conducted by **MD. MAHMUDUL HASAN**, Registration no. **19-10339** under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this study has been dully acknowledged.

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Dedicated

to

My Beloved Parents

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EFFECT OF LEAD TOXICITY ON GROWTH, DEVELOPMENT AND YIELD OF RICE

ABSTRACT

Lead toxicity adversely affects the growth and development of rice plants. An experiment was carried out to evaluate the effect of lead (Pb) stress on morphological, physiological and yield performance of rice plant (*Oryza sativa* L. cv. BRRI dhan29 and cv. BRRI dhan58). The experiment was carried out at the net house of the Department of Agricultural Botany and Plant Physiology Laboratory of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh in Boro season during the period from December, 2020 to May 2021. To know the effects of different lead levels on the morphological, physiological and yield performance of rice plant. The double factor experiment was laid out in a randomized complete block design (RCBD) with three replications and the differences between the means were evaluated by Least Significant Difference (LSD). In this experiment, the treatments consisted of control and three different lead levels viz. Pb_0= without lead (0 mM $PbSO_4$, Lead(II) sulfate), Pb_1= 2 mM $PbSO_4$, Pb_2 = 4 mM $PbSO_4$, Pb_3= 6 mM $PbSO_4$ and two different variety viz. V_1= BRRI dhan29, V_2= BRRI dhan58. The lead treatments were applied at 15 days after transplanting (DAT). The results showed that the no. of non-effective tillers, proline content, no. of unfilled spikelets panicle^{-1} increased under Pb toxicity. Lead stress decreased the morphological parameters (plant height, no. of tillers, leaf area) and the values of physiological (MSI%, RWC%, SPAD value, dry weights) attributes in both rice cultivars. Root, shoot, leaf dry weights, and TDM also decreased in both rice cultivars under Pb stress. Yield attributes (panicle length, no. of effective, no. of filled and 1000 grain wt.) and yield (grain yield) parameters were reduced significantly ($P \leq 0.05$) under lead stress condition. The loss of grain yield for rice variety 1/BRRI dhan29 due to Pb_1, Pb_2 and Pb_3 level of lead was 15.43%, 35.24% and 48.72% respectively over non-lead stressed control. The loss of grain yield for rice variety 2/BRRI dhan58 due to Pb_1, Pb_2 and Pb_3 level of lead was 11.82%, 26.88% and 39.02% respectively over non-lead stressed control. Therefore, it can be concluded Pb-induced toxic effect on morphological and physiological parameters can cause substantial damage of different rice cultivars which can adversely affect yield attributes of rice plant that ultimately decrease the yield of different rice cultivars.

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LIST OF ABBREVIATIONS

%	Percent
@	At the rate of
^{0}C	Degree Celsius
ABA	Abscisic Acid
AEZ	Agro-Ecological Zone
APX	Ascorbate Peroxidase
AsA	Ascorbate
BARI	Bangladesh Agriculture Research Institute
BAU	Bangladesh Agricultural University
BRRI	Bangladesh Rice Research Institute
Cm	Centimeter
Ca	Calcium
CAT	Catalase
CV%	Percentage of Coefficient of Variation
Cys	Cysteine
DAT	Days After Transplanting
DNA	Deoxyribonucleic Acid
DHAR	Dehydroascorbate Reductase
dSm-1	DeciSiemens per metre
EC	Electrical Conductivity
e.g	As for example
et al.	and others
FAO	Food and Agriculture Orzanization
g/gm	Gram
GB	Glycine Betaine
Gly I	Glyoxalase I
Gly II	Glyoxalase II
GPX	Glutathione Peroxidase
GR	Glutathione Reductase
GST	Glutathione S-transferase
GSH	Glutathione
GSSG	Glutathione Disulfide
Ha	Hectare
i.e.	that is
kg	Kilogram
Kg ha^{-1}	Kilogram per hectare
LSD	Least Significant Difference
L	Liter
M	Meter
MDA	Malondialdehyde
MDHAR	Monodehydroascorbate Reductase
MDHA	Monodehydroascorbate
MPa	Megapascal Pressure Unit
ml/L	Milliliter per Liter
MG	Methylglyoxal
Mg/L	Milligram per Liter
MoP	Muriate of Potash

LIST OF ABBREVIATIONS (Continued)

N	Nitrogen
NaCl	Sodium Chlloride
NR	Nitrate Reductase
Nm	Nano Meter
Ng	Nano Gram
P	Phosphorus
Pb	Lead
pH	Hydrogen ion concentration (Negative Logarithm)
PI	Panicle Initiation
Pn	Net Photosynthetic Rate
Pro	Proline
POD	Peroxidase
PS I/II	Photo System I/II
qP	Photochemical Quenching
RCBD	Randomized Complete Block Design
ROS	Reactive Oxygen Species
RWC	Relative Water Content
ppm	Parts Per Million
S	Sulfur
SAU	Sher-e-Bangla Agricultural University
SOD	Superoxide Dismutase
TSP	Triple Super Phosphate
µg/kg	Microgram per kilogram
Zn	Zinc

CHAPTER I

INTRODUCTION

Recent rates of soil contamination with different heavy metals (the non-essential elements for plants) and their entrance in to the agro-ecosystems and transference to human beings through food chain is an alarming situation over the globe. In terrestrial ecosystems, soil act as the main source of heavy metal transference to agricultural products. These metals enter in to the plant systems from soil or from external atmosphere surrounded by the plant and would have serious consequences in crop productivity and grain qualities. Among different heavy metals, lead (Pb), is the second most harmful pollutant after arsenic.

Plant stress is a state where the plant is growing in non-ideal growth conditions that increase the demands made upon it. The effects of stress can lead to deficiencies in growth, crop yields, permanent damage or death if the stress exceeds the plant tolerance limits. Plant stress factors are mainly categorized into two main groups; abiotic factors and biotic factors. The abiotic factors include the different environmental factors that affect plant growth (such as light, water, and temperature), while the biotic factors are the other organisms that share the environment and interact with the plants (such as pathogens and pests). Response to stress usually involves complex molecular mechanisms, including changes in gene expression and regulatory networks.

Rice (*Oryza sativa L.*) belongs to the grass family (Poaceae) and it is economically and socially dominant over all other crops in Bangladesh. More than three billion people consume rice as staple food accounting for about 50-80% of their daily calorie intake (Khush, 2005). In worldwide, 487.76 milion metric tons of rice was produced during the year of 2018-19(October) (USDA, 2018). USDA estimates Bangladesh to produce around 34.4 million tons of rice in MY 2018-19 (October). Among 114 rice producers' countries of the world Bangladesh ranks third (FAO, 2022). The total rice growing area is about 11.38 million hectares, leading to the production of 34.70 million metric tons of rice with an average yield 3.05 t ha^{-1} (DAE, 2015). It is the source of about one third of the total carbohydrate providing considerable amount of recommended Zinc and Niacin. The protein of rice is rich biologically and highly digestive (88%). Being the second most important crop worldwide after wheat it covers almost 90% of area across

Asia alone. There are varieties of use of the crop widely ranging from its use as food in cereals, snacks, brewed beverages, flour, rice bran oil (Gopalan *et al.*, 2007).

Plants normally have three mechanisms of Pb-tolerance i.e., (a) passive mechanisms (plant develops different types of physical barriers against Pb uptake), (b) inducible mechanisms (metal detoxification and its excretion to extra-cellular spaces), and (c) activation of anti-oxidative defense system (which includes both enzymatic and non-enzymatic anti-oxidants) to scavenge ROS (Pourrut *et al.*, 2011; Ashraf *et al.*, 2015). Anti-oxidants, both enzymatic such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), and non-enzymatic such as reduced glutathione (GSH) and oxidized glutathione (GSSG) are involved in direct and/or indirect detoxification of ROS in plants (Mishra and Choudhary, 1998; Mittler, 2002). Except anti-oxidants, plants also accumulate various types of organic compounds or osmolytes proline and soluble sugars to shield essential cellular structures and to maintain cell osmotic potential (Chatterjee *et al.*, 2004; Ali *et al.*, 2014).

Soil is contaminated by heavy metals due to the results of human activities, unscientific agriculture and industrial practices) . Heavy metals may get easily accumulated in many biological systems and cause deleterious symptoms. Heavy metals such as lead (Pb) are of major concern because of their persistence in the environment (Melegy, 2010). Lead is ranked the number one heavy metal pollutant and number two of all hazardous substances by the Agency for Toxic Substances and Disease Registry (Gallardo *et al.*, 2002; ATSDR, 2007). Lead is not biodegradable and is extremely persistent in both water and soil; it can be retained in the environment for 150–5000 years (Saxena *et al.*, 1999). Lead is easily taken by the plants from the soil and is accumulated in different plant tissue. Lead toxicity decreases growth and yield in plants (Shafiq *et al.*, 2008; Kumar and Jayaraman, 2014), disruption of mineral nutrition (Lamhamdi *et al.*, 2013; Naresh kumar *et al.*, 2014), inhibition of photosynthesis (Tian *et al.*, 2014), inhibition of enzyme activity (Malar *et al.* 2014), water imbalance and alterations in membrane permeability (Sharma and Dubey 2005; Israr and Sahi, 2008). Soil contaminated with Pb cause sharp decreases in crop productivity by posing a serious problem for agriculture (Johnson and Eaton, 1980). Rice is one of the most important staple foods in Asia. About 90 percent of the total rice is cultivated in Asia (Salim *et al.*, 2003). In Asian countries, rice (*Oryza sativa*) is a food staple used for daily consumption and provides over 70% of the energy derived from daily food intake (Phuong *et al.*, 1999).

According to Bangladesh Bureau of Statistics (BBS 2004) throughout the country, 22,480 persons were engaged in the battery recharging/recycling establishments and about one-fourth (24.6%) of them are child workers (5-17 years). There is adequate chance of exposure to lead among the workers as the health of them in these establishments is much neglected leading to the risk of lead contamination and development of severe health hazard. Trace metal contaminated sites are of a great concern in this rapidly growing trend of urbanization and industrialization and requires remediation of polluted sites. Organic matter amendments are highly effective because of their inexpensive and ready availability and additional benefits for plant growth and soil properties and the addition of organic matter substantially reduce metal uptake was reported by Bassuk (1986).

Most of the Pb ions absorbed by plants from the soil remain concentrated in the roots, and a small portion is transferred to the stems and leaves. In the plant tissues, active Pb ions exert toxic effects and directly damage the photosynthetic systems; consequently, the developmental processes of the plants, such as growth, mineral absorption, and seed germination, are affected. Santos *et al.* (2015) investigated the response of *Hypnea musciformis* to Pb stress, and reported that the photosynthetic ability, measured in terms of the maximum photosynthetic efficiency of photosystem II (Fv/Fm) and electron transport rate (ETR), of plants under stress was relatively stable, and was not significantly different from that of the plants grown under normal conditions. They speculated that this could be because of an increase in starch synthesis in chloroplasts, which might serve as a stored food, ensuring normal physiological function. However, unlike *Hypnea, ryegrass* (Lolium) responds differently to Pb stress; when exposed to 500 μM Pb, the carotene, chlorophyll a, and chlorophyll b levels in the plants, as well as the photosynthesis rates were significantly lower than in the control plants. Among the parameters studied, the net photosynthesis (Pn) and transpiration rates (Tr) were decreased by 52.9 and 39.4%, respectively. Similarly, under conditions of Pb stress, the growth of *Robinia pseudoacacia* (black locust) seedlings was repressed, and Pb ions were found to be mostly concentrated in the roots. At a Pb concentration of 2000 mg kg^{-1} in soil, the Pb concentration in the root was approximately 400 $\mu g\ g^{-1}$, and the number of chloroplasts and photosynthesis ability decreased significantly; at Pb concentration of 1400 mg kg^{-1} of the soil, the seedling Pn, Fv/Fm, and quantum efficiency of PSII were decreased by 60.62, 29.2, and 55.07%, respectively.

Therefore, it is evident that lead toxicity causes substantial damages to plants. The present study was undertaken with the following objectives-

- To study the effect of different doses of lead on growth and development of rice plant
- To investigate the effect of different doses of lead on yield attributes and yield of rice

CHAPTER II

REVIEW OF LITERATURE

2.1 Rice, the Major Food Crop

Rice is the major food for about 156 million people of Bangladesh. It is a member of the genus Oryza in the family Poaceae and the Oryza genus has many species, of which two diploid species - *Oryza sativa* L. and *Oryza glaberrima* L. are cultivated. In Asia, *Oryza sativa* is most commonly cultivated (Vaughan *et al.*, 2008). The basic chromosome number of rice is n=12 and there is both diploid (2n=24) and tetraploid species (4n=48) (Brar and Khush, 2003). As the agroclimatic conditions of the country are favorable for year-round rice cultivation, Bangladesh has a long history of growing rice. That's why rice is cultivated throughout the country except in the southeastern hilly areas (Shelley *et al.*, 2016). Rice covers a global area of 162.62 million hectares of land producing about 499.37 million tons of crop. This crop is being cultivated across an area of 11.77 million ha yielding about 34.91 million tones in Bangladesh (USDA, 2020). However, the national average rice yield is much lower than that of other rice-growing countries of the world.

Rice, considered as a staple food across major countries of the world, feeds more than half of the world's population (IRRI 2006). The population growth rate is approximately 2 million per year, and if the population growth rate remains same, the total population will reach 238 million by 2050 (Shelley *et al.*, 2016). With the expanding population, the increase in rice production is very important in order to keep in accord to the national food requirement. Due to increased population total cultivable area is decreasing at a rate of more than 1% per year because of construction of houses, roads, industries, etc. Moreover because of climate change like drought, flood, salt stress, extreme temperature stress, and anthropogenic activities cultivated crops face different kinds of adverse conditions and among them Pb stress due to increased industrialization is the most important one. Rice is very sensitive to Pb.

2.2 Heavy metal stress overview

According to a study conducted by Abolghassem Emamverdian (2015) anthropogenic perturbations of biosphere manifested in a broad array of global phenomena including accelerated rate of industrialization, intensive agriculture, and extensive mining accompanied by burgeoning population and rapid urbanization have not only wreaked the havoc on the availability of natural resources but also caused widespread and grave contamination of essential components of life on the planet. Of the implications of human-induced disturbance of natural biogeochemical cycles, accentuated accumulation of heavy metals (HMs) is a problem of paramount importance for ecological, nutritional, and environmental reasons. HMs belong to group of nonbiodegradable, persistent inorganic chemical constituents with the atomic mass over 20 and the density higher than 5 $g \cdot cm^{-3}$ that have cytotoxic, genotoxic, and mutagenic effects on humans or animals and plants through influencing and tainting food chains, soil, irrigation or potable water, aquifers, and surrounding atmosphere. There are two kinds of metals found in soils, which are referred to as essential micronutrients for normal plant growth (Fe, Mn, Zn, Cu, Mg, Mo, and Ni) and nonessential elements with unknown biological and physiological function (Cd, Sb, Cr, Pb, As, Co, Ag, Se, and Hg). Both underground and aboveground surfaces of plants are able to receive HMs. The essential elements play a pivotal role in the structure of enzymes and proteins. Plants require them in tiny quantities for their growth, metabolism, and development; however, the concentration of both essential and nonessential metals is one single important factor in the growing process of plants so that their presence in excess can lead to the reduction and inhibition of growth in plants. HMs at toxic levels hamper normal plant functioning and act as an impediment to metabolic processes in a variety of ways, including disturbance or displacement of building blocks of protein structure, which arises from the formation of bonds between HMs and sulfhydryl groups, hindering functional groups of important cellular molecules superseding or disrupting functionality of essential metals in biomolecules such as pigments or enzymes and adversely affecting the integrity of the cytoplasmic membrane, resulting in the repression of vital events in plants such as photosynthesis, respiration, and enzymatic activities. On the other hand, elevated levels of HMs are associated with the increased generation of reactive oxygen species (ROS), such as superoxide free radicals, hydroxyl free radicals, or non-free radical species (molecular forms) such as singlet oxygen and hydrogen peroxide (H_2O_2) as well as cytotoxic compounds like methylglyoxal (MG), which can cause oxidative stress via disturbing the equilibrium between prooxidant and antioxidant homeostasis within the plant cells. This condition implicates the causation of multiple deteriorative disorders such as, oxidation of protein and lipids, ion leakage, oxidative DNA attack, redox imbalance, and denature of cell structure and membrane, ultimately resulting in the activation of programmed cell death (PCD) pathways. Plants employ various inherent and extrinsic defense strategies for tolerance or detoxification whenever confronted with the stressful condition caused by the high concentrations of HMs. As a first step towards dealing with metal intoxication, plants adopt avoidance strategy to preclude the onset of stress via restricting metal uptake from soil or excluding it, preventing metal entry into plant root. This can be achieved by some mechanisms such as immobilization of metals by mycorrhizal association, metal sequestration, or complexation by exuding organic compounds from root. At next stage, if these strategies fail and HMs manage to enter inside plant tissues, tolerance mechanisms for detoxification are activated which include metal sequestration and compartmentalization in various intracellular compartments (e.g., vacuole) , metal ions trafficking, metal binding to cell wall, biosynthesis or accumulation of osmolytes and osmoprotectants, for example, proline (Pro), intracellular complexation or chelation of metal ions by releasing several substances, for example, organic acids, polysaccharides, phytochelatins (PCs), and metallothioneins (MTs) , and eventually if all these measures prove futile and plants become overwhelmed with toxicity of heavy metal (HM), activation of antioxidant defense mechanisms is pursued. This review has attempted a comprehensive account of past developments and current trends using more than 235 articles in the research on HM poisoning in plants, exploring the response of vital growth, morphological, anatomical, production, and physiological parameters of plants to HM toxicity as well as investigating detoxifying roles of some defense mechanisms adopted by plants in the face of trace element excess.

2.3 Lead (Pb) Stress

According to a study conducted by Murtaza et. al. (2021) found that Lead-Mediated Oxidative Stress Alters the Physiological and Biochemical Properties of Rice. This stress induction, caused by either biotic or abiotic stress stimuli, plants undergo a complex physiological and biochemical reprogramming and redistribution of resources to combat the stress, in our case, in response to lead-induced oxidative stress. As a result, various antioxidant (enzymatic and non-enzymatic) systems are activated, and the synthesis of various biological components is initiated. In the present study, the activity of catalase (CAT) was shown to be significantly induced in all rice cultivars (in both Pb-tolerant and -sensitive rice cultivars) as a result of Pb treatment. Generally, an increase in CAT activity is expected to play a role in lowering ROS overaccumulation, particularly H_2O_2. Here, we observed that the H_2O_2 accumulation pattern was much pronounced in Ilmi (0.6 and 1.2 mM Pb), Yasmen (rice variety) and Amber Barka (rice variety) which were also identified as the most sensitive cultivars regarding their phenotypic responses towards Pb stress. In our recent study, we also observed that CAT activity increased in both drought-tolerant and -sensitive genotypes when Arabidopsis (a model plant for dicots) plants were exposed to drought stress. It is therefore believed that CAT activity alone may not be sufficient to provide the required level of tolerance towards Pb stress. Rather, our study favors the hypothesis that a coordinated action involving various antioxidant (enzymatic and non-enzymatic) systems and well-organized signaling cascades would be more beneficial to plants in providing the expected degree of tolerance, while tending to maintain a balanced reduction–oxidation state within the cells. In addition to CAT, the activity of peroxidase (POD) and that of polyphenol oxidase (PPO) increased concomitantly with an increase in Pb concentration. Furthermore, the Pb-tolerant rice cultivars Tunnae and Mashkab that exhibited a balanced phenotypic growth and improved tolerance towards Pb stress showed a significant increase in POD activity. Moreover, Tunnae showed a significant increase in PPO as well as an increase in superoxide dismutase (SOD) activity.

Initially, we were expecting to see a reduced accumulation level of superoxide anion (O_2^-), particularly in Pb-tolerant cultivars, such as Tunnae and Mashkab, with regard to the increase in SOD activity. Rather, we recorded an increased O_2^- content in both Pb-tolerant and -sensitive rice cultivars. In higher plants, SOD enzymes act as antioxidants and protect cellular components from being oxidized by the reactive oxygen species (ROS). SODs catalyze the conversion of O_2^- into oxygen and hydrogen peroxide (H_2O_2). In the same way, we were expecting to see a high SOD activity concomitant with the increase in O_2^- generation. Under abiotic or biotic stress conditions, the activity of SOD typically increases with the degree of the stress. Here, we recorded a contrasting SOD activity, which did not systematically match the accumulation pattern of O_2^-. It is said that an overaccumulation of ROS in response to abiotic stress may cause oxidation of proteins, inhibition of the activity of enzymes, damage to nucleic acids, and induction of programmed cell death (PCD) that may culminate in cell death. We would then speculate that the observed mismatch between the SOD activity and the accumulation of O_2^- would be caused by disturbed or reduced catalytic ability of SOD to reduce O_2^-

into H_2O_2 and H_2O. From another perspective, O_2^- has been reported as one of the highly reactive free radicals that promote oxidative stress, while interacting with nitric oxide (NO) to generate peroxynitrite ($ONOO^-$). The recorded significant increase in O_2^- at all Pb levels and all tested cultivars would indicate the extent of the oxidative stress induced by Pb treatment. Moreover, the recorded increase in ion leakage would imply that the cell membrane integrity might have been affected by the Pb treatment.

