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THE BIOLOGICAL ABILITY OF *Chara sp.* ALGAE TO ABSORB NICKEL FROM THE ENVIRONMENT

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History	Abstract			
Received: 21 st April 2019 Accepted: 10 th September 2019	Chlorophyta algae (<i>Chara Sp.</i>) were selected and dried. The effective groups of algae responsible for adsorption using infrared (COOH, $C = O$ and CH) were identified and negative groups such as (OH), ($P = O$), (CO ₃), (CH ₂), (Cl) and (Br) on the surface of the algae, and (2) g			
Keywords:	and treated with a series of different concentrations (0.5,10,20,40,100) mg L ⁻¹ of nickel ion			
Dubinin, Nickel, Chlorophyta algae	where the coefficients were incubated at a temperature of (25 ° C). After the incubation period, the equilibrium stabilizers were extracted and the nickel was estimated and mathematically described according to the Equations (single and two surfaces Langmuir, Freundlich, Temkin, Dubinin and Polani) respectively for nickel. It was found that the values of the coefficient of selection were significant for all equations, which gives a clear indication of the possibility of using any of these six equations, but the equation of the two surface Langmuir is the most efficient in the mathematical description of adsorption, In addition, this equation showed a very large correlation between the actual adsorption values and the calculated values of this equation because they have the least standard error (SE) (0.0002) and the highest coefficient of determination (R^2) (1.00), Which earns the advantage in mathematical description on the rest of the equations. The value of the maximum adsorption capacity (Xm) on the first and second surface of algae (1.60) and (7.47) mg. Kg ⁻¹ , while the binding capacity (K) on the first and second surfaces (75.23) and (41.81) L.mg ⁻¹ , respectively.			

SHORT COMMUNICATION

INTRODUCTION

Pollution has been of great concern at the global and local levels as a result of the waste produced by some industrial, household, agricultural, etc., which adversely affect the main aquatic, aerobic and land-based environments and ultimately affect the organism, In this study, the biomass of Chara was used to determine the extent of its adsorption (The accumulation of atoms or liquid molecules called adsorbents on the surface of a solid called the adsorbed) of the Nickel element and is a biological method of pollution treatment known as biotreatment to remove toxic contaminants from the environment [1-3] The use of organisms such as algae, fungi, bacteria and plants in the treatment of toxic pollutants from water is the most appropriate and modern methods for being safe for the environment and low cost compared to physical and chemical methods [4-8].

Chara sp is made of algae (Chlorophyta) which is classified into:

Division: Chlorophyta Class: Charophyceae Order: Charales Family: Characeae Genus: Chara

Chara can be seen by the naked eye as it lives in stagnant freshwater or is installed in soft silt on the bottom of ponds. Many uses of algae are used as a source of protein in food production and oxygen release in wastewater treatments [9]. There are numerous studies of the use of algae or biomass in bioremediation, including [10].

The biomass of chlorophyta, such as *Mugeta sp*, and Cyanophyta, such as *Oscillatoria subrebis*, was taken to detect the biosorbent of heavy metals. It was concluded that the earlier algae have a higher Lead absorption capacity than the less adsorbed Copper element. The results of [11] indicated the susceptibility of the *Scedesmus quadricauda* algae to remove the Nickel element from contaminated water by 98, 53.5, 55.33 and 43% for concentrations 2, 4, 6 and 8 mg.L⁻¹ respectively during eight days of the experiment while less the ability of the algae to remove the

element on the 14th day of the experiment as a result of cell death. [12] studied the efficiency of algae *Scenedesmus quadricauda* and *Chorella vulgaris* in the removal of pollutants from sewage, Nitrogen-NO₃ removal was 89.9%, while phosphorus-phosphate removal was 87.89%. [13] used leaves of lettuce and three forms (fresh, dried and grinding) to remove Lead, Copper and Zinc ions. The type of ground was superior to the other forms in removing the ions above. The results showed that the removal ratio was Lead ions, Copper and Zinc.

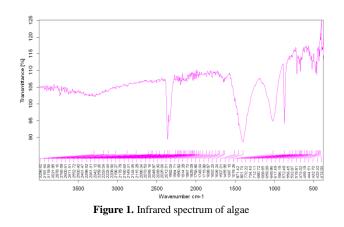
The present study aims to test the effect of primary concentration of (Ni 2 +) at a concentration of (0,5,10,20,40 and100) mg. L $^{-1}$ on the amount of material absorbed on the surface of dried algae.and intended to identify the possibility of removing nickel from contaminated water using algae isolated from the local environment and widespread as it is a modern technology and inexpensive and preserve the environment from pollution.

