

Tolerance and Biosorption of Manganese, Iron and Aluminium by Five *Aspergillus* Species Isolated from Freshwater

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ABSTRACT

Microorganisms play a significant role in bioremediation of heavy metal contaminated soil and wastewater. The present study has been carried out to understand the tolerance potential of different species of *Aspergillus* (*A. flavus*, *A. niger*, *A. nidulans*, *A. oryzae* and *A. terreus*) which were isolated from the Nile water. The degree of tolerance of fungi was measured by minimum inhibitory concentration in the presence of different concentration of metals (Mn, Fe and Al) and compared to a control sample. Results showed variations in the tolerance level of different isolates of *Aspergillus*. *A. niger* and *A. oryzae* were tolerant while *A. nidulans* was the most sensitive species. Removal of manganese, iron, and aluminium using powder and viable *Aspergillus oryzae* biomass was investigated. Metals (Mn, Fe, and Al) removal increased with an increasing dose of biosorbent for the powder and viable biomass within two hours. These results showed that powder and viable *Aspergillus oryzae* biomass can be used for removal of heavy metals from polluted water.

Keywords: *Aspergillus*, *A. oryzae*, bioremediation, heavy metals, resistance.

INTRODUCTION

Contamination of sediments and natural aquatic receptors with different pollutant is a major environmental problem all over the world (Baldrian and Gabriel, 2002; Gavrilesca, 2004; Malik, 2004; Nontokozo *et al.*, 2017). Industrialization and urbanization especially in developing countries have led to the accumulation of heavy metals and petroleum hydrocarbons in the environment (Bamgbose and Sibango, 1998; Byomi *et al.*, 1999; Manay *et al.*, 1999; Ngodigha *et al.*, 1999; Yamasoet *et al.*, 2000; Adeniyi and Folabi, 2002; Monika *et al.*, 2017). Metals that are released into the environment tend to persist indefinitely, accumulating into living tissues and posing a serious threat to the environment and public health. These harmful substances accumulate in crops via food chain; find their way into our bodies, where they can cause a variety of illness. They are cytogenic mutagenic, and carcinogenic in nature and are posing threats to the urban population, which rely on vegetables and foliage crops grown in pre-urban lands. Introduction of heavy metals into environment generally induces morphological and physiological changes in microbial communities (Vadkertiova and Slavikova, 2006; Monisha *et al.*, 2014), hence exerting a selective pressure on the microbiota (Verma *et al.*, 2001). These contaminated sites are the source of metal resistance microorganisms (Gadd, 1993).

Among all living organisms, fungi are very important microorganism; it can tolerate heavy metals to a limit and can also help to remove heavy metals from contaminated soil. Fungi and yeast biomasses are known to tolerate heavy metals (Khan, 2001; Baldrian, 2003; Gavrilesca, 2004; Ding *et al.*, 2016; Fawzy *et al.*, 2017) to achieve implementation of fungal heavy metal biosorption technology in environmental remediation requires

a better understanding of influencing parameters, including initial concentrations, physico-chemical conditions and also contact times. Successful biosorption processes require inexpensive biomaterials display high metal uptake and selectivity based on a biochemical constitution, as well as suitable mechanical properties for applied remediation procedures. In bio-sorption, either viable or powder microorganism or their derivatives are used, which complex metal ions through the action of ligands or functional groups located on the outer surface of the cell (Bolton and Gorby, 1995). Biosorption regarded as physicochemical interactions of metal ions with the cellular compounds of biological species (Kapoor and Viraraghavan, 1997). The mechanism of uptake can be due to ion exchange, chelation, chemical complexation with microbial cell surface groups, adsorption and diffusion through cell wall and membranes (Kuyucak and Volesky, 1988; Churchill *et al.*, 1995; Vaishalya *et al.*, 2015) which differ depending on the species used, the origin and processing of the biomass and solution chemistry. These elements were measured in some areas which related to the company of drinking water and sanitation, and it was higher than values within the standards and specifications to be met in water for drinking and domestic uses. The aim of the present study was to check the tolerance potential of different species of *Aspergillus* to manganese, iron, and aluminium. The removal efficiency of these heavy metals by *A. oryzae* was followed by different factors like growth period and biosorption dose.

