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Toxic effect of Pb, Cd, Ni and Zn on *Azolla filiculoides* in the International Anzali Wetland

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Abstract

The limitation of plant growth in the polluted mediums can be used as a factor to determine of plant tolerance and the toxic effect of these mediums. In this work, the effect of Pb^{2+}, Cd^{2+}, $Ni^{2+}$and Zn^{2+} (individually) on *Azolla filiculoides* growth in the aqueous solution and using this method to water post treatment were studied. During 15 days the biomass the fresh *Azolla* with initial mass of 20 g was grown on the nutrient solution containing these metal ions, each in a concentration 4 mg/l. The presence of these ions, caused about 25%, 42%, 31% and 17% inhibition of biomass growth, respectively, in comparison to *Azolla* control weight which had not heavy metals. The water salinity of 1, 2 and 4 g. NaCl/l decreased the removal of these heavy metals about 4-7%, 20-24% and 40-55%, respectively. The addition of total dissolved solids (TDS) from 50 to 300 ppm. (as $CaCO_3$) into the samples of containing heavy metals increased *Azolla* growth, but decreased the control *Azolla* growth.

Keywords: *Azolla filiculoides, bioaccumulation, living biomass, heavy metals*

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Introduction

Heavy metals are among the most dangerous substances in the environment, because of their high level of durability and harmfulness to live organisms. Biosorption is the accumulation of heavy metals using microorganisms (such as bacteria and fungi) and photosynthetic life (such as algae, aquatic and emergent plants). Biosorption using living aquatic plants (phytoremediation) is a relatively new technology to solve the problem of heavy metal pollution. In the process of phytoremediation pollutants are collected by plant roots and either decomposed to less harmful forms (for example CO_2 and H_2O) or accumulated in the plant tissues. Thus, phytoremediation is environment friendly, inexpensive and can be carried out in polluted places (remediation in situ) plus the products of decomposition do not require further utilization (Roy, *et al.*, 1992, Sternberg and Dorn, 2002).

There are two general mechanisms associated with the separation of dissolved metals from water using aquatic plant biomass. The first is a fast metabolism (within minutes) independent surface reaction that has been modeled as a diffusion process and ends when the soluble metal ions bind or sorb to the outer cell wall of the biomass. The second is a slow metabolism (within hours or days) dependent cellular uptake that has been modeled as a mass transfer process from the outer cell wall to the cell or cell wall interior (Cho*, et al.,*1994 and Axtell*, et al.*, 2003).

The advantage of using living cells over non-living biomass to remove heavy metals is that living cells work as well as dead when the metal concentration is low, and the living cells can generate new biomass through growth allowing the second removal mechanisms to occur. The major disadvantage is the toxic effect the metals can have on the organism; therefore, the using non-living biomass is preferred to remove the high concentration of heavy metals (Wang and Wood, 1984).

Azolla is a small aquatic fern. In fact, it is a symbiotic pair of *Azolla filiculoides* and a heterocystous blue-green alga *Anabaena azollae*. It has been used as a fertilizer in botanical gardens because of nitrogen-fixing capability (Peters and Meeks, 1989). *Azolla* has been used for several decades as green manure in rice fields. On the other hand, it has negative effects on the aquatic ecology due to its capable of colonizing rapidly to form dense mats over water surfaces.

Controlling its reproduction has been deemed necessary in some *Azolla*-abundant areas like South Africa (Ashton and Walmsley, 1976) and the north part of Iran.

In this regard, the development of an *Azolla*-based biosorbent for wastewater treatment, especially in developing countries, may benefit environmental problems, by removing heavy metals from water using this weed (Zhao, *et al.*, 1999).

