



## ***Mucor racemosus* as a biosorbent of metal ions from polluted water in Northern Delta of Egypt**

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### **Abstract**

Twenty samples of polluted water were collected from Damietta's canals and drainages located near the industrial area of New Damietta. Initial concentrations of heavy metals including (zinc, copper and lead) in the polluted water were determined. Fourty–five fungal species were isolated. *Mucor racemosus*, *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Trichoderma koningi* and *Rhizopus oryzae* were isolated frequently. On the basis of its frequency, *Mucor racemosus* was chosen for biosorption studies.

Free and immobilized biomass of *Mucor racemosus* sequestered ions in this decreasing sequence Cu > Zn > Pb. The effects of biomass concentration, pH and time of contact were investigated. The level of ion uptake rose with increasing biomass till 200 mg and then decreased with increasing biomass. The maximum uptake for Cu (60.13 mg/g), Zn (57.67 mg/g) and Pb (21.97 mg/g) respectively occurred at 200 mg/l biomass. The uptake rose with increasing pH up to 5 in the case of Zn and Cu and 4 in the case of Pb. Maximum uptake for all metals was achieved after 15 minutes. Ion uptake followed the Langmuir adsorption model, permitting the calculation of maximum uptake and affinity coefficients. Treatment of *Mucor racemosus* biomass with 0.1 M NaOH at 120°C for 6 h improved biosorbent capacity, as did immobilization with alginate. Immobilized biomass could be regenerated readily with treatment with dilute HCl. The biomass-alginate complex efficiently removed Zn, Cu and Pb from polluted water samples. Therefore, *Mucor racemosus* could be employed either in free or immobilized form as a biosorbent of metal ions in waste water.

**Key words** – alginate – alkali treatment – biosorption – copper – free and immobilized biomass – lead – zinc.

### **Introduction**

The increasing trends towards artificial high life standards are compelling the people towards misuse of resources resulting in environmental degradation at massive scale. Incidentally, increased industrialization has affected the environment through disposal of waste water containing toxic contaminants in the form of metals. This situation is getting more alarming in the last two decades where industrial units are established without environmental impacts assessment and planning. Therefore discharge of heavy metals, their accumulation and contamination has become an environmental, health, economic and planning issue.

Biological methods of metals removal from aqueous solution, known as biosorption have been recommended as cheaper and more effective technique in bioremoval of heavy metals (Apel & Torma 1993, Artola et al. 1997). Biosorption of metals is a property of certain types of microbial biomass that can result in the concentration of metallic elements from relatively diluted solutions (Volesky & Philips 1995). Biosorption has received substantial attention as a potential method for decontamination and recovery of heavy metals from the environment (Lewis & Kiff 1988, Venkateswerlu & Stotzky 1989, Luef et al. 1991). Fungal biomass and seaweed biomass have been found to be excellent biosorbents for sequestering heavy metals (Lewis & Kiff 1988, Holan & Volesky 1994, Volesky & Holan 1995). Fungal biomass has been used to sequester copper, lead, zinc, nickel, cadmium, gold, silver and various actinide elements, such as thorium, uranium and plutonium (Tsezos & Volesky 1981, Gadd & White 1989, Luef et al. 1991, Kapoor & Viraraghavan 1998a & b).

Fungi are known to have good metal uptake systems (Gadd 1986) with metabolism-independent biosorption being the most efficient. The specific mechanism of uptake differs quantitatively and qualitatively according to the species, the origin of the biomass and its processing (Tobin et al. 1984). The hyphal wall was found to be a primary site of metal ion accumulation. This is attributed to several chemical groups (the acetamido group of chitin, amino and phosphate groups in nucleic acids, amino acids, amido, sulfhydryl and carboxyl groups in proteins and hydroxyls in polysaccharides) that might attract and sequester metal ions (Holan & Volesky 1995).

Biomass of fungi such as *Absidia*, *Cunninghamella*, *Mucor* and *Rhizopus*, exhibit excellent metal-ion uptake (Venkateswerlu & Stotzky 1989, Luef et al. 1991, Fourest & Roux 1992, Mueller et al. 1992). This could be due to the high chitin and chitosan content of the cell walls of these fungi (Tsezos & Volesky 1981). To date, research in the area of biosorption suggests it to be an ideal alternative for decontamination of metal containing effluents (Yazdani et al. 2010, Nur Liyana et al. 2011, Nitin et al. 2012, Siddiquee et al. 2013). Generally speaking the biosorption takes place by both living and non-living microbial biomass, but there are differences in the efficiency and mechanisms involved (Park et al. 2005). The efficiency by which dead cells act as sorbent of metal ion may be greater than that of living cells and confirm its wider acceptability. This is due to the argument that dead cells do not have toxicity limitations, no requirement of growth and nutrient media, storage property for extended time period and easy desorption of adsorbed metal ions (Awofolu et al. 2006).

Various physical (heat treatment, autoclaving, freeze drying and boiling) and chemical (acids, alkali and organic chemicals) pretreatment protocols have been developed to convert the viable cells into nonviable (dead) (Loukidou et al. 2003). According to many workers, the chemical pretreatment protocols owing to change in cell wall chemistry of the biosorbent (Kapoor et al. 1999). Yan & Viraraghavan 2000 has reported that alkaline (caustic) treatment could enhance metal binding by biomass.