MATERIALS AND METHODS

Determination of temperature and pH value

Nile water temperature and pH values were measured instrumentally using instrument (Hach hq 430).

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Microorganisms and growth conditions

Fungal strains used in this study including five species of *Aspergillus* (*A. flavus*, *A. nidulans*, *A. niger*, *A. terreus* and *A. oryzae*) were isolated from five sites of fresh water (River Nile at Luxor city area) and identified according to (Raper and Fennell, 1965; Gams *et al.*, 1985; Geiser *et al.*, 2008), were tested for their resistance and growth in the presence of various concentration of metals (Mn, Fe and Al). These microorganisms were grown in Glucose-Czapek's agar medium which consisted of 1L distilled water, 1gm/l K_2HPO_4 , 3.0 g/l $NaNO_3$, 0.5 g/l $MgSO_4$, 0.5 g/l KCl, 0.01 g/l $FeSO_4 \cdot 7H_2O$, 10 g/l glucose and 15 g/l agar. The strains were then allowed to grow at 28°C in an incubator.

Determination of minimum inhibitory concentration (MIC)

The (MIC) is defined as the lowest concentration of metals that inhibit visible growth of isolates. The tolerance of selected isolates to Mn, Fe and Al was determined by dilution method (1, 10, 20, 30, 50, 100, 300, 500 and 1000 ppm of metal ions (Rice *et al.*, 2012), were added separately to glucose Czapek's agar medium. The plates were inoculated with 8 mm agar plugs of young fungal colonies, pre-grown on Czapek's medium for 7 days. Three replicates of each concentrate and controls without metal were used. The inoculated plates were incubated at 28°C for 7-15 days. The effects of heavy metals on the growth were estimated by measuring the radius of the colony extension (mm) against the control for determination of their tolerance.

Preparation of *Aspergillus oryzae* biomass for removal studies

Aspergillus oryzae was selected based on its frequent occurrence during sampling. An inoculum of active growing fungal culture was transferred to 500 ml conical flask containing malt extract-peptone broth medium (Samson *et al.*, 1996) and incubated at 28°C for 15 days. Fungal biomass was harvested and washed twice by sterile bi-distilled water, then this biomass was drained and represented viable biomass. A constant weight of viable biomass was dried at 55°C for 24h and ground with a mortar to make powder biomass.

Effect of biomass concentration

To evaluate the effect of viable and powder biomass on the adsorption behavior of Mn, Fe and Al; 1, 2 and 3 g of biomass were added to separate (250 ml) Erlenmeyer flask. Aliquots (100 ml) of heavy metal concentrations (1, 10, 30, 50, 100, 300, 500 and 1000 ppm) were added to each flask and the flasks were shaken for 60 min. at room temperature before being analyzed.

Contact time

To determine the optimal incubation time, a fixed adsorbent concentration of 1 g of fungal biomass (viable and powder) was added to 100 ml of heavy metal

concentrations with continuous shaking at room temperature before analysis. All samples were analyzed by Dr 5000 automatic adsorption spectrophotometry at wave length (525, 510 nm) for all examined metals.

RESULTS

Physico-chemical characteristics of the Nile water samples showed that; temperatures of all water samples ranged between 27.4°C and 39.9°C. The pH value of the Nile water samples fluctuated between 7.0 and 8.6. Long-time exposure of water and sediment to heavy metals can produce considerable modification of their microbial populations, reducing their activity and their number.

Fungi are natural inhabitants of soil and water; they have greater potential for remediation virtue of their aggressive growth, greater biomass, production and extensive hyphal reach to its habitat. Results of the present study depicted that the tested isolates; *Aspergillus flavous*, *A. nidulans*, *A. niger*, *A. oryza* and *A. terreus* show different tolerance behavior for different heavy metals. Some isolates were sensitive, moderately tolerant and tolerant (Fig. 1). These isolates are tested to determine the minimum inhibitory concentration (MIC) for the metals. The order of toxicity of metals to (*A. flavus*, *A. niger*, *A. oryzae* and *A. terreus*) were Mn > Al > Fe, Mn > Fe > Al, Mn > Fe > Al and Al > Fe > Mn, respectively.

