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Assessment of phyto-filtration and biosorption treatment on the removal of contaminant form wastewater

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Abstract: Nowadays, the environmental pollution resulting from natural resources acquisition is one of the most severe problems. Biosorption is an environmentally friendly and economically attractive technique using low-cost biomasses for contaminants removal. The study on the performance of low-cost biosorbents, such as dried biomasses of Neem leaves (*Azadirachta indica* A. Juss.) and *Azolla pinnata*, in the removal of Ni, Al, Cu, Pb, Fe and Cd ions from aqueous solutions was performed. The optimum concluded conditions were pH: 7 for Ni; 3 for Al; 5 for Cu; 5 for Pb; 3 for Fe and 5 for Cd, contact time: 1 hour, S/L ratio: 1/500 g/ml, shaking rate: 300 rpm and initial metals concentration: 100 mg/l. The higher biosorption capacities were achieved using *Azolla pinnata* biomass that reached 28.22, 28.46, 39.67, 41.27, 35.64 and 21.70 mg/g; whereas, neem leaves achieved lower capacities reached to 14.75, 12.65, 19.60, 35.86, 21.00 and 11.67 mg/g for Ni, Al, Cu, Pb, Fe and Cd, respectively. The biosorption process obeyed the 2nd order kinetic modeling. The isotherm modeling indicated that the biosorption onto *Azolla pinnata* was a multilayer coverage; meanwhile, it was monolayer coverage onto neem leaves. The concluded optimum conditions from batch experiments were applied to treat actual contaminated wastewater samples from Bahr El-Baqar drain. The removal percentage for Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn reached 94.76, 96.66, 90.40, 95.26, 75.51, 86.51, 47.98, 89.10, 93.44 and 88.63 %, respectively using *Azolla pinnata* biomass; whereas, neem leaves achieved removal percentage of 93.60, 98.06, 89.27, 90.95, 60.47, 79.61, 68.91, 36.70, 86.88, 72.03 % and 59.76, respectively. However, neem leaves biomass was mightily recommended to be used in water treatment process because of Mn release from *Azolla pinnata* biomass during water treatment process.

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1. Introduction:

Heavy metals are elements with a specific gravity that is at least five times more than the specific gravity of water **(Charan *et al.*, 2014).** Heavy metals have been excessively released into the environment due to rapid industrialization and have created a major global concern. Lead, cadmium, nickel, copper, zinc, chromium and mercury are often detected in industrial wastewaters, which originate from metal plating, mining activities, smelting, battery manufacture, tanneries, petroleum refining, paint manufacture, pesticides, pigment manufacture, printing and photographic industries, etc., **(Parmar and Thakur, 2013).** Heavy metals enter in tissues through the food chain and accumulate in the body of all living organisms **(Doke *et al.*, 2012)**.

Adsorption is one of the physicochemical treatment processes found to be effective in removing heavy metals from aqueous solutions. An adsorbent can be considered as cheap or low-cost if it is abundant in nature, requires little processing and is a byproduct of waste material from the waste industry. Plant wastes are inexpensive as they have no or very low economic value. There are many reports on aquatic plants and weeds which have been used for bioaccumulation or bioremediation of the sewage wastewater **(Jamuna and Noorjahan, 2009).**

Azolla sp. is an aquatic free floating fern belonging to the family Salviniaceae with high potential for phytoremediation and biosorption programs due to its capacity to accumulate metals such as cadmium, chromium, nickel and zinc **(Benniceli *et al.* 2004, Anitha *et al.*, 2016).** Dry *Azolla* sp. has been shown to bind and concentrate radioactive and heavy metal ions from aqueous solutions over a broad range of concentrations (ppm to ppt) and a wide range of pHs: 2–11 **(Anitha *et al.*, 2016).** Both living as well as the non-viable biomass of *Azolla*sp. can be used for heavy metals removal from wastewater. Today, there is an increasing interest in utilizing *Azolla*sp. biomasses as a decontaminant plant in low-cost wastewater treatment systems **(Rawat *et al.*, 2011)**.

The neem tree (*Azadirachta indica* A. Juss.) has been known as the Wonder Tree for centuries (**Kumar and Navaratnam, 2013)**. This powerful evergreen tree has long been a symbol of health in its native countries, Indian sub content (**Tomar *et al.*, 2008)**. It is one of the very few shade-giving trees that noted for its drought resistance and very high temperatures tolerance. It is a typical tropical to subtropical tree thrives in areas with sub-arid to sub-humid conditions. It can grow in many different types of soil, but it thrives best on sandy and well drained deep soils. The trees are not at all delicate about the water quality and thrive on the merest trickle of water, whatever the quality **(Oboh *et al.*, 2009)**. This makes it a suitable choice for water treatment processes.

Bahr El-Baqar drain is considered as one of the most polluted drains in Egypt. This drain is located in the eastern part of the Nile Delta and receives untreated wastewater starting from east of Cairo and pours into El-Manzala Lake. Nearly 58% of the total wastewater of Bahr El-Baqar drain comes from agricultural drainage, 40% from domestic and commercial drainage and 2% from industrial wastes **(Abdel-Fattah and Helmy, 2015)**. That makes it contaminated with several contaminants including heavy metals.

The objectives of this study were firstly, determining the optimum conditions to biosorb Ni, Al, Cu, Pb, Fe and Cd ions from aqueous solutions via batch biosorption using dried biomasses of Neem leaves and *Azollapinnata*; secondly, application of the optimum conditions in the treatment of actual contaminated wastewater collected from Bahr El-Baqar drain, Alsharqia government, Egypt.

2. Materials And Methods:

2.1. Propagation of *Azolla pinnata* biomass:

Azolla pinnata used in this study was kindly supplied by Agricultural Microbial Department, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt. About 10 g of *Azolla pinnata* was grown in plastic pots (50 cm in diameter and 20 cm depth) containing 1kg soil saturated with 5 liters of tap water. These pots were kept in a greenhouse till *A. pinnata* covered the entire water surface according to **El- Shahat (2016)**. *A. pinnata* was collected and washed gently in running deionized water for several times and then the biomass was air dried.

2.2. Preparation of neem leaves biomass:

Neem leaves biomass was kindly supplied by SEKEM Company. It was thoroughly washed with deionized water to remove any attached dirt and impurities and then the biomass was air dried.