MATERIALS AND METHODS

Chara sp. was collected from the Tigris river and diagnosed according to [14] and dried in the oven 105 °C and then taken 2 g and treated with a series of different concentrations $(0,5,10,20,40,100 \text{ mg.L}^{-1})$ nickel in a single batch method and then incubated the coefficients at 25 °C with all other variables remaining constant. After the incubation period, the stabilizers were extracted and the nickel was determined and mathematically described according to adsorption equations.

Diagnosis of active algae complexes responsible for adsorption using infrared

The active groups on the algae surface responsible for adsorption by using infrared were described in **Figure 1**: COOH, C = O and the CH, and negative groups such as (OH), (P = O), (CO_3) , (CH_2) , (Cl) and (Br). [15]



RESULTS AND DISCUSSION

Table 1 shows a comparison between the amount of adsorbed material on the one hand and the efficiency of adsorption (percentage adsorption) on the other hand with the primary concentration. Note that all studied ions show a constant tendency to remove the water solutions on the surface of the algae. The amount of absorbent material that reflects the amount of bioremediation increases with the increase of the primary concentration of the metal as shown in **Figure 2**.

In this regard, many studies in the field of bioabsorption of algae revealed the mechanism responsible for this reaction, which is the type of reaction between the positive ions of light metals (calcium, magnesium, sodium, potassium) associated with algae and other elements found in water solutions [16].

Table 1. Change the percentage of adsorption with initial concentration at 25° C for nickel ion solution

Initial concentration added (mg.L ⁻¹)	Percentage of adsorption	Amount of absorbent material (mg.kg ⁻¹)
0	-	-11.00
5	31.00	31.00
10	42.10	84.20
20	34.85	139.40
40	45.45	363.60
100	64.85	1297.00
The average	43.65	383.04

There are several factors that influence the process of biological adsorption, such as temperature and pH, but the main factors determining this first process are the electronegativity of electrons. The second factor is the volume of adsorbent ion (the atomic diameter of the adsorbent ion) [17]. Generally the biomass of algae contains alkaline elements (calcium, magnesium, sodium, potassium) are present in normal water. Therefore, when the algae biomass is interacted with heavy elements carried in water. The light metals mentioned above will be released, resulting in an increase in the reaction of water, leading to the formation of light alkaline ions. In addition, the release of these base ions will increase the value of the electrical conductivity of water. The results obtained are consistent with those obtained by other researchers [10, 18] which found that the process of biological adsorption of heavy elements by the mass of algae removed the light elements alkaline (calcium, magnesium, sodium, potassium) to equilibrium solution. The same results were obtained when adopting the technique of columns filled with dry algae that the amount of heavy element adsorbed depends on (1) Equilibrium state of thermally symmetric adsorption, (2) mass transit speed and (3) hydrodynamic factors (adsorption column thickness and duration of contact).

But the efficiency of removal of algae decreases with the increase of intial concentration and this is due to the increase in the intial concentration of a fixed number of effective sites available for adsorption, making the amount of material remaining greater reduces the percentage of this removal on the one hand, On the other hand, increasing the intial concentration of metal ion increases the competition of these ions to correlate to the fixed number of active and empty sites available for adsorption on the surface of a certain weight of algae, thus reducing adsorption efficiency. This is consistent with what [19] to the ability of algae to remove (80%) to (100%) of some heavy metals in less than one day. This reduces the bonding power between the metal ion and the effective location due to the increased coefficient of surface efficiency.

