Biosorption of Cu^{2+} and Zn^{2+} Heavy Metal Ions from Test Solutions Using Green Algae Biosorbent

*Julius Ogwora Maraga
University of Eldoret, Kenya
juliusmaraga@yahoo.com

Philip Kipkoskei Kimaiyo
University of Eldoret, Kenya
philip_kimaiyo@yahoo.com

Prof. Lusweti Kituyi
University of Eldoret, Kenya
joluki@yahoo.com

Prof. Samuel Lutta
University of Eldoret, Kenya
slutta@gmail.com

*Corresponding Author

Abstract: Human activities such as agricultural and industrial operations have led to the accumulation of pollutants for example heavy metals in the environment since they are non biodegradable.. Consequently, there is need to remove these heavy metals from environmental matrices. The different conventional ways of removing heavy metals from the environment are expensive and mostly ineffective. Among these methods, biosorption has been proved to be efficient and cheap in heavy metal removal. Most common marine weeds have been found to be potent biosorbents. This study aimed at examining the capability of green algae in the biosorption of heavy metals from waste waters. The green algae were sampled from the University of Eldoret fish ponds, washed, dried and ground using pestle and mortar. Two selected heavy metal test solutions of Zn^{2+} and Cu^{2+} were used in the study. A 5 gram mass of the algae sample powder was transferred into 250 ml beaker containing a 100 mL solution of specific heavy metal at pH values 4, 7 and 9, and stirred for ten minutes. Ten ml samples were then taken from the reacting vessel at time intervals of 10, 20, 30, 40, 50 and 60 minutes and metal levels measured by the atomic absorption spectrophotometer. The results showed that for each of the two selected metals, the levels significantly increased in the biosorbent faster in the first 10 minutes, while the equilibrium reached after 50 minutes. For Cu^{2+} the maximum biosorption was found to occur at pH 9 and for Zn^{2+} at pH 7 while the least biosorption occurred at pH 4 for Cu^{2+} and for Zn^{2+} at pH 4. The isotherm data fitted Langmuir better than Freundlich from the values of R_2. The R_2 values for pH 4, 7 and 9 ranges, were found to be tending to one, indicating a strong positive relationship between the equilibrium heavy metal ions concentration (Ce) and the metal uptake (Qe). Freundlich, the correlation coefficients, R_2, were found to be less than 0.900, indicating a weak positive relationship between the equilibrium heavy metal ions concentration (Ce) and the metal uptake (Qe). Thus green algae are effective biosorbents for the removal of Zn^{2+} and Cu^{2+} from aqueous solutions. Further studies should be done for other heavy metals by the different algae species at different pH ranges.

Key Words: Heavy metals, Biosorption, Green algae, Pollution

I. INTRODUCTION

Metals are notable for their wide environmental dispersion from industrial waste. Heavy metals have a tendency to accumulate in selected tissues of human body and animals in general. These heavy metals have a high potential being toxic even at relatively minor levels of exposure. The accumulation of heavy metals in the environment over a long period of time can cause both pollution and health risks. Metal poisoning occurs through inhaling the fumes and through ingestion of food contaminated with toxic metals. The heavy metals in soils can be absorbed by plants up to certain levels which are toxic and through ingestion by herbivores or omnivores, metal poisoning takes place. Children are also highly vulnerable to metal poisoning since they interact with toys contaminated with toxic metals (Alkorta *et al.,* 2004). According to the agency for toxic substances and diseases registry based in Atlanta, Georgia, the top four most toxic metals in the environment are lead, mercury, arsenic, and cadmium. Other toxic heavy metals at elevated levels are copper, nickel; zinc, antimony, and selenium. Methods of heavy metal removal from the environment include biosorption, activated sludge process, bio-filter, and anaerobic digestion. Among these methods, biosorption has been proved to be very efficient in heavy metal removal (Pagnanelli *et al.,* 2001).

Biosorption is the process of removing metal ions by means of passive binding to non-living biomass from an aqueous solution. Most common marine weeds have been found to be potent biosorbents (Lewis & McCourt, 2004). This research examined

the capability of green algae in the biosorption of heavy metals from waste waters. The green algae are the large group of algae from which the embryophytes (higher plants) emerged. As such, they form a paraphyletic group, although the group including both green algae and embryophytes is monophyletic.

