BIOACCUMULATION OF HEAVY METALS BY THE WATER FERN *AZOLLA PINNATA*

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Abstract

Azolla pinnata aquatic fronds were tested for their ability to bioaccumulate heavy metals from Yoshida medium supplemented with different heavy metals, i.e. Pb^{+2}, Cd^{+2} and Cu^{+2} at different concentrations 0.0, 0.5, 1.0, 1.5 and 2.0 ppm, at different growth periods of 5, 10, 15, 20, 25 and 30 days. Results revealed that azolla can grow healthy with the accumulation of these metals. The highest fresh and dry weights of azolla were observed after 30 days at 2.0 ppm of Cu^{+2}. These values were significantly higher than those for Pb^{+2} and Cd^{+2} at 1.0 and 0.5 ppm , respectively. Also, azolla fronds exerted higher nitrogenase activity when grown in culture medium without heavy metals, or supplemented with 1.5, 1.5 and 0.5 ppm of Pb^{+2}, Cd^{+2} and Cu^{+2}, respectively. The highest records of nitrogen uptake were obtained, in the presence of Cu^{+2} (2.0 ppm) treatment, while phosphorus uptake increased with increasing the concentrations of Pb^{+2} and Cu^{+2} from 0.5 ppm to 2.0 ppm. On the other hand, phosphorus uptake decreased with increasing the concentrations of Cd^{+2} from 0.5 to 2.0 ppm. Potassium uptake in response to different Cd^{+2} concentrations was lower than the other treatments including the control, from 5 to 30 days of growth periods. The greatest contents of Pb^{+2}, Cd^{+2} and Cu^{+2} were observed in azolla fronds grown in presence of 2.0 ppm of any of these elements and collected after 30 days. Pb^{+2} uptakes were higher at the concentration of 2.0 ppm compared with other concentrations of Cd^{+2} and Cu^{+2}**.** Thus*, Azolla pinnata* showed tolerance to the tested heavy metals at concentration up to 1mg/l that supposed to pollute the medium. Therefore, azolla can serve as a phytoremdiation agent in the heavy metal polluted habitats.

INTRODUCTION

Recently the environment is heavily polluted by various toxic metals, which create danger problems for all living organisms. These metals are retarding farming efficiency and destructing the health of both plants and animals (Varsha *et al.,* 2010).

The capability for removing toxic heavy metals and trace elements from contaminated soils and water by using some plants is a process referred to as phytoremediation. Several terrestrial plants that have been identified in the last two decades as highly effective for absorbing and accumulating various toxic elements are evaluated for their role in the phytoremediation of soils polluted with toxic elements

(Tang *et al*., 2001). Phytoremediation is a new approach that offers more ecological benefits and a cost efficient alternative. It is a cheap method but it requires technical strategy; expert project designers with field experience that choose the proper species and cultivars for particular metals and regions. During various researches, the main focus is to understand the physiological mechanisms of metal absorption, transportation and assimilation; but little is known regarding the genetic basis of hyper accumulation (Pollard *et al*., 2002).

Heavy metal pollutant tolerance of *azolla* may be due to uptake of the metals into cell wall and vacuoles through an intensive carrier transporting system as well as evolution of specific metal resistance enzyme and alteration of cellular metabolites (Forstner and Wittmann, 1981).

The objective of the current work is to investigate the capability of *Azolla pinnata* to accumulat lead (Pb^{+2}) cadmium (Cd^{+2}) and copper (Cu^{+2}) in an enriched metal solution.

MATERIALS AND METHODS

Azolla strain and inocula

Azolla pinnata, used in the present study was kindly provided by Soils, Water and Environ. Res. Inst. (SWERI), Agric. Res. Center (ARC) Giza. The collected azolla fronds were sterilized with mercuric chloride (0.1 %) for 30 sec. according to Vandna and Ashwani (1999) and washed by sterilized water several times. Hundred grams of fresh azolla fronds were plotted between two sheets of tissue paper and then air dried for 30 minutes, afterwards it was used to put in plastic dishes with 40 cm in diameter and 9 cm in depth containing the enriched metal solution of Yoshida medium (Yoshida *et al*., 1976). Also these. Azolla fronds represent the standard inoculum throughout the experiment. Azolla inoculum was used at a rate of 100 gm/m^2

Yoshida medium was supplemented with different concentrations, (0.0, 0.5, 1.0, 1.5, and 2.0 ppm) of either Pb^{+2}, Cd^{+2} or Cu^{+2} in the form of $PbSO_4$, $CdSO_4$ and $CuSO_4$. Five replicates were prepared for each treatment. The azolla fronds in plastic dishes at the rate of 100 g/m^2 were grown under greenhouse conditions. The evaporated water was compensated by adding deionized water when needed. Developed azolla growth was periodically sampled at 0.0, 5, 10, 15, 20, 25 and 30 days to determine fresh and dry weights (El-Shahat, 1997), nitrogenase activity (Hardy *et al*., 1973) and total nitrogen, phosphorus, potassium (Black *et al*., 1965), and the accumulation of Pb^{+2}, Cd^{+2} and Cu^{+2} were determined on dry weight basis by using atomic absorption according to Jackson (1976).Data were statisticaly analyzed for least significant differences at P 0.05 as described by Gomez and Gomez (1984).

