International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 21-06-2021, Accepted: 06-07-2021, Published: 21-07-2021
Volume 6, Issue 4, 2021, Page No. 323-328

Growth regulatory parameters of *Triticum aestivum* L. under lead toxicity

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Abstract
In the terrestrial and aquatic ecosystems the pollution caused by heavy metal accumulation is of major concern. Lead (Pb) is the second most prominent contaminant after arsenic (As). In plants no physiological functioning requires the presence of lead as it is non-essential element. Triticum aestivum L. is one of the major cash crops with high consumption value. The present study enlightened the toxic effect of lead (Pb) on growth regulatory parameters and photosynthetic pigments of test plant in a dose dependent manner. Sterilized seeds were grown under different concentrations of lead acetate salt (800-50 ppm). The result evaluated with reduced germination percentage, retarded root-shoot length and decrease in seed vigorosity as the concentration increases (>100 ppm). The estimation of chlorophyll and carotenoid content also slightly reduced at higher doses of lead salt. Hence, crops cultivated in heavy metal contaminated sites shows poor growth and needs proper monitoring. To overcome this issue, microbial remediation strategies needs to be worked upon along with the application of nanotechnology.

Keywords: lead, heavy metal toxicity, *Triticum aestivum*, seed vigorosity, chlorophyll, carotenoids

Introduction
The major ecological concern is the heavy metal pollution as it poses serious health issues in humans by becoming a part of food chain and its persistent nature [1]. According to the new European REACH regulations, after Arsenic (As) lead (Pb) is the most harmful and chemical of great concern [2]. The toxic level of lead in soil ranges from 400-1000 ppm as per Angelone and Bini [3], and its most common in anthropogenic activities and industrial wastes such as ore, mines, automobiles etc. [1, 4]. The biological systems (both plants and animals) adversely affected by the Pb toxicity which results in severe decrease in growth regulatory parameters such as improper growth, loss of photosynthetic pigments etc. [5, 6]. Although certain plant varieties are Pb tolerant. Interestingly certain pants have developed phytoremedial potential for the clean-up of Pb contaminated sites by accumulating this metal especially in roots at higher doses [1, 5, 7, 8]. Various significant efforts have been made to discover different physiological and biochemical processes in order to make plants adaptable to heavy metal stress and to protect them from severe damages [9]. The extreme production of reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide etc leads to biomolecular damage [10].

Materials and Methods
1. Preparation of heavy metal concentration: The different concentrations (800ppm-50ppm) of Lead acetate trihydrate salt were prepared by dissolving the appropriate amount of salt in sterilized double distilled water. The prepared concentration was mixed in 1% agar and after settlement of the media, sterilized seeds were placed in the media under different experimental setups as Wc (Control), W1 (800 ppm), W2 (400 ppm), W3 (200 ppm), W4 (100 ppm) and W5 (50 ppm).

2. Sterilization of seeds: Seeds of *Triticum aestivum* (var. Arjun) were purchased from certified supplier of Prayagraj district. The healthy seeds were selected and then surface sterilized with 0.2% $HgCl_2$ solution for 2-3 minutes and then soaked in double distilled water overnight. Pots were kept in sterilized condition under standard growth conditions in plant growth chamber under photosynthetically active radiation (PAR) of 150 µmol photons m-2s-1 with 16:8 h day and night regime and 55%± 5 % relative humidity at 25°C ±1°C.

3. Growth regulatory parameters: 2.3.1 Germination percentage: Germination percentage of seeds of each treatment was recorded for 15 days and the rate of germination as Timson's index was calculated using formula [11].

3.1 Timson's index = Σ (G/t); G= germination percentage; t= total period of germination in days.

3.2 Root Shoot Length: The seeds of *Triticum aestivum* that were treated with different concentrations of lead acetate salt ranging from 800-50 ppm were estimated for root shoot length after 5 and 15 days of growth.