The green algae include unicellular and colonial flagellates, most with two flagella per cell, as well as various colonial, coccoid, and filamentous forms, and macroscopic seaweeds (Gupta, Shrivastava and Jain, 2001). In the Charales, the closest relatives of higher plants, full differentiation of tissues occurs. There are about 6,000 species of green algae. Many species live most of their lives as single cells, while other species form colonies, coenobia, long filaments, or highly differentiated macroscopic seaweeds (Jeffrey *et al*., 2004)

A few other organisms rely on green algae to conduct photosynthesis for them. The chloroplasts in euglenids and chlorarachniophytes were acquired from ingested green algae, and in the latter retain a vestigial nucleus (nucleomorph *Valoniopsis pachynema* (Deng *et al.,* 2007). Some species of green algae, particularly of genera *trebouxia* and *pseudotrebouxia* (*trebouxiophyceae*), can be found in symbiotic associations with fungi to form lichens. In general the fungal species that partner in lichens cannot live on their own, while the algal species is often found living in nature without the fungus. *Trentepohlia* is a green alga parasitic on the bark of some trees (Jeffrey *et al*., 2004).

Microorganisms, including algae, bacteria, yeast and fungi can be used as biosorbents for detoxification and recovery of toxic or valuable metals from industrial discharges. One of the most promising biosorbents is ''algae'' (Aksu, 2005). In fact, the uses of algae in the sorption of heavy metals were dated back to 1986 when Mehta & Gaur (2005) reported that there was biosorption of cadmium on the cell of the green microalga *stichococcus bacillaris*. Following this work, there were a number of reports on the sorption of heavy metals by other microorganisms (Lewis & McCourt, 2004).

Domestic and industrial waters are increasingly choking with heavy metal waste; human waste from informal settlements; industrial wastes in the form of gaseous emissions, liquid effluents and solid waste; agro-chemicals in fish ponds, and other wastes especially petro-chemicals and metals from micro-enterprises – the "Jua-kali"; and over-flowing sewers (Golub, 2005). This situation has occasioned spread of water-borne diseases, loss of sustainable livelihoods, loss of biodiversity, reduced availability and access to safe portable water, and the insidious effects of toxic substances and heavy metal poisoning which affects human productivity (Hayes, 2007). A major concern is the level of heavy metals in these waters. Heavy metals enter these waters when industrial and consumer waste, or even from water run – off from neighbouring mining sites drain directly to the water surfaces. These heavy metals bio - accumulate in the sediments and in the water to dangerous levels which pose precarious effects to human health, aquatic life and even the environment (Golub, 2005). The common polluting heavy metals are Pb, Cd, Cu, Zn, Cr and Hg. This has been hypothesized as the core trigger to the numerous heavy metal

poisoning effects noted from the continuous use of boreholes and industrial water (Emsley, 2003). Such effects include memory loss, increased allergic reactions, high blood pressure, depression, mood swings, irritability, poor concentration, aggressive behaviour, sleep disabilities, fatigue, speech disorders, cholesterol, triglycerides, vascular occlusion, neuropathy, autoimmune diseases, and chronic fatigue are just some of the many conditions resulting from exposure to such toxins (John, 2002).

To clean these waters and thus reduce the disparaging effects, various strategies have been put in place to lower the concentration of heavy metals in these waters, sediments and in the aquatic animals which live in these water bodies. This research explored the use of green algae as a possible way of removing these heavy metals from water bodies by biosorption. Biosorption is a property of certain types of inactive, dead, microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solutions. Biomass exhibits this property, acting just as a chemical substance, as an ion exchanger of biological origin. It is particularly the cell wall structure of certain algae, fungi and bacteria which is responsible for this phenomenon. This implies that removal of heavy metals from industrial effluents will be done by biological means which are not harmful to the environment. This was in line with the core objective of this study which was to contribute to the search for less expensive adsorbents and their utilization possibilities for various agricultural waste by-products such as sugarcane bagasse, rice husks, oil palm shells, coconut shell, and coconut husks among the many biosorbents for the elimination of heavy metals from wastewater (Romano & Matteucci, 2007). In this line, the core purpose of this study was to assess the ability of green algae biosorbent to biosorb Cu^{2+} and Zn^{2+} heavy metal ions from test solutions.

