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SHORT COMMUNICATION

HYPERACCUMULATION OF CADMIUM AND NICKEL BY *AZOLLA* SPECIES

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***Azolla,* a small N- fixing and free floating aquatic fern exhibits high growth rate coupled with increased capacity to accumulate nutrients and metal ions in its biomass from flood waters. This trait can be employed for phytoremediation of metal contaminated wastewaters. In the present study, hyper accumulation of toxic heavy metals, Ni (Nickel) and Cd (Cadmium) by three species of *Azolla* (*A. microphylla, A. filiculoides* and *A. pinnata)* resulted in inhibited growth of these species. The concentration of Ni in biomass at applied levels of Ni ranged between 0.7-2.8% of dry weight and for Cd between 0.02-0.2%. Bioconcentration factor (BCF) upto 277 for Cd and 131 for Ni was observed. Metal concentration in biomass increased as metal levels in environment went higher and growth was severely inhibited. The necrosed biomass of *Azolla* was able to accumulate high amounts of Ni and Cd. The studies suggest that *Azolla* biomass can thus be employed for bioconcentration of Ni and Cd from wastewaters.**

Key words: ***Azolla,* heavy metals, phytoremediation, wastewaters.**

The risk of presence of toxic heavy metals in water and soil depends on their bioavailability. Soils usually exhibit higher concentrations of metals than waters because in soils the metals accumulate while in waters they get diluted and even get carried to other sites. The goal of almost every remediation approach is to provide a long term, stable and insoluble species. Many plants hyper accumulate toxic heavy metals and remove them from mobile pools. They can be exploited for remediation of metal contaminated environments. Theoretically, it is possible to extract metals with any of the hyperaccumulators but constraints such as high biomass productivity, cost effective harvest, disposal of metal laden biomass and metal concentration in dry biomass exist (Cunningham and Ow 1996).

Azolla, a small, N- fixing, free floating, aquatic fern has high growth rate coupled with capacity to concentrate and accumulate nutrients and metal ions in its biomass from flood waters. It is distributed world wide and can grow in fresh and polluted waters (Wagner 1997). The wastewater treatment processes employing *Azolla* are advantageous over chemical methods, as they are ecofriendly and effective when concentrations of toxic metals in contaminated waters are low. In diluted wastewaters when metal concentrations are below 100ppm, the chemical methods are ineffective (Volesky 1990). Also, *Azolla* biomass is easily harvestable and decomposable facilitating disposal of metal rich biomass and also metal recovery. Compared to other aquatic plants *Azolla* is more versatile as it can grow in N deficient waters. *Azolla* processes for extraction of heavy metals from wastewaters can operate in both active and passive forms. Studies evaluating the potential of *Azolla spp.* to accumulate heavy metals have been done (Arora and Singh 2001). The ability of *Azolla spp*. to accumulate metals has been attributed to presence of high levels of pectins and polyphosphates. In fact, peptides are involved in metal binding (Sela *et al*. 1988, 1989, Cohen-Shoel *et al*. 2002). The present study evaluates capacity of three *Azolla spp*.

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namely *A. microphylla, A.filiculoides* and *A. pinnata* to hyper accumulate toxic heavy metals Ni and Cd.

The three *Azolla spp*. were obtained from germplasm collection at National Centre for Conservation and Utilisation of Blue Green Algae, Indian Agricultural Research Institute, N.Delhi. They were grown in fedbatch cultures in Espinase & Watanabe (E&W) medium in plastic trays (20x25x5cm) in a polyhouse under controlled temperature of 28±2°C. Medium was replenished every third day. Fresh biomass was harvested on 14th day, thoroughly washed with distilled water and blot dried. This was used as inoculum for metal bioconcentration studies.

The ability of *Azolla spp*. to bioconcentrate toxic metals like Ni and Cd was studied by conducting hydroponic fedbatch experiments. They were grown in 200 ml of E & W medium amended with suitable amounts of $NiCl_2$ (to give 100-500ppm of Ni) or $CdCl_2$ (to give 1- 5 ppm of Cd) in 500ml glass beakers under polyhouse conditions as described above. The biomass was harvested on 7th day as plants showed toxicity symptoms and died. Three replications for each treatment were run and fronds growing in normal E&W medium without heavy metals served as controls. The harvested biomass was washed thoroughly with double distilled water, blot dried and then dried in oven at 40-50°C till constant weights were obtained. Known amount of dry biomass from each replication was digested with triacid (HNO_3+$HClO_4$+H_2SO_4 9:2:1) mixture on a hotplate till a clear solution was obtained and suitably diluted with double distilled water. The levels of Ni and Cd were measured by Atomic Absorption Spectrophotometer (Avanta). The concentration of Ni and Cd in dry biomass was expressed in ppm. The BCF was calculated as described by Boonyapookana *et al.* (2002) and is defined as the ratio of metal concentration in the dry biomass to the initial concentration of metal ion in feed solution. The results were statistically analysed using t- test.

