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Biosorption of Heavy Metals by Dead Biomass of *Mucor Hiemalis* Wehmer and *Trichoderma Viride* Pers. in Separate and Consortium Systems

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Abstract

Mucor hiemalis, *Trichoderma viride* biomasses and their consortium were assayed as biosorbents of heavy metals in liquids. The influence of initial metal concentration, biomass concentration, pH and contact time on their biosorption capacity to Fe, Pb and Cd were investigated in separate and consortium cultures. The increase in biosorption rate occurs with the increase of initial metal ion concentration, as long as binding sites are unsaturated. Sorption isotherms follow the non-linear Langmuir adsorption principle. The maximal uptake of Fe, Pb and Cd (100 ppm) was 50.77±0.78, 45.20±0.50 and 32.69±0.55 mg/g; 63.39±1.74, 57.38±0.40, and 37.03±0.56 mg/g and 70.41±0.43, 61.69±0.46, and 40.13±0.59 mg/g, for *M. hiemalis*, *Tr. viride* and their consortium respectively. Maximum efficiency of biosorption occurs at 200 mg. The highest uptake of Fe, Pb and Cd by M. hiemalis appears at pH 4, while for *Tr. viride* and their consortium the highest uptake occurs at pH 6. Each fungus has a specific contact time for Fe, Pb and Cd max biosorption. The highest uptake was occurred at 15 min for *M. hiemalis*. but for *Tr. viride*, highest uptake occurs at 20 min. In consortium experiment, the highest uptake of Fe and Pb occurs at 20 min but for Cd at 30 min. Generally, biosorption of metal ions proceeds rapidly during the initial 15 min. of contact and then slow down with time and become stable after 30 and 60 min. Based on Qmax values, *Tr. viride*, *M. hiemalis* and their consortium are worthy biosorbents of Fe, Pb and Cd.

Keywords: Initial metal concentration, Biomass concentration, pH, Contact time, sorption isotherms.

Introduction

The presence of heavy metals in an environment generates phenotypic and physiological variations in the living microbiota (**Vadkertiova and Slavikova 2006)**. Several techniques are explored for utilizing associated microbiota within the ecosystem, to decompose, accumulate and/or remove the contaminants **(Khan and Khoo 2000).** Biosorption is considered one of those technologies that contribute to the disposal of dangerous pollutants that are difficult to remove by normal treatment methods. In fact, fungi isolated from polluted places as biosorbents, could biosorbe heavy metals for example **(El-Morsy, 2004; El-Morsy *et al.*, 2013; Abdel-Azeem and Gab Allah, 2010).** Moreover, fungal biomasses could be employed as biosorbents of metal ions in wastewater treatment (**El-Morsy, 2004; Zafar *et al.*, 2007; El-Morsy *et al.*, 2013).** Fungi possess several mechanisms including transformation, precipitation and active uptake to tolerate and detoxify metals **(Gadd 1993; Kapoor *et al.* 1999, Magyarosy *et al.* 2002)**.

Biosorption is an efficient technique for eliminating heavy metals from industrial effluents at a level below 100 mg/L, where other methods are inefficient or costly, by passive binding to various biomasses (**Volesky, 1990; Schiewer and Volesky, 1995).** The uptake of heavy metals by natural substances occurs via various mechanisms including ion exchange, sorption, complexation, chelation, microprecipitation etc. (**Volesky *et al*., 1999; Gadd, 2008).** Biosorbents are complex and variable materials. Microbial biomass can bind comparatively high quantities of metal ions on their cell wall owing to its structural characteristics. The wall structure depends not only on biosorbent species, but also on its culture conditions. Actually, the microbial cell walls, embrace many functional groups such as phosphoryl, carboxyl, amino, ketones, sulfhydryl and hydroxyl groups containing ligands will involve a physico-chemical interaction between the metal ions during the biosorption processes (**Mashitah *et al*., 1999; Arief *et al*., 2008).** The efficacy of biosorption process is mainly affected by the properties of the metal ion, the nature of the biosorbents, and the biosorption environment (**Tobin *et al*., 1984; Sterritt and Lester, 1996; Zamil *et al*., 2009).**

In Egypt, several native fungal taxa were used as biosorbents of heavy metals of them: Aspergillus ustus, A. luchuensis, A. cristatus, A. awamori, Cunninghamella echinulate, Fusarium verticillioides**,** Mucor racemosus**,** M. rouxii, Monacrosporium elegans, Penicillium duclauxi, P. oxalicum, P. lilacinum, Rhizopus oryzae, Rhizopus sp. Saprolegnia delica , Trichoderma viride, Aspergillus sp. AHM69 and Penicillium sp. AHM96 (**El-Morsy, 2004; Ali and Hashem, 2007; El-Gendy et al., 2011; Mahmoud et al., 2011; Hassan and El-Kassas, 2012; El-Morsy et al., 2013; Mahmoud et al., 2013; Saad, 2014**; **Abedin, 2014; Elsayed, 2015; El-Bondkly and El-Gendy, 2022).**

Several studies on the biosorption of heavy metals from solution, industrial and domestic sources have been carried out because of their toxicity and serious ecological impacts. This work aimed to use *Mucor hiemalis* Wehmer and *Trichoderma viride* Pers. in separate and consortium dead biomasses for biosorption of heavy metals to minimize this problem.