Figure (1) showed that the growth of most tested isolates markedly decreased with increasing metal concentration. The growth of *A. flavus* decreased to approximately more than 50% at 300 and 500 ppm of Mn and completely inhibited at 1000 ppm.

Aspergillus nidulans and *A. terreus* sharply decreased by about 60% when they grow at 30 ppm of Fe and completely inhibited at 50, 100, 300, 500 and 1000 ppm. *A. niger* showed exactly contrasting behavior with Fe. Its growth increased at 1, 10 and 30 ppm then decreased with increasing concentration to 50, 100 and 300 ppm. No growth at 500 and 1000 ppm was observed. The growth of *Aspergillus flavus* and *A. oryzae* exhibited considerable tolerance toward aluminum, while higher concentrations of Al (500 and 1000 ppm) failed completely the growth of *A. nidulans* and *A. terreus*.

Influence of biosorbent dose on metals uptake by *A. oryzae*

The uptake of metals by fungal biomass appears to involve a combination of two processes namely bioaccumulation and biosorption. Bioaccumulation is an active metabolism-dependent process, and may include both transport into the cell and partitioning into intercellular components. Biosorption based on the binding of metals to the biomass by processes that do not require metabolic energy.

Manganese, iron and aluminum were investigated by various biosorbent doses (1.0, 2.0 and 3.0 g/l) with different concentrations of metals (1 - 1000 ppm).

Generally, 1 ppm dose of the three studied metals showed the highest adsorption percentage with viable and powder biomass concentrations (Fig. 2).

On the other hand, our results revealed a marked increase in removal of all tested metals by the increa-

sing of biosorbent mass (Fig. 2). The maximum removal efficiency was observed at 3.0 g of powder biomass 58, 69, 71.9% for 1.0 ppm of Mn, Fe and Al ions respectively. While with viable biomass, it was slightly lower (51, 56, 69%) for Mn, Fe and Al, respectively.