The non-living *Azolla filiculoides* has been shown to be able to effectively adsorb Cr (III), Cr (VI), zinc (II) and nickel (II) from solutions and electroplating effluent (Zhao, *et al.*, 1997, 1998 and 1999) and gold (III) from aqueous solution (Antunes, *et al.*, 2001). We had shown that the removal of heavy metals could be increased by activation of the non-living *Azolla filiculoides* using $H_2O_2/MgCl_2$ (Taghi Ganji**,** *et al.*, 2005).

The kinds of living biomass also have been shown to be able to effectively remove heavy metals. This process decreases the growth ability of biomass that it depends on the toxic quantity of each heavy metal ion. For instance, *Azolla caroliniana* can remove Hg (II), Cr (III) and Cr (VI) from municipal waste water (Bennicelli, *et al.*, 2004). *Microspora* and *Lemna minor* also to be able to remove Pb^{2+} and Ni^{2+} from aqueous solution (Axtell, *et al.* 2003).

In this study, the toxic effect of Pb^{2+}, Cd^{2+}, $Ni^{2+}$and Zn^{2+} on the living *Azolla filiculoides* by determining of the biomass growth and the presence effect of water's NaCl and total dissolved solids (TDS) in this process were studied.

Materials and Methods

The experiment was performed in a number of flasks as batch biosorption experiments. 4 ml IRRI solution as a commercial nutrient without nitrates was added to each jar (because *Azolla* used nitrogen provided by the cyanobacteria *Anabaena azollae*) (Ladha, *et al.*, 1992).

IRRI medium contained K_2SO_4 (174 µg/ml), $CaCl_2$ (147 µg/ml), $MgSO_4$ (169 µg/ml), H_3PO_4 (144 µg/ml), Fe chelate (3 µg/ml), NaH_2PO_4 (138 µg/ml), $CuSO_4$ (0.16 µg/ml), $MnCl_2$ (3.6 µg/ml), $ZnSO_4$ (0.4 µg/ml), $NaMoO_4$ (0.8 µg/ml), H_3BO_3 (5.6 µg/ml), $CoCl_2$ (0.1 µg/ml) and glucose (500 µg/ml).

The Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} (metals under experiments) stock solutions were prepared by dissolving their corresponding the salts of $Pb(NO_3)_2$, $CdCl_2.2.5H_2O$, $NiCl_2$ and $ZnSO_4$ (analytical grade from Merck) in deionised water. TDS also was provided by dissolving the salts of $Na_2SO_4.10H_2O$, $CaCl_2.2H_2O$, $MgCl_2.6H_2O$and $NaHCO_3$ (Merck) in deionised water. The heavy metal solutions (volume 3 l) were introduced with known concentrations (C_o) 4 mg/l into the flasks (each solution contained one metal ion) except one of the flask that was used as a control. Viz. the control solution, containing only nutrient medium and biomass.Fresh *Azolla filiculoides* (as living biomass) was collected from the surface of the Anzali International Wetland in the north part of Iran. The amounts of *Azolla* (20 g.) were washed with deionised water for 1 min and were then added to each flask.

During 15 days as the experiment period the following parameters were maintained: pH 7.0 ± 0.2, water temperature (25 ± 2 °C), photoperiod 16/8 (8 h. by day and night under light of fluorescent lamp), agitation rate 50 rpm for 6h in each day. At an interval of 48 h, the biomass obtained was collected, weighed (fresh mass) and at the end of 15 day of cultivation it was dried at 70 °C until no further weight loss within 8 days (as dry mass). Dried matter of *Azolla filiculoides* was digested with 0.2 M. HNO_3 for determination of metal content in biomass. The analysis of heavy metal content in the solution and biomass were performed by a Shimadzu Model AA-680 Flame Atomic Absorption Spectrophotometer (Japan).

Results

Azolla growth

Heavy metals as the growth inhibitors

As shown in Figure 1, fresh *Azolla* mass was increased during experiment period. The initial *Azolla* mass and metals concentration were 20 g. and 4 mg/l (for each heavy metal, individually), respectively.