Materials and methods

Preparation of free fungal biomass

Trichoderma viride and *Mucor hiemalis* were assayed for their potency in biosorption of metal ions in **single** and **consortium** cultures. They were cultured on potato dextrose broth (PDB) at 28 ^oC for 7 days on a rotary shaker at 180 rpm. Produced pellets were washed twice by using sterile double distilled water then drained and dried at 60 ^oC for 24 h to constant weight and ground within a mortar before determination of its metal biosorption potentiality **(Barclay *et al*., 1998).**

Optimization of factors affecting the biosorption of heavy metals

Initial metal ion concentration

Initial metal ion concentration (50,100,200, 300 ppm) of iron, lead, and cadmium was assayed by using constant died biomass of 200 mg/l of both taxa in **single** and **consortium** cultures. The reaction flasks were then left for 15 min on a rotary shaker at 180 rpm at 28 ^oC. Samples were taken immediately following exposure of the mycelium to the metal solution. A triplicate set of flasks was carried out for each treatment and the average values were calculated after filtration **(Barclay *et al*., 1998).**

Biomass concentration

Dead fungal biomasses of 50, 100, 200, and 300 mg/l in a **single** and **mixed** sample were added separately in 250 ml Erlenmeyer flasks containing 50 ml of each heavy metal (iron, lead, and cadmium) in concentrations of 100 ppm. The flasks were then left for 15 minutes on a rotary shaker at 180 rpm at 28 ^{o}C. Samples were taken immediately following exposure of the mycelium to the metal solution. The experiment was done in triplicate. The average values were calculated after filtration and analyses. **(Barclay *et al*., 1998).**

pH Sorption efficiency of metal ions (Iron, Lead, and Cadmium) by bio-sorbents died biomasses in **single** and **mixed** experiments was carried out at different pH values of 2, 3, 4, 5, 6 and 7. A constant bio-sorbents biomass of 200 mg/l was added to 50 ml (100 ppm) of heavy metal solution (iron, lead, and cadmium). To avoid shifts in pH, the pH was readjusted with 0.1N HCL and 0.1N NaOH after every addition. In case of lead, pH was adjusted with 0.1 N HNO_3 and 0.1 N NH_4OH. The reaction flasks then were agitated on a rotary shaker at 180 rpm for 15 min at 28^o C. Experiments were performed in triplicates. The average values were calculated after filtration and analyses **(Barclay *et al*., 1998).**

Contact time

A fixed bio-sorbent biomass of 200 mg/l of in a **single** and **mixed** cultures was added to 250 ml flasks containing 50 ml (100 ppm) of heavy metal solution (iron, lead, and cadmium). The experiments were then agitated on a rotary shaker at 180 rpm for 15 min at 28^{o}C. Triplicate samples were taken at 5, 10, 15, 20, 30, 60 and 120 min respectively. Experiments were performed in triplicates. The average values were calculated after filtration and analyses **(Barclay *et al*., 1998).**

Biosorption mechanism

The amount of metal adsorbed by a biosorbent can be assessed by determination of the metal ion uptake (q) that can be calculated from equation ($q = V (C_i-C_f) /M$)**Volesky and Holan (1995**. The sorption isotherm can be expressed by plotting **q** versus **C_f**. (**Langmuir, 1918) and** the linear isotherm equation is $q = Q_{max}\ bc_f /1+bc_f$. Where: Qmax is the maximum amount of metal per gram of biomass corresponding to saturation of the adsorption sites, b is the dissociation constant and Cf is the final metal ion concentration (ppm).

Results and Discussion:

The potential of uses fungal biomasse as bisorbent agents for the removal of heavy metals from polluted waters has been widely recognized where fungi can be easily grown and reproduced in considerable amounts using relatively unsophisticated and inexpensive culture media, environmental compatibility, high adsorption capacity, no sludge production, and their special mechanical properties in large-scale production. (**Dusengemungu et al., 2020; Tamjidi et al., 2023**). They are effective biosorbent due to their adsorption capacity through one mechanism or combinational processes into the fungal cells Thus, fungal uses as biosorbent for bioremoval of metal ions from aqueous solutions is economic ecofriendly valuable technique **(Viraraghavan and Srinivasan 2011).**

Effect of Initial metal concentration:

Sorption isotherms of Fe, Pb and Cd by *M. hiemalis, Tr. viride,* and their consortium are illustrated in Fig. 1a, b & c and appear to follow the typical nonlinear Langmuir adsorption pattern**.** The absorption increases when the initial metal ion concentration rises up, if binding sites are not saturated. The maximal uptake of Fe, Pb and Cd occurred at 100 ppm for *M. hiemalis, Tr. viride,* and mixed of both fungi (*M. himalis + Tr. viride)*.

