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Biosorption of Pb, Cd, Cu and Zn from the wastewater by treated *Azolla filiculoides* with $H_2O_2/MgCl_2$

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Abstract

The adsorption of heavy metals onto treated *Azolla filiculoides* by $H_2O_2/MgCl_2$, as a cosmopolitan free-floating waterfern, was investigated from aqueous solutions in the batch biosorption experiments. The maximum uptake capacities of the collected *Azolla* from rice field at the optimal conditions for Pb, Cd, Cu and Zn ions were approximately 228, 86, 62 and 48 mg/g (dry *Azolla*), respectively. On the other hand, the maximum uptake capacities of the collected *Azolla* from the Anzali International Wetland in the north part of Iran at the same conditions for these heavy metals were about 124, 58, 33 and 34 mg/g (dry *Azolla*), respectively. Such decrease of uptakes is due to the pollution of Anzali International Wetland, which reduces the capacity uptake of metals. The recovery of biosorbed heavy metals from the rice field *Azolla* was carried out by HCl and NaCl desorbents that the recovery of 64- 86% and 51-72% was occurred, respectively.

Key words: *Azolla filiculoides, batch biosorption, heavy metals*

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Introduction

The different methods are used for the removal of heavy metals as important contaminants in water and wastewater. In the chemical methods, to effectively decrease of heavy metals to acceptable levels require a large excess of chemicals, which increase the costs because of generating the voluminous sludge (Spearot and Peck, 1984). On the other hand, a number of methods exist for the removal of heavy metals from liquid waste when they are present in high concentrations. These include methods such as precipitation, evaporation, electroplating, ion exchange and membrane processes (Matheickal and Yu, 1999). In the meantime, biosorption of heavy metals from aqueous solutions is a relatively new technology for the treatment of industrial wastewater. The major advantages of biosorption technology are their effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent materials (Volesky, 1990 and 1994). Furthermore, biosorption methods often provide better results than activated carbon and natural zeolites and are comparable to synthetic ion exchange resins (Matheickal and Yu, 1997).

An adsorbent material (biosorbent), both living and nonliving, is derived from suitable biomass can be used for the effective removal and recovery of heavy metal ions from wastewater streams (Niu, *et al*., 1993 and Muraleedharan, *et al*., 1995). These include bacteria (Ozdemir, *et al*., 2003), fungi (Fourest, *et al*., 1994), yeast (Volesky, *et al*., 1993), marine algae (Kaewsarn, 2002) and others.

Biosorption in natural or uncontrolled situations typically involves a combination of active and passive transport mechanisms starting with the diffusion of the metal ion to the surface of the microbial cell (Donmez, *et al*., 1999). The batch biosorption of the metals on biomass were best described by Langmuir isotherms (Feng and Aldrich, 2003).

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). *Azolla* has been shown to be able to effectively adsorb hexavalent and trivalent chromium, 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).

The initial binding and exchange of heavy metal ions to insoluble constituents in the *Azolla* matrix most probably involves cell wall charged groups (such as carboxyl and phosphate). Pectin and Cellulose are important polysaccharides constituent of plant cell walls, made of fragments of polygalacturonic acid chains, which interact with Ca^{2+} and Mg^{2+} (as the important exchanged ions with heavy metals) to form a three dimensional polymer by $(-COO)_2Ca$ and or $(-COO)_2Mg$ bindings as the ion exchanging bases (Jauneau, *et al.*, 1997; Kamnev, *et al.*, 1998 and Iijima, *et al.*, 2002). K^+ and Na^+ are mostly present in *Azolla* cell as soluble salts (Cohen-Shoel, *et al.*, 2002 *a*). In this study, biomass oxidation in the presence of $MgCl_2$ in the activating process of the nonliving *Azolla filiculoides* for batch biosorption of Pb^{2+}, Cd^{2+}, Cu^{2+} and Zn^{2+} (separately) were considered. The effects of HCl and NaCl as recovery agents also were investigated.

