Abstract - The present study is focused on to examine the effects of phosphorus on physiological, photosynthetic pigments and oxidative status in Vetiver grass under lead (Pb) treatment. The root and shoot lengths of vetiver seedlings decreased gradually with the increase of lead concentrations (100, 200, 400, 600, 800 and 1000 mg/l). The growth rate was declined to 22.8 % and 21.1 % in root and shoot length respectively compared to the control. The addition of phosphorus (P) significantly increased the root (13.0 %) and shoots (19.3 %) lengths in vetiver seedlings at 1000 mg/l Pb + 680 mg/l P treatment compared to the control. The seedling biomass varied from 3.4 to 4 g in shoot and 2.5 to 3.1 g in root compared to the control at 1000 mg/l Pb + 680 mg/l P. Photosynthetic pigments including chlorophyll a, b and carotenoid content were decreased with increasing level of Pb concentrations in the growth medium. The addition of P significantly increased the photosynthetic pigments content in vetiver seedlings under the Pb with P treatment compared to the control. Antioxidative enzyme (SOD, CAT and POX) activities were gradually increased with increase in the lead concentrations. The addition of phosphorus (P) significantly increased the antioxidative enzyme activities in vetiver seedlings at 1000 mg/l Pb + 680 mg/l P compared to the control plants. These results suggested that the addition of P in the medium is found to be the reason for increase in the plant growth and antioxidative enzyme activities in vetiver seedlings under Pb treatment.

Keywords - Heavy metal, Vetiveria zizanioides L., Antioxidative enzymes, Lead, Photosynthesis.

I. INTRODUCTION

Lead (Pb) heavy metal pollution is one of the major ecological concerns due to its impact on plant productivity, human and animal health through the food chain and its high persistence in the environment [1]. Lead originates from various sources like mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts, metal plating and finishing operations, fertilizers, pesticides and from additives in pigments and gasoline [2].

Phosphorus (P) is an important macronutrient that constitutes vital molecules such as nucleic acid, phospholipids and sugar phosphates in all living organisms, which can exert influences on heavy metal accumulation and its relevant mechanisms in hyperaccumulators based on the interaction between P and heavy metals [3].

Heavy metals generally cause damage to plants, either directly or indirectly by triggering an increased level of production of reactive oxygen species (ROS). These ROS include superoxide radical (O$_2^•$-), hydroxyl radical (OH$^-$) and hydrogen peroxide (H$_2$O$_2$) that are produced as byproducts during membrane linked electron transport activities as well as by a number of metabolic pathways. ROS damages cell membranes, nucleic acids and chloroplast pigments. Plants have antioxidant systems to protect them against oxidative damage. Those detoxification processes are complex and highly compartmentalized in plant cells. The level of ROS in plant is controlled by an antioxidative system that consists of antioxidative enzymes like Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX), and peroxidase (POX) and non-enzymatic low molecular mass antioxidants. SOD is a major scavenger of superoxide anion free radical, which is converted into hydrogen peroxide (H$_2$O$_2$) and oxygen (O$_2$). CAT localized in peroxysomes, scavenges H$_2$O$_2$ by converting it to H$_2$O and O$_2$. POD reduces H$_2$O$_2$ using several reductants of phenolic compounds. The two enzymes APX and Glutathione S-transferase (GST) play a pivotal role in scavenging ROS and maintaining the level of antioxidants ascorbate and glutathione [4]. Pb toxicity inhibits chlorophyll synthesis by causing an impaired uptake of essential elements such as Mg and Fe [5] and even accelerates the decomposition of chlorophyll [6]. Like the chlorophyll contents, the activity of ALAD decreases in plant leaves when exposed to higher levels of heavy metals [7]. Vetiver grass (Vetiveria zizanioides) is a tall (1 – 2 m), fast-growing, perennial tussock grass. It has a long (3 - 4 m), massive and complex root system, which can penetrate to the deeper layers of the soil [8]. The present study is focused on to examine the effects of phosphorus on physiological, photosynthetic pigments and oxidative status in Vetiver grass under lead (Pb) treatment.
Plant growth condition and lead treatment

Vetiver plants were collected and washed with tap water to remove the soil particles. Plants were transferred into plastic cups containing Hoagland nutrient solution (full strength) and provided proper aeration continuously. Subsequently, plants were treated with different concentrations of lead (0, 100, 200, 400, 600, 800, and 1000 mg/l), with phosphorus and without phosphorus. While medium without Pb served as control.

