



***Cladophora* sp. Biosorption of Metal-contaminated Water**

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Abstract

Artisanal and Small-scale Gold Mining (ASGM) activities employing the mercury amalgamation technique have resulted in a significant drop in the quality of water from Ngwabalozi River, Zimbabwe. Mercury levels as high as 0.31 mg L^{-1} have been reported in its waters and the low river pH (pH 3) favors proliferation of microorganisms responsible for methylmercury (a potent neurotoxin) formation. Thus, removal of mercury from contaminated systems is a priority. Therefore, the aim of this study is to develop a low-cost batch reactor for the removal of mercury from mercury contaminated acidic waters to meet the World Health Organization (WHO) standard of 0.006 mg L^{-1} using *Cladophora* sp. algae. Optimum reactor parameters were determined by studying the effect of contact time, pH, initial mercury concentration and the presence of competing cations on the adsorption of mercury by *Cladophora* sp. The mass of algae required in a 1000 L batch reactor was then determined at the optimum adsorption conditions for the single and multi-component solution systems. A rapid uptake of mercury by *Cladophora* sp. algae was displayed. More than 99 % of the mercury in solution was removed within the first five minutes of contact and equilibrium was attained after ten minutes. High adsorption capacities (up to 805 mg kg^{-1} at pH 3) were obtained at the optimum mercury concentration of 1.0 mg L^{-1} . Competitive adsorption studies showed that the selectivity of cations by *Cladophora* sp. was in the following order: $\text{Hg}^{2+} > \text{Fe}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+}$. Based on the optimized reactor conditions, nearly 4.8 kg of *Cladophora* sp. per 1000 L batch is required to treat the water in Ngwabalozi River for a mercury only solution. However, for a multi-component system, the mass of *Cladophora* sp. required was more than triple (17.1 kg) the mass required in a single component solution.

Keywords: Biosorption; Algae; Mercury; Batch reactor; Acidic water

Introduction

Abject poverty has fueled a rise in Artisanal and Small-scale Gold Mining (ASGM) activities in countries such as Zimbabwe, Mozambique and Colombia. These miners prefer mercury for their activities due to its high availability and ease of use as the amalgamation process does not require any sophisticated equipment [1]. Exposure is inevitable as mercury vapour is inhaled when the gold is smelted from the gold-mercury amalgam [2]. Mercury is also dumped in the environment during mining, increasing mercury emissions. Zimbabwe has roughly 300,000 people involved in mercury-related illegal gold mining with an estimated mercury use of about 25 Mg a⁻¹ [3]. Pollution of rivers and other potable water sources is inevitable since ASGM is usually located very close to water sources, for example, Ngwabalozi River where mercury concentrations as high as 0.31 mg L⁻¹ have been reported [4]. The low pH of the river water (pH 3) favors the proliferation of anaerobic microorganisms responsible for Hg methylation into methylmercury (CH₃Hg⁺). Methyl mercury is a very toxic and soluble form of mercury, which is also easily taken up by plants and animals [5]. Therefore, mercury removal before its biological methylation is important to prevent mercury hazards to the ecosystem. Unfortunately, conventional technologies used to remove heavy metal pollutants from aqueous media are, in general, characterized by high capital and operational costs [6-9], and therefore unsuitable for developing nations such as Zimbabwe. Thus, the aim of this study is to develop a low-cost batch reactor for removal of mercury to meet the World Health Organization (WHO) standard of 0.006 mg L⁻¹ [10] using algae. Use of an alga (*Cladophora* sp.), an abundant natural biosorbent, is cheaper than industrial synthetic adsorbents, and was hence tested in this study.

Materials and methods

1) Reagents

Reagents used for the sorption studies were of analytical grade, except for standards used for quantification purposes which were high purity reagents (i.e. certified “heavy metals free”). All instrument standard solutions were prepared on the day of use. Deionized water with an electrical resistivity of 18.2 MΩ cm (Millipore, USA) was used for reagent preparation.

2) Algae sampling

A rigorous cleaning procedure as described by Monperrus et al. [11] was implemented for all vessels used in this study. Freshwater living filamentous green algae was randomly picked from Alexander Dam in Springs (Gauteng Province, South Africa) using nitrile gloves. Foreign material such as papers, grass and plant leaves which were attached to the algae were removed prior to further treatment. Algae were placed into polyethylene plastic containers containing water from the dam in which they were growing. The containers were tightly sealed and placed in refrigerated bags for transportation to the laboratory where some of the algae were treated by washing thoroughly with tap water and rinsed with deionized water until all foreign material and yellow ageing parts were removed. The rest of the algae which was not to be used immediately was stored in a refrigerator at 4 °C.

2.1) Algae characterization

Identification of the freshwater algae was carried out under a light microscope (Olympus Instruments, Melville, NY, USA) equipped with a DMX 1200 digital camera at 200 X magnification (Nikon, Japan). The various functional groups present on the algae surface before batch adsorption tests were determined using Fourier Transform Infrared (FTIR) analysis. FTIR spectra were measured directly from *Cladophora* sp. using a Tensor 27 (Bruker, Germany) device in the range of 4,000 to 400 cm⁻¹.