2.3. Biosorption experiments (batch procedures):

Metals stock solutions of 1000 mg/l were prepared by dissolving metallic salts in demineralized water. The used salts; $NiCl_2.6H_2O$, $Al_2(SO_4)_3.(NH_4)_2SO_4.24H_2O$, $CuCl_2.2H_2O$, $PbCl_2$, $FeCl_3$ and $CdCl_2$ were analytical grade (A.R) reagents purchased from Aldrich Chemical Company, Germany. The other chemicals were reagent grade and used without further purification. All batch experiments were carried out in triplicate. Each experiment conditions are mentioned in the tables' remarks. Unless otherwise stated, 0.1 g of biomasses was shaken with 25 ml of metal aqueous solution at room temperature for 2 hours. The solutions were filtered and the remained metal concentrations were analyzed using Inductively Coupled Argon Plasma, iCAP 6500 Duo (Thermo Scientific, England). Multielement certified standard solution of 1000 mg/l (Merck, Germany) was used as stock solution for instrument calibration. Diluted solutions of HCl and NaOH were used to adjust pH of metals aqueous solutions using pH meter. The maximum pH values were7 for Ni; 3 for Al; 5 for Cu; 5 for Pb; 3 for Fe and 5 for Cd, as metal precipitation as hydroxides occurred at higher pH values. Desorption of heavy metals ions from biomasses was performed via shaking with 0.01 M $Na_2EDTA.2H_2O$ at 300 rpm for 1 hour and Solid/liquid ratio of 1/100 g/ml.

2.4. Application of biomasses in the treatment of actual wastewater in Bahr El-Baqar drain:

According to heavy metals concentrations, three wastewater samples were chosen from ten collected ones from Bahr El-Baqar drain during March 2019. Sites were located between latitudes and longitudes of 30^0 38' 18.6" N and 31^0 41' 51.6" E (site 1), 31^0 01' 13.2" N and 32^0 12' 12.9" E (site 2) and 30^0 11' 0.5" N and 32^0 12' 13.7" E (site 3). Neem leaves and *A. pinnata* biomasses were applied to treat wastewater samples under the concluded optimum conditions from the batch experiments (contact time: 1 hour, S/L ratio: 1/500, shaking rate: 300 rpm, room temperature). No pH adjustment was done in order to avoid the precipitation of some metals. Heavy metals were determined by inductively coupled argon plasma (iCAP 6500 Duo, Thermo Scientific, England).

2.5. Calculations and data evaluation:

2.5.1. Biosorption equations:

The amount of biosorbed heavy metal to biosorbents was expressed according to **Vitor and Corso (2008)** and **Kumar *et al.* (2005)** using the following equation:

$$q = [(C_i – C_f) V]/M$$

Where; (q) is the amount of sorbed metal onto the unit amount of the biomass (mg/g), (C_i) is the initial concentration of the heavy metal in aqueous solution (mg/l), (C_f) is the final (remained) concentration of the heavy metal in aqueous solution (mg/l), (V) is the volume of the metal aqueous solution (l), (M) is the biomass weight (g).

2.5.2. Kinetic modeling equations:

The linearized form of the first order model is assumed to be:

$$\mathbf{Log\ (q_e-q_t) = Log\ Q_e - (K_{1,ad}/2.303)\ t}$$

Where; (q_e) is the experimental amount of adsorbed metal at equilibrium (mg/g), (q_t) is the experimental amount of adsorbed metal at time "t" (mg/g), (Q_e) is the calculated amount of adsorbed metal at equilibrium (mg/g), ($K_{1,ad}$) is the rate constant of first order adsorption (min^{-1}), (t) is the time (min.). Linear plots of (Log (q_e-q_t)) versus (t) indicate the applicability of this kinetic model where ($K_{1,ad}$) and (Q_e) can be determined from the slope and intercept of the obtained straight lines, respectively.

The linearized form of the second order model is assumed to be:

$$\mathbf{t/q_t = 1/(K_{2,ad}\,Q_e^2) + (1/Q_e)\ t}$$

Where; ($K_{2,ad}$) is the rate constant of second order adsorption (g mg^{-1} min^{-1}). The plot of (t/q_t) versus (t) should give a straight line

2.5.3. Equilibrium modeling equations:

The linearized Langmuir equation assumes the form:

$$\mathbf{C_e/q_e = 1/(Q_e\,K_L) + (1/Q_e)\,C_e}$$

Where; (C_e) is the metal concentration in aqueous solution at equilibrium (mg/l), (q_e) is the experimental amount of adsorbed metal at equilibrium (mg/g), (Q_e) is the calculated amount of adsorbed metal at equilibrium (mg/g), (K_L) is the Langmuir constant (l/mg). Values of (Q_e) and (K_L) can be determined from the slope and intercept of the obtained straight line, respectivelyof plotting (C_e/q_e) versus (C_e).

The linearized logarithmic Freundlich equation assumes the form:

$$\mathbf{Log\ q_e = Log\ K_F + (1/n)\ Log\ C_e}$$

Where; (K_F) is the Freundlich constant indicating adsorption capacity, (n) is the Freundlich constant indicating adsorption intensity. Values of (n) and (K_F) can be determined from the slope and intercept, respectively of the obtained straight lineof plotting (Log q_e) versus (Log C_e) **(Salima *et al.*, 2013** and **Farah and El-Gendy, 2013).**

2.5.4. Desorption equations:

The amount of desorbed element from biomass (de-loaded) using Na_2EDTA was expressed according to **Bulgariu and Bulgariu (2014)** and **Kanwal *et al.* (2013).**

$$\mathbf{Desorption\ \% = (q_{desorbed}/q_{sorbed})\ X\ 100}$$

Where; (q) is the amount of desorbed or sorbed metal onto the unit amount of the biomass (mg/g).

2.5.5. Resorption equations:

The amount of resorbed element to biomass (re-loaded) was expressed according to **Aboulsoud (2008)**

$$\mathbf{Resorption\ \% = (q_{resorbed}/q_{sorbed})\ X100}$$

Where; (q) is the amount of resorbed or sorbed metal onto the unit amount of the biomass (mg/g).

2.5.6. Wastewater treatment equations:

$$\mathbf{Removal\ (\%) = (C_i - C_f/\ C_i)\ X\ 100}$$

Where; (C_i) is the initial element concentration before treatment, and (C_f) is the final element concentration after treatment.

