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Phytoremediation of Wastewater for Irrigation Purpose Using Azolla

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REMEDIATION of contaminated water using plants, or phytoremediation, is one of the most promising new technologies for remediation. This work shows that Azolla aquatic plants, *Azolla pinnata* and *Azolla filiculoides,* are capable of causing a marked decline in nitrogen and certain heavy metals in mixed wastewater (industrial, sewage and agricultural wastewater) of the Shibin-Alqunatir drain, Qalubia Governorate. Results reveal that total uptake of N, Fe, Zn, Cu and Mn by Azolla tissues increased with time up to three weeks. For *Azolla filiculoides,* such increases reached 4.15, 2.5, 3.27, 3.80 and 4.33 fold that of the Hoagland nutrient solution (control) for N, Fe, Zn, Cu and Mn, respectively. *Azolla pinnata* showed a similar trend for such elements reaching 4.39, 2.91, 3.15, 3.43 and 3.20 fold that of the control, respectively. The elements concentrations in wastewater were markedly decreased after removing of Azolla at the end of the experiment. For *Azolla filiculoides* decreases were 56.4, 91.0, 41.5, 82.5, 37.7,12.1,46.7 and 67.2 % of the initial concentration of N, Fe, Zn, Cu, Mn, Co, Cd and Ni, respectively. *Azolla pinnata* decreases were 53.7, 92.7, 83.0, 59.1, 65.1,95.0, 90.0 and 73.1 %, respectively. Depending on the biological accumulation factor (BAF), values of Fe, Cu, Co and Cd showed higher response for phytoremediation than Zn, Mn and Ni.

Keywords: Azolla , Removal, Nitrogen, Heavy metals,Wastewater.

The reuse of wastewater for irrigation became a very important policy to maximize the use efficiency of the limited water resources and to achieve the extension programs of the agricultural development. Wastewater may contain some elements in high levels. Some of the heavy metals present in water are toxic to plants since they interfere with the effect of some enzymes and may cause lethal effect on the embryos and metabolic process. Also there could be a delay in seed germination and root growth (Bohn *et al.*, 1985).

Phytoremediation is a form of bioremediation, or use of biological processes to detoxify a site. Phytoremediation, specifically, is the use of plants to remove pollutants from the environment or to render them harmless (Raskin, 1996). Several species of aquatic plants have been shown to have the ability to grow in contaminated water and actually extract the pollutant from the growth medium, *i.e., Alyssum heldreichii* and *Thlaspi caerulescens* (Brooks, 1998) and water hyacinth, *Eichhornia crassipes* (Salati, 1987). These plants can function in several ways. Some plants can hyperaccumulate toxic trace elements in their tissues. Others can convert the pollutants to less toxic compounds and volatilize them (Terry & Zayed, 1994 and Brooks, 1998). Some aquatic plants' roots can filter contaminants from water (Brooks & Robinson, 1998). Some hyperaccumulators of one metal can hyperaccumulate other metals if they are present; for example, copper or cobalt hyperaccumulators can hyperaccumulate both. Other hyperaccumulators take up only a specific metal, even if others are present (Brooks, 1998). Biosorption consists of several mechanisms, mainly ion exchange, chelation, adsorption, and diffusion through cell walls and membranes (Dushenkov *et al.*, 1995) which differ are depending on the used species, the origin and processing of the biomass and solution chemistry. *Azolla pinnata* showed a remarkable ability of taking up Cd from the external medium. Total Cd taken up by the test plant, surface adsorption was about 90%. Cd adsorption did not occur at a constant rate. However, an equilibrium was reached in 2 hr (Gaur & Noraho 1995). The critical concentration of iron for growth of Azolla was 20 µg Fe/L as reported by Rains and Talley (1979) or 50µg Fe/L (Yatazawa *et al.*, 1980).

The objective of this study is to investigate the removal of nitrogen and certain heavy metals from wastewater or render them harmless for irrigation purposes with application of Azolla as the biosorbent.

Material and Methods

Water samples were collected in different seasons, *i.e.*, January, April, June and October in 2000 and 2001 from mixed wastewater (industrial, sewage and agricultural wastewater) of Shibin-Alqunatir drain, Qalubia Governorate. Water samples were taken from midstream water about 30 cm beneath water surface. All water samples were analyzed for pH, EC, soluble ions, boron, nitrogen, phosphorus, heavy metals (Fe, Mn, Zn, Cu, Cd, Co and Ni) and biological oxygen demand (BOD) (Table 1).

