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Phytoremediation of Heavy Metals (Zn and Pb) and its Toxicity on *Azolla filiculoides*.

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Abstract: ***Heavy Metal that enters into the environment through various sources cause adverse effect on living organisms. One of the methods of removing these pollutants from water and soil is the use of plants (phytoremediation). There are many plants (hyperaccumulators) which have the ability to accumulate large amounts of heavy metals. One of them is the aquatic fern Azolla filiculoids, which can bind some heavy metals. The aim of the study was the effect of heavy metals on metabolic parameters like Starch, Reducing sugar and Non reducing Sugar and total soluble sugar of Azolla plant during phytoremediation. Azolla plant were harvested in nutrient medium containing 2.5%, 5% and 10% Individually heavy metals (Zn and Pb) containing containers. After 30 days of harvesting it was observed that biochemical parameters were greatly affected by Zinc and Lead. Starch, Reducing sugar and Non reducing Sugar and soluble sugar content of plant were highly reduced in Zn containing nutrient medium in comparision to Pb.***

Keywords: *Azolla filiculoids*, Total Soluble Sugar, Reducing Sugar, Non Reducing Sugar, Starch, Phytoremediation

1. Introduction

Environmental pollution increases due to increased urbanization and industrialization. Toxic waste originating from industrial unit, Automobile engines and domestic operators disposed in to the component of our environment such as soil, air and water. These toxic substances are through water or Soil ultimately enters in to the food chain and cause health hazards. One of the inorganic compounds that pollute environment are heavy metals, which are added through human factors or other geophysical modifications. Some of the metals are essential for the growth and development of living organisms. However, many heavy metals are highly toxic when the concentration exceeds certain limits (M.M. Al-Subu *et. al.*,1996). Phytoremediation is one of the methods of lowering the content of heavy metals in the environment. There are many plants, which can absorb extremely high amounts of heavy metals. They are called hyperaccumulators and they are exploited to clean up the environment (Evanko and Dzombak, 1997). Alfalfa (*Medicago sativa* L., *Trifolieae*) is highly adept at binding lead and cadmium (Gardea-Torresdey *et al.*,1996). Also *Thlaspi caerulescens* Presl. (*Brassicaceae*) can bind large amounts of Zinc (Nedelkoska and Doran, 2000). Hop (*Humulus lupulus* L., *Cannabinaceae*) can be used to remove lead ions from contaminated water (Gardea-Torresdey *et al*., 2002) and the willow (*Salix* sp. L. *Salicaceae*) can bind cadmium (Klang-Westin and Perttub, 2002). *Taraxacum officinale* Weber (*Asteraceae*) and *Ambrosia artemisiifolia* L. (*Asteraceae*) have the ability to remove Pb, Zn and Cd from soil (Pichtel *et al.*, 2000). *Phaseolus vulgaris* L. (*Fabaceae*) could be used to absorb pollutants from water contaminated with lead (Piechalak *et al*., 2002) and Indian mustard (*Brassica juncea* L., *Brassicaceae*) is a high-biomass Pb accumulator (Gleba *et al.*, 1999). Also duckweed (*Lemna minor* L., *Lemnaceae*) can effectively remove lead from water (Rahmani and Sternberg, 1999).

The aquatic fern *Azolla filiculoides*. (*Azollaceae*) is a small plant, common in many parts of the world, especially in tropical environments (Watanbe *et al.*, 1992). *Azolla* is a good bioremediation and can be used for the treatment of wastewater such as industrial effluents, sewage water etc. and it is eco-friendly and effective. *Azolla* sps. can bind metals like Zn, Pb, Cu, Cd, Au, Ni, Sr, Cr and Hg (Gaur and Noraho, 1995;Sanyahumbi *et al.*, 1998; Antunes *et al*., 2001; Cohen-Shoel *et al*., 2002; Bennicelli *et al*., 2004). Z.Stepniewska,et al(2005) has also described the potential of *Azolla caroliniana* for the removal of Pb and Cd from wastewaters.

The aim of the research presented in this paper was to study the effect of Pb and Zn in various concentration in Shive & Robbins medium-1 nutrient solution on metabolic parameter (Starch, Total Soulable Sugar, Reducing sugar and Non reducing Sugar.) of *Azolla filiculoides*.in order to study its capability for removal of Lead and Zinc from waste water.

