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# ROLE OF EXTRACELLULAR POLYMERIC SUBSTANCES IN ADSORPTIVE BINDING OF HEAVY METALS ON TO *MICROSYSTIS AERUGINOSA*

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**Abstract**

The role of extracellular polymeric substances (EPS) from the cyanobacterial species *Microcystis aeruginosa* in cadmium, copper, lead, and zinc biosorption is reported in the present work. The biosorption capacity of whole biomass (*Microcystis aeruginosa)* and biomass without EPS was investigated by optimizing different experimental conditions like biomass dose, time of contact, pH of suspension, and initial heavy metal ion concentration. FTIR spectra showed that different functional groups like carboxyl, hydroxyl and amide groups are present in biomass with and without EPS which scavenge heavy metals. The Langmuir model was found to be most suitable fit for the biosorption data, with Cd, Cu, Pb, and Zn ions showing maximum removal capacity of 125.0, 71.42, 90.90, and 56.0 mg per gram of whole biomass and 62.50, 50.0, 58.33, and 48.33 mg per gram of biomass without EPS respectively. The Pseudo 2$^{nd}$ order kinetic model explains the biosorption data very well for all heavy metals with regression coefficient values of 0.99-1.0 for both the biomasses. The whole biomass intact with EPS showed a greater potential to scavenge heavy metals than the biomass without it. Thus, the role of EPS was demonstrated to be very essential in heavy metal adsorption onto *M. aeruginosa* biomass.

**Key words:** Extra cellular polymeric substances, *M. aeruginosa*, Biosorption, EPS, Heavy metal, FTIR.

## Introduction

Heavy metal contamination has been recognized as an important pollution factor worldwide. Heavy metals like lead, copper, cadmium, and zinc are present naturally in earth crust and are released in the environment through soil erosion, metal corrosion, sediment re-suspension, volcanic activity, and geological weathering (yang *et al*., 2005). Industrial effluents produced from mining processes, metal plating, tanneries, battery manufactures, and smelting cause heavy metal contamination of the water resources (Shallari., 1998). This polluted water becomes a source of dangerous heavy metals in the food chain, causing teratogenic alterations in animals, plants, and humans (Surana *et al*., 2019). Heavy metal exposure in humans can harm several organs such as the kidneys, brain, liver, skin, and pancreas. (Lesmana *et al*., 2009). Therefore, these harmful heavy metals need to be eliminated from wastewater. Many traditional approaches like ion-exchange, chemical adsorption, membrane ultra-filtration, and chemical precipitation have been developed and applied to remove toxic heavy metals from the wastewater (Singh *et al*., 1998). However, cost inefficiency, incomplete removal, and toxic slurry generation are the limiting factors for the feasibility of these physico-chemical methods (Chandra *et al*., 2020, Kratochvil & Volesky, 1998). Biosorption has been demonstrated to be a potent, resilient, reusable, and cost-effective alternative to chemical techniques for heavy metal remediation, even when they are at extremely low concentrations (Saha *et al*., 2010; Yoshida *et al*., 2006). Different biological materials have been investigated and utilized for heavy metal removal such as yeast, fungi, algae, and bacteria, but algae have been reported among one of those biomaterials that have higher capacity of heavy metal removal because its cell wall is composed of large number of polymers. Bioremediation of heavy metals using algal biomass is an economic and cost-effective approach as algal biomass can be conveniently grown because of their simple and cheaper nutritional demand. Algal biomass is available round the year and even dead algal biomass can also be used where no oxygen or nutrient supply is required (Darda *et al*., 2019). Moreover, the biomass can be reactivated and reused by desorption of attached metal ions. The heavy metal adsorbed onto algae can be recovered, regenerated, and recycled. Thus, algal bioremediation has emerged as greener and eco-friendly approach (Pahlavanzadeh *et al*., 2010).

Cultivation of algal biomass can be achieved in conventional open pond system, but a better feasible yield can be achieved by using photobioreactors under controlled conditions. The commercial scale cultivation of algal biomass is economically feasible for production of nutraceuticals, biofuels, bioenergy, and some other value-added products being focused along with bioremediation of heavy metals as a co-production application (Ramasamy *et al*., 2020; Zeraatkar *et al*., 2016).

Among different groups of algae, on the basis of heavy metal accumulation, cyanobacteria have been suggested as attractive biosorbent because of their ubiquitous presence in nature, simple nutritional demand, large mucilage volume, tolerance, and good metal sorption capacity (Gupta & Rastogi, 2008). Many cyanobacterial species like *Spirulina, Dunaliella, Nostoc, Anabaena,* and *Synechococcus* have been cited for efficient biosorption of heavy metals (Abdel-Aty *et al*., 2013). One of the major cyanobacterial species that form nuisance freshwater cyanobacterial blooms is *Microcystis aeruginosa* (Stone, 2011). *M. aeruginosa* biomass, as alive microbial or dead biomass, has been studied extensively in the past and found to effectively bioaccumulate heavy metals. *M. aeruginosa,* like other microorganisms, develop net-like protective layer (biofilm) consisting of many biopolymers (ter Laak *et al*., 2009). Major part of organic portion (50%-90 %) of biofilm is constituted by extracellular polymeric substances (Donlan & Costerton, 2002). Extracellular

polymeric substances (EPS) are huge high-molecular weight natural polymers that are released into the surroundings by microorganisms and mainly consist of exopolysaccharides, proteins, humic substances, and traces of uronic acid and nucleic acids etc. These biopolymers in the EPS sequester heavy metals through their sorption properties (Joubert *et al*., 2006). Moreover, the amount of EPS contents increases as the *Microcystis* bloom develop and hence, its impact on aquatic quality is also enhanced. Over the past two to three decades, heavy metal binding by microbial biomass in terms of its interaction with EPS polymers has been studied and reported. It was reported that the presence of EPS in biofilms enable them to remove most of the heavy metals (90%) (Chen *et al*., 1995). The inclusion of proteins in the EPS of *Chroococcus sp*. increased Hg adsorption (Song *et al*., 2014). In a research work *M. aeruginosa* was used to decontaminate wastewater through biosorption of lead and cadmium (Cheraghpour *et al*., 2020). Zhang *et al*., (2014) demonstrated the correlation between shaking speed and contact of adsorbate i.e. (Cd and Cu) and digested aerobic activated sludge. They also showed that neutral pH enhanced the adsorption capacity. The *Microcystis* blooms in freshwater lakes have been recognized as ecological problem worldwide (Lizhen *et al*., 2018) and cycle of heavy metals in aquatic environment is highly influenced by the bioaccumulative capacity of biomass of bloom-forming Cyanobacteria (*M. aeruginosa*). The role of EPS in Cd (II) binding by bacteria (*B. subtilis* and *P. putida*) has been reported that the presence of EPS enhances the adsorption capacity of bacteria (Wei *et al*., 2011). To better understand the destiny of heavy metals in eutrophic waters, the bioaccumulation potential of heavy metals by *Microcystis* biomass should be explored (Baptista & Vasconcelos, 2006).