To withstand abiotic stresses, plants activate various adaptive response mechanisms that include the accumulation of solutes as well as proteins, in addition to the enzymatic antioxidant system. Here, we recorded a significant reduction in total protein content in all rice cultivars, and at all Pb levels. Another non-enzymatic antioxidant acting as an osmoprotectant, which generally accumulates in response to abiotic stress, is proline, also known as the stress amino acid. Recent studies reported that proline was differentially accumulated when rice cultivars were exposed to Pb and copper (Cu) stress. Our data indicated that the accumulation of proline was much higher in the Pb-tolerant rice cultivars Tunnae and Mashkab under 1.2 mM Pb treatment, therefore suggesting a possible role of proline in the adaptive response mechanism towards Pb stress tolerance in rice.

Many studies have reported a change in the accumulation of photosynthetic pigments, such as chlorophyll, in response to abiotic stresses. Under normal growth conditions of plants, chlorophylls contribute to nutrition and energy acquisition for plants to complete their life cycle. Therefore, the recorded reduction in chlorophyll would reflect a disturbed photosynthetic process and energy supply within the cell due to the Pb toxicity, which in turn would have an effect on the synthesis of soluble sugars. Here, our findings revealed that the soluble sugars, such as sucrose, glucose, and fructose, were differentially affected under Pb stress. For instance, we observed a significant increase in sucrose content, particularly with the 1.2 mM Pb application. Under the same conditions, glucose and fructose levels were shown to be significantly reduced, suggesting a prevalent role of sucrose over glucose and fructose in the adaptive response mechanism towards Pb tolerance. Similarly, previous reports revealed a variation in the production of carbohydrates, a change in the sugar metabolism, and aggregation of various osmolytes in response to heavy metal stress.

It appears that each antioxidant system taken independently may not be enough to provide the required level of tolerance towards lead (Pb) stress. Rather, all the results put together suggest that the tolerance to lead (Pb)-induced oxidative stress would be a consequence of synergetic actions of both enzymatic and non-enzymatic antioxidant systems within the cell that help maintain a balanced reduction–oxidation state and an optimum growth and productivity of rice plants.

After industrial revolution and ever-increasing urbanization, there has been a consistent addition of heavy metals (HMs) in the environment, which is now becoming a challenge to produce from the contaminated soil (Govil *et al.*, 2008; Granero and Domingo 2002). Industrial processing, mining, automobiles, use of synthetic fertilizers, and other agro-chemicals are building pools of various heavy metals of which lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg), are of major concerns (Huang *et al.,* 2007; Li *et al.,* 2008).

Recent rates of soil contamination with different heavy metals (the non-essential elements for plants) and their entrance in to the agro-ecosystems and transference to human beings through food chain is an alarming situation over the globe (Abrahams, 2002; Anjum *et al.*, 2016). In terrestrial ecosystems, soil act as the main source of heavy metal transference to agricultural products. These metals enter in to the plant systems from soil or from external atmosphere surrounded by the plant and would have serious consequences in crop productivity and grain qualities. Among different heavy metals, lead (Pb), is the second most harmful pollutant after arsenic and recently listed as “the chemical of great concern” according to the new European REACH regulations (Pourrut *et al.*, 2011). It severely affects normal plant metabolism, morph-physiological features and crop growth and productivity (Sharma and Dubey, 2005; Ashraf *et al.*, 2015). It often leads to diminished growth, deformation of cellular structures, ion homeostasis, reductions in chlorophyll biosynthesis, hormonal imbalance andinducese over-production of reactive oxygen species (ROS) in plants (Shahid *et al.*, 2011; Kumar *et al.*, 2012). Pb, being non-redox metal, cause ROS production that led to oxidative stress within plant cells (Singh *et al.*, 2010). Once produced, these ROS readily attacks to biological structures and biomolecules and results in metabolic dysfunction (Clemens, 2006). Plants normally have three mechanisms of Pb-tolerance i.e., (a) passive mechanisms (plant develops different types of physical barriers against Pb uptake), (b) inducible mechanisms (metal detoxification and its excretion to extracellular spaces), and (c) activation of anti-oxidative defense system (which includes both enzymatic and non-enzymatic anti-oxidants) to scavenge ROS (Pourrut *et al.*, 2011; Ashraf *et al.*, 2015). Anti-oxidants, both enzymatic such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), and non-enzymatic such as reduced glutathione (GSH) and oxidized glutathione (GSSG) are involved in direct and/or indirect detoxification of ROS in plants (Mishra and Choudhary, 1998; Mittler, 2002). Except anti-oxidants, plants also accumulate various types of organic compounds or osmolytes proline and soluble sugars to shield essential cellular structures and to maintain cell osmotic potential (Chatterjee *et al.*, 2004; Ali *et al.*, 2014).

2.4 Pb stress in Bangladesh

Lead toxicity has increased in Bangladesh due to increased disposal of municipal and industrial solid and liquid wastes, vehicle exhausts to the soil (Kibria, 2013). With sharp rise in the use of motor vehicles, use of lead acid battery (LAB) has also increased in Bangladesh and as a result, manufacturing of LAB has also increased proportionally (Ahmad *et al.*, 2014). Workers in these industries are at constant risk of exposure of lead and thus increasing lead content I soil (Ahmad *et al.*, 2014). When lead present at an elevated level in soil, are absorbed by the root system, accumulate in different parts of plants, reduce their growth and impairs metabolism. Lead pollution coats the surface of the leaves and reduces the amount of light reaching it and as a result the growth of crops has become stunted or killing the plants by reducing photosynthesis, inhibiting respiration, encouraging an elongation of plant cells influencing root development by causing pre-mature aging (Chamon *et al.*, 2005a).

Sewage waters are used to irrigate rice fields which are usually mixed with irrigated water; however, those are not treated because of the lack of infrastructure and facilities for sewage treatment (Nawaz *et al.*, 2006). In such conditions there is a strong need to investigate the effect of lead on rice seed germination and seedling and plant growth by evaluating various physiological and biochemical attributes of *Oryza sativa*.

2.5 Lead effect on Growth

Plant roots rapidly respond to absorbed Pb, through a reduction in growth rate and change in branching pattern. Several workers have a reported the inhibition of root growth at 10^{-2} to 10^{-6} M Pb concentration or at a soil Pb content above 10 mg/Kg

(Breckle, 1991). At lower concentrations of Pb, development and extension of the main route is affected much more than the lateral roots (Obroucheva et. al., 1998). Goodbold and Kettner (1991) reported that *Picea abies* plans when exposed to Pb at concentrations of 0.1 to micro M shows increased Pb content in roots with increasing Pb supply. A four-week exposure of the growing plants to 0.5 micro M Pb reduced to the growth of primary, secondary and tertiary roots. The initiation of lateral roots appeal to be more sensitive to be then the growth of primary roots. In *Zea mays* seedlings, Obroucheva *et al.* (1998) observed strong inhibition of primary root growth and a shorter branching zone with more compact lateral roots occupying opposition March closed to the root tip compared with roots grown in the absence of Pb. It appears that the inhibition of road growth and Pb toxicity is as result of Pb induced inhibition of cell division in root tips (Eun *et al.*, 2000). When the effect of different concentrations of PB nitrate was started on the road growth cell division chromosome morphology and the nucleus of onion reduction in root growth my topic is regularities and chromosome thickness where observed (Wierzbicka, 1994). Microtubules of different regions of the root meristem and in different states of the cell cycle show differential susceptibility. These effects do not appear to be a general phenomenon common to toxic metal sins Al and Cu at a concentration that decreases root growth to a comparable level did not show similar mental detrimental effects on microtubules (Eun *et al.*, 2000). Based on such observations it is suggested that the damaged to microtubule by PB is an important component of Pb induced injury in plants (Eun *et al.*, 2000).

Pb decreases seed germination, length and biomass of root and shoot of rice seedlings (Mishra and Choudhuri, 1998). Trace metal contaminated sites are of a great concern in this rapidly growing trend of urbanization and industrialization and requires remediation of polluted sites. Organic matter amendments are highly effective because of their inexpensive and ready availability and additional benefits for plant growth and soil properties and the addition of organic matter substantially reduce metal uptake was reported by Bassuk (1986).

2.6 Specific Ion effect

Lead is the major component of those nutritional elements by seedlings and plants and causes deficiencies or adverse ion distribution within the plant (Trivedi and Erdei, 1992). Research about the effect of heavy metals on plant growth has been well documented (Qin *et al.*, 2000; Kang *et al.*, 2002), including effects of lead pollution on under extreme conditions (Laraque and Trasande, 2005; Clemens, 2006).

2.7 Nutritional imbalance

Lead toxicity in soil impaired plant nutrition, affects plant nutrient relationship (Gopal and Rizvi 2008), and changes internal nutrient ratios among the plant tissues (Kabata-Pendias and Pendias, 1992). Research done so far regarding lead toxicity and its relation with plant mineral nutrition is not enough to make a definite conclusion about lead mechanism of nutrition imbalance; but it can be stated from the previous work that lead influences mineral uptake by plants. It restricts the entry of divalent cations such as Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, and Mn^{2+} and anions like NO^{3-} in various plants including rice (Chatterjee *et al.*, 2004). Two mechanisms might be involved in reduced uptake of mineral nutrients. First mechanism known as physical depends on size of metal ions and second is the chemical one that might be due to metal-induced changes in the cell metabolism by disrupting cell membrane and alteration in enzymatic activities. Efflux of K^+ ions from roots showed sensitivity of K^+-ATPase and -SH groups of plasma membrane to Pb is an example of the second type of mechanism (Sharma and Dubey, 2005). However, it is hard to conclude that reduction in ion uptake is due to blockage of nutrients through roots, reduction in translocation from roots to shoots, or alteration in distribution pattern of metal ions in the plants. Mostly, lead effects on mineral accumulation in aerial plant parts are similar and follow a common trend while in roots, it varies among plant species to species and amount of lead in the soil/rhizosphere (López *et al.*, 2007; Gopal and Rizvi, 2008). Reduced mineral uptake caused by Pb might be due to competition between metal ions of similar size to lead like K^+ ions with Pb (almost similar atomic radii) or fluctuations in plant physiological functions (Sharma and Dubey, 2005). Further, phosphorous is negatively correlated with soil Pb contents (Paivoke, 2002). Pb lowers the nitrate uptake from the soil but did not cause nitrogen efflux out of the plant cells. The reduction in nitrogen contents in plants exposed to Pb stress might be induced by the reduced nitrate reductase activity (the rate-limiting enzyme in nitrate assimilation) (Xiong *et al.*, 2006; Sengar *et al.*, 2009). A significant reduction in nitrate in paddy soils and nitrate reductase activity in rice seedlings under lead-polluted medium were also reported by Tariq and Rashid (2013) and Sharma and Dubey (2005). However, Burzynski and Grabowski (1984) concluded that decline in nitrate uptake might be due to moisture stress caused by Pb in the soil.

So, it is hard to conclude regarding nutrient relation with Pb toxicity as much work is still needed to explore the uptake and translocation mechanisms of nutrients, and plant-nutrient relation grown in lead-contaminated soil.

2.8 Oxidative stress

HMs toxicity including lead (Pb) quite often leads to the generation of reactive oxygen species (ROS) in rice that might be generated during cell metabolic processes, may appear as a byproduct of reduced form of molecular oxygen O_2 or due to excitation of highly energized molecules. The ROS may include superoxide anion radicals ($O^{2\bullet-}$), hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), and alkoxy radicals (RO) (Munné-Bosch and Penuelas, 2003). The over production of these species in plants is regarded as oxidative stress, a renowned feature of Pb stress (Liu *et al.*, 2008; Yadav, 2010). However, the degree of damage due to ROS depends on metal stress level, its type and form, plant nature, and time of exposure, etc. ROS react with cell membranes, organelles, and biomolecules like proteins, lipids, chloroplasts, nucleic acids (DNA or RNA), and diminished cell normal functions, induced cell abnormalities and death (Clemens, 2006; Singh *et al.*, 2010). Lead impaired cell membrane structures by interfering with lipid bilayer, changing its composition and causing leakage of K^+, as polyunsaturated fatty acids and their esters are highly vulnerable to ROS (Liu *et al.*, 2008; Gupta *et al.*, 2009). ROS forms reactive aldehydes by eliminating H_2 from unsaturated fatty acids and thus imparts cell membrane by distorting lipid bilayer (Mishra *et al.*, 2006). Pb ions thus cause lipid peroxidation and reduce saturated fatty acids while enhances unsaturated fatty acid contents of plasma membranes in many plant species including rice (Singh *et al.*, 2010). ROS reacts with bisallylic hydrogens present on polyunsaturated fatty acids in three different steps: (a) initiation (lipid radical formation), (b) progression (lipid peroxyl radical formation as a product of lipid radical + oxygen reaction), and (c) termination (production of non-radical product after molecular reactions with lipid peroxyl radicals) (Bhattacharjee, 2005). Resultantly, these changes in lipid membranes cause cell abnormalities by disrupting cellular membranes (Gupta *et al.*, 2009), cell organelles like mitochondria, peroxisomes (Małecka *et al.* 2008), and chloroplastic ultra structures (Hu *et al.*, 2007). Lipid peroxidase activity was increased more than 170 % when rice seedlings were exposed to Pb $(NO_3)_2$ for 20 days (Verma and Dubey, 2003).

2.9 Effect on Germination Stage

In *Triticum aestivum* and *Cucumis sativus*, lead toxicity decreases seed germination rate, length and weight of fresh and dry mass of roots and shoots (Munzuroglu and Geckil, 2002). These effects can be attributed to the fact that lead obstructed the absorption of seed germination and seedling growth in rice (He, 1990; Cai*et al.*, 2002). Pb decreases seed germination, length and biomass of root and shoot of rice seedlings (Mishra and Choudhuri, 1998). Rice seed germination rate and the amount/quality of chlorophyll decrease remarkably with increasing lead concentration. The decrease in grain yield of rice due to lead toxicity was reported by Chatterjee *et al.*, (2004).

Rice seed germination rate and the amount/quality of chlorophyll decrease remarkably with increasing lead concentration. The decrease in grain yield of rice due to lead toxicity was reported by Chatterjee *et al.* (2004).

Lead adversely affects seed germination, root/shoot ratio, and their fresh and dry weight in rice (Mishra and Choudhari, 1998). The effects were more adverse at higher concentrations of Pb2+ (Zeng *et al.*, 2006). A significant inhibition in morphological response and photosynthetic pigments with higher bioaccumulation of Pb in roots and ultrastructural aberrations in pollens were observed in two contrastive rice cultivars Pokkali and IRRI-112 against Pb stress (Arce and Yllano, 2008). Pb^{2+} at 0.5 to 1 mM significantly inhibited rice root length (about 40 %) in 20-day-old rice seedlings demolished viability of rice root cells and promoted cell death by triggering ROS production (Verma and Dubey, 2003). Yang *et al.* (2000) found a rapid development of adventitious roots and 10-fold higher root biomass with low Pb and higher oxalate contents in tolerant rice cultivars than sensitive ones which suggest that oxalate compounds are involved in reduced uptake of Pb. They further accentuated that exogenous feeding of oxalate to the growing medium ameliorate the Pb-induced root inhibition in rice. Pb inhibits rice seedling growth and viability and upregulates ROS production; however, enhanced Ca^{2+} accumulation, myelin basic protein (MBP) kinase (a signaling pathway for systematic responses under stress conditions) activities, calcium-dependent protein kinase (CDPK) (involved in rapid biochemical activation under abiotic stresses) inhibits Pb-induced cell death and mitogen-activated protein (MAP) kinase activation (one of the main pathways by which extracellular stimuli are transduced into intracellular responses) (Huang and Huang, 2008).

2.10 Effect on Plant morphology

Although Pb is not an essential element for plants, it is easily taken up by plants from the soil and accumulated in different organs. After being taken up by roots, the localization of Pb is greater in roots than in other parts of the plants (Sharma and Dubey, 2005). Pb binds strongly to the carboxyl groups of galacturonic acid and glucuronic acid in the cell wall, which restricts its transportation via apoplast (Rudakova *et al.*, 1988). Pb toxicity causes serious damage to seedling growth and chlorosis in leaves (Burton *et al.*, 1984, Hossain *et al.*, 2007).

Different parts of the plant absorb different quantities of heavy metals; the highest were shown in roots and leaves and the least amount were found in fruits and seeds reported by Natasa *et al.* (2015). The different accumulation rate among different parts of the rice plant was most probably caused by the restricted translocation of toxic metals between roots and shoots (Brekken and Steinnes, 2004). The restriction in the translocation of lead from roots to shoots and other above ground parts can be explained by a metal exclusion mechanism in roots by which plants avoid damages in their photosynthetic processes (Borisev *et al.*, 2008). It was assumed that mycorrizhal associations can greatly reduce metal translocation from roots to shoots by binding metals on cell wall components or intracellular immobilization (De Maria *et al.*, 2011).

2.11 Effect on Plant Physiology

It adversely affects plant's morpho-physiological and biochemical processes such as seed germination and seedling growth, plant phenology, and root/shoot ratio; disrupts cell membrane permeability, photosynthesis, plant respiratory processes, chlorophyll contents, chloroplastic lamellar organization and cell division; and cause growth and developmental abnormalities as well as ultrastructure changes (Dogan et al,. 2009; Ling and Hong, 2009; Gupta *et al.*, 2009, 2010; Maestri *et al.*, 2010).

Lead pollution coats the surface of the leaves and reduces the amount of light reaching it and as a result the growth of crops has become stunted or killing the plants by reducing photosynthesis, inhibiting respiration, encouraging an elongation of plant cells influencing root development by causing pre-mature aging (Chamon *et al.*,2005).

2.12 Effect on Photosynthesis

It has been reported that lead deposition in leaves of rice decreased the concentrations of chlorophyll contents. Lead produced highly significant effects on shoot, root lengths and seedling dry biomass of *Lythrum salicaria* (Juseph *et al.*, 2002). Waste water, which is used for irrigation. According to Sara Awan *et al.* (2015) lead stress negatively affected the vigor of seedlings and biochemical attributes such as ion, chlorophyll, nitrogen and protein contents. All the varieties can tolerate low concentrations of Pb but at higher concentration of 500 and 1000 ppm, it causes inhibitory effects. The effect of Pb is different in each variety.

Lead stress negatively affected the vigor of seedlings and biochemical attributes such as ion, chlorophyll, nitrogen and protein contents (Awan *et al.*, 2015). All the varieties can tolerate low concentrations of Pb but at higher concentration of 500 and 1000 ppm, it causes inhibitory effects. The effect of Pb is different in each variety. SB is found to be more sensitive at higher Pb concentrations.

Lead, being a heavy metal, interacts with photosynthetic machinery during plant development, i.e., hyper-accumulation in leaves and then subsequent partitioning in to various leaf tissues, e.g., stomata and leaf mesophyll, interacts with cytosolic enzymes and their functions, chloroplast and acyl lipid membranes, interfere with PS II and PS I, as well as interferes at molecular level with normal physio-anatomical and biochemical functions (Prasad and Strzałka, 1999). Lead toxicity inhibited photosynthesis adversely by distortion of chloroplast structure, reduction in rate of chlorophyll, plastoquinone and carotenoid synthesis, disruption of electron transport chain, cause CO2 deficiency by stomatal closure, and reduction in the enzymatic activity of Calvin cycle (Xiong *et al.*, 2006; Singh *et al.*, 2010). It also causes changes in lipid composition of thylakoid membranes in the chloroplasts (Stefanov *et al.*, 1995). Pb diminished chlorophyll production by reduced uptake of Mg and Fe (essential components of chlorophyll) (Burzynski, 1987).