3.3 Seed Vigor Index (SVI): The seed vigor index (SVI) of the seedlings was estimated after 15 days [12].
SVI = total root length + total shoot length x Seed germination percent.

3.4 Photosynthetic pigments content: The chlorophyll content of wheat was determined by the formula provided by Arnon [13]. For the estimation of carotenoid content, absorbance was recorded at 645 nm [14]. For the estimation of chlorophyll and carotenoid content grown seedlings were treated with the prepared concentration of lead acetate in pot condition, ranging from 800-50 ppm under the experimental setups Wc, W1, W2, W3, W4 and W5.

3.5 Statistical analysis: The experiment was conducted in triplicates. The data obtained was analyzed by analysis of variance (ANOVA) followed by Least square difference (LSD) at the level of $P<0.05$ by using SPSS 16.0.

Results

The various growth parameters were observed in *Triticum aestivum* grown under lead toxicity at different concentrations ranging from 800-50 ppm in Wc to W5 experimental setups.

1. Seed germination: Germination parameters were investigated to gather information about the tolerance level of *Triticum aestivum* against lead toxicity. Maximum germination was recorded for Wc (Control) followed by W5 (50 ppm). Initially, 3 days old seedling was able to germinate at 800 ppm but after 15 days of treatment was unable to tolerate lead toxicity and therefore the germination percentage declined drastically (Table 1).

2. Timson's Index: At higher concentration of 800 ppm (W1) no value was recorded after 15 days of treatment. As the concentration lowers down to 50 ppm (W5) increased values of Timson's index was found however comparatively less than control (Wc) (Table 2).

3. Root Shoot Length: Root shoot length was found to be higher in W5, which was high when compared to control (without lead). At higher concentration of lead acetate at 800-200 ppm in setup W1, W2 and W3 salt no data was recorded for root shoot length as they were not measurable (Table 3).

4. Seed Vigor Index: For setup W5 (50 ppm) highest value of seed vigor index was recorded which was high when compared to control (Table 4). No values were recorded for W1 (800 ppm), W2 (400 ppm) and W3 (200 ppm) as the seeds were not able to sustain at these concentrations.

5. Photosynthetic pigments content: Highest total chlorophyll, Chl*a* and Chl*b* value was recorded for W2 whereas high carotenoid content was found in case of W5. All the values of chlorophyll pigments were high when compared to control. At higher concentration of 800 ppm low carotenoid content was recorded for W1 (Table 5).

Table 1: Seed germination percentage after 3 and 15 days in *Triticum aestivum* under lead acetate treatment.

Treatments	Germination Percentage	
	3 Days	15 Days
Wc (Control)	40±0.2	73.33±11.547.1
W1 (800 ppm)	60±0.2	0
W2 (400 ppm)	53.33±0.115	26.66±11.54701
W3 (200 ppm)	46.66±0.23	33.33±11.54701
W4 (100 ppm)	26.66±0.115	33.33±11.54701
W5 (50 ppm)	53.33±0.23	53.33±11.54701

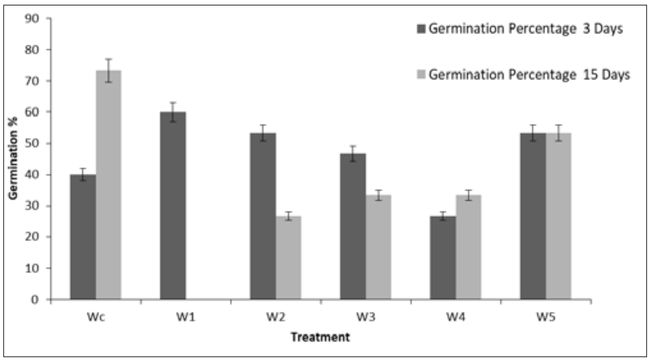

Fig 1: Germination percentage of *T. aestivum* calculated after 3 and 15 days of treatment.