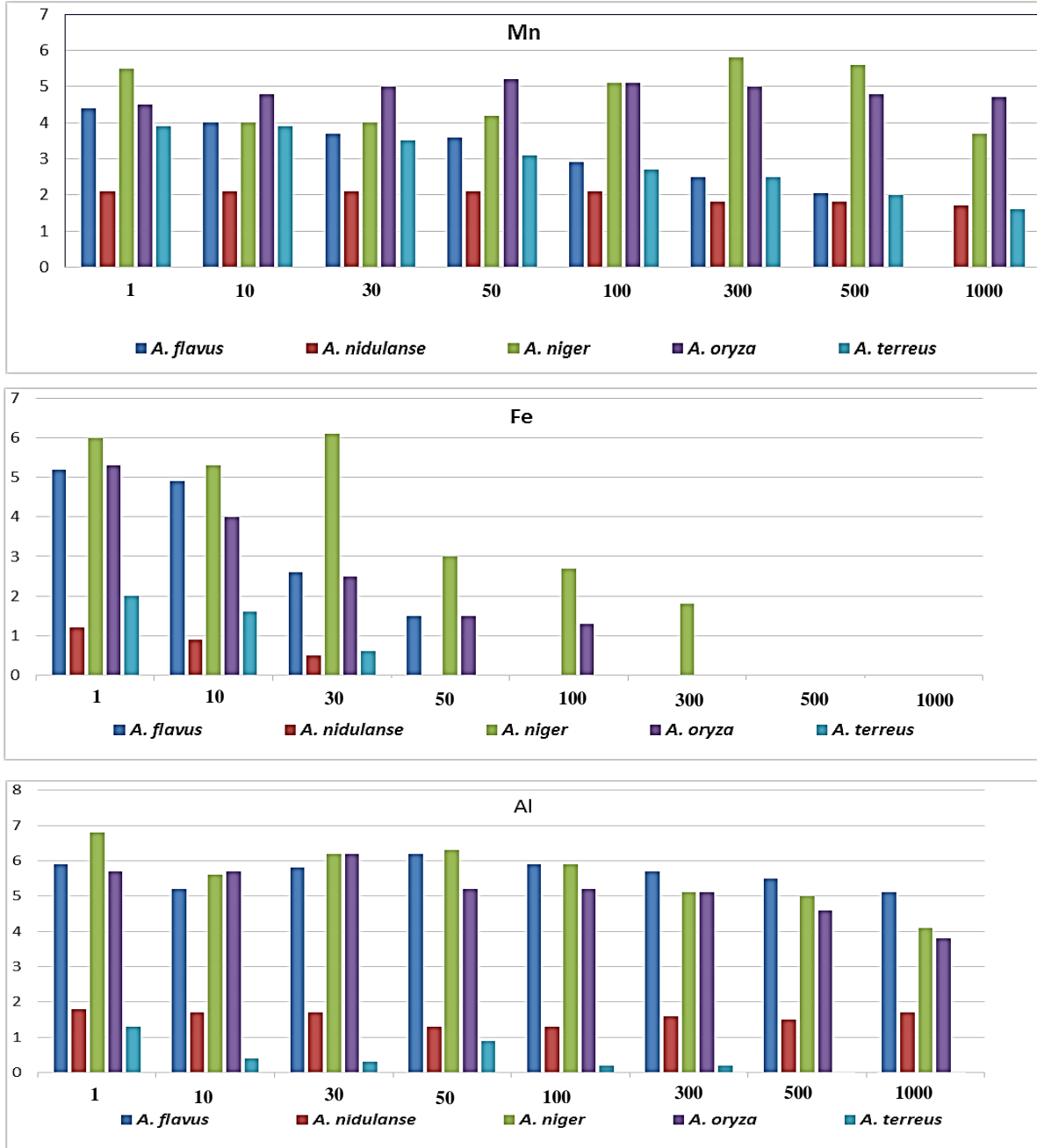


Figure (1): Effect of different concentrations of heavy metals (Mn, Fe and Al) on growth