The main reasons of reduced photosynthetic activity due to Pb may be the affinity of lead for protein N and S ligands; cause damage to chloroplastic ultrastructure (Islam *et al.*, 2007); hindrance in the electron transport chain (ETC) reactions (Qufei and Fashui, 2009); breakdown of chlorophyll contents by enhanced activity of chlorophyllase (Liu *et al.,* 2008); inhibition and substitution of Mg and Fe by Pb (divalent cations) in chlorophyll (Chatterjee *et al.* 2004; Cenkci *et al.*, 2010); reduced activities of ferredoxin NADP+ reductase as well as delta-aminolevulinic acid dehydratase at the source of chlorophyll production (Gupta *et al.*, 2009); reduced CO_2 levels due to stomatal closure (Romanowska *et al.* 2006); plastoquinone and carotenoid inhibition (Chen *et al.,* 2007; Cenkci *et al.,* 2010); and inhibited enzymatic catalysis of Calvin cycle (Liu *et al.,* 2008). The effect of Pb toxicity on photosynthetic machinery varies with rice genotype. Normally, chlorophyll b is more prone to distortion under Pb-stressed conditions than chlorophyll a (Xiong *et al.,* 2006). Pb effects on PS I, cytochrome b/f complex, as well as donor and acceptor sites of PS II. Further, it was reported that electron transport of PS II is more susceptible to Pb than PS I (Sersen *et al.,* 1998). Pb displaces Cl−, Ca2+ and Mn2+ from oxygen evolving complex of PS II and cause its dissociation (Rashid *et al.*, 1991). In vitro conformational changes have been observed in the subunits of light-harvesting chlorophyll (LHC) following Pb binding (Ahmed and Tajmir-Riahi 1993). The process of chlorophyll cessation is catalyzed by chlorophyllase, pheophorbide oxygenase, red chlorophyll catabolite reductase, and Mg-dechelatase that dissociates it in to magnesium, phytol, and product of porphyrin after primary cleavage, that is also responsible for chlorophyll bleaching (loss of green color) (Harpaz-Saad *et al.*, 2007). However, in a study conducted by Bazzaz *et al.* (1975), it was reported that inhibition of photosynthesis due to Pb may be due to stomatal closure and not because of direct effect of Pb on photosynthesis.

2.13 Effect on Yield

Rice growth and yield reduced when exposed to lead. Pb^{2+} in the soil (1000 ppm) not only reduced rice biomass but also paddy yield about 12% (Gu *et al.,* 1989). In a study conducted by Xie and Huang (1994), they found that lead, even at 2500 ppm, did not affect the growth of a hybrid rice genotype named BShan you 63. Moreover, increased rice biomass and yield in rice at 100 ppm has also been reported by (Wang *et al.*, 1997). Conversely, Li *et al.* (2007) found significant reductions in rice growth and yield at 1200 mg of lead kg^{-1} of soil than control and found lower Pb^{2+} contents in rice grains than root, shoot, and leaves. About 1216, 179, and 62 times higher lead contents were recorded in roots, stems, and leaves, respectively, than grains at maturity (Liu *et al.,* 2003). So, a great variation exists among lead concentration within plant parts. Normally, lead concentration is recorded from maximum to minimum in the following order: root > shoot > ear (up to heading stage) > grain (at ripening stage). The degree of variation among these parts significantly depends on soil lead concentration and plant growth stages. Furthermore, grain lead concentration significantly correlates with shoot and ear lead contents at heading stage (Liu *et al.,* 2013). A positive correlation between grain yield vs straw yield and grain yield vs spikelets per panicle exhibited that if paddy yield reduced due to lead toxicity, then rice biomass will also reduce under Pb toxic conditions whereas relative changes of grain yield have a significant correlation with changes in spikelet numbers produced.

CHAPTER III

MATERIALS AND METHODS

The pot experiment was conducted from December 2020 to May 2021 comprising of collection of seed, raising of seedlings, growing and experimentation, data collection, compilation, etc. to study the effect of Pb in growth, development and yield of rice. A brief of soil, climate, materials and methods used for conducting the experiment is presented below.

3.1 Location of the experimental site

The experiment was set at the Net House and Plant Physiology Laboratory of the Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. The location of the pot experiment at 240 75′ N latitude and 900 50′ E longitude at the elevation of above 18m of sea level and it was under the Agro-Ecological Zone-28, namely Madhupur Tract. For better understanding the experimental location, the Map of AEZ of Bangladesh has been added in Appendix IV.

3.2 Characteristics of Soil that used in Pot

The soils used in pot were collected from the experimental field of Department of Agricultural Botany, SAU, Dhaka. The pot experiment was conducted by using typical rice growing silty loam soil having non-calcarious properties. The soil was Deep Red Brown Terrace Soil under Tejgaon Series belonging to the Agro-Ecological Zone of Madhupur Tract. The soil for the pot was collected from 0-15 cm depth. The collected soil was pulverized followed by the removal of weeds, stubble, brick pieces, insects, etc. The soil was then sun dried, crushed and passed through a 2 mm sieve. After that the soils were mixed up properly and 400 g soil was taken for initial physical and chemical analysis. The morphological properties of this soil have been presented in Appendix II and the physio-chemical properties in Appendix III.

3.3 Climate

The site of the study was characterized by a subtropical monsoon climatic zone. Moderately low temperature along with moderate rainfall prevailed during the period from November to April. The cool and dry weather prevailed during January to March with the mean temperature 27.6°C. Temperature during February to April was moderately hot but highly humid along with moderate to high rainfall.

3.4 Planting Material

Oryza sativa L. cv. BRRI dhan29 and cv. BRRI dhan58 was used as test crop which is a stress tolerant rice variety recommended for cultivation in Boro season. These varieties were developed at Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur and was released for farmers use in 1994 and 2012. Plant height of BRRI dhan 29 is 95 cm, medium slender and white, planting season is Robi, Boro (late October to mid-November), Yield is 7.5t/ha. Plant height of BRRI dhan58 is 100-105 cm, in vegetative stage size and shape taller than BRRI dhan29, grain as like BRRI dhan29 but slight slender,1000-grain weight 24 g, ripe grain colour as like straw colour, life time 150-155 days. Planting season is Boro season, seedling in seed bed late November to Mid-December, yield is 7-7.5 t/ha.

3.5 Treatments

The pot experiment consisted of two factors as shown below:

Factor A: Different levels of Lead (PbSO4, Lead (II) Sulfate) with irrigation water

i. Pb_0 = 0 mM $PbSO_4$, Lead(II) sulfate

ii. Pb_1 = 2 mM $PbSO_4$

iii. Pb_2 = 4 mM $PbSO_4$

iv. Pb_3 = 6 mM $PbSO_4$

Factor B: Name of two varieties

i. V_1 = *Oryza sativa* L. cv. BRRI dhan29

ii. V_2 = *Oryza sativa* L. cv. BRRI dhan58

There were following 8 treatment combinations:

1. V_1Pb_o/ C = *Oryza sativa* L. cv. BRRI dhan29 + 0 mM $PbSO_4$
2. V_1Pb_1 = *Oryza sativa* L. cv. BRRI dhan29 + 2 mM $PbSO_4$
3. V_1Pb_2 = *Oryza sativa* L. cv. BRRI dhan29 + 4 mM $PbSO_4$
4. V_1Pb_3= *Oryza sativa* L. cv. BRRI dhan29 + 6 mM $PbSO_4$
5. V_2Pb_o/ C = *Oryza sativa* L. cv. BRRI dhan58 + 0 mM $PbSO_4$
6. V_2Pb_1= *Oryza sativa* L. cv. BRRI dhan58 + 2 mM $PbSO_4$
7. V_2Pb_2= *Oryza sativa* L. cv. BRRI dhan58 + 4 mM $PbSO_4$
8. V_2Pb_3= *Oryza sativa* L. cv. BRRI dhan58 + 6 mM $PbSO_4$

3.5.1 Lead treatment

There were four lead levels including control which were prepared by adding respected amount Lead ($PbSO_4$, Lead (II) Sulfate) to the soil/pot as water dissolved solution. The lead treatments were Pb_0 (Control), Pb_1 (2 mM $PbSO_4$), Pb_2 (4 mM $PbSO_4$) and Pb_3 (6 mM $PbSO_4$). To spread lead homogeneously in each pot, lead was dissolved in water and were given to pots as irrigation water for proper lead imposition. When no lead added it termed as control (C) Pb_0.The lead treatments were applied at 15 days after transplanting (DAT).

3.6 Experimental Design and Layout

The experiment was laid out in a Randomized Complete Block Design (RCBD) as two factorial arrangements with three replications. The experimental area was divided into three equal blocks. Each contain 8 pots where 8 treatment combinations were allotted randomly. There were total 24 (8×3) pots in the experiment. The layout of the experiment has been shown in Appendix V.

3.7 Collection of Planting Material

Seeds of *Oryza sativa* L. cv. BRRI dhan29 and *Oryza sativa* L. cv. BRRI dhan58 were used as planting material, which were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur.

3.8 Pot Preparation

Plastic pots were used in this experiment. The diameter of each pot was 35 cm (14 inches) at the top and 15 cm (6 inches) at the bottom. The depth of each pot was 30 cm (12 inches). The collected soil was sun dried, crushed and passed through a sieve to remove weeds, stubble, brick pieces, insects, etc. The dry soil was then thoroughly mixed up with well rotten cow dung (75 g for 12 kg soil) before filling the pots. Each pot was filled up with 12 kg soil on 20 January, 2021 and all experimental pots received recommended doses of N, P and K fertilizers. After that the pots were pre-labeled for each treatment combination and placed at the net house of the Department of Agricultural Botany. At last, measured water was added to bring soil at field capacity condition.

3.9 Manure and Fertilizer Application

Well rotten cow dung at the rate of 12.5 ton ha^{-1} mixed up with soil before filling the pot. The following fertilizers i.e., urea, triple super phosphate (TSP), muriate of potash (MoP), gypsum and $ZnSO_4$ were used as sources of nitrogen, phosphorus, potassium, sulfur and zinc were applied at a rate recommended by BARI for the variety BRRI dhan67 shown in tabular form below.

Table 3.1. Manures and Fertilizers applied for the experimental pot

Manures and Fertilizers	Dose $bigha^{-1}$	Dose ha^{-1}	Dose Pot^{-1}	Application (%)			
				Basal	15 DAT	35 DAT	55 DAT
Cow dung	---	12.5 Ton	75 g	100	---	---	---
Urea	36 kg	269 kg	1.62 g	---	33.33	33.33	33.33
TSP	13 kg	97 kg	0.66 g	100	---	---	---
MoP	16 kg	120 kg	0.72 g	100	---	---	---
Zypsum	13 kg	97 kg	0.58 g	100	---	---	---
$ZnSO_4$	1.5 kg	11 kg	0.066 g	100	---	---	---

The weight of 1 ha soil at the depth of 15 cm is considered approximately 2 million kg of soil. According to the above rate, manures and fertilizers were calculated as per pot that contained 12 kg soil. The whole amounts of TSP, MoP, Zypsum and ZnSO4 were applied during the final pot preparation. Urea was applied in three equal splits at 15, 35 and 55 days after transplanting (DAT).

3.10 Seedbed Preparation

Wet seedbed was prepared by December 7, 2020 and sprouted seeds were sown on December 8, 2020 following the recommendation of BRRI (BRRI 1995).

3.11 Seedling Raising

A very common procedure was followed in raising of seedlings i.e., the seeds were soaked for 48 hours and then washed properly in fresh water and after that incubated for sprouting. The sprouted seeds were sown in the wet seedbed on December 8, 2020.

3.12 Uprooting and Transplanting of Seedlings

Healthy and uniform seedlings of thirty days old were uprooted carefully from the seedbed and were transplanted in the experimental pots at the rate of single seedling hill^{-1} on January 27, 2021 maintaining three seedlings in each pot. The seedbed was watered before uprooting the seedlings from the seedbed to minimize the root damage. The seedlings were watered after transplanting in the pot for their better establishment. After one week of transplanting all the experimental pots were checked for any missing hill, which was filled up with extra seedlings.

3.13 Intercultural Operations

After transplantation of seedlings, different intercultural operations like weeding, irrigation, plant protection measures etc. were accomplished for better growth and development of the seedlings.

3.13.1 Weeding and Irrigation

The hand weeding was done as when necessary to keep the experimental pots free from small aquatic weeds. Irrigation was done whenever necessary but the frequency of irrigation became less in harvesting stage. Irrigation was done at evening as salt was applied with irrigation water.

3.14 General observation of the experimental pots

The plants were under regular observation and the plants looked normal green except the plants treated with salt. No lodging was observed but the maximum tillering, panicle initiation, and flowering stages were not uniform.

3.15 Detection of maximum tillering and panicle initiation stage

Maximum tillering and panicle initiation stages were detected through regular inspection. When the number of tillers hill^{-1} reached the highest number and after that decreasing in trend, was considered as maximum tillering stage. When a small growth at the top of upper most nodes of main stem was noted like a dome was considered as an indication of the beginning of panicle initiation stage. But these stages were not uniform and were varied with treatments.

3.16 Harvesting

The crops were harvested at maturity when 80-90% were turned into straw colored on May 28, 2021. The crop was cut at the ground level and pot wise crop was bundled separately, tagged and brought to the threshing floor. The grains were then sun dried to a moisture content of 12% and straw was also sun dried properly. The grain and straw yields and different plant physiological parameters were recorded after harvesting.

3.17 Data Collection

The data on the following parameters were collected from each treatment.

A. Morphological parameters

- Plant Height (cm)
- No. of Tillers Plant^{-1}
- Leaf Area (cm^2)

B. Physiological parameters

- SPAD Value
- Leaf Membrane Stability Index (%MSI)
- Relative Water Content (%RWC)
- Dry Weight of Root
- Dry Weight of Stem
- Dry Weight of Leaf
- Total Dry Matter (TDM)
- Proline Content

C. Yield contributing and other parameters

- Panicle Length (cm)
- No. of Effective Tillers Plant^{-1}
- No. of Non-Effective Tillers Plant^{-1}
- No. of Filled Grains Panicle^{-1}
- No. of Unfilled Grains Panicle^{-1}
- 1000 Grain Weight (g)

D. Yields

- Grain Yield Plant^{-1}

3.18 Detailed Procedures of Recording Data

A brief outline of the data collecting procedure followed during the experiment is given below:

3.18.1 Plant Height (cm)

Plant height was measured in centimeter from 30 days after transplanting (DAT) at 15 days interval up to 120 DAT, beginning from the top surface level of the pot to the tip of the longest leaf at booting and flowering stage and at maturity stage, from the top surface level of the pot to the tip of the tipper end of the longest panicle.

3.18.2 Number of Tillers Plant^{-1}

Tillers, which had at least one visible leaf were counted from 30 days after transplanting (DAT) at 15 days' interval up to 120 DAT.

3.18.3 Leaf Area (cm^2)

Leaf area was measured in centimeter^2 by non-destructive method at heading stage.

3.18.4 Leaf Membrane Stability Index (MSI%)

The plasma membrane stability or intactness was estimated through the leakage of electrolytes. Fresh leaf trips (0.2 g) of uniform size were placed in test tubes, containing 10 ml distilled water and kept for 30 minutes in water bath at 40 °C for measuring the initial electrolyte conductivity (C1). The final electrolyte conductivity (C2) was measured after boiling the plant samples for 15 minutes at 100 °C. MSI was calculated as-

$$\text{MSI} = \left(1 - \frac{C1}{C2}\right) \times 100$$

3.18.5 Relative water content (%RWC)

Relative water content (%RWC) was measured according to the following method suggested by Barrs and Weatherley (1962). From each experimental pot three leaves were randomly selected and cut with scissors. Fresh weight (FW) of leaf laminas were taken and then immediately floated on distilled water in a Petri dish for 4 hours in the dark. After drying excess surface water with paper towels turgid weights (TW) were measured. Then the sample was oven dried at 80 °C for 48 hours and dry weights (DW) were measured. RWC% was calculated by the following formula:

$$\text{RWC}(\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

3.18.6 SPAD Value

Leaf chlorophyll content was measured by using a hand-held chlorophyll content SPAD meter (SPAD 502, Konica Minolta, Japan). At each evaluation the chlorophyll content was measured five times from three randomly selected leaves at different positions plant^{-1} and the average was used for analysis.

3.18.7 Dry Weight of Root

After harvesting, roots of the plants were very carefully separated from the soil and then sun dried. Then they were sliced into small pieces to put into pre-labeled envelop and placed in oven for 72 hours at 70 °C. After oven drying the samples were put into desiccators to cool down at room temperature. Then dry weight of root was taken.

3.18.8 Dry Weight of Stem

After harvesting, stems of the plants were separated from the leaf and then sun dried. Then they were sliced into very thin pieces to put into pre-labeled envelop and placed in oven for 72 hours at 70 °C. After oven drying the samples were put into desiccators to cool down at room temperature. Then dry weight of stem was taken.

3.18.9 Dry Weight of Leaf

After harvesting, leaves of the plants were collected and sun dried. Then they were sliced into small pieces and were put into pre-labeled envelop and placed in oven for 72 hours at 70 °C. After oven drying the samples were put into desiccators to cool down at room temperature. Then dry weight of the sample was taken.

3.18.10 Total Dry Matter (TDM)

The plant parts i.e., roots, stems, leaves and panicles were detached from each other and were kept separately in oven for 72 hours at 70 °C. The oven dried samples of these plant parts were weighted for dry matter production. The total dry matter production was calculated from the summation of dry matter produced by the above-mentioned plant parts and grain weight per plants in gram.

3.18.11 No. of Effective and Non-Effective Tillers Plant^{-1}

The total number of tillers plant-1 was counted from the experimental pots at maturity and were grouped into effective (panicle bearing tillers) and non-effective tillers plant^{-1}.

3.18.12 No. of Filled Grains and Unfilled Grains Panicle^{-1}

Each grain was tested for whether it was filled or not by pressing the grain between the forefinger and the thumb. In case of more than 5 effective tillers plant^{-1}, grains of 5 randomly selected panicles of each experimental pot were counted and then the average number of filled and unfilled grains for each panicle was determined. In case of less than 5 effective tillers plant^{-1}, grains of all the panicles plant^{-1} were counted and then the average number of filled and unfilled grains for each panicle was determined.

3.18.13 Thousand Grain Weight (g)

200 clean sundried grains were counted from the seed stock obtained from the sample plants and weighed by using an electronic balance and then multiplied by 5.

3.18.14 Grain Yield Plant^{-1}

The grains plant^{-1} was separated by threshing and then properly sun dried and weighed to get grain yield plant^{-1}.

3.19 Statistical Analysis

The recorded data of different parameters were statistically analyzed to get the level of significance using the Statistix 10 computer package program. Analysis of variance was calculated following two factors randomized complete block design. The mean differences among the treatments were compared by least significant difference (LSD) test at 5% level of significance.

CHAPTER IV

RESULT AND DISCUSSION

Effect of lead toxicity on growth, development and yield in the present study were presented in the tables and figures and discussed. A summary of the analysis of variance (ANOVA) with regards to all the studied parameters has been shown in Appendices VI to XII. The results obtained in the experiment were presented and discussed under the following sub-headings.

4.1 Results

4.1.1 Plant Height

Subjecting rice plants to lead stress caused a significant ($P \leq 0.01$) reduction in plant height (cm) at 30 DAT, 60 DAT, 90 DAT and, at harvest compared to the control treatment without lead stress (Figure 4.1.1 and Appendix VI). A clear difference was noticed between the plants grown under lead stress conditions and control conditions. For, the Variety 1 at 30 DAT, 60 DAT, 90 DAT and harvest time with 2mM lead levels (Pb_1 treatment) plant height was decreased by 6.13%, 4.47%, 3.92%, 3.43%, with 4 mM lead levels (Pb_2 treatment) by 17.48%, 12.76%, 11.18%, 9.79% and with 6 mM lead levels (Pb_3 treatment) by 29.98%, 22.33%, 20.74%, 16.80% respectively, when compared to control treatment. Fig.4.1.1 showed that plant height decreased gradually with increasing lead stress.

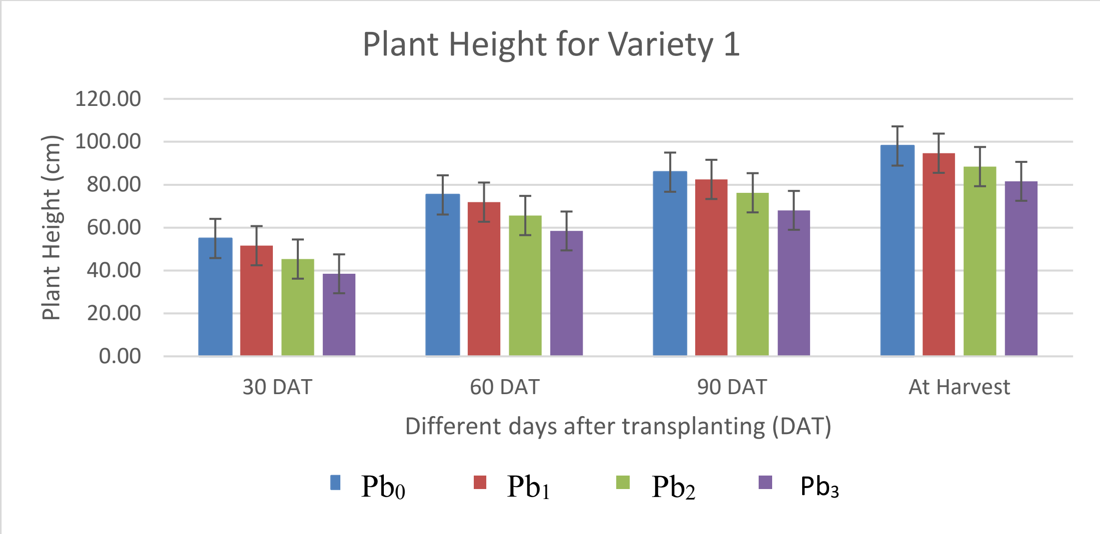

Figure 4.1.1. Effect of different lead concentrations on the plant height of rice variety 1 at different days after transplanting (LSD $_{(0.05)}$ = 2.60, 1.86, 1.80 and 1.43 at 30, 60, 90 DAT and at harvest, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

For variety 2 rice plants to lead stress also caused a significant ($P \leq 0.01$) reduction in plant height (cm) at 30 DAT, 60 DAT, 90 DAT and, at harvest compared to the control treatment without lead stress (Figure 4.1.2). A clear difference was noticed between the plants grown under lead stress conditions and control conditions. For, the Variety 2 at 30 DAT, 60 DAT, 90 DAT and at harvest with 2 mM lead levels (Pb_1 treatment) plant height was decreased by 7.66%, 5.69%, 5.02%, 4.42%, with 4 mM lead levels (Pb_2 treatment) by 18.05%, 13.41%, 11.83%, 10.41% and with 6 mM lead levels (Pb_3 treatment) by 28.94%, 22.35%, 18.97%, 17.02% respectively, when compared to control treatment. Fig.4.1.2 showed that plant height decreased gradually with increasing lead stress.