The aim of the present study is to investigate the capability of whole cell *M. aeruginosa biomass* intact with EPS relative to that of EPS depleted *M. aeruginosa* biomass for removal of heavy metal ions like Cd (II), Cu(II), Pb(II), and Zn(II). Although the role of EPS in heavy metal binding by some bacteria has been previously explored but this study is the first report of influence of *M. aeruginosa* EPS on its biosorption capacity. The contribution and relative importance of EPS in heavy metal scavenging capacity of *M. aeruginosa* was evaluated by optimizing different physico-chemical conditions like temperature, pH, contact time, and biomass dosage for maximum removal of heavy metal ions from contaminated ground water. Two sets of experiments were conducted for comparative study through biosorption experiments by utilizing the whole cell biomass of *M. aeruginosa* in one set of experiments and EPS depleted biomass of *M. aeruginosa* in second set of experiments. The outcomes of these comparative investigation and characterization of EPS would reveal that extracellular polymeric substances (EPS) of *M. aeruginosa* have a significant potential to be used as biosorbent for the cost-effective removal of heavy metals (Cd, Cu, Pb, and Zn) from contaminated water. The findings of this study could help better understand the impact of cyanobacterial blooms on heavy metal (Cd, Pb, Cu, and Zn ions) behavior in water sources.

## Material and Methods

**Reagents:** Standard solutions of heavy metal ions (cadmium, copper, lead, and zinc) of 1000 $mgL^{-1}$ concentration were purchased from Merck, Germany for Atomic Absorption Spectrophotometer. Stock solution was used for the preparation of working standard solution of desired concentrations of metals using deionized water for dilution. Standard alkaline (NaOH) and acidic ($HNO_3$) solutions were used for the adjustment of the pH of solutions. All chemicals in this investigation were of analytical grade.

**Equipment:** The pH of all solutions was measured by using a pH meter (Digital pH meter, Knick 647, Berlin Germany). Flame Atomic Absorption Spectrophotometer (FAAS) (Unicam, 969-UK) was used for the heavy metal analysis under standard conditions. FTIR spectra of biomass were recorded by using FTIR spectrophotometer (Agilent, Cary 630 FTIR). The sonicator (Elma, E 30 H) was used to agitate the biomass to remove EPS.

**Cultivation of whole *M. aeruginosa* biomass:** The strain of cyanobacteria, *M. aeruginosa*, was obtained from Chinese Academy of Science, China. The cultures were incubated at 25°C in BG-11 medium (Sigma-Aldrich, Germany) at pH 7.1, under a light–dark cycle (12hr-12hr), irradiated with a cool light intensity of 50 μmol $m^{-2}$ $s^{-1}$ for 25 days (Rzymski *et al*., 2014). After incubation, the biomass was collected by filtration (Whatman filter paper, grade 1), washed with deionized water, and lyophilized. The lypholized biomass was ground, passed through the mesh of 250 μm, and stored for biosorption experiments. The whole biomass was abbreviated as **WB**.

***M. aeruginosa* biomass without EPS:** After harvesting the above microbial biomass was suspended in deionized water, agitated in an ultra-sonic bath sonicator for half an hour then centrifuged at 12000 rpm for 15 minutes. The microbial pellet was separated from the supernatant containing EPS. The same process was repeated to ensure the maximum removal of EPS. The biomass without EPS was lyophilized, ground and passed through 250 μm, and stored for biosorption experiments. This biomass without EPS was abbreviated as **BWE**.

**FTIR analysis:** FTIR study was conducted to identify the functional groups present on the surface of *M. aeruginosa* cell responsible for metal sorption. WB and BWE were subjected to Fourier Transform Infrared (FTIR) spectroscopy for the detection of important functional groups. The FTIR spectra were run within a wavelength range of 4000-400 $cm^{-1}$. The peaks of FTIR spectra were indexed to understand the nature of functional groups possibly involved in heavy metal binding.

**Point of zero charge:** For the determination of isoelectric point/point of zero charge, 50 mL of $KNO_3$ (0.1 M) was taken in a series of flasks and pH was adjusted from 1 to 12 using 0.1 M HCl or NaOH. Then 0.1 g of freeze-dried biomass (WB or BWE) was added in each flask separately. Then $N_2$ gas was bubbled through these flasks to stabilize

the pH and flasks were immediately capped, flasks were shaken on orbital shaker for 48 hours. After this, the final pH ($pH_f$) of each solution was measured. The point of zero charge was determined from the graph of initial pH ($pH_i$) versus ΔpH ($pH_f$-$pH_i$).

**Adsorption studies:** The adsorption capacity of *M. aeruginosa* (WB) was investigated through biosorption experiments by optimizing the initial concentration of metal, biomass dose, pH, and contact time. The kinetics and equilibrium were studied by conducting batch experiments using Erlenmeyer flasks (250 mL) containing 100 mL of metal solution at room temperature. At the end of the experiment, the clear supernatant having residual metal concentration was obtained after filtration. The clear supernatant was collected, acidified with nitric acid, and stored till further analysis in the refrigerator. All experiments were run in triplicate, the results shown were mean values and standard deviation (SD) was shown as error bars in respective figures. The same set of biosorption experiments were conducted using the BWE. In each set, a control was processed having only metal ion solution without biomass.

**Metal concentrations analysis:** The concentration of remaining heavy metal was determined using Flame Atomic Absorption Spectrophotometer under standard conditions for cadmium, copper, lead, and zinc. Standard analytical solutions (Merck, Germany) were used for calibration. Equation 1 was used to calculate the quantity of heavy metal being adsorbed per gram of dry biomass (Eq. 1).

$$q_{eq} = \frac{(C_o - C))V}{M} \qquad \text{Eq 1}$$

where; $q_{eq}$ is the quantity of heavy metal that is adsorbed per unit mass of the adsorbent (mg/g), $C_o$ is initial and C is the final metal ion concentrations ($mgL^{-1}$), V is the volume of metal solution (L), and M is the amount of the biomass (g). The percentage removal efficiency (R %) of heavy metal was calculated by using the following equation 02.

$$R(\%) = \frac{(C_o - C)}{C_o} \text{ x100} \qquad \text{Eq 2}$$

Langmuir (Equation 3) and Freundlich (Equation 4) adsorption isotherm models were used for biosorption data evaluation.

$$q_e = \frac{b\, q_{max} C_e}{1 + bC_e} \qquad \text{Eq 3}$$

where $q_{max}$ is the maximum concentration and $q_e$ are the equilibrium concentration of metal adsorbed in mg per gram of dry biomass, $C_e$ is the metal ion concentration at equilibrium ($mgL^{-1}$) and b is the Langmuir constant.

$$q_e = K_f C_e^{1/n} \qquad \text{Eq 4}$$

where n and $K_F$ are the Freundlich constants.

**Effect of biomass quantity:** The influence of biomass quantity on heavy metals removal efficacy was investigated by adding different amounts of biosorbent (0.5 – 5.0 $gL^{-1}$) keeping all other parameters constant. The residual metal ion concentration was calculated as above.

**Effect of initial concentration of metal:** The concentration of initial metal was increased from 5,10,20,40,60, 80,100,120,140,160 $mgL^{-1}$to optimize the metal initial concentration for maximum adsorption on biomass. Solutions of different metal concentrations with a constant quantity of biomass were shaken at speed of 120 rpm using orbital shaker at 25 ± 2 °C while all parameters were kept constant. The remaining concentration of metal, after removal of biomass, was calculated to determine the metal concentration presenting maximum adsorption.

**Effect of pH:** The impact of initial pH of metal ion solution on the sorption of metal ions by natural biomass was evaluated. Acids ($HCl/HNO_3$;1.0 M) or alkali (NaOH; 1.0 M) was used to adjust the pH (2-8) of metal solution keeping the biomass dose and heavy metal concentration, and contact time constant. Suspension of biomass and metal solution was stirred for 30 min at 25 ± 2°C using an orbital shaker. The residual metal concentration was measured to explore the influence of pH on heavy metal binding by biomass.