2.6. Statistical analysis:

All determinations were made in triplicate for all assays. Data were subjected to an analysis of variance (ANOVA) with statistical significance at $P<0.05$ being tested using the Duncan's Test **(Waller and Duncan, 1969)**. Mean having the same alphabetical letters in the same column are not significant at P (significance probability value) =0.05 level.

3. Results And Discussion:

3.1. Effect of pH:

pH of the medium is one of the most important parameters affecting the metal ions biosorption from aqueous solutions. Both the electrical charges of the biosorbent cell wall components and the ionic forms of the metal ions in solution depend on the solution pH **(Shaaban *et al.*, 2017)**. The biosorption of studied metals increased with increasing pH to give the highest significant values at pH 7 for Ni, 3 for Al, 5 for Cu, 5 for Pb, 3 for Fe and 5 for Cd **(Table 1)**. Heavy metals precipitated at pH values over the highest studied ones. Both biomasses followed the same behavior, whereas *A. pinnata* was more efficient as biosorbent than neem leaves for all studied metals. The decrease of biosorption levels by lowering pH can be explained by competition between (H^+) and metal ions for capturing same active sites. The higher the pH value, the higher the dissociation since free sites for metals binding can be produced **(Ahmady-Asbchin & Omran, 2012)**.

3.2. Effect of solid/liquid ratio:

The lower the proportion of the biosorbent used compared to the amount of solute or water, the more indicative of the economic success of the water treatment process. Biosorbents dosage strongly affects the biosorption process as it provides the binding sites for metal biosorption. The increase of the biosorbent dose while other factors are constant increases the biosorption of metal ions due to greater surface area which in turn increases the number of available binding sites. As shown in **Table (2)**, S/L ratio of 1/500 g/ml was the best one that provided the highest significant biosorption values for both biosorbents. At lower concentrations of the biosorbents, the quantity of metal biosorbed per unit weight of the biosorbent is high. Conversely, at high concentration of the biosorbent, the amount of metal ion biosorbed per unit weight decreases. This is because of lower sorbate (i.e. metal ions) to binding site ratio due to less possible interaction to binding sites and the insufficient amount of solute present for complete distribution onto the available binding sites **(Kanamarlapudi *et al.*, 2018)**.

Table (1): Interaction effect between biosorbent type and pH.

Biosorbent	Ni		Al		Cu		Pb		Fe		Cd	
	pH	**Biosorption (mg /g)**	**pH**	**Biosorption (mg /g)**	**pH**	**Biosorption (mg /g)**	**pH**	**Biosorption (mg /g)**	**pH**	**Biosorption (mg /g)**	**pH**	**Biosorption (mg /g)**
Azolla pinnata	**1**	3.80 ^{g}	**1**	2.38 ^{e}	**1**	8.81 ^{e}	**1**	8.06 ^{e}	**1**	4.51 ^{e}	**1**	3.31 ^{e}
	3	14.53 ^{c}	**2**	9.94 ^{b}	**3**	29.70 ^{b}	**3**	22.50 ^{c}	**2**	25.51 ^{b}	**3**	11.69 ^{b}
	5	17.87 ^{b}	**3**	16.58 ^{a}	**5**	31.50 ^{a}	**5**	30.20 ^{a}	**3**	25.65 ^{a}	**5**	14.39 ^{a}
	7	18.40 ^{a}										
Neem leaves	**1**	2.13 ^{h}	**1**	0.40 ^{f}	**1**	7.23 ^{f}	**1**	5.10 ^{f}	**1**	2.49 ^{f}	**1**	2.95 ^{f}
	3	6.78 ^{f}	**2**	2.66 ^{d}	**3**	11.61 ^{d}	**3**	21.37 ^{d}	**2**	6.78 ^{d}	**3**	7.07 ^{d}
	5	7.66 ^{e}	**3**	4.45 ^{c}	**5**	11.77 ^{c}	**5**	24.67 ^{b}	**3**	9.94 ^{c}	**5**	7.91 ^{c}
	7	9.44 ^{d}										

(Remarks: values with different letters in a column are significantly different at the 0.05 level, experiment conditions: initial metal concentration: 100 mg/l, contact time: 2 hours, S/L ratio: 1/250 g/ml, shaking rate: 200 rpm, room temperature)

Table (2): Interaction effect between biosorbent type and solid/liquid ratio.

Biosorbent	S/L ratio (g/ml)	Biosorption (mg /g)					
		Ni	**Al**	**Cu**	**Pb**	**Fe**	**Cd**
Azolla pinnata	**1/100**	6.80 ^{e}	10.14 ^{c}	10.09 ^{e}	8.97 ^{e}	10.23 ^{d}	6.36 ^{e}
	1/250	18.40 ^{b}	16.58 ^{b}	31.50 ^{b}	30.20 ^{c}	25.65 ^{b}	14.39 ^{b}
	1/500	24.55 ^{a}	26.16 ^{a}	37.21 ^{a}	40.34 ^{a}	30.54 ^{a}	19.50 ^{a}
Neem leaves	**1/100**	3.37 ^{f}	1.95 ^{f}	4.95 ^{f}	7.78 ^{f}	4.81 ^{f}	3.45 ^{f}
	1/250	9.44 ^{d}	4.45 ^{e}	11.77 ^{d}	24.67 ^{d}	9.94 ^{e}	7.91 ^{d}
	1/500	13.82 ^{c}	8.09 ^{d}	18.39 ^{c}	33.93 ^{b}	18.90 ^{c}	10.59 ^{c}

(Remarks: values with different letters in a column are significantly different at the 0.05 level, experiment conditions: pH: 7 for Ni; 3 for Al; 5 for Cu; 5 for Pb; 3 for Fe; 5 for Cd, initial metal concentration: 100 mg/l, contact time: 2 hours, shaking rate: 200 rpm, room temperature).

3.3. Effect of contact time:

The time required for the biomass in reaching the maximum metal removal capacity is typically an economically important factor. The rapid metal removal reflects the economical application of the biosorbent in the treatment of actual contaminated water samples. In case of both biomasses, a two-stage kinetic behavior was evident, a very rapid initial removal over a few minutes followed by a long period of much slower removal **(Table 3)**.

Table (3): Interaction effect between biosorbent type and contact time.