Table 2: Timson's index calculated after 3, 7 and 15 days in *T. aestivum* under lead acetate treatment.

Treatment	Timson's Index		
	3 Days	5 Days	15 Days
Wc (Control)	13.78±0.83	8.26±0.46	5.03±0.17
W1 (800 ppm)	27.67±0.61	12.44±0.40	0.19±0.03
W2 (400 ppm)	19.25±0.13	10.81±0.13	1.85±0.07
W3 (200 ppm)	17.62±0.18	9.55±0.19	2.22±0.03
W4 (100 ppm)	9.11±0.39	5.77±0.77	2.22±0.03
W5 (50 ppm)	18.51±0.13	11.4±0.13	3.54±0.01

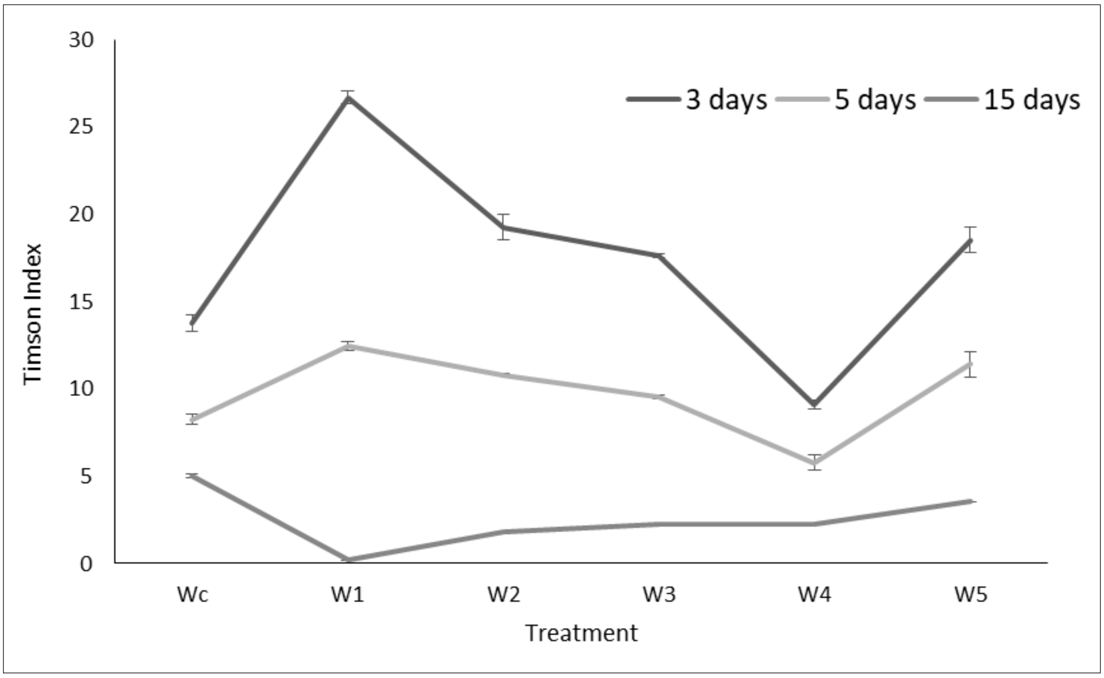

Fig 2: Timson's index calculated for *T. aestivum* after 3, 5 and 15 days of treatment.

Table 3: Effect of lead acetate on growth of *T. aestivum* under the treatment lead acetate.