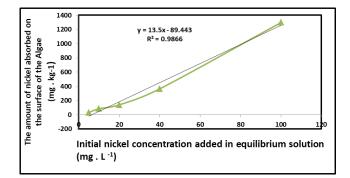


Figure 2. Relationship between concentration of initial nickel in the equilibrium solution and the amount obtained on the surface of the algae at $25 \text{ }^{\circ}\text{C}$

It is known that the process of removal of heavy metals by algae can be attributed to different mechanics, where these heavy elements are linked through several mechanisms to eliminate the toxic effects of some heavy metals and vary the resistance mechanisms depending on the type of algae and type of metal has shown some of their ability to produce extracellular compounds have the ability to bind to metal [20, 21]. Metals and their ions have the ability to bind with functional groups or bonds found in bioactive molecules such as (PO4, COOH, NH4, OH and SH) and convert them into forms that can not enter the cell or reduce the efficiency of the ion or by controlling the permeability of the cell membrane Or having effective sites on the cell wall to bind these metal ions to the cell wall. These mechanisms used by algae to resist the toxic effect of metals or prevent them from entering the cell known as exclusion or dimensions [13, 22, 23].

Many algae have the ability to remove intracellular toxicity by forming peptide compounds rich in SH called Y-Glutamyl-cystinil in the case of acute concentrations of minerals. At the level of the sub-lethal concentrations can be the compounds (Y-Glutamyl- cystinil- cystinil) (GSH). In both cases these compounds are peptide or protein phytochelating compounds and is rich in the amino acid cysteine, linking the metal ions with their molecular structures at the sites (SH) within their chemical composition, thus producing the metal complexes [24, 25]. The status of accumulation may give solutions by exploiting microscopic organisms that have the adaptive ability to tolerate these high percentages of pollutants and their concentration in their bodies and thus work to rid the environment from them. This calls for serious consideration of using these organisms as biological treatments or as evidence of pollution by algae, which have a large variety of species and genus, which gives a wide spectrum of sensitivity or tolerance to these pollutants, but must provide certain organisms or strains of laboratory use by finding pure isolates [26]. Some types of algae are used in the manufacture of products to remove mercury from groundwater [27], which makes bio-absorption an important role in solving the problem of water pollution from the heavy elements resulting from industrial activities as they have the ability to do so, as confirmed by many international studies In this regard [11, 28-32].

Mathematical description of adsorption equations

The ability of green algae to adsorb nickel was tested by thermal symmetric dynamic equilibrium. In order to obtain the biological adsorption constants that reflects the properties of the soluble surface and its tendency or the degree of attraction of heavy ions to it as a treatment method for the disposal of heavy elements in heavy water. Table 2 shows the values of the factor (R^2) and the standard error (SE) of the adsorption equations obtained by applying single and two surface Langmuir, Freundlich, Temkin, Dubinin and Polani respectively for nickel . It is clear from Table 2 that the values of the coefficient of determination were significant for all equations, which gives a clear indication of the possibility of using any of these six equations. However, the two surface Langmuir equations are the most efficient in the mathematical description of adsorption. The superiority of the two surface Langmuir equations in the mathematical description of the process of removing (biological adsorption) nickel from the surface of the algae was illustrated in Figure 3 of the two surface Langmuir equations.

Equati	\mathbf{R}^2	SE	
Langmuir with one surface		0.95	0.003
Langmuir with two	First surfrace	1.00	0.000
	Second surfrace	1.00	0.0003
Surreco	The average	1.00	0.0002
Freundlich		0.95	0.16
Dubinin	0.95	0.16	
Temkin	0.65	360.42	
Polani	0.71	0.89	

Table 2. Standard error (SE) and the determination factor (R^2) for nickel absorption experiments

Nickel absorbed on the surface of the algae

This is consistent with what referred by [33 -38], the process of biosorption of heavy metals by microorganisms passes through two stages. The first is rapid uptake due to surface adsorption on cellular wall components followed by slow absorption due to the ionic transition across the cell membrane to cytoplasm cells. The surface cells of these organisms consist of polysaccharides, proteins and lipids. Therefore, the biological absorption processes have many constants that depend on the speed of biosorption, such as the structural properties of the adsorbent material and the organic surface of the biosorpante, For example, the composition of proteins, the density of the surface charge, the surface area and the topography influence factors in the process of adsorption, as well as the amount of adsorben and the duration of contact and the degree of interaction of the medium and temperature and the initial concentration of metal ion has a significant impact on the speed of biosorption process.