The control *Azolla* grows with the higher rate so that after 15 days, its fresh weight was 56.3 g., while at same time, the *Azolla* mass in the containing samples containing Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} were 42.3 g., 32.7 g., 38.6 g. and 46.8 g., respectively.

In other words, at the end of experiment period (after 15 days), the presence of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} caused a distinct limitation of *Azolla filiculoides* growth relative to a control sample about 25%, 42%, 31% and 17% less growth, respectively. It is considerable that Zinc and nickel are both essential trace elements, required in only small amounts to perform various coenzyme and regulatory functions unlike lead and cadmium (Salt and Prince, 2002). According to Figure 1 is appeared that the toxic effect of heavy metals on *Azolla filiculoides* growth is the following arrangement: $Cd^{2+} > Ni^{2+} > Pb^{2+} > Zn^{2+}$.

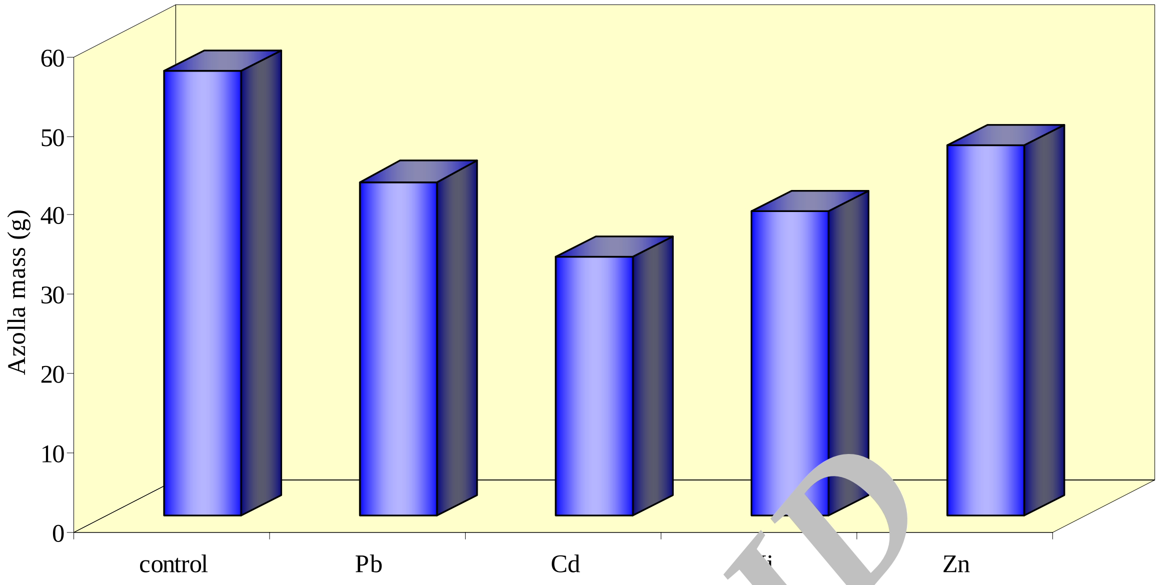

Figure 1: Effect of heavy metals on Azolla growth after 15 days

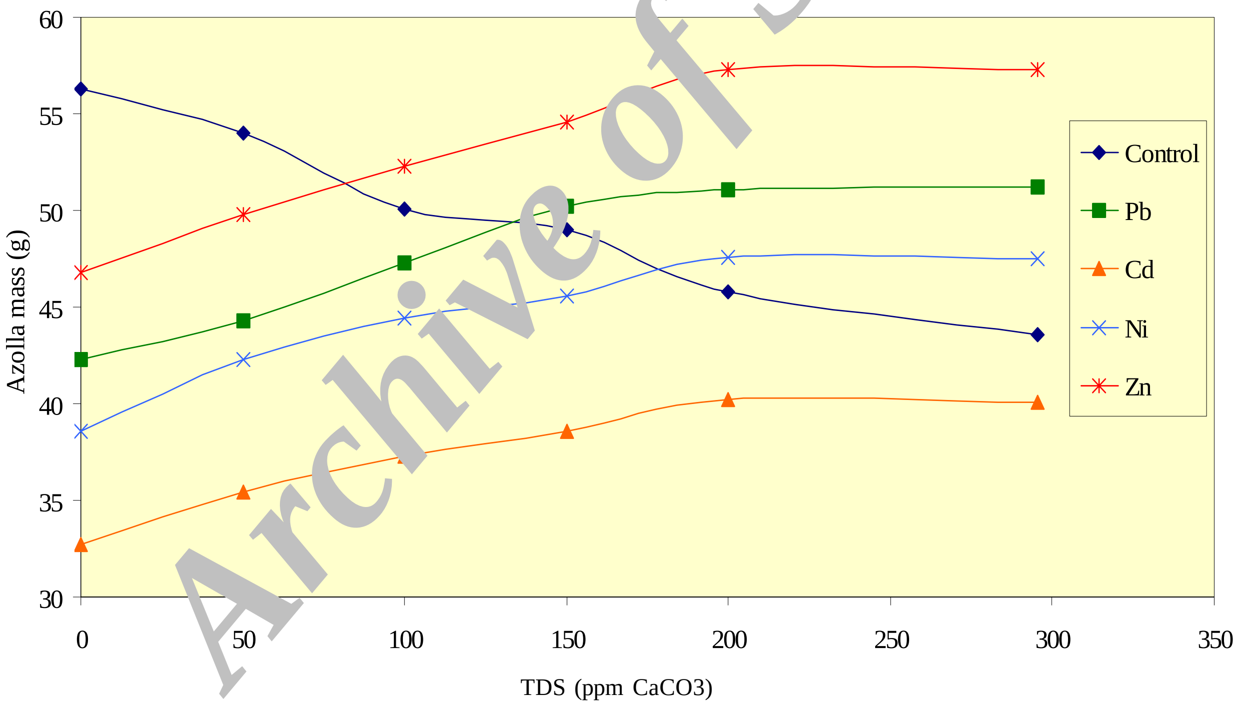

Figure 2: Effect of TDS on Azolla growth after 15 days

Effect of water's TDS

Figure 2 shows the effect of TDS addition into samples on the biomass growth. This TDS consisted of Ca^{2+}, Mg^{2+}, Na^{+}, HCO_3^{-}, Cl^{-} and SO_4^{2-} ions. The relation of each ion value to other ions value was selected about same at the different quantities of TDS. The comparisons were performed with due attention to the *Azolla* mass in the control and samples of without TDS after 15 days (section 1.1).

