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Original Research Article

Effect of Variable Lead Concentrations on Biochemical Properties of Two Varieties of *Triticum aestivum* L. (Wheat): A Comparative Study

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A B S T R A C T

A pot-culture experiment was conducted to study the effects of lead (Pb) (applied as lead acetate) on two varieties of *Triticum aestivum* L. (Wheat): PBW-373 and PBW-343. Lead was applied to wheat seedlings at 0, 2, 4 and 8 g/kg quantity of lead acetate mixed with soil. Lead also showed deleterious effects on the production of photosynthetic pigments, carbohydrates; soluble protein in all variants but Pb toxicity increased the Proline accumulation in leaves of plants as the result of lead toxicity to plants. This work represents the effect of variable lead concentrations on biochemical properties of two wheat varieties and we could conclude that PBW 343 got more toxicity as compared to PBW 373 hence it has the tolerance capacity.

Keywords

Lead stress, Biochemical analysis, wheat, lead effect on wheat, abiotic stress, and lead stress tolerance

Introduction

Biotic stress includes the damage caused by different life forms e.g. pathogens like virus, bacteria, fungi etc., and insects and pests. Abiotic stress refers to the impact of environmental factors like climatic factors, edaphic factors, environmental pollution etc. Heavy metals and salinity in the environment are on the increase, affecting crop productivity. Lead is known to cause a broad range of toxic effects in living organism, including those that are morphological, physiological, and biochemical in origin (Srivastava, *et al.*, 2015). Ratlam (Madhya Pradesh), Bandalamottu Mines (Andhra Pradesh), Vadodara (Gujarat) and Kobra (Chhattisgarh) these are four site of lead in India. These places are highly contaminated with lead so cannot be used for farming as lead is highly toxic heavy metal; only lead tolerant crops can be grown at these places.

With this work we tried to find out if wheat can tolerate the lead concentration and can be grown in lead contaminated soil. To study this, we performed a pot experiment and give different lead concentration to two randomly selected wheat varieties PBW 343 and PBW 373 followed by different biochemical analysis.

Materials and Methods

For the experiment seeds of two different varieties of T. aestivum viz. PBW 343 and PBW 373 were obtained from Krishi Vigyan Kendra, Dariyapur, Rae Bareli, Uttar Pradesh. The germinated seeds of these two varieties were grown in soilrite medium and their leaves were harvested after 45 days of germination. The various chemicals used in this study were of analytical grade and procured from Hi-media. Lead acetate was used for lead source to the plants

Formula: $Pb(C_2H_3O_2)_2$
Molecular mass: 325.29 g/mol

A pot experiment was conducted for this work. Pots were filled with 250 g soilrite. The soilrite was treated with lead acetate to provide four concentrations of lead in the toxicity range (0, 2, 4 and 8 g/kg soilrite). Pots without lead treatment served as control for both varieties. The seeds of wheat were surface sterilized by 1%sodium hypochlorite for 1 min and after 1 min seeds were washed twice with distilled water. The sterilization process was followed by sowing 25 seeds per pots. Seed germination was seen within 3 to 5 days. Water was regularly given to all pots for proper growth of seedlings for 40 to 45 days (Divya *et al.,* 2018). After 45 days of treatment some biochemical parameters were studied:

Lead Acetate Treatment to *Triticum aestivum*

Pots were filled with 250 g soilrite. The soilrite was treated with lead acetate to provide four concentrations of lead in the toxicity range (0, 2, 4 and 8 g/kg soilrite). Pots without lead treatment served as control for both varieties. The seeds of wheat were surface sterilized by 1% sodium hypochlorite for 1 min and after 1 min seeds were washed twice with distilled water. The sterilization process was followed by sowing 25 seeds per pots. Seed germination was seen within 3 to 5 days. Water was regularly given to all pots for proper growth of seedlings for 40 to 45 days. After 45 days of treatment following biochemical parameters were measured (Akinci, et. al., 2010):

Determination of Biochemical Parameters of Both Varieties

Determination of chlorophyll content (Cenkci *et al.,* 2010)

Chemicals

Acetone (80%): 80ml of acetone was diluted with 20 ml distilled water and volume was made up to 100 ml.