TABLE 1. Chemical composition of the collected water samples.

Parameter	January	April	June	October	means ± S.d
pH	8.15#	6.89	7.25	7.61	7.48 ± 0.54
EC, dS. m^{-1}	1.64	0.88	2.64	2.03	1.80 ± 0.74
Ca^{2+}	89.0	122.4	144.0	117.4	118 ± 22.63
Mg^{2+}	33.1	35.4	44.0	34.0	36.63 ± 5.01
Na^{+}	198.3	270.9	334.0	252.5	264 ± 55.97
K^{+}	21.1	23.8	29.3	22.6	24.20 ± 3.57
Cl^{-}	271.6	390.5	514.0	363.2	385 ± 100
HCO_3^{-}	201.9	294.0	316.6	286.1	275 ± 50.19
CO_3^{2-}	0.00	0.00	0.00	0.00	0.00
SO_4^{2-} ▼	164.5	173.4	214.7	166.7	180 ± 23.56
NO_3-N	50.69	54.4	56.93	54.04	54.02 ± 2.56
NH_4-N mg/L	17.82	18.13	23.25	22.83	20.51 ± 2.93
PO_4^{3-} ▲	0.31	0.34	0.49	0.46	0.40 ± 0.09
B	0.33	0.43	0.8	0.53	0.52 ± 0.20
Fe	3.87	8.39	10.49	8.83	7.90 ± 2.83
Zn	0.15	0.31	1	0.67	0.53 ± 0.38
Cu	1.69	2.28	3.86	2.92	2.69 ± 0.93
Mn	0.67	0.99	1.46	1.13	1.06 ± 0.33
Co	0.08	0.1	0.23	0.16	0.14 ± 0.07
Cd	0.08	0.11	0.14	0.02	0.09± 0.05
Ni	0.39	0.58	0.98	0.72	0.67 ± 0.25
Biologicaloxygen demand (BOD)	543	567	682	594	597 ± 60.69
Total suspended matter	3326	3247	2445	2876	2974 ± 403
SAR	4.54	5.53	6.23	5.26	5.39 ± 0.70
Class of salinity	Increasing problem	Increasing problem	Increasing problem	Increasing problem	
Class of alkalinity	Increasing problem	Increasing problem	Increasing problem	Increasing problem	

Each value in the table represents a mean of water samples taken in two years time.

Azolla was propagated in a nursery by growing 10 g of Azolla in plastic dishes (35 cm diameter and 10 cm depth) containing one kg of soil, clay, *Vertic torrifluvents* and three litters of tap water. Dishes were kept in the greenhouse till the growing Azolla covered the surface of the water. Azolla plants were harvested and washed gently in running tap water using a screen of 0.2 mesh. The washed Azolla samples were put in a 0.01N mercuric chloride for one minute, then washed several times with deionized water and air dried on filter paper from one to two hours. Two grams fresh weight of Azolla per dish (22.5 cm in diameter and 10 cm deep) were used as a standard inocula. Two Azolla species, *i.e., Azolla pinnata* and *Azolla filiculoides* were grown for four weeks on the following solutions:

i. 1/5 Hoagland's nutrient solution (control). Stock solution consists of 224, 235, 160, 62, 32, 24, 1.77, 0.27, 0.11, 0.131, 0.032, 0.05 and 1.12 mg/L of N, K, Ca, P, S, Mg, Cl, B, Mn, Zn, Cu, Mo and Fe, respectively. The pH and EC values of this solution were 6 and 0.8 dS/m, respectively.

ii. The collected wastewater of Shibin-Alqunatir drain. Three replicates were made for each treatment and the inoculated dishes were kept under greenhouse conditions at a constant volume throughout the experimental period by compensating the evaporated water by adding distilled water when necessary. Developed Azolla cultures were periodically sampled after 7, 14, 21 and 28 days to record dry weight, total nitrogen and heavy metals contents. Also water samples were taken prior to Azolla removal at the end of the experiment (4 weeks).

