Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 479-483
Received: 12-11-2017
Accepted: 14-12-2017

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Comparative study on the Effect of Different Lead Concentrations on Two varieties of *Triticum aestivum* L. (Wheat)

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Abstract
Lead (Pb) is the most common heavy metal contaminant in the terrestrial and aquatic ecosystems. It enters the ecosystem through various natural and anthropogenic sources. Plants absorb Pb from their environment; but it is not an essential element. Wheat (*Triticum aestivum* L.) is a main link in tropic chain; and it is one of the economically important food crops. Wheat is also the main pathway for ingestion of the pollutant by humans and animals through food and feed. The present work discusses how Pb affects nutrient uptake and metabolism in wheat, Pb gets accumulated in a dose-dependent manner in wheat plant, and causes toxicity in the plant. A pot-culture experiment was conducted to study the effects of Pb (applied as lead acetate) on two wheat varieties: PBW-373and PBW-343. Lead was applied to wheat seedlings at 0, 2, 4 and 8 g/kg quantity of lead acetate mixed with soil. The shoot length was observed to be decreased slowly with increasing concentration and at the highest concentration of Pb, the root length was decreased by four times of control in both variety. It decreased the growth and yield of plant as well as different parameters of growth measurements. Both varieties PBW 373 and PBW 343 has a great capacity to accumulate lead which shows their tolerance against lead but PBW 373 has a higher capacity of accumulation as compared to PBW 343.

Keywords: Lead, Wheat, Source of lead, Mechanism of toxicity, pot experiment, lead accumulation

Introduction
Wheat (*Triticum aestivum* L.) is a staple food of overgrowing world population. Millions of tons of wheat are grown each year throughout the globe. Yield of wheat is low, due to different reasons such as drought, salinity, water logging, lack of balanced nutrition of the crop, infertile soils and contaminated soils with various contaminants including chemicals and heavy metals. The over use of natural resources and rapid industrialization in developing countries have further increased the contamination of agricultural soils with metals. Heavy metal contamination is harmful to wild life, humans and agricultural products produced. Selected heavy metals are essential micronutrients, for example, as they act as cofactors of key metabolic enzymes, but when their concentration becomes high in soil, they become more toxic to the plants. The sources of lead (Pb) indeed are from numerous daily used common products in diverse locations, some of them quite unexpected (Srivastava *et al*., 2015) [7]. Lead adsorption onto roots has been documented to occur in several plant species. Once adsorbed onto the rhizoderm roots surface, lead may enter the roots passively and follow translocating water streams. High level of lead is inhibitor for seed germination and different growth parameters of plants; it reduces germination by lowering the enzymatic activity and growth by slow the rate of cellular division and expansion. Various physiological processes such as water transport, photosynthesis, respiration, metabolism of carbohydrates are inhibited by lead.
This paper represents how the increasing concentration of lead alters the photosynthetic pigments of two different wheat varieties viz. PBW 373 and PBW 343 as a comparative study.

Material and Method
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. After 45 days of treatment following parameters were measured:

1. Determination of Lead Accumulation in Two Varieties of Triticum Aestivum Lead Accumulation in Wheat

1.1 Lead accumulation in Triticum aestivum

The plant and soilrite samples were sent to ICRISAT-Hyderabad for accumulation studies. There, samples were dried and finely ground before determining lead in the plant (roots plus shoot) and soilrite samples. The ground plant samples were weighed in digestion tubes and treated with concentrated nitric acid in the presence of an oxidizing agent hydrogen peroxide. The digested samples were analyzed for lead contents using Inductively Coupled Plasma (ICP) – Optical Emission Spectrometer (OES).A detailed description of the method is available in an earlier paper (Shirisha *et al*., 2014) [6]. The results are expressed on dry wt. basis. Accumulation of lead in samples = lead concentration (µg/g) in samples x their biomass.

1.2 Determination of Bioaccumulation factor (BF)

The BF of *Triticum aestivum* was calculated with the following formula as per Zhao *et al*. (2003):

BF= [Pb concentration in plant]/ [Pb concentration in soilrite culture]

2. Determination of Physiological Parameters of Both Varieties

2.1 Quantitative determination of germination percentage, root and shoot length

Germination percentage of wheat seeds was determined by using the following equation:

Germination percentage = Total number of seeds germinated/total number of seeds sown x100

Root length (cm) = measured at the time when the plants of two wheat varieties grown under four lead treatments were harvested.

