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Effect of Heavy Metal Lead (PB) Stress of Different Concentration on Wheat (*Triticum aestivum* L.)

Khizar Hayat Bhatti, Sehrish Anwar, Khalid Nawaz, Khalid Hussain, Ejaz Hussain Siddiqi, Raja Usman Sharif, Aqsa Talat and Aneela Khalid

Department of Botany, Institute of Chemical and Biological Sciences, University of Gujrat, Gujrat-50700, Gujrat, Pakistan

Abstract: A pot experiment was conducted to study the adverse effects of Lead (Pb) on two wheat varieties i.e. Chakwal-97 and Sehar-2006. Plants were treated with Pb at 0, 40 and 60 ppm solution levels. Pb reduced the morphological parameters such as shoot/root length, shoot fresh/dry weights, number of tillers. Pb stress also decreases the photosynthetic pigments such as chl a, chl b but carotene contents were increased. Na^+, K^+ ion contents were also decreased by Pb. So Pb as a heavy metal has detrimental effect on wheat growth and development.

Key words: Heavy metal · Lead · Wheat · Growth · Photosynthetic pigments

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a potential source of food for overgrowing world population. Millions of tons of wheat are grown each year throughout the globe [1]. In Pakistan, it has central position in the cereal crops and occupies about 66% of the annual food cropped area, providing about 60% calories of the average diet as a major source of food [2, 3]. Yield of wheat is low, due to different reason such as salinity, drought, water logging, lack of proper nutrients, infertile soils and heavy metals [4].

The over use of natural resources and rapid industrialization in developing countries have increased the addition of heavy metals into the soil [5]. Heavy metal pollution is a major environmental problem because their contamination is harmful to wild life, humans and agriculture [6]. Heavy metals are essential micronutrients, for example, as cofactors of key metabolic enzymes, but when their concentration become high in soil, they become most toxic to the plants [7].

Lead (Pb) is a major anthropogenic pollutant that has been accumulated in different aquatic and terrestrial ecosystems since the industrial revolution. It is one of the most widely distributed heavy metal that is highly destructive to plants and most difficult to control [8-10].

Naturally, Pb is present in soil, sea water, lakes and rivers. It is also a component of lead batteries, rubber, paints, metal products (steel and brass) and dusts [11]. Besides natural sources, exhaust fumes of automobiles, chimneys of factories, mining, effluents from storage battery, smelting of Pb ores, fertilizers, additives in pigments, metal plating and pesticides are also major sources of Pb [12]. Due to low solubility and strong binding capacity with soil colloids, Pb has long residence time in soil, causing a large number of direct and indirect effects on plant growth and metabolism. It induces many biochemical and structural changes in biological systems [13, 14]. Pb in soil not only changes the soil microorganism activities and resulted in soil fertility deterioration, but also directly affects the physiological processes and main symptom of Pb stress includes leaf chlorosis [15, 16]. Decrease in the root hair development, water potential, plant hormones and stunted growth in plants are caused by the deposition of Pb [17, 18]. Oxidative stress in plants is caused by Pb [19]. Different reactions are shown by plants against Pb stress. Some of them are sensitive and the others are more tolerant [20].

The objective of the study is to examine the morphological, biochemical and physiological effects of heavy metal Pb stress on two wheat varieties.

Corresponding Author: Khizar Hayat Bhatti, Department of Botany, Institute of Chemical and biological sciences, University of Gujrat, Gujrat-50700, Gujrat. Pakistan, E-mail: khizar.hayat@uog.edu.pk.

MATERIALS AND METHODS

Two wheat varieties Sehar-2006 and Chakwal-97 were used in the experiment to study the effects of Pb. Experiment was performed in Botany lab and nursery of University of Gujrat, Hafiz Hayat Campus, Gujrat. Seeds were sterilized with sodium hypochlorite and washed by distilled water. Tap water was used to wash the sand. Seeds of each variety were sown in sand in plastic pots on 27th February 2012. For each variety, 15 plastic pots were used. Tap water was given regularly to plants for irrigation during course of study. After 7 days of sowing, Hoagland solution was given for better germination and growth.

Pb treatments were given in the form of Pb acetate as follows:

To (control) = 0 ppm, T1= 40 ppm, T2 = 60 ppm

The experiment was laid down in a completely randomized design (CRD) with 5 replicates (n=5) for each treatment of both varieties. After 15 days of treatment, following parameters were measured.