As can be seen, the control *Azolla* growth was decreased by increasing TDS, so that using TDS of 50 up to 300 ppm (as $CaCO_3$) decreased *Azolla* growth in quantities of 2.3 g. (4.0%) to 12.7 g. (22.5%), respectively. It may be due to the much more presence of cations and anions that have inhibitor effect in the nutrient uptake, especially, carbohydrates (Ladha, *et al.*, 1992). On the other hand, the increasing of TDS increased the *Azolla* samples growth which containing heavy metal ions. In other words, using TDS of 50 up to 300 ppm (as $CaCO_3$) increased *Azolla* growth in quantities of 2.0-8.9 g. (4.7-21.0%), 2.7-7.4 g. (8.2-22.6%), 3.7-8.9 g. (9.5-23.0%) and 3.0-10.5 g. (6.4-22.4%) in Pb^{2+}, Cd^{2+}, $Ni^{2+}$and Zn^{2+} solutions, respectively.

Heavy metals uptake by *Azolla*

Water post treatment

As can be seen from Figure 3, the removal of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} (with initial concentration of 4 mg/l) after 15 days reached to about 61%, 57%, 68% and 74%, respectively. This experiment shows that the living *Azolla filiculoides* can be used to purification of polluted water by these heavy metals and at the mentioned conditions.