Materials and Methods

Preparing and treating of *Azolla*

Fresh *Azolla* (as raw living biomass) was collected from the surface of rice fields and Anzali Wetland in the south shores of Caspian Sea, Gilan province, the north part of Iran. 2 g. of *Azolla* was washed three times with deionised water (each time 100 ml. for 30 min.) and was air-dried in sunlight. *Azolla* (as raw non-living biomass) then was sieved to particles of 1.5-2.0 mm. before use. *Azolla* was treated as follows: the *Azolla* samples (each sample 2 g.) were soaked in 2 M. $MgCl_2$ at presence of 30 ml. H_2O_2 8 mM., as the optimal values for 12 h. under 125 rpm. and away from the light at pH 7. The samples were washed with deionised water. Because *Azolla* by alkali washing at pH 10-11 loses the minor quantities of its exchanger cations such as Mg^{2+} (Cohen, *et al.*, 2002 *b*), our samples were soaked in NaOH solution at pH 10.5 (as activation pH) for 6 h. The treated *Azolla* samples were washed three times with deionised water (each time 100 ml. for 0.5 min.) to removal excess Mg ions (unadsorbed) from the *Azolla*. These activated *Azolla* samples were then dried in oven at 60 ºC for 8 h. The pH of Samples was adjusted by using 0.1 M. NaOH and 0.1 M. HCl.

Experimental work

The Pb^{2+}, Cd^{2+}, Cu^{2+} and Zn^{2+} stock solutions were prepared by dissolving their corresponding analytical grade salts of $Pb(NO_3)_2$, $CdCl_2{\cdot}2.5H_2O$, $CuCl_2{\cdot}5H_2O$ and $ZnSO_4$ (Merck) in distilled water. The ion concentrations in stock solutions were about 4 g/l. For obtaining of adsorption isotherms, a series of flasks (250 ml., as batch biosorption experiments) were prepared containing heavy metal solutions (100 ml.) of known concentrations (Co) varying from 0.38 to 4.6 mM. by diluting of stock solutions with distilled water. For the study of batch biosorption, separately and/or comparative study, each solution contained one metal ion. The mounts of activated *Azolla* (200 mg.) were added to the flasks (biomass dose 2 g/l) and the mixtures agitated on a rotary shaker for 10 h. The pH of sample solutions were adjusted by using 0.1 M. HCl or 0.1 M. NaOH during the equilibrium period, at the obtained optimal values for each heavy metal viz. pHs 5.5, 6, 5.5 and 6.7 for removal of Pb^{2+}, Cd^{2+}, Cu^{2+} and Zn^{2+}, respectively. The experiments were performed at room temperature (22 ± 2°C). In the temperature range of 10-50°C there are no, or very minor changes to the surface and chemistry of the groups involved in sequestering the metal ions from solution (Sanyahumbi, *et al.*, 1998). The biomass was removed by filtration through a 0.45 µm. membrane filter (Millipore) and the filtrate was measured for ion content (Ce) by a Shimadzu Model AA-680 Flame Atomic Absorption Spectrophotometer (Japan).

Results

The optimization of the biosorption pH and the used $H_2O_2/MgCl_2$ (as biomass treatment agents) were performed for the rice field *Azolla filiculoides* and these results were then used both for the fixing of adsorption isotherms by the wetland and the rice field *Azolla filiculoides.*

Effect of pH on biosorption processes

Figure 1 shows the biosorption of Pb^{2+}, Cd^{2+}, Cu^{2+} and Zn^{2+} (Co was 1mM for each metal ions) were increased up to pH 5.5, 6, 5.5 and 6.7, respectively. The decreasing of biosorption levels by lowering pH can be explained due to competition between protons and metal ions for the capturing same sites, which at low pHs, metal ions do not successful. Also it may be explained dues to the dissociation of formed

three-dimensional structure from pectin to the monomers by hydrolysis of polysaccharides at the acidic pHs. In the recent case, the trace quantities of biosorbed heavy metals in all probability are desorbed from the monomers, easily.