Water characteristics were determined using standard methods outlined by Jackson (1958); Sauter & Stoub (1990);Watanabe & Olsen (1965) and Baruah & Barthakur (1997). Total heavy metals contents of Fe, Zn, Cu, Co, Cd and Ni were determined after digestion with a hydrofluoric/ perchloric acids mixture (Chapman and Pratt, 1961). The dried plant samples were digested in concentrated H_2SO_4 and H_2O_2 at 400C° for N determination. Heavy metals content of water as well as digestion solutions of Azolla samples were analyzed using an Atomic Absorption Spectrophotometer.

Results and Discussion

Table 2 shows dry weight of different Azolla species grown on Hoagland's nutrient solution and wastewater at different periods of Azolla growth. Dry matter of the two Azolla species increased with time recording the highest values after 21 days from inoculation. Results reveal that the growth of two species of Azolla in the wastewater was comparable to that of in Hoagland's nutrient solution. The dry matter accumulation after 21 days of *Azolla filiculoides* and *Azolla pinnata* in wastewater reached 172 and 158% of that of Hoagland's nutrient solution, respectively. Increasing values of Azolla dry weight, grown on wastewater, could be due to higher nutrients content of wastewater compared to Hoagland's solution. However, the relative increase in growth per week for Azolla grown on Hoagland's nutrient solution was higher for the third week. It then tended to decrease. Meanwhile, those for Azolla grown on wastewater were higher during the first two weeks. The decline in increase % after two weeks of growth could be due to increasing nitrogen and heavy metals content of the used wastewater. Moore (1969) reported that Azolla is sensitive to changes and deficiencies in the supply of plant nutrients. For optimal growth, the fern requires all the macro- and micronutrients, essential for normal plant growth. Only low concentration was tolerated and the growth pattern was similar in the presence of 0, 10 or 20 ppm of NH_4^+. El-Haddad *et al.* (1988) found that application of 40 ppm urea-N secured the highest growth yield of *Azolla pinnata* and *Azolla filiculoides*. In presence of NH_4^+ –N, the highest growth yield was secured at 40 and 10 ppm for *Azolla pinnata* and *Azolla filiculoides*, respectively. *Azolla filiculoides* resulted higher dry weight compared to *Azolla pinnata* at any time of growth. Lumpkin (1987) reported that under favorable conditions both the fronds and the roots of some species start, almost doubling in weight after every 3-5 days. As the young plant grows in size, it also develops more roots. They are almost fully-grown in 15-20 days and have roots of 4-5 cm or more full- grown roots.

TABLE 2. Dry weight and growth increase % of the two Azolla species grown on 1/5 Hoagland's nutrient solution and wastewater as affected by period of inoculation.

Growth period, days	Azolla filiculoides		Azolla pinnata	
	Dry weight g/dish	Growth increase, % #	Dry weight g/dish	Growth increase, %
		1/5 Hoagland's nutrient solution		
0	0.10		0.09	
7	0.14	40.00	0.12	33.33
14	0.21	50.00	0.18	50.00
21	0.36	71.43	0.31	72.22
28	0.32	-11.11	0.27	-12.90
		Wastewater		
0	0.10		0.09	
7	0.23	130.00	0.24	166.67
14	0.58	152.17	0.49	104.17
21	0.98	68.97	0.80	63.27
28	0.92	-6.12	0.74	-7.50

Growth increase %: mean the percentage of increase in Azolla dry weight for one week.

Concentrations of N, Fe, Zn, Cu and Mn in Azolla tissues are illustrated in Fig 1. Data reveal that the content of these elements in Azolla tissues increased with time up to three weeks. Concentration of Fe in the two Azolla species reached the maximum after 21 days from inoculation. Manganese recorded the highest value after 28 days. Elements concentration increased in Azolla tissues when wastewater was used as a growth medium. Such increases recorded 90, 29, 56, 77and 95% more than the Hoagland's nutrient solution for N, Fe, Zn, Cu and Mn respectively, after 21 days for *Azolla filiculoides*. *Azolla pinnata* showed a similar trend for such elements recording 51, 60, 72, 64 and 109% increases above the control treatment. EL-Shahat (1997) obtained similar data concluding that the concentration of Fe and Zn in Azolla tissues increased along with time. However, concentrations of Zn and Cu increased in case of *Azolla pinnata* even after 28 days while, they reached to the maximum after 21 days from inoculation for *Azolla filiculoides* then tended to decrease. This may be due to differences between the two Azolla species, differences in uptake, translocation and utilization of such metals. It seems that heavy metals contents of Azolla, may reflect the changes and status of water. It is worth to mention that concentrations of Fe, Zn, and Mn reached the highest values in *Azolla pinnata* as they reached 30.8,62.6and 213 % of those of *Azolla filiculoides* after three weeks from inoculation, respectively. While,Concentrations of N and Cu reached the highest values in *Azolla filiculoides:* 4.1 and 26.0% of those of *Azolla pinnata,* respectively. EL-Shahat (1997) studied the uptake of Fe and Zn by *Azolla pinnata* grown on solutions enriched with 1, 2, 3, 4, 5, 6, 7, 8, 9 and10 mg/L for Fe and 0.01, 0.02, 0.04 and 0.08 mg/L for Zn and he found that the uptake rate of both metals was the highest with high concentrations.