Determination of Morphological Parameters: Two plants were taken from each replicate to measure morphological parameters. Root length, shoot length were measured of each replicate with the help of scale. Root and shoot fresh weights were also measured with help of analytical balance. Then, they were oven-dried at 65°C for one week and their dry weights recorded. Numbers of tillers were also noted.

Determination of Chlorophyll Contents: Chlorophyll a, chlorophyll b and carotenes were determined according to Arnon method (1949). 0.5 g fresh weight of leaf was ground with 2ml alcohol, then 5 ml more alcohol was added and after keeping overnight in test tubes, OD (optical density) was measured with the help of spectrophotometer.

Chlorophyll a, chlorophyll b and carotenes were measured by following formulas:

- Chlorophyll a: 12.7 × OD (663) – 2.69 × OD (645) mM
- Chlorophyll b: 22.9 × OD (645) – 4.68 × OD (663) mM
- Carotenes: OD (480) + 0.114 × OD (663) – (0.638) × OD (645) mM

Determination of Na^+ and K^+ Ion Contents: Samples were taken after digestion and got the reading from flame photometer. Standard solutions of different concentration of testing nutrients (Na+, K+) were made. Standards were made of 50,100,150,200,250 ppm. Reading of standards from flame photometer was taken.

Statistical Analysis: Statistical analyses were carried out by using analysis of variance (ANOVA) and Microsoft Excel 2003.

RESULTS

Heavy metal pollution is a worldwide environmental problem, causing destructive effects on plants. When heavy metals are present in excess quantity, they become toxic and reduce the growth of plants. Pb is a heavy metal which is most difficult pollutant to control. It produces structural and biochemical changes in plants. In present experiment, effect of Pb was studied. Pb decreased the morphological characteristics such as shoot/ root lengths, fresh and dry weights of root and shoot, number of tillers and concentration of chlorophyll a, chlorophyll b and carotene contents were also affected by Pb.

Shoot Length (cm): Data showed that Pb treatments (40 and 60 ppm) decreased shoot length (cm) in both wheat varieties as compared to control. However, at 60 ppm, Pb caused significant reduction (p=0.001) in shoot length in them than of control. However, the difference in the varieties was least significant (p=0.05) regarding shoot length under lead stress. Nevertheless, Chakwal-97 exhibited higher shoot length values than of Sehar-2006 under the Pb treatments (Fig.1).

Root Length (cm): Results showed that Pb treatments (40 and 60 ppm) decreased root length in both wheat varieties as compared to control. However, 60 ppm solution had significantly (p=0.001) reduced shoot length under Pb stress (Fig. 2). There is no significant (ns) variation in both varieties of wheat.

Shoot and Root Fresh Weights: Results showed that Pb had decreased the shoot fresh weight of both wheat varieties significantly (p=0.001) and Pb treatment had reduced the root fresh weight (g) of both wheat varieties significantly (p=0.001) at both treatments (40 and 60 ppm) as compared to control. However, 60 ppm has more pronounced effect on root fresh weight. There is least

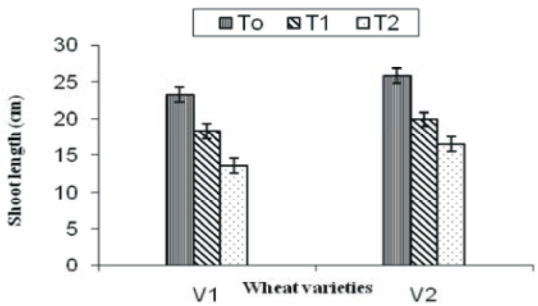

Fig. 1: Shoot length of two wheat varieties under Pb stress when plants were 35 days old Where, To = 0, T1= 40 and T2= 60 ppm, respectively (V1=Sehar-2006, V2=Chakwal-97).

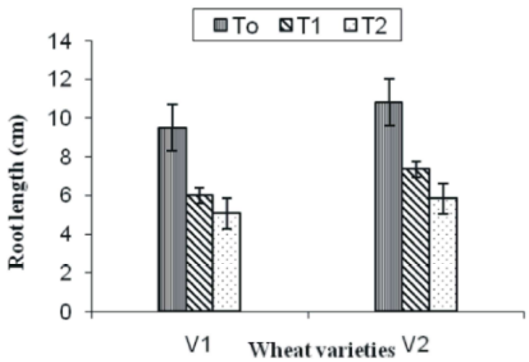

Fig. 2: Root length of two wheat varieties under Pb stress when plants were 35 days old (T1=40 and T2= 60 ppm).

