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Uptake of lead on plant H Vulgare at different intervals and doses

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Abstract
In the present manuscript uptake of Pb (II) and toxic effect of the metal on some biochemical parameters in Barley (H. Vulgare) were studied. The uptake of Pb (II) by the plant gradually increased with increase in concentration of lead nitrate in the irrigation solution. Maximum accumulation of the metal was noticed within 10 days. Maximum removal about (85%) of the metal was recorded below 5µg/ml and 15 µg/ml Pb (II) promoted senescence of barley plant by decreasing chlorophyll, activity of protease, catalase and increasing proxidase activity acid pyro phosphatase and the rate of acid to alkaline pyro phosphatase, protein, free amino acid DNA and RNA over their respective control values But below 10 µg/ml these constituents were least affected.

Keywords: H. Vulgare, chlorophyll, metal, DNA, RNA, Concentration

Introduction
The untreated developmental activities such as industrialization and urbanization carried out during the past few year have given rise to serious problem of environment contamination. The water environment can generally be characterized as a dilute aqueous solution containing a large variety of organic and inorganic chemical species dissolved and in suspension and include a variety of plant and animal life. A Generally increase in the level of heavy metals poses a pervasive threat to ecosystem. Among the heavy metals such as Cr (VI) in the environment as well as in plant is now becoming a major cause of environmental pollution. In spite of the use of many physico-chemical methods aquatic plants have recently drawn attention of scientists all over the world as an effective tool for the removal of heavy metals pollutants from water bodies [1-4]. Attempt were made in the laboratory to develop methods for the removal of the metals from water bodies using aquatic plants [5, 6]. In the present investigation an attempt has, therefore been made to assessed toxic effect of the metal on changes in some biochemical parameters such as protease, amino acid, DNA, RNA and activities of enzymes such as protease, catalase, peroxidase acid and alkaline pyrophosphatase in plant Barley.

Material and Method
Seeds of culture Jyoti of the crop H. Vulgare were sown up in 22 cm. diameter earthen pot in the glass house at 25±ºC for 5 to 10 days, seedlings raised were transplanted at 2 leaf stage in the pot, maintaining a density of 16 seedling/m [2]. Plants were irrigated at weekly intervals with tap water and different doses of lead nitrate solutions around Hindu College Moradabad Campus (U.P.) India. The soil used for culture experiment related to energy conservation, transformation and utilization subject to analysis [7].
The mature plants were used during the investigation, twelve plastic tumblers (25 liters) were filled each with 5 liters of culture medium. The culture medium of pH 7.5 (below) was prepared [8]. From the stock solution of lead nitrate, different volumes of solutions were added to the culture medium separately to maintain different concentration of Pb (II) (1.0, 1.5 to 15 µg/ml).
To check the loss of the metal from experimental system due to evaporation and absorption on the walls of the containers control experiments without the plant were performed simultaneously with the experimental running time. For chlorophyll estimation weighted leaf samples from control are the different Pb (II) treatments were homogenized in 1 ml 80% acetone and filtered through whatman paper. The absorbance was measured at two wavelength 645 & 665 nm and the quantification of chlorophyll was made [9].

Barley plants (100 gm) were floated in each tumbler they were cultured in a natural environmental condition during winter session. Analysis of intake of Pb(II) and toxic effects of the metal on some biochemical parameters were made with the treated plants estimation of protein, free amino acid estimated spectrophotometrically [10, 11] DNA, RNA were extracted and estimated by methods given in Markham and Burton [12, 13].

Enzyme assays the protease, catalase and peroxidase were extracted in phosphate buffer (pH-6.5). Extraction and measurement of the activities of acid and alkaline Pyro phosphatases were done [14]. Each experiment was carried out 8 times and the mean values were taken.

Result and Discussion

H. Vulgare plants, water and soil samples were collected from selected area and were analyzed for lead (II) and it was found that they were almost free from detectable Pb (II) contamination below 1.0 µg/ml level Pb (II) exposure results in a dose dependent damage to the plant (Table-1). In barley plants exposed to 15 µg/ml, there is a clear growth inhibition, whereas fresh weight (FW) and dry weight (DW) decreased by only 28% and 30%, respectively at 15 µg/ml Pb (II) with their respective control. The growth inhibition was similar to that previous reported as earlier [15]. Pb (II) is not probably so acutely toxic to the plant because the plant survived for five days even at 150 µg/ml of the metal the study was under taken upto 11 days and it was found that the plants were affected to a greater extent at and above 15.0 µg/ml Pb (II). The uptake of the metal increased gradually with increase in concentration and duration of contact time, through the plant look up maximum Pb (II) with in one day, while the % age removal decreased with increase in concentration. The plant removed completely the metal from 1.0 and 1.5 µg/ml culture medium within 10 days and the kinetic study indicated that the mode of uptake was possibly absorption [16].