Sample preparation

1gm of leaf sample was taken into a clean pestle and mortar and was grinded into a fine pulp with the addition of 20 ml acetone. The samples were then centrifuged at 5000 rpm for 5 minutes in cooling centrifuge at 4^0C. The supernatant was collected and transferred to a 100 ml volumetric flask.

The residue was grinded with 20 ml of 80% acetone, centrifuged and transferred to the same volumetric flask. This procedure was repeated until the colorless residue was obtained.

Calculation of result

The volume of the sample was made up to 100ml with 80% acetone. The absorbance was recorded in a U.V spectrophotometer at 645nm and 663 nm. The chlorophyll content was expressed in O.D units as mg/fresh weight of leaves. The result was calculated by the following formula:

Total chlorophyll (µg/ml) = 20.2 (A_{645}) + 8.02 (A_{663})

Chlorophyll a (µg/ml) = 12.7 (A_{663}) - 2.69 (A_{645})

Chlorophyll b (µg/ml) = 22.9 (A_{645}) - 4.68 (A_{663})

Determination of total carbohydrates

Chemicals

2.5 N HCl: 7.84ml of deionized water was added to 2.6 ml of concentrated HCl. Anthrone reagent: 200mg of anthrone was dissolved in 100 ml of ice-cold 95% H_2SO_4. This was prepared fresh before use.

Standard glucose: Stock- 100 mg glucose was dissolved in 100 ml of water. Working standard- 10 ml of stock was diluted to 100ml distilled water. This was stored in refrigerator after adding a few drops of toluene.

Sample preparation

100 mg of leaf sample was taken into a boiling tube and then hydrolyzed by keeping in a boiling water bath for 3 hours with 5ml of 2.5N HCl. It was then cooled to room temperature. It was neutralized with solid Na_2CO_3. The volume was made up to 100ml and then it was centrifuged. Supernatant was collected and 0.5 ml and 1 ml of aliquots were taken for analysis.

Calculation of result

Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standards where '0' served as blank. Volume was made up to 1ml in all the tubes including the sample tubes by adding distilled water. Anthrone reagent was added and then it was heated for 10 minutes in boiling water bath. It was cooled down rapidly and green to dark green colour was read at 630 nm. From the standard graph the amount of carbohydrate present in the sample was calculated. The formula used was:

Amount of carbohydrate present in 100mg of the sample = (mg of glucose / Volume of test sample) X 100

Determination of proline content

Chemicals

3% Salfosalicyclic acid: 3ml of salfosalicyclic acid was mixed with 97 ml distilled water.

Glacial acetic acid: 6.5 ml glacial acetic acid was mixed in 3.5 ml distilled water.

Ninhydrin**:** 0.25 gm ninhydrin in 10 ml distilled water.

Toluene

Sample preparation

1 gm of leaf samples were extracted in 5 ml of 3 % salfosalicyclic acid at 95^0C for 15 minutes. After filtration, 2 ml of supernatant was transferred to a new tube containing 2 ml of acetic acid and 2 ml of acidified ninhydrin reagent.

Calculation of result

After 30 minutes of incubation at 95^0C, samples were kept at room temperature for 30 minutes and 5 ml of toluene was added to the tube with shaking at 150 rpm to endure red product. The absorbance of toluene layer was determined at 532 nm. The proline content was calculated by the standard graph.

Determination of protein content (Garcia *et al.*, 2006)

The method developed by Lowry *et al.*, (1951) and is sensitive enough to give a moderately constant value.

Chemicals and Reagents

Phosphate buffer: 0.1 M, pH 7.6

Solution A: Alkaline Na_2CO_3 reagent: 2.0 gm Na_2CO_3 dissolved in 0.1N sodium hydroxide (NaOH) and volume was made to 10 ml with 0.1 N NaOH.

Solution B: Copper sulphate reagent: Prepared 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartarate solution.

Solution C: Folin's reagent: The reagent was diluted appropriately so that it is 1N in respect of its acid content.

Alkaline copper sulphate reagent: 1 ml of solution B was added to 50 ml of solution A. This mixture was unstable and was prepared fresh.

20% (w/v) TCA: 20gm of TCA was dissolved in 100 ml of distilled water.

Acetone

NaOH

Bovine serum albumin (BSA): 100µg/ml solution in distilled water.