2. Material and Methods

Plants were collected from natural pond of Rajendra Agriculture University, Pusa, Bihar. The pond was provided with fresh water without additional fertilizers. *Azolla* plants were maintained and cultured in the GDM College, Kankarbagh, Bihar, India under control condition of temperature (27^0C in Day and 22^0C in night) and constant day length (16 hrs light and 8 hrs dark). Six containers were taken and 1000 ml of Shive and Robbin medium-I was poured in each container. Three containers contained 2.5%, 5%, and 10% of Zinc and the other three contained the said percentage of Lead and a separate container was taken as control. 250gm of fresh plant were harvested in each seven containers for 30 days. After 30 days of harvesting plants were shed dried for biochemical estimation.

Preparation of Reagents for Biochemical Estimation
2.5N HCL

Anthrone reagent: Dissolve 200mg anthrone in 100ml of ice cold 95% conc. H_2SO_4 (Prepare freshly).

Harding reagent: 12gm Sodium potassium tarterate dissolved in 1000ml Distil Water (D.W.)

Nelson reagent: Prepared by dissolving 50gm ammonium molybdate in 900ml D.W. To this 42ml of H_2SO_4 were added slowly. 6gm hydrated Sodium Arsenate were dissolved in 50 ml D.W. and was added to the ammonium molybdate and H_2So_4 solution.

Iodine Solution: For preparing 100ml of 0.1M solution , 3gm of potassium iodide (KI) was moisten with few drops of water in a beaker. Measured out 2.54gm of iodine and add to the moisten potassium iodide. Add a small volume of water and stir. Pour the solution in a graduated cylinder and add D.W to make the 1000ml. of it.

Test for Sugar:

Preparation of extraction solution; 100mg of dried sample was taken in a test tube , 6-7 ml of 80% ethanol was added. The sample was heated in a water bath at 80^0C for 30 minutes and centrifuged at 3000 rpm for 5 minutes. The supernatant was collected in a flask and 80% ethanol was added to a final volume of 50ml.The residue was used for starch estimation.

Test for Total soluble sugar: 1ml of extraction solution was taken and 1.5ml of water was added, followed by 6.5 ml of anthrone reagent. The sample was mixed and incubated at room temperature for 15 minutes to allow colour development. Absorbance at 620nm. was read on spectrophotometer.

Test for Non reducing sugar: For the test of Non Reducing Sugar 1ml of extraction solution was taken in a clean test tube and heated in a boiling water bath until it condenses to 0.05-0.1ml. Now, 0.1ml of 30% KOH was added and incubate in a boiling water bath for 10 mins. The solution was cooled down to room temperature, 3ml of anthrone reagent was added and incubated at room temperature for 10-15 mins. The absorbence was read at 620nm.

Test for reducing sugar: 2gm of dried sample were taken in a test tube 10ml of 2% oxalic acid added and covered with aluminium foil to reduce evaporation and heated in a water bath at 80^0C for 25 minutes. After cooling, 0.2ml of extraction solution taken in a clean test tube and made final volume 1ml. 1ml of copper reagent and 1ml of Harding's reagent was added and mixed well. Test tube was heated in a boiling water bath for 10 minutes. Then, 1ml of Nelson's reagent was added, and left for few minutes for colour development. Absorbance was read at 600nm.

Test for Starch: The residual sugar was rinsed with 20-30ml D.W in 50ml flask. The solution was gelatinized in a boiling water bath for 15 minutes. 0.1ml of Iodine solution was added and absorbance was taken at 610nm. Same protocol was followed for the detection of total soluble sugar, non reducing sugar, reducing sugar and starch in the plants grown in three different concentrations of Pb , Zn and control

3. Results and Discussion

Results in Table 1 Shows that Total Soluble Sugar, Non Reducing Sugar, Reducing Sugar and Starch was high in control in comparison to Zn and Pb supplemented plants. From the present investigation, it is clear that the *Azolla filiculoides* were sensitive and severely affected by zinc and lead in nutrient medium and biochemical datas also confirms that the effect on plant is directly proportional to the concentration of heavy metals which might be due to higher uptake of heavy metals and its harmful nature.