2.2) Algae culture

The algae samples were grown in an algal culture medium for 2-3 d and then acclimated in distilled water at room temperature and natural light for a day before use for experiments [12]. The Acidic Bold-Basal Medium (ABM) algae culture was prepared as described by Culture Collection of Algae and Protozoa (CCAP) Laboratory [13]. The solution was then autoclaved using a precise shaking incubator WIS-30 (Daihan Scientific Co., Ltd, Korea) at 121 °C and 1,000 Pa for 15 min. This was done in order to sterilize the culture media by inactivating microbial life such as bacteria [14].

3) Batch adsorption tests

All batch experiments were conducted in triplicates and for each set a control which contained the metal contaminant but lacked algae were setup so as to determine if anything besides algae caused the observed effects. Stock solutions of 10 mg L⁻¹ of Hg²⁺, Co²⁺, Cu²⁺, Zn²⁺ and Fe²⁺ were prepared by weighing appropriate amounts of the following salts: HgSO₄, Co(NO₃)₂·6H₂O, Cu(NO₃)₂·3H₂O, Zn(NO₃)₂·6H₂O and FeSO₄. Appropriate aliquots were taken from these standards for subsequent dilution to the desired concentration level. To prevent precipitation of the metals, prepared solutions were acidified using 1 % ultrapure HNO₃ (v/v) and stored in a refrigerator at 4 °C prior to the relevant experiments. Single component batch tests were performed in 250 mL conical flasks and a solution volume of 200 mL was used and competitive adsorption tests were performed in 500 mL Erlenmeyer flasks and a solution volume of 500 mL was used. At different equilibrium time intervals, 2 mL of the sample volume was withdrawn for analysis. This volume was such that the total volume drawn was less than 10 % of the initial volume used so as to minimize the change in the ratio between the metal concentration and the

sorbent mass [15]. Of the 2 mL volume collected during the batch tests, 500 µL were used for analysis. This volume was placed in acid washed 15 mL centrifuge tubes and made up to 10 mL. The dilution factor was calculated and taken into account in determining the actual mercury concentration from the results obtained from the mercury analyzer. In the batch adsorption tests, the effects of pH, initial Hg concentration, contact time and the presence of competing cations were investigated.

3.1) Effect of initial concentration

The effect of initial Hg concentration was investigated by using a stock solution of 10 mg L⁻¹ to make working solutions of 0.25, 0.50, 0.75, 0.85 and 1.0 mg Hg L⁻¹. An algae adsorbent mass of 2.0±0.05 g was used and the agitation rate was fixed at 150 rpm. The effect of initial Hg concentration was studied at ambient temperature. The contact time was varied from 0-120 min.

3.2) Effect of pH

The effect of pH on the adsorption of Hg by *Cladophora* sp. algae was investigated at the optimum concentration (1.0 mg Hg L⁻¹) by adjusting the pH of the solution to pH 3.0, 6.5 and 8.5 by using either 0.1 M NaOH or HNO₃ at ambient temperature. An algae mass of 2.0±0.05 g was used; the contact time was varied from 0-120 min at a fixed agitation rate of 150 rpm.

3.3) Effect of contact time

The effect of contact time on the adsorption of Hg by algae was investigated at the optimum pH (pH 3), optimum concentration (1.0 mg Hg L⁻¹) at ambient temperature. An algae mass of 2.0±0.05 g was used at a constant agitation rate of 150 rpm. The contact time was varied from 0 to 120 min and the Hg concentration in solution as well as the Hg adsorbed by algae (i.e. bioavailable Hg) was determined at the end of each contact time. The obtained equilibrium capacities (q_e) were then plotted against the equilibrium time for kinetic modeling.

3.4) Effect of competing cations

Competitive adsorption tests were performed by using the same procedure as that for Hg adsorption on algae in a single component solution. The effect of Hg^{2+} , Fe^{2+} , Co^{2+} , Zn^{2+} and Cu^{2+} cations on the adsorption of Hg by algae in a multicomponent solution was investigated for an algae mass of 4.0 ± 0.05 g, cation concentration of 1.0 mg L^{-1} , optimum pH (pH 3) and ambient temperature. The agitation rate was maintained at 150 rpm whereas the contact time varied from 0 to 120 min.

4) Sample preparation

After batch adsorption tests, the experimental solution containing algae was filtered through a $0.45 \mu\text{m}$ filter paper (ACE, South Africa). The treated algae were then freeze dried in a lyophilizer (Labconco, USA) at -40°C for 2 d. After drying, the samples were crushed into a fine powder using liquid nitrogen and stored in a cool dry place until further use.

5) Analytical method

A mass of 0.25 ± 0.005 g of homogenized dry algae samples were weighed using an analytical balance (Precisa 180A, Switzerland) and placed into acid washed digestion tubes (PTFE-TFM liners) into which 16 mL HNO_3 and 4 mL H_2O_2 were added before being placed into the Multiwave 300 microwave digester (Anton Paar, Switzerland). The digested samples were then transferred into 50 mL acid washed volumetric flasks and the volumes were completed with distilled water after which the samples were kept at 4°C until metal analysis. Total Hg in water and digested algae samples were determined using an automated Flow Injection-Cold Vapour Atomic Absorption Spectrometry system (FI-CVAAS) FIMS 400 (Perkin Elmer, USA) whereas the concentrations of competing cations (Fe^{2+} , Co^{2+} , Cu^{2+} , and Zn^{2+}) were obtained by using an Inductively Coupled Plasma-Optical Emission

Spectroscopy (ICP-OES) instrument (Spectro Genesis, Germany). All measurements were performed in triplicates and the mean value was reported. A certified reference material (BCR 482 Lichen) was also prepared in triplicate using the above mentioned digestion procedure and analysed to validate the used methodology.