Table 1: Pb (II) treatment of Barley shown change in nutrients given as % of conc. And % change of total amount per plant % as well as effect on change of the total amount of chlorophyll (% chl.)

Pb (II) µg/ml	Na % Conc.	%	K % Conc.	%	Ca % Conc.	%	P % Conc.	%	Mg % Conc.	%	Fe % Conc.	%	Cu % Conc.	%	Zn % Conc.	%	Mn. % Conc.	Chl. %
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.0	-30	-60	-17	-54	-13	-51	-13	-50	-54	-73	-44	-71	-51	-19	-54	-50	-31	-63
1.5	-38	-72	-32	-70	-45	-74	-35	-71	-71	-86	-52	-80	-55	-38	-76	-50	-36	-83
15	-76	-91	-61	-88	-70	-89	-52	-88	-84	-91	-63	-89	-67	-76	-92	-75	-51	-92

The metal taken up by the plants is probably oxidized to Pb (IV) and forms stable complexes with carboxylic group of protein molecules present in this plant itself [17].

Chlorosis was associated with reduced leaf chlorophyll content. In barley seedling concentration of chlorophyll a and b were already significantly lowered at 1.0 µg/ml Pb (II) and this effect was even more pronounced at 1.5 and 15 m g/ml Pb (II) Fig. (1). In addition, chlorophyll a and b contents per plants presented a large reduction in barley seedling Fig. (2), Where as the diminution is by a factor 13-14 for 15 µg/ml: Pb (II). There is a good correlation between the reduction of chlorophyll and Mg content, especially in the case of treatment Table (1). Previous authors showed that lead stress results in a heavy reduction of chlorophyll owing to both chlorophyll disorganization and diminution in the amount of thylakoids and grana and dried inhibition of chlorophyll synthesis, as well as changes of chlorophyll structure owing to replacement of key nutrients (Mg, Fe and Cu) by Pb (II) as reported earlier [18].

From the above facts it can be concluded that fresh weight and per plant chlorophyll a and b were affected by increasing in concentration of Pb (II) solution and seedling growth was also affected with less seed germination.

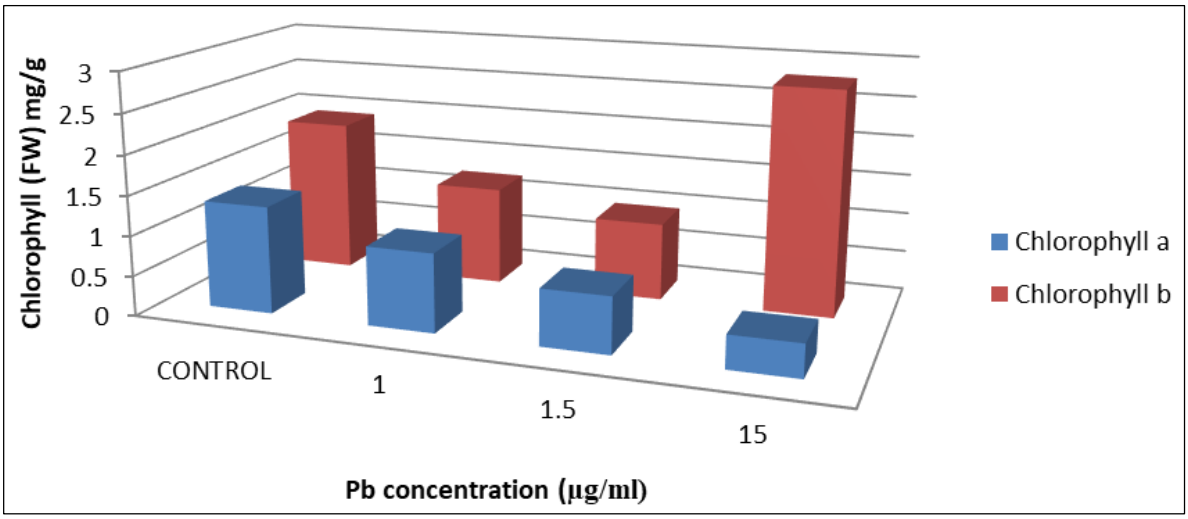

Fig 1: Toxic effect of Pb (II) on concentration of chlorophyll a and b mg/g fresh weight