**Kinetic study:** kinetics of metal adsorption was studied by conducting the batch equilibrium experiments. The kinetics of metal adsorption were investigated by agitating the suspension in Erlenmeyer flasks (250 mL) at 120 rpm for a contact time of 15 min to 3 hrs. while keeping all other parameters constant. Suspensions were filtered and residual metal concentration was determined as above.

## Result and Discussions

**Characterization of microbial biomass:** The FTIR spectra of *M. aeruginosa* showed its complex nature and presence of various functional groups. The FTIR spectrum of WB and BWE is presented in (Fig. 1). In the spectrum of WB a strong broadband at 3274 $cm^{-1}$ represented the presence of bonded –NH and –OH groups in alcohols, carboxylic acids, and phenols. The large band width was due to different degree of hydrogen bonding in the biomass. The peak at 2922.2 $cm^{-1}$ was due to the asymmetric stretching vibration of aliphatic chain (C-H). The carboxylate ion produces a strong band at 1636 $cm^{-1}$ due to symmetric stretching vibrations of C=O and other band at 1541.3 $cm^{-1}$ was due to the bending vibrations of (N-H) group and stretching vibrations of C-N in amide group (Salman *et al*., 2010). These two bands confirmed the presence of proteins in the biomass (Yee *et al*., 2004). A strong band observed at 1027.8 $cm^{-1}$ was attributed to the stretching vibrations of ether groups (-C-O-C) (Bashardoost *et al*., 2010). All the bands in FTIR spectrum of WB suggested the presence of carboxylic acids, proteins, alcohols, reducing sugars, and polysaccharides. In the FTIR spectrum of BWE, all the above mentioned bands were present but showed a little shift i.e.; bonded –OH (3274 $cm^{-1}$ to 3260 $cm^{-1}$), asymmetric stretching vibration of aliphatic chain C-H (2922.2 $cm^{-1}$ to 2910 $cm^{-1}$), carboxylate ion -C=O (1636 $cm^{-1}$ to 1654 $cm^{-1}$), amide group -NH (1541.3 $cm^{-1}$ to 1507.7 $cm^{-1}$), and ether C-O-C (1027.8 $cm^{-1}$ to 1022.7 $cm^{-1}$).

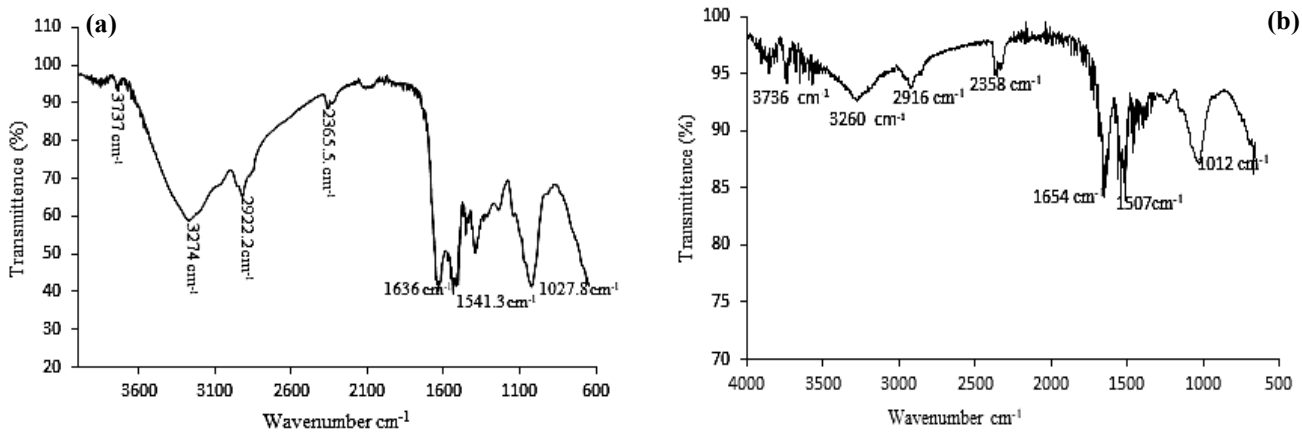


Fig. 1. FTIR spectra of (a) WB and (b) BWE.

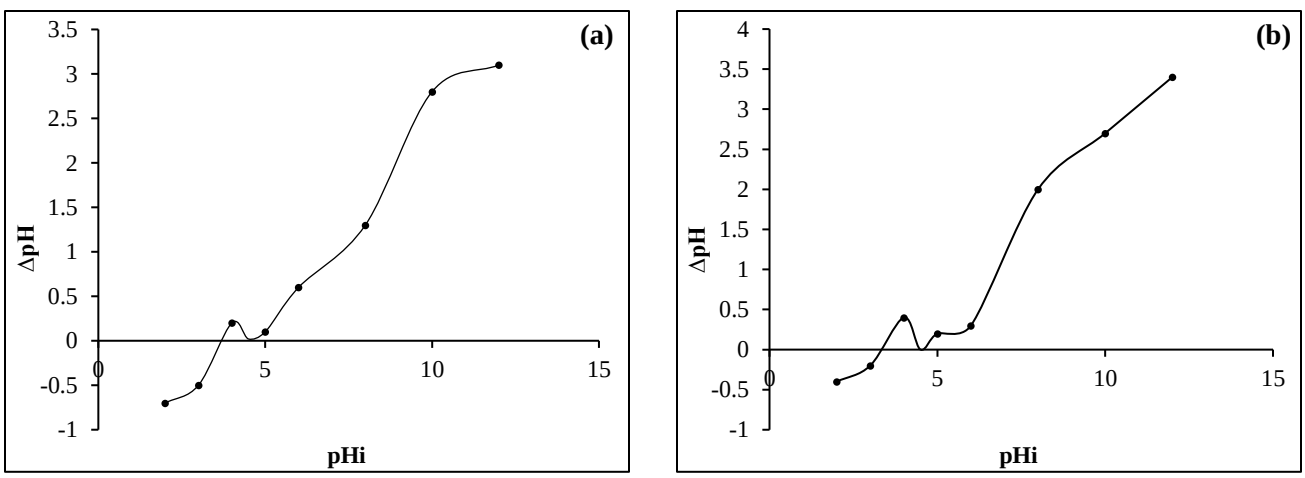


Fig. 2. Point of zero charge of (a) Whole biomass of (WB); (b) Biomass without EPS (BWE).

The change in position and shape of the bonded –OH band (3260 $cm^{-1}$) showed that some of the compounds responsible for hydrogen bonding had been removed or their concentration was reduced in the biomass. In the spectrum of BWE, shift in absorption frequencies and different fingerprint region confirmed that after the removal of EPS the nature of the biomass was changed. The peak positions in both the spectra did not have much difference implying that the general composition of EPS and the cell wall were likely to be identical. The removal of EPS did not influence much the chemistry of functional groups of cell wall. These findings supported prior research by Fang *et al*., who discovered that EPS intact and EPS-free bacteria produced identical FTIR spectra (Fang *et al*., 2011).