6) Data evaluation

6.1) Extraction efficiency

The extraction efficiency (r) of Hg by algae refers to the maximum amount of Hg ions removed from solution compared to the initial concentration of Hg ions. The mass balance equation used to calculate the extraction efficiency is shown in Eq. 1 [12]:

$$r = (C_0 - C_t / C_0) \times 100\% \quad (\text{Eq. 1})$$

Where r is the extraction efficiency (%), C_0 is the initial cation concentration (mg L^{-1}), C_t is the equilibrium cation concentration (mg L^{-1}).

6.2) Kinetic models

(1) Pseudo 1st order model (Eq. 2)

$$\log(q_e - q_t) = \log q_e - (k_1 / 2.303)t \quad (\text{Eq. 2})$$

A plot of $\log(q_e - q_t)$ against time t , gives the values of q_e and k_1 on the intercept and slope of the plot respectively. Where; q_e (mg g^{-1}) is the adsorption capacity at equilibrium, q_t (mg g^{-1}) is the adsorption capacity at time t and k_1 (L min^{-1}) is the rate constant [16].

(2) Pseudo 2nd order model (Eq. 3)

$$t/q_t = 1/k_2 q_e^2 + (1/q_e)t \quad (\text{Eq. 3})$$

A plot of t/q_t against t gives a straight line and the values of q_e and k_2 are determined from the slope and intercept of the plot respectively. Where q_e (mg g^{-1}) is the adsorption capacity at equilibrium, q_t (mg g^{-1}) is the adsorption

capacity at time t and k_2 ($\text{g mg}^{-1} \text{ min}^{-1}$) is the rate constant [17].

(3) Intraparticle diffusion model (Eq. 4)

$$q_t = K_{id} t^{0.5} \quad (\text{Eq. 4})$$

A plot of q_t against $t^{0.5}$ gives a straight line, which, if not passing through the origin indicates that pore diffusion is not the rate limiting step for the adsorption of Hg^{2+} on *Cladophora* sp. algae. Where; q_t (mg g^{-1}) is the adsorption capacity at time t and K_{id} ($\text{mg g}^{-1} \text{ min}^{-0.5}$) is the rate constant for intraparticle diffusion [18].

6.3) Equilibrium adsorption capacity

The equilibrium adsorption capacities (q_e) were determined using the following mass balance equation:

$$q_e = V(C_o - C_e)/m \quad (\text{Eq. 5})$$

Where; q_e (mg g^{-1}) is the amount of metal adsorbed per gram of adsorbent (algae), V (L) is the volume of media, C_o (mg L^{-1}) is the initial metal ion concentration in media, C_e (mg L^{-1}) is the equilibrium metal ion concentration, m (g) is the mass of adsorbent (algae) used [12].

6.4) Equilibrium isotherm models

(1) Langmuir equilibrium isotherm model (Eq. 6)

$$q_e = (q_m b C_e) / (1 + b C_e) \quad (\text{Eq. 6})$$

Where; q_e (mg g^{-1}) is the amount of metal adsorbed per gram of adsorbent (algae), q_m (mg g^{-1}) is the amount of adsorption corresponding to complete monolayer coverage (i.e. maximum adsorption capacity), b (L mg^{-1}) is the adsorption constant related to binding energy and C_e (mg L^{-1}) is the equilibrium cation concentration in media [19].

(2) Freundlich equilibrium isotherm model (Eq. 7)

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e \quad (\text{Eq. 7})$$

A plot of $\ln q_e$ against $\ln C_e$ gives a straight line and the values K_f and n are determined from the intercept and slope of the graph respectively. Where q_e (mg g^{-1}) is the amount of metal adsorbed per gram of adsorbent (algae), C_e (mg L^{-1}) is the equilibrium cation concentration in media, K_f and n are temperature dependent Freundlich constants related to adsorption capacity and intensity respectively. For favorable adsorption, the values of n must be between 0 and 1 [20].

7) Equilibrium adsorption isotherm for multi-component system

(7.1) Extended Langmuir equilibrium isotherm model

The extended Langmuir equilibrium isotherm model for adsorption in a multicomponent system is given by:

$$q_{ei} = q_m b C_e / 1 + \sum_{i=1}^n b_i C_{ei} \quad (\text{Eq. 8})$$

Where q_{ei} (mg g^{-1}) is the amount of metal adsorbed per gram of adsorbent (algae), q_m (mg g^{-1}) is the amount of adsorption corresponding to complete monolayer coverage (i.e. maximum adsorption capacity), b (L mg^{-1}) is the adsorption constant related to binding energy and C_{ei} (mg L^{-1}) is the equilibrium concentration of the cations in media [21].

(7.2) Extended Freundlich equilibrium isotherm model

$$q_{ei} = k_{fi} \sum_{i=1}^n C_{ei}^{1/n_i} \quad (\text{Eq. 9})$$

Where q_{ei} (mg g^{-1}) is the amount of metal adsorbed per gram of adsorbent (algae), C_{ei} (mg L^{-1}) is the equilibrium concentration of

cation in media, k_{fi} $\text{mg g}^{-1} (\text{mg L}^{-1})^n$ is a temperature dependent Freundlich constants related to adsorption capacity and n_i is a temperature dependent Freundlich constants known as the adsorbent intensity [21].