Shoot length (cm) = measured at the time when the plants of two wheat varieties grown under four lead treatments were harvested.

2.2 Determination of Growth Ratio Percentage (GR)

The growth ratio of plant was calculated with the following formula:

GR= [Plant biomass with Pb]/ [Plant biomass without Pb] x 100

2.3 Determination of Pb tolerance index (TI)

The tolerance index of plant is calculated with the following formula:

TI= [Root length with Pb]/ [Root length without Pb]

Results

Lead accumulation in plants

The amount of lead accumulated in complete *T. aestivum* plant biomass was increased with the increasing concentration of lead in the growing medium (Fig.1.1). Accumulated lead concentration is expressed in µg/g in fig 1.1. According to the results presented in figure 1.1, lead accumulation was directly proportional to the increasing concentration of lead treatment. It was increased by 63% in PBW 373 whereas 30% lead accumulation was observed in PBW 343 as compared to control. That means both wheat varieties accumulated lead but the capacity of lead accumulation was different in both varieties. PBW 373 showed 50 fold more accumulation of lead as compared to PBW 343. According to this result PBW 373 is a good lead accumulator. Earlier studies by Lopez *et al*., 2009 [4] found the related results of lead accumulation in alfalfa plant.

Determination of Bioaccumulation Factor

Bioaccumulation factor of both varieties increased with increasing lead concentration (Fig 1.2). According to figure 1.2, bioaccumulation factor was increased in both varieties, the BF of PBW 373 was increased by 60% and 65% increase in BF of PBW 343 was seen as compared to control of both varieties (Wierzbicka *et al*., 2007) [8].

Determination of Physiological Parameters

Quantitative Determination of Germination Percentage, Root Length and Shoot Length

According to Lin *et al*., 2007 the first symptom of Pd toxicity is seen on growth of the plants. These were the changes observed in *T. aestivum* with increasing lead concentration as compared to control:

Germination percentage

The germination of seeds under the increasing concentrations of lead in soilrite was determined after sowing 25 seeds per pot. The results for two wheat varieties at four lead concentrations are shown in Figure 2.1.1.

It is clear that as compared to control (C), the germination of seeds in both varieties decreased. 57% decrease in seed germination of PBW 343 variety and 50% decrease in seed germination of PBW 373 variety was observed. As discussed earlier in figure 1.1 PBW 373 showed a good accumulation capacity and in figure 1.2 its bioaccumulation factor was less than other variety PBW 343. In figure 2.1.1 the decrease in seed germination was less in PBW 373 as compared to PBW 343. According to all results presented till this section PBW 373 variety showed tolerance as it accumulated more lead still showed less decrease in the seed germination as compared to PBW 343.

Root length

The root length under the increasing concentration of lead in soilrite was observed to be decreased. The results for the two *T. aestivum* varieties at four lead concentrations are shown in Figure 2.1.2.

The Pb concentration in the growing medium decreased the root length of both *T. aestivum* varieties significantly. Root length was 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 (Nakano et. al. 1981) [5]. According to the figure 2.1.2, with respect to control 75% decrease in root length of PBW 373 was seen whereas in PBW 343 it was 52% at the highest concentration of lead acetate (8 g/kg). The decrease in root length was more in PBW 373 as it accumulated lead 50 times more than PBW

343 (figure 1.1), so the roots are highly affected in this variety as compared to other one.

Shoot Length

The shoot lengths of both varieties growing under the increasing concentration of lead in soilrite were observed to be decreasing. The results for the both *T. aestivum* varieties at four lead concentrations are shown in Figure 2.1.3.

Pb has inhibitory effect on morphological parameters of *T. aestivum* varieties in present study. Shoots were negatively affected by increasing concentration of lead, same results were seen in earlier study by Akinci *et al*., 2010 [1] for tomato crops. 70% decrease in roots length of PBW 373 was seen whereas 80% decrease was observed in PBW 343 as shown in figure. 2.1.3. This decrease was 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 (Egley *et al*., 1983) [2].

Growth Measurement

Growth measurement in both varieties is done by calculation growth ratio, Pb tolerance index and bioaccumulation factor as follows:

Growth Ratio Percentage

The growth ratio percentage of both varieties decreased with the increasing lead concentration as shown in figure 2.2.1.