Biosorbent	Contact time (min)	Biosorption (mg /g)					
		Ni	**Al**	**Cu**	**Pb**	**Fe**	**Cd**
Azolla pinnata	**1**	13.61 ^{e}	13.59 ^{e}	18.94 ^{e}	20.67 ^{g}	15.97 ^{e}	8.26 ^{e}
	5	14.35 ^{d}	13.95 ^{d}	21.49 ^{d}	21.88 ^{f}	17.95 ^{d}	10.57 ^{d}
	15	14.71 ^{c}	14.47 ^{c}	23.58 ^{c}	23.78 ^{d}	19.28 ^{c}	11.61 ^{c}
	30	15.84 ^{b}	15.50 ^{b}	27.31 ^{b}	27.98 ^{b}	22.32 ^{b}	12.78 ^{b}
	60	18.35 ^{a}	16.65 ^{a}	31.86 ^{a}	30.23 ^{a}	25.74 ^{a}	14.41 ^{a}
	120	18.40 ^{a}	16.58 ^{a}	31.50 ^{a}	30.20 ^{a}	25.65 ^{a}	14.39 ^{a}
Neem leaves	**1**	7.17 ^{j}	1.04 ^{j}	7.53 ^{j}	19.19 ^{i}	5.09 ^{j}	3.97 ^{j}
	5	7.32 ^{i}	2.25 ^{I}	8.72 ^{I}	20.08 ^{h}	5.51 ^{i}	4.64 ^{i}
	15	7.34 ^{h}	2.70 ^{h}	9.46 ^{h}	22.77 ^{e}	7.44 ^{h}	5.23 ^{h}
	30	7.63 ^{g}	3.46 ^{g}	10.59 ^{g}	23.70 ^{d}	8.52 ^{g}	6.60 ^{g}
	60	9.40 ^{f}	4.47 ^{f}	11.91 ^{f}	24.76 ^{c}	9.96 ^{f}	7.90 ^{f}
	120	9.44 ^{f}	4.45 ^{f}	11.77 ^{f}	24.67 ^{c}	9.94 ^{f}	7.91 ^{f}

(Remarks: values with different letters in a column are significantly different at the 0.05 level, experiment conditions: pH: 7 for Ni; 3 for Al; 5 for Cu; 5 for Pb; 3 for Fe; 5 for Cd, initial metal concentration: 100 mg/l, S/L ratio: 1/250 g/ml, shaking rate: 200 rpm, room temperature)

The removal equilibrium is achieved after 60 minutes and no significant increase was observed afterwards. The fast biosorption kinetics observed firstly is typical for biosorption of metals involving no energy-mediated reactions, where metal sequester from solute is due to physicochemical interactions between biomass and metal solution **(Akhtar *et al.*, 2008)**. The further slow period may be attributed to the transformation of the metal ions finally into biosorbent cells by purely chemical strategy **(Li *et al.*, 2006)**. This result reflects the stability of metal binding to the biomass and therefore insures that the captured heavy metals are not easily released in water in case of normal contact.

3.3.1. Kinetic modeling:

The biosorption capacities of metal ions at time intervals (derived from the contact time data) are evaluated by studying the first and second order kinetic models. The first order model considers that the rate of occupation of adsorption sites is proportional to the number of unoccupied sites, while the second order model is based on the fact that metal ions displace alkaline earth ions from the biosorbent adsorption sites **(Cruz *et al.*, 2004)**. The linearized first and second order kinetic models are represented in **Figures (1 and 2)**. Whereas **Table (4)** represents the correspondent (Q_e) values along with the correlation coefficient (R^2) associated at each linearized model. In spite of the magnitude of (R^2) high values of the first order model, the calculated maximum capacities (Q_e) are shown to be far less than the experimental ones ($q_{e\ (exp)}$). These results reflect the failure of the first order model to describe biosorption of studied metals by both biomasses. On the other hand, the comparison between ($q_{e\ (exp)}$) and (Q_e) derived from the second order model shows the fitting of studied metals biosorption by both biomasses to this model. Also, the (R^2) values that are nearly approach unity reveal that metal removal process follows the second order kinetic model. These results reflect the participation of displacement of alkaline earth ions from the biomasses binding sites by metal ions (ion exchange) more than the occupation of metal ions in the unoccupied sites (chelation) **(Aksu, 2001; Cruz *et al.*, 2004** and **Tüzün *et al.*, 2005)**.

3.4. Effect of initial metal concentration:

The initial metal concentration provides an important driving force to overcome all mass transfer resistances of metal ions between the solute and biosorbent. Hence, a higher initial concentration of metal ions will enhance the biosorption process till equilibrium is attained **(Aksu, 2001)**.

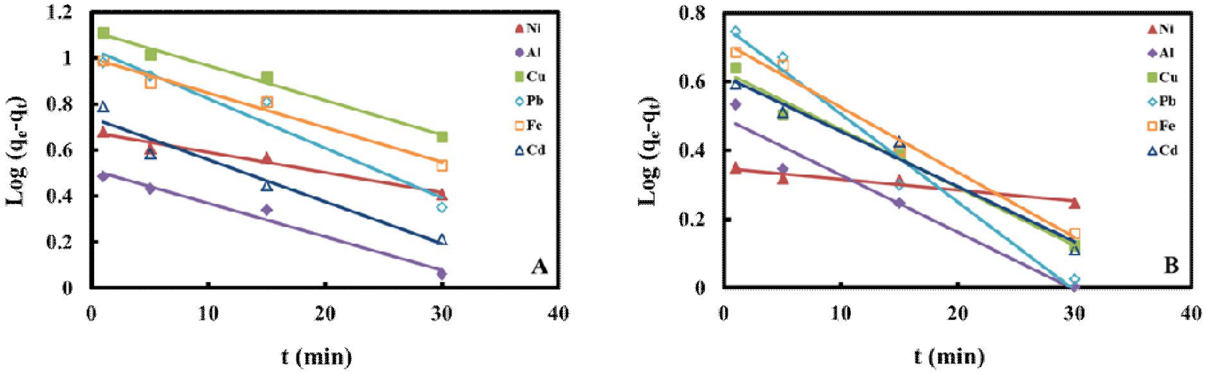

Fig. (1): Linearized first order kinetic model for metals biosorption by: (A) *Azolla pinnata,* (B) Neem leaves.