7.3) Sips equilibrium isotherm model

The Sips equilibrium isotherm model for adsorption in a multicomponent system is given by

$$q_{ei} = K_s C_e^\gamma / 1 + \sum_{i=1}^n a_{si} C_{ei}^{\gamma_i} \quad (\text{Eq. 10})$$

Where q_{ei} (mg g^{-1}) is the amount of metal adsorbed per gram of adsorbent (algae), K_s (L g^{-1}) is the Sips isotherm constant, a_{si} (L mg^{-1}) and γ [–] are the Sips isotherm constants and C_{ei} (mg L^{-1}) is the equilibrium concentration for the cation in media [21].

8.2) Multi component system

Competitive adsorption test results were used in designing the batch reactor for a multicomponent adsorption system. Only the cation(s) which showed a significant effect on the adsorption of Hg^{2+} on *Cladophora* sp., were considered. Experimental data was fitted on the extended Langmuir, Freundlich and Sips equilibrium isotherm models. Since iron is an essential element in human nutrition, its equilibrium concentration was set at 0.3 mg L^{-1} in the model. The best fit multi-component equilibrium isotherm model equation was determined by using linear regression. The value of q_e was determined by using Solver on Microsoft Excel.

Results and discussion

Method validation results are detailed in the supplementary material.

8) Batch reactor design

In order to determine the mass of algae required in the batch reactor at different initial Hg^{2+} concentrations, two scenarios were considered: (1) single component system (Hg^{2+} only) and (2) multicomponent system (Hg^{2+} adsorption in the presence of competing cations). The following assumptions were made: (1) equilibrium is reached in the batch adsorber, (2) the equilibrium saturation capacities are correlated by the Langmuir equation; (3) equilibrium Hg^{2+} concentration is 0.006 mg L^{-1} [10]; (4) 1,000 L is to be treated per batch (5) maximum retention time is 10 min (optimum adsorption time).

8.1) Single component system

Mass of algae required in a single component system: Substituting Eq. 5 into Eq. 6 yields Eq.11.

$$m(\text{kg}) = [V(C_o - C_e)(1 + bC_e)/1000q_m bC_e] \quad (\text{Eq. 11})$$

1) Algae structure

Observations made on algae under a light microscope (Figure 1) showed that the algae collected from Alexander Dam was filamentous, with cells joined from end to end. Though this alga was not branched like the common *Cladophora* sp. (e.g. *Cladophora glomerata*), its hair-like nature and rough texture fitted well the description of *Cladophora* sp. In addition, the order of *Cladophora* sp., *Cladophorales*, stipulates that it is filamentous, with cells joined end to end in definite series, with or without branching of multinucleate cells [22].

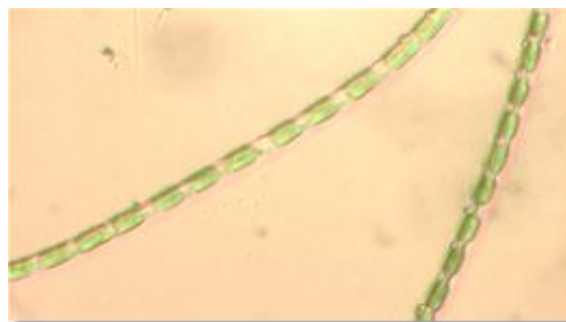


Figure 1 Algae structure.

2) Algae functional groups

From the FTIR spectrum of *Cladophora* sp. the functional groups present in algae were O-H (alcohol) observed at 3,300-3,600 cm^{-1} , N-H (amine) observed at 3,200-3,500 cm^{-1} and ν (C=O) observed at 1,780-1,680 cm^{-1} for carboxylic acids (Figure 2). For algae species, this band is shifted to lower frequencies at 1,700-1,600 cm^{-1} [23]. It has been reported that the majority of the algae functional groups responsible for metal sorption such as the carboxyl are acidic and are available at low pH [24]. Also, at high pH values, the surface charge of algae is negative due to the presence of the hydroxyl (-OH) functional group thus facilitating metal adsorption due to attraction between the negative algae surface charge and the positively charged metal cation. Therefore, with reference to the FTIR results obtained, most of the metal adsorption is expected to occur at acidic and alkaline conditions.

3) Effect of pH

pH plays a major role on the adsorption of heavy metals by algae as it influences the solution chemistry of the metals, the activity of functional groups (carboxylate, phosphate, thiol and amino groups) on the cell wall as well as the competition of cations for the binding sites [25-26]. Maximum Hg^{2+} adsorption (adsorption capacity of 805 mg kg^{-1}) was observed at pH 3 (Figure 3(a)), which is also algae point of

zero charge [27]. Due to the high adsorption capacity observed, it was concluded that under the given experimental conditions, the surface charge of alga at the point of zero charge developed as negative, thus more binding sites such as the carboxyl functional group, which are acidic, were available for mercury adsorption (Hg^{2+}). Though low pH values have been reported to result in a decrease in metal sorption due to the increased concentration of the hydrogen ions (H^+) which are preferentially adsorbed onto the alga surface compared to cations [24] the results obtained were in agreement with the findings of Le Faucheur et al. [28] who concluded that Hg^{2+} uptake by algae seems to be stimulated by proton addition after investigating the influence of pH on Hg^{2+} uptake by a green, unicellular alga, *Chlamydomonas reinhardtii*. Therefore, pH 3 was chosen as the optimum pH.