Effect of H_2O_2 and $MgCl_2$ on *Azolla* treatment

As can be seen from Figure 2, the use of $MgCl_2$ in the activation (treatment) process even in absence of H_2O_2 increases the removal of heavy metals in the biosorption process. Co was 1 mM. for each metal ions and biosorption pH was kept at the obtained optimal values from Figure 1. The used H_2O_2 volume was 30 ml. The increasing heavy metals biosorption in consequence of the rising $MgCl_2$ concentration can be due to Mg^{2+} replacement as an ion exchanger, instead of the *Azolla* removed ions. Because, it had been showed the lose of various quantities of exchanger ions viz. Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} from the raw nonliving *Azolla* in consequence of washing by the solutions with neutral pH (Cohen, *et al*., 2002 *a*). Therefore *Azolla* loses the quantities of exchanger ions, as the result of washing by deionised water (pH 7) in the preliminary stage, prior to experimental work in the present study. On the other hand, the increasing of used H_2O_2 in the treatment process in absence of $MgCl_2$ has not remarkable effect on the removal of heavy metals in the biosorption process. But the using H_2O_2 increases heavy metal biosorption, remarkably in cases when Mg ions as the exchanger ions are increased, proportionately. The highest metals removal was obtained in consequence of using 2 M. $MgCl_2$ and 30 ml. H_2O_2 8 mM. by the treated *Azolla* at the optimal biosorption pHs for each heavy metal ion (Figures 2a and 2b). It may be explained as follows: the cell wall polysaccharides such as cellulose and pectin can be oxidized using H_2O_2 as oxidant agent (Shao, *et al.*, 2003; Pacoda and Montefusco, 2004 and Robert and Barbati, 2002). Therefore in the treatment process, H_2O_2 can be converts the more hydroxyl groups of cell wall polysaccharides of *Azolla* such as cellulose and pectin to free carboxyl groups. This state increases $(–COO)_2Mg$ bindings, as the ion exchanging bases

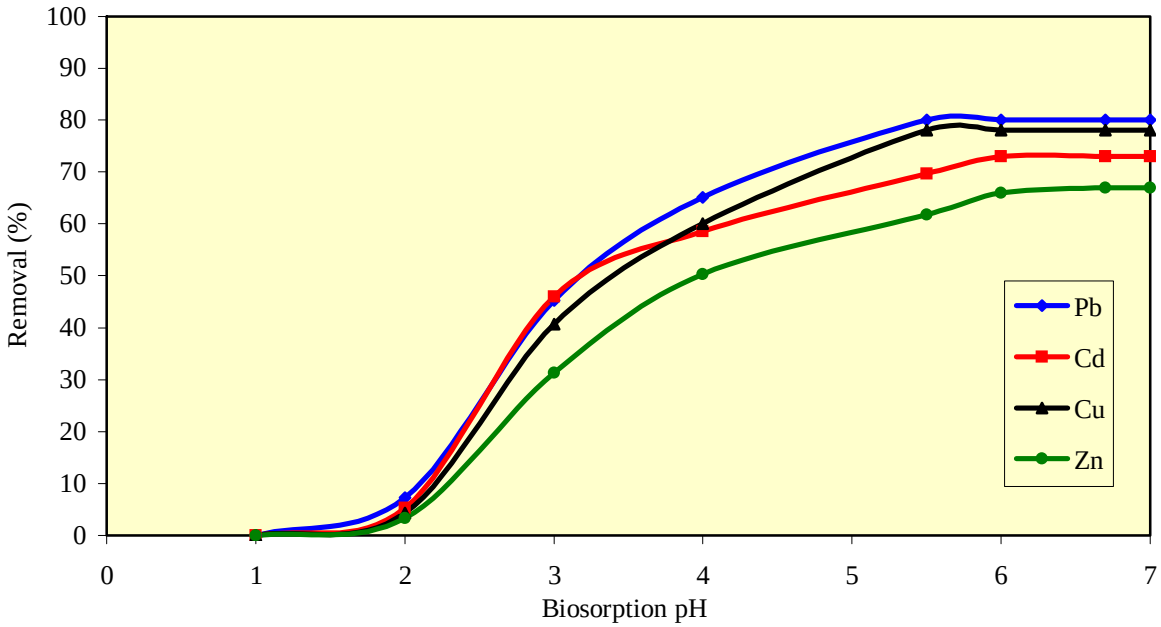

Figure 1: Effect of process pH on the removal percentages

at presence of $MgCl_2$ in the treated *Azolla* cell biomass (Zhao and Duncan, 1997 and 1998 and Davis and Volesky, 2003) which is written as:

$$qe = Qmax\ bCe / (1 + bCe)$$

where qe is the concentration of metals in the sorbent phase viz. biomass (mmol/g dry biomass), Ce is the equilibrium metals concentration or unadsorbed (mM.) that is obtained at the end of biosorption process. Q_{max} (mmol/g dry biomass) and b (1/mM) are the maximum adsorption capacity and a measure of adsorption energy and/or the sorption binding constant, respectively.