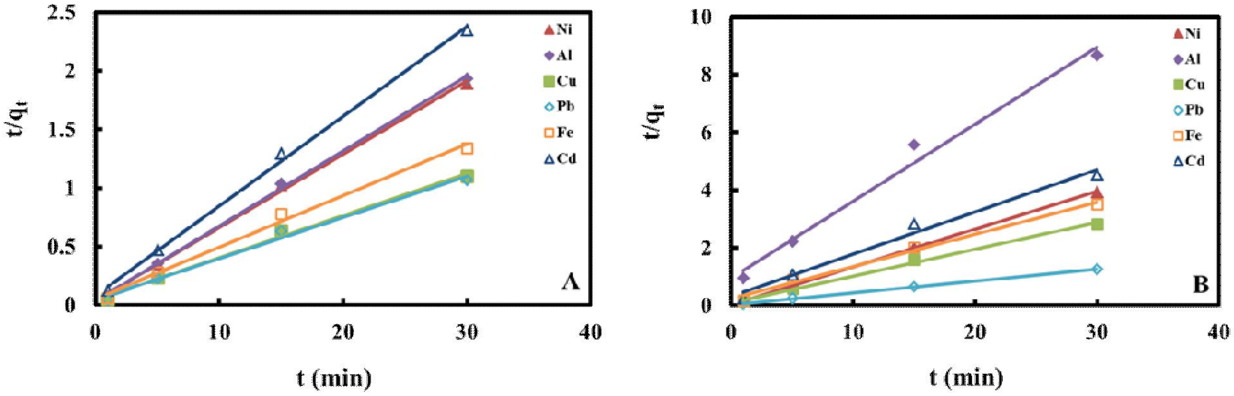

Fig. (2): Linearized second order kinetic model for metals biosorption by: (A) *Azolla pinnata,* (B) Neem leaves.

Table (4): First and second order kinetic parameters for metals biosorption.

Biosorbent	Metal	First order parameters			Second order parameters			$q_{e\ (exp)}$ (mg/g)
		$K_{1,\ ad}$ (S^{-1})	$Q_{e\ (calc.)}$ (mg/g)	R^2	$K_{2,\ ad}$ ($g.mg^{-1}.S^{-1}$)	$Q_{e\ (calc.)}$ (mg/g)	R^2	
Azolla pinnata	Ni	3.38×10^{-4}	4.75	0.9671	1.90×10^{-3}	15.95	0.9985	18.35
	Al	5.57×10^{-4}	3.26	0.9766	1.98×10^{-3}	15.63	0.9986	16.65
	Cu	5.76×10^{-4}	13.09	0.9866	4.49×10^{-4}	27.86	0.9936	31.86
	Pb	8.25×10^{-4}	10.96	0.9514	4.32×10^{-4}	28.49	0.9916	30.23
	Fe	5.76×10^{-4}	9.95	0.9773	5.90×10^{-4}	22.73	0.9936	25.74
	Cd	6.99×10^{-4}	5.50	0.9465	1.18×10^{-3}	13.07	0.9979	14.41
Neem leaves	Ni	1.23×10^{-4}	2.23	0.9199	8.71×10^{-3}	7.65	0.9996	9.40
	Al	6.41×10^{-4}	3.13	0.949	1.24×10^{-3}	3.75	0.9856	4.47
	Cu	6.49×10^{-4}	4.26	0.9811	1.45×10^{-3}	10.78	0.9965	11.91
	Pb	9.83×10^{-4}	5.79	0.9751	1.04×10^{-3}	24.10	0.9993	24.76
	Fe	7.25×10^{-4}	5.178	0.9894	8.85×10^{-4}	8.94	0.992	9.96
	Cd	6.18×10^{-4}	4.13	0.9733	1.15×10^{-3}	6.81	0.9821	7.90

As shown in **Table (5)**, the metals biosorption capacities increased with increasing their initial concentrations to give the highest significant biosorption values at 100 mg/l where equilibrium is attained and no higher significant increase was achieved afterwards.

Table (5): Interaction effect between biosorbent type and initial metal concentration.

Biosorbent	Metal concentration (mg/l)	Biosorption (mg /g)					
		Ni	Al	Cu	Pb	Fe	Cd
Azolla pinnata	0.5	0.122 ^{m}	0.121 ^{m}	0.122 ^{m}	0.123 ^{h}	0.127 ^{i}	0.121 ^{i}
	1	0.244 ^{k}	0.242 ^{k}	0.245 ^{k}	0.247 ^{g}	0.249 ^{h}	0.241 ^{h}
	5	1.220 ^{i}	1.210 ^{i}	1.223 ^{i}	1.224 ^{f}	1.223 ^{g}	1.214 ^{g}
	10	2.433 ^{g}	2.404 ^{g}	2.443 ^{g}	2.446 ^{e}	2.445 ^{f}	2.405 ^{f}
	25	6.046 ^{e}	5.936 ^{c}	6.078 ^{e}	6.085 ^{d}	6.084 ^{e}	5.905 ^{e}
	50	11.858 ^{b}	11.406 ^{b}	12.075 ^{b}	12.075 ^{c}	11.988 ^{b}	10.988 ^{b}
	100	19.800 ^{a}	16.730 ^{a}	31.910 ^{a}	30.260 ^{a}	25.260 ^{a}	14.760 ^{a}
	200	20.051 ^{a}	17.008 ^{a}	32.474 ^{a}	30.724 ^{a}	25.724 ^{a}	14.974 ^{a}
	500	20.255 ^{a}	17.000 ^{a}	32.063 ^{a}	31.563 ^{a}	26.063 ^{a}	15.063 ^{a}
Neem leaves	0.5	0.119 ^{n}	0.117 ^{n}	0.117 ^{n}	0.123 ^{h}	0.122 ^{i}	0.122 ^{i}
	1	0.237 ^{l}	0.234 ^{l}	0.235 ^{l}	0.245 ^{g}	0.245 ^{h}	0.244 ^{h}
	5	1.186 ^{j}	1.167 ^{j}	1.180 ^{j}	1.222 ^{f}	1.221 ^{g}	1.219 ^{g}
	10	2.373 ^{h}	2.336 ^{h}	2.369 ^{h}	2.443 ^{e}	2.443 ^{f}	2.413 ^{f}
	25	5.925 ^{f}	3.596 ^{f}	5.894 ^{f}	6.083 ^{d}	6.080 ^{e}	5.903 ^{e}
	50	8.330 ^{d}	4.284 ^{e}	9.262 ^{d}	12.005 ^{c}	8.738 ^{d}	7.525 ^{d}
	100	9.250 ^{c}	4.545 ^{d}	11.703 ^{c}	24.760 ^{b}	9.950 ^{c}	8.010 ^{c}
	200	9.267 ^{c}	4.699 ^{d}	12.000 ^{c}	24.724 ^{b}	10.474 ^{c}	8.224 ^{c}
	500	9.543 ^{c}	4.787 ^{d}	11.611 ^{c}	24.813 ^{b}	10.063 ^{c}	8.063 ^{c}