4) Effect of initial Hg concentration

As the concentration of Hg^{2+} ions increased so was the amount of Hg^{2+} ions adsorbed. This can be attributed to the increasing concentration of Hg^{2+} ions in solution that competed for a finite number of binding sites on the algae surface. A maximum adsorption capacity of 805 mg kg^{-1} was attained at an initial concentration of 1.0 mg L^{-1} and optimum pH 3 (Figure 3(b)).

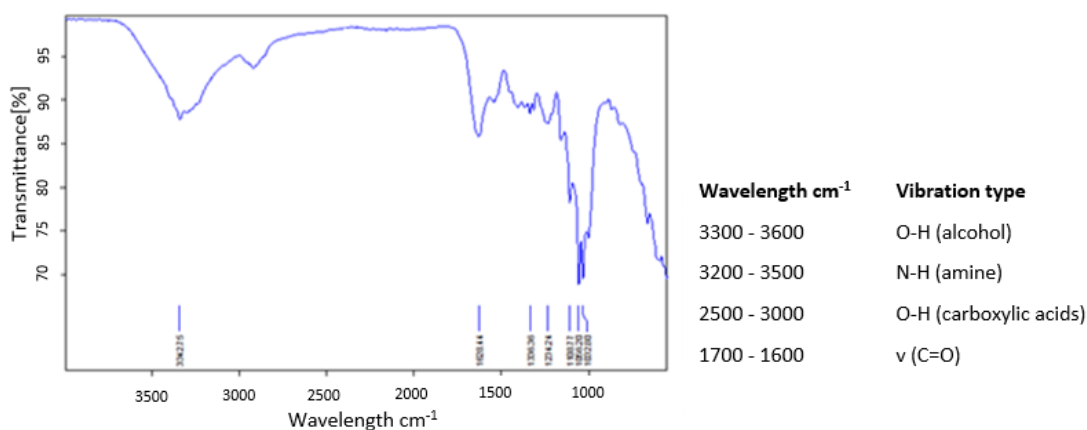


Figure 2 FTIR spectrum of *Cladophora* sp.

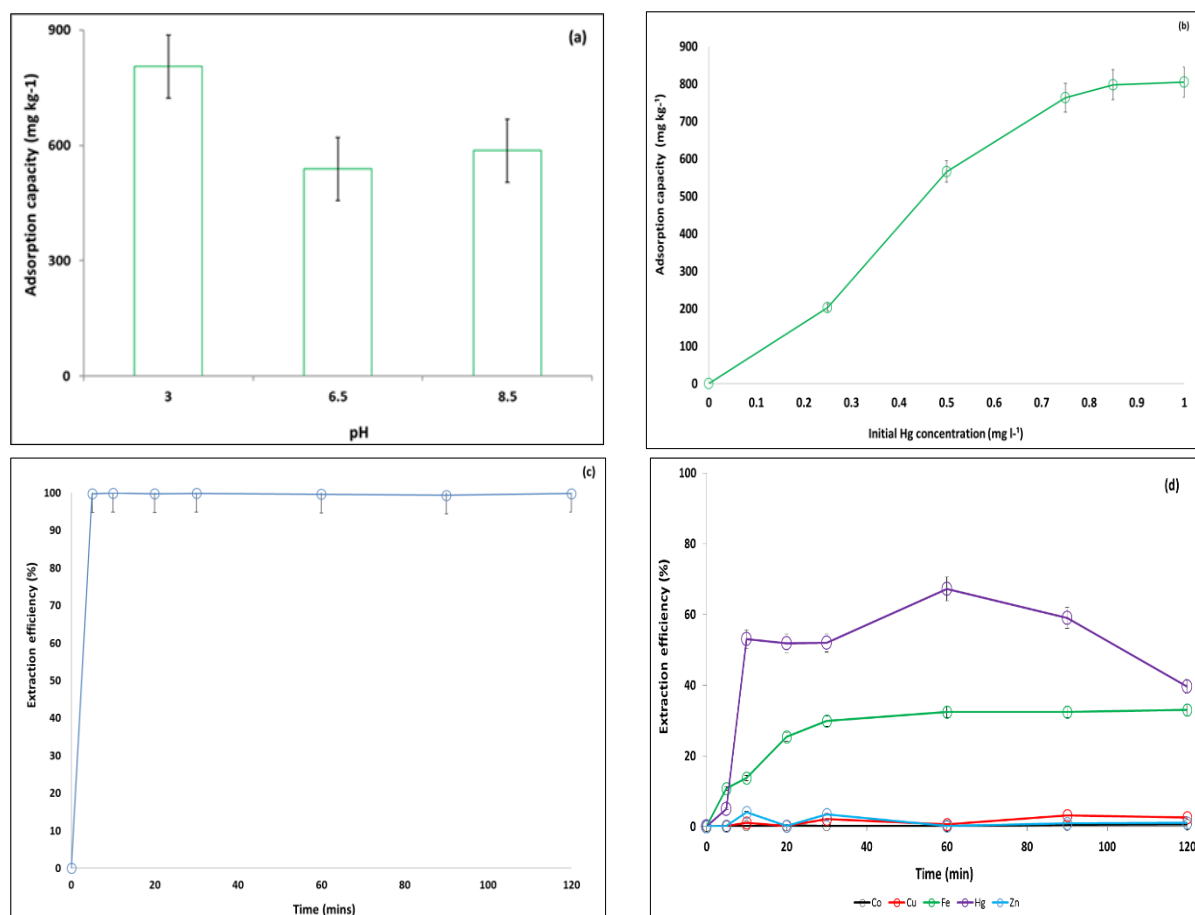


Figure 3 Effects of (a) pH, (b) concentration, (c) contact time and (d) competing cations on the adsorption of mercury on *Cladophora* sp. algae. Error bars represent the standard deviation of triplicate measurements.

