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THE INFLUENCE OF HEAVY METALS ON GROWTH AND DEVELOPMENT OF WATER FERN, *AZOLLA SPS*

RIMJHIM SHEEL[1], MONI ANAND[2] & KUMARI NISHA[3]

[1,2]Department of Botany, Ganga Devi Mahila College, Kankarbagh Patna, Bihar, India

[3]Department of Biotechnology, College of Commerce, Rajendra Nagar, Patna, Bihar, India

ABSTRACT

Rapid industrialization increases pollutants on earth, like heavy metals which causes number of disease in plants and animals. Several water ferns are capable of accumulating heavy metals, one of which is *Azolla* species. *Azolla* species are useful in the detoxification of industrial effluent. Roots of *Azolla* plant absorb Heavy Metals like Zn and Pb from water. Hence, Azolla species can be used for phytoremediation. The aim of this study was to assess the influence of heavy metal on the growth and development of water fern *Azolla*. Plants were grown in nutrient medium for 1 month containing heavy metals Zn 10% and Pb 10% separately. These Heavy metals transfer from root to the shoot which results in reducing the concentration of essential nutrients such as K^+, Na^+ and Mg^{++} in plants and inhibits normal growth. Result of the experiment also shows that the high concentration of Zinc is more effective in retarding the growth of *Azolla sps.* Then Lead.

KEYWORDS: Increases Pollutants on Earth, Number of Disease in Plants and Animals, Several Water Ferns are Capable of Accumulating Heavy Metals

INTRODUCTION

Continuous industrialization and urbanization have resulted in excess levels of heavy metals in the biosphere (Lu et al., 2004). Toxic levels of some heavy metals appear as a result of environmental pollution due to removal from automobile traffic, industries, mining and agricultural wastes (Oncel et al., 2000). Aquatic ferns has an ability to take up heavy metals hence, makes them an interesting research material in the treatment of industrial effluents and sewage waters (Andra et al., 2010). Zinc and lead compounds are widely used in various industries to make paint, rubber, dyes, detergents, ointment and wood preservatives. Some amount of zinc is also released into the environment by natural processes, but most comes from human activities like waste burning, steel production, coal burning and mining (Andra et al., 2010). Heavy metals plays an important role in many biochemical reactions within the plants (Lu et al.,2004) as essential micronutrients. Several plant species, such as, Water Lilies (*Nymphaea spontanea*) (Choo et al., 2006), Parrot Feather (*Myriophylhum aquaticum*), Watermint (*Mentha aquatic*) (Kamal et al., 2004) Water Lettuce (*Pistia stratiotes*) (Mishra et al., 2008) and Creeping Primrose (*Ludwigina palustris*), have been studied to determine their potential in accumulating heavy metals.

The water fern *Azolla* is dichotomously branched free floating fast growing aquatic fern grow on moist soils and marshy ponds. The shape of Indian species is typically triangular measuring about 1.5 to 3.0 cm in length, 1 to 2 cm in breadth. In the dorsal leaf lobe there is an ellipsoidal cavity filled with gases is lined with mucilage (T.A. Lumpkin. et.al.,

1980) which contains the cyanobiont *Anabaena azollae* (G.A. Peters et.al., 1980) and a gram positive non-nitrogen fixing bacteria (B. Hates et.al., 1980) identified as *Arthrobacter* species (M. Grilli Caiola et.al., 1988). *Azolla* is worldwide distributed and have been intensively studied due to their high growth rates combined with high bioremediation efficiency. *Azolla* species are rich in proteins, essential amino acids, vitamins A, B12, beta-carotene, growth promoter intermediates and minerals like potassium, phosphorus, calcium, copper, iron and magnesium. These nutrient values, together with the low lignin content makes them a useful nutrient feed for livestock. Due to rich in Phosphorus and Nitrogen, *azolla* has been successfully used as a green manure in rice fields in India and other places and as a feed supplement for aquatic and terrestrial animals (T.S. Marwaha. et.al., 1992 and E.V.D. Teckle-Haimanot et.al., 1995).

The present investigation demonstrates the effect of heavy metals (Zinc and Lead) on the morphological characteristic of *Azolla filiculoides* plants during phytoremediation when the heavy metal concentrations are high.

MATERIAL AND METHODS

Plants were collected from natural pond of Rajendra Agriculture University, Pusa, and Bihar. The pond was provided with fresh water without additional fertilizers. *Azolla* plants were maintained and cultured in the Ganga Devi Mahila 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). Three containers were taken and 1000ml of Shive and Robbin's medium-1 (Sheel and Sinha 2003) was poured in each container. First container containing 10% Zinc, second containing 10% Lead and third was taken as Control. Five healthy Plantlets of 1cm in length and approx. 70mg of weight were selected and grown in each container containing the above solutions.

Plants were harvested under suitable condition for 1month and data concerning morphological character (length and weight) were measured after 7 days of interval.