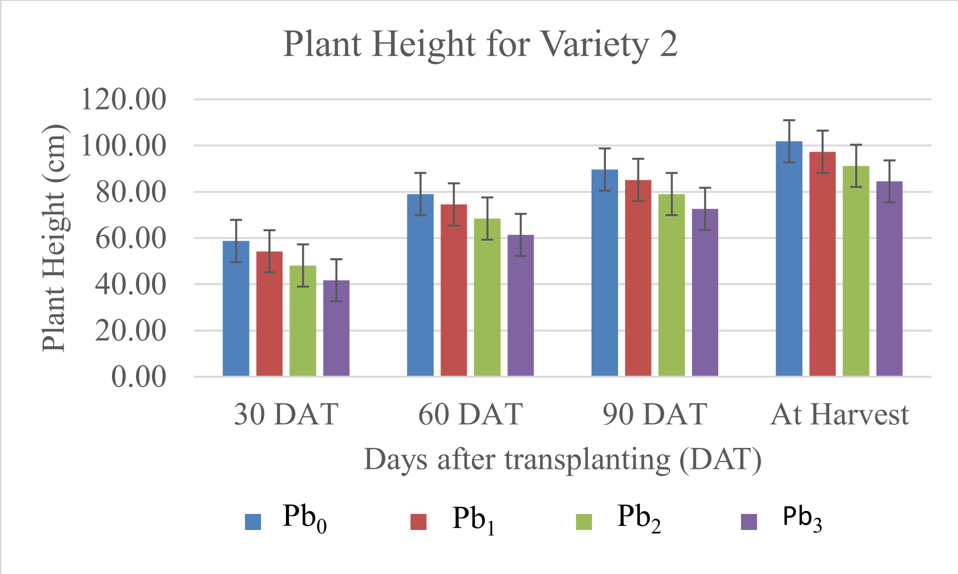

Figure 4.1.2. Effect of different lead concentrations on the plant height of rice variety 2 at different days after transplanting (LSD $_{(0.05)}$ = 2.60, 1.86, 1.80 and 1.43 at 30, 60, 90 DAT and at harvest, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Plant height of rice plants decreased significantly under lead stress (Table 4.1 and Appendix VI)

Table 4.1. Interaction effect of different Lead (Pb) concentrations and rice varieties on the plant height at different days after transplanting

Treatment	**Plant Height (cm)**			
	30 DAT	**60DAT**	**90DAT**	**At Harvest**
V_1Pb_0	54.933 b	75.233 b	85.833 b	98.03 b
V_1Pb_1	51.567 c	71.867 c	82.467 c	94.67 c
V_1Pb_2	45.333 e	65.633 e	76.233 e	88.43 e
V_1Pb_3	37.867 g	56.833 g	66.100 g	79.97 g
V_2Pb_0	58.733 a	79.033 a	89.633 a	101.83 a
V_2Pb_1	54.233 b	74.533 b	85.133 b	97.33 b
V_2Pb_2	48.133 d	68.433 d	79.033 d	91.23 d
V_2Pb_3	41.733 f	61.367 f	72.633 f	84.50 f
LSD $_{(0.05)}$	2.38	2.32	2.11	2.39
CV (%)	2.60	1.86	1.80	1.43

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

The key factor in Pb-induced toxicity in plants is its transportation to various parts of the plants via vascular bundles (Ashraf *et al.*, 2017). However, the adverse effects of Pb depend on the exposure time, concentration and intensity, plant stage, and its availability in different parts of the plant. The previous findings suggest that HMs including Pb, have toxic effects on various physio-biochemical processes at different stages of rice growth and development (Ashraf *et al.*, 2017; Xie *et al.*, 2018). There are some research findings where Pb-induced growth reduction in rice was reported. Rice growth and yield reduced when exposed to lead in the soil (1000 ppm) and reduction of paddy yield was about 12 % (Gu *et al.,* 1989). Li *et al.* (2007) found significant reductions in rice growth and yield at 1200 mg of lead kg^{-1} of soil than control and found Pb^{2+} contents in rice grains than root, shoot, and leaves. Pb (1.2 mM) induced significant reduction in agronomic traits such as plant height, number of tillers per plant were recorded in different rice cultivars (Ashraf *et al.,* 2015). Altered plant water relation, reduced nutrient uptake, destruction of photosynthetic pigments, reduction of photosynthetic rate are common reasons for growth reduction under Pd toxicity.

4.1.2 No. of Tillers Plant^{-1}

Tiller formation in rice is a very important agronomic trait for grain production and number of tillers provide valuable information about the stress profile of a plant under abiotic stress (Suzuki *et al.*, 2005). The number of tillers plant^{-1} were significantly ($P \leq 0.01$) reduced by increasing level of lead (Figure 4.2.1 and Appendix VII). Pb_1 Pb_2 and Pb_3 treatment reduced tiller number by 13.20%, 25.38% and 44.71% respectively for variety 1.

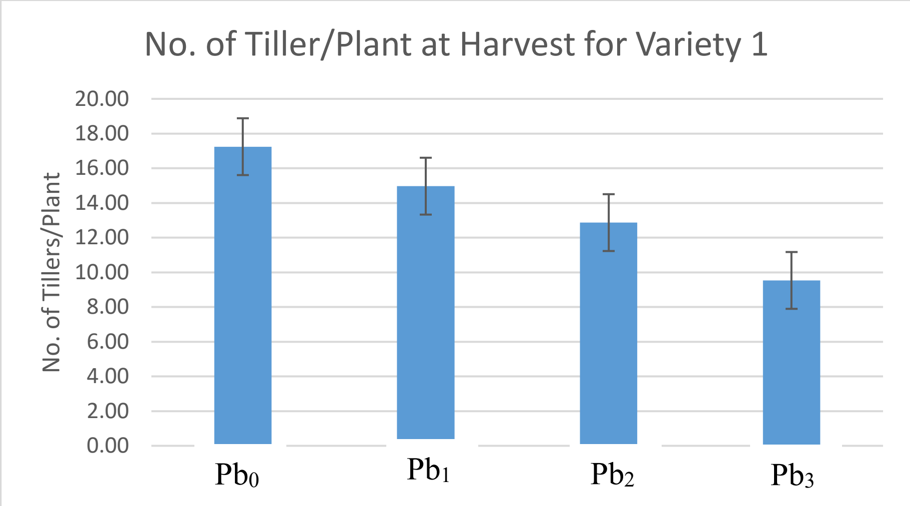

Figure 4.2.1. Effect of different lead concentrations for rice variety 1 on number of tiller plant^{-1} (LSD $_{(0.05)}$ = 0.92)

For Variety 2 tiller number per plant at harvest was decreased by 7.69% 15.57% and 32.42% respectively when compared to control (Figure 4.2.2 and Appendix VII). In respect of lead effect, the result showed that the number of tillers plant^{-1} was greatly affected even at Pb_1 and Pb_2 treatment but the maximum reduction in the number of tillers plant^{-1} was found at Pb_3 treatment.

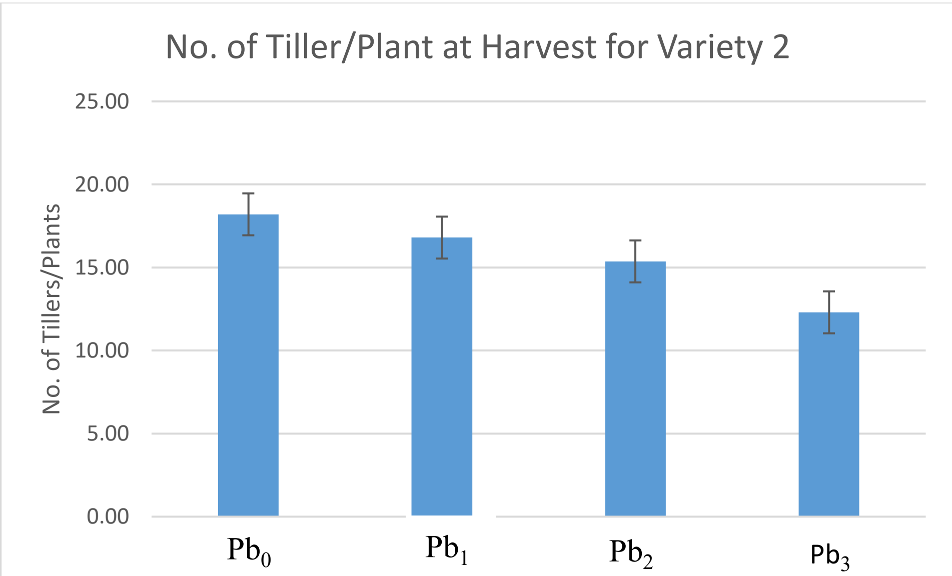

Figure 4.2.2. Effect of different lead concentrations for variety 2 on number of tiller plant^{-1} (LSD $_{(0.05)}$ = 0.92)

Results showed that total number of tillers plant^{-1} was significantly lowered by increasing the concentration of lead (Table 4.2 and Appendix VII)

Table 4.2. Interaction effect of different lead concentrations and rice varieties levels on the number of tillers plant^{-1} of rice at harvest

Treatment	Number of Tillers Plant^{-1} at Harvest
V_1Pb_0	17.24 b
V_1Pb_1	14.97c
V_1Pb_2	12.87 d
V_1Pb_3	9.53 e
V_2Pb_0	18.20 a
V_2Pb_1	16.80 b
V_2Pb_2	15.37 c
V_2Pb_3	12.30 d
$LSD_{(0.05)}$	0.92
CV (%)	3.58

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD

Lead toxicity reduces nutrient uptake, decreases assimilate translocation to different parts. Reduction of tiller number has been recorded under Pb stress. The study of the present experiment also showed similar result. Reduction in tiller number was higher with the increase of Pb concentration. Pb (1.2 mM) induced significant reduction in agronomic traits such as plant height, number of tillers per plant were recorded in different rice cultivars (Ashraf *et al.,* 2015). No. of tiller of rice plant decreased under various concentration of Pb (50, 100, 150 and 200 mg kg^{-1}) stress (Jasmin *et al.,* 2015).

4.1.3 Leaf Area (cm^2)

The most immediate response to lead stress was the decrease in the expansion rate of the leaf surface area. The leaf area of rice plants was significantly ($P \leq 0.01$) affected by lead stress (Figure 4.3.1 and Appendix VII). The highest leaf area was observed with control (Pb_0) treatment while the lowest was observed with Pb_3 treatment 9.28%, 20.13% and 31.28% reduction in leaf area was noticed for variety 1 in lead-affected Pb_1 , Pb_2 and Pb_3 plants respectively when compared to control.

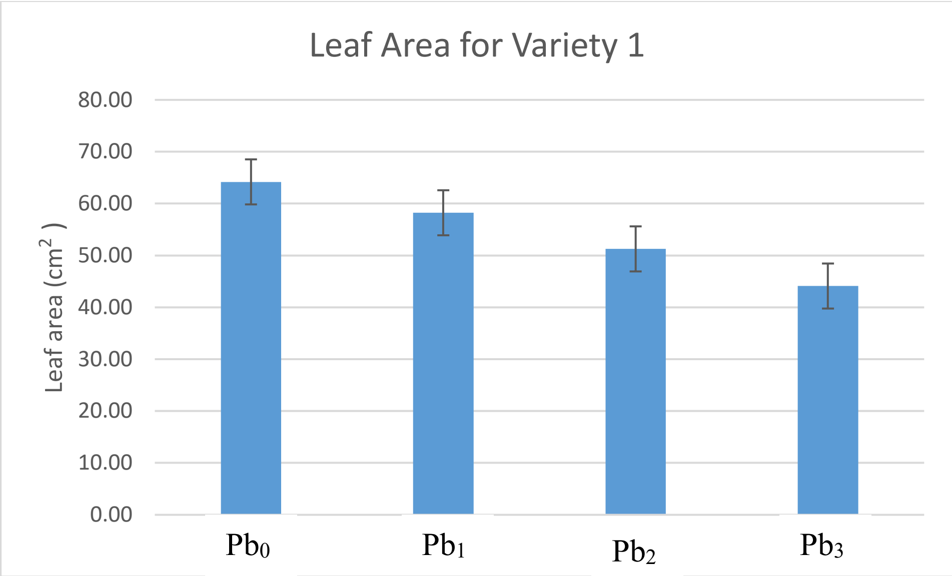

Figure 4.3.1. Effect of different lead concentrations on the leaf area of variety 1 rice (LSD $_{(0.05)}$ = 2.62 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

For variety 2 response to lead stress was a decrease in the expansion rate of the leaf surface area. The leaf area of rice plants was significantly ($P \leq 0.01$) affected by lead stress (Figure 4.3.2 and Appendix VII). The highest leaf area was observed with control (Pb$_0$) treatment while the lowest was observed with Pb$_3$ treatment 10.63%, 20.54% and 29.32% reduction in leaf area was noticed for variety 2 in lead-affected Pb$_1$, Pb$_2$ and Pb$_3$ plants respectively when compared to control.

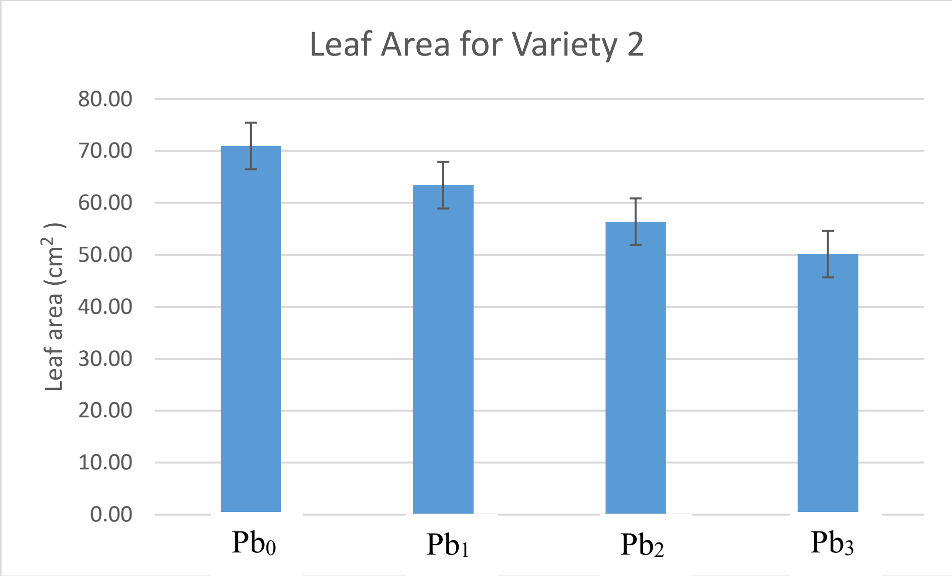

Figure 4.3.2. Effect of different lead concentrations on the leaf area of variety 2 rice (LSD $_{(0.05)}$ = 2.62 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Statistical analysis has shown that lead reduced the leaf area of rice plants significantly (Table 4.3 and Appendix VII).

Table 4.3. Interaction effect of different lead concentrations and rice varieties on the leaf area

Treatment	Leaf Area (cm^2)
V_1Pb_0	64.19 b
V_1Pb_1	58.23 c
V_1Pb_2	51.27 d
V_1Pb_3	44.11 e
V_2Pb_0	70.97 a
V_2Pb_1	63.42 b
V_2Pb_2	56.40 c
V_2Pb_3	50.16 d
LSD$_{(0.05)}$	2.62
CV%	2.61

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

Lead stress decreased leaf area of the rice seedlings in the present study. The more the concentration the more the decrease in leaf area was demonstrated. The Caryopses of rice (*Oryza sativa* cv. Koshihikari) was placed at different concentrations of $Pb(NO_3)_2$ like 1 - 30 µM Pb up to 5 days. The length of leaf, length of coleoptile decreased under those stresses (Hossain *et al.,* 2015). Kulaz *et al.* (2021) noticed decrease in leaf area index when soybean plants were exposed to of lead (0, 25, 50, 75, 100 mg L^{-1}).

4.1.4 Leaf Membrane Stability Index (MSI%)

One of the major influences of lead stress include changes in membrane permeability leading to destabilization of membrane proteins. That's why, electrolyte leakage was measured to determine the leaf cell membrane stability index (MSI%). Membrane stability index (MSI%) of variety 1 plants decreased significantly ($P \leq 0.01$) with the increment of lead stress (Figure 4.4.1 and Appendix VII). MSI% decreased by 4.83%, 17.65% and 29.23% under Pb_1, Pb_2 and Pb_3 lead stress respectively compared to control. The exposure of rice plants to lead stress reduced the MSI% and higher reduction was found at Pb_3 treatment.

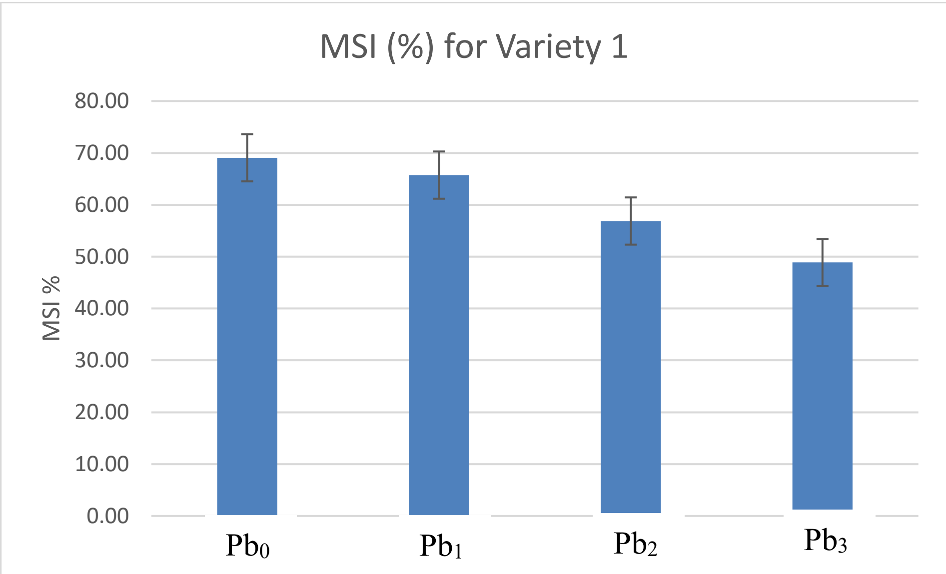

Figure 4.4.1. Effect of different lead levels on the membrane stability index (MSI%) of rice variety 1 ($LSD_{(0.05)} = 6.85$ and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

For variety 2 membrane stability index (MSI%) of plants decreased significantly ($P \leq 0.01$) with the increment of lead stress (Figure 4.4.2 and Appendix VII). MSI% decreased by 4.42%, 16.23% and 26.49% under Pb_1, Pb_2 and Pb_3 lead stress respectively compared to control. The exposure of rice plants to lead stress reduced the MSI% and higher reduction was found at Pb_3 treatment.