The alkaline treatments, including sodium hydroxide, potassium hydroxide, alkaline detergents or other alkaline reagents ruptures the cell walls of microbes and exposes additional functional groups for metal ion binding. The residual alkalinity may result in the hydrolysis of certain metals, thus enhancing the biosorption capacity of biomass (Brierley 1990). According to Huang (1996) acid-washing process have better results over other treatments as this treatment may dissolve polysaccharide components in the outer cell wall layer of the biosorbent, thus producing additional binding sites. It is obvious that many different and challenging contributions have been made on the path to develop biosorption by the biosorbent.

Therefore the aim of this study was to isolate fungi from polluted water to find a new biosorbent agent. The ability of free and immobilized biomass of this biosorbents to sequester zinc (Zn), copper (Cu) and lead (Pb) was investigated.

## Materials and Methods

### Sampling procedures and isolation methods

Twenty water samples were collected in May 2010 from polluted water of Damietta's drainages and canals located near the industrial area of New Damietta in clean, sterilized 1 L screw-cap glass bottles. To each sample, 372 mg/l of EDTA (ethylene diamine-tetraacetic acid disodium salt) was added as a chelating agent to reduce toxicity in samples laden with heavy metals. Other twenty samples were also collected in clean, sterilized screw-cap glass bottles for the determination of heavy metals concentration in the polluted water samples.

Examination of samples began immediately after return to the laboratory. For isolation of fungi a millipore filter paper (0.45  $\mu$ m pore size) technique was used to concentrate fungal propagules from the polluted water. The fungal propagules were resuspended in sterilized bottles containing 50 or 100 ml of sterilized water, and 1 ml from each dilution was transferred to a clean sterilized petri-dish (three replicates) contained streptopenicid (0.35g/ml), rose bengal (0.035gm/ml) glucose (10 gm/l), peptone (5gm/l) and agar (15gm/l) (Cooke 1963). The plates were incubated at 28°C for 4–7 days. The relative frequency of occurrence was calculated as the number of species isolated from each sample divided by the total number of samples. The isolated species were classified as very frequent (>20%), frequent (10-20%), or infrequent (<10%) as adapted from (Tan and Leong 1989). Sporulating isolates were identified using specific media that were used for identifying sporulating isolates. Non-sporulating strains were grouped as mycelia sterilia according to similarities in colony morphology (Taylor et al. 1999).

### Metal biosorption by free fungal biomass

*Mucor racemosus* was selected based on its frequent occurrence in sampling. The fungus was subcultured on Potato Dextrose broth at 28°C for 7 days on a rotatory shaker at 180 rpm. Fungal pellets were washed twice in sterile double distilled water, drained and dried at 60°C to constant weight and ground with a mortar and pestle before determination of metal biosorption (El-Morsy 2004).

### Effect of initial metal concentration

To evaluate the effect of initial metal ion concentration ( $C_i$ ) on adsorption behaviour of Zn, Cu and Pb by dried mycelial biomass, aliquots of 10, 50, 70, 100, 150, 200, 300, 400 and 600 ppm concentration of zinc sulfate, copper sulfate and lead acetate solutions were added to 250 ml Erlenmeyer flasks with a fixed biomass of 200 mg/l. The pH was adjusted to 4 with 0.1N HCl and 0.1N NaOH. The samples were mixed well by shaking. For sorption isotherm experiments, flasks were agitated on a rotatory shaker (180 rpm) at room temperature until no additional metal was removed (3-5 h). The samples were filtered through 0.45  $\mu$ m millipore filters. Triplicate samples were analyzed by A Perkin-2380 atomic absorption spectrophotometry. Samples also were taken from experimental controls, which contain no biomass (El-Morsy 2004).

### Effect of biomass concentration

To evaluate the effect of biomass concentration on the adsorption behaviour of Zn, Cu and Pb. Biomass concentrations of 100, 200 and 300 mg/l were added to 250 ml Erlenmeyer flasks separately. Aliquots (50 ml) of heavy metal solution (100, 200 and 300 mg/l) were added to each flask, and the flasks were left 15 minutes on a rotatory shaker at 180 rpm at room temperature before being analyzed as above (El-Morsy 2004).

### Effect of pH

A Sorption of metal ions by dried mycelial biomass was studied at pH values of 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6 and 6.5. The samples were shaken at 180 rpm using a rotatory shaker at room

temperature. A fixed biomass of 200 mg/l was added to 50 ml of heavy metal solution containing Zn, Cu and Pb at an initial concentration of 200 mg/l for 15 minutes. To avoid shifts in pH due to biomass addition, the pH was adjusted with 0.1N HCl and 0.1N NaOH after the solution had been in contact with the adsorbent. In the case of Pb, pH was adjusted with 0.1 N HNO<sub>3</sub> or 0.1N NH<sub>4</sub>OH. Triplicate samples were analyzed as above (El-Morsy 2004).

### **Time of contact**

To determine the optimal incubation time, a fixed adsorbent concentration of 200 mg/l of fungal biomass was added to 50 ml of heavy metal solution containing an initial metal concentration of 200 mg/l of Zn, Cu and Pb. Three samples were taken at 5, 10, 15, 25, 35, 60, 120 and 1440 minutes at room temperature (El-Morsy 2004).