**Point of zero charge:** The point of zero charge is regarded as a key property of biosorbent as its role is very crucial to decide ionization of biomass. The isoelectric point, or point of zero charge, was calculated in order to explain the probable behavior of biomass toward metal ion biosorption as a function of solution pH. The isoelectric point of WB and BWE was determined to be 4.5 (Figs. 2a & 2b). This suggested the existence of weak acidic functional groups in biomass. A solution with a pH less than 4.5 protonates the biomass, resulted in a positively charged surface. Hence, biosorption in an acidic media was reduced. A pH greater than 4.5 will result in a negatively charged surface showing enhanced biosorption of heavy metal ions. Thus. the WB & BWE have acidic surface that may be due to contribution of carboxylic acid groups in amino acids of proteins. This finding was also supported by the FTIR spectra of both WB and BWE showing the presence of carboxylic acids in both biomasses.

**Effect of biomass dose:** The influence of biomass dose on biosorption of heavy metals (cadmium, copper, lead, and zinc) was investigated by adding different amounts of biomass i.e., from 0.5 to 5 $gL^{-1}$ (Fig. 3). The findings clearly showed that percent removal was highly dependent on biomass dosage. In case of cadmium, as the amount of WB was increased from 0.5 $gL^{-1}$ to 1.0 $gL^{-1}$, the percent removal was also increased from 27.4% to 60.2%. After that further increase in WB dose (1.5 $gL^{-1}$) did not affect much the percent removal (50%) and even a decrease in percent removal (47% to 41.6%) was observed as the WB amount was further increased (2.5-5.0 $gL^{-1}$). Likewise, percent removal of all metal ion increased by increasing dose of WB from 0.5 to 1.0 $gL^{-1}$ i.e., 40.4% to 53.2% (Cu (II)), 37.5% to 58% (Pb (II)), and 40.4% to 50.5% (Zn (II)). Same trend in percent removal of metal ions was depicted by BWE, but percent removal efficiency was smaller compared to that depicted by the WB i.e., from 17.8% to 53.7% (Cd (II)), 37.7% to 47% (Cu (II)), 31.5% to 51.5% (Pb (II)), 28% to 48.9% (Zn (II)).

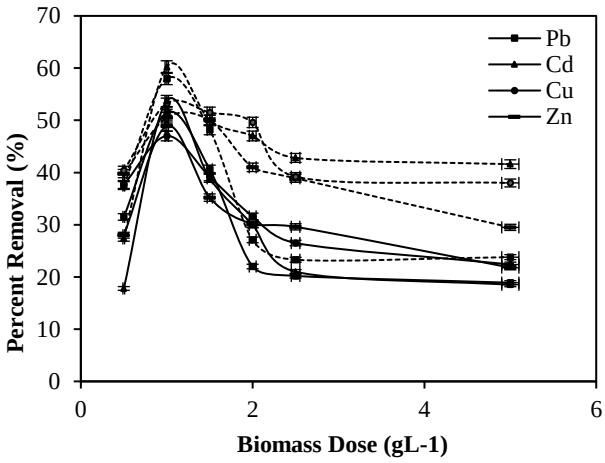


Fig. 3. Effect of amount biomass dosage on percent removal of Cadmium, Copper, Lead, and Zinc. The dotted lines represent the WB and the solid line present the BWE ($C_o$= 20 $mgL^{-1}$, pH=5.0 for Lead, Cadmium and 6.0 for Copper and Zinc. Time of contact = 60 min for Cadmium, Copper, Lead, and 45 min for zinc).

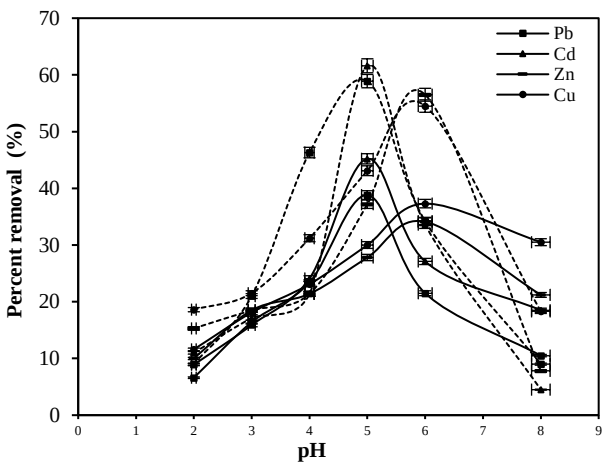


Fig. 4. Effect of pH on percent removal of WB (dotted lines) and BWE (solid lines) for cadmium, copper, lead, and zinc. ($C_o$= 20 $mgL^{-1}$, Contact time = 60 min for Cd, Cu, Pb ions and 45 min for Zinc ions, biomass dose= 1 $gL^{-1}$).

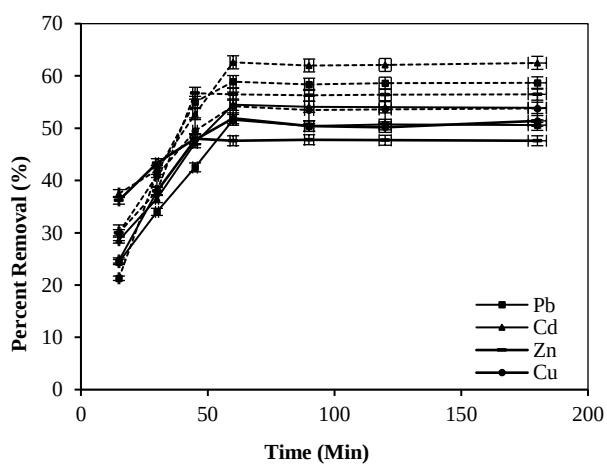


Fig. 5. Effect of contact time on biosorption studies of WB (dotted lines) and BWE (solid lines) for cadmium, copper, lead, and zinc ions. ($C_o$= 20 $mgL^{-1}$, pH=5.0 for Pb & Cd ions and 6.0 for Zn & Cu ions, biomass dose= 1 $gL^{-1}$).

Further increase in biomass quantity had a very small impact and even higher amounts showed a decreased percent removal capacity. The same behavior was expressed by the WB as well as BWE. The maximum increment found for cadmium (60.2%), was possibly due to higher charge density. When the biomass dose was low, surface-active sites were freely available and got saturated very fast showing a higher $q_{eq}$ value but as the amount of biomass was increased the effective sites were reduced because of partial aggregation of biomass and hence biosorption was decreased (Karthikeyan *et al*., 2007). The biomass quantity of 1.0 $gL^{-1}$ was selected for further experiments as further increase in biomass dose did not cause an increase in percent removal efficiency. The decrease in percent removal of heavy metals by BWE i.e., 10.79% for Cd, 11.65% for Cu, 11.20% for Pb, and 3.16% for Zn, strongly confirmed the fact that EPS contains many polymeric compounds with functional groups that helped to scavenge heavy metal ions showing a better percent removal capacity.

**Effect of pH:** It is well-established fact that pH affects the adsorption of heavy metal. The pH of metal solution was varied (2-8) to investigate its impact on metal uptake by the WB and BWE. The biosorption capacity of both WB & BWE showed an ascending trend as the pH was increased initially (Fig. 4). Lead and Cadmium showed highest percent removal when pH was adjusted at 5 while zinc and copper showed best results at pH 6.0. However, as the pH was raised further, the % removal of all heavy metal ions declined considerably for both the WB and the BWE. When we used WB as biosorbent, the $q_{eq}$ values were increased as we increased the pH of metal solution from 1 to 5 i.e., 27.5 to154 mg/g for Cd and 22.5 to 147.25mg/g for Pb. While copper and zinc showed maximum $q_{eq}$ as the pH was increased from 1 to 6.0 (46.5 to 136.25mg/g (Cu(II)) and 38.25mg/g to 141.25mg/g (Zn(II)). Further increase in pH up to 8.0 depicted a dramatic decrease in $q_{eq}$ for all heavy metal ions i.e., 11.25mg/g (Cadmium), 45.75 mg/g (Copper), 22.50 mg/g (lead), and 19.50mg/g (Zinc). Same trend was observed with BWE, however, decrease in metal uptake capacity was much sharper by the WB as compared to the BWE. Metal uptake depends on the composition of biomass cell wall (nature of functional groups) as well as the chemical behavior of metal ion. Less metal uptake at acidic pH supports the fact that Hydrogen ion also approach the active sites of biomass and their availability for metal ions is restricted. Increased density of positive charge on active sites produces repulsive forces restricting the metal cation approach. Furthermore, cation uptake is also reduced as the anion uptake is accelerated simultaneously (Rai *et al*., 1996).