Treatments	Root Length		Shoot Length	
	5 Days (Mean)	15 Days (Mean)	5 Days (Mean)	15 Days (Mean)
Wc (Control)	5±0.00	5.6±0.2	5.5±2.598	4.3±0.1
W1 (800 ppm)	0	0	0	0
W2 (400 ppm)	0	0	0	0
W3 (200 ppm)	0	0	0	0
W4 (100 ppm)	0	9.2±0.1527	0.5±0.288	2.8±0.1
W5 (50 ppm)	4.233±0.2516	11.7±0.2081	8.1±0.793	14.7±0.1

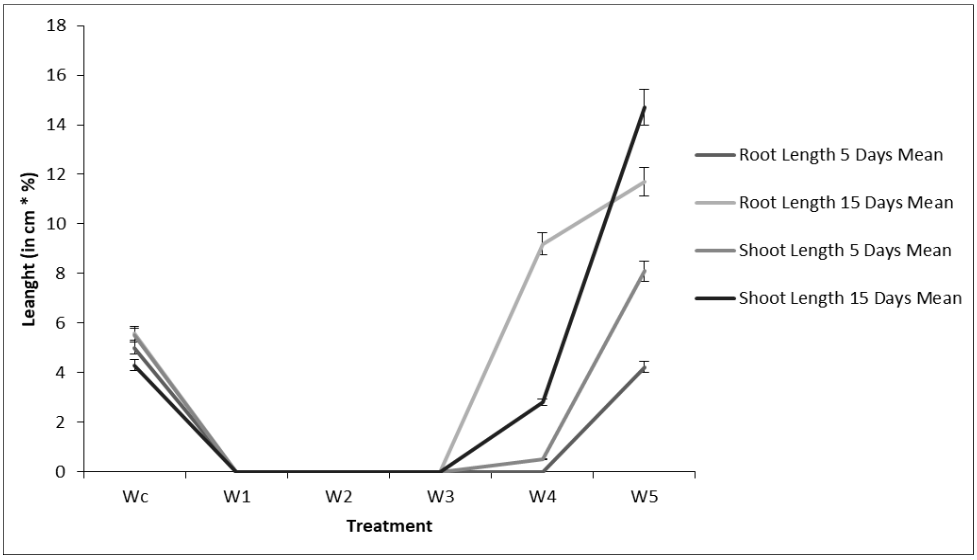

Fig 3: Root Shoot Length of *T. aestivum* calculated after 5 and 15 days of treatment.

Table 4: Seed Vigor Index calculated in *T. aestivum* under the treatment lead acetate.

Treatments	SVI (in cm* %)	
	5 Days (Mean)	15 Days (Mean)
Wc (Control)	725.967 ± 19.401	386.6667 ± 9.015
W1 (800 ppm)	0	0
W2 (400 ppm)	0	0
W3 (200 ppm)	0	0
W4 (100 ppm)	402.182 ± 1.924	13.33 ± 0
W5 (50 ppm)	1409.69 ± 13.421	666.625 ± 5.33

