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Biosorption of Heavy Metals Contaminating theWonderfonteinspruit Catchment Area using *Desmodesmus sp.*

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***Abstract*—**A vast array of biological materials, especially algae have received increasing attention for heavy metal removal. Algae have been proven to be cheaper, more effective for the removal of metallic elements in aqueous solutions. A fresh water algal strain was isolated from Zoo Lake, Johannesburg, South Africa and identified as *Desmodesmus sp*. This paper investigates the efficacy of *Desmodesmus sp.*in removing heavy metals contaminating the Wonderfonteinspruit Catchment Area (WCA) water bodies. The biosorption data fitted the pseudo-second order and Langmuir isotherm models. The Langmuir maximum uptakes gave the sequence: Mn^{2+}>Ni^{2+}>Fe^{2+}. The best results for kinetic study was obtained in concentration 120 ppm for Fe^{3+} and Mn^{2+}, whilst for Ni^{2+} was at 20 ppm, which is about the same concentrations found in contaminated water in the WCA (Fe^{3+}115 ppm, Mn^{2+} 121 ppm and Ni^{2+} 26.5 ppm).

***Keywords*—**Biosorption, Green algae, Heavy metals, Remediation.

I. Introduction

THE first goldfields of the Witwatersrand basin were discovered in 1886, in a place called *Highveld* [1]. Only a year later, gold mining reached the Wonderfonteinspruit Catchment Area (WCA), situated 30 km west of Johannesburg. Gold-mining in the WCA inspired extensive urbanisation, transforming largely rural and under-developed areas into densely-populated regions. Westonaria, Randfontein and Carletonville are some of the towns which owe their very existence to gold mining in the WCA [1]. Although mining became the most importance source of direct or indirect income in the area, it also brought with it the curse of environmental degradation. It is now the major cause of environmental devastation after about 120 years of mining. The consequence of mine closure was not only observed in large-scale land degradation, but also in widespread pollution of surface water and groundwater in this area. Thus, clean-up methods must be developed in order to remove heavy metals from contaminated water bodies in this area.Methods for removing metal ions from aqueous solutions mainly consist of physical, chemical and biological technologies. However, chemical precipitation and electrochemical treatment are ineffective, especially when metal ion concentration in aqueous solution is between 1 and 200 mg/L [2]. This process also produces a large quantity of sludge required to be treated with great difficulty. In recent years microbial processes have started to be used in the cleanup of radioactive and metallic contaminants from soil and water through biotransformation, biodegradation and biomineralization [3], [4], [5] and [6]. Depending on the site and its contaminants, bioremediation may be safer and less expensive than alternative conventional solutions [7]. Biological remediation techniques, such as the use of algae, offer the potential for highly selective removal of toxic metals; in addition, they can also be used both *in situ* and *ex situ*. The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms. The process continues until equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. Metal ions in solution bind passively onto algal cells and this occurs mainly on functional groups present on the cell walls. Possible biosorption mechanisms are: chemisorption, complexation, surface adsorption-complexation, ion exchange and microprecipitation [8]. Physical sorption is due to weak Van der Waals forces, whereas chemical sorption is due to electron exchange and formation of chemical bonds. There are several chemical groups that would attract and sequester the metals in biomass: acetamido groups of chitin, amino and phosphate groups in nucleic acids, amido, sulphhydryl, carboxyl groups in proteins and hydroxyls in polysaccharide. However, it does not necessarily mean that the presence of some functional group guarantees biosorption, perhaps due to steric, conformational or other barriers.

In this work, the biosorption of some (iron, manganese and nickel) of the metals contaminating the WCA on live algal cells is investigated. The maximum metal uptake and the biomass affinity for a certain metal will be obtained from the sorption isotherms: Langmuir and Freundlich. Two different models will be used to describe the biosorption kinetics of the biomass: the pseudo-first order model proposed by Lagergren and the pseudo-second order model proposed by Ho [9] and [10]. The algae was sampled from Zoo Lake, Johannesburg, South Africa, it was identified by CTA-extraction techniqueand characterised using Fourier transform infrared spectroscopy (FTIR) to determine possible metal binding mechanisms.

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II. MATERIALS AND METHODS

2.1 Preliminary study: Determination of heavy metals contaminating the WCA waters

Sampling of the WCA took place in the following locations: Hippo Dam; Donaldson Dam; Aviary Dam and Robinson Lake, see Fig. 1 (a) – (d). This exercise helped in estimating how severe the pollution is within the area. When the metal concentrations present in the water were known, solutions with similar concentrations were prepared using reagents of analytical grades, for the laboratory experiments. Robinson Lake showed to be the worst affected of the four water bodies, with results showing that Manganese (Mn – 121 ppm), Nickel (Ni – 27 ppm), Uranium (U – 2.01 ppm) and Iron (Fe – 115 ppm) were present in the lake in concentration way higher than the regulated limit by the Department of Water Affairs (DWAF). Fe, Mn, Ni, and U exceed the regulated limit by 576, 1211, 177, and 5048 times; respectively. Robinson Lake had the lowest measured pH at 2.4 followed by Hippo and Aviary Dams, at 3.98 and 3.1; respectively. An acceptable pH level was found in Donaldson Dam at 6.84, however the dam was found to have alarmingly high dissolved U, which still makes the dam hazardous for domestic and recreational use. Therefore since metals concentrations are relatively high in Robinson Lake; it was used as a reference when preparing metal solutions for laboratory experiments.