### **Alkali-treatment of the biosorbent**

To generate ionic sites without significant modification of the cell wall structure before sorption, the mycelium was treated with 0.1 M NaOH at 120°C for 6 h and filtered through 0.45 μm millipore filter paper. The treated mycelium then was washed several times to reach neutral pH and oven dried at 60°C. After that, Aliquots of 10, 50, 70, 100, 150, 200, 300, 400 and 600 ppm were added to 250 ml Erlenmeyer flasks with a fixed biomass of the alkali-treated biosorbent (200 mg/l). The flasks then were agitated on a rotatory shaker (180 rpm) for 15 minutes at room temperature. The pH was adjusted to 4 in the case of Pb and 5 in the case of Zn and Cu (El-Morsy 2004).

### **Metal biosorption by alkali treated-immobilized fungal biomass**

To each well of a percolating plate, 200 mg of alkali-treated biosorbent was added, followed by a drop of 4% sodium alginate of high viscosity. A drop of 0.25 M CaCl<sub>2</sub> was added to each well separately to form beads. The beads were collected and air dried to yield pellets. Dried pellets, each containing 200mg biosorbent biomass were added to conical flasks containing aliquots (50ml) of 10, 50, 70, 100, 150, 200, 300, 400 and 600 mg/l concentrations of zinc sulfate, copper sulfate and lead acetate. A constant pH of 4 in the case of Pb and 5 in the case of Zn and Cu and a time of contact of 15 minutes were used for all metal ions at room temperature. After measuring the residual metal concentration ( $C_f$ ) in the solution, the beads were collected and regenerated using diluted acid (0.1 M HCl) followed by 3% bicarbonate. A control experiment was carried out using mycelium free alginate pellets.

### **Biosorption of Zn, Cu and Pb from polluted water**

10 Samples were collected from canals located near the industrial area of New Damietta in July 2012. The Samples contained metal ions at different concentrations. Immobilized fungal biomass (three pellets) was added to 250 ml Erlenmeyer flasks containing 100 ml of polluted water sample without adjustment of pH. In a second experiment, the test was carried out at pH 4 and pH 5. The flasks then were agitated on a rotatory shaker (180 rpm) for 15 minutes at room temperature. The residual metal ion concentration was determined by A Perkin-2380 atomic absorption spectrophotometry.

### **Biosorption mechanism**

To determine the quantity of metal that can be attracted and retained in an immobilized form, it is customary to express metal uptake ( $q$ ) by the biosorbent as the amount of metal adsorbed per unit of biomass. The calculation of the metal uptake (mg/g dry biosorbent) is based on the material balance of the sorption system. The amount of metal adsorbed by the biosorbent from solution can be estimated from this formula:  $q = V (C_i - C_f) / M$  (Holan and Volesky, 1994) where  $q$  is the metal ion uptake (mg/g),  $C_i$  is the initial metal ion concentration (ppm),  $C_f$  is the measured final concentration of the metal ion in the solution (ppm),  $V$  is the liquid sample volume (ml) and  $M$  is the starting biosorbent weight (mg).

The sorption–isotherm relationship can be expressed mathematically by plotting  $q$  versus  $C_f$ . This first was done in the classical work of Langmuir (1918) who studied activated carbon adsorption. The linear form of the Langmuir isotherm equation is represented by this equation:

$$q = Q_{\max} bC_f / 1+bC_f.$$

Where  $Q_{\max}$  is the maximum amount of metal per gram of biomass corresponding to saturation of the adsorption sites. The dissociation constant ( $b$ ) is a coefficient related to the affinity between metals and biomass.

## Results

### Occurrence of fungi in the polluted water

Fourty-five fungal species were isolated from polluted water at Damietta's canals and drainages located near the industrial area of New Damietta (Table. 1). The majority of species were mitosporic (37 species of hyphomycetes and three species of agonomycetes). Zygomycetes were represented by three species while yeast was represented by two species. The frequent species were *Mucor racemosus* (50%) followed by *Aspergillus flavus* (48%), *A. niger* (36%), *A. fumigatus* (30%), *Trichoderma Koningii* (30%) and *Rhizopus oryzae* (29%).

### Influence of biomass, pH and time of contact on metal uptake

Metal uptake varied with biomass concentration, pH and time of contact. The level of ion uptake rose with increasing biomass till 200 mg and then decreased with increasing biomass (Fig. 1. a). Uptake rose with increasing pH up to 4 in the case of Pb and 5 in the case of Zn and Cu (Fig. 1. b). The maximum uptake of all metals was achieved after 15 minutes and decreased promptly (Fig. 1. c).