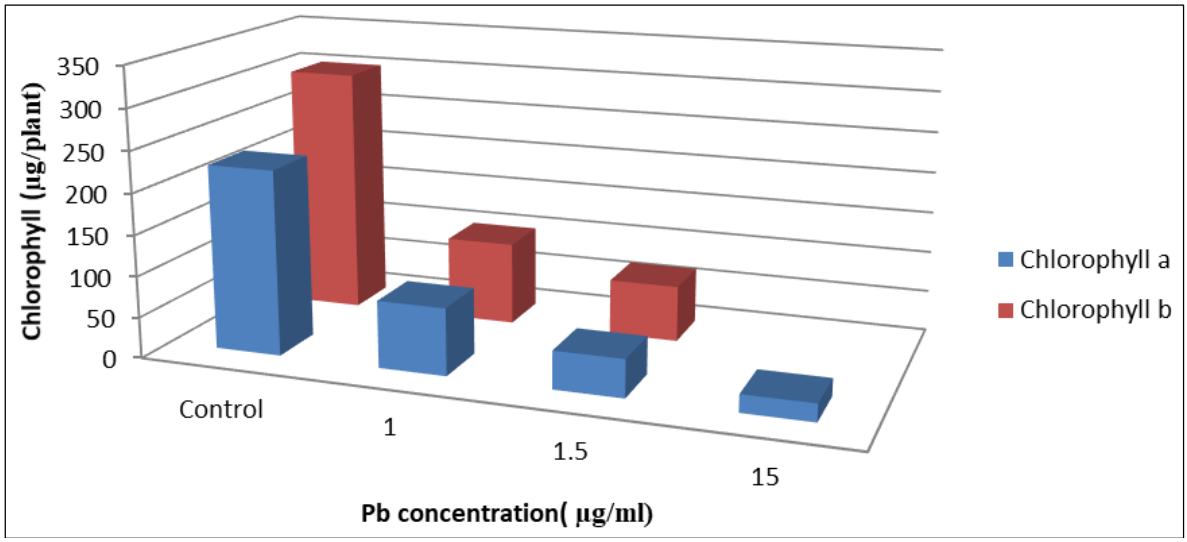

Fig 2: Toxic effect of Pb(II) on concentration of chlorophyll a and b (µg/plant)

The toxic effect of Pb(II) on the protease, catalase, peroxidase acid and alkaline pyrophosphatases after five days of contact as shown in Fig. 3 in all the treatment activities of protease, catalase and alkaline pyrophosphatases decreased while an opposite trend in the activities of peroxidase and acid pyrophosphatase and ratio of the acid to alkaline pyrophosphatase activities were observed (*viz*. 0.51, 0.52, 0.50, 0.59, 0.75) with increasing concentration of Pb(II).