The negative charge on biomass active sites was increased with the increase of pH attracting the positive metal ions and hence, imparting higher biosorption capacity. At higher pH metal hydroxide complexes are formed that is not adsorbed by active binding sites of biomass and hence, metal uptake by biomass is decreased. Metal speciation in solution is also pH dependent. Solubility of metal ions is greater in acidic solution than in alkaline solutions. Metals are easily oxidized in acidic solution forming a cation. Metals are not reactive towards

bases, but they may get precipitated as hydroxide in alkaline solutions.

The Ksp values of precipitation of metal ion as hydroxides is a key to determine the pH for precipitation of metal ions. The pH of precipitation from Ksp values of metal hydroxides at metal ion concentration of 10 $mgL^{-1}$ was found to be 9.2 for Cd (II), 6.0 for Cu(II), 8.7 for Pb(II), and 7.4 for Zn(II). When the pH of respective metal solution was increased above its pH of precipitation, the metal solubility was decreased as it would be precipitated as metal hydroxides i.e., $M(OH)_2$.

The WB and BWE showed maximum biosorption capacity for Pb(II) & Cd(II) at pH 5 and for Cu(II) & Zn(II) at pH 6. These optimized pH values for maximum metal removal are quite below the pH of precipitation for each metal ion. This fact is also supported by experimental results showing that biosorption capacity of WB & BWE for all metal ions is reduced at higher pH of solution as the availability of these metals is restricted due to their precipitation as hydroxides prior to adsorption by biomass. The critical role of pH for metal adsorption suggested that the active biosorbent sites on biomass were probably the weak carboxyl groups and to some extent the hydroxyl groups present in polysaccharides. The percent removal by WB (61.6% for Cd, 54.5% for Cu, 58.9% for Pb, 56.5% for Zn) was higher at optimum pH than shown by BWE (45.2% for Cd, 34% for Cu, 38.8% for Pb, and 34.1% for Zn. This increment in percent removal by WB (26.62% for Cd, 31.55% for Cu, 34.12% for Pb, and 39.82% for Zn) supported this probability as WB contained the carboxyl, amide and hydroxyl groups in large proportion compared to BWE where some of these active groups had been removed as EPS. So, the WB showed much higher efficiency for heavy metal removal than the BWE.

**Effect of contact time and adsorption kinetics:** biosorption is highly dependent on time of contract between metal ion and biosorbent for its successful completion. (Fig. 5) shows the influence of contact time on the % removal capacity of *M. aeruginosa.* It is clear that as contact time rises, biosorption of all heavy metal ions increases till equilibrium is reached. The maximum $q_{eq}$ value was attained in 60 minutes for cadmium (62.6 mg/g (WB),54.5 mg/g (BWE)), copper (54.2 mg/g (WB), 51.30 mg/g (BWE)), lead (58.9 mg/g(WB),51.90 mg/g (BWE)), while $q_{eq}$ value for zinc (56.70 mg/g (WB), 48 mg/g (BWE)) was attained after 45 minutes of contact. After an equilibrium was achieved, biosorption of all heavy metal ions by both biomass (WB and BWE) was not affected. Two-step kinetic behavior was observed in this biosorption, a very rapid uptake within a few initial minutes and then slower uptake for a long time up to 60 minutes. In all cases, almost 80% of the total percent removal capacity was achieved within 60 minutes by cadmium, copper, lead, and 45 minutes by zinc ions. In a study of *Chlorella Vulgaris* for nickel uptake showed rapid adsorption within 10 minutes and equilibrium was achieved in 60 min (Aksu, 2002). Matheickal & Yu., 1999 reported 90% of total cadmium removal by *Durvillaea potatorum* within half an hour and a very slow removal thereafter. This rapid uptake of metal ions makes this process more efficient and cost-effective in terms of operation.

The similar trend in biosorption behavior was depicted by both biomass (WB and BWE) but WB showed higher % removal capacity than BWE. Percent removal by the WB for cadmium (62.60%), copper (54.2%), lead (58.9%), and zinc (56.7%) was higher compared to that shown by the BWE i.e., 54.50% (cadmium), 51.9% (copper), 51.3% (lead), and 48% (zinc). This decrease in percent removal capacity of BWE (14.86% (Cd (II)), 18.12% (Cu(II)), 14.81 (Pb(II)), and 4.43% (Zn(II)) confirmed the fact that concentration of some functional moieties responsible for heavy metal binding was decreased in biomass as they were removed as EPS, hence the efficiency of biomass was decreased.

Kinetics of biosorption process is one of the important theoretical tools to decide the adsorption mechanism either as chemical reaction or mass transfer to collect optimized operating conditions (Alyüz & Veli, 2009). Pseudo-first-order (PFO) and the pseudo second-order kinetic (PSO) are the mostly used kinetic models in batch systems to explain the biosorption kinetics. The linearized forms of PFO and PSO models are given in Eq 5 & 6 respectively.

$$\ln(q_e - q_t) = \ln q_e - k_1 t \qquad \text{Eq 5}$$

$$\frac{1}{q_t} = \frac{1}{k_2 {q_e}^2} + \frac{t}{q_e} \qquad \text{Eq 6}$$

The quantity of metal ions adsorbed, in mg/g, at equilibrium and at time t is represented by qt and qe, respectively, and the PFO and PSO reaction rate constants are $k_1$ ($min^{-1}$) and $k_2$ ($gmg^{-1}\ min^{-1}$). The biosorption data of all the four metal ions (Cd,Cu,Pb,and Zn ions) at initial concentration of 20 $mgL^{-1}$ was investigated by using both the kinetic models. The biosorption data fits best to a kinetic model when a linear fit is obtained with $R^2$ value equal to or greater than 0.98 and the $q_{cal}$ is equal or very close to the $q_{exp}$. Tables 1 and 2 show the values of important parameters for both the kinetic models. It could be inferred that the biosorption data of WB fitted better to the PSO model ($R^2$ =0.994, 0.996, 0.992, 0.99 for cadmium, copper, lead, and zinc ions respectively) than the PFO model ($R^2$ =0.87, 0.75,0.77, and 0.55 for cadmium, copper, lead, and zinc ions respectively).