Figure 4 shows the effect of water salinity (NaCl) on the removal of heavy metal (C_o of 4 g/l) by living *Azolla* after 15 days. As can be seen, the addition of 1, 2 and 4 g NaCl /l (Merck) decreases the removal of heavy metal ions. These removal percentages are as follows, respectively: Pb^{2+} about 57%, 41% and 22%; Cd^{2+} about 51%, 34% and

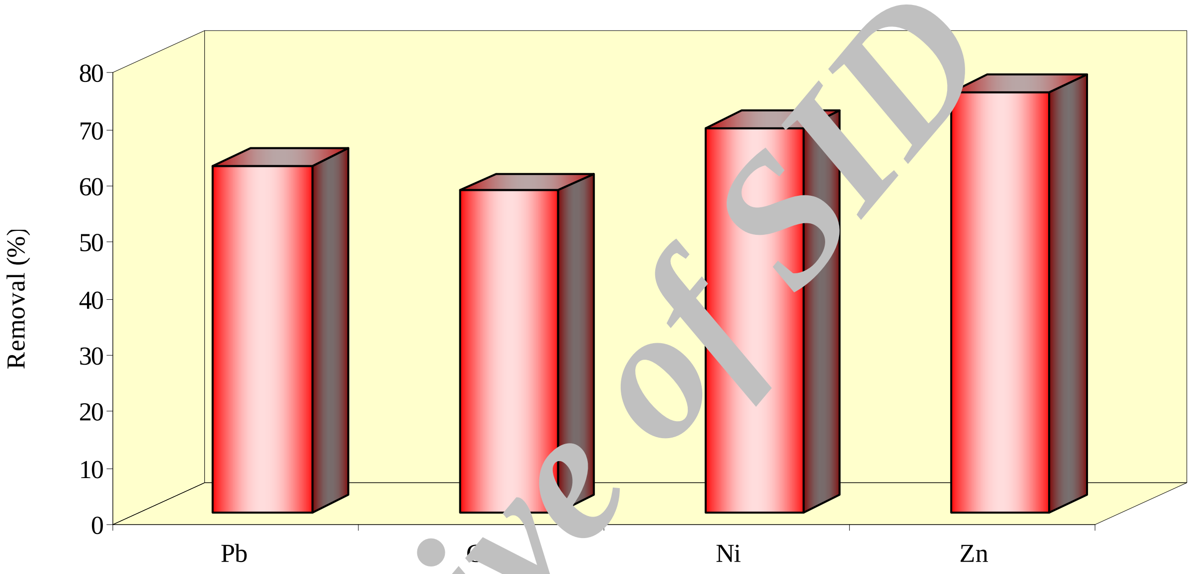

Figure 3: Ability of living Azolla in the removal of heavy metals after 15 days

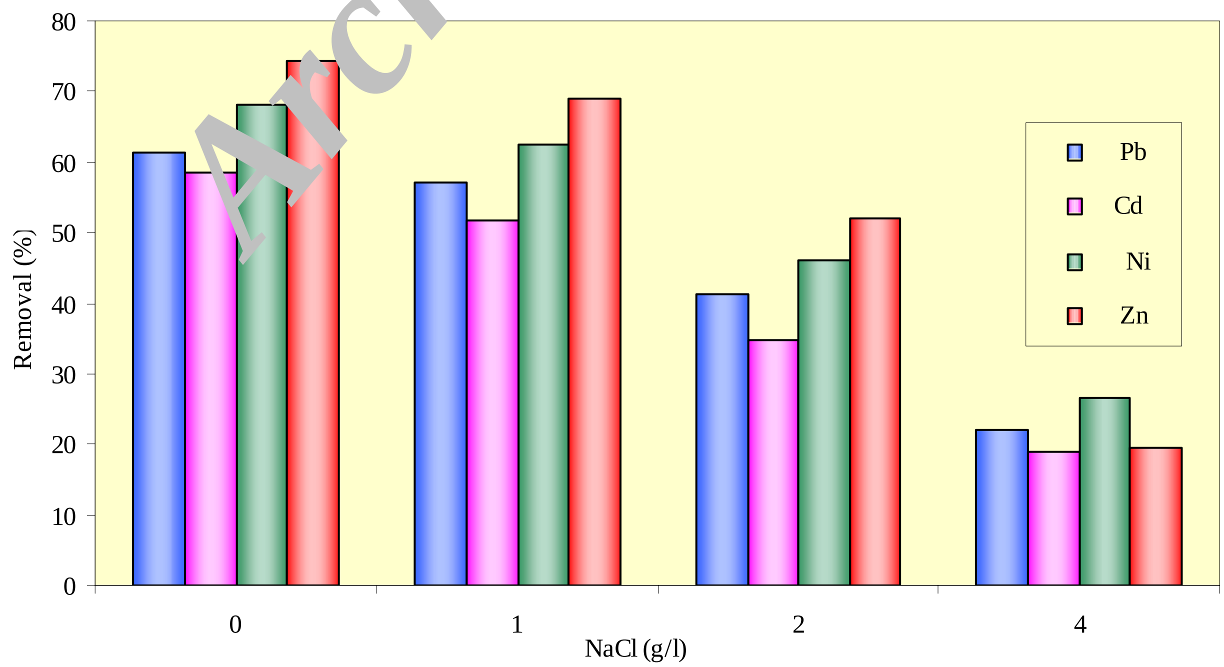

Figure 4: Effect of water salinity (NaCl) on the removal of heavy metals by living Azolla after 15 days