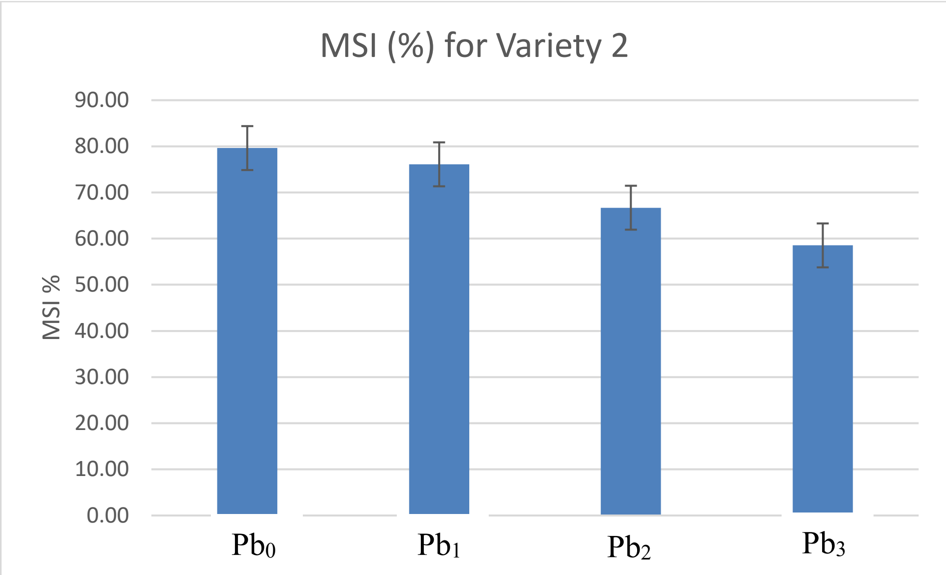

Figure 4.4.2. Effect of different lead levels on the membrane stability index (MSI%) of rice variety 2 (LSD $_{(0.05)}$ = 6.85 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Statistical analysis has shown that lead reduced the MSI% of rice plants significantly (Table 4.4).

Table 4.4. Interaction effect of different lead concentrations and rice varieties on MSI%

Treatment	MSI%
V_1Pb_0	69.06 b
V_1Pb_1	65.72 b
V_1Pb_2	56.87 c
V_1Pb_3	48.87 d
V_2Pb_0	79.62 a
V_2Pb_1	76.10 a
V_2Pb_2	66.70 b
V_2Pb_3	58.33 c
LSD(0.05)	6.85
CV%	6.00

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

Heavy metal toxicity including Pb quite often leads to the generation of reactive oxygen species (ROS) in rice that might be generated during cell metabolic processes, may appear as a byproduct of reduced form of molecular oxygen O_2 or due to excitation of highly energized molecules. The ROS may include superoxide anion radicals (O_2^-), hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), and alkoxy radicals (RO) (Munné Bosch and Penuelas 2003). The over production of these species in plants is regarded as oxidative stress, a renowned feature of Pb stress (Yadav 2010). Lead impaired cell membrane structures by interfering with lipid bilayer, changing its composition and causing leakage of K^+, as polyunsaturated fatty acids and their esters are highly vulnerable to ROS (Liu *et al.,* 2008; Gupta *et al.,* 2009). ROS forms reactive aldehydes by eliminating H_2 from unsaturated fatty acids and thus imparts cell membrane by distorting lipid bilayer (Mishra *et al.*, 2006). Pb ions thus cause lipid peroxidation and reduce saturated fatty acids while enhances unsaturated fatty acid contents of plasma membranes in many plant species including rice (Singh *et al.,* 2010). Therefore, cell membrane damage is one of the common damaging effects under stress condition. In the present study, Pb stress caused membrane damage which is evident from decreased MSI. The data represent that MSI decreased with the increase of Pb concentration in the soil. Increased superoxide anion generation and electrolyte leakage was observed in rice plant under Pb stress (Khan *et al.,* 2021). Rice plant was grown in different Pb concentration like 0.1, 0.3, 1, 10 μM for three days. Cell wall extensibility of apical region of rice root decreased gradually with the increase of Pb concentration (Hossain *et al.,* 2015). Increased production of hydrogen peroxide (H_2O_2) and malanodialdehyde (MDA) recorded in scented rice plants when were exposed to 400, 800, and 1,200 ppm of Pb (Ashraf *et al.*, 2017).

4.1.5 Relative Water Content (RWC%)

A characteristic symptom of lead stressed rice plants was tissue dehydration and that was exhibited as leaf relative water content (RWC %) reduction, compared with non-lead stress treatment (Figure 4.5.1 and Appendix VII). Increased lead level caused significant ($P \leq 0.01$) reduction in leaf relative water contents (RWC%). For, variety 1 RWC% was reduced 4.89%, 14.83% and 25.05% compared to the control plants in the plants treated with Pb_1 Pb_2 and Pb_3 level of lead respectively.

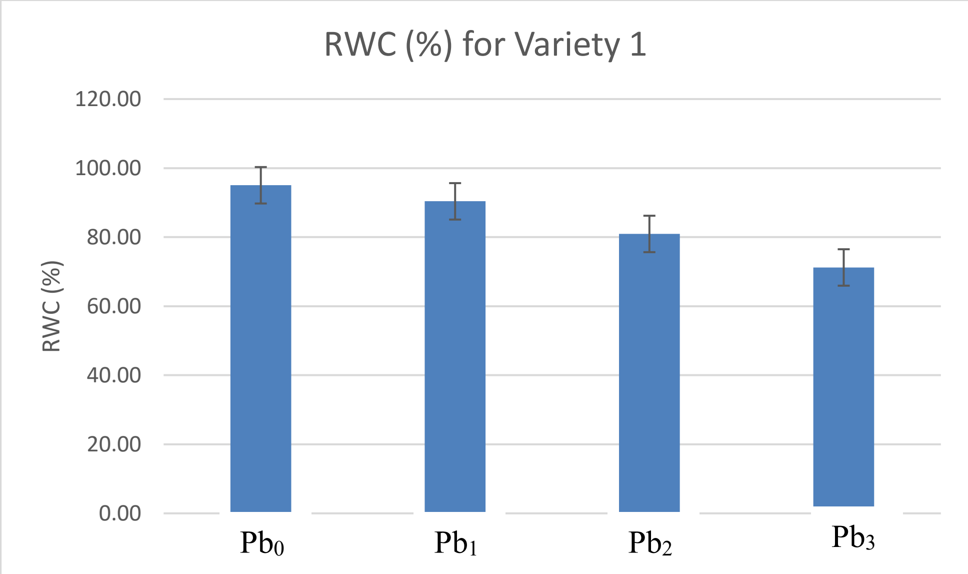

Figure 4.5.1. Effect of different lead concentrations on the relative water content (RWC %) of rice variety 1 (LSD $_{(0.05)}$ = 2.90 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD).

A characteristic symptom of lead stressed rice plants was tissue dehydration and that was exhibited as leaf relative water content (RWC %) reduction, compared with non-lead stress treatment (Figure 4.5.2 and Appendix VII). Increased lead level caused significant ($P \leq 0.01$) reduction in leaf relative water contents (RWC%). For variety 2 RWC% was reduce by 3.31%, 11.21% and 20.48% compared to the control plants in the plants treated with Pb_1 Pb_2 and Pb_3 level of lead respectively.

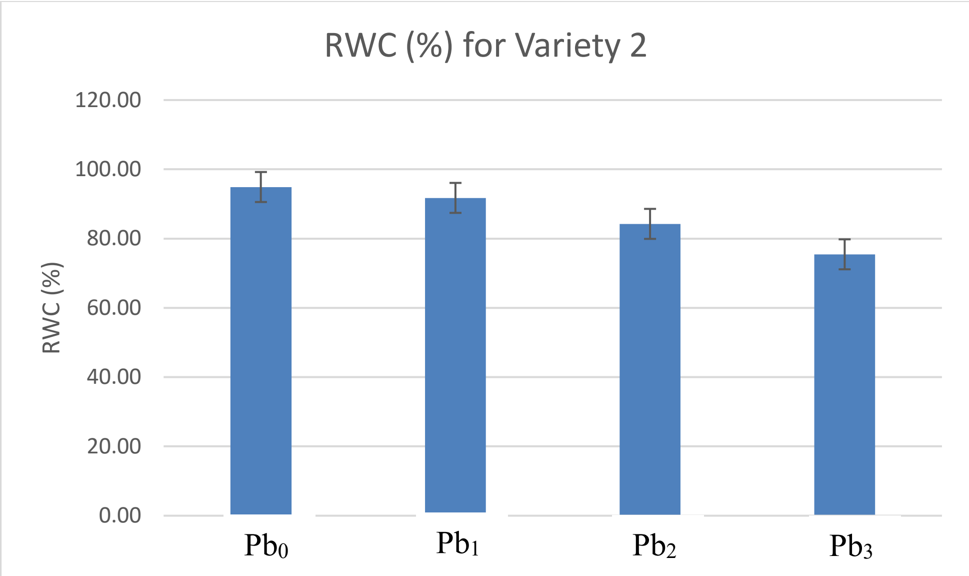

Figure 4.5.2. For variety 2 Effect of different lead concentrations on the relative water content (RWC %) of rice (LSD $_{(0.05)}$ = 2.90 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD).

Table 4.5. Interaction effect of different lead concentrations and rice varieties on RWC%

Treatment	RWC%
V_1Pb_0	95.02 a
V_1Pb_1	90.37 b
V_1Pb_2	80.93 d
V_1Pb_3	71.22 f
V_2Pb_0	94.89 a
V_2Pb_1	91.75 b
V_2Pb_2	84.25 c
V_2Pb_3	75.46 e
$LSD_{(0.05)}$	2.90
CV%	1.94

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

Heavy metal including Pb causes physiological drought due to alteration in osmotic potential near root zone. As a result, plants under heavy metal/Pb stress suffered from physiological drought and showed decreased water content (Nahar *et al.*, 2016). Mung bean plants showed reduced RWC under cadmium stress (Nahar *et al.,* 2016). Lead affected plants of the present study showed reduced RWC. This reduction was higher in higher concentration of Pb.

4.1.6 Proline Content

Lead stress caused a substantial increase ($P \leq 0.01$) for leaf proline content (Figure 4.6.1 and Appendix X). For variety 1 proline content was low in control that is Pb_0. Under Pb_1 Pb_2 and Pb_3 Proline content increased by 83.02%, 286.60% and 476.99% respectively. The percent increase of proline content proportionally increased with the increase of lead stress.

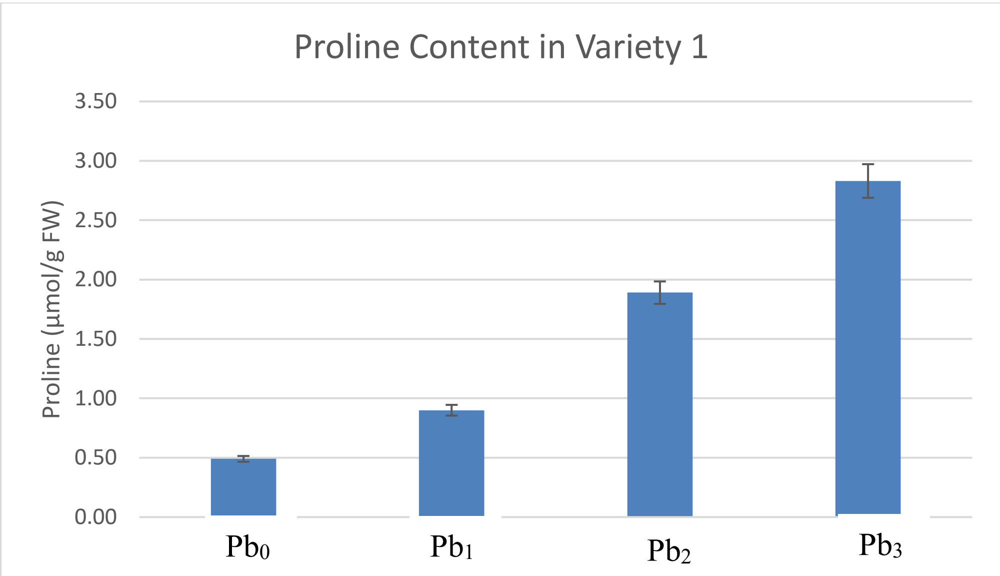

Figure 4.6.1. Effect of different lead concentrations on the proline content of rice variety 1. (LSD $_{(0.05)}$ =0.52 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

For variety 2 lead stress also caused a substantial increase ($P \leq 0.01$) for leaf proline content (Figure 4.6.2 and Appendix X). For variety 2 proline content was low in control that is Pb_0.Under Pb_1 Pb_2 and Pb_3 Proline content increased by 77.55%, 259.81% and 518.98% respectively. The percent increase of proline content proportionally increased with the increase of lead stress.

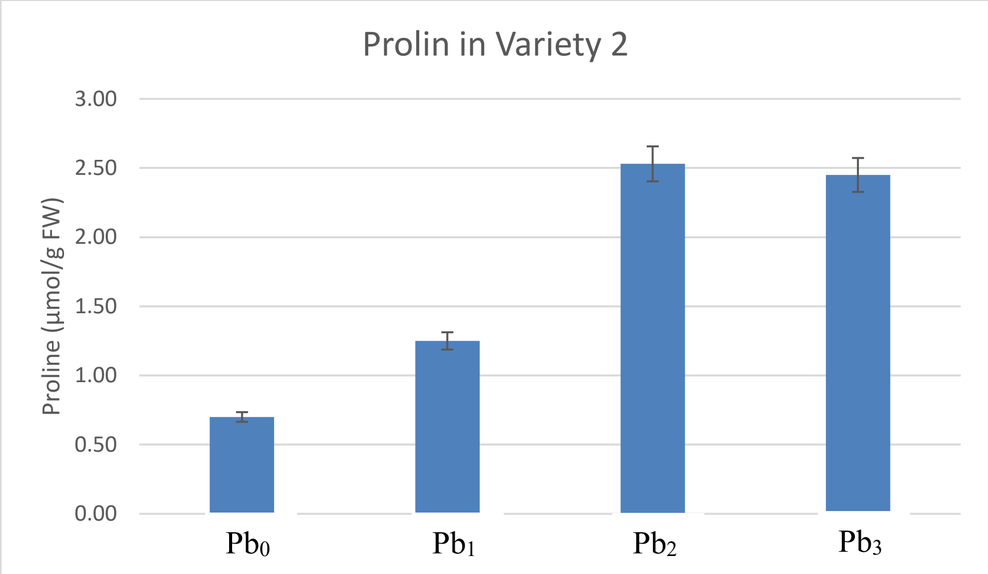

Figure 4.6.2. Effect of different lead concentrations on the proline content of rice variety 1. (LSD $_{(0.05)}$ =0.52 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD).

Table 4.6. Interaction effect of different lead concentrations and rice varieties on Proline Content

Treatment	Proline Content
V_1Pb_0	0.49e
V_1Pb_1	0.89de
V_1Pb_2	1.89c
V_1Pb_3	2.82b
V_2Pb_0	0.70e
V_2Pb_1	1.24d
V_2Pb_2	2.52b
V_2Pb_3	4.34a
$LSD_{(0.05)}$	0.52
CV%	15.96

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

Proline, a multifunctional amino acid, significantly increases during abiotic stress conditions and is well known for its role in the adaptive response mechanism toward abiotic stress tolerance. Proline helps is osmoregulation which can improve the water uptake and water status under stress condition including Pb stress (Chun *et al.*, 2018). Khan *et al.* (2021) observed a significant increase in the activity of proline in rice cultivars. In response to Pb stress, increased Pro in X-Jigna (66.79 and 219.02%), followed by Furat (70.56 and 166.53%), and Amber 33 (56.48 and 125.34%), while Ediget showed the lowest level (41.62 and 62.45%), under 0.6 mM and 1.2 mM Pb treatments, respectively (Khan *et al.,* 2021). In an experiment, different Pb levels: 0 (CK), 100, 300, 500, 700, and 900 mg/kg soil were applied in rice plants grown in pot. Lead toxicity at higher levels impaired plant physiological parameters. Pb induced proline accumulation (Zeng *et al.,* 2007).

4.1.7 SPAD Value

SPAD is a convenient tool to estimate the absolute values of chlorophyll per unit leaf area. Lead stress caused a substantial decline ($P \leq 0.01$) for leaf chlorophyll content (Figure 4.7.1 and Appendix VIII). For variety 1 SPAD value was high in the control both at 60 DAT and 90 DAT. Under Pb_1 and Pb_2 lead stress, SPAD values reduced by 6.55% and 13.26% at 60 DAT and 3.62% and 11.54% at 90 DAT respectively when compared to control. But under Pb_3 stress, there was a 20.90% and 19.73% reduction over control in SPAD values at 60 DAT and 90 DAT respectively. The percent reduction of total chlorophyll content proportionally increased with the increase of lead.

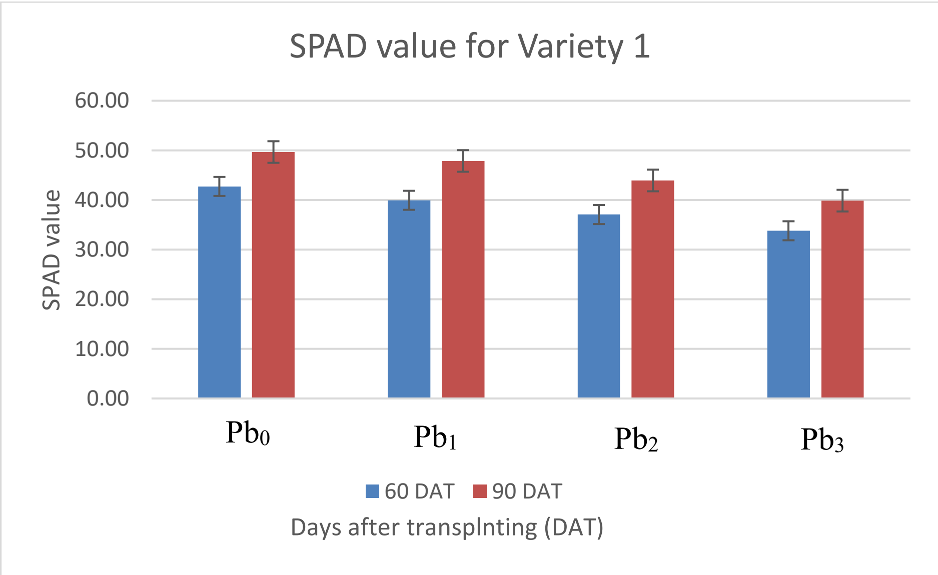

Figure 4.7.1. Effect of different lead concentrations on the chlorophyll content (SPAD value) of rice variety 1 at different days after transplanting ($LSD_{(0.05)}$ = 1.80 and 2.26 at 60 and 90 DAT, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

For variety 2 leaf chlorophyll content (SPAD) was high in the control both at 60 DAT and 90 DAT. Under Pb_1 and Pb_2 lead stress, SPAD values reduced by 6.32% and 11.69% at 60 DAT and 4.92% and 11.92% at 90 DAT respectively when compared to control. But under Pb_3 salinity stress, there was a 18.68% and 18.99% reduction over control in SPAD values at 60 DAT and 90 DAT respectively. The percent reduction of total chlorophyll content proportionally increased with the increase of lead.

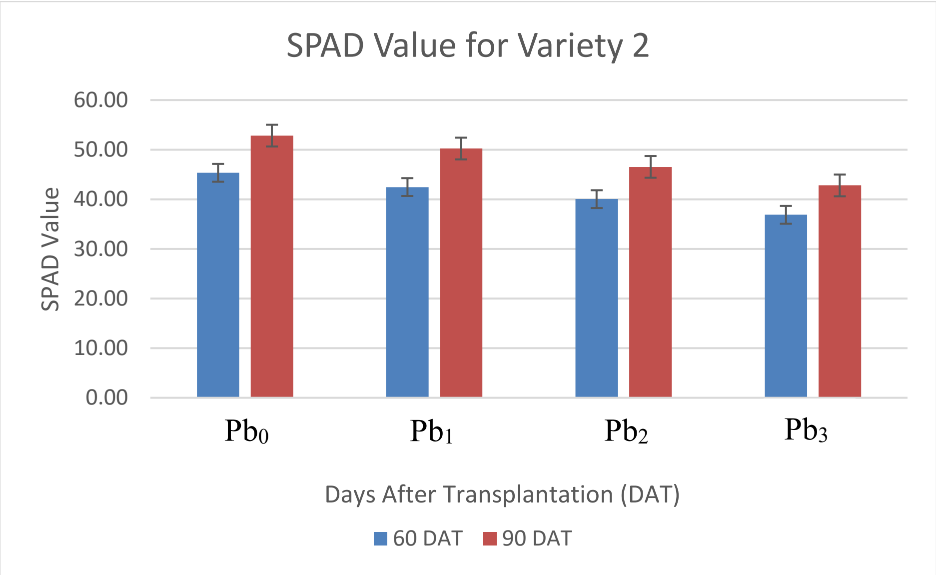

Figure 4.7.2. For variety 2 Effect of different lead concentrations on the chlorophyll content (SPAD value) of rice at different days after transplanting (LSD (0.05) = 1.80 and 2.26 at 60 and 90 DAT, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Table 4.7. Interaction effect of different lead concentrations and rice varieties on SPAD Value

Treatment	SPAD Value	
	60 DAT	90 DAT
V_1Pb_0	42.73 b	49..67 bc
V_1Pb_1	39.93 c	47.87 cd
V_1Pb_2	37.07 d	43.93 e
V_1Pb_3	33.80 e	39.87 f
V_2Pb_0	45.33 a	52.83 a
V_2Pb_1	42.47 b	50.23 b
V_2Pb_2	40.03 c	46.53 d
V_2Pb_3	36.87 d	42.80 e
$LSD_{(0.05)}$	1.80	2.26

CV%	2.58	2.76

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

Heavy metal stress including the Pb destroy photosynthetic pigments and photosynthetic activity.