The results indicated that both metals (Ni and Cd) severely inhibited growth of all the three species. The fronds showed toxicity symptoms by third day and by 7th day the biomass was almost dead. Cd (1ppm) allowed little growth and *Azolla spp.* produced about 40-60% of control biomass in presence of 1ppm Cd while at 5 and 10 ppm concentration, there was no growth. Ni (100-500 ppm) proved toxic and no growth was seen. After seven days the biomass harvested was dead. With increasing levels of Cd and Ni in growth medium, increased amounts of Cd and Ni accumulated in dry biomass of *Azolla spp.* (Table 1 and 2). *A.pinnata* showed highest concentration of Cd (2759 ppm) and highest BCF among the three species (Table 1). *A. filiculoides* showed highest conc. of Ni (28443 ppm) and BCF ranged from 32-131 for the three species (Table 2). As evident from the concentration of Ni and Cd in dry biomass, *Azolla spp*. can concentrate Ni upto about 3% and Cd upto about 0.2% on dry weight basis. Thus *Azolla spp.* can be classified as hyper accumulators of Ni and Cd and can be used for concentration and recovery of metals from wastewaters.

Many higher plants are known as hyperaccumulators of metals but their large scale use for bioremediation is not realized because of slow growth rate and limited biomass production (Black 1995). The *Azolla spp.* being small, free floating with high growth rate (the doubling time ranges between 2-7 days) has greater potential (Wagner 1997). In earlier studies *Azolla pinnata* and *A. filiculoides*

Table 1. Bioconcentration of cadmium (Cd) by *Azolla* spp. after seven days of incubation.

	Azolla microphylla		***A. filiculiodes***		***A. pinnata***	
Cd conc. in medium (ppm)	**Cd content in dry biomass (ppm)**	**BCF**	**Cd content in dry biomass (ppm)**	**BCF**	**Cd content in dry biomass (ppm)**	**BCF**
Control	4.78 ± 0.02		1.68 ± 0.02		3.25 ± 0.95	
1	232.6 ± 39.7	232	168.3 ± 1.6	168	277.1 ± 22.8	277
5	897.4 ± 45.2	179	1175.9 ± 18.0	235	1153.4 ± 121.6	243
10	1805.5 ± 145.8	181	2608 ± 16.1	260	2759.7 ± 181.8	275

Values are mean of triplicate ± S. D.

Table 2. Bioconcentration of nickel (Ni) by *Azolla spp*. after seven days of incubation.

	Azolla filiculoides		*Azolla microphylla*		*Azolla pinnata*	
Ni conc. in the medium (ppm)	**Ni content in dry biomass(ppm)**	**BCF**	**Ni content in dry biomass(ppm)**	**BCF**	**Ni content in dry biomass(ppm)**	**BCF**
Control	47.06± 13.53		36.5 ±6.8		49.16 ± 21.11	
100	13016.13 ±1951.74	131	8517.43 ±2134.2	85	7217.16 ±148.53	72
200	17429 ±1506.25	88	13732.93 ±969.27	69	9442.56± 362.4	47
300	23382.53 ±2439.80	78	17172.03 ±1160.28	57	10610.3 ±86.40	35
400	25764.06 ± 2297.4	64	19563.2 ± 2961.39	50	14015.33 ±2520.65	35
500	28443.63 ±1369.48	57	21785.43 ±1578.40	46	16252.46 ± 4251.22	32

Values are mean of triplicate ± S. D.

have been shown to absorb, Cr, Pb, Cd, Zn, Ur and other heavy metals and also show tolerance to these heavy metals when they are present in low concentrations (1-10 ppm) and *Azolla* biomass adsorbs heavy metals (Sarkar and Jana 1985, Jain *et al*. 1990, Gaur and Noraho 1995 and Zhao and Duncan 1997). The *Azolla spp.* used in this study were able to tolerate 1 ppm of Cd and not higher concentrations but Ni at 100 ppm was toxic and no growth occurred. However, the necrosed biomass concentrated high levels of these metals. Large scale cultivation and harvest of these *Azolla spp*. is easy and *Azolla* farming can be done in fresh or waste waters bodies, in cemented tanks and in fields. Therefore, these *Azolla spp*. can be employed to design simple process for making waste waters reusable for irrigation purposes.

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