Sample preparation

1 gm of sample was taken and macerated in pestle mortar in 5 ml of phosphate buffer. The homogenate was centrifuged at 8000 rpm for 20 minutes. The supernatant was collected and extraction was repeated 4-5 times. The supernatants were combined and the volume was made to 50 ml with phosphate buffer.1 ml of the above was taken and to it 1 ml of 20% TCA was added. It was then kept for half an hour and centrifuged at 8000 rpm for 20 minutes. The pellet was washed with acetone twice and it was again centrifuged. Supernatant was then discarded.

Calculation of result

Dissolved the pellet in 5ml of 0.1N NaOH and mixed well till it got dissolved. Took 1ml of above solution and added to it 5 ml of freshly prepared alkaline copper sulphate reagent. It was mixed properly and after 10 minutes, 0.5 ml of Folin's reagent was added. It was then left for 30 minutes to develop the colour. The absorbance was recorded at 660nm after setting the instrument with reagent blank as 0.1 N NaOH. In another set of test tubes suitable aliquots of BSA was taken. The total volume was made to 1 ml with 1 N NaOH and the colour was developed. Standard curve of absorbance at 660nm vs. µg of BSA was drawn. From this standard graph the amount of protein in the sample was determined.

Results and Discussion

Determination of Biochemical Parameters

Effect of Lead on Chlorophyll

The amount of chlorophyll a, b and total chlorophyll decreased under the increasing concentration of lead in soilrite. The results for the two wheat varieties at four lead concentrations are shown in Figure 1 (a) and (b) for PBW 373 and PBW 343 respectively.

In this study, Pb addition reduced the photosynthetic pigments (chlorophyll a and chlorophyll b) significantly (Fig. 1 'a' and

'b'). Chlorophyll contents were reduced with increasing concentration of Pb because Pb prevents the incorporation of Fe (iron) in phytoporphyrin ring of chlorophyll molecule and this leads to reduction in chlorophyll contents. Pb is known to inhibit chlorophyll synthesis either due to impaired uptake of Mg and Fe by plants (Malanga G *et al.*, 1995) or because of increased chlorophyllase activity (Monk LS *et al.*, 1989). Heavy metal stress such as caused by Pb reduced photosynthetic pigments by either reducing their synthesis or enhancing the process of biodegradation (Lozano R *et al.*, 1996). 57% decrease in chlorophyll was seen in PBW 373 and 58% decrease in chlorophyll was seen in PBW 343.

Effect of Lead on Carbohydrate content

The amount of carbohydrate was reported to decrease under the increasing concentration of lead in soilrite. The effect of various concentrations of lead on both varieties is presented in Figure 2.

In the present investigation, total carbohydrate of both varieties of wheat plant was negatively affected by the increasing concentration of lead in soilrite medium as presented in figure 2. Some studies found that treatment of plant with lead increased respiration rates of its organ and reduced the photosynthetic rates. The negative effect of lead on carbon metabolism is a result of their possible interaction with the reactive centre of ribulose bisphosphate carboxylase. In the present study 53% decrease in carbohydrates was seen in PBW 373 where as 65% decrease in carbohydrates was seen in PBW 343.

Effect of Lead on Proline

Proline is one component of the non-specific defense systems towards lead toxicity. It alleviates metal toxicity by acting as a metal chelator and as a protein stabilizer (Sharma and Dubey, 2005). Increasing lead in the growing medium increased the proline contents in the leaves of the two wheat varieties used in the study (Fig. 3).

Proline concentration increased in leaves of both varieties of wheat exposed to increasing lead concentrations. In wheat plant, proline concentration peak was significantly increased at 8 g/kg lead acetate in both varieties PBW 373 and PBW 343 by 81.35% and 93% respectively as compared to control.

Both varieties showed significant increase but PBW 343 was increased 10 folds of PBW 373. Proline increases as the result of lead toxicity to plants; present work shows that PBW 343 got more toxicity as compared to PBW 373.

Effect of Lead on Protein Content

Lead damages the proteins and inhibits protein synthesis also. With the increasing concentration of lead, protein contents are decreased due to toxic the results on the effects of increasing concentrations of Pb on protein concentration are shown in Fig. 4 (a) and (b).