5) Effect of contact time

The effect of contact time on the adsorption of Hg²⁺ by *Cladophora* sp. was investigated at the optimum pH (pH 3) and optimum concentration of 1.0 mg L⁻¹. The concentration of Hg in solution rapidly decreased within the first 5 min, attaining more than 99 % Hg removal and equilibrium was attained after 10 min which was chosen as the optimum contact time (Figure 3(c)). Mechanism of heavy metal uptake consists of passive and active uptake [24, 29]. The biosorption mechanisms are further classified according to its dependence on cell metabolism and to the location within the cell and the metal removed, although most of them are not yet fully understood [30]. The rapid uptake of Hg by algae is of great importance in environmental

applications and the observed short retention times will result in huge operation cost savings.

6) Effect of competing metal cations

The removal efficiency of Hg²⁺ decreased from greater than 99 % in a single component system to 67.2 % in a multi component system (Figure 3(d)). However, this extraction efficiency is still high compared to the results obtained by other scholars, for example, Plaza et al. [31] who studied the biosorption of Hg in the presence of Zn, Cd and Ni using a brown algae biomass and observed a significant decrease of Hg uptake by more than 50 %. Therefore, *Cladophora* sp. can be still used as a biosorbent material in environmental applications. The selectivity of cations by *Cladophora* sp. was in the following order:

$\text{Hg}^{2+} > \text{Fe}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+}$. The difference in sorption affinities may be attributed to differences in electronegativity of the atoms which also follows the same order. Therefore, the greater the electronegativity or ionic radii the higher the sorption affinity, explaining the high extraction efficiency of Hg^{2+} obtained in the presence of competing cations [32-34]. The observed trend for Hg^{2+} adsorption is most likely to be a result of adsorption and desorption occurring simultaneously. However, further work must be carried out in order to validate this assumption.

The extraction efficiency of Hg^{2+} by *Cladophora* sp. was compared with other adsorbents reported in literature at different

conditions (Table 1). Generally, for algae adsorbents, high extraction efficiencies in the range of 90-98 % were reported at low pH (pH: 4-5) [12, 25, 35] and shorter retention times in the range of 30-90 min [25, 35]. These reported results were close to the results obtained in this study. Though the cation removal efficiencies of the other adsorbents such as activated carbon from carbonized eucalyptus wood, rice husk ash and 2-mercaptobenzimidazole loaded natural clay were also high (> 90 - > 99 %), they are most efficient at high initial mercury concentrations (40-100 mg L^{-1}) [36-38], thus they are not suitable for the intended purpose of this study, since Hg is present in very low concentrations in the environment.

Table 1 Comparison of Hg^{2+} extraction efficiencies for different adsorbents

Adsorbent	Hg^{2+} extraction efficiency (%)	Conditions	Reference
<i>Cladophora</i> sp. algae	> 99	pH: 3 Room temperature Initial Hg^{2+} concentration: 1.0 mg L^{-1} Equilibrium contact time: 10 min	This study
<i>Cladophora fracta</i> algae	98	pH: 5 Temperature: 18 °C Initial Hg^{2+} concentration: 1.0 mg L^{-1} Equilibrium contact time: -	[12]
Activated carbon from carbonized eucalyptus wood	> 90	pH: 3 Temperature: 25 °C Initial Hg^{2+} concentration: 40 mg L^{-1} Equilibrium contact time: -	[38]
Rice husk ash	98	pH: 6 Temperature: 25 °C Initial Hg^{2+} concentration: 100 mg L^{-1} Equilibrium contact time: 3 h	[37]
Filamentous algae <i>Spirogyra</i> sp.	90	pH: 4 Temperature: 25 °C Initial Hg^{2+} concentration: 1.0 mg L^{-1} Equilibrium contact time: 30 min	[25]
2-mercaptobenzimidazole loaded natural clay	> 99	pH: 4-8 Temperature: 30 °C Initial Hg^{2+} concentration: 50.0 mg L^{-1} Equilibrium contact time: 8 h	[36]
<i>Sargassum Glaucescens</i> biomass	94.5	pH: 5 Room temperature Initial Hg^{2+} concentration: 0.2 mg L^{-1} Equilibrium contact time: 90 min	[35]

7) Kinetic modeling

7.1) Pseudo first and second order kinetic models

The pseudo second order model (Figure 4(b)) showed the best linearization with $R^2 = 1$ compared to the pseudo first order model ($R^2 = 0.9223$) (Figure 4(a)). Thus, chemisorption is the rate limiting step in the interaction between Hg^{2+} ions and *Cladophora* sp. In addition, the calculated adsorption capacity (q_e) for the pseudo second order model is 833 mg kg^{-1} and this is very close to the experimental value of 805 mg kg^{-1} .

