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Lead Induced Toxic Effects on the Growth and Metabolism of Sunflower (*Helianthus annus* L.) Plants

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Abstract

Stimulation at lower concentration & suppression at higher conc. of lead was recorded in sunflower plants. Chlorophyll & Sugar conc. was found to be decreased at higher doses while a slight enhancement was observed in protein concentration. Elevation in Catalase activity was observed at 1mM conc. of lead acetate while the activity of Peroxidase enzyme was found to be stimulated at all the doses of lead.

Keywords: Lead, Toxic Effects, Growth & Metabolism, *Helianthus annus L*.

Introduction

Heavy metals are broadly grouped into essential & non-essential heavy metals. Essential metal deficiency or excess beyond certain threshold limits give rise to detrimental effects in plants & animals. Non-essential heavy metals are beneficial when their intake by plants is in very low amounts but excess of non-essential heavy metals causes deleterious effects on the growth & metabolism of plants. Sometimes they are reported to be accumulated in the edible portions such as seeds & grains etc. Sometimes they are accumulated in plants & animals through food- chain as a result of biomagnification. Nature has gifted resistance power in plants, animals & human beings and they can prevent excessive accumulation of potentially toxic metal species but beyond a certain limit they lose their resistance power and metal species get accumulated causing dangerous effects.

Review of Literature

Hasset et.al.(1976) determined that substantial lead particulate matter was deposited on plant surfaces and was dependent upon the characteristics of the leaf surface as well as on the wind speed. Carlsson et.al.(1996) reported that lead particulates are deposited naturally on plant leaf surfaces with pubescent leaves accumulating 17 times more than those with smooth surfaces. Barman & Bhargava,1997 reported that accumulation of heavy metals from soil to plant parts did not follow any particular pattern and varied with respect to metal & plant parts. Godbold & Huttermann et.al.1985, Breckle,1991,Nies,1999,reported that solubility and mobility of metals is affected by absorption, desorption and complexation processes which are dependent on soil types. R. Naresh et.al. 2003, observed that toxic metals such as lead in milk of dairy cows affected with Mastitis, a global problem in dairy cows which adversely affected the quality of milk. According to M.N. Al. Kathiri et.al. 1997, Parley, an important cattle crop of South Arabia was found to be contaminated with lead and ultimately affecting the health of people who consume milk and meat. Jerusa Simone et.al,2006 reported that Sunflower plant contaminated with four metal ions (Cd,Cu,Pb & Zn) decreases height & mass by 35% & 40% respectively as compared to control plants. U.S. Department of health and human services, 1992 reported that exposure to lead can severely damage the brain and kidney and can cause miscarriages in pregnant women. According to Rajeev Gopal & Neena Khurana,2011 degree of oxidative damage in Sunflower plants assessed by the manifestation of external visual toxicity effects, tissue conc. & alteration in biochemical parameters were found to be in the order Cd>Cr>Ni>Co>Pb. Nada Elloumi, et.al. 2016 observed that increased concentration of heavy metals like Pb in soil due to sewadge sludge amendment leads to increased root& shoot length, leaves number, biomass & antioxidative activity of Sunflower. Kamil M.Al-Jobori et.al.2019 reported that Pb content in the plants increased linearly with the Pb conc.with the highest content in roots. So awareness about the Pb-toxicity is the acute demand of present time throughout the world.

Objective of the Study

As we already know that plants are producers and require certain heavy metals for their proper growth but excessive amount of these metals cause toxic effects on plants.These metals by food chain & biomagnification can cause deleterious effects on all living-beings.The present study was based to observe toxic effects of Pb on morphological & physiological parameters of Sunflower plants.

Materials and Methods

Experiment was an example of hydroponics and plants were cultured in glass or plastic petridishes. The first step in these experiments was to collect glass or plastic petridishes of equal shape & size. Now these petridishes were de-contaminated with the help of HCl & distilled water. After de-contamination, petridishes were kept in oven to make it moisture free. Now these petridishes were lined with high quality of Whattman filter paper to maintain proper moisture for the growth & development of plants. Controlled plants were provided with basal nutrient solution whereas test plants were treated with different doses of lead along with basal nutrient solution. The experiment was carried out in replicates,in the temperature range of 20-30°C.Plants were observed daily for some abnormal changes in the form of chlorosis, necrosis, browning of leaf tissues, reduction in growth and other typical toxic symptoms concerned with Pb exposure .

The basal nutrient solution was prepared using the method given by Hewitt, 1963. Macronutrient and Micronutrient solutions were prepared by using A.R.(Analytical Reagent) grade chemicals. Controlled plants were provided only basal nutrient solution while in test plants graded levels of Pb-acetate solutions were superimposed on basal nutrient solution and were supplied in the doses of 0.25mM, 0.5mM, 1mM solutions. Nutrient solution was changed regularly leaving enough nutrient solution to cover a height of about 2 mm. As far as morphological parameters were concerned, number of plant branches were counted in each petridish and their mean values were taken. Plants were then harvested for taking fresh & dry matter yield. For evaluating the fresh weight of plants they were taken out from petridishes, washed with running water & followed by distilled water. After taking fresh weight of plants they were cut into pieces & kept in oven at 70°C.After 3 days, the dry weight of each plant was measured with the help of electronic balance. For measuring metabolic parameters, fresh leaves were ground with sand in ice-chilled pestle and mortar kept in ice-bath.1gm of leaf tissue was extracted in 10 ml. of glass distilled water. The homogenate was filtered through two folds of muslin cloth with the help of Buchner funnel and stored at freezing temperature in refrigerator. Leaf extract was used for estimation of various metabolic parameters. Chlorophyll conc. was measured by the method of Petering, 1940. Protein & total sugar were estimated by the method of Lowry et.al. 1951 & Dubias, et. al.1956, respectively.The activity of anti-oxidative enzymes viz-Catalase was measured by the method of Bisth,1972, a modified method of Euler & Josephson,1927 while that of Peroxide was measured by the modified method of Luck,1963.