(Remarks: values with different letters in a column are significantly different at the 0.05 level, experiment conditions: pH: 7 for Ni; 3 for Al; 5 for Cu; 5 for Pb; 3 for Fe; 5 for Cd, contact time: 2 hours, S/L ratio: 1/250 g/ml, shaking rate: 200 rpm, room temperature)

3.4.1. Isotherm modeling:

From the view point of application, it is important to develop an appropriate mathematical model for describing the equilibrium behavior of a biosorbent-metal system in order to guide the further treatment process for real contaminated water. Each adsorption isotherm is characterized by certain constants express the surface properties and affinity of the sorbent and can also be used to calculate the biosorption capacity of biomass.

The linearized Langmuir and Freundlich adsorption isotherms corresponded to metals biosorption by biomasses are represented in **Figures (3 and 4)**. Whereas **Table (6)** represents the correspondent constants along with the correlation coefficients (R^2) associated at each linearized model. As can be observed, the high (R^2) values that nearly all approach unity reveal that both Langmuir and Freundlich models were suitable for describing biosorption equilibrium of metals under study in case of *Azolla pinnata* whereas Langmuir model only was suitable in case of neem leaves. This finding indicates that probably the sorption onto *Azolla pinnata* was a multilayer coverage “heterogeneous sorption”;

meanwhile, the sorption onto neem leaves was monolayer coverage “homogeneous sorption” **(Bulgariu, and Bulgariu, 2014)**. This finding may help to explain the higher biosorption capacity of *Azolla pinnata* biomass than neem leaves one as multilayer coverage acts to biosorb higher number of metals ions.

The calculated (Q_e) values from Langmuir model match with the experimental ones ($q_{e\ (exp)}$) for both biomasses. These results reflect the applicability of biosorption in the treatment of water samples that contain low and high concentrations of metal ions. The higher (K_F) calculated values, indicating high adsorption capacities, are found to be proportional to the actual high biosorption capacities ($q_{e\ (exp)}$) and vice versa. Also, (n) values that are greater than unity indicate the favourability of the biomass to biosorb metals under study from water **(Aboulsoud, (2008), Salima *et al.* (2013), Farah and El-Gendy (2013)).**

3.5. Effect of shaking rate:

Shaking rate can promote the biosorption process as it controls the mobility of sorbate ions and subsequently affects the binding chance to sorbent binding sites. As shown in **Table (7)**, the biosorption capacity was directly proportional to shaking rate where the highest significant biosorption capacities were achieved at the highest shaking rate (300 rpm). Increasing the shaking rate escalate the mobility of the sorbate particles on the sorbent's surface thus increasing the removal percentage **(Garba *et al.*, 2016).**

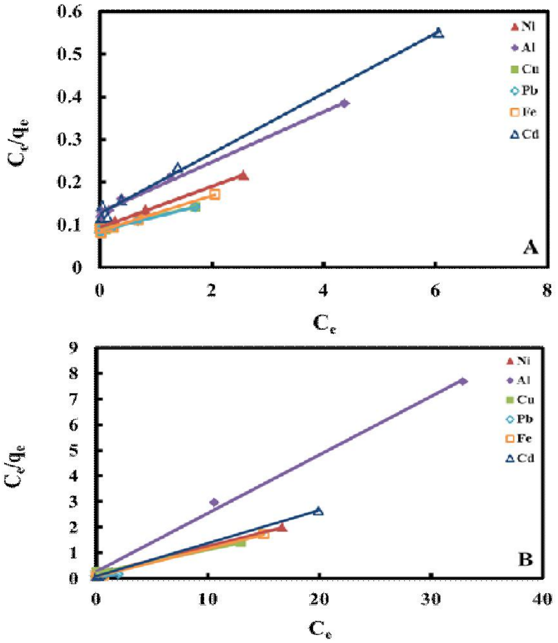

Figure (3): Linearized Langmuir adsorption isotherms for metals biosorption by: (A) *Azolla pinnata,* (B) Neem leaves.

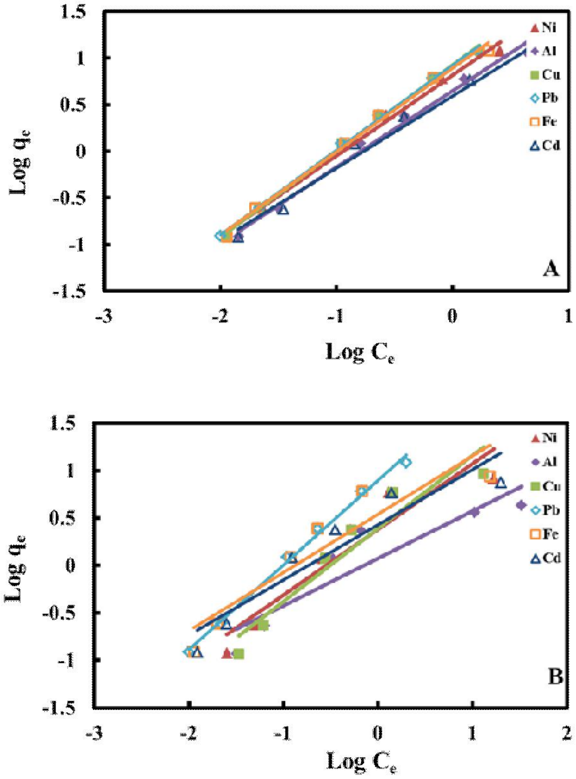

Figure (4): Linearized Freundlich adsorption isotherms for metals biosorption by: (A) *Azolla pinnata,* (B) Neem leaves.