The main reasons of reduced photosynthetic activity due to Pb may be the affinity of lead for protein N and S ligands; cause damage to chloroplastic ultrastructure (Islam *et al.,* 2007); hindrance in the electron transport chain (ETC) reactions (Qufei and Fashui 2009); breakdown of chlorophyll contents by enhanced activity of chlorophyllase (Liu *et al.* 2008); inhibition and substitution of Mg and Fe by Pb (divalent cations) in chlorophyll (Cenkci *et al.,* 2010); reduced activities of ferredoxin $NADP^+$ reductase as well as delta-aminolevulinic acid dehydratase at the source of chlorophyll production (Gupta *et al.,* 2009); reduced CO_2 levels due to stomatal closure (Romanowska *et al.*, 2006); plastoquinone and carotenoid inhibition (Cenkci *et al.,* 2010); and inhibited enzymatic catalysis of Calvin cycle (Liu *et al.,* 2008). Different research findings demonstrated reduction of photosynthetic pigment levels as one of the common stress response under heavy metal stress including Pb. Soybean plants were imposed with various level of lead (0, 25, 50, 75, 100 mg L^{-1}). Reduction of leaf greenness was recorded with reduction of SPAD value under those lead stress (Kulaz *et al.,* 2021). Scented rice plants were exposed to 400, 800, and 1,200 ppm of Pb and reduction of chlorophyll *a* and chlorophyll *b* was demonstrated (Ashraf *et al.*, 2017). In the present study it has been demonstrated that various concentration of Pb decreased the SPAD value indicating the reduction of leaf greenness, i.e. the photosynthetic pigment level. Khan *et al.* (2021) observed significant reduction of chlorophyll content Amber 33 rice cultivar (21.75 and 37.65%), and Ediget (23.04 and 33.17%), while X-Jigna (10.09 and 15.21%), and Furat (8.94 and 15.95%), showed the least reduction under both 0.6 mM or 1.2 mM Pb treatments, respectively (Khan *et al.,* 2021).

4.1.8 Dry Weights of Root, Shoot, Leaf and Total Dry Matter (TDM) plant^{-1}

Dry matter estimation is regarded as a valuable index for monitoring vegetative growth of the rice plant (Hakim *et al.*, 2014b). Total dry matter (TDM) is defined as the sum total of root, shoot, and leaf dry weight. The total dry matter (TDM) was significantly

($P \leq 0.01$) influenced under different levels of lead (Figure 4.8.1 and Appendix IX). For variety 1- Root, shoot, leaf dry weights, and TDM decreased by 7.38%, 15.95%, 8.27%, and 10.37% respectively in lead-stressed plants (Pb_1) when compared to unstressed control. Further, a dramatic reduction of dry weights of root, shoot, leaf, and TDM with Pb_2 lead stress was 20.49%, 29.65%, 26.92%, and 26.84% respectively and for Pb_3 lead stress was 33.61%,40.09%, 41.15%, and 39.83% respectively when compared to control plants without lead stress. A maximal reduction in root, shoot, leaf dry weights, and TDM was observed at Pb_3 treatment.

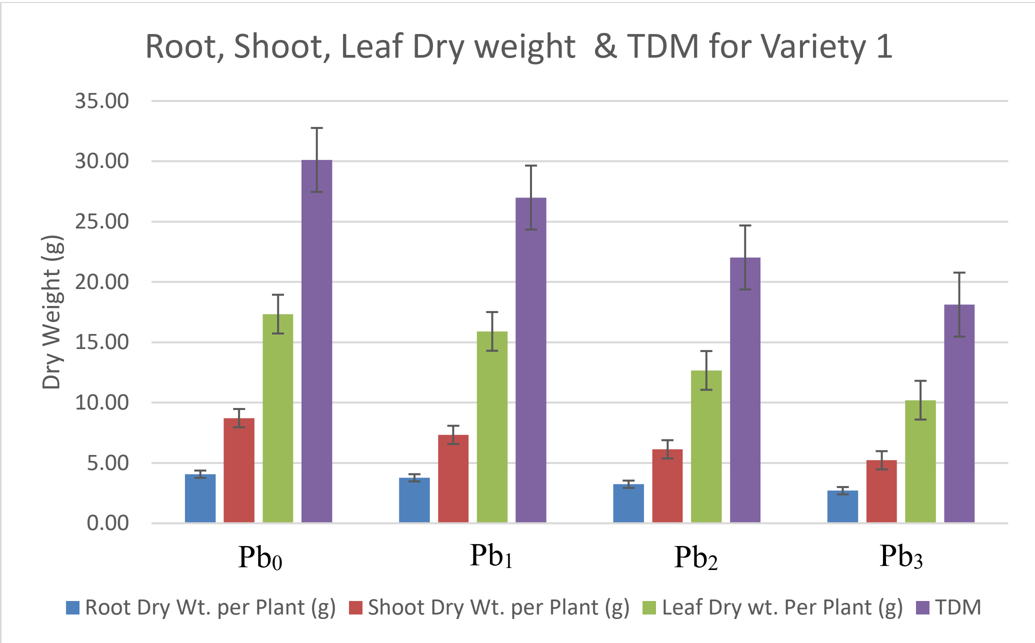

Figure 4.8.1. Effect of different lead concentrations on the dry weights of root, shoot, leaf and total dry matter (TDM) plant^{-1} for variety 1 of rice (LSD $_{(0.05)}$ =0.59, 0.69, 2.22 and 2.00 for dry weights of root, shoot, leaf and total dry matter (TDM), respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

The total dry matter (TDM) was significantly ($P \leq 0.01$) influenced under different levels of lead (Figure 4.8.2 and Appendix XIII). For, variety 2- Root, shoot, leaf dry weights, and TDM decreased by 9.21%, 8.98%, 7.70%, and 8.26% respectively in lead-stressed plants (Pb1) when compared to unstressed control. Further, a dramatic reduction of dry weights of root, shoot, leaf, and TDM with Pb2 lead stress was 22.37%, 18.60%, 19.11%, and 19.42% respectively and for Pb3 lead stress was 34.87%,31.64%, 32.51%, and 32.60% respectively when compared to control plants without lead stress. A maximal reduction in root, shoot, leaf dry weights, and TDM was observed at Pb3 treatment.

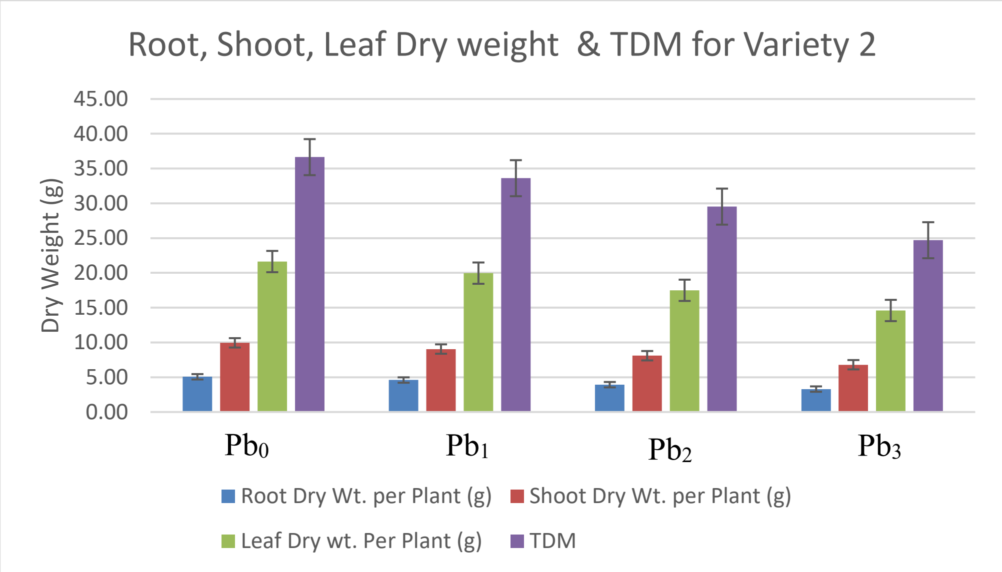

Figure 4.8.2. Effect of different lead concentrations on the dry weights of root, shoot, leaf and total dry matter (TDM) plant^{-1} of rice for variety 2 (LSD $_{(0.05)}$ =0.59, 0.69, 2.22 and 2.00 for dry weights of root, shoot, leaf and total dry matter (TDM), respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Table 4.8. Interaction effect of different lead concentrations and rice varieties on root, shoot, leaf and total dry matter (TDM) plant^{-1}

Treatment	Root dry wt. plant^{-1} (g)	Shoot dry wt. plant^{-1} (g)	Leaf dry wt. plant^{-1} (g)	Total dry matter (TDM) plant^{-1} (g)
V_1Pb_0	4.07 bc	8.71 bc	17.33 b	3.11 c
V_1Pb_1	3.77 cd	7.32 d	15.90 bc	26.99 d
V_1Pb_2	3.23 de	6.13 e	12.67 d	22.03 f
V_1Pb_3	2.70 e	5.22 f	10.20 e	18.12 g
V_2Pb_0	5.07 a	9.95 a	21.63 a	36.65 a
V_2Pb_1	4.60 ab	9.05 b	19.97 a	33.62 b
V_2Pb_2	3.93 c	8.10 c	17.50 b	29.53 c
V_2Pb_3	3.30 d	6.80 de	14.60 cd	24.70 e
$LSD_{(0.05)}$	0.59	0.69	2.22	2.00
CV%	8.80	5.17	7.80	4.12

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

Different growth parameters decreased under Pb stress. In the present study, dry weight of root, shoot, leaf and total dry matter decreased under Pb stress. Decreased plant height, leaf area, tiller number were recorded in Pb affected rice plants. This reduction was due to disturbed physiology of rice plants under Pb stress. There are various research findings which showed similar results with the present study. Fresh wt. (g pot^{-1}), dry wt. (g pot^{-1}) and length (cm pot^{-1}) of rice plant decreased under Pb stress (Jasmin *et al.*, 2015). Fresh and dry wt. of root and shoot decreased when the rice plants were subjected to various concentration of Pb (0, 250, 500, 1000, or 2000 ppm $PbCl_2$ solution) (Awan *et al.,* 2015).

4.1.9 Panicle Length (cm)

As higher panicle length could provide a higher number of grains, so panicle length is regarded as an important yield contributing character. Results revealed that the panicle length of rice was also significantly ($P \leq 0.01$) affected by various levels of lead (Figure 4.9.1 and Appendix X). Here, for variety 1 lead stress caused about 6.87%, 21.01% and 36.19% downfall in the length of panicle with Pb_1, Pb_2 and Pb_3 treatments respectively when compared to control plants without lead stress (Pb_0).

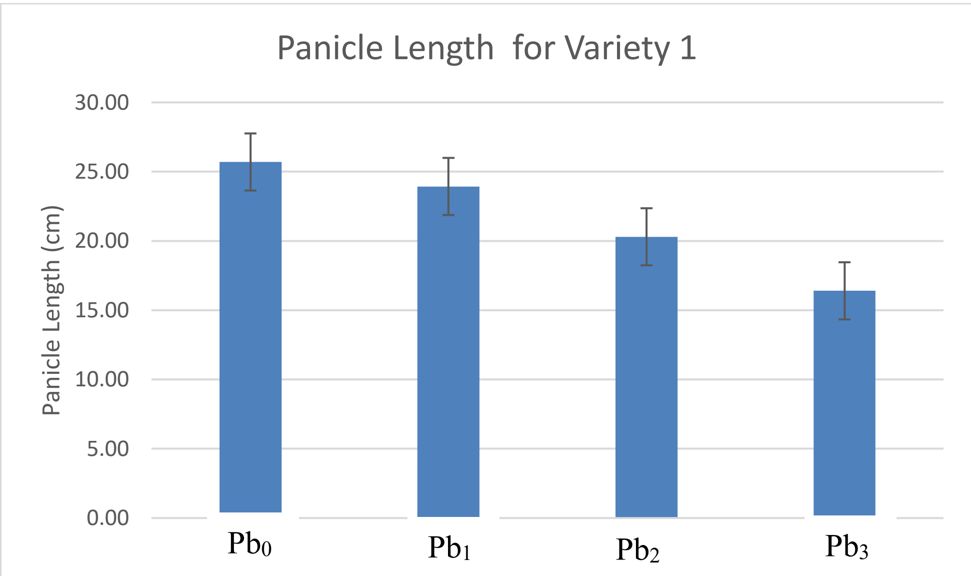

Figure 4.9.1. Effect of different lead concentrations on the panicle length of rice for variety 1 ($LSD_{(0.05)}$ =1.67 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

As higher panicle length could provide a higher number of grains, so panicle length is regarded as an important yield contributing character. Results revealed that the panicle length of rice was also significantly ($P \leq 0.01$) affected by various levels of lead (Figure 4.9.2 and Appendix IX). Here, for variety 2 lead stress caused about 5.76%,18.18% and 28.49% downfall in the length of panicle with Pb_1, Pb_2 and Pb_3 treatments respectively when compared to control plants without lead stress (Pb_0).

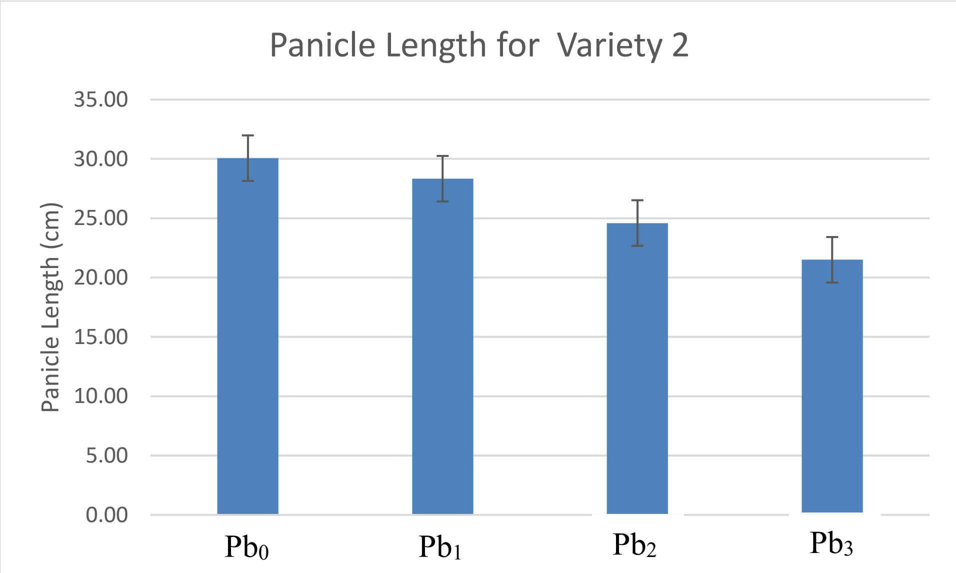

Figure 4.9.2. Effect of different lead concentrations on the panicle length of rice for variety 2 ($LSD_{(0.05)}$ =1.67 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Table 4.9. Interaction effect of different lead concentrations and rice varieties on panicle length

Treatment	Panicle length (cm)
V_1Pb_0	25.70 c
V_1Pb_1	23.93 d
V_1Pb_2	20.30 e
V_1Pb_3	16.40 f
V_2Pb_0	30.06 a
V_2Pb_1	28.33 b
V_2Pb_2	24.60 cd
V_2Pb_3	21.50 e
$LSD_{(0.05)}$	1.67
CV%	4.00

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

Lead (50, 100, 150 and 200 mg kg^{-1}) was applied on rice (*Oryza sativa*) and reduction of panicle length together with shoot and root length reduction were recorded (Jasmin *et al.*, 2019).

4.1.10 No. of Effective Tillers Plant^{-1}

The yield of rice plants is mostly dependent upon the number of effective tillers i.e., panicle-bearing tillers plant^{-1}. Rice plants were significantly ($P \leq 0.01$) influenced by lead stress in terms of effective tiller production. For variety 1 No. of effective tillers plant-1 gradually decreased with increased levels of lead (Figure 4.10.1 and Appendix XI). In case of Pb1, Pb2 and Pb3 treatment 12.53%, 31.09% and 45.94% reduction of effective tillers was observed respectively compared to non-lead stress condition (control).

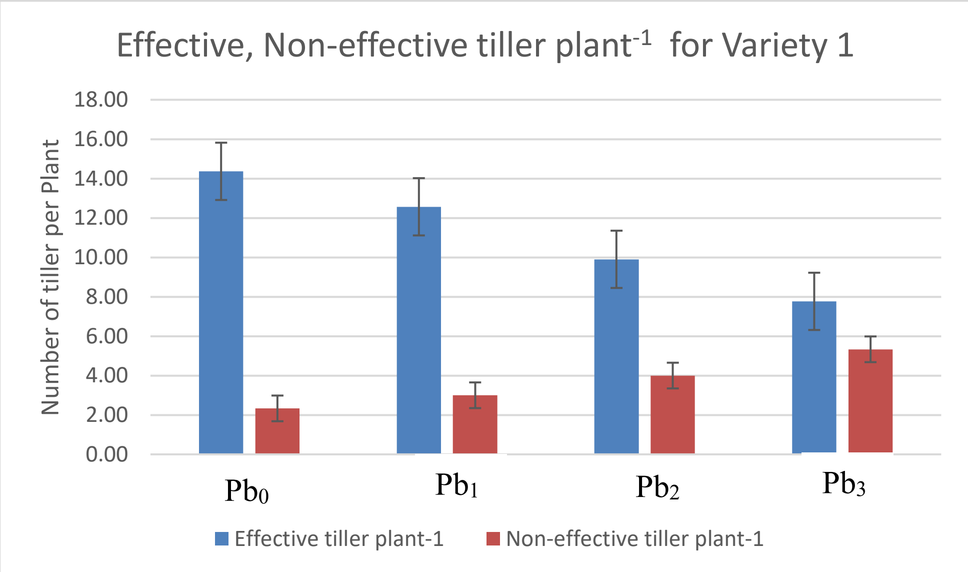

Figure 4.10.1. Effect of different lead concentrations on the number of effective and non-effective tillers plant^{-1} of rice variety 1 (LSD $_{(0.05)}$ = 1.41 and 0.64 for number of effective and non-effective tillers, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD).

For variety 2 No. of effective tillers plant^{-1} gradually decreased with increased levels of lead (Figure 4.10.2 and Appendix IX). In case of Pb1, Pb2 and Pb3 treatment 11.55%, 28.12% and 40.78% reduction of effective tillers was observed respectively compared to non-lead stress condition (control).

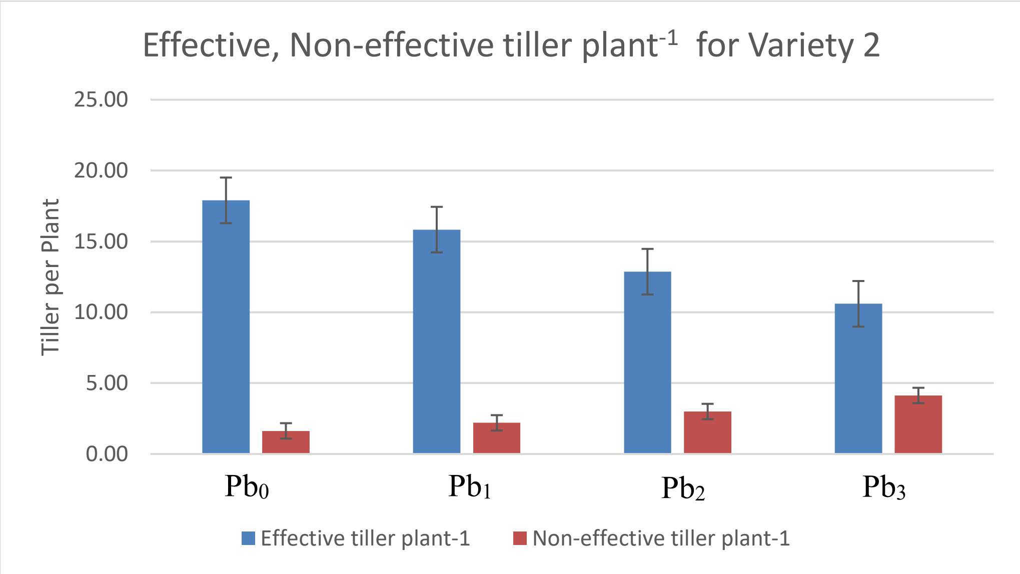

Figure 4.10.2. Effect of different lead concentrations on the number of effective and non-effective tillers plant-1 of rice variety (LSD (0.05) = 1.41 and 0.64 for number of effective and non- effective tillers, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD).