### Biosorption mechanism

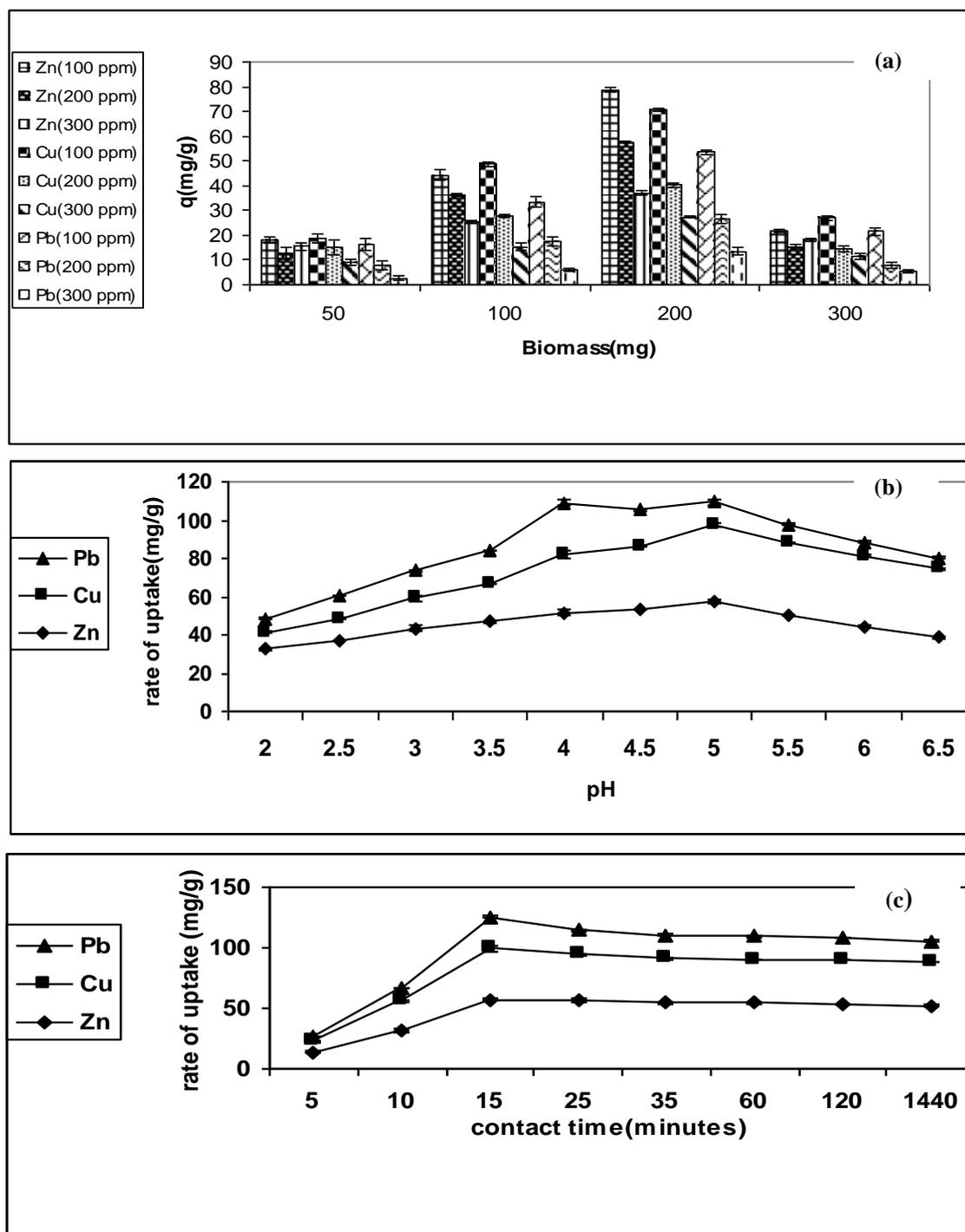
Sorption isotherms of Zn, Cu and Pb by dead biomass of *M. racemosus* are shown in (Table. 2). Sorption isotherms represented the distribution of metal ions between aqueous and solid phases (biomass) when the concentration increases as long as binding sites are not saturated. These isotherms permit the calculation of the adsorption capacities and dissociation constants of metal ions. The maximum uptake of Cu (60.13 mg/g), Zn (57.67 mg/g) and Pb (21.97 mg/g) occurred at a biomass of 200 mg/l.

### Metal biosorption by immobilized fungal biomass

Uptake of Zn, Cu and Pb increased after treatment with 0.1 M NaOH at 120°C for 6 h at biomass of 200 mg/l. Accordingly, NaOH pretreatment was employed in experiments designed to test the efficiency of alginate-immobilized biomass in enhancing metal uptake. Alginate beads have the capacity to be laden and remove metal ions from metal solutions and water samples polluted with heavy metals. The best rate of uptake of alginate beads ( $q$ ) occurs at 100 ppm for Zn and Pb and at 150 ppm for Cu. In all heavy metals under study biosorption increased till a definite heavy metal concentration after which rate of uptake began to decrease by alginate beads. Results revealed a marked increase in uptake of all tested metals by the alkali treated alginate-immobilized biomass over free biomass in enhancing metal uptake. The best rate of uptake by alkali treated alginate-immobilized *M. racemosus* for Zn occurred at 100 ppm (111.58 mg/g), Cu occurred at 150 ppm (121.33 mg/g) and Pb occurred at 100 ppm (96.15 mg/g) (Fig. 2).

### Biosorption of Zn, Cu and Pb from polluted water

Alkali treated alginate-immobilized biomass showed strong ability for removal of metal ions from polluted samples in the order Pb > Cu > Zn at pH 4 while at pH 5 Cu > Zn > Pb (Table. 3).



**Fig.1** – Effect of biomass (a), pH (b), and contact time (c) by free biomass of *M. racemosus*. Each value is the average of three replicates.