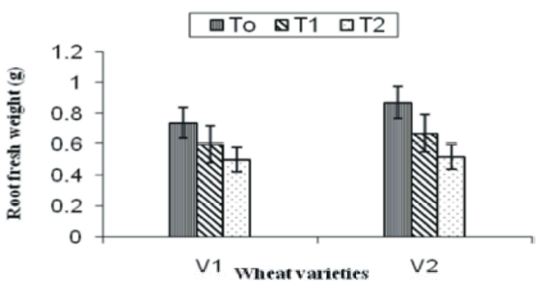

Fig. 3: Root fresh weight of two wheat varieties after treatment with Pb when plants were 36 days old (T1=50 and T2=100 mM).

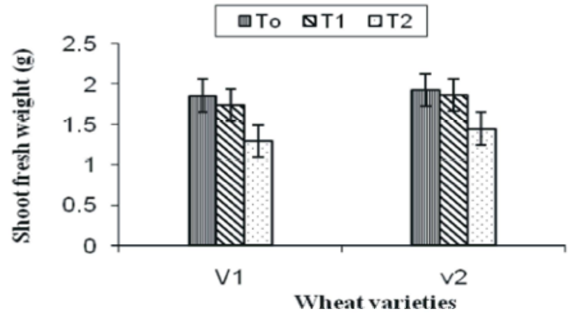

Fig. 4: Shoot fresh weights of two wheat varieties under Pb stress when plants were 36 days old, where (T1=40 and T2=60 ppm) respectively.

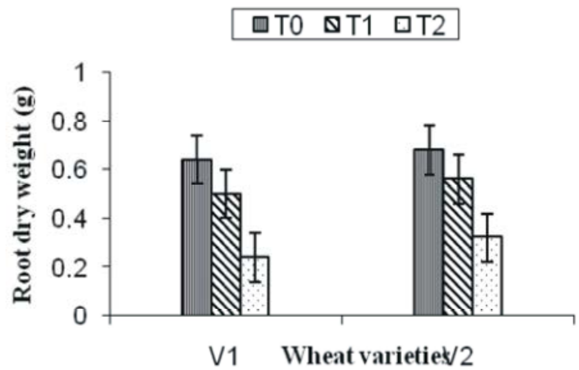

Fig. 5: Root dry weight of two wheat varieties under Pb stress when plants were 39 days old. And T1=40 and T2=60 ppm.

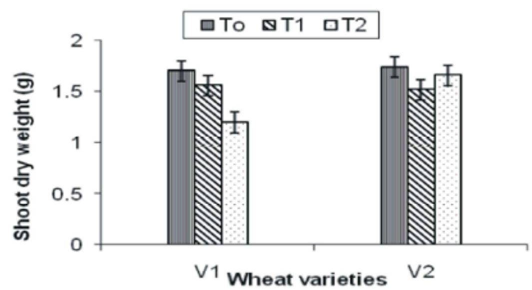

Fig. 6: Shoot dry weight of two wheat varieties under Pb stress when plants were 39 days old (T1=50 and T2=100 mM) respectively.

significant (p=0.05) variation in both varieties in root fresh weight under Pb stress. Chakwal-97 has only slightly more root fresh weight as compared to Sehar-2006 (Fig. 3).

Shoot and Root Dry Weights: Data showed that Pb treatment had significantly (p=0.001) reduced the root dry weight (g) in both varieties at both treatments (40 and 60 ppm). A treatment of 60 ppm had more decreasing effect on root dry weight and Pb treatment had also reduced the shoot dry weight (g) least significantly (p=0.05) at both treatments (40 and 60 ppm) as compared to control (Fig. 5,6).

Number of Tillers: Pb treatment reduced the number of tillers in both wheat varieties at both treatments (40 and 60 ppm) as compared to control. However,

Fig. 7: Number of tillers of two wheat varieties under Pb stress when plants were 36 days old (T1=40 and T2=60 ppm).