II. METHODOLOGY

Sample and Sampling Preparation: The green algae (*spirogyra sp.*) samples were obtained from the University of Eldoret fish ponds where they were found floating. They were collected and dried using direct sunlight for three hours after which they were put into polythene bags and transferred to the laboratory.

Sample Preparation: The collected green algae were washed with distilled water for several times to remove all the dirt particles. The cleaned algae were then dried in a hot air oven (Memmert UNB 300, *Germany*) at 60 ^{0}C for 24 hrs. The dried green algae were then ground using a pestle and mortar and then sieved using +60 mesh sieve to a constant particle size. The powdered green algae were then stored in plastic bottles.

Preparation of Heavy Metals Solutions: The reagents for preparing standard solutions of Cu^{2+} and Zn^{2+} were of analar grade, procured from Sigma Aldrich Company in form of $CuCl_2.2H_2O$ and $Zn(NO_3)_2.6H_2O$ salts, respectively. 1000ppm stock solution was prepared for each of the two heavy metals using the procedure outlined by Karthikeyan *et al.* (2007).

Biosorption Experiments: A 5 gram mass of the green algae sample powder were measured accurately and transferred into

250 mL beaker containing a 100 mL of specific heavy metal at pH 4 and then stirred for about ten minutes. In order to determine heavy metal ion concentration biosorbed from the solution, 10 mL samples were taken out from the reacting vessel, filtered through whatman cellulose filter paper at specific time intervals of 10, 20, 30, 40, 50 and 60 minutes before measurement by the AAS machine, and the results were then tabulated. The same procedure was repeated for pH 7 and 9. The difference between the initial and remaining metal concentration was assumed to be taken up by the biosorbent.

Data Analysis: Data collected was processed using Ms-Excel program. To test the adsorption kinetics, the data was subjected to Langmuir and Freundlich isotherms to determine which model fitted best to the adsorption data.

III. RESULTS AND DISCUSSION

Effect of pH and Contact Time on Metal Uptake by Green Algae

(i) Effect of pH
Effects of pH on metal uptake by immobilized green algae cells involved biosorption experiments at pH values of 4, 7 and 9 with the heavy metal ions Cu^{2+} and Zn^{2+} at respective initial concentrations in ppm of 0.05, and 7.00 due to variation of the metallic detective limits.

Figures 1 and 2 belolw show that the levels of the metal biosorbed initially increased steadily and rapidly reaching equilibrium after sometime. The increase of biosorption at initial stages could be due to the enormous initial binding sites on the surface of the algae. With time, these active sites were filled up, which led to eventual reduction in the uptake of the metal ions from the aqueous solution. These observations agree with those reported by Chojnacka *et al.* (2005). The amount of metal ions remaining in solution, therefore decreased, but afterwards, remained constant because the number of binding sites became saturated with the metal ions, resulting in almost zero rate of adsorption and desorption. It was also clearly seen that for Cu^{2+}, pH 9 favoured its biosorption followed by pH 7 while pH 4 gave the lowest. Biosorption for the Zn^{2+} metal ions was similar except the suitability of pH was not consistent. Generally high pH values favoured the biosorption of the metal ions. This is due to surface of the algae which at a higher pH become negatively charged, while at a low pH value, it is positively charged (Hajar, 2009).
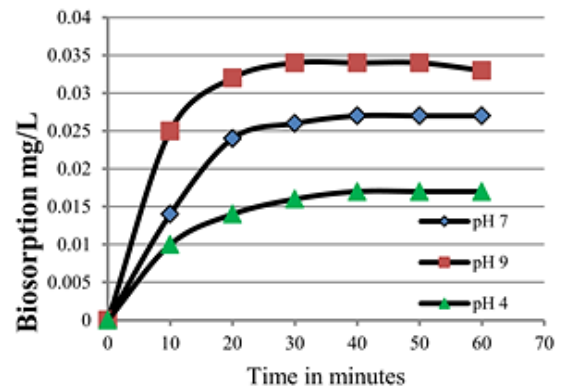