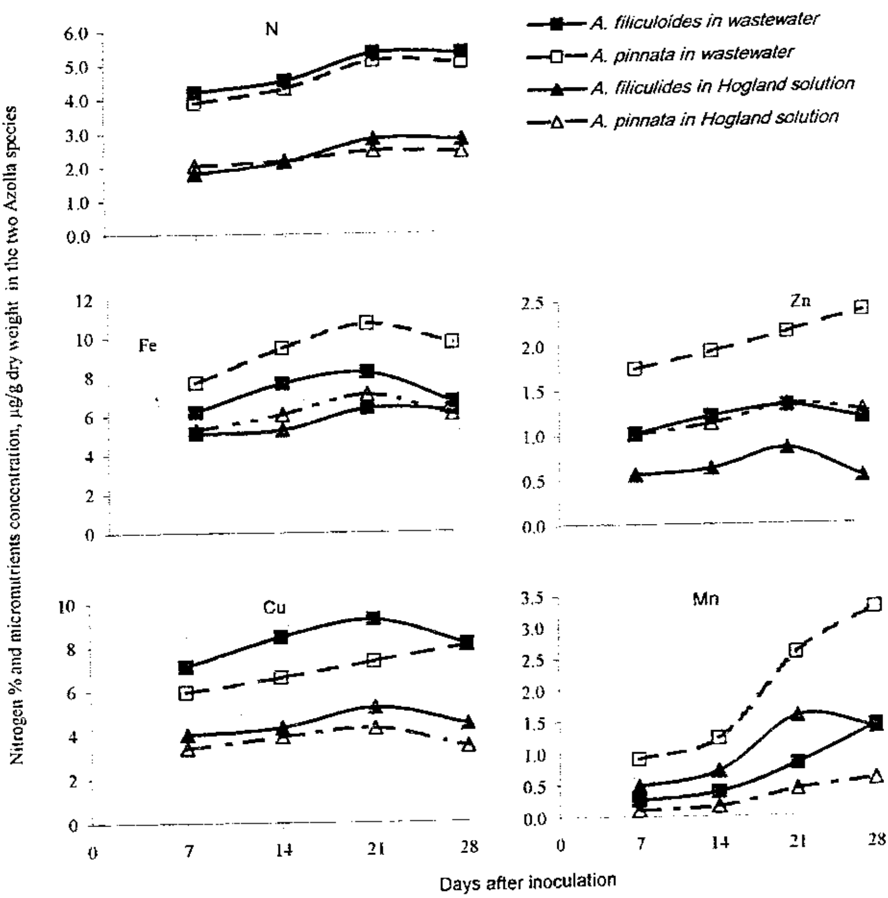

Fig.1. Concentration of N, Fe, Zn, Cu and Mn in different Azolla species as affected by period of growth.

Total uptake of the studied nutrients reached the highest values after 21 days of Azolla growth (Fig. 2). Depending on Azolla dry weight and concentration of such nutrients after 21 days, it is clear that normal growth of Azolla showed usual levels of such nutrients uptake, as described by Moore (1969) and Yatazawa *et al.* (1980). However, *Azolla filiculoides* inoculation in wastewater resulted in accumulating higher amounts of N, Fe, Zn, Cu and Mn, reaching 4.15, 2.5, 3.27, 3.80 and 4.33 fold compared to the Hoagland nutrient solution (control), respectively. Those for *Azolla pinnata* were 4.39, 2.91, 3.15, 3.43 and 3.20 fold, in the same order. Uptake of N and Cu increased due to inoculation of *Azolla filiculoides* compared with *Azolla pinnata*, while Zn, Mn and Fe showed a contrary trend.