3.6. The maximum biosorption capacity under the optimum conditions:

The concluded optimum conditions from the above discussed experiments were applied together to detect the maximum capacity of each biomass **(Table 8)**. The higher biosorption capacities were achieved using *Azolla pinnata* biomass that reached 28.22, 28.46, 39.67, 41.27, 35.64 and 21.70 mg/g for Ni, Al, Cu, Pb, Fe and Cd, respectively. The biosorption capacity was decreasing in the order of Pb > Cu > Fe > Al ≥ Ni > Cd. Whereas, neem leaves achieved lower capacities reached to 14.75, 12.65, 19.60, 35.86, 21.00 and 11.67 mg/g for Ni, Al, Cu, Pb, Fe and Cd, respectively. The biosorption capacity was decreasing in the order of Pb > Fe > Cu > Ni> Al > Cd.

3.7. Reusability and regeneration of biomasses:

Principally, recovery of metals from biomasses is mandatory to prevent the recycling of the heavy metal in the environment **(Yadav *et al.*, 2014).** Biotechnological exploitation of biosorption technology for heavy metals removal depends on the efficiency of the regeneration of biosorbent after metal desorption. Moreover, recovery of biosorbent apart from loaded biomass without damaging its capacity is a very important aim for the success of the biosorption technology development and an important feature for its possible utilization in continuous systems in industrial processes **(Deng *et al.,* 2006)**. The optimal eluent must be effective, cheap, non-damaging to the biomass and non-polluting **(Aboulsoud, 2008)**. The optimum conditions concluded from the previous experiments were conducted as initial biosorption data for this experiment **(Table 8)**.

Table (6): Langmuir and Freundlich equilibrium parameters for metals biosorption:

Biosorbent	Metal	Langmuir parameters			Freundlich parameters			$q_{e\,(exp)}$ (mg/g)
		K_L (l/mg)	Q_e (mg/g)	R^2	K_F	n	R^2	
Azolla pinnata	**Ni**	0.512	20.8	0.9928	6.544	1.16	0.998	19.86
	Al	0.457	17.0	0.9893	4.411	1.22	0.9937	16.58
	Cu	0.357	31.7	0.985	8.43	1.09	0.9967	31.50
	Pb	0.380	30.6	0.9845	8.413	1.10	0.9976	30.20
	Fe	0.467	24.6	0.9808	7.737	1.12	0.9935	25.65
	Cd	0.557	14.2	0.9742	3.912	1.30	0.9938	14.39
	Ni	0.512	20.8	0.9928	6.544	1.16	0.998	19.86
Neem leaves	**Ni**	0.646	9.1	0.9955	2.404	1.44	0.8946	9.44
	Al	0.870	4.4	0.9978	1.195	2.00	0.848	4.45
	Cu	0.433	11.0	0.9829	2.471	1.30	0.9157	11.77
	Pb	0.474	24.9	0.9934	7.897	1.12	0.9949	24.67
	Fe	1.510	9.1	0.9992	3.486	1.63	0.8664	9.94
	Cd	1.451	7.8	0.9997	2.698	1.72	0.8841	7.91

Table (7): Interaction effect between biosorbent type and shaking rate.

Biosorbent	Shaking rate (rpm)	Biosorption (mg /g)					
		Ni	Al	Cu	Pb	Fe	Cd
Azolla pinnata	**0**	15.91 ^{c}	12.60 ^{c}	30.37 ^{c}	28.58 ^{d}	24.39 ^{c}	11.63 ^{d}
	200	18.40 ^{b}	16.58 ^{b}	31.50 ^{b}	30.20 ^{b}	25.65 ^{b}	14.39 ^{b}
	300	19.86 ^{a}	18.22 ^{a}	34.17 ^{a}	31.63 ^{a}	26.63 ^{a}	15.43 ^{a}
Neem leaves	**0**	8.09 ^{f}	3.75 ^{f}	7.34 ^{f}	21.95 ^{f}	8.73 ^{f}	6.97 ^{f}
	200	9.44 ^{e}	4.45 ^{e}	11.77 ^{e}	24.67 ^{e}	9.94 ^{e}	7.91 ^{e}
	300	10.73 ^{d}	5.58 ^{d}	17.90 ^{d}	29.40 ^{c}	12.43 ^{d}	11.98 ^{c}

(Remarks: values with different letters in a column are significantly different at the 0.05 level, experiment conditions: pH: 7 for Ni; 3 for Al; 5 for Cu; 5 for Pb; 3 for Fe; 5 for Cd, initial metal concentration: 100 mg/l, S/L ratio: 1/250 g/ml, contact time: 2 hours, room temperature).

The desorption capacity using 0.01M $Na_2EDTA.2H_2O$ ranged from 17.40 to 71.01 % in case of *Azolla pinnata* and ranged from 4.28 to 21 % in case of neem leaves. EDTA forms very stable complexes with the transition metals **(Abo Farha *et al.*, 2010)**; therefore it is able to sequester metal ions from biosorbent surface. The low concentration of Na_2EDTA solution was mild on biosorbent tissues thus it didn't cause a harm to biosorbent.

The resorption capacity ranged from 37.33 to 98.74 % in case of *Azolla pinnata* and ranged from 33.31 to 99.33 % in case of neem leaves. A possible cause of reduction in the resorption capacity of biomasses can be attributed to the adverse effect of the eluent on the binding sites of the biosorbent cell wall components **(Tüzün *et al.*, 2005).** Also, accumulation of remaining metal molecules inside the biomass acts to decrease the further resorption efficiency in the next cycle **(Shaaban *et al.*, 2017)**.

Table (8): Biosorption-desorption-resorption capacities of metals for one cycle under the optimum concluded conditions.