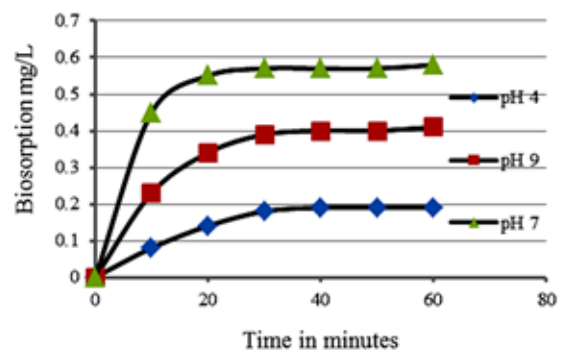Figure 1: Cu^{2+} biosorbed with time at different fixed pHs

Figure 2: Zn^{2+} biosorbed with time at different fixed pHs

(ii) Effect of Contact Time

Figures 1 and 2 show that the biosorption efficiency of the two heavy metal ions by green algae as a function of contact time increased with the rise in contact time till equilibrium was reached. The fast initial metal biosorption rate could be attributed to the surface binding while the slower sorption that followed could be as a result of the interior penetration (Bishnoi & Pant, 2004).

Different kinds of functional groups, with different affinities to the metal ions, are usually present on the biomass surface. The active binding groups with higher affinities are firstly occupied (Deng *et al.,* 2007). According to these results, a contact time of 60 min was set in order to ensure attainment of equilibrium conditions. These findings agree with those reported by Maheswari *et al.* (2008) while Karthikeyan *et al.* (2007) found a significant fraction of the total Cu^{2+} uptake was achieved within 30 minutes.

(iii) Biosorption at Specific pH Values for Metal Ions

Figures 3, 4 and 5 show the comparison of metal ions at pH values of 4, 7 and 9. It was observed that, Zn^{2+} were biosorbed mostly while Cu^{2+} were the least.
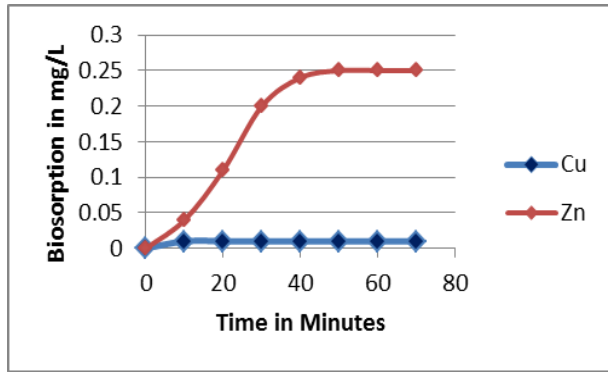
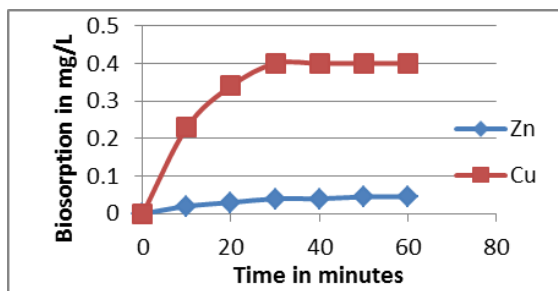**Fig 3: Variation of biosorption of metal ions with time at pH 4**

Fig 4: Variation of biosorption of metal ions with time at pH 7
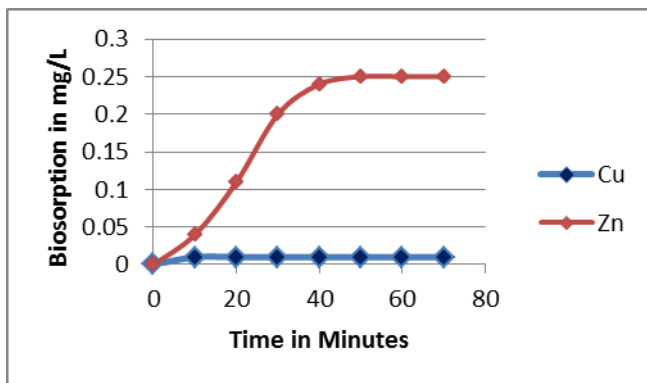