of five species of *Aspergillus* .

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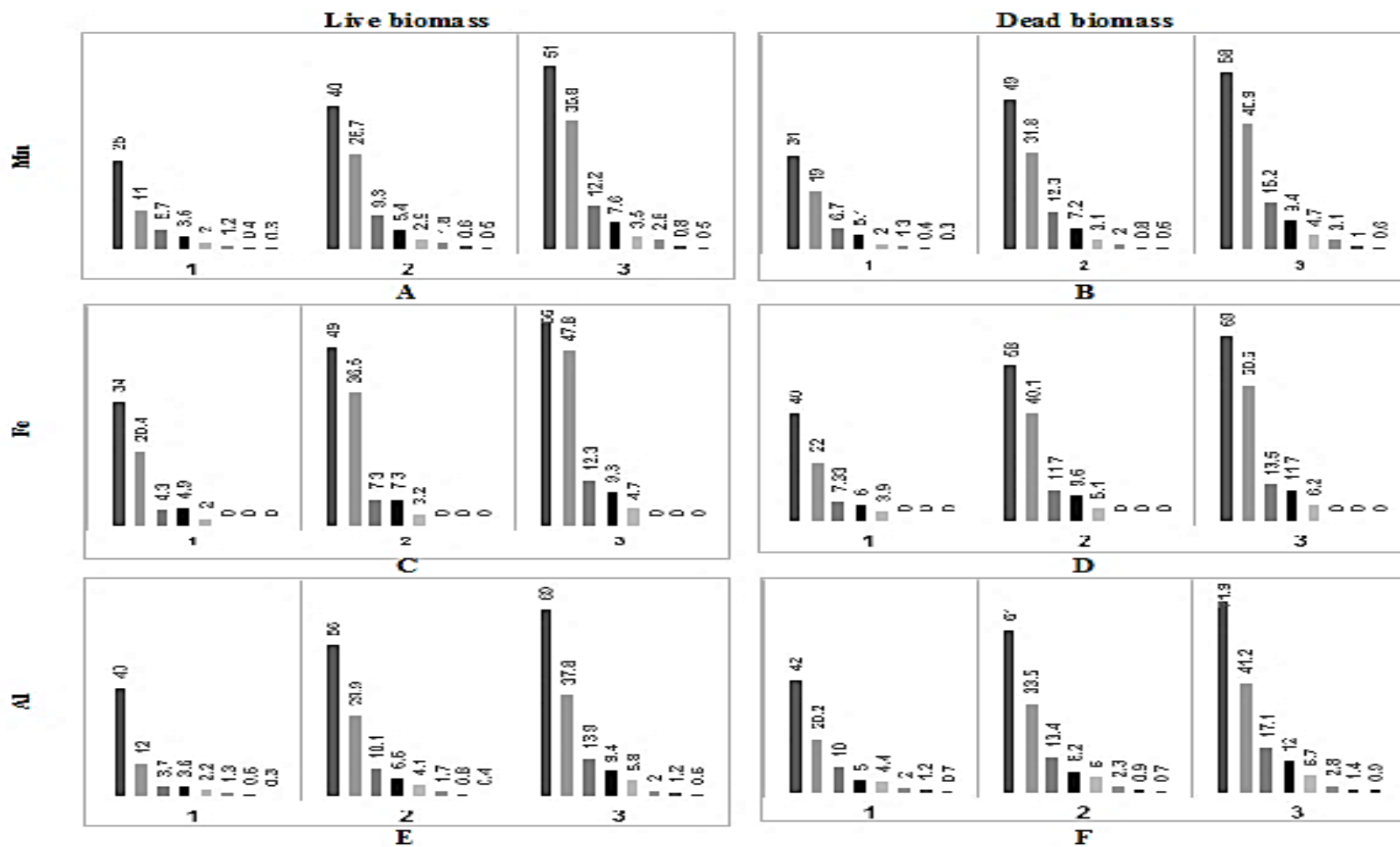