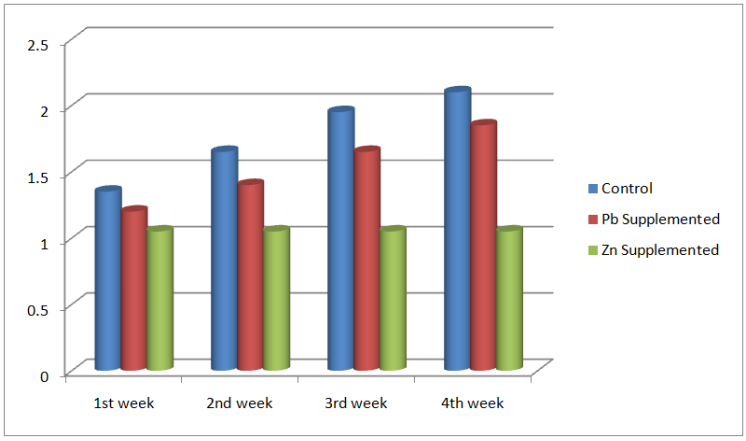
RESULTS AND DISCUSSIONS

Data concerning length of plantlets used in research are presented in Table-1 (average length of five plantlets in cm) and in Table-2, data concerning average weight of five plantlets in mg are presented. On the basis of the data presented in Table –1 and Table- 2, Histogram-1 and Histogram –2 are also presented for length and weight of plantlets respectively. The average growth rate of plant length and weight are recorded weekly for 1 month. It was observed that medium containing heavy metals (Zn and Pb) inhibit the normal growth of plant. After 1month of harvesting, the average increase in length of five plantlets in control was 1.10cm and fresh weight increased to 78.2mg. The average length of Pb supplemented plantlets was 0.85cm and weight was 24.65mg, whereas the average length in Zn supplemented plantlets were slightly observed in first week and it remain almost the same in second, third & fourth week. In 4^{th} week, the Zn supplemented plantlets became dry and fragile and gradual decrease in weight was recorded in second, third and fourth week which is presented in Table –2.

Table: 1

Different Solutions/Length in cm	1st Week	2nd Week	3rd Week	4th Week
Control	1.35	1.65	1.95	2.10
10% Pb Supplemented	1.23	1.40	1.65	1.85
10% Zn Supplemented	1.05	1.05	1.05	1.05

Average increase in length in cm of plantlets after 7days in Control, Pb & Zn supplemented solutions



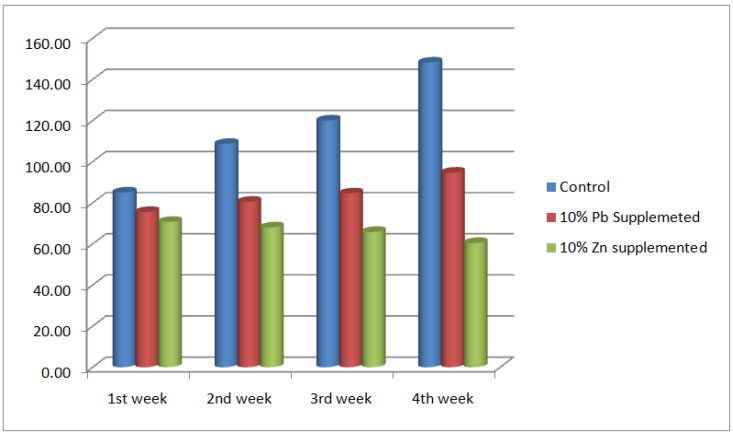
Histogram: 1

Weekly growth in length in cm. of plantlets in different solutions

Table: 2

Different Solutions/ Weight in mg	1st week	2nd week	3rd week	4th week
Control	85.00	108.6	120.10	148.20
10% Pb supplemented	75.50	80.50	84.55	94.65
10% Zn supplemented	70.65	68.00	65.80	62.40

Average weight of plantlets in mg after 7 days of interval, in different solutions



Histogram: 2

Weekly increase/ decrease in weight in mg of plantlets in Control, Pb & Zn supplemented solutions.

Azolla shows maximum efficiency of accumulation of Zn and Pb up to 4% of concentration (Deval et. al.2012). From the present investigation, it is clear that the growth of *Azolla* was sensitive and severely affected at higher concentration, especially at 10% of Zn and Pb in nutrient medium. In the third week, the clorosis begins in Zn supplemented plantlets and in fourth week the tip of the plantlets became dry. Which might be due to higher uptake of heavy metals and harmful nature of effluent? Zn and Pb both are essential and beneficial micronutrients for human bodies and plants because these are the elements, which are useful in biochemical reactions of the body. But completely utilization is not possible when it is in excess quantity, because it induces toxic environmental factor in the living body (Xiaomei *et*

al., 2004). Excess Zinc transfer from root to the shoot results in reducing the concentration of essential nutrients such as K^+, Na^+ and Mg^{++} in plants and inhibits normal growth (Lumpkin and Plucknett, 1980). Heavy metals are mobile and bio accessible. Their accumulation in soils and uptake by plants can easily reach through food chain to animals. Pb uptake in the roots of water fern was higher than in the stem and leaves (Erzsebet Buta et.al 2011.). The accumulation of Pb is higher in the case of a low concentration (below 4%). In the stem and leaves the translocation of Pb was lower than roots. So, the accumulation rate in plants decreased with increasing concentration (Erzsebet Buta et.al. 2011). The high dose (10%) of lead and Zinc negatively influenced the process of growth of stem and leaves.

CONCLUSIONS

It can be conclude that *Azolla* plant absorb high quantity of Zn and Pb which affect their growth and development. Zinc accumulates in large quantity in shoot and leaves where as Pb concentration is high in root. It also has been concluded that high concentration of Zn in shoot inhibits growth of leaves and stem but high Pb concentration in root slightly inhibits the growth of leaves or stem.

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