The protein concentration was decreased with the increasing concentration of lead.81.4% reduction was observed in PBW373 at the highest concentration of lead but 84% reduction was observed in PBW373 at the highest concentration of lead as compared to control.

The decrease in protein concentration is observed because lead damages the genetic material which causes disturbance in central dogma so proteins get damaged due to higher concentration of lead.

Fig.1 Effect of different concentrations of lead on chlorophyll of PBW 373(a) and PBW 343 (b)

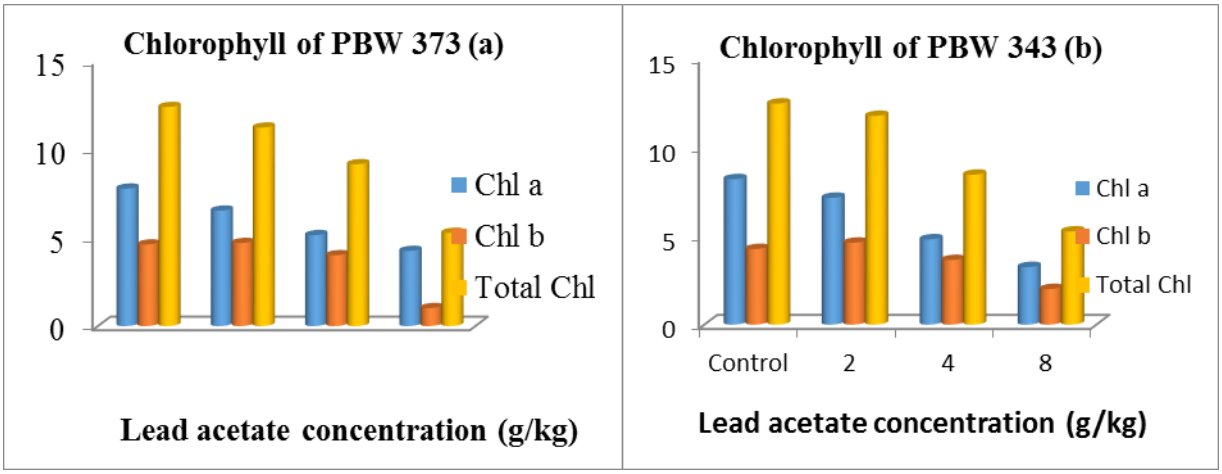


Fig.2 Effect of different concentration of lead on carbohydrate contents

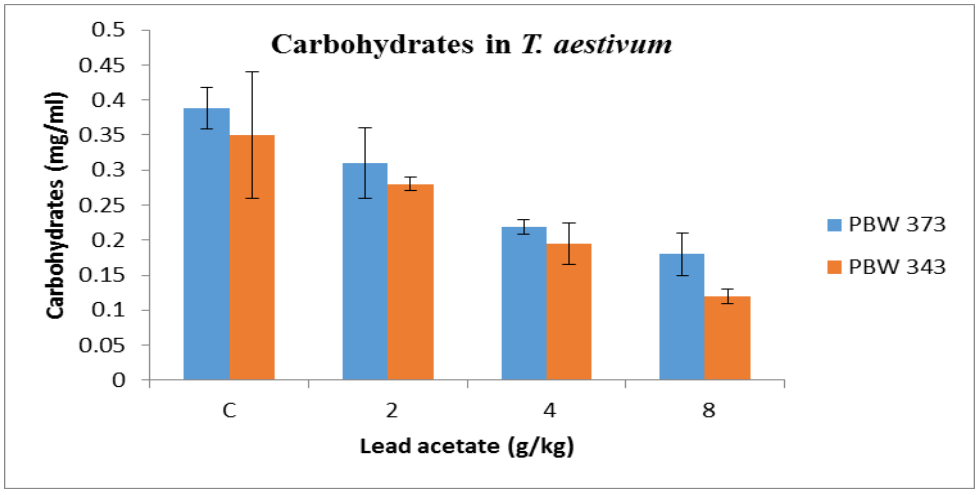


Fig.3 Effects of different concentrations of lead on Proline contents in the leaves