Table 4.10. Interaction effect of different lead concentrations and rice varieties on effective tillers plant^{-1}

Treatment	Effective tillers plant^{-1}
V_1Pb_0	14.37 c
V_1Pb_1	12.57 d
V_1Pb_2	9.90 e
V_1Pb_3	7.77 f
V_2Pb_0	17.90 a
V_2Pb_1	15.83 b
V_2Pb_2	12.87 d
V_2Pb_3	10.60 e
$LSD_{(0.05)}$	1.41
CV%	6.32

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.11 No. of Non-Effective Tillers Plant^{-1}

Less number of non-effective tillers plant^{-1} is a positive attribute towards higher grain yield. But different levels of lead increased the no. of non-effective tillers plant^{-1} significantly ($P \leq 0.01$). The no. of non-effective tillers plant-1 ranged from 1.5 to 6.4. Pb_3 in variety 1 treatment had the highest no. (6.4) of non-effective tillers plant^{-1} and control had the lowest no. (1.5) of non-effective tillers plant^{-1} (Figure 4.10.1 and Appendix XI).

For variety 1 in case of Pb_1, Pb_2 and Pb_3 no. of non-effective tiller increased 28.57%, 71.43% and 128.57% compared to the control treatment.

For variety 2 in the case of Pb_1, Pb_2 and Pb_3 no. of non-effective tiller increased 34.69%, 83.67% and 153.06% compared to the control treatment.

Table 4.11. Interaction effect of different lead concentrations and rice varieties on non-effective tillers plant^{-1}

Treatment	Non-effective tillers plant^{-1}
V_1Pb_0	2.33 d
V_1Pb_1	3.00 c
V_1Pb_2	4.00 b
V_1Pb_3	5.33 a
V_2Pb_0	1.63 e
V_2Pb_1	2.20 de
V_2Pb_2	3.00 c
V_2Pb_3	4.13 b
$LSD_{(0.05)}$	0.64
CV%	11.48

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

The control plants showed the lowest no. of non-effective tillers plant^{-1}. Exposure of rice plant to Pb stress increased the no. of non-effective tillers plant^{-1}. There might have developmental disorder of rice plants under Pb stress due to which no. of non-effective tillers plant^{-1} increased under Pb stress. According to the findings of Ashraf et. al. (2017) tillers/pot decreased in different rice cultivars subjected to Pb stress. Productive tillers/pot also decreased under Pb stress. No. of tiller/3 plant No. of Panicle/3 plant and No. of grain/panicle decreased under Pb toxicity (Jasmin *et al.,* 2015).

4.1.12 No of Filled Spikelets Panicle^{-1}

Rice grain yield is closely related to the number of filled spikelets panicle^{-1}. No. of filled spikelets panicle^{-1} decreased significantly ($P \leq 0.01$) with the increase of lead level (Figure 4.12.1 and Appendix XI). The highest number of filled grain panicle^{-1} was counted at control condition in Pb_0 in variety 2 that is 149.5 and the lowest number of filled grain per panicle was recorded at the Pb_3 in variety 1 level of lead that is 69. A decrease of 12.04%, 21.91% and 38.09% number of filled spikelets panicle^{-1} was observed with Pb_1, Pb_2 and Pb_3 level of lead respectively for variety 1 compared to control (no lead).

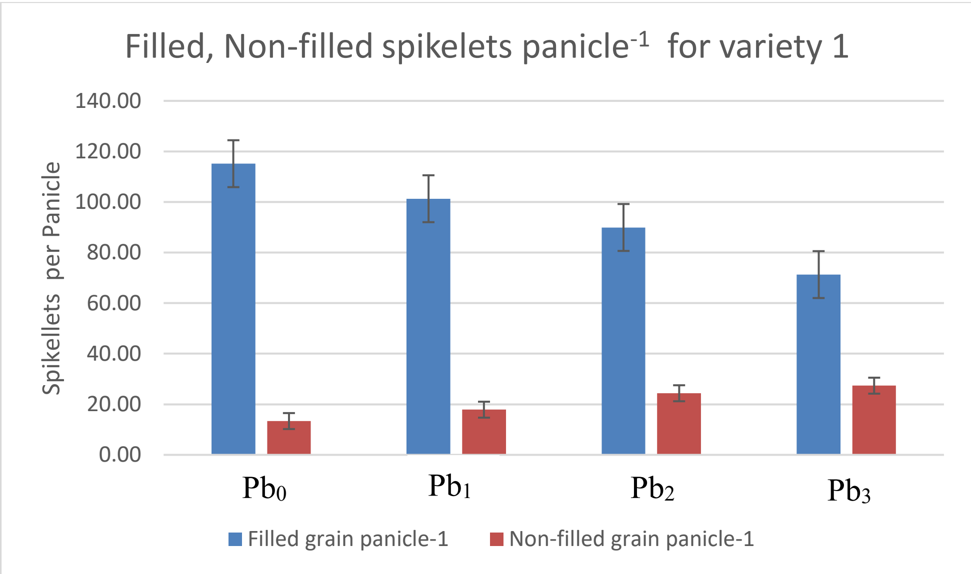

Figure 4.12.1. Effect of different lead concentrations on the number of filled and unfilled spikelets panicle^{-1} of rice (LSD $_{(0.05)}$ = 8.72 and 2.25 for number of filled and unfilled grains for variety 1, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

A decrease of 7.07%, 16.94% and 25.05% number of filled grains panicle^{-1} was observed with Pb_1, Pb_2 and Pb_3 level of lead respectively for variety 2 compared to control (no lead).

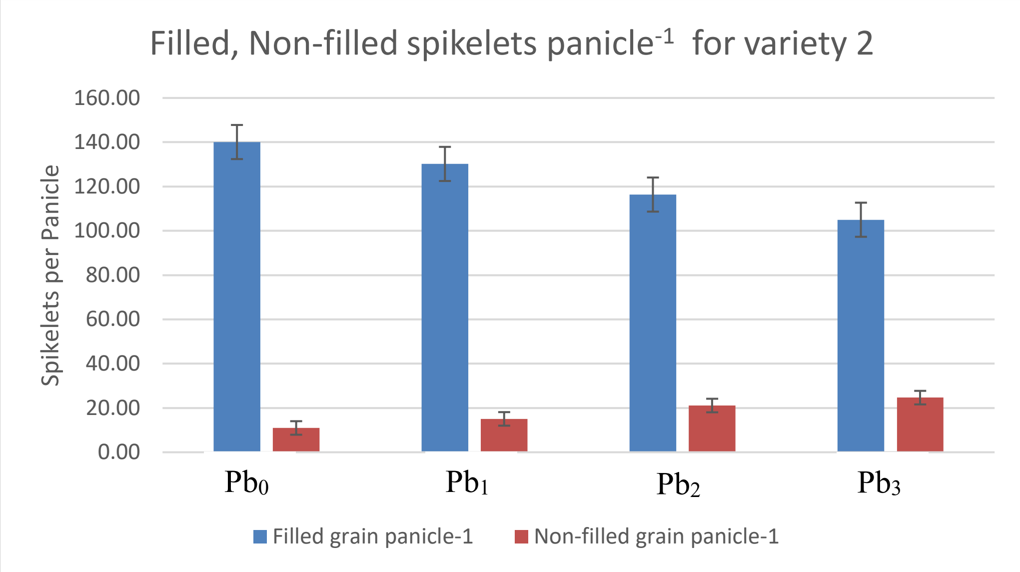

Figure 4.12.2. Effect of different lead concentrations on the number of filled and unfilled spikelets panicle-1 of rice (LSD (0.05) = 8.72 and 2.25 for number of filled and unfilled grains for variety 2, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD).

Table 4.12. Interaction effect of different lead concentrations and rice varieties on filled grain panicle^{-1}

Treatment	Filled spikelets panicle^{-1}
Pb_0V_1	115.17 c
Pb_1V_1	101.30 d
Pb_2V_1	89.93 e
Pb_3V_1	71.30 f
Pb_0V_2	140.10 a
Pb_1V_2	130.20 b
Pb_2V_2	116.37 c
Pb_3V_2	105.00 d
$LSD_{(0.05)}$	8.72
CV%	4.58

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.13 No. of Unfilled Spikelets Panicle^{-1}

In rice, less number of unfilled spikelets panicle^{-1} is a desirable attribute to get higher grain yield. Due to lead stress, panicles produced sterile spikelets with partial or complete grain loss. A gradual increase in the number of unfilled grains panicle^{-1} in variety 1 was observed in Pb_1, Pb_2 and Pb_3 treatment where 33.67%, 82.29% and 104.74% increment was observed respectively compared to unstressed control (Figure 4.12.1 and Appendix XI).

A gradual increase in the number of unfilled grains panicle^{-1} in variety 2 was observed in Pb_1, Pb_2 and Pb_3 treatment where 37.39%, 92.40% and 124.92% increment was observed respectively compared to unstressed control.

Table 4.13. Interaction effect of different lead concentrations and rice varieties on unfilled spikelets panicle^{-1}

Treatment	Unfilled spikelets panicle^{-1}
V_1Pb_0	13.37 e
V_1Pb_1	17.87 d
V_1Pb_2	24.37 b
V_1Pb_3	27.37 a
V_2Pb_0	10.97 f
V_2Pb_1	15.07 e
V_2Pb_2	21.10 c
V_2Pb_3	24.67 b
$LSD_{(0.05)}$	2.25
CV%	6.64

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

No. of tiller/3 plant, No. of Panicle/3 plant, No. of grain/panicle decreased in rice plant subjected to Pb stress (Jasmin *et al.,* 2015).

Pb (1.2 mM) induced significant reduction in agronomic traits such as plant height, number of tillers per plant, number of panicles per plant, and number of spikelets per panicle in all the rice cultivars was evaluated along with the accumulation of superoxide ions (O_2-), protein, proline, chlorophyll, sucrose, glucose, and fructose contents in all the rice cultivars (Ashraf *et al.*, 2017). No. of tiller/3 plant No. of Panicle/3 plant No. of grain/panicle 1000 grain wt. (g/pot) decreased in rice under Pb stress (Jasmin *et al.*, 2015).

Variations in the agronomic traits of the tested rice cultivars (X-Jigna, Ediget, Furat and Amber 33) to different Pb treatments (0.6 mM and 1.2 mM) resulted decrease in plant height, number of tillers per plant, number of panicles per plant, number of spikelets per panicle, and Biomass (dry weight) (Khan *et al*, 2021).

1.1.14 Thousand Grain Weight (g)

To study the effect of lead stress on rice yield, the average weight of 1000 grains grown under control and, three different lead levels were measured. 1000 grain weight significantly ($P \leq 0.01$) decreased with the increase of lead levels (Figure 4.14.1 and Appendix XII). For variety 1 a gradual decrease in 1000 grain weight was observed with the increase in lead concentration and it was 16.67%, 25.43% and 35.34% with the treatment Pb_1, Pb_2 and Pb_3 respectively compared to non-lead stress control.

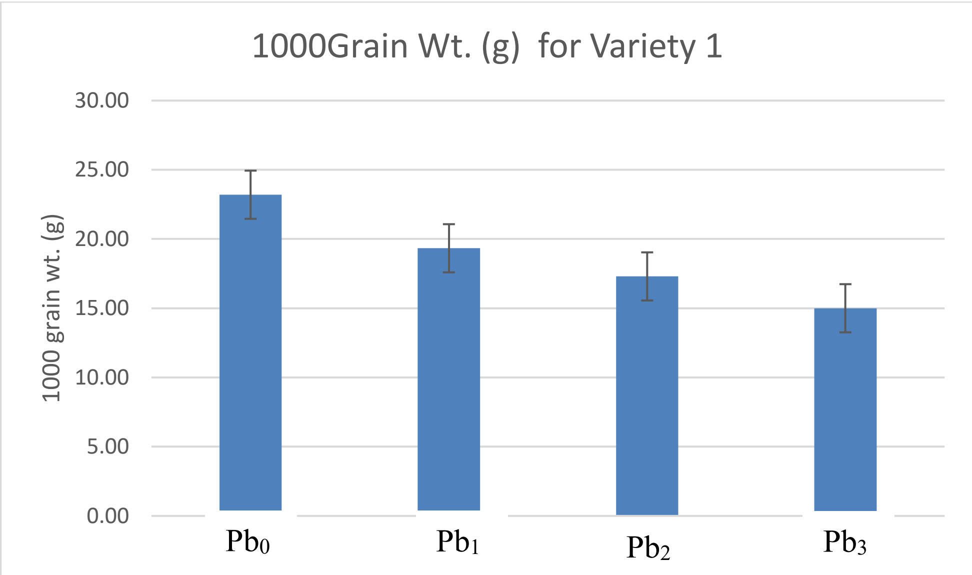

Figure 4.14.1. Effect of different lead concentrations on the 1000 grain weight of rice variety 1 (LSD $_{(0.05)}$ = 2.45 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

For variety 2 a gradual decrease in 1000 grain weight was observed with the increase in lead concentration and it was 9.07%, 24.85% and 36.98% with the treatment Pb_1, Pb_2 and Pb_3 respectively compared to non-lead stress control.

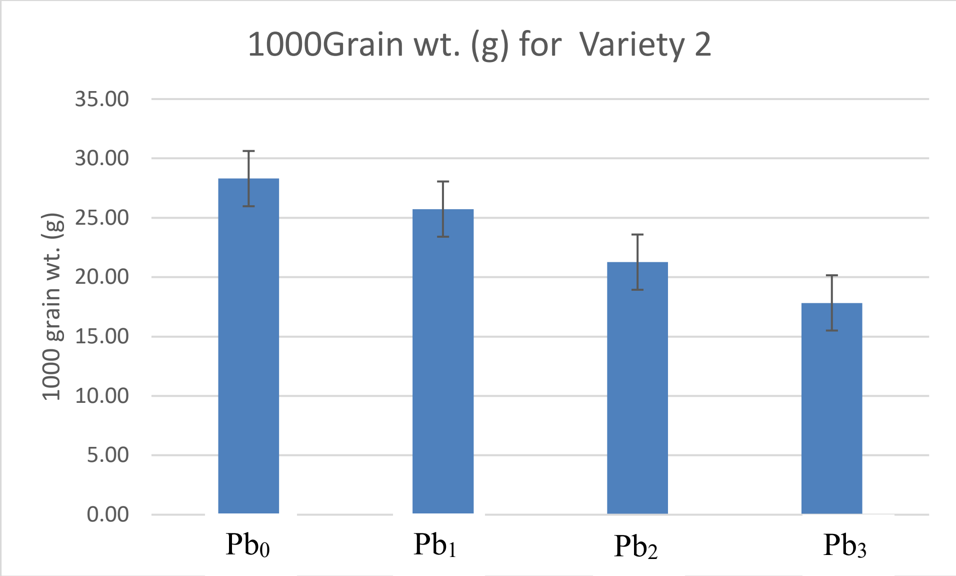

Figure 4.14.2. Effect of different lead concentrations on the 1000 grain weight of rice variety 2 (LSD $_{(0.05)}$ = 2.45 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Table 4.14. Interaction effect of different lead concentrations and rice varieties on thousand grain weight

Treatment	Thousand grain weight (g)
V_1Pb_0	23.20 c
V_1Pb_1	19.33 de
V_1Pb_2	17.30 ef
V_1Pb_3	15.05 f
V_2Pb_0	28.30 a
V_2Pb_1	25.73 b
V_2Pb_2	21.27 cd
V_2Pb_3	17.83 e
$LSD_{(0.05)}$	2.45
CV%	6.66

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

Decreasing the photosynthesis rate and hindering the assimilate translocation Pb stress decreases the grain size. The physiological processes of plants are hampered under Pb stress which eventually exerts adverse effect on growth and developmental process of plant. The 1000-grain wt. decreased in rice plant in the present study under Pb stress. Jasmin *et al.* reported reduced 1000 grain wt. (g pot^{-1}) in rice affected by Pb (Jasmin *et* *al.,* 2015). Different cultivars of scented rice plants were subjected to various level of 400, 800, and 1,200 ppm of Pb; all the doses decreased 1000-grain wt.

4.1.15 Grain Yield Plant^{-1}

The ultimate desirable product of yield components of rice is grain yield. Lead stress led to significant ($P \leq 0.01$) reduction in the grain yield plant^{-1} with the most drastic reduction being observed at Pb_3 treatment (Figure 4.15.1 and Appendix XII). The loss of grain yield for rice variety 1 due to Pb_1, Pb_2 and Pb_3 level of lead was 15.43%, 35.24% and 48.72% respectively over non-lead stressed control.

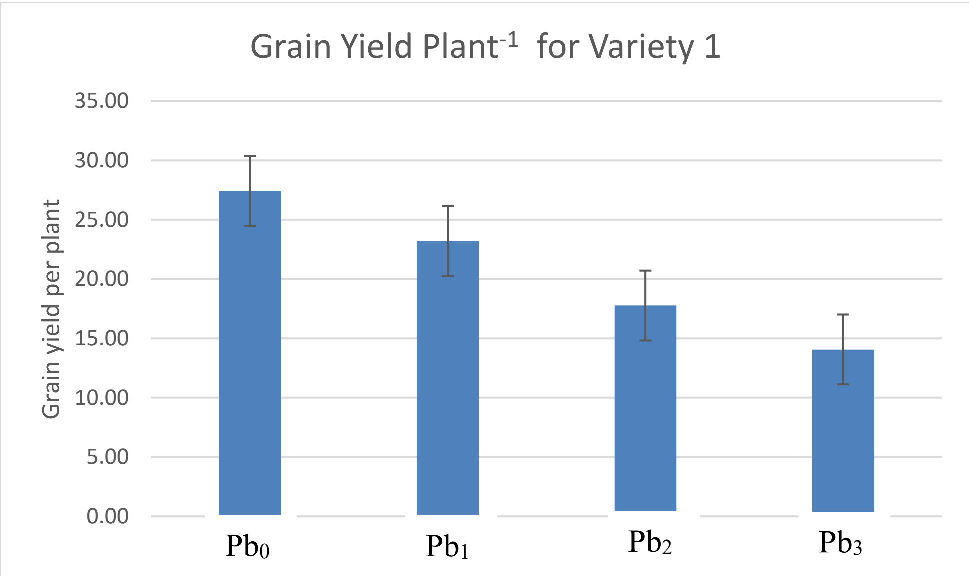

Figure 4.15.1. Effect of different lead concentrations on the grain yield plant^{-1} of rice variety 1 (LSD $_{(0.05)}$ = 1.46 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

The loss of grain yield for rice variety 2 due to Pb_1, Pb_2 and Pb_3 level of lead was 11.82%, 26.88% and 39.02% respectively over non-lead stressed control.

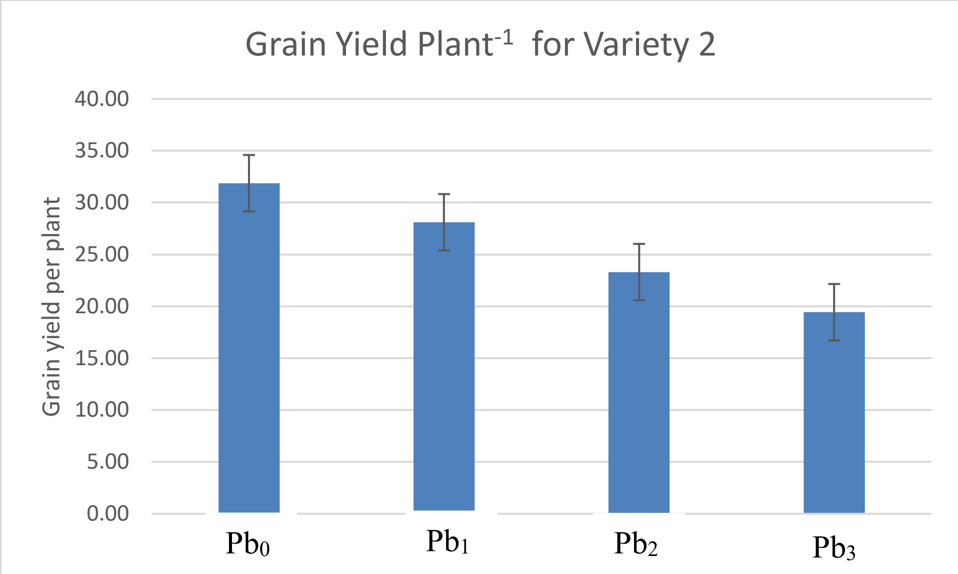

Figure 4.15.2. Effect of different lead concentrations on the grain yield plant^{-1} of rice variety 2 (LSD $_{(0.05)}$ = 1.46 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

Table 4.15. Interaction effect of different lead concentrations and rice varieties on grain yield plant^{-1}

Treatment	Grain yield plant^{-1}
V_1Pb_0	27.43 b
V_1Pb_1	23.20 c
V_1Pb_2	17.77 e
V_1Pb_3	14.07 f
V_2Pb_0	31.87 a
V_2Pb_1	28.10 b
V_2Pb_2	23.30 c
V_2Pb_3	19.43 d
$LSD_{(0.05)}$	1.46
CV%	3.61

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

Grain yield of rice is dependent on different yield attributes. Effective tiller plant^{-1}, no. of filled spikelets panicle^{-1}, 1000-grain wt. etc. were the measured yield attributes of the present study. Lead stress adversely affected the reproductive development and decreased the parameters of yield attributes of rice in the present study. Finally, the yield reduction was noticed is Pb affected rice plants. Li *et al.* (2007) found significant reductions in rice growth and yield at 1200 mg of lead kg^{-1} of soil than control and higher found Pb^{2+} contents in rice grains than root, shoot, and leaves. Rice growth and yield reduced when exposed to lead in the soil (1000 ppm) and reduction of paddy yield was about 12 % (Gu *et al.,* 1989). Different cultivars of scented rice plants were subjected to various level of 400, 800, and 1,200 ppm of Pb. All Pb levels reduced the yield and yield components of all rice cultivars; nonetheless such reductions were observed highest in Xinagyaxiangzhan (69.12%) than Meixiangzhan-2 (58.05%) and Basmati-385 (46.27%) cultivars. Lead stress also deteriorated the grain quality (Ashraf *et al.,* 2017).