Langmuir equation transforms to the linearized form:

$$Ce/qe = Ce/Qmax + 1/(Qmax\ .b)$$

that Q_{max} and b are found from the slop and intercept of Ce/qe vs. Ce linear plot such that Q_{max} =slope^{-1} and b = (intercept $^{-1}$.slope).
qe is given from the following relation (Arica, *et al.*, 2003):

qe = (Co – Ce) / Biomass dose

where Co is the initial concentration of the metal ions (mM) that is provided from 0.38 to 4.6 mM. and biomass dose is 2g/l in this study. As can be seen from Figures 3a and 3b, the removal percentages by wetland *Azolla* are less than those for rice field *Azolla*. This reduces of the removal percentages were about of between 23–27 % for the initial concentrations of 1mM for each ion.

Table 1, shows the values of Q_{max}, b and correlation (r^2) for the biosorption of heavy metals by the rice field and Anzali International Wetland *Azolla*.

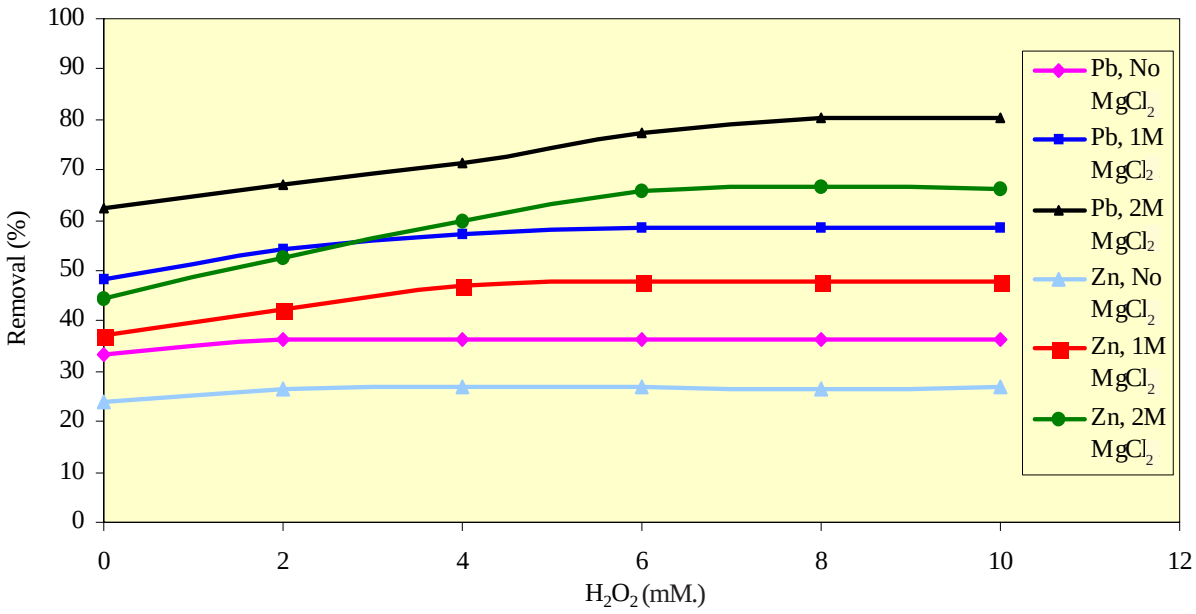

Figure 2a: Effect of used $H_2O_2/MgCl_2$ in Azolla treatment on the Pb^{2+} and Zn^{2+} biosorption percentages