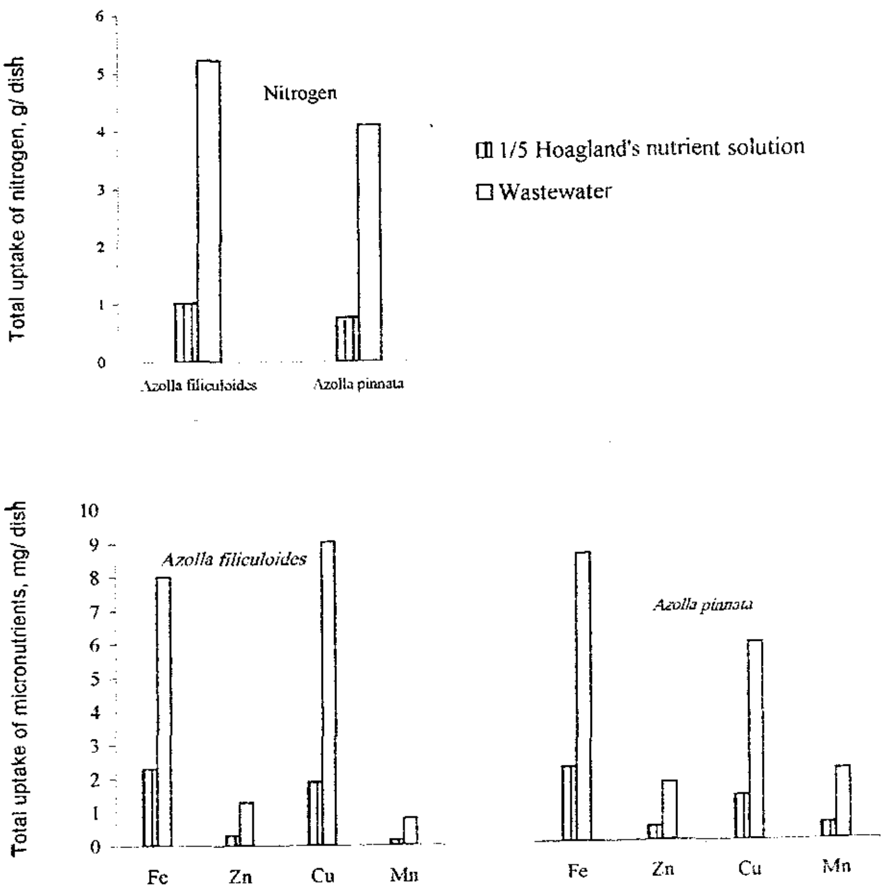

Fig. 2. Total uptake of nutrient elements after 21 days from Azolla inoculation.

Elements concentrations in wastewater, after removal of Azolla (28 days from inoculation) are shown in Table 3. Concentration of different nutrients in wastewater after *Azolla filiculoides* removal decreased by about 43.6, 34.7, 91.0, 41.5, 82.5, 37.7,12.1,46.7 and 67.2 % for NO^-_3, NH^+_4, Fe, Zn, Cu, Mn, Co, Cd and Ni, respectively, from initial concentration in wastewater Table, 1 while, incase of *Azolla pinnata* decreases were 32.4, 26.1, 92.7, 83.0, 59.1, 65.1,95.0, 90.0 and 73.1 %, respectively.

TABLE 3. Elements concentrations (mg/L) in wastewater after 28 days from Azolla inoculation.