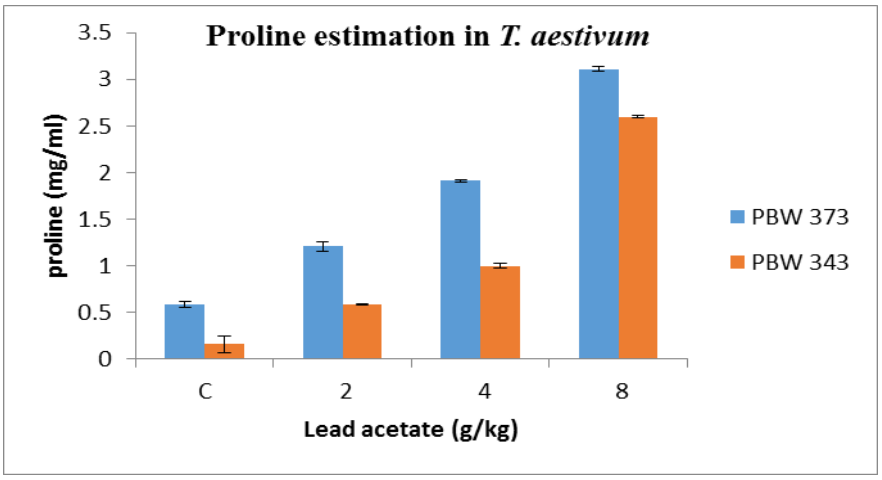
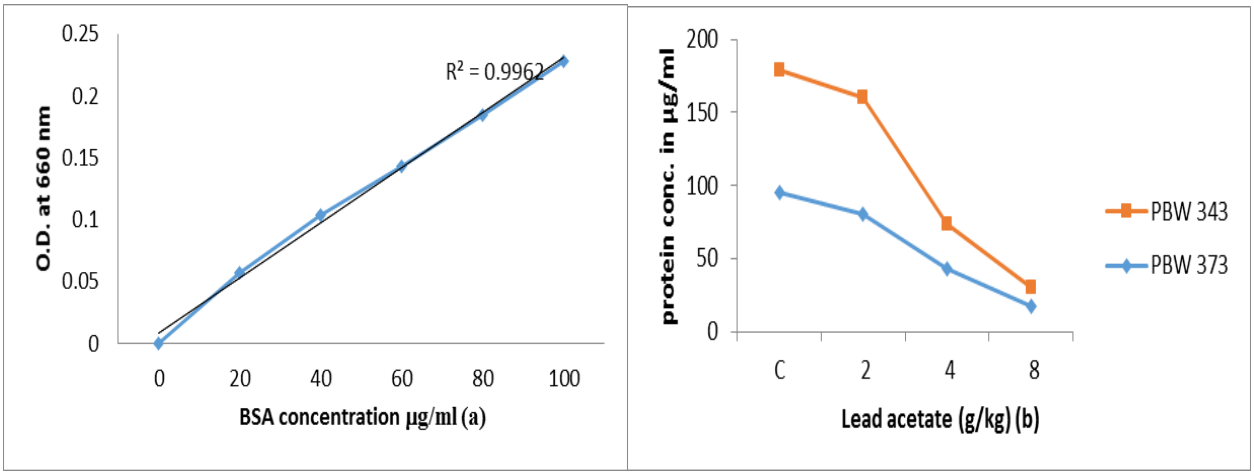


Fig.4 Standard curve of BSA (a) and Effect of different concentration of lead on protein concentration (b)



It has been observed in the present study that lead stress showed deleterious effects on the production of photosynthetic pigments, carbohydrates and soluble protein in all variants but the proline content got increased in leaves with the increasing concentration of lead acetate.

A drastic change was observed in chlorophyll of both varieties, which was highly reduced in PBW 373 by 76%, this decrease was 30 folds of chlorophyll of PBW 343 whereas 53% decrease in carbohydrates was seen in PBW 373 where as 65% in PBW 343. In wheat plant, proline concentration peak was significantly increased in PBW 343 12 fold of PBW 373 at 8 g/kg lead acetate.

There was no significant difference between both varieties in respect to their protein content but lead showed toxic effects on protein of both varieties. After finding all the results we could find that lead is highly toxic to wheat plant which was obvious but the aim of this study was to compare both of the varieties with respect to its tolerance capacity. Overall these results showed that PBW 373 had Pb tolerance capacity as compared to PBW 343.

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