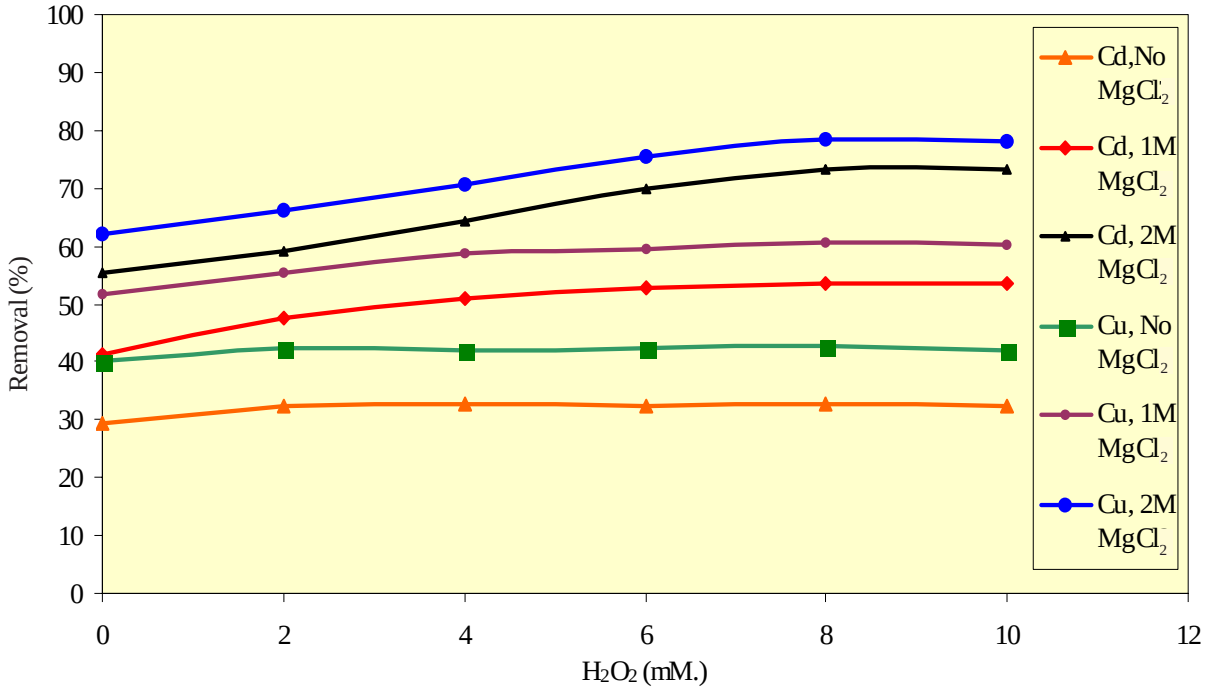

Figure 2b: Effect ofused $H_2O_2/MgCl_2$ in Azolla treatment on the Cd^{2+} and Cu^{2+} biosorption percentages

Recovery of heavy metals from *Azolla*

The effect of two desorbents (HCl and NaCl) was studied. The desorption of metal ions was carried out from each 2 g. of rice field *Azolla*, which had been used in the biosorption of heavy metals with Co 1mM., in this study. It was performed by 300 ml. HCl and NaCl (0.2 M.) for 6 h. in a batch system, separately. The recovery percentage is obtained from the following relation (Zhao, *et al*., 1999 and Arica, *et al*., 2003):

Recovery (%) = (Desorbed / Adsorbed) 100

that the "desorbed" is the concentration and/or the mass of metal ions after the desorption and the "adsorbed" is equal to (Co–Ce) and/or (m_o–me) for each recovery process. m_o and me are the heavy metals mass in the aqueous solution, before and after the biosorption, respectively.

As can be seen from Table 2, the recovery of heavy metals by the both desorbents from *Azolla* was performed the following arrangement: Zn^{2+} > Cu^{2+} > Cd^{2+} > Pb^{2+}. It was occurred by 0.2 M. HCl about 86%, 73%, 72% and 64%, respectively. While the recovery of these metal ions by 0.2 M. NaCl was occurred about 72%, 62%, 60% and 51%, respectively.