### Discussion

This study surveyed fungi from polluted water with the goal of identifying new species with metal-ion biosorbent potential. Forty-five species were isolated. The most frequent species were *Mucor racemosus*, *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Trichoderma koningii* and *Rhizopus oryzae*. *Mucor racemosus* is the most frequent fungus in water samples which are polluted with heavy metals. This suggests that *M. racemosus* may be heavy metal-resistant fungus as a subject for the studies reported here.

**Table1** Fungi isolated from a polluted water of Damietta's canals and drainages located near the industrial area of New Damietta.

Species		% of occurrence
<b>Hyphomycetes :-</b>		
<i>Aspergillus sulphureus</i>	( Fresenius ) Thom and Church	16
<i>A. candidus</i>	Link	18
<i>A. flavus</i>	Link	48
<i>A. fumigatus</i>	Fresenius	30
<i>A. niger</i>	Van Tiegh	36
<i>A. terreus</i>	Thom and Church	13
<i>A. clavatus</i>	Desmazieres	13
<i>A. cervinus</i>	Massee emend - Neill	18
<i>A. carneus</i>	(Van Tiegh ) Bloohwitz	15
<i>A. terricola</i> Var. <i>Americana</i>	(Marchal)	12
<i>A. wentii</i>	(Wehmer)	10
<i>A. flavipes</i>	(Brain.Et Sart.) Thom et Church	14
<i>Alternaria citri</i>	Elliss et pierree apud.	15
<i>Acremonium guillemattii</i>	(W.Gams)	12
<i>Ac. charticola</i>	(Lindau) Gams	13
<i>Ac. falciform</i>	(Carrion) Gams	15
<i>Ac. strictum</i>	Gams	7
<i>Ampulliferina fagi</i>	M.B.Ellis Spec.nov	9
<i>Botryotrichum piluliferum</i>	Sacc.et March	8
<i>Curvularia pallescens</i>	Boedijin	9
<i>Fusarium moniliforme</i>	Sheldon	11
<i>F. oxysporium</i>	Sehlecht	14
<i>Phialophora richardisae</i>	Nannf	9
<i>Phialophora. cyclaminis</i>	Beyma	7
<i>Penicillium corylophilum</i>	Dierckx	17
<i>P. chermesinum</i>	Biourge	17
<i>p. piscarium</i>	Westling	16
<i>p. camembetri</i>	Thom	15
<i>p. purpurogenum</i>	Stoll	16
<i>p. citrinum</i>	Thom	17
<i>p. notatum</i>	Westling	15
<i>p. simplicissimum</i>	(Oud.)Thom	16
<i>p. candidum</i>	Link	17
<i>p. brevis compactum</i>	Dierckx	19
<i>p. janthinellum</i>	Biourge	18
<i>Trichoderma koningii</i>	Oud	30
<i>Trichoderma piluliferum</i>	Webster and Rifai	27
<b>Agonomycetes:-</b>		
<i>Demateaceous sterile mycelium</i>	brown	11
<i>D. sterile mycelium</i>	black	12
<i>Hyaline sterile mycelium</i>		11
<b>Zygomycota:-</b>		
<i>Mucor racemosus</i>	Fresenius	50
<i>Rhizopus oryzae</i>	Went et Prinsen	29
<i>Syncephalastrum racemosus</i>	Scholer	12
<b>Yeast:-</b>		
<i>Geotrichum candidum</i>	Link:Fr	12
<i>Trichosporon cutaneum</i>	(De.Beurmann) Ota.	14

The isolated species were classified as very frequent (>20%), frequent (10-20%), or infrequent (<10%) as adapted from (Tan and Leong 1989).