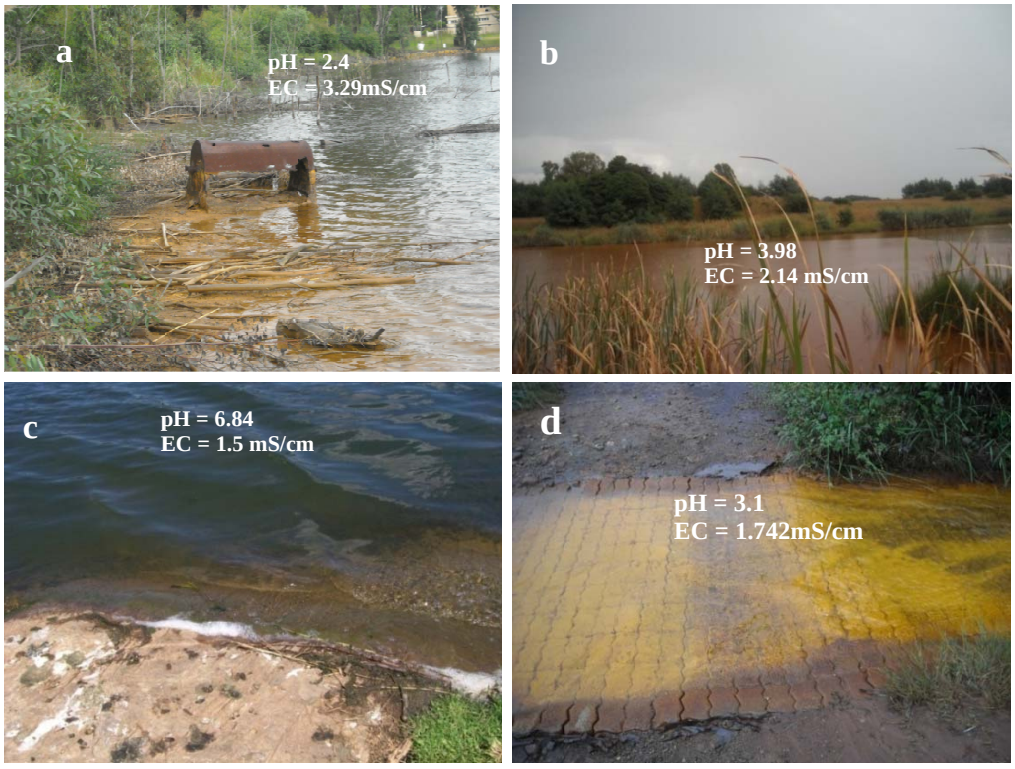


Fig. 1 (a) Robinson Lake; (b) Hippo Dam; (c) Donaldson Dam, (d) Aviary Dam

2.2 Characterization of fresh water algae and culturing

Samples of fresh water algae were collected from Zoo lake ponds, Johannesburg in South Africa. CTA-extraction based on [11], for the purpose of algal identification was done for the sample.

- *Zoo lake algal identification procedure*

The identification of the Zoo Lake algae was carried out by RuhanSlabbert from the University of Stellenbosch. The barcoding cytochrome c oxidase subunit1 (COI or coxl) region and internal transcriber region 2 (ITS2) were amplified in an attempt to identify theunknown algae sample. Primer pair Gaz F1/Gaz R1 was used (Table 1). The Polymerase Chain Reaction (PCR) reactants were prepared as follows: 1 x Epicentre Failsafe Premix A (Separations), 0.5µM of each primer, 0.25U Failsate Enzyme and 20 ng algae DNA.

The PCR was performed in a Geneamp 9700 (Applied Biosystems) with the following conditions stage 1: 95 ºC for 5 minutes, stage 2: 40 cycles consisting of 95 ºC at 30 seconds, stage 3: 45 ºC at 50 minutes 72 ºC for 60 seconds and stage 4: the final extension of 72 ºC for 10 minutes.

PCR purification was done using the NucleoFast Purification System (Separations). Sequencing was performed with each primer (Gaz F1/Gaz R1) and BigDye Terminator V1.3 (Applied Biosystems) followed by electrophoresis on the 3730xl DNA Analyser (Applied Biosystems).

TABLE I
PRIMER SEQUENCES USED FOR ALGAE IDENTIFICATION

Primer Name	Region	Primer Sequence	Reference
Gaz F1	COI	TCAACAAATCATAAAGATA TTGG	[11]
Gaz R1		ACTTCTGGATGTCCAAAAA AYCA	
ITS 03F-800	ITS	CGATGAAGAACGYAGCGA	[11]
ITS 05R-700		TACTTGTTCGCTATCGGTCT CT	

Metal ions concentrations in solution were determined by atomic adsorption spectrophotometry (Spectro AA, 55B Varian). Adsorbate was aspirated into the spectrophotometer, where metal ions present in solution were ionised in an air and acetylene flame environment until a corresponding concentration was recorded on the screen. The wavelengths and slit widths were varied to suit the optimum working range required for the particular metal ion. Before every run, the equipment was calibrated using known high grade standard solutions and the appropriate lamp until a curve was obtained, which was in agreement with the calibration curves in the AAS working manual.