The growth ratio was decreased with the increasing lead concentration. 68% decrease and 55% decrease were observed in PBW 373 and PBW 343 respectively. The decrease in the growth ratio of PBW 373 was 20 fold to PBW 343 at the highest concentration of lead as shown in figure 2.2.1. This drastic decrease in PBW 373 is related to the lead accumulated by this variety, as it accumulated high amount of lead so its growth ratio decreased more than PBW 343 at the highest concentration of lead as compared to control of both varieties.

Pb tolerance index (TI)

The tolerance index of plants of various crops is very useful in determining their tolerance against lead or any other heavy metal toxicity. The tolerance index of both varieties of *T. aestivum* decreased with the increasing lead concentration in the growing medium (Fig. 2.2.2).

The tolerance index was decreased with the increasing lead concentration. 76% decrease and 53% decrease were seen in PBW 373 and PBW 343 respectively.

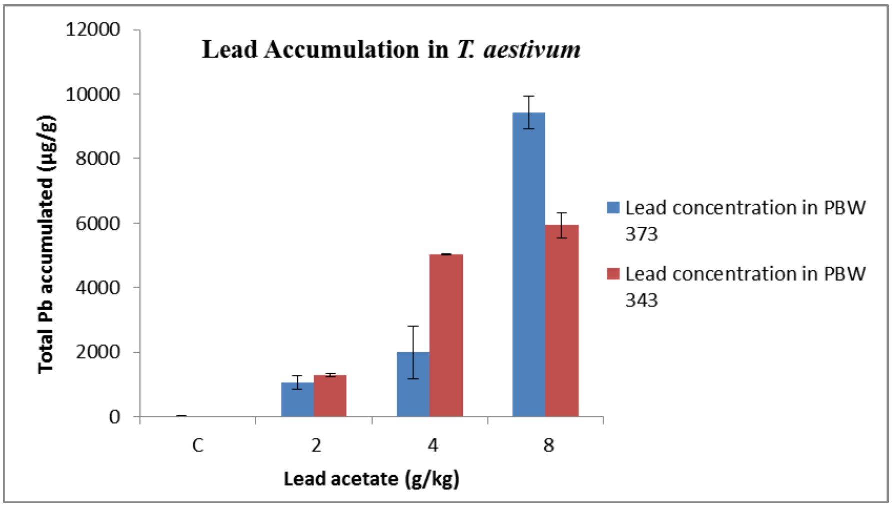

Fig 1.1: Lead accumulation (µg/g) in two varieties of *T. aestivum*

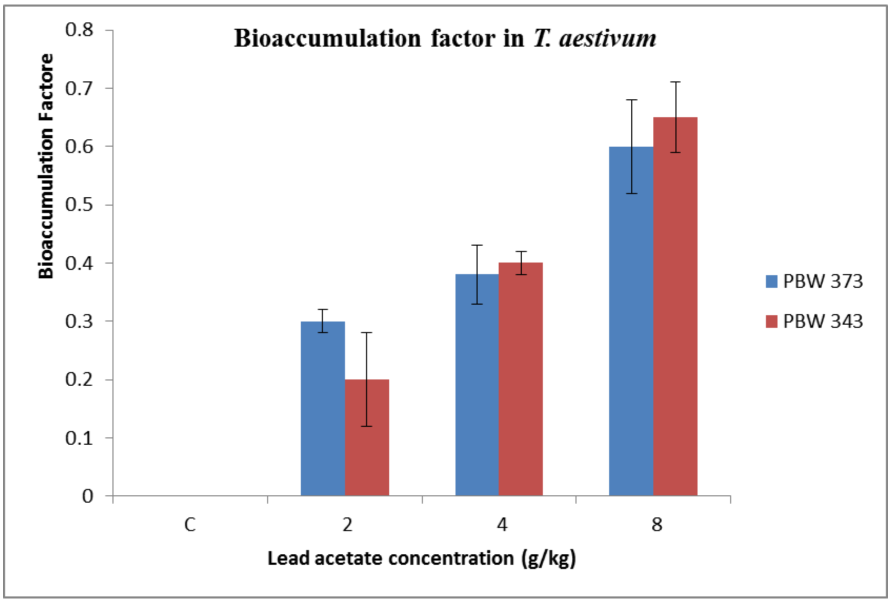

Fig 1.2: Effect of different concentrations of lead on Bioaccumulation factor

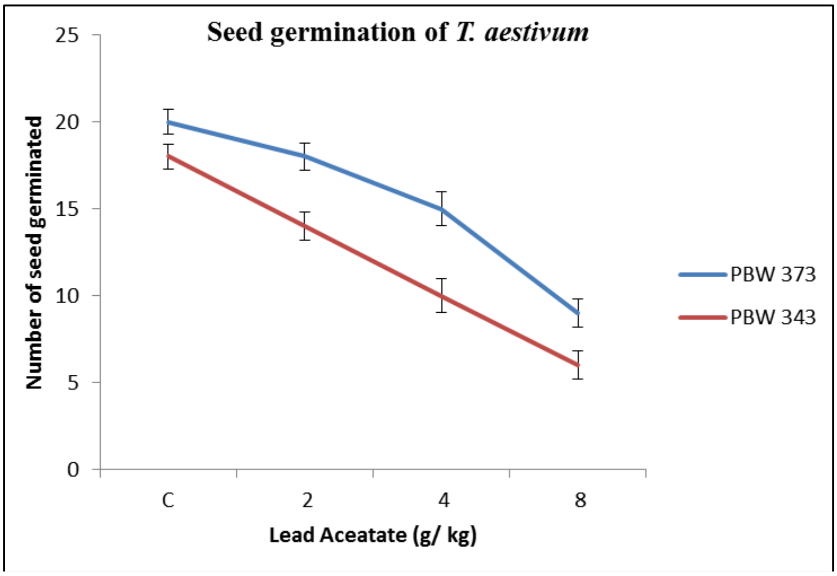

Fig 2.1.1: Effect of different concentrations of lead on seed germination

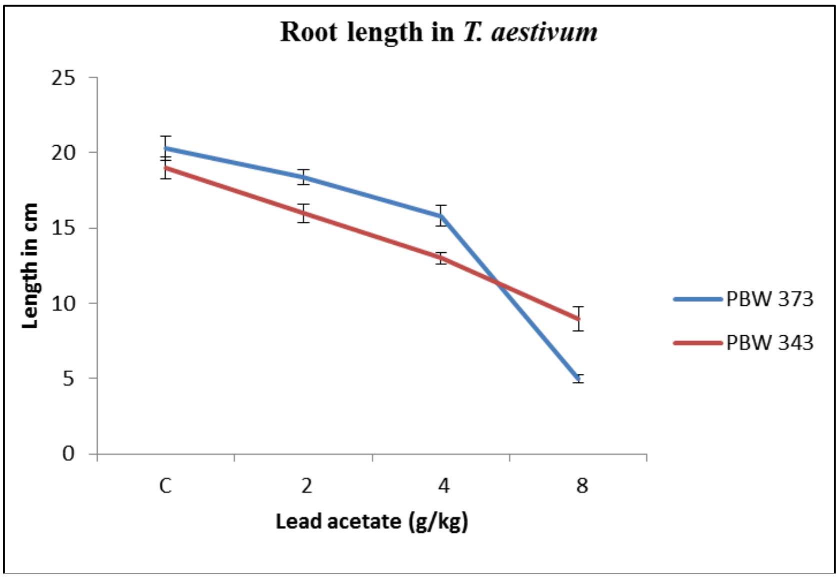

Fig. 2.1.2: Effect of different concentrations of lead on root length

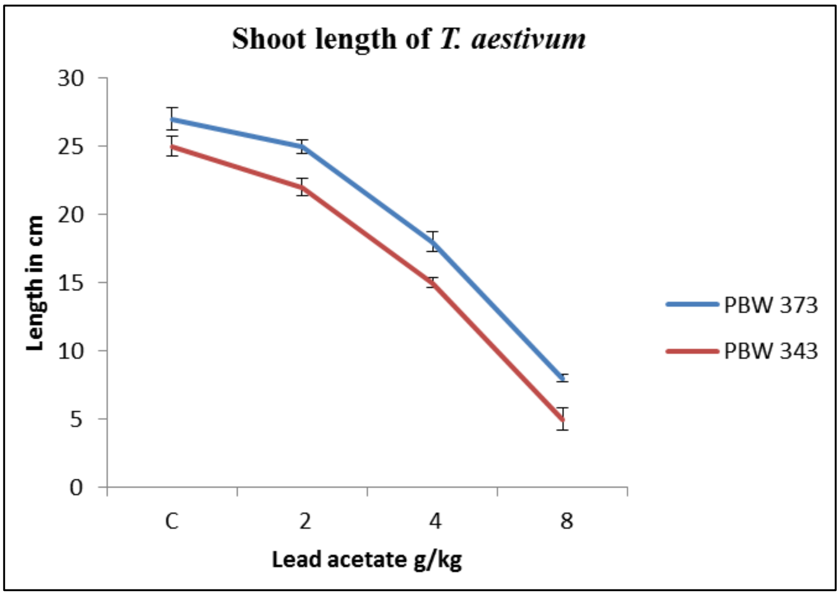

Fig 2.1.3: Effect of different concentrations of lead on shoots length

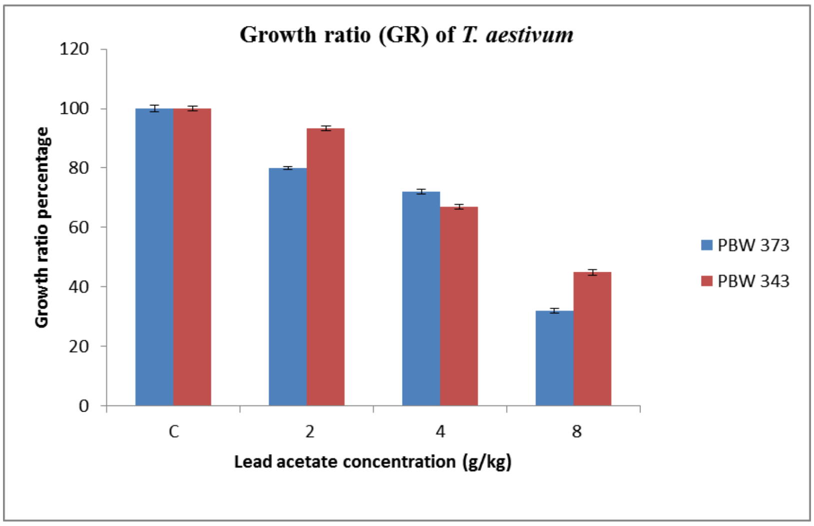

Fig 2.2.1: Effect of different concentrations of lead on Growth ratio percentage