Treatment 1: Control,
Treatment 2: Control + phosphorus,
Treatment 3: Different concentrations of lead (Pb),
Treatment 4: Different concentrations of lead with phosphorus (Pb + P),

After 16 days of treatment, plants were removed from the hydroponic solution and thoroughly rinsed with tap water and distilled water. Shoot and root tissues were collected separately, weighed and used for determining the antioxidative enzyme activity and photosynthetic pigments.

Determination of photosynthetic pigments

The photosynthetic pigments (chlorophyll a,b and Car) were determined according to the method of Arnon [9]. Briefly, fresh leaves (100 mg) were homogenized in 80% (V/V) ice cold acetone and centrifuged at 5000 rpm for 5 min. The supernatant was collected and pellet was re-extracted twice 2 ml of 80% acetone. The absorbance of the supernatant was measured using Double beam UV-visible spectrophotometer.

II. BIOCHEMICAL ANALYSIS

Extraction of enzymes

Fresh leaves or roots (0.1g) were homogenized in a pre-chilled mortar pestle using under ice -cold conditions with 1 ml of 50 mM phosphate buffer (pH 7.5) containing 0.5 mM EDTA. The homogenate was centrifuged at 10,000 rpm for 10 min and supernatant was used to measure the activities of SOD, CAT, and POX.

Assay of Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium (NBT) method as described by Dhindsa et al., [10]. The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 13.33 mM methionine, 2.25 mM NBT, 0.1 mM EDTA, 50 mM NaCO₃, 60mM riboflavin and enzyme extract (0.1mL). Riboflavin was added last, and the glass test tubes were shaken and placed under fluorescent lamps. The reaction occurred for 15 min and was then stopped by switching off the light. The absorbance was measured at 560 nm. Blanks were run in the same manner, but without light and enzyme extract, respectively. One unit of SOD activity was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under the assay conditions.

Assay of Catalase (CAT) activity

CAT activity was measured according to the method of Aebi [11]. The reaction mixture (3 ml) contained 50 mM phosphate buffer (pH 6.1), guaiacol (16 mM), H₂O₂ (2 mM), enzyme and distilled water. The oxidation of guaiacol was measured by the decrease in absorbance at 470 nm for 1 min using UV-visible spectrophotometer.

Assay of Peroxidase (POX) activity

POX activity was measured using the method of Castillo et al., [13]. The reaction mixture (3 ml) contained 50 mM phosphate buffer (pH 6.1), guaiacol (16 mM), H₂O₂ (2 mM), enzyme and distilled water. The oxidation of guaiacol was measured by the decrease in absorbance at 470 nm for 1 min using UV-visible spectrophotometer.

Statistical analysis

For statistical validity, each treatment was made in 3 replicates for estimating enzyme activity and photosynthetic parameters, biomass, root and shoot length measurement. The analysis of variance (ANOVA) was performed using SAS program (SAS Institute, 1989). The mean differences were analyzed by Student- Newman-Keuls Test at the P<0.05 significance level.

III. RESULTS AND DISCUSSION

Effects of Pb and P addition on the Plant growth and biomass of Vetiver seedlings

Lead causes many changes in physiological and biochemical damage in growing plants. In the present study root and shoot lengths of vetiver seedlings decreased gradually with the increase of lead concentrations (100 to 1000 mg/l) in growth medium. The growth rate was declined to 22.8 % and 21.1 % in root and shoot length respectively when compared to the control. The addition of phosphorus (P) significantly increased the root (13.0 %) and shoots (19.3 %) lengths in vetiver seedlings at 1000 mg/l Pb + 680 mg/l P treatment compared to the control (Figuer-1 a, b). Plant biomass that denotes plant growth is an important factor for successful application of phytoextraction since its effectiveness depends on both plant biomass and elemental concentrations in a plant. The shoot and root biomass of vetiver seedlings were increased with increase in different concentrations of lead with phosphorus in the growth medium (Fig-2 a, b). The seedling biomass varied from 3.4 to 4 g in shoot and 2.5 to 3.1 g in root compared to the control at 1000 mg/l Pb + 680 mg/l P. Similar results reported that biomass of Alfalfa plants with phosphate/citric acid mixtures were higher than control. So, phosphate/citric acid mixtures can be used for enhancing Alfalfa plants to accumulate biomass as a remediation strategy for soil contaminated with heavy metals [13]. Phosphorus can help plants to accumulate biomass and initiate tolerance to
metals when availability of heavy metals are found in soil and water bodies [14].