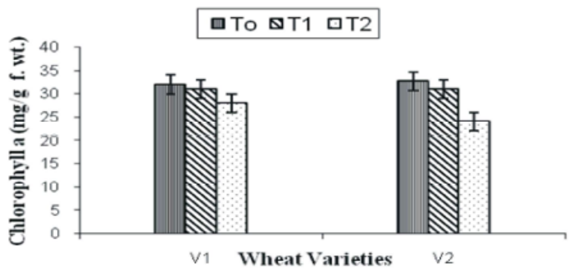

Fig. 8: Chlorophyll a content of two wheat varieties under Pb stress when plants were 38 days old and T1=40 and T2=60 ppm.

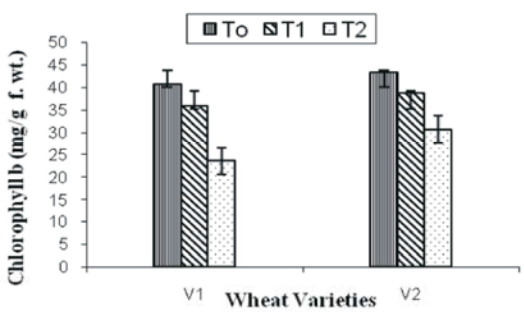

Fig. 9: Chlorophyll b contents of two wheat varieties under Pb stress when plants were 38 days old (T1=40 and T2=60ppm).

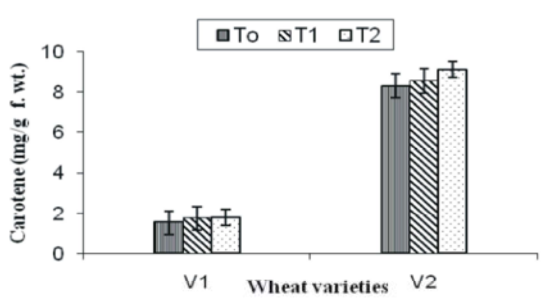

Fig. 10: Carotene contents of two wheat varieties under Pb stress when plants were 38 days old, (T1=40 and T2=60 ppm).

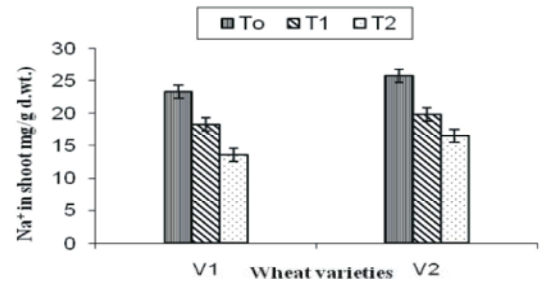

Fig. 11: Na^+ ion concentrations in shoot of wheat varieties under Pb stress when plants were 45 days old [T1=40 and T2=60ppm respectively].

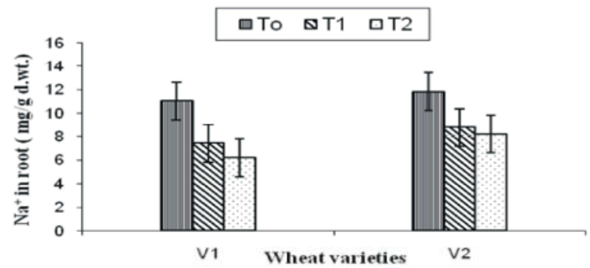

Fig. 12: Na^+ ion concentrations in root of wheat varieties under Pb stress when plants were 45 days old [T1=40 and T2=60ppm respectively].

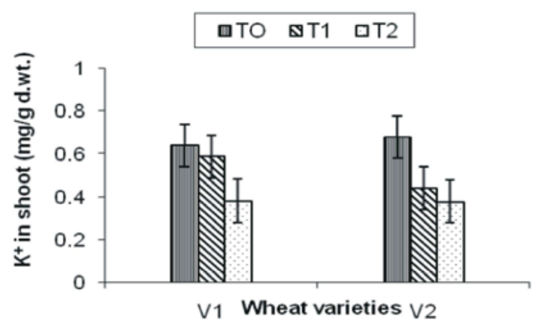

Fig. 13: K^+ ion concentrations in shoot of wheat varieties under Pb stress when plants were 45 days old [T1=40 and T2=60ppm respectively].