For *M. hiemalis* the highest uptake of Iron, Lead and Cadmium were 53.45±1.11, 45.20±0.50 and 32.69±0.55 mg/g respectively **(Fig. 1, a)**. For *Tr. viride* the highest uptake of Fe, Pb and Cd were (63.39±1.74, 57.38±0.40, and 37.03±0.56 mg/g respectively **(Fig. 1, b)**. Whereas the maximal uptake of Fe, Pb and Cd of the consortium biomasses were 70.41±0.43, 61.69±0.46 and 44.60±0.55 mg/g respectively **(Fig. 1, c)**. In fact, the maximal uptake is in the following order: consortium (*M. hiemalis + Tr. viride).> Tr. viride> M. hiemalis* and mixed of both fungi. These results can be attributed to the increase of competition between ions for the vacant binding sites and the lack of active sites on the biomass at higher concentrations **(El-Gendy and El-Bondkly 2016)**. Alike, **Tamjidi et al. (2021)** stated that at high concentrations, the number of active sites and the sorption rate will decrease. Moreover, the sharp rise in uptake by mixed biomasses may be a result of the increases of functional groups in the wall that acts as cationic exchanger and provide abundant sources of metal binding sites **(Gadd 2010).** A single reagent grade was used in all tests to minimize the availability of metal concentrations and to avoid competitive reduction of mixed metals on biosorbent **(Ruthven, 1984)**.

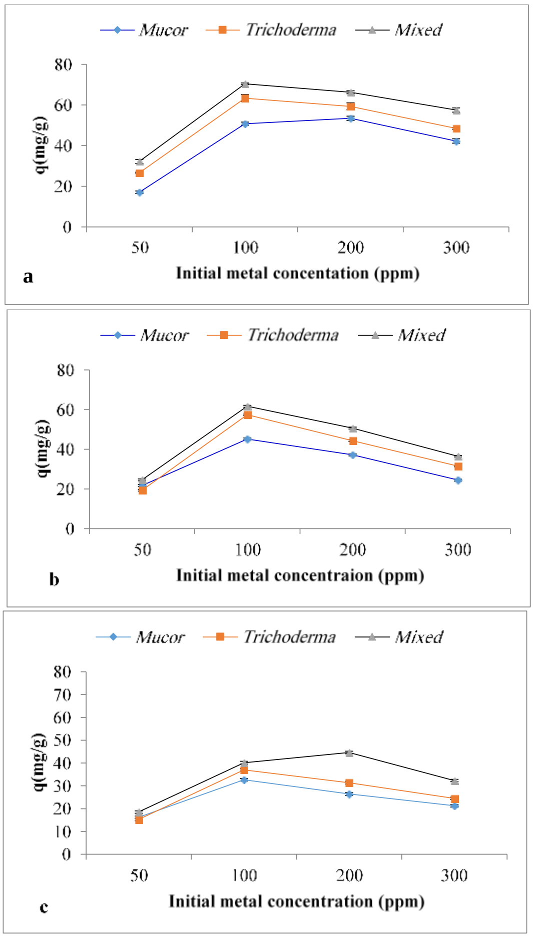

Figure 1. Effect of **initial metal ion concentration** on biosorption of heavy metals by separate and mixed dead biomasses of *Mucor hiemalis* and *Trichoderma viride*. (a); Iron, b; Lead, c; Cadmium

Effect of biomass concentration:

Biomass (Biosorbent) is very important in the process of biosorption **(Asgher and Bhatti 2010).** The biosorption capacity depend on the biomass that it is a positive relationship, where with the biomass increase the surface area increases and more vacant sites become available for more metal ions **(Gong *et al*. 2005; Tamjidi and Ameri, 2020; Tamjidi et al., 2021)**. The effectiveness of biosorption of Iron, Lead and Cadmium occurred at 200 mg in separate and consortium dead biomasses. In case of *M hiemalis* the maximum Fe, Pb and Cd uptake was 46.35±0.61, 40.25±0.51 and 48.62±0.52 mg/g respectively **(Fig. 2a)**. Whereas for *Tr. viride*, the maximum uptake for Fe, Pb and Cd was 59.41±0.56 mg/g, 57.54±0.49 mg/g and 37.47±0.59 mg/g respectively **(Fig. 2 b)**. Moreover, the highest uptake was recorded by consortium biomasses for Fe (66.25±1.23 mg/g), Pb (62.32±0.42 mg/g) and Cd (40.46±0.57 mg/g) **(Fig. 2 c).** It is also noticed that the uptake level decreases when the biomass concentration increases over 200 mg **(Fig. 2a, b, c).** This probably owing to the interference between binding sites and inadequacy of metal ions (**Hajahmadi *et al*. 2015) or** restriction of the access of metal ions to that sites (**Fourest and Roux, 1992).**