FT-IR was the preferred method for infrared spectroscopy in this study. An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. All materials have a unique combination of atoms; therefore no two compounds produce the exact same infrared spectrum. The size of the peaks in the spectrum is a direct indication of the amount of material present. The functional groups essential for biosorption process of fresh water biomass will therefore be characterised using FT-IR.

All vessels used for culturing were autoclaved (HA-300 MD, HICLAVE, autoclave) at 0.1MPa and 190 ºC, to inactivate any microbials. The fresh water algae's natural environment was resembled by using Beijerinck medium for culturing as obtained from (Barsanti&Gualtieri, 2006). The media was prepared from premixed stock solutions in a 1L erlenmeyer flask. CO_2 gas was bubbled at the rate of 50 ml/min into the culturing medium for an hour daily. The bubbling of CO_2 is necessary as it is required for photosynthesis to occur, also CO_2 gas bubbling ensures that the CO_2/HCO^{-3} balance is maintained, which is essential for algae growth. Sylvania, Gro-lux (F35W/GRO-T8) lights were used for illumination during the culturing process. Agitation / mixing were induced by a MS 300, Boeco Germany, magnatic stirring plate. The successive growth of the algae population under batch culture conditions was monitored using a 4802 UV / VIS Double beam spectrophotometer. The Beijerinck medium was further modified to culture stock algae utilized for batch experiments with different metal concentrations. Ni^{2+},Mn^{+2} andFe^{3+} salts were omitted in micronutrients used to prepare media for stock algae Ni^{2+}, Mn^{2+} and Fe^{3+} respectively.

2.3 Biosorption isotherm

The experiments were carried out with 0.01 g initial biomass, at initial pH values of 2.4 and at different initial metal concentrations: 5, 10, 25, 50, 100 and 130 mg/L. Tests were run for 120 minutes on an orbital shaker, enough time to reach equilibrium. After 120 minutes elapsed, the possible change of metal concentrations in solution was then measured using an AAS. The experimental results were fitted to the Langmuir and Freundlich sorption isotherm models. Equilibriumisotherm models are usually classified into the empirical equations and the mechanistic models.Equilibrium isotherm models are usually classified into the empirical equations and the mechanistic models. The mechanistic models are based on mechanism of metal ion biosorption, which are able not only to represent but also to explain and predict the experimental behavior [13], [14]. These models can provide information of metal uptake capacity and difference in metal uptake between various species [15], [13], [16]. The competence of these equations to interpret the sorption experimental data can be observed from its parameter values (*n* for Freundlich and *b* for Langmuir), which both represent the relative magnitude and diversity of energies associated with a particular sorption process in a certain range where the initial assumptions are still valid.

Langmuir model was derived on assumptions that: (i) maximum adsorption corresponds to a saturated monolayer of adsorbate molecule on the adsorbent surface; (ii) the adsorption sites and reactions have a constant free-energy change (ΔG^{o}_{ads}) for all sites; and (iii) there is no transmigration of adsorbate in the plane of the surface [17]. With Langmuir two parameters will be analysed to evaluate the efficiency of the system: q_{max}, maximum adsorption capacity (mmol/g) and *b*, the energy of adsorption (L/mg). This model has the form as shown in Eqs 1 and 2:

$$qe = \frac{bq\max Ce}{1+bCe} \quad (1)$$

$$\frac{Ce}{qe} = \frac{Ce}{q\max} + \frac{1}{bq\max} \quad (2)$$

Where *qe*, is the amount adsorbed at equilibrium and q_{max}, is the Langmuir constant, which is equal to the adsorption capacity. The parameter*b*represents the Langmuir sorption equilibrium constant and *Ce* is the equilibrium concentration. The Freundlich isotherm is based on these assumptions: (i) the adsorbent has a heterogeneous surface energy, where different sites could have different site energies, as opposed to the Langmuir assumption of constant site energy; (ii) the site

energies for adsorption follow a Boltzmann distribution and the mean site energy = ΔH^{o}_{M} and; (iii) the change in adsorption site entropy increases linearly with increase site enthalpy ($-\Delta H^{o}_{ad}$) [18].The Freundlich equation can be written in the following form (Eq. 3):

$$\log Qe = \log K_f + \frac{1}{n}\log Ce \qquad (3)$$

Where, K_f and n are the Freundlich constants. The parameter K_f indicates the Freundlich adsorption capacity, while the parameter n characterizes the heterogeneity of the system. The parameter n is usually greater than unity. A larger *n* value, means that the system is more heterogeneous, which usually results in non-linearity of the adsorption isotherm.