RESULTS and DISCUSSION

Fresh weight

Results revealed a significant increase in fresh weights (Table 1) with increasing the growth period up to 30 days. On the contrary, increasing the concentrations up to 2.0 ppm of Pb^{+2} and Cd^{+2} decreased the fresh weights of Azolla. The highest Azolla fresh weight of 8049.33 gm/m ^{2} was due to exposing azolla to 2 ppm Cu^{+2} up to 30 days compared to all other tested treatments. All Cu^{+2} applied concentrations increased Azolla fresh weight to be significantly higher than those recorded by the control treatment. Rauf *et al*. (2009) reported that copper (Cu^{+2}) is an essential plant micronutrient and often occurs in high concentrations in the aquatic ecosystems. In addition, Cu has spread contamination level due to its use as a mineral pesticide in agriculture (Ahmad *et al*., 2008). Khosravi *et al*. (2005) found that the presence of Pb^{+2}, Cd^{+2}, Ni and Zn in nutrient solution of Azolla caused approximately 25, 42, 31 and 17 % inhibition of biomass growth, respectively, compared with that of Azolla in heavy metals lacking medium.

Generally, the effect of Pb^{+2} and Cd^{+2} was more or less harmful rather than Cu^{+2} to azolla growth.

Dry weight

The obtained results showed a significant increase in dry weight of Azolla (Table 2) with increasing growth periods up to 30 days. On the contrary, different concentrations of Pb^{+2} and Cd^{+2} decreased significantly Azolla dry weight compared to control. These decreases were noticed along with increasing metal concentration from 1 up to 2 ppm to both Pb^{+2} and Cd^{+2}. Regarding Cu^{+2} effect on Azolla dry weight, results revealed that Azolla dry weight responded positively to the increase of Cu^{+2} concentration from 1 to 2 ppm (123.38 gm/m^2). The highest Azolla dry weight of 340.04 gm/m^2 was from 2 ppm Cu^{+2} treatment at 30 days compared to the other tested treatments. Wang and Chen (2006) suggested that metal ions are adsorbed first to the cell surface by interactions with metal-functional groups as carboxyl, phosphate, hydroxyl, amino, sulphur, sulphide group…etc., and then penetrate the cell membrane and enter into the cell. On the other hand, all Cu^{+2} applied concentrations at all incubation periods increased azolla dry weight compared to control treatment.

Table 1.

Table 2.

Nitrogenase activity

The different applied metal concentrations exhibited different effects on nitrogenase activity of *A. pinnata* (Table 3). However, the highest values of nitrogenase activity were 21.94, 18.42 and 14.60 µmol C_2H_4/gm dry wt/hr at concentrations of 1.5, 1.5 and 0.5 ppm of Pb^{+2}, Cd^{+2} and Cu^{+2} at 25 , 20 and 25 days of growth , respectively. These findings were observed by El-Berashi (2008) who reported that the highest value records of nitrogenase activity were 21.80, 19.83, 13.53 and 19.42 µmole C_2H_4/gm dry wt./hr with concentrations of 1.0, 1.0, 5.0 and 3.0 ppm of Pb, Zn, Cu and Mo after 2, 5, 5 and 10 days of growth periods, respectively. The authoress also found that N-uptake of *A. pinnata* in presence of Pb , Zn, Cu and Mo with respective concentrations of 4, 4, 2 and 5 ppm were 4864.8, 7709.9, 6079.2 and 2174.5 mg/m^2 at 25, 25, 25 and 20 days growth period, respectively.

Nitrogen uptake

The mean values of N-uptake (Table 4) increased with prolonged growth periods. Different concentrations of Pb^{+2} and Cd^{+2} tended to decreased significantly the N-uptake compared to control treatment. These decreases were noticed along with increasing metal concentration from 1 up to 2 ppm. In respect to Pb^{+2} and Cd^{+2}. Results of Cu^{+2} effects on N-uptake by azolla fronds revealed that it was positively responded to the increase of Cu^{+2} concentrations up to 2 ppm. The highest values of nitrogen uptake were obtained with 2.0 and 0.0 ppm Cu^{+2}, 0.5 ppm $Pb^{+2,}$ and 0.5 ppm Cd^{+2} given 9572.31, 8721.32, 7174.12 and 7088.41 mg/m^2 respectively, at 30 days of growth periods.

Table 3.

Table 4.

Phosphorus uptake

P-uptake (Table 5) increased with increasing the growth periods up to 30 days with Pb^{+2}, Cd^{+2} and Cu^{+2}, respectively. Maximum uptake of phosphorus by azolla fronds was obtained after 30 dayes of growth at 2.0 ppm of Pb^{+2} (742.67 mg/m^2) and at 2.0 ppm of Cu^{+2} (2943.42 mg/m^2) while P-uptake showed a lower value (1595.80 mg/m^2) at 2.0 ppm after 30 days of growth period.