Fig 5: Variation of biosorption of metal ions with time at pH 9

At the optimum pH of around 6.5, the anion population reached saturation point hence no more metal uptake took place. These findings agreed with those of Salman and Mehdi (2011). These figures showed consistent biosorption of the metal ions at all the pH values of 4, 7 and 9, without changing the order of biosorption. The initial metal ion biosorption increased during the first few minutes and then leveled off after sometime. This is in agreement with the results reported by Sheng *et al.* (2004)

illustrating the biosorption mechanism takes place in two stages. In the first stage pseudo-equilibrium was reached, while in the second stage, a slow decrease of metal uptake took place. This decrease was, however, as a result of the metal ion crossing through the cell wall, causing intracellular accumulation since the number of binding sites of the ligands on the algae decreased (Bothwell *et al.,* 2003).

The result of the effect of pH on biosorption capacity, involving the uptake of Cu^{2+} and Zn^{2+}, onto green algae as a function of pH shows that most biosorption generally occurred within 7 ± 2 pH values for the two metal ions giving the optimum pH value of 6.5. It can be seen that Cu^{2+} was least biosorbed while Zn^{2+} was the highest.

It has been reported that biosorption of heavy metal ions is dependent on the pH of the solution as it affects the degree of ionization of the biosorbent (Davis *et al*., 2003). The pH of the solution influences both metal binding sites on the cell surface and the chemistry of metal in solution. It was evident from Figure 4.8 that as the pH of the heavy metal solution increased from 4 to 6.5, the biosorption capacity of the green algae also increased rapidly reaching an optimum value before registering a decline with one exception of Zn^{2+}. The decreasing biosorption level at a lower pH was due to competition between protons and metal ions for the same sites of the alginate polymer.

Increased proton charge (H^+) density on the sites of biomass surface at low pH values (pH 4) restricted the approach of metal cations as a result of repulsive force. In contrast, when the pH value increased, biomass surface was more negatively charged, ($R-COO^-$), and the biosorption of the metal ions with positive charge reached optimum. The decrease in biosorption at higher pH values, that is more than pH 9, may be attributed to the formation of anionic hydroxide complexes of the metal ions and their competition with the active sites. These results were in agreement with those reported by Davis *et al*. (2003).

The order of biosorption was, $Zn^{2+} > Cu^{2+}$ at all the pH values investigated. These findings were similar to those reported by Hajar (2009). In the biosorption phenomenon, pH value affects two aspects; metal ion solubility and biosorbent total charge, since protons can be adsorbed or released (Romera *et al.,* 2007). This behaviour will depend on the functional groups present on the alga cell wall, which in turn determine the state according to the following equations, 1 and 2;

$$B\text{-}H \rightleftharpoons B^- + H^+ \quad .... \; 1$$
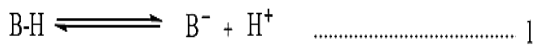
Therefore,

$$K_a = \frac{[B^-][H^+]}{[B\text{-}H]} \quad \text{and}$$

$$pKa - pH = \frac{\log[B^-]}{[B\text{-}H]} \quad .... \; 2$$

For pH values lower than pKa, equilibrium 1 shifts to the left, implying that more protons are consumed and pH increases until its value equals pKa. When the pH of the medium is higher than pKa, the opposite will happen, equation 2. At higher pH, the number of negatively charged active sites increased, facilitating a higher electrical attraction to positively charged metal ions. However, most metal ions were highly biosorbed at pH values > 4, these findings are supported by Romera *et al.* (2007) and Gupta & Rastogi (2008).

Biosorption Isotherms

Table 1, 2, 3 and 4 shows Langmuir and Freudlich isotherm data for Zn and Cu respectively.

Table 1: Langmuir Isotherm data for Zn^{2+}

Ce	Qe	Ce/Qe
0.468	4.673	0.1001
0.463	4.744	0.0976
0.397	6.011	0.066
0.371	6.541	0.0567
0.335	7.271	0.0461
0.335	7.289	0.046

Table 2: Langmuir Isotherm data for Cu^{2+}

Ce	Qe	Ce/Qe
0.043	0.22	0.195
0.042	0.219	0.192
0.035	0.4	0.088
0.031	0.42	0.074
0.029	0.48	0.06
0.025	0.56	0.045