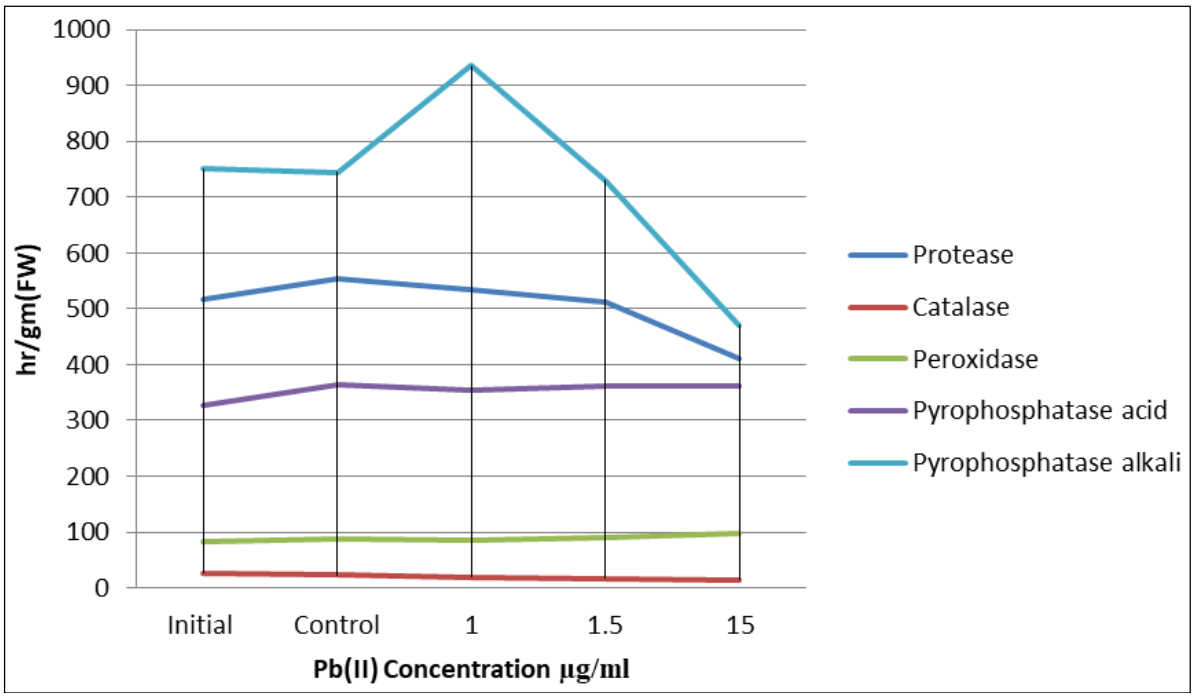

Fig 3: Toxic effect of Pb (II) on changes in the activities of protease, catalase, peroxidase acid and alkaline activities pyrophosphatase in the plant H. Vulgare after different duration of contacts.

The toxic effect of the metal on changes in protein and free amino acid contents [19] are given in Fig. (4&5) while in those in DNA and RNA in Fig. (6) after 5 and 10 days of contact. Protein content decreased slightly at 15 µg/ml, however the value remain almost the same as that in control at 1.0 and 1.5 µg/ml of the free amino acid content remained almost unchanged with that of control even after 10 days of contact with the gradual increasing concentration Pb (II) in all the treatments except at 15 µg/ml, where as the value decreased slightly with time. So the Pb (II) exerted its adverse influence more with 15 µg/ml. But DNA content remained almost same as that in control in all the treatment at 15 µg/ml the RNA content decreased and 1.5 or 1.0 µg/ml of the metal the values were gradually increasing A decrease in RNA content with time at 15 µg/ml was because Pb (IV) probably produced due to oxidation of tetravalent metal was lead with RNA prevents its degradation by RNA.

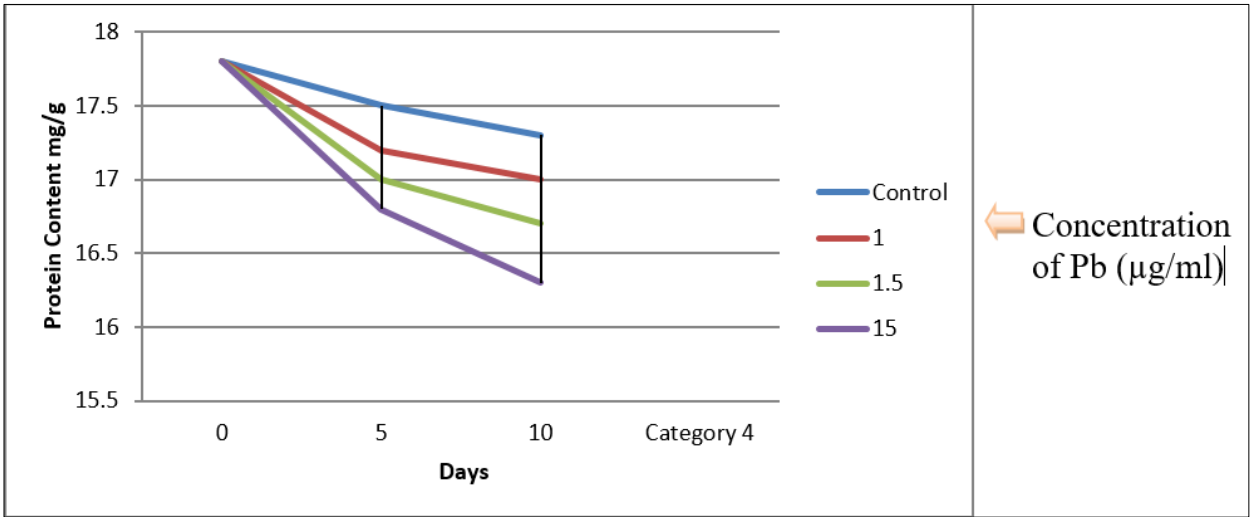

Fig 4: Toxic effect of Pb (II) on changes in protein content mg/g fresh weight in H. Vulgare (whole plant) after different durations of contact.