Potassium uptake

Data presented in Table 6 showed an increase of K-uptake with increasing the growth period up to 30 days in the presence of any of $Pb^{+2,}$ Cd^{+2} and Cu^{+2}. On the other hand, different concentrations of Pb^{+2}, Cd^{+2} and Cu^{+2} increased significantly the values of K uptake. The application of 2.0 ppm Pb^{+2} or Cu^{+2} to the growth medium increased significantly K-uptake to higher values (6361.60 and 6229.06 mg/m^2 for Pb^{+2} and Cu^{+2}, respectively) at 30 days of growth periods. In the Cd^{+2} treatments, K-uptake at 30 days growth period was the lower compared with pb^{+2} and Cu^{+2} values. Costa *et al*. (1999) stated that the biomass analysis of azolla showed that nitrogen, phosphorous, potassium and organic content could favor the use azolla as biofertilizer especially when it is growing in domestic waste waters.

Table 5.

Table 6.

Heavy metals accumulation

Results revealed that the Pb^{+2}, Cd^{+2} and Cu^{+2} accumulated in azolla fronds (Table 7) after 30 days of growth period were highly significant compared with the control treatment. The highest accumulations of Pb^{+2}, Cd^{+2} and Cu^{+2} were 270.11, 255.20 and 209.50 mg/m^2, respectively, at the concentration of 2.0 ppm at 30 day growth period. Taghi ganji *et al*. (2005) concluded that, the removal of heavy metal ions (Pb^{+2}, Cd^{+2}, Cu^{+2} and Zn^{+2}) throughout *azolla* fronds depends on the treatment conditions of biomass and the biosorption process. Other studies showed that the contents of metals determined in the solution medium decreased whereas accumulation of the metals inside *Azolla* tissues was increased (Stepniewska *et al*., 2005). On the other hand, the aquatic fern azolla has been reported to accumulate high concentrations of heavy metals and metalloids (3-4 mgl^{-1}) from aqueous media (Rai and Tripathi, 2009). Sastry *et al*. (2005) reported that, the content of Pb^{+2} was up to 416 mg/kg dry weight and cadmium up to 259 mg Cd^{+2}/kg dry weight of Azolla. They suggested that, *Lemna minor* and *A. Pinnata*, can take up copper and decrease its concentration in the medium, therefore, they can effectively used for the treatment of waste water by developing the appropriate technology.

Table 7.

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المعالجة الحيوية للتلوث بالعناصر الثقيله باستخدام نبات الازولا

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استخدام النباتات من أكثر التقنياتِ الجديدةِ الوَاعِدةِ لمعالجةِ المياه الملوثة. فى هذه الدراسه تم استخدام *Azolla pinnata* سرخس مائى قادر على امتصاص بعض العناصر الثقيله فى بيئه يوشيدا التى تحتوى على الرصاص و الكادميوم والنحاس بتركيزات 0.0 ، 0.5، 1.0، 1.5 ، 2.0 جزء فى المليون. واختبرت *Azolla pinnata* على قابليتها لامتصاص هذة العناصرعلى فترات مختلفة 5 ، 10 ، 15، 20، 25، 30 يوم . اوضحت النتائج ان الأزولا تستطيع ان تنمو جيدا أثناء امتصاصها لهذة العناصر من البيئة.وأن أعلى أوزان للأزولا الطازجه والجافه ظهرت بعد 30 يوم فى وجود عنصر النحاس بتركيز 2 جزء فى المليون . هذه النتائج كانت اعلى من نتائج عنصرى الرصاص والكادميوم عند تركيزات 1، 0.5 جزء فى المليون على التوالى .

الأزولا التى نمت فى بيئه لاتحتوى على عناصر ثقيله أظهرت معدل تثبيت للنيتروجين أعلى ويليها التى تم دعمها بـــ 1.5 ، 1.5، 0.5 جزء فى المليون من الرصاص والكادميوم والنحاس على التوالى و أعلى معدل لامتصاص النيتروجين ظهر عند تركيز 2 جزء فى المليون للنحاس و الممتص من الفوسفور يزداد بزياده تركيز الرصاص و النحاس من 0.5 جزء فى المليون الى 2.00 جزء فى المليون . على الجانب الاخر الممتص من الفوسفور يقل بزياده تركيز الكادميوم من 0.5 الى 2 جزء فى المليون والممتص من البوتاسيوم فى التركيزات المختلفه من الكادميوم كان أقل من المعاملات الأخرى بما فيها معامله الكنترول فى الفتره من 5 إلى 30 يوم من النمو. اعلى مستوى من الرصاص والكادميوم والنحاس ظهر فى الازولا التى نمت فى تركيز 2 جزء فى المليون من كلا والتى تم جمعها بعد 30 يوم بينما كان الممتص من الرصاص اعلى عند تركيز 2 جزء فى المليون مقارنه بالتركيزات الاخرى للكادميوم والنحاس لذلك يمكن القول أن الأزولا اظهرت القدرة على تحمل تركيزات للعناصر الثقيله المختبرة حتى 1 ملليجرام /لتر لكل عنصر من العناصر الملوثه وكذلك تنقية المياه من بعض العناصر الثقيلة.