**Table 1. Critical parameters of Pseudo 1st order kinetic model.**

| Metal ion | Whole biomass | | | | | | | Biomass without EPS | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp. $q_{eq}$ (mg/g) | Cal. $q_{eq}$, mg/g | $K_1$ | $R^2$ | Intercept | Slope | D (%) | Exp. $q_{eq}$ (mg/g) | Cal. $q_{eq}$, mg/g | $K_1$ | $R^2$ | Intercept | slope | D (%) |
| Cd (II) | 62.6±0.20 | 4.34 | 0.015 | 0.87 | 1.468 | -0.015 | -93.06 | 54.5±0.36 | 3.33 | 0.011 | 0.87 | 1.204 | -0.011 | -93.87 |
| Cu (II) | 54.2±0.26 | 3.21 | 0.011 | 0.75 | 1.165 | -0.011 | -96.79 | 51.3±0.61 | 3.23 | 0.009 | 0.69 | 1.17 | -0.009 | -93.77 |
| Pb (II) | 58.9±0.26 | 3.67 | 0.013 | 0.77 | 1.301 | -0.013 | -93.76 | 51.9±0.81 | 3.46 | 0.01 | 0.67 | 1.241 | -0.01 | -93.25 |
| Zn (II) | 56.7±0.56 | 2.62 | 0.012 | 0.55 | 0.965 | -0.012 | -95.37 | 48.0±0.66 | 1.81 | 0.008 | 0.50 | 0.593 | -0.008 | -96.23 |

**Table 2. Critical parameters of Pseudo 2nd order kinetic model.**

| | Whole biomass | | | | | | | Biomass without EPS | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Metal ion | Exp. $q_{eq}$ (mg/g) | Cal. $q_{eq}$, mg/g | $K_2$ | $R^2$ | Intercept | Slope | D (%) | Exp. $q_{eq}$ (mg/g) | Cal. $q_{eq}$, mg/g | $K_2$ | $R^2$ | Intercept | Slope | D (%) |
| Cd (II) | 62.6±0.20 | 67.56 | 0.0012 | 0.994 | 0.174 | 0.015 | 7.93 | 54.5±0.36 | 58.82 | 0.0014 | 0.993 | 0.209 | 0.017 | 7.93 |
| Cu (II) | 54.2±0.26 | 57.47 | 0.0012 | 0.996 | 0.160 | 0.017 | 6.03 | 51.9±0.61 | 55.55 | 0.0016 | 0.993 | 0.205 | 0.018 | 7.04 |
| Pb (II) | 58.9±0.26 | 64.51 | 0.0012 | 0.992 | 0.197 | 0.015 | 9.53 | 51.3±0.81 | 56.18 | 0.0012 | 0.991 | 0.258 | 0.018 | 9.51 |
| Zn (II) | 56.7±0.56 | 60.97 | 0.0015 | 0.990 | 0.173 | 0.016 | 7.54 | 48.0±0.66 | 48.78 | 0.0076 | 1 | 0.056 | 0.021 | 1.62 |

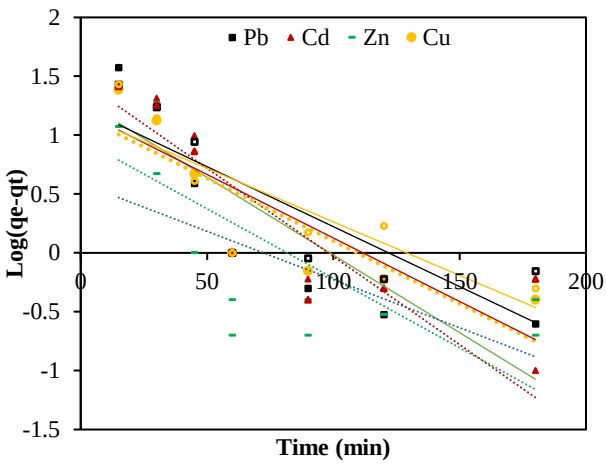


Fig. 6. Linear expression of PFO kinetics of biosorption for cadmium, copper, lead, and zinc ions. (Contact time = 60 min for cadmium, copper, lead ions and 45 min for zinc, pH=5.0 for Pb & Cd ions and 6.0 for zinc & copper ions, biomass dose= 1 $gL^{-1}$). Dotted lines show the PFO data of WB and solid lines show that of BWE.

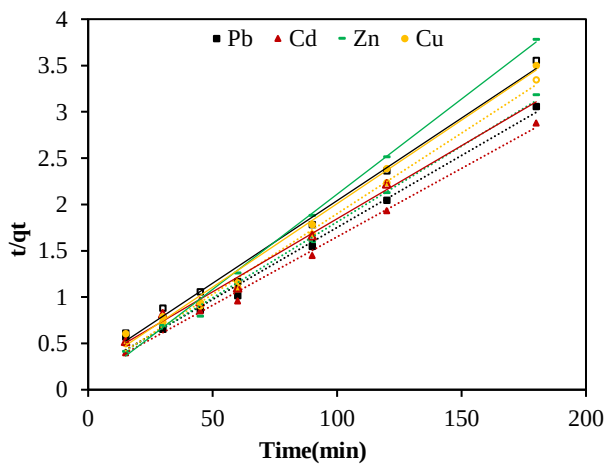


Fig. 7. Linear expression of PSO kinetics of biosorption for cadmium, copper, lead, and zinc ions. (Contact time = 60 min for cadmium, copper, lead ions and 45 min for zinc, pH=5.0 for Pb & Cd ions and 6.0 for zinc & copper ions, biomass dose= 1 $gL^{-1}$). Dotted lines show the PSO data of WB and solid lines show that of BWE.

The percent deviation values of all four heavy metals for PSO kinetic model were much smaller than those calculated for PFO kinetic model for WB (Tables 1 & 2). The same trend was observed with the biosorption data of BWE i.e., it also fits to the PSO kinetic model better than the PFO kinetic data.

Figures 6 & 7 present the linear plot of PFO and PSO kinetic data. The figures clearly show that the kinetic data of all heavy metals (By both WB and BWE) fitted better to PSO kinetic model.

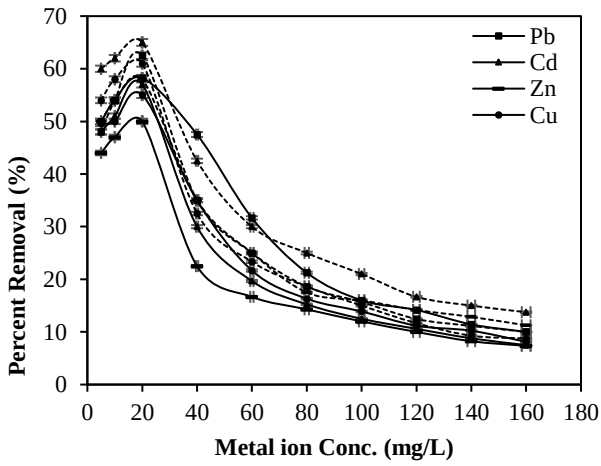


Fig. 8. Influence of initial metal ion concentration on biosorption of WB (dotted lines) and BWE (solid lines) for cadmium, copper, lead, and zinc ions. (Contact time = 60 min for cadmium, copper, lead ions and 45 min for zinc, pH=5.0 for Pb & Cd ions and 6.0 for zinc & copper ions, biomass dose= 1 g/L).