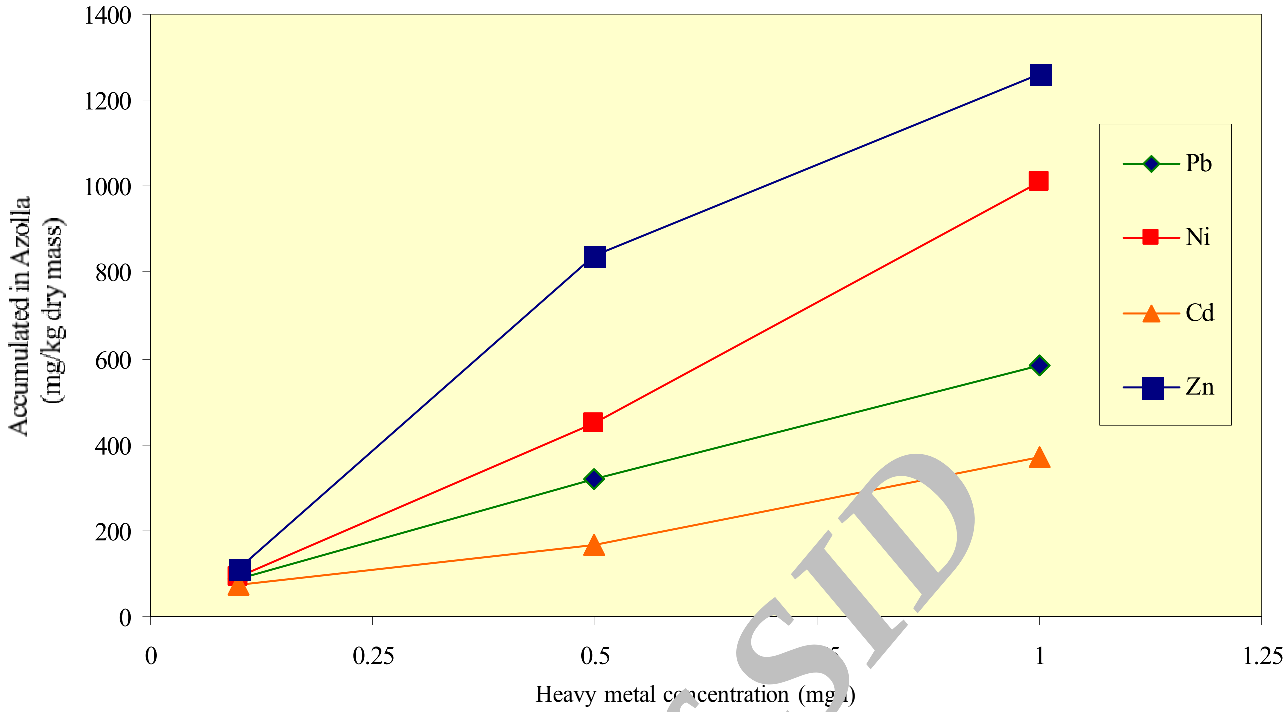

Figure 5: Accumulation of heavy metals by living Azolla after 15 days

19%; Ni^{2+} about 62%, 46% and 26%; Zn^{2+} about 69%, 52% and 19%.

Accumulation in *Azolla* mass

Figure 5 shows the contents of metals under examination in *Azolla filiculoides* (dry mass). To do this, the initial concentrations (C_0) were selected 0.1, 0.5 and 1 mg/l. and the biosorption time was 15 days. Heavy metal contents in biomass were determined by digestion of *Azolla* dried mass with 0.2M HNO_3. Pb^{2+} content was on the level 86, 320 and 586 mg/kg (dry mass) for these initial concentrations, respectively. The other metal ions were accumulated in the following amounts: 75, 165 and 371 mg. Cd^{2+}/kg (dry mass); 93, 450 and 1010 mg. Ni^{2+}/kg (dry mass); 110, 841 and 1260 mg.

Zn^{2+}/kg (dry mass) for the same initial concentrations of metal ions, respectively. It had been shown that plant cadmium uptake was metabolically mediated, and appears to be competitive with zinc uptake (Grant, *et al.*, 1998).

Discussion and Conclusion

The results obtained suggest that the living *Azolla filiculoides* has the capacity to accumulate large quantities of the heavy metals such as Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+}. This study showed that *Azolla filiculoides* has growth ability in the solutions containing these metal ions with the initial concentrations of 4 mg/l. within 15 days, although its growth was limited relative to *Azolla* control. The heavy metals concentration in solution can be modeled using the following material balance describing the time dependency of concentration (as a slow metabolism) with a first order differential equation as (Rahmani and Sternbetg, 1999):

$$\partial Cs/\partial t = -m_x r_s/V$$

where Cs is the metal ion concentration (mg/l.), m_x the wet (fresh) mass of plant (g), r_s the consumption rate of metal ion (mg metal ion /g biomass /h), and V the volume of water in experiment (l), that

$$r_s = \varepsilon_s (-r_x)$$

where r_x is the disappearance rate of biomass (g biomass /g biomass /h), and ε_s the amount of metal ion per unit biomass (mg metal ion / g biomass).

using TDS up to 300 ppm. (as $CaCO_3$) increased *Azolla* growth up to 21.0%, 22.6%, 23.0% and 22.4% in Pb^{2+}, Cd^{2+}, $Ni^{2+}$and Zn^{2+} solution, respectively. It can be due to the less diffusion of heavy metal ions into the biomass cells because of the presence and diffusion of ions with less harm (TDS) for the biomass growth. On the other hand, the addition of NaCl decreases the removal of heavy metal ions that can be due to the higher mobility and diffusion of Na^+ and Cl^- relative to heavy metal ions (Rai and Rai, 2003).

Moreover, it was determined that the more growth of biomass was nearly led to more removal. The toxic effect of the individual metal ions was the following arrangement: Cd^{2+} > Ni^{2+} >Pb^{2+}> Zn^{2+}, respectively.

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