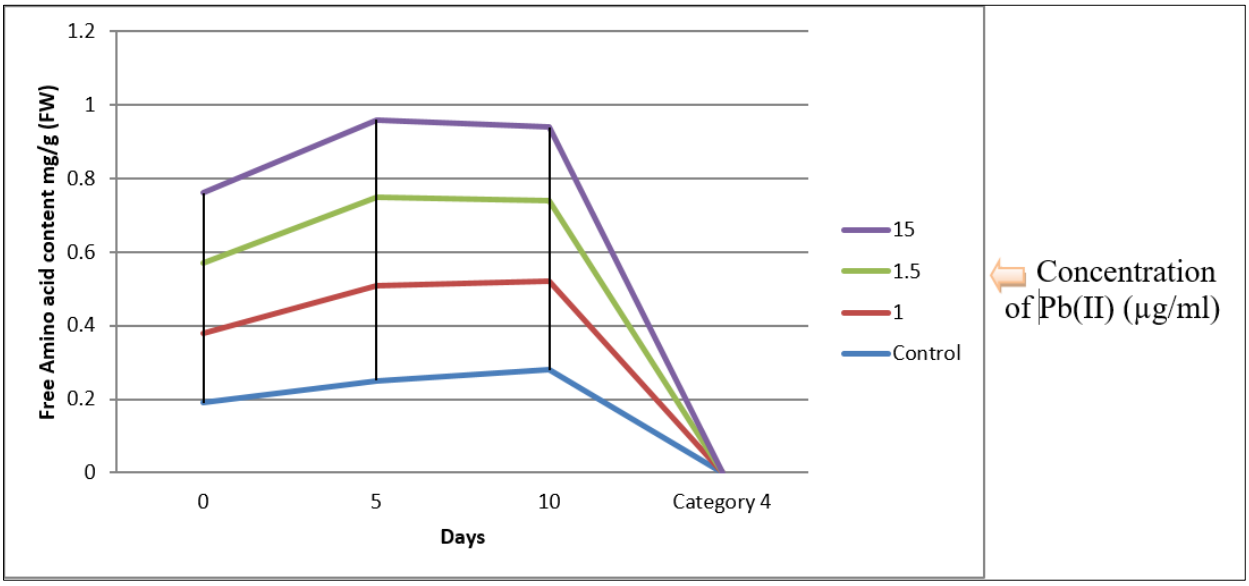

Fig 5: Toxic effect of Pb (II) on changes in free amino acid content mg/g fresh weight in H. Vulgare after different duration of contact.

The increasing activity of enzyme may be due to senescence of the plant and increased activity of number of hydrolytic enzymes [20]. A decrease in enzymatic activity may be due to formation of protein complex with Pb (IV) changing the conformation and solubility of protein.

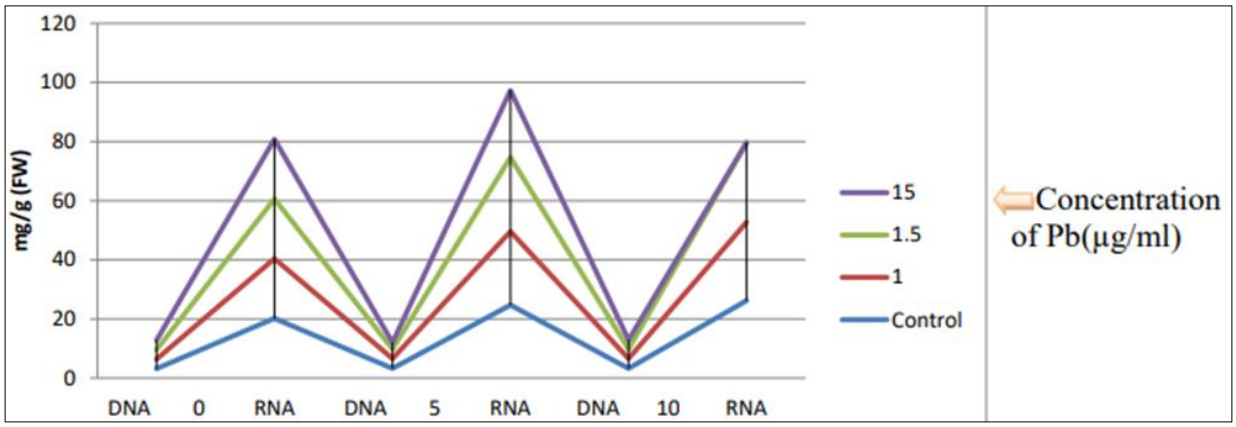

Fig 6: Toxic effect of Pb (II) on change in DNA and RNA content in Barley after different duration of contact

Conclusion

In conclusion lead is non-essential element for plant although, it accumulates in different parts of plant and causes negatively affects various physiological processes such as ATP production, lipid peroxidation, DNA and RNA damage by over production of RDS. In addition lead strongly inhibits seed germination, root elongation, seedling development, plant growth, transpiration, chlorophyll production and water

and protein content. Therefore, this is responsibility of we people, government and various environmental agencies to control heavy metal production.

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