Discussion and Conclusion

The removal of heavy metal ions (Pb^{2+}, Cd^{2+}, Cu^{2+} and Zn^{2+}) by collected *Azolla filiculoides* in the batch experiment in the fixed *Azolla* dose depends on the treatment conditions of biomass and the biosorption process. In the treatment of *Azolla*, the kind of activator material (mineral and oxidant agents) and activation pH were important. The best removal results were obtained when *Azolla* was treated by 2 M. $MgCl_2$ and 30 ml. H_2O_2 8 mM. at pH 7 for 12 h. and it was then washed by NaOH solution at pH 10.5 for 6 h. pHs 5.5, 6, 5.5 and 6.7 for the biosorption of Pb^{2+}, Cd^{2+}, Cu^{2+} and Zn^{2+} were obtained as the optimal values, respectively. The maximum uptake capacities (Q_{max}) of Pb, Cd, Cu and Zn ions by the rice field *Azolla filiculoides* in the optimal conditions were about 228, 86, 62 and 48 mg/g (dry *Azolla*), respectively (Table 1). For instance, Q_{max} for Cd^{2+} and Cu^{2+} in the one of the studies by a biomass adsorbent in the batch biosorption were approximately 53 and 36 mg/g (dry biomass), respectively (An Ong, *et al*., 2005).

Table 1a: Rice field Azolla

	Qmax (mmol/g)	b (l/mM)	r^2
Pb^{2+}	1.10	5.21	0.99
Cd^{2+}	0.77	5.51	0.98
Cu^{2+}	0.98	5.34	0.98
Zn^{2+}	0.73	4.70	0.97

Table 1b: Rice field Azolla

	Qmax (mmol/g)	b (l/mM)	r^2
Pb^{2+}	0.60	2.74	0.99
Cd^{2+}	0.52	2.20	0.97
Cu^{2+}	0.52	2.68	0.98
Zn^{2+}	0.52	1.42	0.94