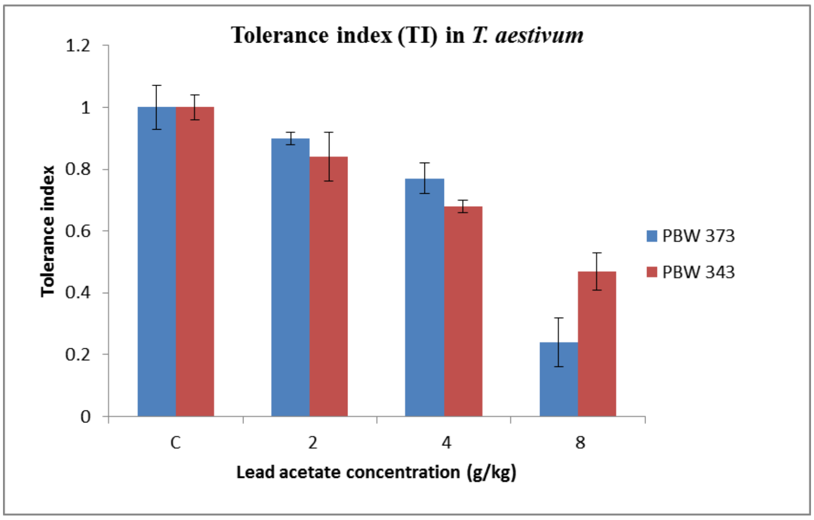

Fig 2.2.2: Effect of different concentrations of lead on Tolerance Index (TI)

Conclusion

Based on data, it is suggested that Pb as a heavy metal has toxic effects on plant morphological parameters. It decreased the growth and yield of plant as well as different parameters of growth measurements. Both varieties PBW 373 and PBW 343 has a great capacity to accumulate lead which shows their tolerance against lead but PBW 373 has a higher capacity of accumulation as compared to PBW 343.

Future aspects

PBW 373 showed higher capacity than PBW 343 so there is a need to detect that particular gene which is responsible to provide this strength to the plant and it is also to be studied if the accumulated lead is available in gains of the wheat plant as well.

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