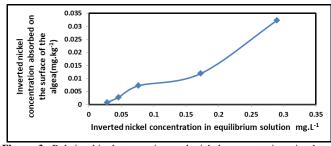


Figure 3. Relationship between inverted nickel concentrations in the equilibrium solution as a function of the inverted concentration

The largest adsorption of nickel on the surface of the algae and its energy

Single and two surface Langmuir equation is based on the assumption that intermolecular forces are rapidly decreasing with distance and predictability of mono-layer coverage of the ion adsorbent on the surface of adsorbent material also assumes that adsorption occurs on specialized and homogeneous sites of adsorbent material. The values of the adsorption constants for the linear formula of the Langmuir equation by algae have been extracted from the relativist and their tendency. The values of the strong determinants obtained for this equation lead us to conclude that the mass of algae used in adsorption is strongly subject to this equation in the adsorption process. The maximum adsorption capacity on the first surface of algae was 75.23 L. mg⁻¹ as shown in **Table 3**.

Table 3. Values of the two surface Langmuir equation for nickel absorption on the surface of the algae

x _{m2}	K ₂	x _{m1}	K ₁
mg .kg ⁻¹	L. mg ⁻¹	mg .kg ⁻¹	L. mg ⁻¹
7.47	41.81	1.60	75.23

While the maximum adsorption capacity on the second surface of algae was 7.47 mg.kg⁻¹, while the binding capacity on the second surface of algae was 41.81 L. mg⁻¹ as shown in **Table 3**. This confirms that there are two surfaces in the process of adsorption biological or so-called biological removal as a means of disposal of heavy metals in the solutions of water and this is due to the presence of an effective substance in the body of algae used and this is consistent with [18] referred to the two surface Langmuir equation of the lead element, where they obtained the values of adsorption capacity for two types of green algae ranging from (14.9) to (160.3) while the energy values of the link ranged from (0.02) to (0.024). While [27] had the largest adsorption capacity of algae (19.36, 16.17, 116.35) mg.kg⁻¹ for cadmium, nickel and lead respectively.

The researchers also pointed to the success of the equations of Langmuir, Freundlich, Temkin, Dubinin in the mathematical description of the process of adsorption biological, and these results are consistent with the results reached [39- 41]. [42] obtained the superiority of the equation of Langmuir on Freundlich in the mathematical description of the process of adsorption of heavy metals by algae. Our results (Table 1) have shown a difference in the behavior and quantities of adsorption per ion on the surface of the algae. This difference is due to the

physical properties of each ion (electrolysis, radius and ionization effort), thus it can be concluded that the use of algae is necessary in removing heavy metals from water before being used in agriculture.

REFERENCES

[1] Mahmmoud,I.M; Salwan,W.Y; Muwafaq,A.R. (2018) Physiological and Biochemical Changes Analysis to Labors Blood Samples in Plastics Recycling Factory in Mosul-Iraq. *Journal of Global Pharma Technology*. 10 (6): 281-288

[2] Abbas, M.H.H. (2007). Bioremediation of agricultural soils polluted with heavy metals and organic compounds. Ph.D. Thesis, Fac. Agric., Benha Univ., Egypt.

[3] Kumar, A.; B.S. Bisht; V.D. Joshi and T. Dhewa (2011) Review on Bioremediation of Polluted Environment: A management Tool. *International Journal of Environmental Sciences*. 1(6); 1079-1093

[4] Al-Azzawi, Souad Ghali Kazem (2006) The use of some algae in the treatment of industrial waste water for Hilla textile factory. Master of Science, Department of biology, college of Science, University of Babylon.

[5] Bulgariu, D. and L. Bulgariu(2012) Equilibrium and kinetics studies of heavy metal ions biosorption on green algae waste biomass. *Bioresour. Technol.* 103:489-493

[6] Kelly-Vargas, K.; M. Cerro-Lopez; S. Reyna-Tellez; E.R. Bandala and J.L. Sanchez Salas (2012) Biosorption of heavy metals in polluted water using different waste fruit cortex. *Phys. Chem. Earth.* 39: 26-39

[7] Sousa, F.W.; M.J. Sousa; R.N. Isadora; I.R.N.Oliveira; A.G. Oliveira; R.M.Cavalcante; P.B.A.Fechine; Neto VOS; D. de Keukeleire and R.F. Nascimento (2009). Evaluation of a low-cost adsorbent for removal of toxic metal ions from wastewater of an electroplating factory. *J. Environ. Manag.* 90: 3340–3344

[8] Talaat, R. A. (2012). Environmental and Bacteriological Study of Sewage Water for Masab Qara Saray in Mosul City and Some Treatment Techniques. Master of Science, Department of biology, college of Education, University of Mosul.