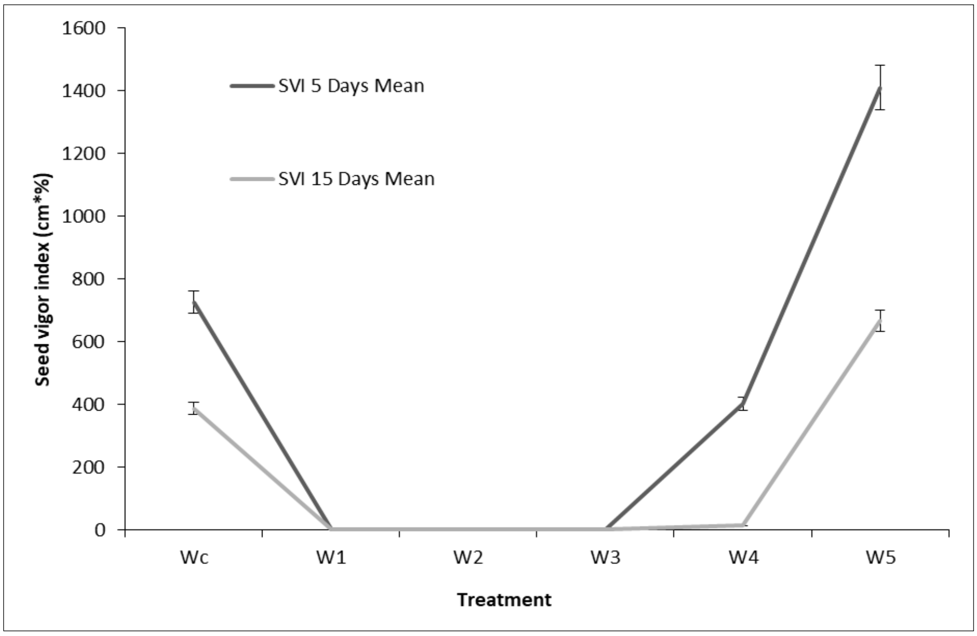

Fig 4: Seed Vigour Index of *T. aestivum* after 5 and 15 days of treatment.

Table 5: Chlorophyll estimation of *T. aestivum* under the treatment of lead acetate.

Treatments	Chl a (mg/ml)	Chl b (mg/ml)	Total Chl (mg/ml)	Carotene (mg/ml)
Wc (Control)	1.606333±0.035	2.840427±0.063	4.445193±0.099	0.14925±0.018
W1 (800 ppm)	1.847703±0.014	3.85422±0.038	5.699853±0.052	0.15718±0.021
W2 (400 ppm)	3.737903±0.033	7.174273±0.070	10.90827±0.104	0.35028±0.009
W3 (200 ppm)	1.686897±0.021	3.533727±0.025	5.218727±0.046	0.05805±0.001
W4 (100 ppm)	3.259717±0.005	6.211233±0.010	9.467567±0.016	0.22058±0.001
W5 (50 ppm)	1.588617±0.055	4.197993±0.100	5.784427±0.155	0.4979±0.003

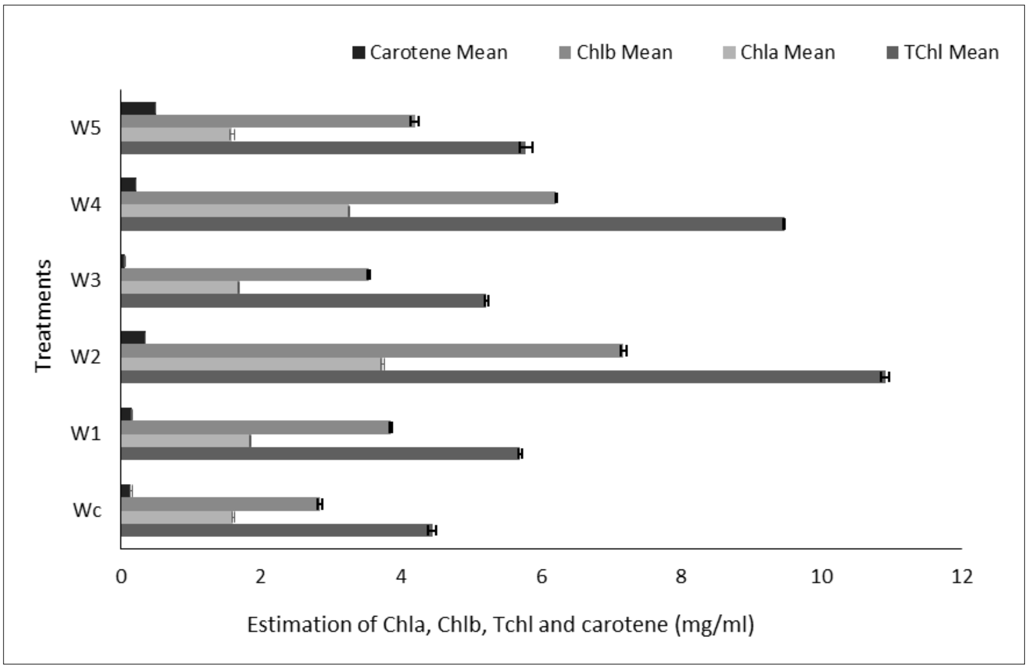

Fig 5: Estimation of Chl a, Chl b and Total Chlorophyll content in *Triticum aestivum*.