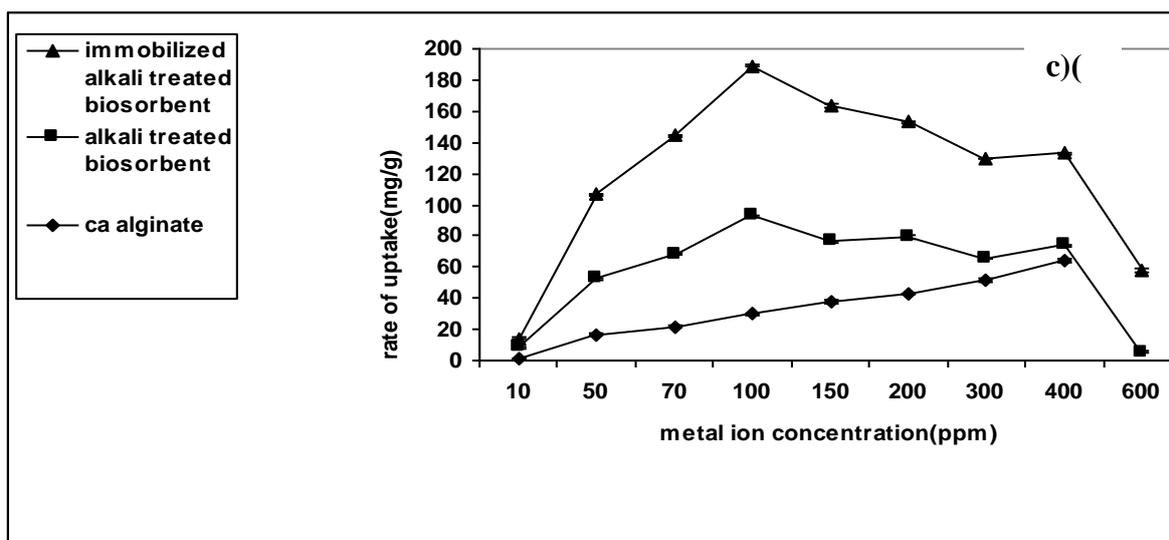
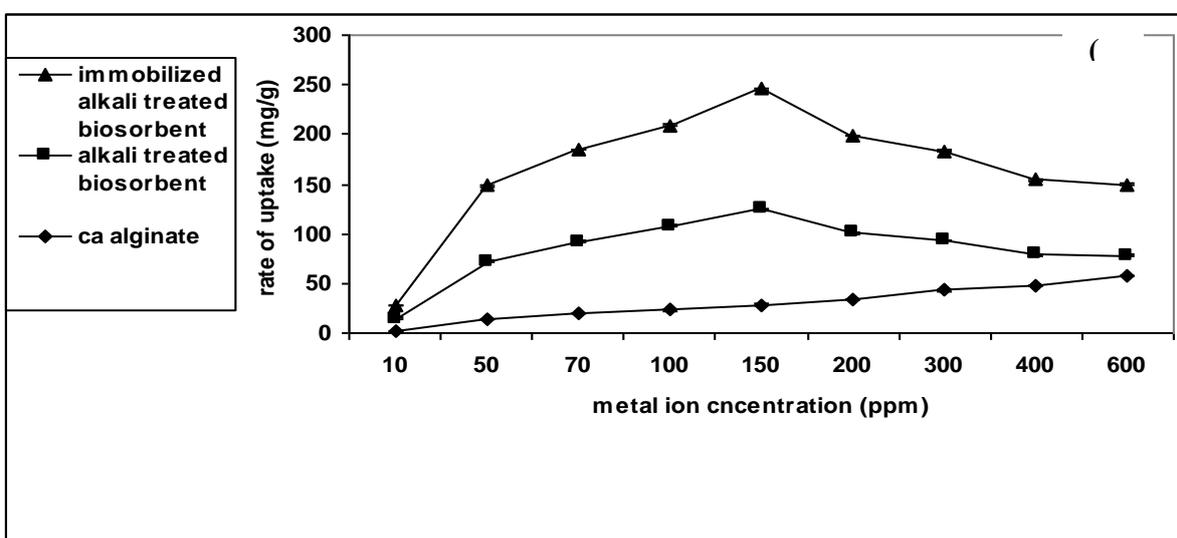
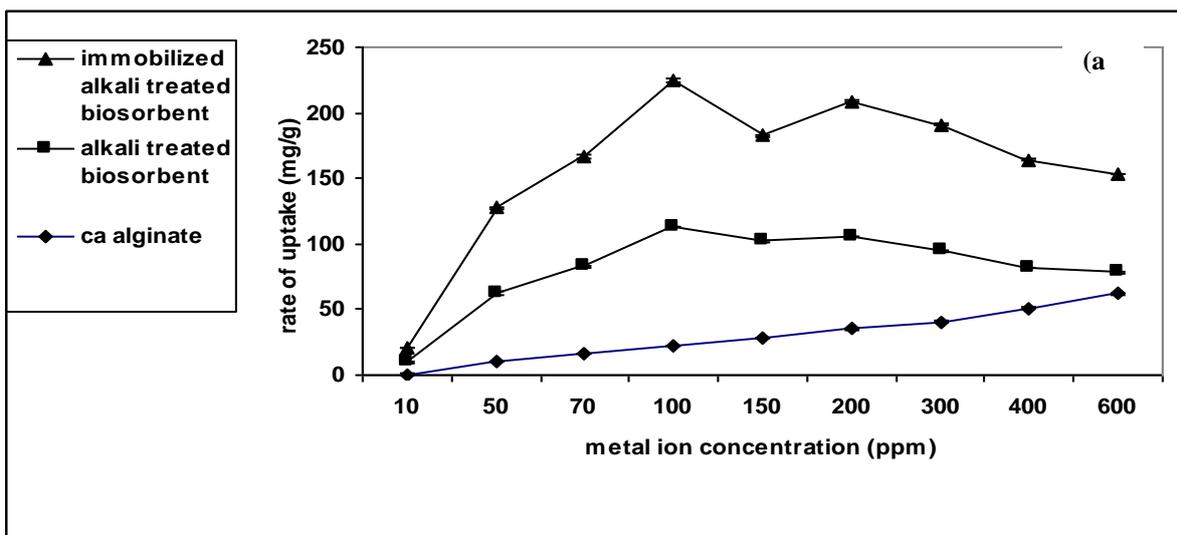


Fig. 2 – Biosorption capability of ca alginate, alkali treated and alkali treated alginate-immobilized *Mucor racemosus* for (a) Zn, (b) Cu and (c) Pb.