[9] Graham, L.E. and L.W. Wilcox (2000). Algae. Prentice-Hall, Inc. upper Suddle River, NJ07458. Wisconsin University.

[10] Sulaymon, A. H.; A. A. Mohammed and T. J. Al-Musawi (2013). Multicomponent Biosorption of Heavy Metals Using Fluidized Bed of Algal Biomass. *Journal of Engineering*. 4(19): 469-484

[11] Al-Mayaly, I. K.(2009). Removal of Nickel ions by *Scenedesmus quadricauda* from contaminated water under laboratory conditions. *Iraqi Journal of Science*. 50(4): 458-461

[12] Kamel, Ruwaida Fahim and Sabri, Anmar Wahbi and Abdalbaki, Basmat Jameel (2010). Effect of physical factors (temperature, intensity of light) on the efficiency of Chaldean *Scenedesmus quadricauda, Chorella vulgaris* in the removal of contaminants. *Baghdad Science Jour.* 7 (2): 918-926

[13] Shartooh, S. M.; S. A. Kasim; R.H. Obaid; A. A. Hadi and A. A. Abdulmajeed (2014). Lettuce leaves as biosorbent material to remove heavy metal ions from industerial wastewater. *J. Baghdad for Sci.* 11(3):1164-1170

[14] Prescott, G.W. (1979). How To Know The "Freshwater Algae". Third Ed, Univ. of Montana, USA, p.293

[15] Iqbal, M.; A. Saeed and S.I. Zafar (2009). FTIR spectrophotometry, kinetic and adsorption isotherms modeling, ion exchange and EDX analysis for understanding the mechanism of Cd²⁺and Pb²⁺ removal by mango peel waste. *J. Hazard Mater.* 164: 161–171

[16] Naja, G. and B. Volesky (2006) Behavior of mass transfer zone in a biosorption column. *Environ. Sci. Technol.* 40(12): 3996-4003

[17] Abdul-Ameer, Youssef Kazem and Merza, Director of Mubarak (2010) Study of adsorption of tripodrolidine and mitochloropramide hydrochloride from its water solutions on the surfaces of bentonite and kaolin. *Journal of Science of Mustansiriya*. 21(5): 102-114

[18] Wang, D.; E. McLaughlin; R. Pfeffer and Y.S. Lin (2011) Aqueous phase adsorption of toluene in a packed and fluidized bed of hydropholic aerogels. *Chemical Engineering*. 168: 1201-1208

[19] Kassim, T.I.; S.A.W. Al-Rikabee and G.H. Al-Rubaiee (2006) Ability of Cyanophyceae species (*Oscillatoria pseudogeminata* and *Spirulina major*) in reduction of some pollutants from wastewater treatment plant, South Baghdad. *Euro-Arab Environment Conference & Exhibition* 2006, 612-621

[20] Farhan, A. M.; A. H. Al-Dujaili and A. M. Awwad (2013). Equilibrium and kinetic studies of cadmium (II) and lead (II) ions biosorption onto *Ficus carcia* leaves. *International Journal of Industrial Chemistry*. 4(24):1-8

[21] Kamsonlian, S.; S. Suresh ; C. Majumder and S. Chand (2011). Characterization of Banana and Orange Peels: Biosorption Mechanism. *Intern. J. Sci. Technol. & Manag.* 2 (4): 1-7

[22] Al-Qahtani, K. M. (2012). Biosorption of Binary Mixtures of Heavy Metals by *Medicago sativa*. World Appl. Sci. J. 16 (3): 465-473

[23] Lee, Y.-C. and S.-P. Chang (2011). The biosorption of heavy metals from aqueous solution by Spirogyra and Cladophora filamentous macroalgae. *Bioresource Technology*. 102: 5297–5304

[24] Abdullah, Lena Saeed Mohammed (2006). The ability of heavy metals to bind to blue-green algae, responsible genes and optimal applications in the biological treatment of contaminated water for agricultural use. PhD thesis, Institute of Arab Research and Studies, Department of Environmental Studies, Alexandria University, Egypt. [25] Cobbett, C.S. (2000). Phytochelatins and their role in Heavy Metal Detoxification. *Plant Physiology*. 123: 825-832

[26] Hala, Ali Arshad (2000). Effect of some heavy metals on some of the vital activities in the cyanobacteria *Anabena oryzae* stabilized atmospheric nitrogen. Master Thesis, University of Tikrit, Iraq.