Discussion

1. Effect of Pb concentration on growth parameters: Maximum seed germination was recorded for Wc (Control) followed by W5 at 50 ppm (Table 1). In plants the toxic effect of lead results in inhibited root growth because of water uptake inhibition [15, 16, 17]. As roots are the primary organ for the absorption it causes accumulation at higher concentration and low translocation to shoots [1, 16, 17].

Maximum value for root shoot length was found in case of W5 which was high when compared to control i.e. without lead (Table 3). No value was recorded for root shoot length at 800-200 ppm of lead acetate (Figure 3). Other authors have also reported the same trend for different plants [18, 19]. The accumulation of lead in roots shows that roots may play a part in partial barrier system against Pb translocation to shoot [1, 20], signifying that rates of lead uptake and translocation are low [21].

In case of *Pluchea. sagittalis* these data signify that shoot appeared to be the primary site of Pb toxicity. The similar results were recorded for *Iris* sp. exposed to Pb concentration of 10 mMol/L for 28 days [22]. In the present work the growth of wheat plant was negatively affected by the toxic effect of lead and this result was in accordance with Gopal and Rizvi [23], Mishra *et al.* [18] and Zhou *et al.* [24].

In the present study, seed vigorosity was decreased at the higher concentration in W1 at 800 ppm of Pb and the root discoloration has been observed and this result was in accordance with Gopal and Rizvi [23] and Gupta *et al.* [25].

2. Effects of Pb on Chlorophyll and carotenoid content

As per Grata˜o *et al.* [9] in higher plants, the level of photosynthetic pigments is a consistent marker of lead (Pb) toxicity. In case of *Triticum aestivum*, for W2 highest value of total chl, chl*a* and chl*b* was recorded on the other hand high carotenoid content was recorded for W5. In case of W1 (800 ppm) low carotenoid content was found (Table 5).

In the presence of heavy metals the reduced rate of chlorophyll content is credited to inhibition of chlorophyll biosynthesis and reduced activity of d-ALA-D [26]. According to Goncalves *et al.* [27], several terrestrial plants that have been exposed to various metals showed reduced rate of chlorophyll along with the altered activity of d-ALA-D [28].

The data of the chlorophyll content of the wheat seedlings exposed to differing concentrations of metals were also shown in Table 5. The decreased content of chlorophyll in plant parts is accredited to heavy metal accumulation [29]. In the present study, chlorophyll (a+b) content declined progressively with increasing concentrations of heavy metals. In wheat seedlings, a slight decrease in the content of chlorophyll was reported after lead accumulation. This result was found in accordance with Shakya *et al.* [30] and Schützendübel and Polle [31]. The toxic effect of lead causes reduced rate of photosynthesis, chlorophyll content and antioxidant enzyme [32].

Conclusion

The present study suggests that in *Triticum aestivum* the higher concentration of heavy metal (Lead) shows detrimental effect on growth regulatory parameters that includes germination percentage, root-shoot length, seed vigor index (SVI), chlorophyll and carotenoid content. The cultivation of cash crops at heavy metal contaminated sites may results in reduced crop production and accumulated content of toxic metal in such crop plants after entering in food chain will cause severe health ailments. In order to overcome the issue of lead toxicity in soil it is therefore suggested to ensure the soil testing of the field before the cultivation of crops so that it could prevent the incurrent loss and would thereby make agricultural practices more advanced and cost-effective.

Acknowledgements
Thanks are due to Prof. G. Kumar, Head department of Botany, University of Allahabad for providing Laboratory facilities and UGC for financial assistance.

Conflict of Interests
Authors are not having any conflict of interests.

Author's contributions
Experimental design and data evaluated by AT and SJ, paper writing by PM, Paper content evaluated by AD.

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