2.4 Kinetic study

The biosorption experiments were performed with monometallic solutions prepared from stock solutions of 1000 mg/L using chemical reagents of analytical grade: $FeCl_3.6H_2O$; $MnCl_2.3H2O$ and $NiCl_2$. The initial pH of the solutions (~2.4) was adjusted with concentrated HCl. Kinetic studies were carried out on an orbital shaker at 180 rpm with 50 ml of algae solution in 250 mL Erlenmeyer flask and 5, 20 and 120 mg/L initial concentrations of the metals, at room temperature. Samples of the metal solution were removed at different time intervals: 5, 10, 15, 30, 60 and 120 minutes. The pH and the biomass concentration were measured per time interval.

In order to achieve the proper design of an adsorber, the adsorption equilibria need to be supplemented with adsorption kinetics. Several kinetic models, namely pseudo-first and second order, saturation type, Weber and Moris, as well as the Elovich model, are available. Pseudo-first order kinetic model is given as Eqs. (4) and (5), whilst Pseudo second-order kinetic models are given as Eqs. (6) and (7).

$$\frac{dQ}{dt} = k(Q_e - Q) \qquad (4)$$

which can be integrated to give,

$$\ln(Q_e - Q) = \ln Q_e - kt \qquad (5)$$

And,

$$\frac{dQ}{dt} = k(Q_e - Q)^2 \qquad (6)$$

which can be integrated to give,

$$\frac{t}{Q} = \frac{1}{kQ_e^{\,2}} + \frac{1}{Q_e}t \qquad (7)$$

Where, Q_e is the amount adsorbed at time t. Parameter k is a reaction constant for pseudo-first and pseudo-second order. These equations were developed based on the sorption capacity of the solid phase. From a literature survey, it seems that the pseudo-second order model is more prevalent in comparison to the pseudo-first order model, as a significant disagreement between pseudo-first order model prediction and experimental data has been observed. Further, this trend suggests that the ratelimiting step in heavy metal biosorption is chemisorption, which involves valence forces through the sharing or exchange of electrons between sorbent and sorbate, complexation, coordination and/or chelation, rather than physisorption.

III. RESULTS AND DISCUSSION

3.1 Characterization of fresh water algae

Zoo Lake algae was found to have a percentage identity of 100 % to that of genus *Desmosdesmus sp. / Scenedesmus sp.* After visual inspection of the unknown sample under a microscope and comparing it to the genus on the internet it was found to be a good match in terms of size and form (Fig. 2).

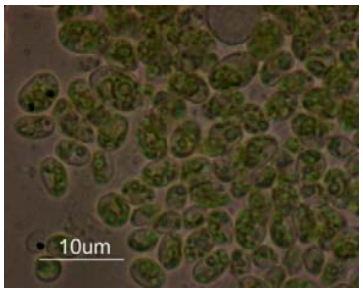


Fig.2 View of *Desmodesmus sp.* under light microscope(Taken by Edmore Kativu, Witwatersrand University)

This genus is ubiquitous in freshwater habitants as single to 32-celled coenobia and is phenotypically plastic [19]. The Zoo Lake algae was therefore used for all the experimental work in this study.

The spectral data were processed using OPUS software. Infraredspectra were recorded in the region of 500 – 4000 cm^{-1} at a resolution of 4 cm^{-1}. Fig. 3 shows functional groups present on the algae cell walls of the Zoo Lake algae. As shown in Fig. 3, the algae sample had at least six functional groups, namely: Alcohols, esters, ethers (C-O – 1045.427 cm^{-1}); Amines (C-N – 1190.089 cm^{-1}); Alkanes (C-H – 1473.628 cm^{-1}); Carboxylic acids (C=O – 1645.29 cm^{-1}); Alkynes (C=C – 2129.43 cm^{-1}) and hydroxyls (O-H) – 3373.52 cm^{-1}. The class of compounds present in the algae are therefore: carbohydrates; fatty acids; proteins; organic acids; nucleic acids; lipids and polysaccharides.