Photosynthesis system is very important for plant growth and biomass production. Effect of Pb and P on photosynthetic pigments content is presented in figure 3 (a, b and c). The level of chlorophyll a, b and carotenoids content were gradually reduced up to 600 mg /L Pb compared to the control. The increased concentration of photosynthetic pigments content were 136.5 % (Chl a), 143.2 % (Chl b) and 246.1% (Car) at 800 mg /L Pb, respectively when compared to the control. The addition of phosphorus (P) the Chl a, b and Car content were significantly found to be in increasing trend with increasing Pb concentrations with P compared to the control.
Fig. 3 Effects of Pb and P addition on the photosynthetic (a) Chl a, (b) b, and (c) Car pigments of vetiver seedlings

Fig. 4 Effects of Pb and P on antioxidative enzyme (SOD, CAT, and POX) activities of vetiver seedlings
Heavy metals induce oxidative stress by generation of superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (HO$^-$), and singlet oxygen (O$_2$^•), collectively termed as Reactive Oxygen Species (ROS) [15]. Therefore, the induction of antioxidant enzymes including SOD, CAT, APX and POX is an important protective mechanism to minimize oxidative damage in polluted organisms [16]. Super oxide dismutase (SOD) considered playing a key role in cellular defense mechanisms against Reactive Oxygen Species (ROS). The effects of Pb on SOD are illustrated in Fig 4 (a). SOD activity of Vetiver leaves was found to be increased significantly (P<0.05) up to 600 mg L$^{-1}$ Pb level, followed by a decrease from 800 to 1000 mg L$^{-1}$ Pb of treatments, compared to their respective controls. Due to the addition of phosphorus (P), this enzyme activity was increased by 96.6 % at 1000 mg L$^{-1}$ Pb with P concentration compared to the control. The SOD activity decreases the risk of OH radical formation which may cause severe damage to membranes, proteins, and DNA [17]. The changes that occurred in catalase activity under Pb and P treatment is presented in Fig 4 (b). Compared to the control, CAT activity was increased with increasing Pb concentrations with P in leaves of Vetiver plants after 16 days of exposure, compared to the respective controls.

The activity of POX leaves of Vetiver plants grown in different concentrations of Pb and Pb + P containing hydroponic solutions is represented in Fig 4 (c). POX activity in leaves was found to increase significantly (P<0.05) up to 600 mg l$^{-1}$ and Pb + P treatments than their corresponding controls. POX activity was decreased at 800 to 1000 mg l$^{-1}$ and Pb + P treatments compared to the control. The increased SOD, CAT, APX and POX activities in Vetiver seedlings may be considered as circumstantial evidence for tolerance mechanisms developed by this plant species. [18].

IV. CONCLUSION

This study investigated the effects of phosphorus and lead on the physiological and biochemical changes in Vetiver seedlings. The shoot and root biomass of vetiver seedlings were increased by increasing the different concentrations of lead with phosphorus in the growth medium. It is hypothesized that phosphorus can promote plant to accumulate biomass and initiate tolerance to metals even if there is availability of metals in the growth medium. The maintenance of high antioxidative enzymes activities were observed along with the increased Pb with P concentration, influence a strong internal detoxification mechanisms inside plant cells. These results suggested that the addition of P in the medium is found to be increase the plant growth and antioxidative enzyme activities in vetiver seedlings under Pb treatment. Thus, Vetiver plants seem suitable for use as a phytoremediator in heavy metals contaminated soil and water bodies.

REFERENCES