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was carried out to assess the effect of lead on morphological, physiological and yield performance of rice plant (*Oryza sativa* cv. BRRI dhan29 and cv. BRRI dhan58). The experiment was carried out at the net house of the Department of Agricultural Botany and Plant Physiology Laboratory of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh in Boro season during the period from December, 2020 to May 2021. The double factor experiment was laid out in a randomized complete block design (RCBD) with three replications and the differences between the means were evaluated by Least Significant Difference (LSD). In this experiment, the treatments consisted of control and three different lead levels viz. Pb_0= without lead (0 mM $PbSO_4$, Lead(II) sulfate), Pb_1= 2 mM $PbSO_4$, Pb_2 = 4 mM $PbSO_4$, Pb_3= 6 mM $PbSO_4$and two different variety viz. V_1= BRRI dhan29, V_2= BRRI dhan58. The lead treatments were begun at 45 DAT.

Then data was recorded on plant height (cm), no. of tillers plant^{-1}, leaf area (cm^2), leaf membrane stability index (MSI%), leaf relative water content (RWC%), chlorophyll content (SPAD reading), dry weights of root, shoot, leaf and total dry matter (TDM), proline content, panicle length (cm), no. of effective and non-effective tillers plant^{-1}, no. of filled and unfilled spikelets panicle^{-1}, 1000 grain weight (g) and grain yield. The collected data were statistically analyzed for evaluation of the treatment effect and a significant variation among the treatments was found while different lead levels and variety were applied in different combinations.

The result of the experiment revealed that almost all the morphological, physiological and yield contributing characters were decreased significantly except no. of non-effective tillers, no. of unfilled spikelets panicle^{-1} due to imposition of lead. Plants grown in control condition (without lead) performed best in recording the morphological, physiological and yield contributing characters of rice whereas the lowest data of all parameters was recorded from 6 mM treated plants. Lead stress causes oxidative stress and this is indicated by reduction of membrane stability index value under Pb stress in both the cultivars. Proline content increased in both rice varieties with the increase of Pb doses whereas the RWC decreased gradually with the increase of Pb doses which indicates the lead-induced oxidative stress. For variety 1 leaf chlorophyll content (SPAD) was high in the control both at 60 DAT and 90 DAT. Under Pb_1 and Pb_2 lead stress, SPAD values reduced by 6.55% and 13.26% at 60 DAT and 3.62% and 11.54% at 90 DAT respectively when compared to control. But under Pb_3 stress, there was a 20.90% and 19.73% reduction over control in SPAD values at 60 DAT and 90 DAT respectively. For variety 2 leaf chlorophyll content (SPAD) was high in the control both at 60 DAT and 90 DAT. Under Pb_1 and Pb_2 lead stress, SPAD values reduced by 6.32% and 11.69% at 60 DAT and 4.92% and 11.92% at 90 DAT respectively when compared to control. But under Pb_3 stress, there was a 18.68% and 18.99% reduction over control in SPAD values at 60 DAT and 90 DAT respectively. In fact, there was a gradual decrease of most of the parameters with the increase of lead dose. For variety 1- Root, shoot, leaf dry weights, and TDM decreased by 7.38%, 15.95%, 8.27%, and 10.37% respectively in lead-stressed plants (Pb_1) when compared to unstressed control. Further, a dramatic reduction of dry weights of root, shoot, leaf, and TDM with Pb_2 lead stress was 20.49%, 29.65%, 26.92%, and 26.84% respectively and for Pb_3 lead stress was 33.61%,40.09%, 41.15%, and 39.83% respectively when compared to control plants without lead stress. A maximal reduction in root, shoot, leaf dry weights, and TDM was observed at Pb_3 treatment. For, variety 2- Root, shoot, leaf dry weights, and TDM decreased by 9.21%, 8.98%, 7.70%, and 8.26% respectively in lead-stressed plants (Pb1) when compared to unstressed control. Further, a dramatic reduction of dry weights of root, shoot, leaf, and TDM with Pb2 lead stress was 22.37%, 18.60%, 19.11%, and 19.42% respectively and for Pb3 lead stress was 34.87%,31.64%, 32.51%, and 32.60% respectively when compared to control plants without lead stress. A maximal reduction in root, shoot, leaf dry weights, and TDM was observed at Pb3 treatment.

The results showed that all the morphological, physiological and yield attributes varied to a considerable extent under lead stresses which render the lower yield. While the no. of non-effective tillers plant^{-1}, days to flowering, no. of unfilled spikelets panicle^{-1} was increased in response to lead stress. The highest number of filled grain panicle^{-1} was counted at control condition in Pb_0 in variety 2 that is 149.5 and the lowest number of filled grain per panicle was recorded in variety 1 at the Pb_3 level of lead that is 69. A decrease of 12.04%, 21.91% and 38.09% number of filled spikelets panicle^{-1} was observed with Pb_1, Pb_2 and Pb_3 level of lead respectively for variety 1/BRRI dhan29 compared to control (no lead). A decrease of 7.07%, 16.94% and 25.05% number of filled grains panicle^{-1} was observed with Pb_1, Pb_2 and Pb_3 level of lead respectively for variety 2/BRRI dhan58 compared to control (no lead). The loss of grain yield for rice variety 1/BRRI dhan29 due to Pb_1, Pb_2 and Pb_3 level of lead was 15.43%, 35.24% and 48.72% respectively over non-lead stressed control. The loss of grain yield for rice variety 2/BRRI dhan58 due to Pb_1, Pb_2 and Pb_3 level of lead was 11.82%, 26.88% and 39.02% respectively over non-lead stressed control.

Further studies are required for understanding the intrinsic reason behind Pb-induced toxic effect on morphological, physiological and yield performance of rice under lead stress. Different combinations of lead and other varieties may be included for further study.

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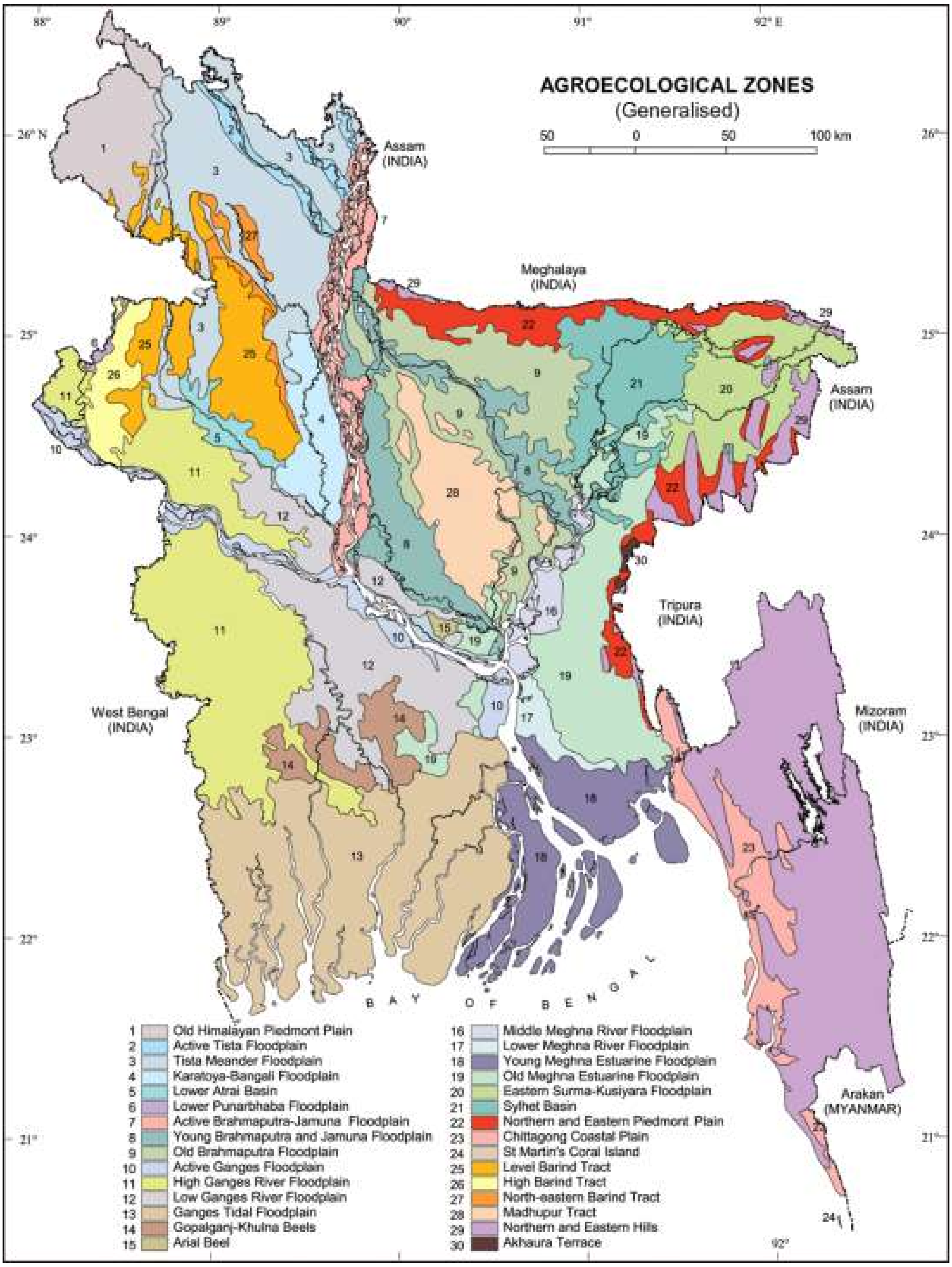
Appendix I. Experimental location on the map of Agro-Ecological Zones of Bangladesh



Appendix II. Morphological Characteristics of the Experimental Field

Morphology	**Characteristics**
Location SAU Farm,	Dhaka
Agroecological zone	Madhupur Tract (AEZ- 28)
General Soil Type	Deep Red Brown Terrace Soil
Parent material	Madhupur clay
Topography	Fairly level
Drainage	Well drained
Flood level	Above flood level
Soil series	Tejgaon

(SAU Farm Dhaka)

Appendix III. Physical and Chemical properties of the initial soil sample

Characteristics	**Value**
Particle size analysis	
% Sand (2.0-0.02 mm)	22.53
% Silt (0.02-0.002 mm)	56.72
% Clay (<0.002 mm)	20.75
Textural class	Silt Loam
pH (1: 2.5 soil- water)	5.6
Bulk Density (g/cc)	1.45
Particle Density (g/cc)	2.52
Organic carbon (%)	0.47
Organic matter (%)	0.81
Total N (%)	0.05
Available P (ppm)	18.1
Available K (meq/100g soil)	0.10
Available S (ppm)	2.006

(SAU Farm, Dhaka)

Appendix IV. Maximum and minimum monthly temperature (°C), relative humidity and rainfall during December, 2020 to May, 2021 at the farm of SAU

Name of the Months	**Average air temperature (^{O}C)**		**Relative Humidity (%)**	**Rainfall (mm)**
	Maximum	**Minimum**		
December, 2020	31	18	63	1.9
January, 2021	28	16	61	3.5
February, 2021	27	13	57	12.3
March, 2021	34	15	57	8.1
April, 2021	34	16	57	73.4
May , 2021	35	20	66	178.5

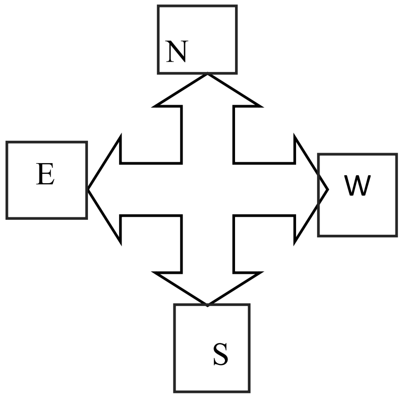
(Weather station, Sher-e-Bangla Agricultural University, Dhaka-1207)

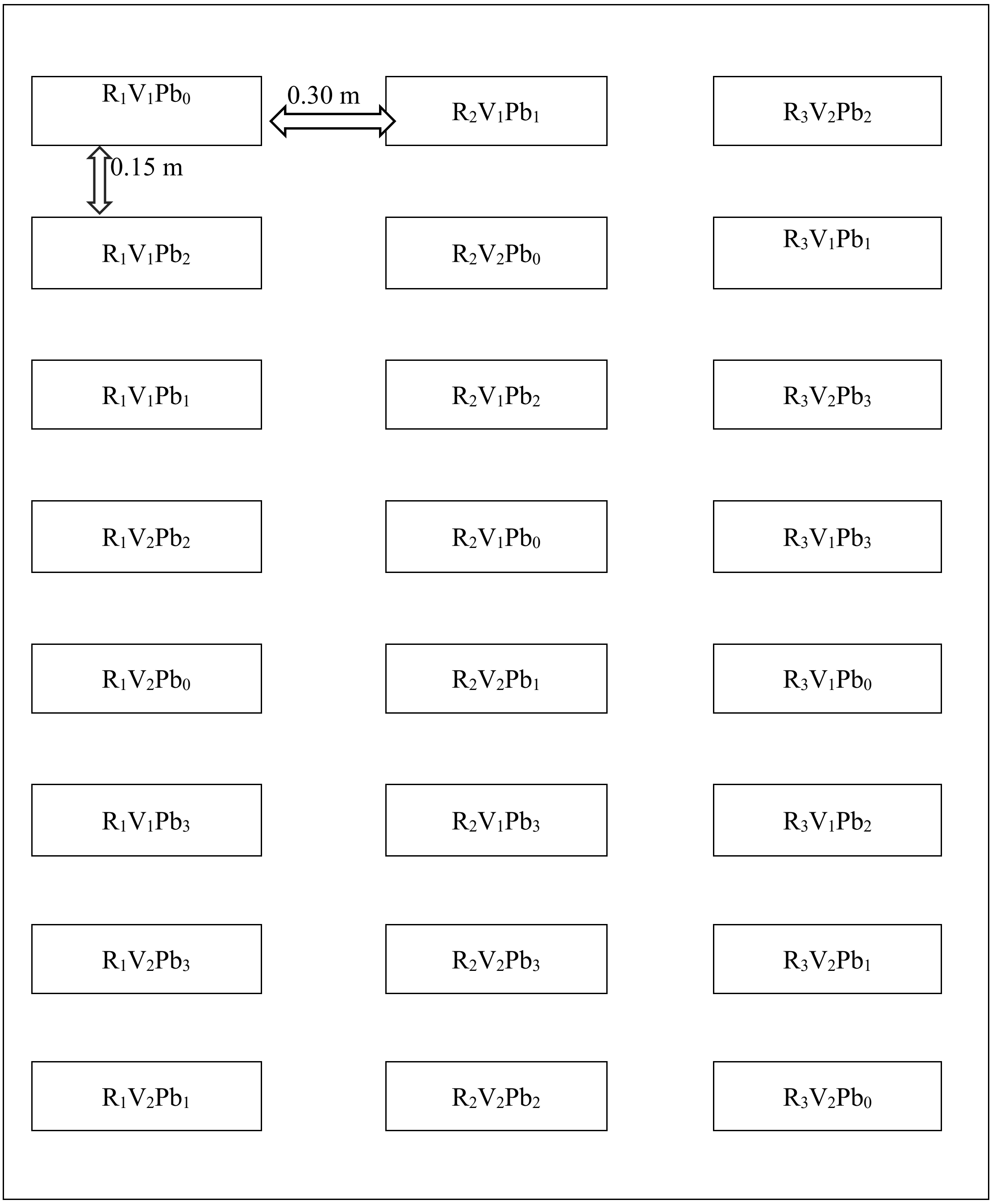
Appendix V . Layout of the experiment

Pot Size : 30 cm × 35 cm

Pot to pot distance : 0.15 m

Block to block distance : 0.30 m





Appendix VI Table Analysis of variance of the data on plant height as influenced by lead effect on 2 varieties of rice

Source of Varience	df	Mean square of plant height at different days after transplanting (DAT)			
		30	60	90	At Harvest
Replication	2	28.01	37.46	40.61	36.99
Tratment	3	332.66**	371.23**	384.75**	358.04**
Variety	1	64.68**	71.42**	93.61**	71.42**
Treatment*Variety	3	0.61^{NS}	1.16^{NS}	4.83^{NS}	1.16^{NS}
Error	14	1.84	1.75	1.46	1.86

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix VII Table Analysis of variance of the data on tiller plant^{-1}, leaf area, MSI%, RWC% as influenced by lead effect on 2 varieties of rice

Source of Varience	df	Mean square of			
		Tiller plant^{-1} at Harvest	Leaf area	MSI%	RWC%
Replication	2	8.12	411.88	1.02	8.07
Tratment	3	50.35**	466.93**	520.11**	553.32**
Variety	1	24.34**	201.05**	613.49**	29.11**
Treatment*Variety	3	0.97*	0.92^{NS}	0.27^{NS}	5.75^{NS}
Error	14	0.27	2.23	15.31	2.75

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix VIII Table Analysis of variance of the data on SPAD value at 60 DAT and 90 DAT as influenced by lead effect on 2 varieties of rice

Source of Varience	df	Mean square of SPAD value	
		60 DAT	90 DAT
Replication	2	24.17	27.12
Tratment	3	82.78**	114.35**
Variety	1	46.76**	45.90**
Treatment*Variety	3	0.10^{NS}	0.18^{NS}
Error	14	1.05	1.66

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix IX Table Analysis of variance of the data on dry weight of root, shoot, leaf and TDM as influenced by lead effect on 2 varieties of rice

Source of Varience	df	Mean square of dry weight of			
		Root	Shoot	Leaf	TDM
Replication	2	1.24	0.41	5.01	0.48
Tratment	3	2.83**	12.17**	58.93**	164.59**
Variety	1	3.68**	15.89**	116.16**	278.32**
Treatment*Variety	3	0.04 NS	0.14 NS	0.15 NS	0.31 NS
Error	14	0.11	0.15	1.60	1.30

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix X Table Analysis of variance of the data on proline content and panicle length as influenced by lead effect on 2 varieties of rice

Source of Varience	df	Mean square of	
		Proline content	Panicle length
Replication	2	0.42	65.02
Tratment	3	10.64***	94.90**
Variety	1	2.75***	123.76**
Treatment*Variety	3	0.51***	0.21 NS
Error	14	0.08	0.91

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix XI Table Analysis of variance of the data on effective tillers plant^{-1},non-effective tillers plant^{-1} , filled spikelets panicle^{-1} and unfilled spikelets panicle^{-1} as influenced by lead effect on 2 varieties of rice

Source of Varience	df	Mean square of			
		Effective Tillers Plant^{-1}	Non-Effective Tillers Plant^{-1}	Filled Spikelets Panicle^{-1}	Unfilled Spikelets Panicle^{-1}
Replication	2	6.20	3.24	176.05	6.75
Tratment	3	56.27**	8.56**	1722.55**	231.61**
Variety	1	59.53**	5.13**	4870.65**	46.76**
Treatment*Variety	3	0.14 NS	0.07 NS	22.10 NS	0.19NS
Error	14	0.64	0.13	24.80	1.65

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix XII Table Analysis of variance of the data on 1000 grain weight and grain yield plant^{-1} as influenced by lead effect on 2 varieties of rice

Source of Varience	df	Mean square of	
		1000 Grain Weight	Grain Yield Plant^{-1}
Replication	2	8.71	10.95
Tratment	3	97.73**	192.61**
Variety	1	125.58**	153.52**
Treatment*Variety	3	3.50 ^{NS}	0.37^{NS}
Error	14	1.95	0.69

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

PLATES

Plate I. Seedbed Preparation

Plate II. Uprooting of seedling from seed bed

Plate III. Pot Preparation

Plate IV. Transplanted seedling in experimental pot   Plate V. Watering plants

Plate VI. Collection of Data

Plate VII. Collection of Data