Biosorbent	Metal	Biosorption (mg/g)	Desorption (%)	Resorption (mg/g)	Resorption (%)
Azolla pinnata	**Ni**	28.22	53.09	27.87	98.74
	Al	28.46	17.40	15.20	53.41
	Cu	39.67	46.62	31.61	79.68
	Pb	41.27	49.10	31.3	75.85
	Fe	35.64	38.12	13.30	37.33
	Cd	21.70	71.01	9.47	43.64
Neem leaves	**Ni**	14.75	5.88	11.48	77.86
	Al	12.65	4.28	5.80	45.85
	Cu	19.60	6.74	16.48	84.06
	Pb	35.86	11.01	35.62	99.33
	Fe	21.00	9.17	6.995	33.31
	Cd	11.67	21.73	10.14	86.93

(Remarks: values are the average of 3 replicates, experiment conditions: pH: 7 for Ni; 3 for Al; 5 for Cu; 5 for Pb; 3 for Fe; 5 for Cd, contact time: 1 hour, S/L ratio: 1/500 g/ml, shaking rate: 300 rpm, initial metals concentration: 100 mg/l, room temperature, eluent: 0.01M $Na_2EDTA.2H_2O$, elution S/L ratio: 1/1000 g/ml, elution time: 1 hour, shaking rate: 300 rpm)

Table (9): Concentrations of trace elements and heavy metals in Bahr El-Baqar drain wastewater before and after treatment using *Azollapinnata* and neem leaves biomasses:

Sample No.	Treatment		pH	Al	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	V	Zn
1	**Before**		7.5	8.585	0.0298	N.D	0.0232	0.149	3.821	0.3811	N.D	N.D	0.1303	0.0259	0.1764
	Azolla pinnata	**After**	7.6	0.4495	0.0055	N.D	0.0011	0.0704	0.5154	2.038	N.D	N.D	0.0142	0.0017	0.0555
		Removal (%)	-1.33	94.76	81.54	-	95.26	52.75	86.51	-434.77	-	-	89.10	93.44	68.54
	Neem	**After**	7.6	0.5493	0.0098	N.D	0.0021	0.0719	1.116	0.1185	N.D	N.D	0.0171	0.0208	0.1697
		Removal (%)	-1.33	93.60	67.11	-	90.95	51.74	70.79	68.91	-	-	86.88	19.69	3.80
2	**Before**		7.5	1.513	0.2214	0.0177	0.0136	0.1862	1.971	0.3292	N.D	0.1065	N.D	0.027	0.3136
	Azolla pinnata	**After**	7.6	0.3528	0.0074	0.0017	0.0014	0.0456	0.4263	1.977	N.D	0.0554	N.D	0.008	0.0618
		Removal (%)	-1.33	76.68	96.66	90.40	89.71	75.51	78.37	-500.55	-	47.98	-	70.37	80.29
	Neem	**After**	7.6	0.4182	0.0043	0.0019	0.0027	0.0736	0.5816	0.1263	N.D	0.0723	N.D	0.0107	0.1262
		Removal (%)	-1.33	72.36	98.06	89.27	80.15	60.47	70.49	61.63	-	32.11	-	60.37	59.76
3	**Before**		7.6	5.918	N.D	N.D	0.0138	N.D	5.547	0.5995	N.D	0.0654	0.5791	0.059	0.6448
	Azolla pinnata	**After**	7.7	0.5479	N.D	N.D	0.0104	N.D	0.9232	2.250	N.D	0.0381	0.117	.0088	0.0733
		Removal (%)	-1.32	90.74	-	-	24.64	-	83.36	-275.31	-	41.74	79.80	85.08	88.63
	Neem	**After**	7.7	1.146	N.D	N.D	0.0121	N.D	1.131	0.1983	N.D	0.0414	0.283	0.0165	0.2922
		Removal (%)	-1.32	80.64	-	-	12.32	-	79.61	66.92	-	36.70	51.13	72.03	54.68
Guidelines *				5	0.01	0.05	0.1	0.2	5	0.2	0.01	0.2	5	0.1	2

(Remarks: * Recommended limits for constituents in reclaimed water for irrigation **(Adopted from Rowe and Abdel-Magid, 1995)**, violating concentrations are shaded, experiment conditions: contact time: 1 hour, S/L ratio: 1/500 g/ml, shaking rate: 300 rpm, room temperature, no PH adjustment was done)

3.8. Application of biosorption optimum conditions in the treatment of contaminated wastewater from Bahr El-Baqar drain:

Biomasses of *Azolla pinnata* and neem leaves were applied to treat three wastewater samples from Bahr El-Baqar drain **(Table 9)**. Quality of wastewater samples was assessed according to recommended limits for constituents in reclaimed water for irrigation **(Rowe and Abdel-Magid, 1995)**. Only the violating concentrations are mentioned in the following discussion. In general, *Azolla* was more efficient than neem leaves in lowering metals concentrations in wastewater samples as did before in aqueous solutions, except Mn that *Azollapinnata* acted to increase its concentration in all samples by percentage ranged from 275 to 500 %. This can be attributed to the high content of Mn in *Azolla pinnata* tissues, as it contains 2418 ppm of Mn as estimated by **Anitha *et al.* (2016)** in comparison with 1569 ppm of Fe, 325 ppm of Zn, 9.1 ppm of Cu, 8.11 ppm of Co, 5.06 ppm of Cr, 5.33 ppm of Ni, 8.1 ppm of Pb and 1.2 ppm of Cd. **Ganji *et al.*, (2005)** stated that binding or ion-exchange of heavy metal ions is possible during biosorption using *Azolla* sp. due to the presence of charged groups such as carboxyl and phosphate in the *Azolla* matrix. These results coincide with kinetic modeling studies in the current study that reflect the participation of ion exchange more than chelation in the biosorption process.

The removal percentage reached 94.76, 96.66, 90.40, 95.26, 75.51, 86.51, 47.98, 89.10, 93.44 and 88.63 % for Al, Cd, Co, Cr, Cu, Fe, Ni, Pb, V and Zn, respectively using *Azolla pinnata* biomass. Whereas, neem leaves achieved removal percentage reached93.60, 98.06, 89.27, 90.95, 60.47, 79.61, 68.91, 36.70, 86.88, 72.03 % and 59.76 for Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn, respectively. Except for Mn in case of *Azolla*, all violating concentrations were lowered to be less than the guidelines in the case of both biosorbents. A slight increase in samples pH (1.33 %) was equally observed in case of both biomasses, this may be attributed to the favorability of H^+ binding to active sites **(Sarı and Tuzen, 2009)**.

Conclusions:

Although dried biomass of neem leaves showed significantly lower biosorption capacities of heavy metals than dried biomass of *Azolla pinnata,* neem leaves biomass is mightily recommended to be used in the water treatment process. This is because of Mn release from *Azollapinnata* biomass during the water treatment process. Neem leaves are eco-friendly, low-cost, natural, and reusable tool showing promising results in improving quality of water resources and elimination of the environmental pollution.

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