[27] Barkly, N. P. (2004). Extraction of mercury from ground water using immobilized algae. U.S.E. Environmental protection Agency, Cincinnati Ohio, USA.

[28] Ali, A.H.(2011). Performance of Adsorption/Biosorption for Removal of Organic and Inorganic Pollutants. Ph.D. Thesis, University of Baghdad, College of Engineering

[29] Najah, Adnan and Baznjana, Raghad (2012). The use of baking yeast in biological treatment to remove heavy metals from wastewater. *Najah University Journal of Research (Medical Sciences)*. 102: 26-118

[30] Rathinam,A.;B. Maharshi;S.K. Janardhanan ; R.R. Jonnalagadda and B.U. Nair (2010). Biosorption of cadmium metal ion from simulated wastewaters using *Hypnea valentiae* biomass: A kinetic and thermodynamic study. *Biores. Technol.* 101: 1466–1470

[31] Sulaymon, A.H.; S.E. Ebrahim; S.M. Abdullah and T. Al-Musawi (2010). Removal of Lead, Cadmium, and Mercury Ions Using Biosorption. *Desalination and Water Treatment*. 24: 344-352

[32] Wang, J. and C. Chen (2009). Biosorbents for heavy metals removal and their future. *Biotechnol. Advances*. 27: 195-226

[33] Bayo, J. (2012). Kinetic studies for Cd (II) biosorption from treated urban effluents by native grapefruit biomass (*Citrus paradisi* L.): The competitive effect of Pb (II), Cu (II) and Ni (II). *Chemical Engineering Journal*. 191: 278-287

[34] Fathi, A.A.; M.M. Azooz and M.A. Al-Fredan (2012) Abolishing toxicity of copper by some environmental factors using green alga *Chlorella vulgaris. Am. J. Environ.* Sci. 8: 633-641

[35] Gupta, V. K. and A. Rastogi (2008). Biosorption of lead (II) from aqueous solutions by non-living algal biomass *Oedogonium* sp. and *Nostoc* sp.: A comparative study. Colloids and Surfaces B: *Biointerfaces* 64:170-178

[36] Montazer-Rahmati, M.M.; P.Rabbani; A. Abdolali and A.R. Keshtkar (2011). Kinetics and equilibrium studies on biosorption of cadmium lead, and nickel ions from aqueous solutions by intact and chemically modified brown algae. *J. Hazard. Mater.* 185: 401-407

[37] Reddy, D.H.K.; Y. Harinath; K. Seshaiah and A.V.R.Reddy (2010). Biosorption of Pb (II) from aqueous solutions using chemically modified *Moringa oleifera* tree leaves. *Chem. Eng. J.* 162: 626–634

[38] Yin, P. ; Z. Wang ; R. Qu ; X. Liu ; J. Zhang and Q. Xu (2012) Biosorption of Heavy Metal Ions onto Agricultural Residues Buckwheat Hulls Functionalized with 1-Hydroxylethylidenediphosphonic Acid. J. Agric. Food Chem. 60: 11664-11674

[39] Almasi, A.; M.Omidi; M. Khodadadian; R.Khamutian and M.B. Gholivand (2012) Lead (II) and cadmium (II) removal from aqueous solution using processed walnut shell: kinetic and equilibrium study. *Toxicol. Environ. Chem.* 94: 660–671

[40] Babarinde, N.A.A. (2011) Kinetic, equilibrium and thermodynamic of the biosorption of Pb (II), Cd (II) and Cr (III) by neem leaf. *J. Innovative Res. Eng. Sci.* 2: 291-306

[41] Chakravarty, P.; N.S. Sarma and H. Sarma (2010). Biosorption of cadmium (II) from aqueous solution using heartwood powder of *Areca catechu. Chem. Eng. J.* 162:949–955

[42] Al-Khazragy,S.M.; H.K. Hussein and N.K. Shareef (2005) Efficacy of *Spirogyrra* spp. in Adsorption of Cadmium, Cobalt, Copper, Iron, Lead, Mercury, Nickel and Zinc Ions from Aqueous Solution. *Journal of the University of Kerbala*. 3(11): 1-15