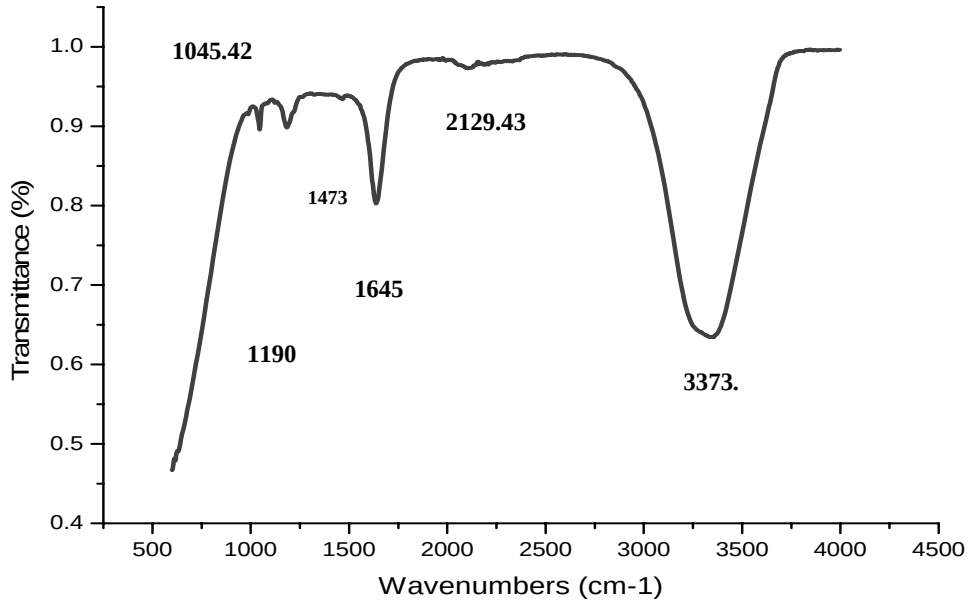


Fig.3 FTIR spectra of *Desmosdesmus sp.*

3.2 Biosorption isotherms

The biosorption isotherms represent the relationship between the amounts of solute adsorbed by a unit mass of solid sorbent and the amount of solute remaining in the solution at equilibrium. The parameters determined for the biosorption of Fe^{3+}; Mn^{2+} and Ni^{2+} on algae cells at different initial concentrations are tabulated in Table 2.

TABLE II
CHARACTERISTIC PARAMETERS AND COEFFICIENTS OF THE EXPERIMENTAL DATA ACCORDING TO LANGMUIR EQUATION

Metal	Qmax(mg/g)	b (1/mg)	R^2
Fe^{3+}	1.523	0.1675	0.9758
Mn^{2+}	144	0.086	0.8366
Ni^{2+}	71.94	0.101	0.9445

The values of R^2 indicate that the Langmuir model can be best used to describe the biosorption of Fe^{3+} and Ni^{2+} on algae cells. The maximum sorption capacity Q_{max}, is a function of many parameters such as pH and temperature, it provides a good measure for comparing the efficiency of different sorbents in removing a given metal. Therefore maximum biosorption capacities of algae cells for Fe^{3+}, Mn^{2+}, and Ni^{2+}were, 1.523 mg/g; 144 mg/g and 71.94 mg/g, respectively. According to *b* (1/mg) parameter the affinity of metals on algae cells produced this sequence: $Mn^{2+} > Ni^{2+} > Fe^{3+}$.

The sorption equilibrium constant; *b,* which is a measure of heat of adsorption is utilised to calculate dimensionless separation parameter R_L [20]. Weber and Chakarborti [20] expressed characteristics and the feasibility of Langmuir isotherm in terms of dimensionless constant separation factor R_L, which is defined in Eq. 8.

$$R_L = \frac{1}{1 + bC_0} \tag{8}$$

Where *b* is the Langmuir constant and C_0 is the initial concentration of Fe^{3+} and Ni^{2+}. The value of separation factor, R_L provides information about nature of adsorption [21]. The initial concentration used to calculate this value in this experiment was 5 mg/L. In the current study, the average value of separation parameter is found to be 6.153 and 0.664; for Fe^{3+} and Ni^{2+}, respectively. Ni^{2+} represented a favourable and reversible adsorption on algal cells, but Fe^{3+} adsorption on algal cells was found to be unfavourable.

TABLE III
DETERMINATION OF THE SEPARATION FACTOR

R_L value	Type isotherm	R_L (Fe^{3+})	R_L (Ni^{2+})
$R_L > 1$	Unfavourable	6.153	-
$R_L = 1$	Linear and reversible	-	-
$0 < R_L < 1$	Favourable and reversible	-	0.664
$R_L = 1$	Irreversible	-	-

The biosorption isotherm of metals with algal cells was also investigated using the Freundlich model. Table 4, summarises the parameters obtained from the equation. The Freundlichisotherm is among the earliest empirical equations employed to predict adsorption equilibrium data. According to

the K_f parameter, biosorption of metals on algae cells is produced following the sequence: Ni^{2+}> Mn^{2+}> Fe^{3+}.

TABLE IV
CHARACTERISTIC PARAMETERS AND COEFFICIENTS OF THE EXPERIMENTAL DATA ACCORDING TO FREUDLICH EQUATION

Metal	K_f	n	R^2
Fe^{3+}	0.011	0.221	0.9376
Mn^{2+}	2.73	1.145	0.9863
Ni^{2+}	4.01	1.626	0.7988

According to the K_f parameter, biosorption of metals on algal cells produced the following sequence: Ni^{2+}> Mn^{2+}> Fe^{2+}. According to the equilibrium constants, the metals affinities for algal cells followed the sequence: Ni^{2+}> Mn^{2+}> Fe^{2+}. A similar order can be deduced from the rule of Irving Williams [8]. The rule describes the stability of complexes between metal cations and oxygen donor groups (such as carboxyl and hydroxyl groups) [22]. The equilibrium constant, increases with bond strength [23] [24], thus Ni^{2+}formed the most stable complex with alga whilst Fe^{3+} the weakest. The steps correspond to the dissociation of the complexes formed between metals in solution and water hydronium ions followed by the interaction of metal with algal functional groups [25].