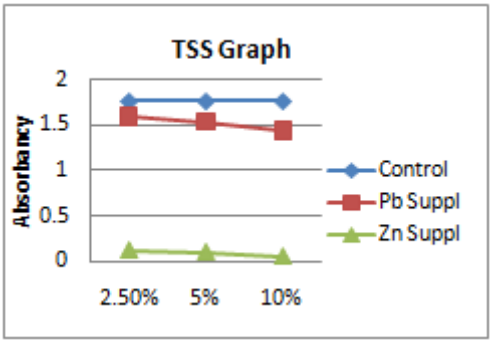
According to Thayaparan.M, Iqbal.S.S,et al.(2013) The uptake ability and the Bio concentration factor of *Azolla sps.* for lead and Zinc increased with the increase of concentration in the growth medium. *Azolla* can absorb maximmum at only 4%. But its uptake capacity significantly increased with the increase of exposure time.

Table 1

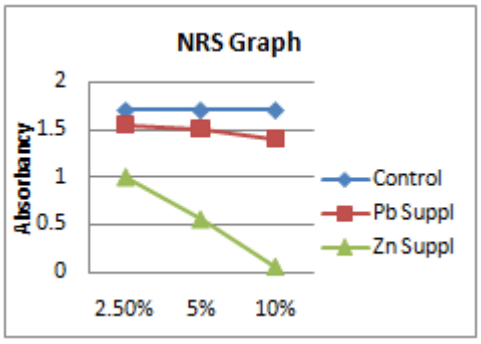
Biochemical test	Control	Pb Supplemented			Zn Supplemented		
		2.5%	5%	10%	2.5%	5%	10%
TSS	1.77	1.60	1.54	1.44	0.12	0.10	0.05
NRS	1.70	1.55	1.5	1.4	1	0.55	0.05
RS	0.07	0.05	0.04	0.04	0.02	0.01	0
Starch	1.57	1.44	1.38	1.30	0.11	0.10	0.05

Note -Table:1 Shows O.D of TSS and NRS at 620nm, RS at 600nm and Starch at 610nm

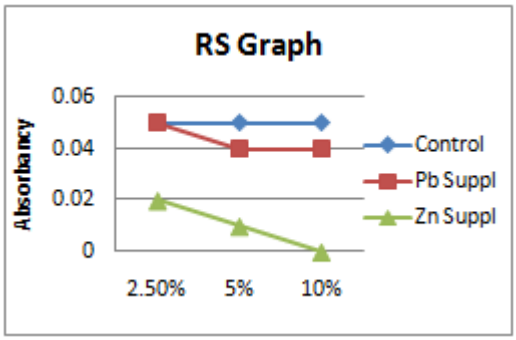
Zinc is more effctive then Lead because Zinc easily moves from root to the shoot which results in reducing the concentration of essential nutrients such as K^+, Na^+ and Mg^{++} in plants and inhibits biochemical reaction of plant (Lumpkin and Plucknett, 1980). As Zinc is mobile and bioaccessible metal, its accumulation in soil and plants can reach the food chain easily. Pb uptake in the roots of water fern was higher than in the stem and leaves (Erzsebet Buta et.al 2011.). In stem and leaves the translocation of Pb was lower than roots (Erzsebet Buta et.al., 2011). So, it showed less effect then Zn. The high dose (10%) of lead and Zinc negatively influenced the metabolic process of plant.



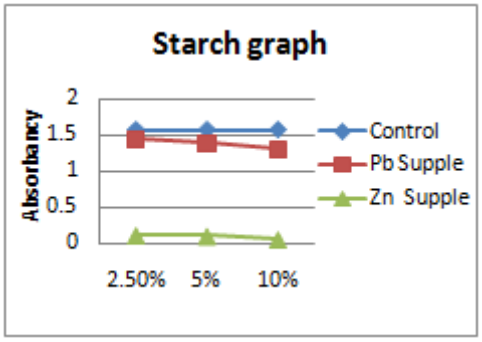
Graph of Total Soluble Sugar concent- ration at 620nm



Graph of Non Reducing Sugar at 620nm



Graph of Reducing Sugar concentration at 600nm



Graph of Starch concentration at 610nm

4. Conclusion

It can be conclude that *Azolla filiculoides* absorbed high quantity of Zn and Pb which affects the biochemical parameters. Both heavy metals reduces the concentration of biochemical parameters, But Zn greatly influence the metabolic pathway of *Azolla* plant in comparision to Pb. It Reduces the concentration of Total Soluble Sugar, Non Reducing Sugar, Reducing sugar and Starch content of the plant . It also has been concluded that high concentration (10%) of Zn and Pb highly inhibits above mentioned biochemical parameters in comparision to low concentration (2.5%)

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