7.2) Intraparticle diffusion model

The Intraparticle diffusion model exhibited two distinct regions: (1) initial pore diffusion due to external mass transfer effects ($R^2 = 1$)

followed by (2) intraparticle diffusion ($R^2 = 0.2349$) (Figure 5) [39]. However, the y-intercept does not pass through the origin, therefore, pore diffusion is not the only rate limiting step.

8) Equilibrium adsorption isotherm models

8.1) Single component system

The data obtained from the study of the effect of initial Hg^{2+} concentration was used to fit both the Langmuir and Freundlich equilibrium isotherm models as shown in Table 2. The Langmuir equilibrium isotherm model best represented the results with an $R^2 = 1.00$ compared to $R^2 = 0.02$ in the Freundlich isotherm. The maximum adsorption capacity (q_e) of *Cladophora* sp. assuming a monolayer surface was found to be $1,010 \text{ mg kg}^{-1}$.

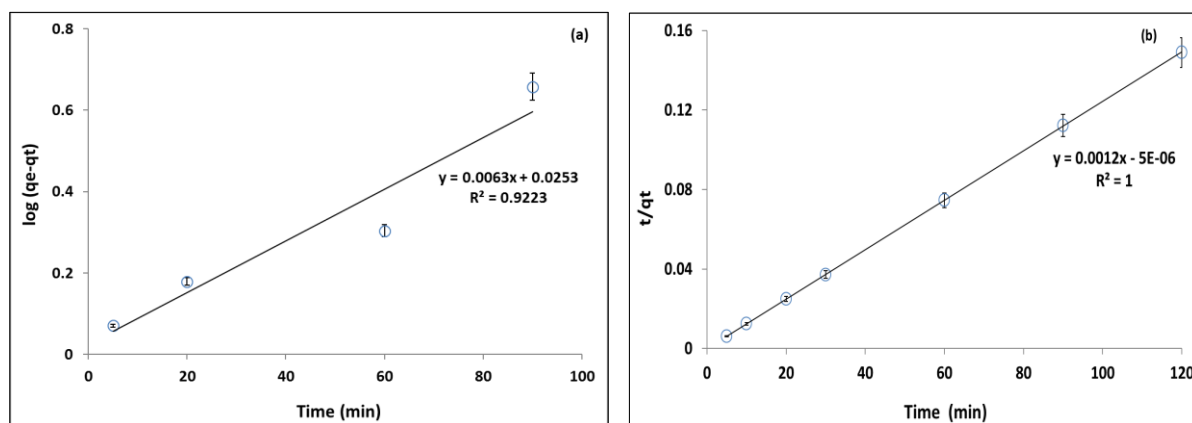


Figure 4 (a) Pseudo first and (b) pseudo second order kinetic models.

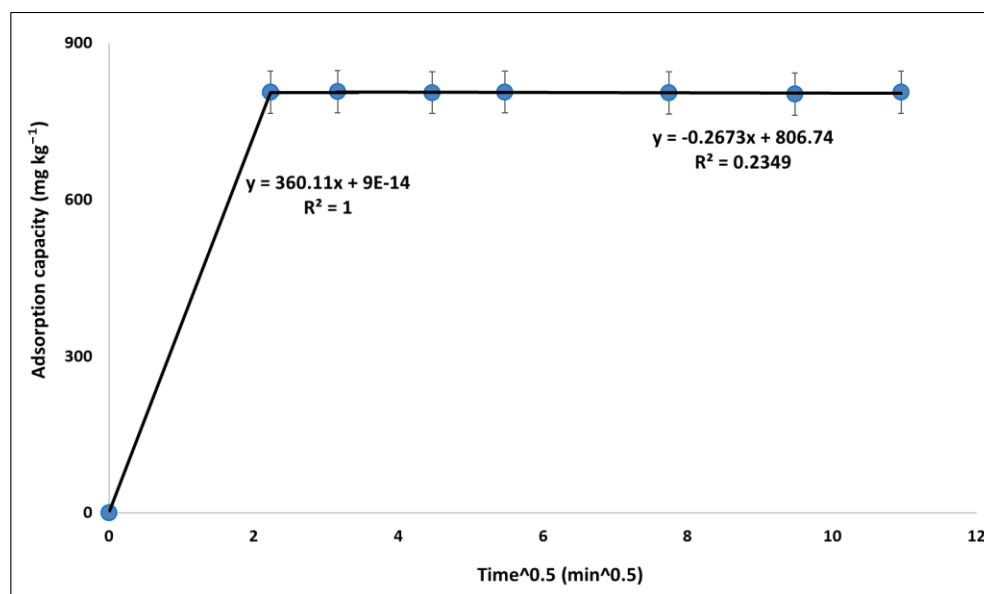


Figure 5 Intraparticle diffusion model.

Table 2 Coefficients of Langmuir and Freundlich isotherms

Langmuir isotherm			Freundlich isotherm		
R^2	q_m (mg g ⁻¹)	b (L mg ⁻¹)	R^2	K_f (L g ⁻¹)	n (-)
1.00	1.01	11.14	0.02	0.55	27.93

8.2) Multi-component system

Competitive adsorption studies showed that Fe²⁺ is the major competitor in the adsorption of Hg²⁺ on *Cladophora* sp. algae compared to (Cu²⁺, Zn²⁺ and Co²⁺). Therefore, assuming that the effect of other cations is negligible, equilibrium isotherm models were studied for a binary system (Hg²⁺ and Fe²⁺) only. The Sips equilibrium isotherm model best described the results with an R^2 value of 0.932 compared to the Extended Langmuir ($R^2 = 0.839$) and Extended Freundlich ($R^2 = 0.718$) equilibrium isotherm models (Table 3).