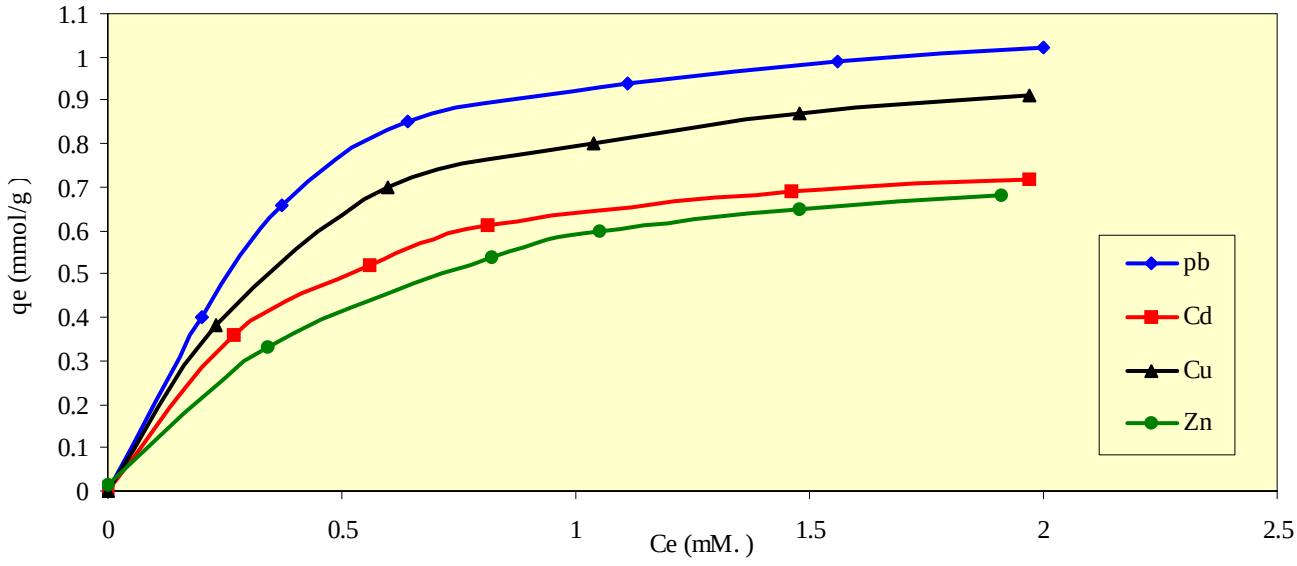

Figure 3a: Adsorption isotherms for rice field *Azolla*

Table 2: Heavy metals recovery by two desorbents

	me (mg)	0.2 M. HCl Desorbent		0.2 M. NaCl Desorbent	
		Desorbed (mg)	Recovery (%)	Desorbed (mg)	Recovery (%)
Pb^{2+}	41.44	106.94	64.52	85.02	51.29
Cd^{2+}	30.34	59.57	72.43	49.26	60.03
Cu^{2+}	14.60	35.83	73.28	30.54	62.46
Zn^{2+}	22.23	37.26	86.32	31.15	72.16

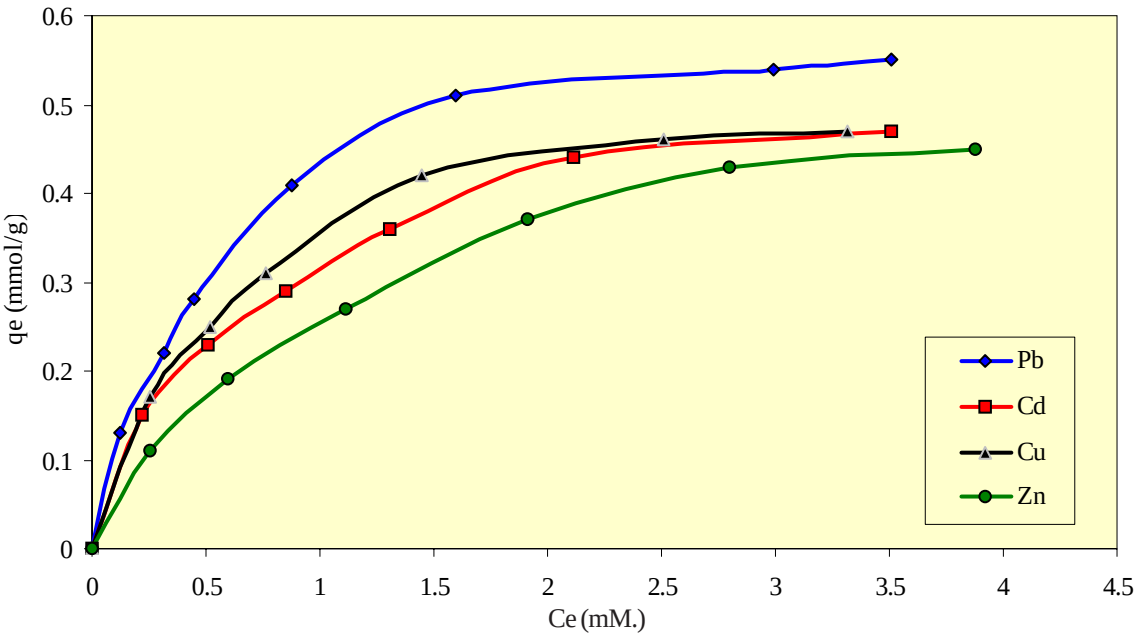

Figure 3b: Adsorption isotherms for Anzali Wetland Azolla

In compared with the values of Qmax in this study is appeared that the treated rice field *Azolla* with H_2O_2/$MgCl_2$ can be considered as a biomass with the suitable ability for biosorption among of other types of biomass.

Figure 3b shows adsorption isotherms of Pb, Cd, Cu and Zn ions by the wetland *Azolla*. The obtained Qmax values for these heavy metals by the Anzali Wetland *Azolla* in the same conditions were about 124, 58, 33 and 34 mg/g (dry *Azolla*). The considerable difference between the obtained Qmax values by wetland *Azolla* with the obtained Qmax values by rice field *Azolla* can be evaluated because of the pollution of wetland mater due to discharging the kinds of wastewater to the Caspian Sea and the Anzali Wetland. Consequently the uptake capacity of heavy metals by *Azolla* is reduced by reason of the adsorption of wastewater contaminants. The study of heavy metals recovery shows that the ability of proton in the exchanging and recovery is more than Na^+.

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