NO^-_3	NH^+_4	Fe	Zn	Cu	Mn	Co	Cd	Ni
Azolla filiculoides								
30.46	13.39	0.71	0.31	0.47	0.66	0.123	0.048	0.22
Azolla pinnata								
36.50	15.16	0.58	0.09	1.10	0.37	0.007	0.009	0.18

The ratio between concentrations of heavy metals in Azolla and their concentrations in water indicates a biological accumulation factor (BAF). This factor reflects the affinity of the studied aquatic plants to a specific element or a pollutant. High (BAF) values express the high affinity of such aquatic macrophytes to bioaccumulation specific elements. Apart from the plant species, the BAF depends on the metal concentration in the water itself, *i.e.*, it increases with increasing metal concentration in the water (Webber, 1973). Data in Table 4 show that the BAF for all heavy metals, except for Cu, increased incase of *Azolla pinnata* more than *Azolla filiculoides*. Values of BAF varied widely from one metal to another for the two Azolla species. The (BAF) showed the following order: Cu> Fe > Ni > Zn > Cd > Mn> Co for *Azolla filiculoides*. For *Azolla pinnata* the order was Co > Cd > Zn > Fe > Ni> Mn > Cu. It appears that with *Azolla filiculoides,* Cu and Fe reached the highest values of BAF, indicating its high affinity for bioaccumulation. Cobalt and Cd reached the highest values of BAF with *Azolla pinnata*.

TABLE 4. Values of biological accumulation factor (B.A.F.) for the studied heavy metals.

Fe	Zn	Cu	Mn	Co	Cd	Ni
Azolla filiculoides						
9.37	3.76	17.06	2.16	1.79	3.13	7.72
Azolla pinnata						
16.69	27.88	7.28	8.86	100.00	45.56	14.00

In general, it can be noticed that the two Azolla species can remove the studied elements from polluted water of the Shibin-Alqunatir drain. *Azolla filiculoides* was beneficial for removing N and Cu, whereas *Azolla pinnata* was superior for removing Fe, Zn, Mn, Co, Cd and Ni elements from the polluted water. On the other hand, depending on BAF values, Fe, Cu, Co and Cd showed higher response for phytoremediation than Zn, Mn and Ni.

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استخدام نبات الأزولا في معالجة ماء الصرف بغرض استخدامه في الري

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يعتبر استخدام بعض النباتات ذات المقدرة على تراكم بعض المواد الغير مرغوب فيها إحدى التقنيات الحديثة المستخدمة في معالجة ماء الصرف ، وفى هذا البحث تم استخدام صنفين من نبات الأزولا هم (*Azolla pinnata and Azolla filiculoides*) لدراسة مدى قدرتهما على تقليل تركيز النيتروجين وبعض العناصر الثقيلة الموجودة بمياه مصرف شبين القناطر بمحافظة القليوبية والذي يحتوى على مياه مخلوطة من صرف صحي ، زراعي ، صناعي وقد أوضحت النتائج مايلى:

كانت الكميه الممتصة من عناصر النيتروجين، الحديد، الزنك، النحاس، المنجنيز عند تلقيح ماء الصرف **بصنف** *Azolla filiculoides* ٤,١٥ ، ٢,٥٠ ، ٣,٢٧ ، ٣,٨٠ ، ٤,٣٣ مره قدر الممتص من المحلول المغذى (١/٥ هوجلاند) على الترتيب. بينما كانت الزيادات المقابلة في حالة التلقيح بصنف *Azolla pinnata* ٤,٣٩ ، ٢,٩١ ، ٣,١٥ ، ٣,٤٣ ، ٣,٢٠ مره على الترتيب مما انعكس على الكميه المتبقية من تلك العناصر بماء الصرف بعد انتهاء التجربة حيث وصل تركيز النيتروجين، الحديد، الزنك، النحاس، المنجنيز، الكوبالت، الكادميوم، النيكل إلى ٥٦,٤ ، ٩١,٠ ، ٤١,٥ ، ٨٢,٥ ، ٣٧,٧ ، ١٢,١، ٤٦,٧ ، ٦٧,٢ ٪ من تركيز هذه العناصر في بداية التجربة في حالة تلقيح الماء **بصنف** *Azolla filiculoides* بينما انخفضت تلك العناصر إلى ٥٣,٧ ، ٩٢,٧ ، ٨٣,٠ ، ٥٩,١ ، ٦٥,١ ، ٩٥,٠ ، ٧٣,١ ٪ في حالة تلقيح الماء بصنف *Azolla pinnata* . وباستخدام عامل التراكم الحيوي (BAF) للتعرف على كفاءة الأزولا في التخلص من العناصر الثقيلة وجد أن الأزولا لها المقدرة على التخلص من الحديد ، النحاس، الكوبالت، الكادميوم بكفاءة اكبر من عناصر الزنك ، المنجنيز والنيكل.