Moreover, $q_{cal}$ values for WB produced by PSO kinetic model are 67.56 (cadmium), 57.47 (Copper), 64.51 (lead), 60.97 (zinc) which were closer to the $q_{exp}$ values i.e., 62.6±0.20, 54.2±0.26, 58.9±0.26, and 56.7±0.56 for cadmium, copper, lead, and zinc ions respectively compared to that produced by PFO kinetic model. All $q_{cal}$ values are represented as mg/g. Similarly, the $q_{cal}$ values produced by PSO kinetic model were closer to $q_{exp}$ values for BWE compared to that produced by PFO kinetic model. Another important tool to demonstrate the fitting of biosorption data to particular kinetic model is the percent deviation value calculated as in eq. 7.

$$D(\%) = \left(\frac{q_{e(cal)} - q_{e\,(exp)}}{q_{e\,(exp)}} * 100\right) \qquad \text{Eq 7}$$

**Biosorption isotherm & effect of initial concentration of metal ion:** The metal biosorption capacity of biomass (WB and BWE) was investigated by gradually changing the concentrations of metal from 5 $mgL^{-1}$to 160 $mgL^{-1}$, while rest of the parameters were kept constant (Fig. 8). The WB showed an increase in metal uptake by increasing the initial cadmium concentration from 5 $mgL^{-1}$ to 20 $mgL^{-1}$ (60% to 65%). however, as the concentration was further increased to 80 $mgL^{-1}$, 25% reduction in metal removal (%) capacity was depicted. Further increase in concentration imparted very little effect on percent removal by WB. Similarly, the WB showed maximum metal uptake at 20 $mgL^{-1}$ concentration for all heavy metals; copper (61%), lead (62.50%), zinc (58%). The same trend was shown by all four heavy metals against the BWE but efficiency for metal

uptake was reduced. i.e., cadmium (57%), copper (55%), lead (58.25%), and zinc (50%). The metal uptake at low metal concentration is small because the mass transfer coefficient is quiet low at solid-liquid interface of biomass-metal solution. Higher concentration of metal provides much stronger driving force and hurdles regarding mass transfer at interface of biomass-metal ion solution are defeated (Edris *et al*., 2014) and an increase in metal uptake is observed. At higher initial metal concentration, the number of heavy metal ions become relatively high compared to available adsorption sites. Overcrowding of metal ions caused desorption, hence the removal (%) of heavy metal ion was decreased. The same trend in metal sorption of lead and cadmium ions was also reported (Sari & Tuzen, 2008).

Equilibrium adsorption isotherms are best analytical tool to describe the sorption capacity of biomass. Affinity and surface characteristics of biomass are expressed by definite constants that are characteristics of these isotherms. The Langmuir and Freundlich isotherms models were used to evaluate experimental data and their calculated constant were given in (Tables 3 and 4).

Figs. 9a and 9b present the non-linear Langmuir and Freundlich isotherm respectively for the absorption of cadmium, copper, lead, and zinc using WB and BWE as biosorbent. The experimental $q_{eq}$ values are presented as the markers while the calculated $q_{eq}$ for WB are presented by dotted lines and for BWE by solid lines in both the figures 9a and 9b**.** The figure clearly shows that the adsorption data of all heavy metals (By both WB and BWE) fitted better to Langmuir compared to Freundlich isotherm.

The data was fitted best to Langmuir isotherm model for both the WB and BWE with $R^2$ values of 0.99, 0.99, 0.99, and 0.98 (WB) and 0.99, 0.99, 0.98, and 0.99 (BWE) for cadmium, copper, lead, and zinc respectively (Tables 3 & 4). The Langmuir adsorption isotherm postulates the monolayer adsorption of metals i.e., metal ion is adsorbed only on effective binding sites available on biomass surface and one active site holds one metal ion at a time. An efficient biosorbent should produce less value of Langmuir constant (b) and higher value of $q_{max}$ (Fourest & Roux, 1992).

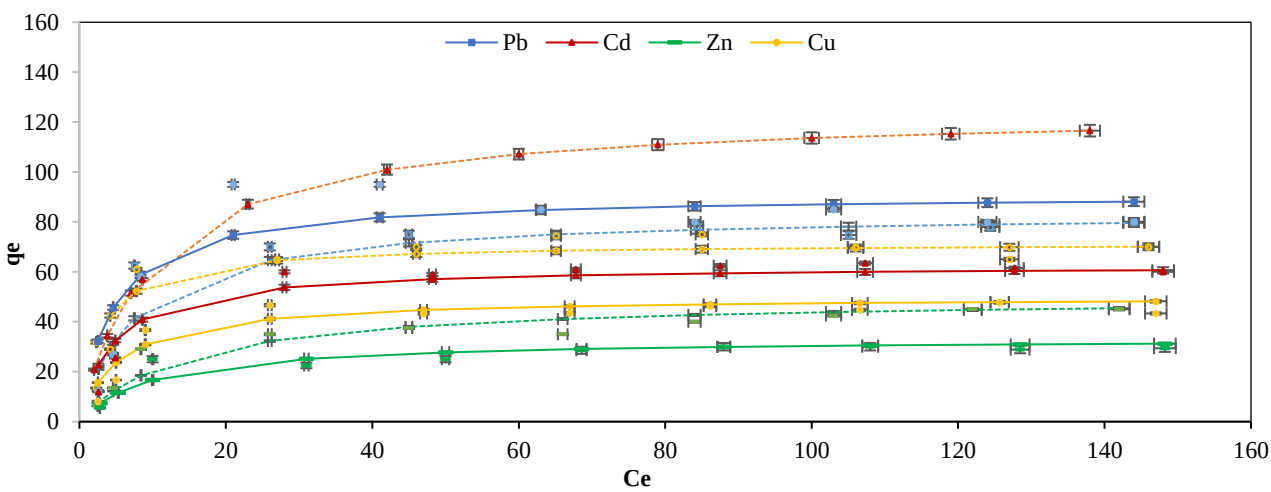


Fig. 9a. Non-linear Langmuir isotherm for biosorption for cadmium, copper, lead, and zinc ions. (Contact time = 60 min for cadmium, copper, lead ions and 45 min for zinc ions, pH=5.0 for Pb & Cd ions and 6.0 for zinc & copper ions, biomass dose= 1 $gL^{-1}$). Dotted lines and hollow marks show the data of WB and solid lines and marks show that of BWE.

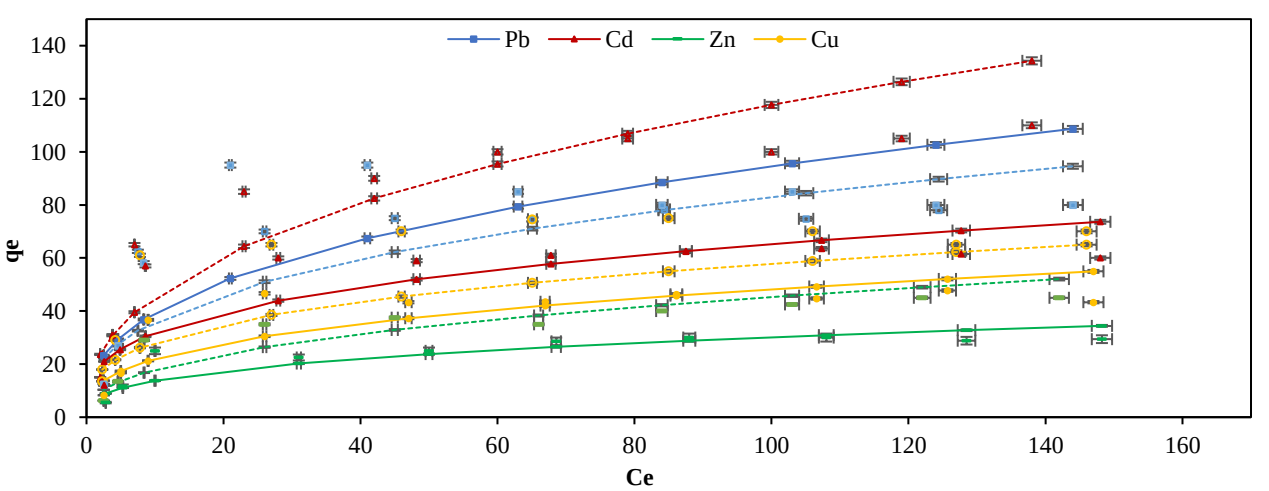


Fig. 9b. Non-linear Freundlich isotherm for biosorption for cadmium, copper, lead, and zinc ions. (Contact time = 60 min for cadmium, copper, lead ions and 45 min for zinc ions, pH=5.0 for Pb & Cd ions and 6.0 for zinc & copper ions, biomass dose= 1 $gL^{-1}$). Dotted lines and hollow marks show the data of WB and solid lines and marks show that of BWE.