3.3 Kinetic study

Fig. 4, shows the evolution of metal uptake during the biosorption of Fe^{3+}, Mn^{2+} and Ni^{2+}, with algal cellsin different batch solutions. The biomass in contact with each metal solution decreased the metal concentration until an equilibrium concentration was reached after two hours. The maximum adsorption capacity of Fe^{3+}, Mn^{2+} and Ni^{2+} are shown in Fig. 4. The experimental data were fitted to the pseudo first and pseudo second order kinetic model, only pseudo first-order and second-order fitted linearly for the experimental data (Fig.5 and 6). The plots were for initial concentration of 120 mg/L. Although both plots fitted linearly for the experimental data, the best fit was however observed for pseudo second order. This is due to the correlation coefficient (R^2) being better at 0.999, 0.9787 and 0.9753 for Fe^{3+}, Mn^{2+} and Ni^{2+}, respectively. The pseudo first order however had R^2 of 0.369, 0.196 and 0.539 for Fe^{3+}, Mn^{2+} and Ni^{2+}, respectively (Table 5). The corresponding kinetic parameters derived from these models are shown in Table 5 and 6. Since the best fit was observed in the pseudo-second order, this indicates that the biosorption occurred in at least two steps.

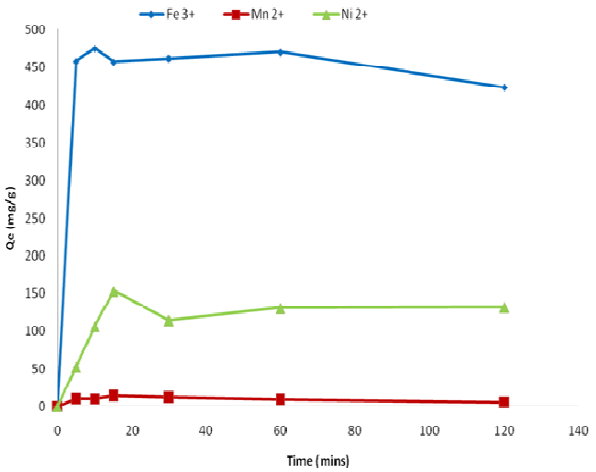


Fig. 4 Evolution of metal uptake of iron, manganese and nickel

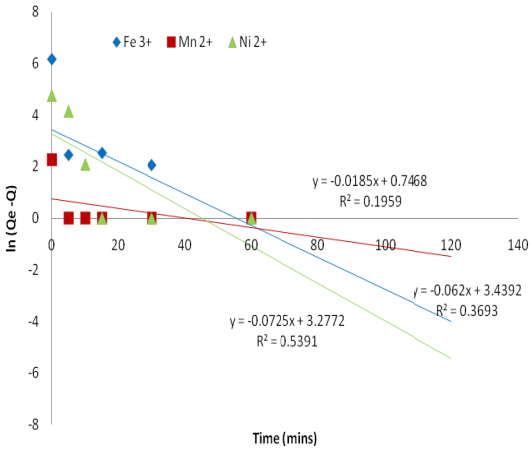


Fig. 5 Pseudo first-order plot for metals in solution with initial concentration of 120 mg/L.

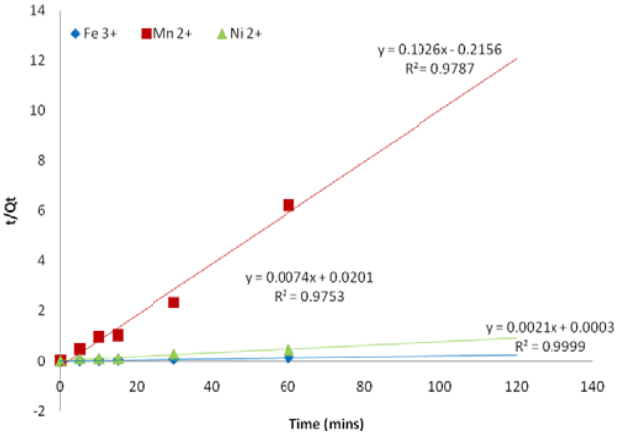


Fig. 6 Pseudo second-order plot for metals in solution with initial concentration of 120 mg/L.

The steps correspond to the dissociation of the complexes formed between metals in solution and water hydronium ions followed by the interaction of metal with algae functional. The kinetic uptake (K_f) for second order at 120 mg/L, followed the sequence: Ni^{2+}>Fe^{3+}> Mn^{2+}, which is inversely proportional to cation size (Ni^{2+} 0.124 nm; Fe^{3+} 0.135 nm, and Mn^{2+} 0.140 nm). The sequence of kinetic rates (n) for secondorder at 120 mg/L is inconsistent with metal uptakes: Mn^{2+} > Fe^{3+}> Ni^{2+}.