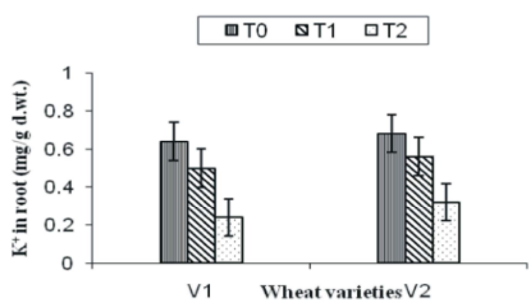

Fig. 14: K^+ ion concentrations in root of wheat varieties under Pb stress when plants were 45 days old [T1=40 and T2=60ppm respectively].

at 60 ppm solution there is more reduction in number of tillers (p=0.05). There is least significant (p=0.05) variation in both varieties. Chakwal -97 has only slightly more number of tillers as compared to Sehar- 2006 (Fig.7).

Photosynthetic Pigments: Pb decreased the chl. a and chl. b contents significantly (p=0.05) at 60 ppm solution level. There is no significant (ns) variation in both varieties (Fig.8, 9). However, carotene contents were increased after Pb treatments. There is highly significant (p=0.001) variation in both varieties. Chakwal-97 has high concentration of carotene as compared to Sehar- 2006 (Fig.10).

Na^+ and K^+ Ion Contents in Shoot: Pb had decreased the K^+ and Na^+ ion contents in shoot of both wheat varieties.

Na^+ and K^+ Ion Contents in Root: Pb had decreased the Na^+ and K^+ ion contents in root of both wheat varieties.

DISCUSSION

Heavy metal pollution has become a worldwide environmental concern because of its non reversible and long term adverse effects [21]. When heavy metals are present in high concentration, they produce toxic symptoms in plants. Pb is a widely distributed heavy metal which is most difficult pollutant to control. It produces structural and biochemical changes in plants [22-24].

Pb has inhibitory effect on morphological parameters of wheat varieties in present study. Shoot length decreased when Pb treatment was applied. It decreased due to reduction of meristematic cells in the shoot region by the accumulation of Pb. These findings are similar to another study in which there was also reduction in shoot length of wheat due to Pb contaminated soil [25]. Pb has also decreased the root length of both wheat varieties significantly. Root length is reduced due to inhibition of cell division in meristematic zone of root. Pb is a powerful inhibitor of root growth and accumulates largely in the roots [26, 27]. These results correlates with another study in which root length of wheat was reduced due to heavy metal Pb. It inhibits the growth of plants by alteration in enzyme activity and induction of oxidative stress [28]. Pb reduced the root and shoot fresh weight of both wheat varieties in this experiment. These results correlates to earlier study in which high concentration of Pb decreased the root and shoot fresh weight in wheat [29]. Pb is very toxic heavy metal which has detrimental effect on yield and biomass characteristics like fresh shoot and root weights, dry weights of both wheat varieties due to changes in metabolism and physiology of plants due to Pb. It also decreased the root dry weights of both root and shoot in sunflower [30].

In the present experiment, Pb has reduced the photosynthetic pigments (chlorophyll a and chlorophyll b) significantly. Chlorophyll contents are reduced by high concentration of Pb, because Pb prevents the incorporation of Fe (iron) in phytoporphyrin ring of chlorophyll molecule, so cause reduction in chlorophyll contents [31, 32]. Pb is known to inhibit chlorophyll synthesis either due to impaired uptake of Mg and Fe by plants [33]. Or because of increased chlorophyllase activity [34]. Similar results were observed in earlier study in which Pb stress decreased photosynthetic pigment amounts of the wheat leaves [35]. Heavy metal stress such as Pb caused reduction in photosynthetic pigments by either reducing their synthesis or enhanced biodegradation [36, 37]. Our data suggest that Pb has increased the carotene contents in Chakwal 97. The increase in carotene contents in plants due to heavy metal stress may be a strategy adopted by plants to alleviate the toxic effects of free radicals generated under heavy metal toxicity [38]. Carotene increment occurs under Pb stress in okra (*Hibiscus esculentus*) seedling [39].

CONCLUSION

Based on data, it is suggested that Pb as a heavy metal has toxic effects on plant morphological and biochemical parameters. It decreased the growth, yield, photosynthetic pigments and ion contents.

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