Result & Discussion

Effect on growth parameters & visible symptoms

The growth of sunflower plants was observed to be drastically affected by different doses of Pb-acetate treatment. The reduction in growth was found to be dose dependent. Excessive chlorotic symptoms were observed & the symptom was directly proportional to the dose of Pb-acetate treatment. Basal leaf shedding was also observed at higher doses of lead. Fresh and dry weight of plants were also found to be decreased. In fact overall growth of plants was found to be adversely affected by higher doses of Pb-acetate solution. Reduced growth of plants at higher doses of lead was already reported by number of workers (Dixit et.al. 2001, Liao et.al. 2003 & Singh et.al. 2006).Factors responsible for reduced growth of plants might be associated with abnormal transport of some essential nutrients such as Zn, K and Fe. In some cases, displacement of chemically related metal ions with heavy metals might be a responsible factor for suppressed growth of plants.Tsui, 1948 reported that deficiency of Zn due to excess amount of lead might be a reason of suppressed growth as Zn is reported to be involved in the reduction of Auxin through its involvement in the synthesis of tryptophan, a precursor of Auxin.

Effects on Metabolic Parameters

Concentration of chlorophyll & total sugar were found to be significantly reduced at higher doses of Pb-acetate solution as compared to controlled plants. Baryla et.al. 2001,reported that chlorosis might be attributed to reduced chlorophyll density & associated pigments. Yang et.al. 1989, observed reduced sugar conc. perhaps due to interference of lead in $PSII$ which result in reduced sugar photosynthesis in Spinach plants. Protein concentration was observed to be reduced at lower doses while it was found to be elevated at higher doses of lead.

Bisht et.al., 1976 observed decreased protein content as a result of heavy metal toxicity. They were of the view that diverse effects of heavy metal toxicity in enzymes, accumulation of non-protein nitrogen including individual amino acids & decrease in protein nitrogen content of plants subjected to excess conc. of heavy metals suggest that cellular concentration of heavy metal may determine a normal balance of the functional protein & other cellular metabolism. The activity of anti - oxidative enzyme catalase was found to be reduced at lower doses but was found to be elevated at higher doses. The activity of peroxidase enzyme enhanced at all the doses of lead i.e. 0.25mM, 0.5mM &1mM.Higher POD (Peroxidase) activity reflects more serious damages happened on plant organs. Liao et.al. (2003) observed increased peroxidase activity in *Vicia faba* plant in heavy metal stress condition. Enhanced activity of these anti- oxidative enzymes might be due to the generation of metal ion induced H_2O_2(Reactive oxygen species).An elevated activity of anti-oxidative enzymes indicates the excessive heavy metal stress conditions in plants and the changes in the activity of enzymes can be correlated with the plant species & heavy metal type. A decrease in Catalase activity was also reported by Somashekaraiah et.al. 1992. Catalase activity might be suppressed due to supply of iron for the synthesis of Catalase enzyme. This might be due to the fact that during heavy metal stress conditions, the transport of essential elements get blocked which might have resulted into reduced catalase activity.

Table-1

Lead Induced effects on the metabolic parameters of Sunflower (*Helianthus annus* L.) plants

S.No	Treatments	mg. of chlorophyll/ gm fresh weight	µg Protein/ gm fresh wt.	mg. of sugar/ gm. fresh wt.
1.	Control	0.751± 0.031	158.43±2.3764	1.0563±0.01875
2.	0.25 mM $Pb(CH_3COO)_2$	0.492±0.0275	141.399±1.9803	0.9875±0.0125
3.	0.5 mM $Pb(CH_3COO)_2$	0.554±0.0115	113.277±4.7528	0.912±0.0125
4.	1.0 mM $Pb(CH_3COO)_2$	0.438 ± 0.0195	176.649±3.1685	0.8188±0.01875
	CD Value at 5% P	**0.0776**	**20.517**	**0.113**

Table-2

Effect of Various Treatments of lead on the activity of anti-oxidative enzymes viz. Catalase & Peroxidase in Sunflower (*Helianthus annus* L.) plants

S.No	Treatments	Catalase Activity(µ moles H_2O_2 split/ 100 mg fresh wt.	Peroxidase activity (OD/100mg.fresh wt.)
1.	Control	17.5±2.500	0.26±0.06
2.	0.25 mM $Pb(CH_3COO)_2$	17.5±2.500	0.42±0.06
3.	0.5 mM $Pb(CH_3COO)_2$	10.0±0.000	0.88±0.06
4.	1.0 mM $Pb(CH_3COO)_2$	27.5±2.500	0.49±0.02
	CD Value at 5% P	**14.73**	**0.348**

Values represent the mean ±SD of two replicates.

CD represents cricitical difference between two observations.

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