TABLE V
KINETIC PARAMETERS FOR THE BIOSORPTION OF Fe^{3+}, Mn^{2+} and Ni^{2+}WITH *Desmodesmus sp.* FOR A BINARY SYSTEM

Metal ions	Parameters	Initial concentration Pseudo 1st order		
		5 mg/L	20 mg/L	120 mg/L
Fe^{3+}	R^2	0.651	0.104	0.3693
	k	0.0447	0.0238	0.0062
	Q_e	9.136	6.205	31.162
Mn^{2+}	R^2	0.5741	0.9342	0.1959
	k	0.0046	0.2858	0.0185
	Q_e	6.594	400	2.110
Ni^{2+}	R^2	0.784	0.857	0.5391
	k	0.223	0.1279	0.0725
	Q_e	26.61	36.039	26.5

TABLE VI
KINETIC PARAMETERS FOR THE BIOSORPTION OF Fe^{3+}, Mn^{2+} and Ni^{2+}WITH *Desmodesmus sp.*FOR A BINARY SYSTEM

Metal ions	Parameters	Initial concentration Pseudo2nd order		
		5 mg/L	20 mg/L	120 mg/L
Fe^{3+}	R^2	0.9001	0.9654	0.9999
	k	0.013	0.05	0.015
	Q_e	8.5837	31.15	476.19
Mn^{2+}	R^2	0.9404	0.9436	0.9787
	k	0.0093	0.9836	0.9753
	Q_e	12.82	99.01	13.3
Ni^{2+}	R^2	0.9749	0.9836	0.9753
	k	0.02	0.07	0.003
	Q_e	11.83	34.8	135.135

Fig. 7, shows the evolution of metal concentration during the biosorption of Fe^{3+}, $Ni^{2+}$and Mn^{2+} with *Desmodesmus sp.* in the same batch system. The biomass in contact with each metal solution decreased the metal concentration until an equilibrium concentration was reached after two hours. Mn^{2+}, Fe^{3+} and Ni^{2+} showed a decrease in solution in 5 minutes, with Fe^{3+} decreasing from 115 mg/L to 2.23 mg/L ; from 121 mg/L to 31.787 mg/L and 26.5 mg/L to 22.62 mg/L for Fe^{3+}, Mn^{2+} and Ni^{2+}, respectively. After 5 minutes it is then assumed that most of the functional groups available sites were occupied following the fluctuating behaviour of Mn^{2+} removal over two hours. Ni^{2+} was biosorbed at 14.605 mg/g with Fe^{3+} at 523 mg/g and Mn^{2+} at 364.73 mg/g (Fig. 8). It is observed that the biosorption capacity of the algal cells is in agreement with the metal rate removal in solution. The preference or selectivity of metals by algae is administered by the number of functional groups present in the algae cell walls.

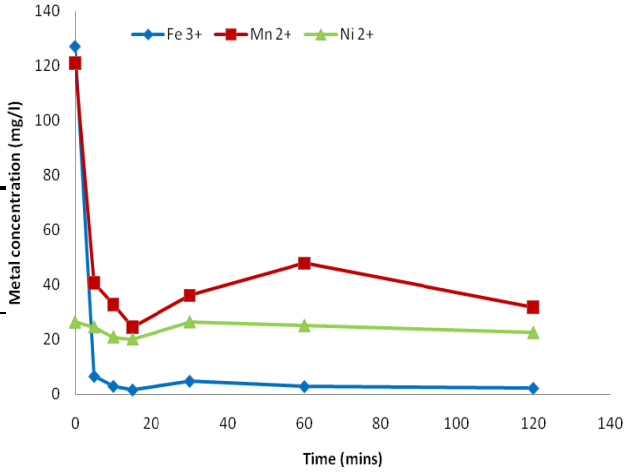


Fig. 7 Evolution of metal concentration as a function of time in a ternary metal solution.

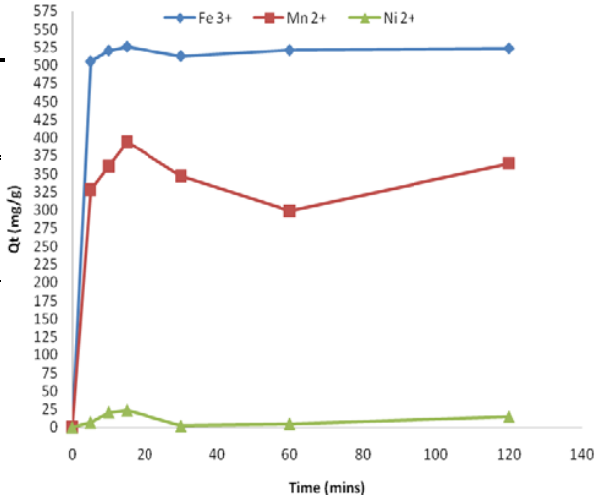
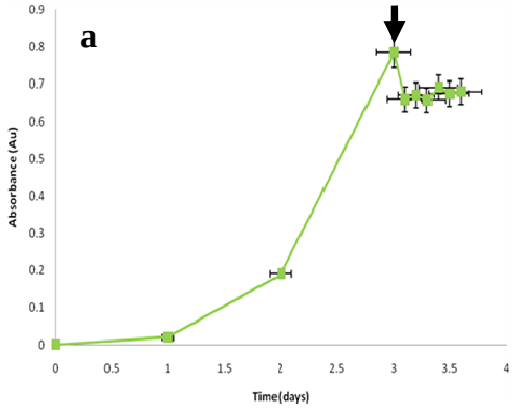
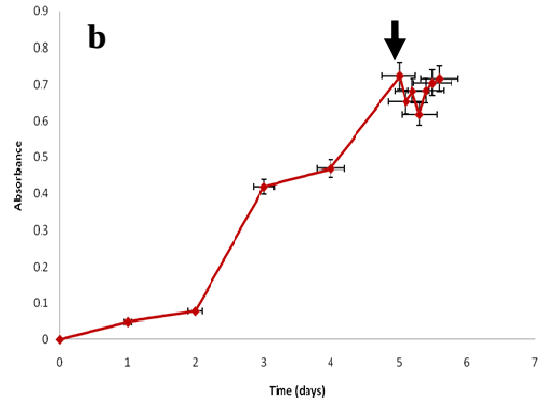