**Table 3. Parameter of Langmuir isotherm regarding the adsorption of lead, cadmium, copper, and zinc.**

| Metal ion | Whole biomass | | | Biomass without EPS | | |
|---|---|---|---|---|---|---|
| | $q_{max}$, mg/g | b, L/mg | $R^2$ | $q_{max}$, mg/g | b, L/mg | $R^2$ |
| Cd | 125.00 | 0.100 | 0.99 | 62.50 | 0.22 | 0.99 |
| Cu | 71.42 | 0.30 | 0.99 | 50.00 | 0.18 | 0.99 |
| Pb | 90.90 | 0.133 | 0.99 | 58.33 | 0.22 | 0.98 |
| Zn | 56.00 | 0.074 | 0.98 | 48.33 | 0.100 | 0.99 |

**Table 4. Parameters Freundlich isotherm regarding the adsorption of lead, cadmium, copper, and zinc.**

| Metal ion | Whole biomass | | | Biomass without EPS | | |
|---|---|---|---|---|---|---|
| | $K_F$ | N | $R^2$ | $K_F$ | N | $R^2$ |
| Cd | 17.18 | 2.43 | 0.83 | 15.64 | 3.22 | 0.68 |
| Cu | 13.87 | 3.22 | 0.69 | 10.07 | 2.94 | 0.72 |
| Pb | 15.80 | 2.77 | 0.72 | 16.44 | 2.63 | 0.68 |
| Zn | 7.17 | 2.50 | 0.81 | 6.29 | 2.94 | 0.76 |

The maximum metal adsorption capacity ($q_{max}$) for WB, by using Langmuir adsorption model, were calculated to be 125 mg/g (cadmium), 71.42 mg/g (copper), 90.90 mg/g (lead), and 56 mg/g (zinc). The $q_{max}$ value of BWE were 62.50 mg/g, 50.0 mg/g, 58.33 mg/g, and 48.33mg/g for cadmium, copper, lead, and zinc ions respectively. *M. aerugonisa* showed much higher biosorption capacity for all the metals than many other biological materials reported earlier. Biosorption capacity of some biological materials already reported is shown in (Table 5).

The above comparison supported our findings that *M. aerugonisa* (WB) had high potential for heavy metal removal from aqueous systems. The fitness of data to Langmuir isotherm model also supported the uniform distribution of binding sites having uniform energy on biomass surface and metal ions were adsorbed in monolayer and did not compete with each other in single metal ion system. Moreover, the biosorption capacity of WB is higher than that of BWE supporting the fact that EPS contained many compounds having active binding sites for heavy metals. On the basis of all above discussion a possible mechanism has been proposed as follows:

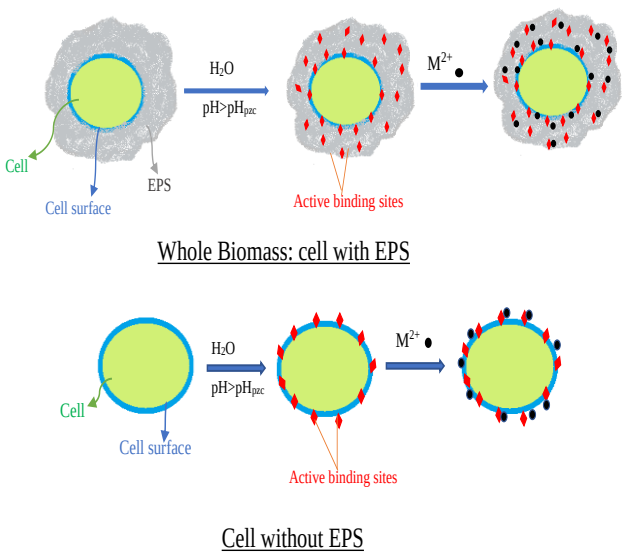


Fig. 10. proposed mechanism for EPS-metal ion binding mechanism.

**Table 5. Comparison of metal intake capacity of *M. aerugonisa* with that of different biological materials.**

| Biomass | Biosorption capacity (mg/g) | | | | |
|---|---|---|---|---|---|
| | Cadmium | Lead | Coper | Zinc | Reference |
| *B. subtilis* | - | - | 29.62 | - | Fang *et al*., 2011 |
| *Caulerpa lentillifera* | 4.70 | 28.70 | - | - | Pavasant *et al*., 2006 |
| *Chlorella minutissima* | 11.10 | 9.74 | - | - | Roy *et al*., 1993 |
| *Gracillaria* sp. | 0.30 | 0.45 | 0.59 | 0.40 | Sheng *et al*., 2004 |
| *Mucor rouxii* | 8.50 | 35.70 | - | - | Yan & Viraraghavan, 2003 |
| *Padina* sp. | 0.75 | 1.25 | 1.14 | 0.81 | Sheng *et al*., 2004 |
| *P. putida* | - | - | 15.72 | - | Fang *et al*., 2011 |
| *Rhizopus arrhizus* | 27.0 | 56.00 | - | - | Fourest & Roux, 1992 |
| *Sargassum* sp. | 0.76 | 1.16 | 0.99 | 0.50 | Sheng *et al*., 2004 |
| *Syzygium cumini* | - | 32.50 | - | - | King *et al*., 2007 |
| *U. lactuca* | *29.2* | 34.7 | - | - | Sari & Tuzen, 2008 |
| *Ulva* sp. | 0.58 | 1.46 | 0.75 | 0.54 | Sheng *et al*., 2004 |
| *M. aerugonisa* (WB) | 62.60 | 58.90 | 54.20 | 56.70 | Present study |
| *M. aerugonisa* (BWE) | 54.50 | 51.30 | 51.90 | 48.0 | |

## Conclusion

In current study the role of EPS of *M. aeruginosa* for biosorption of heavy metal (Cd, Cu, Pb, and Zn ions) was investigated. Our investigation showed that whole *M. aeruginosa* biomass bearing EPS had much higher capability to remove significant amounts of heavy metal ions from contaminated aqueous solutions compared to BWE. The presence of EPS significantly enhances the biosorption capacity of microbial biomass. FTIR showed the presence of functional groups in the biomass that were responsible for metal binding. However, the binding active sites for EPS and cell wall were almost same. The biosorption efficiency of both biomasses is dependent on time of contact of metal and biomass, pH of suspension, amount of biomass dose, and initial concentration of heavy metal. The biosorption process followed the PSO kinetics and Langmuir model was best fit for the biosorption data demonstrating metal ion adsorption in a monolayer at current experimental conditions. Thus the *M. aeruginosa* bearing EPS has a significant potential to be used as biosorbent for the cost effective and efficient removal of heavy metals from contaminated/ wastewater and the EPS in the biofilm around the cell can be considered as an outer shield that protect the cell from the surrounding heavy metal exposure.

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