Figure. (2): Effect of the fungal biomass (Live and Dead) and dose on the biosorption percentage of Mn. (a and b), Fe (c and d) and Al (e and f) by *A. oryzae*

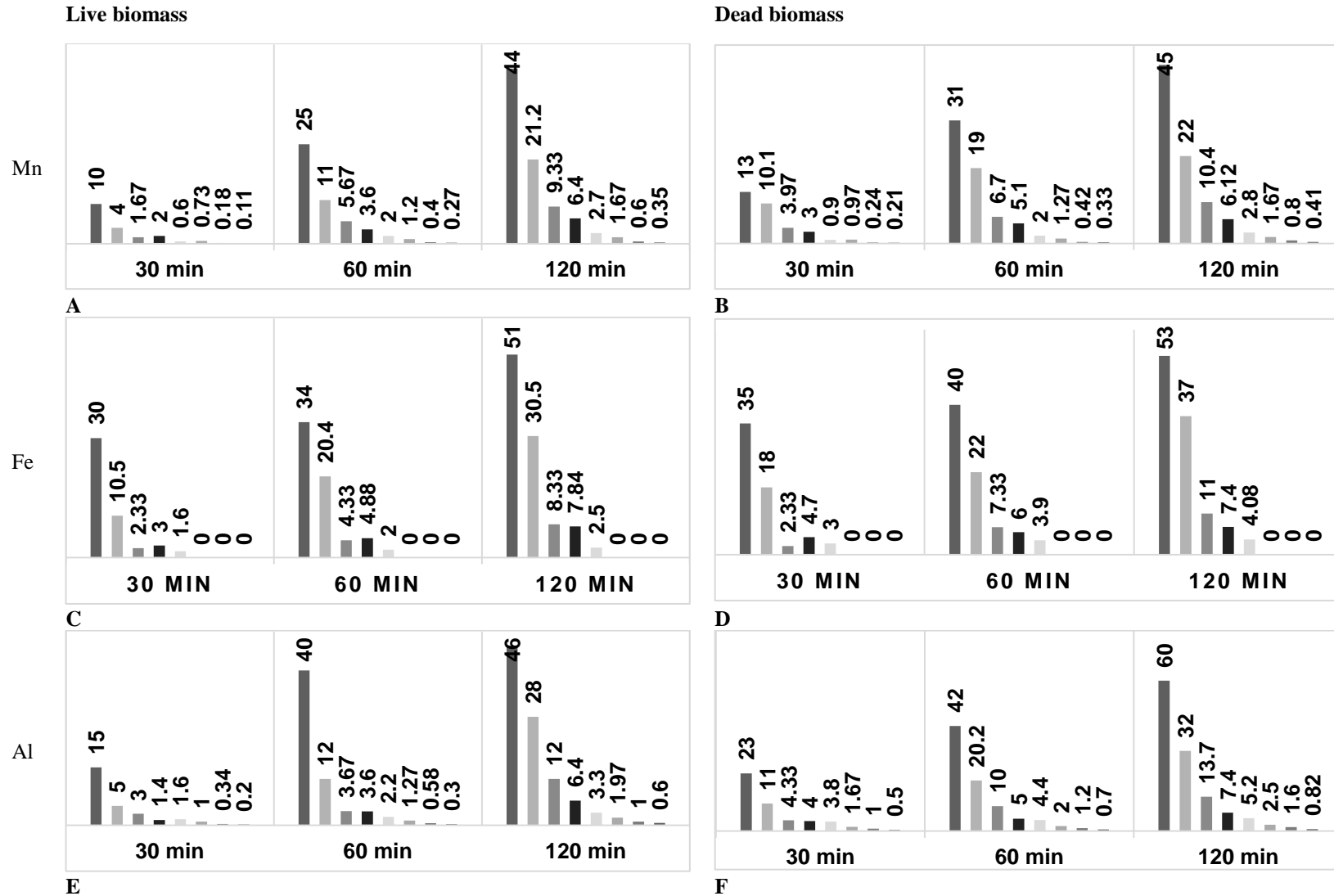


Figure (3): Effect of the fungal exposed time (30, 60 and 120 min) contact on the percentage of Manganese (a and b), Iron (c and d) and Aluminum (e and F) biosorption by *A. oryzae*.

Effect of contact time on the metal uptake by *A. oryzae*.

The results illustrated in figure (3), indicated that the level of ion uptake rose with increasing time contact. Uptake of Mn, Fe and Al ions by viable biomass of *A. oryzae* increased with increasing contact time from 30 min to 120 min. High removal rates of 44%, 51% and 46% for Mn, Fe and Al ions, respectively were recorded at 120 min when the metal concentration maintained at 1.0 ppm. Biosorption percentage decreased with increasing metals concentration with low removal rates 0.11%, 1.6%, 0.2% for Mn, Fe and Al ions respectively, at 30 min when the metal concentration maintained at (1000 ppm for Mn and Al) and at metal concentration 100 ppm for Fe. On the other hand, removal rate by powder biomass of *A. oryzae* was higher than that of viable biomass with increasing contact time from 30 min to 120 min. High removal rate (45%, 53%, 60%) for (Mn, Fe and Al ions) respectively, at 120 min when the metal concentration maintained at 1.0 ppm. Biosorption percentage also decrease with increasing metals concentration, low percentage of biosorption was clearly observed at 30 min with concentration (1000 ppm for Mn and Al) and at metal concentration 100 ppm for Fe the percentage estimate (0.5%, 3% and 0.5%) for (Mn, Fe and Al ions) but this percentage of biosorption more than Viable biomass.

The biosorptive capacity of fungal cells powder has been studied extensively in comparison to living cells. Systems using living cells are likely to be more sensitive to metal ion concentration (toxicity effects) and adverse operating conditions (pH and temperature). Furthermore, constant nutrient supply is required for systems using living cells (increased operating cost for waste streams devoid of nutrients) and recovery of metals and regeneration of biosorbent is more complicated for living cells. The biosorptive capacity of powder cells may be greater than that of living cells. Use of powder biomass in industrial applications offers certain advantages over living cells.

DISCUSSION

Heavy metals contamination has become a serious threat for the environmental safety, pressurizing soils, water streams, atmosphere and living systems. Generation of large quantities of toxic effluents containing metals, metalloids, lanthanides and radio-nuclides are deteriorating biodiversity, ecosystem and human safety. Fungi are versatile group as they can adapt and grow under various extreme condition of pH, temperature and nutrient availability, as well as high metal concentrations (Anand *et al.*, 2006). They offer the advantage of having cell wall material which shows excellent metal binding properties (Gupta *et al.*, 2000). Describing the ability to grow at high metal concentrations, (Malik, 2004) distinguished tolerant to more rapidly comparing with that of higher concentrations (Ahalya *et*