Fig. 8 Metal uptake capacity of *Desmodesmus sp*.

Desmodesmus sp. has six functional groups from the IR characterisation: ester (C-O), amines (C-N), alkanes (C-H), carboxyls (C=O), hydroxyls (O-H) and alkyne (C≡C). Ester and amides are ligands of Class B (which form strong bonds with hard ions), whereas carboxyls and hydroxyls are ligands of Class A (form strong bonds with soft ions). Fe^{3+}, Mn^{2+} and Ni^{2+} fall under the category of *borderline / intermediate* ions. These ions therefore will form not so strong bonds with Class A and B ligands. Live algae cells induce metabolism dependant biosorption, where metal removal could be attributed to extra/intra cellular accumulation, precipitation or cell surface sorption.

It is well known that algae cells exposed to heavy metals may suffer serious morphological and biochemical alterations [26]. The effect of Fe^{3+}; Mn^{2+} and Ni^{2+} (with initial concentration of 120 mg/L) on the growth of green microalgae *Desmodesmus sp.* was studied. Metal solutions were introduced to the algae medium and algae cell growth was monitored over 5, 10, 15, 30, 60 and 120 minutes.The growth curves of algae cells are shown in Fig. 9 (a), (b) and (c); were the arrow indicates the point at which metal solutions were introduced in the algae system. The curves show that algae growth was remarkably reduced in the first 5 minute of the metal present in solution. This behaviour can be attributed to the presence of high concentrations of Fe^{3+}, Mn^{2+} and Ni^{2+}in solution. Metal toxicity to algae occurs by affecting their metabolic processes, through protein denaturation by the blockage of functional groups, displacing an essential metal, modification of the active conformation of the molecule or by rupture of cellular and organellar membrane integrity [27][28][29].





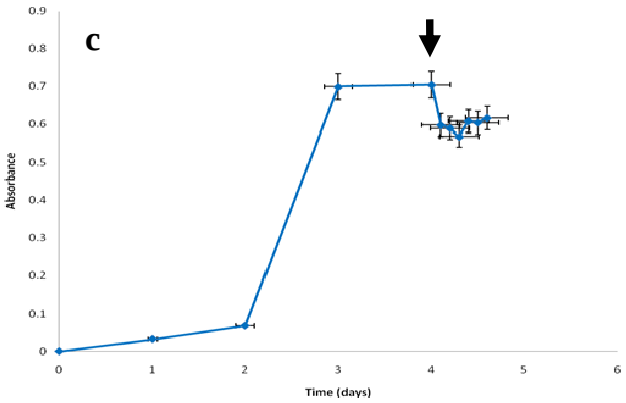


Fig. 9 Effect of (a) Nickel, (b) Manganese and (c) Iron, on growth rate of algae cells.

IV. Conclusions

- The biosorption of iron, manganese and nickel with green alga *Desmodesmus sp.* fitted linearly to the pseudo-second order kinetic model, indicating that the process occurred in at least two steps. Iron had the fastest biosorption kinetics. Nickel had highest metal uptake. The kinetic uptakes and rates were inversely proportional to the size of metal ions.
- The biosorption isotherms of iron, manganese and nickel fitted the Langmuir model best. The metal uptake sequence was different to that obtained in the kinetic experiments. According to the equilibrium constants, the affinity of metals for the biomass followed the sequence: $Mn^{2+} > Ni^{2+} > Fe^{2+}$.

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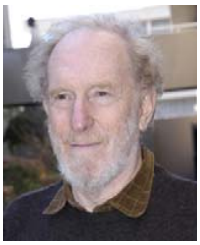

Prof. David Glasser is a co-founder and director of COMPS. He oversees the overall commercial activities and development strategies of the Centre, and liaises with Wits University's leadership and industry partners to promote COMPS. He supervises the Centre's academic and consultant research teams, and supervises 40 PhD and MSc students. Prof. Glasser has been awarded an A1 scientist status by South Africa's National Research Foundation. He was Wits University's Dean of the Faculty and served on the university's council. He was President of the South African Institute of Chemical Engineers, from which he received the SAIChE Gold Medal in 2000. He was the inaugural recipient of the Harry Oppenheimer Memorial Award (Gold Medal and Fellowship) to pursue research in Australia and USA in 2002.

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