9) Batch reactor design

The calculated mass of algae required for the single and binary component systems are detailed in the following sections.

9.1) Single component system

The mass of algae required to reduce the different initial mercury concentrations to the WHO standard of 0.006 mg L⁻¹ increased with an increase in initial Hg concentration (Table 4). About 4.8 kg of *Cladophora* sp. algae per 1000-L batch is required to treat the

water in Ngwabalozi River which has an initial Hg concentration of 0.31 mg L⁻¹. All R_L values obtained for Hg²⁺ adsorption onto algae were in the range of 0.08 to 0.26 (Table 4). These results show that favourable adsorption of Hg²⁺ will occur at the different model scenarios.

9.2) Binary system

Using the results in Table 3, batch reactor design for a binary system was based on the Sips equilibrium isotherm model. The obtained non-linear regression equation is:

$$q_{eHg} = \frac{3.48C_{eHg}}{1+5.07C_{eHg}+0.15C_{eFe}^{0.044}} \quad (\text{Eq. 12})$$

Substituting Eq.13 into Eq.6, the mass of algae required at different model scenarios in a binary system is given in Table 4. The mass of algae required in a binary system was more than triple the mass of algae required in a single component system for all model scenarios. For the binary system, about 17.1 kg of *Cladophora* sp. algae per 1000-L batch is required to achieve the WHO standard of 0.006 mg L⁻¹ in Ngwabalozi River.

Table 3 Coefficients of extended Langmuir, extended Freundlich and Sips equilibrium isotherms

Extended Langmuir equilibrium isotherm		Extended Freundlich equilibrium isotherm		Sips equilibrium isotherm	
q_m (mg g ⁻¹)	0.790	kf_1 (l g ⁻¹)	0.511	y_2 (-)	0.044
b_1 (L mg ⁻¹)	4.415	n_1 (-)	0.040	as_2 (l mg ⁻¹)	0.1495
b_2 (L mg ⁻¹)	0.020	n_2 (-)	1.00	y_1 (-)	1.000
				as_1 (l mg ⁻¹)	5.073
				K_s (l g ⁻¹)	3.483
R^2 (-)	0.839	R^2 (-)	0.718	R^2 (-)	0.932

Table 4 Mass of algae required for the different model scenarios

$C_o (Hg^{2+}/Fe^{2+})$ (mg L ⁻¹)	$C_e (Hg^{2+})$ (mg L ⁻¹)	$C_e (Fe^{2+})$ (mg L ⁻¹)	Mass (kg) (Single component system)	Mass (kg) (Binary system)	R_L $1/(1+bCo)$ (Single component system)
0.25	0.006	0.25	3.85	13.70	0.26
0.31	0.006	0.30	4.80	17.10	0.22
0.50	0.006	0.30	7.80	27.70	0.15
0.70	0.006	0.30	10.96	41.70	0.11
0.85	0.006	0.30	13.33	50.10	0.10
1.00	0.006	0.30	15.70	55.80	0.08

Conclusion

Zimbabwe is a low-income nation whose waste management practices and technologies are still at their infancy. Its dilapidated economy makes conventional and passive heavy metal removal technologies from contaminated waters impossible due to their high capital and maintenance costs respectively. The persistence of Hg in the environment, its biogeochemical transformations in aquatic environments, especially the production of methylmercury a known neurotoxin which has a great tendency to biomagnify within the different trophic levels of the aquatic food chain; makes mercury removal from polluted potable water sources a priority. For poverty stricken communities located downstream the ASGM activities; where river and stream water is the only source of potable water for domestic use, people and animals are at risk of Hg exposure through methylmercury contaminated fish and water consumption as well as through dermal contact. The abundance, adaptability, low cost, easy cultivation and harvest of *Cladophora* sp. makes them suitable candidates for heavy metal removal from contaminated waters in poor nations such as Zimbabwe, hence, they were tested in this study. Batch adsorption test results revealed that *Cladophora* sp. algae can be used as a low cost but highly effective sorbent material for the removal of Hg from contaminated waters in both single and multi-

component solutions. More than 99 % extraction efficiency was observed at a Hg concentration of 1.0 mg L⁻¹ within 10 min at pH 3 in a single component solution. At these optimum conditions, about 4.8 kg of *Cladophora* sp. algae is required to treat 1000 L of Hg contaminated water in a batch reactor. The rapid uptake of Hg and the high extraction efficiency displayed will result in huge cost savings during industrial and domestic applications and further treatment of the water for Hg removal is not required. However, for a multi-component system, a maximum Hg extraction efficiency of 67.2 % was observed after a contact time of 60 min, for an initial cation (Hg^{2+} , Fe^{2+} , Cu^{2+} , Zn^{2+} and Co^{2+}) concentration of 1.0 mg L⁻¹ and pH 3. The observed extraction efficiency is still high compared to other studies in literature, for example, Le Faucheur et al [28]. The adsorption selectivity of cations by *Cladophora* sp. was in the order: $Hg^{2+} > Fe^{2+} > Cu^{2+} > Zn^{2+} > Co^{2+}$. In addition, about 17.1 kg of *Cladophora* sp. is required for Hg adsorption from a multicomponent solution in a 1000 L batch reactor.

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