**Table 2** Uptake Capacities of various heavy metals by *Mucor racemosus* derived from the Langmuir equation ( $q = Q_{\max} bC_f / (1 + bC_f)$ )

Parameters/metal	Zn	Cu	Pb
A.W.(g)	65.39	63	207.19
Q (mg/g)	57.67	60.13	21.97
Q(mmole/g)	0.88	0.95	0.11
b(mM)	0.3	0.46	2.05
Affinity (1/b)	3.3	2.17	0.49

Q= maximum absorption capacity, b= dissociation constant, A.W= Atomic Weight

### Biosorption by free biosorbent and effect of biomass concentration

Fourest & Roux 1992 reported that metal-ion uptake per gram of biosorbent increases as long as the biosorbent is not saturated. However, uptake values also depend on the nature and the origin of the biosorbent itself (Luef et al. 1991). In the present study, uptake of Zn, Cu and Pb by free biosorbent in solution varied depending on the initial metal concentration, biomass concentration, time of contact and pH. With *M. racemosus*, the optimal time for biosorption was 15 minutes after contact. This result is similar to that obtained by Volesky & Philips 1995 (*Saccharomyces cerevisiae*), El-Morsy 2004 (*Cunninghamella echinulata*), who reported that most metal biosorption was achieved in 5–15 minutes, followed by residual and slower additional metal deposition (Tsezos & Volesky 1981), conceivably indicating a secondary metal binding mechanism. Alike, the maximum uptake by *M. racemosus* occurred at pH 4 for Pb and at pH 5 for Cu and Zn with uptake falling with rising pH. The effects of pH on the biosorbent capabilities of fungal biomass appeared to vary with assay conditions, the particular metal ion and fungal species. Analogous to results reported here, pH between 4 and 5 was reported as optimal for biosorption of Zn and Cu by *Saccharomyces cerevisiae* (Tsezos & Volesky 1981, Tobin et al. 1984, Volesky & Philips 1995) found that pH near 4 was optimal for metal uptake by *Rhizopus arrhizus*. In contrast, Luef et al. 1991 reported that biosorption of Zn by mycelium of *A. niger*, *Penicillium chrysogenum* and *Clavicipis paspali* rose with increasing pH up to 9. Fourest and Roux 1992 reported that the optimal uptake of Zn and Pb by *Rhizopus arrhizus* was achieved at a neutral pH and pH 5, respectively. Lewis & Kiff 1988 found that acidic pH reduced metal biosorption by the latter, a reduction that could be attributed to the precipitation of metal ions.

### Biosorption mechanism

The sorption process involves biomass as a solid phase and a liquid phase containing metal ions, with ion distribution between solid and liquid phases determined by the affinity of biomass for metals. The quality of the biosorbents material is evaluated in terms of how much metal it can attract and retain in an immobilized form. It is customary to determine metal uptake (q) by the biosorbents as the amount of metals bound by the unit of biomass. Sorption isotherm followed the typical Langmuir adsorption pattern (Ruthven 1984). The results presented here for *M. racemosus* are consistent with the Langmuir – isotherm model (Fourest & Roux 1992).

Alkali treatment of biosorbents pretreatment of the biomass was performed following the work of (Kapoor & Viraraghavan 1997, 1998 a & b, Kapoor et al. 1999). Alkali treatment improved the capacity of the *M. racemosus* biosorbents to chelate metal ions, especially the higher biomass of 200 mg/l. The higher affinity might be attributed to the chitin and chitosan content of the fungus cell wall, exposed after NaOH treatment. NaOH appears to remove amorphous polysaccharides from the cell wall, generating accessible space within the  $\beta$ -glucan–chitin skeleton and hence permitting metal ions to precipitate on this surface (Tsezos & Volesky 1981, Fourest & Roux 1992). Similarly formaline pretreatment significantly improved the surface active site that actually participates in Cu biosorption (Kapoor & Viraraghavan 1997, 1998 a & b, Kapoor et al. 1999).

**Table 3** Biosorption efficiency (%) of Zn, Cu and Pb from polluted water by alginate pellets of alkali treated-immobilized *Mucor racemosus*.

Parameters/metal	Zn	Cu	Pb
pH=4	80.55	81.11	96.15
pH=5	95.04	95.88	83.25
Control (without adjustment)	pH 56.65	63.10	39.37

Kapoor et al. 1999 reported that both Langmuir and Freundlich isotherm data at pH 5 at Cu biosorption by NaOH pretreated *Aspergillus niger* biomass were statistically significant at 95% confidence level. The alkaline treatments including sodium hydroxide, potassium hydroxide, alkaline detergents or other alkaline reagents ruptures the cell walls of microbes and exposes additional functional groups for metal ion binding. The residual alkalinity may result in the hydrolysis of certain metals, thus enhancing the biosorption capacity of the biomass (Brierley 1990). The biomass of *Aspergillus niger* pretreated with Na<sub>2</sub>CO<sub>3</sub> (0.2 N) exhibited the maximum biosorption capacity (20.82 mg/g) for Cu.

Treatment of *A. niger* with NaOH (0.2 N and 0.5 N ) yield equivalent adsorption capacity (18.81 mg/g) the biosorption capacity of (16.5 mg/g ) recorded for fungal biomass pretreated with NaHCO<sub>3</sub> (0.2 N ) and detergent was observed to reduce up to 22% in comparison to maximum recorded potential of >37% in alkali pretreatment (Javaid et al. 2011).

Baik et al. 2002 reported that *Aspergillus* sp. has the ability to absorb maximum level of Cu when treating the cell fraction with NaOH. Volesky & Philips 1995 explained this is due to microbial biomass consisting of poor mechanical strength and little rigidity. However, biosorbents are hard enough to withstand and the application of pressures, water retention capacity, porous and transport to metal ion sorbate species.