Table 3: Freundlich data for Cu^{2+}

TIME	Ce	Qe	logCe	logQe
10	0.039	0.22	-1.409	-0.657
20	0.031	0.38	-1.509	-0.42
30	0.03	0.4	-1.523	-0.397
40	0.029	0.42	-1.537	-0.376
50	0.026	0.48	-1.585	-0.318
60	0.022	0.56	-1.657	-0.251

Table 4: Freundlich data for Zn^{2+} at pH 4

TIME	Ce	logCe	Qe	logQe
10	0.6225	-0.2059	0.0775	-1.111
20	0.6072	-0.2167	0.0928	-1.032
30	0.5554	-0.2554	0.1446	-0.8398
40	0.516	-0.2874	0.184	-0.7352
50	0.5135	-0.2894	0.1865	-0.7293
60	0.5048	-0.2969	0.1952	-0.7095

The data fitted best the Langmuir isotherm than the Freundlich since the correlation coefficients, R^2, at pHs 4 and 7 for the Langmuir were greater than 0.900. This shows that the sorption of these metal ions had a monolayer coverage on the surface of the algae. The values k are related to the strength of adsorbent-sorbate affinity, and at both pH values, k values were above unity which showed strong adsorbent-sorbate equilibrium. This implied that the sorption of heavy metal ions on the algae took place at the functional group binding sites strongly as is supported by Alkorta *et al.* (2004).

The extent of biosorption and the mechanisms involved are also known to be influenced by pH, initial metal concentration, and contact time with the green algae biomass. Metal ions are important, as the associated properties of ionic charge, size and solubility determine how effectively a particular metal is absorbed and how likely it will be displaced by a more preferred competitor (Salman & Mehdi, 2011). The data was fitted to the Langmuir model at pH 9 too and the correlation coefficient, R^2, was greater than 9.2 for all the 4 metal ions as shown in Table 4.5. This agrees with the work reported by Bulgariu *et al.* (2009). At low pH, the cell binding sites are closely linked with H^+ ions making them unavailable for metal cations. However, with an increase in pH, there is an increase in the number of ligands with negative charges which results in increased binding for cations. pH is an important parameter for adsorption of metal ions from aqueous solution because it affects the adsorption of the metal bindings to the ligands on the algae (Hamdy, 2000). The maximum monolayer coverage capacities (Q_{max}) from the Langmuir isotherm model were generally greater than one, increasing with pH. This indicated that biosorption was favourable and the data fitted well to Langmuir isotherm model. The n values between 1 and 10 represent a greater biosorption capacity; demonstrated by the three pH values of 4, 7 and 9. The

magnitude of k_f and n shows easy separation of heavy metal ion from wastewater and high adsorption capacity, respectively. The k_f values at pH 7 were high compared to pHs of 4 and 9 indicating that biosorption occurred optimally around this pH value of 6.5 also supported by Davis *et al*. (2003).

The value of n, which is related to the distribution of bonded ions on the sorbent surface, is found to be almost unity for the algae, indicating that a greater adsorption of the two heavy metals was quite favorable (Fraile *et al*., 2005). It was noted that the R^2 values for the Langmuir were much greater than those for the Freundlich at pH values of 7 and 9. Thus biosorption followed a surface monolayer adsorption mechanism. However at pH 4, the two mechanisms seemed to have played a role in the metal ion biosorption, and their R^2 values were close for both isotherms.

IV. CONCLUSION AND RECOMMENDATIONS

Conclusion

- Contact time influenced the biosorption of Cu^{2+} and Zn^{2+} ions
- From the results, maximum biosorption of Cu^{2+} and Zn^{2+} was generally observed near pH 7 (optimum pH value recorded at pH 6.5).
- The order of biosorption was $Zn^{2+} > Cu^{2+}$
- The equilibrium data for Cu^{2+} and Zn^{2+} ions followed the Langmuir isotherm.
- Green algae biomass effectively biosorbed metal ions
- The initial metal ion concentration influenced the order of biosorption.

General Recommendations

Green algae powder which can be obtained cheaply and is easily available should be used as a biosorbent for the removal of heavy metals from contaminated waste waters. A further study on other pH values at different contact times on different metal pollutants is recommended. It is also prudent that studies be carried out on other alga species to establish the best bio sorbent.

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