al., 2005). They were marked increase in removal of all tested metals by the increasing of biosorbent mass during these investigation, the results were in harmony with the previous work by: Jayaraman and Arumugam (2014) which recorded that; increase of the availability of free adsorption sites and the adsorption surface area help in removal of metals, during their work in Metal tolerance analysis of micro fungi isolated from metal contaminated soil and waste water. The maximum removal efficiency was observed at 3.0 g of powder biomass 58, 69, 71.9 % for 1.0 ppm of Mn, Fe and Al ions respectively. While with viable biomass, it was slightly lower (51, 56, and 69 %) for Mn, Fe and Al, respectively. Similar enhancement in powder mass of *A. oryzae* was a feasible and favorable process in adsorption of aluminum ions by Omeike *et al.* (2013)

Effect of contact time on the metal uptake by *A. oryzae*.

The uptake of Mn, Fe and Al ions by viable biomass of *A. oryzae* increased with increasing contact time from 30 min to 120 min. The maximum amounts were recorded at 120 min when the metal concentration maintained at 1.0 ppm. Biosorption percentage decreased with increasing metals concentration with low removal rates for tested metals at 30 min at concentration (1000 ppm for Mn and Al) and at concentration 100 ppm for Fe. High removal rate by powder biomass of *A. oryzae* was higher than that of viable biomass with increasing contact time from 30 min to 120 min. Use of powder biomass in industrial applications offers certain advantages over living cells. The powder biomass can be procured from industrial sources as a waste product from established fermentation processes (Kapoor and Viraraghavan, 1997)

CONCLUSIONS

According to our knowledge, limited work in *A. oryzae* adsorption ability to aluminum and almost no previous work in the ability of this fungus adsorbent to iron and manganese. Thus, *A. oryzae* biomass adsorption potency is a promising ecofriendly tool to clean up water from manganese, iron and aluminum *Aspergillus oryzae* appeared to be a suitable candidate for wastewater bioremediation. Further research will assist optimization of final biosorption capacity to improve the economic sustainability and practicalities of large-scale implementation of fungal heavy metal bioremediation. Finally, we conclude that, *Aspergillus* is a potential method to clean environment from toxic element with a promised feedback to use powder biomass to avoid any contamination by fungus.

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التحمل و الامتصاص الحيوي للمنجنيز و الحديد و الالومنيوم بواسطة خمسة أنواع من فطر الأسبرجيليس المعزولة من المياه العذبة

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الملخص العربي

تلعب الكائنات الحية الدقيقة دورا هاما في المعالجة الحيوية للتربة ومياه الصرف الصحي الملوثة بالمعادن الثقيلة. وقد أجريت هذه الدراسة لمعرفة مدى قدرة الأجناس المختلفة من فطرة *الأسبرجيلوس* (*أسبرجيلوس* فلافوس، *أسبرجيلوس* نيجر، *أسبرجيلوس* نيدويولانس، *أسبرجيلوس* أوريزي و *أسبرجيلوس* تيريوس) لمقاومة العناصر الثقيلة و ذلك للعينات التي تم عزلها من مياه نهر النيل. وقد تم قياس درجات المقاومة المختلفة للفطريات قيد الدراسة عن طريق قياس تثبيط النمو للفطريات باستخدام تركيزات مختلفة لبعض المعادن مثل (المنجنيز، الحديد و الألمنيوم) ومقارنتها بالعينة المحكمة. كما أظهرت النتائج أن هناك تفاوت في مستوى مقاومة العزلات المستخدمة في الدراسة لهذه العناصر، و كان فطري *أسبرجيلوس* نيجر و *أسبرجيلوس* أوريزا لهما القدرة على المقاومة في حين فطر *أسبرجيلوس* نيدويولانس كان هو الأكثر حساسية. بالإضافة لذلك تم إختبار قدرة فطر *أسبرجيلوس* أوريزي (مسحوق الفطريات و الفطريات الحية) على إزالة العناصر المختبرة (المنجنيز، الحديد و الالومنيوم) من المياه الملوثة خلال فترات زمنية مختلفة، حيث أظهرت هذه الدراسة مقدرة الفطر على ازالة العناصر المستخدمة بكميات كثيرة في غضون ساعتين و ذلك بزيادة كمية الفطر.