Physical and chemical treatments used to enhance the metal uptake capacity of the biomass, which led to removal, hiding or exposing chemical groups that binding or exchange with the adsorbed metal ions (Saleh et al. 2009). Similar enhancement in metal uptake capacity of the fungal biomass regarding alkali pretreatment was recorded by (Yan &Viraraghavan 2000, El-Morsy 2004, Das et al. 2007). It could be due to chemical modifications of the cell wall components. The modification of biomass probably destroys autolytic enzymes that cause purification of biomass and remove lipids and proteins that mask the reactive sites (Muraleedharan &Venkobachar 1990).

### Immobilization of the biosorbents

Immobilization has been reported to enhance the capacity of fungal biomass for chelating metal ions (Lewis & Kiff 1988, Yousef 1997). Here, alginate immobilization of alkali treated biosorbents beads resulted in a nearly two fold increase in metal-ion uptake over free biosorbents. Alginate carboxyl groups are known to play an important role in metal binding (Kuyucak & Volesky 1989). Previous studies have revealed a high capacity of alginate-biomass beads to remove metal ions from polluted water (El-Morsy 2004).

In this study, up to 96.15%, 81.11 % and 80.55% of Pb, Cu and Zn were removed at pH 4 respectively. While at pH 5 up to 95.88 %, 95.04 % and 83.24% of Cu, Zn and Pb were removed respectively. Kapoor & Viraraghavan 1998b used *A. niger* biomass immobilized in polysulfone polymer in the form of spherical beads to remove Zn, Cu and Pb ions from industrial waste water. A packed bead column containing *A. niger* beads removed 58 % Pb, 38 % Cu and 16 % Zn from the culture in 12 h. Biomass immobilized on polyacrylamide similarly was non immobilized biomass for metal biosorption (Yousef 1997). The uptake of the heavy metals (Cu, Co and Fe) was examined in the immobilized column experiments (*Rhizopus delemar* immobilized on polyurethane foam). More than 92% heavy metal removal from a mixed solution was achieved during the 5 cycles (Kolishka Tsekova & Galin Petrov 2002). More recently, biosorption of metals by

immobilized cell system has been used efficiently for removal of metals from industrial effluent (Gadd & White 1993, Gulay et al. 2003, Yakup et al. 2004, Sun et al. 2010, Hemambika et al. 2011, Tan & Ting. 2012, Ahemad & Kibret 2013). Cells with such abilities are immobilized either as entrapped biomass or as a biofilm to form a system for treating waste water known as a bioreactor (Qureslii et al. 2001). Immobilized cells of *Kluyveromyces marxianus* allowed for high removal capacity for Cu, Co and Zn where 1.6, 1.5 and 1.3 fold increase were recorded compared to free cells (Yousef 1997).

The biosorption of immobilized cells of *Aspergillus* sp. was 60.94% of Cu, *Penicillium* sp. was 97.21% of Cd and *Cephalosporium* sp. was 73.27% of Pb; whereas the dead cells of *Aspergillus* sp. was 46.91% of Cu, *Penicillium* sp. was 95.27% of Cd and *Cephalosporium* sp. was 70.67% of Pb. All these results reveal that the adsorption capacities of the immobilized fungal cells were greater than that of dead cells (Hemambika et al. 2011). Johncy Rani et al. 2010 found similar adsorption capacity of Cu, Cd and Pb in the immobilized cells of *Bacillus* sp., *pseudomonas* sp. and *Micrococcus* sp. respectively. Leusch et al. 1995 explained this is because dead fungal cells consist of small particles with low density, poor mechanical strength and little rigidity. Hence, the immobilization of biomass is necessary on before subjecting to biosorption. Holan & Volesky 1994 explained that immobilized cells offers many advantages including better reusability, high biomass loading and minimum clogging in continuous flow systems. Adsorption of heavy metals was also dependent on cell density in calcium alginate beads. The results obtained for the uptake of heavy metals under study show us that the metal accumulation by *Mucor racemosus* is a chemical, equilibrated and saturable mechanism. Thus, adsorption increases when the initial metal concentration rises as long as the binding sites are not saturated.

Zygomycetes sp such as *Mucor* sp, *Rhizopus arrhizus* and *Absidia orchidis* generally are reported to be efficient biosorbent agents (Lewis & Kiff 1988, Fourest & Roux 1992, Mueler et al. 1992, Holan & Volesky 1995). The work reported here demonstrates *M. racemosus* to be as effective as *Cunninghamella echinulata* and *Rhizopus* sp. This based on the finding that immobilized biomass of *C. echinulata* was able to remove ions from a natural environment up to 95 % (El-Morsy 2004). It was also found that maximum copper removal by *Rhizopus arrhizus* of 98% was obtained using the biosorption kinetics of copper under optimum conditions. (Preetha & Viruthagiri 2007).

## Conclusion

Biomass of *M. racemosus* efficiently is able to remove Zn, Cu and Pb from a solution. This capacity was enhanced when the biomass was alkali treated and immobilized. The fungus in its immobilized form also was able to remove ions from a natural environment up to 96 %, therefore the fungus is proposed as an effective biosorbent for removal of heavy metals in waste water treatment.

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