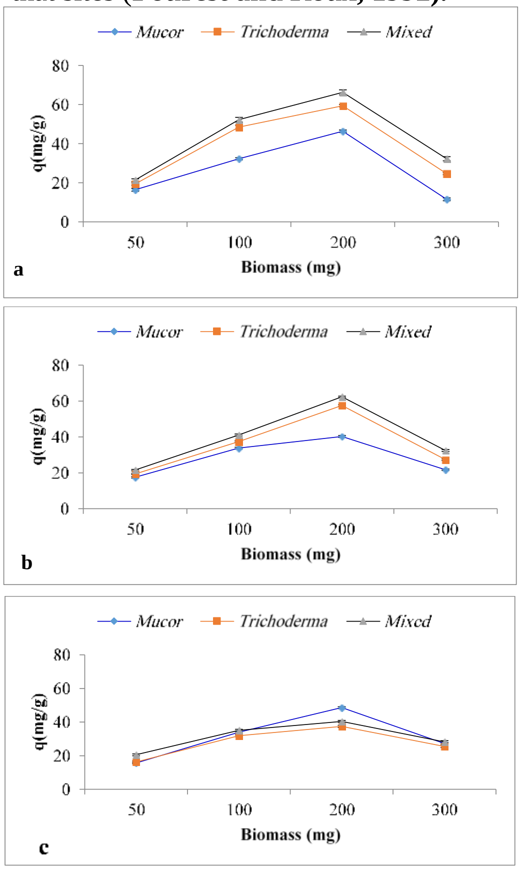

Figure 2. Effect of **biomass concentration** on biosorption of heavy metals by separate and mixed dead biomasses of *Mucor hiemalis* and *Trichoderma viride*. (a); Iron, b; Lead, c; Cadmium

Effect of pH:

pH is of prime importance in the biosorption processes. In fact, initial pH is significant in commencing any biosorption experiment. Figure 3a, b, c demonstrated the influence of pH on the biosorption of Iron, Lead and Cadmium by testing dead biomasses of fungal species.

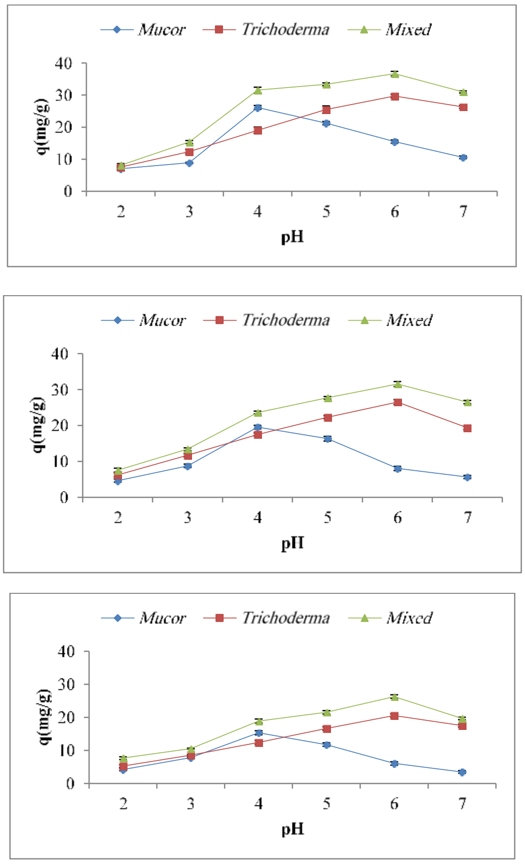

Figure 3. Effect of **pH concentration** on biosorption of heavy metals by separate and mixed dead biomasses of *Mucor hiemalis* and *Trichoderma viride*. (a); Iron, b; Lead, c; Cadmium

For *M. hiemalis* the highest uptake of Iron, Lead and Cadmium were 26.15±0.61, 19.55±0.50, and 15.38±0.46 mg/g respectively at pH 4 and the lowest uptake 7.09±0.40, 4.65±0.42, at pH 2, 3.53±0.46 at pH 7. Likewise, *Trichoderma viride* revealed the highest uptake of Iron (29.72±0.51 mg/g), Lead (26.52±0.67 mg/g) and Cadmium (20.58±0.71 mg/g) occurs at pH 6 and the lowest uptake of Iron (7.57±0.50), Lead (6.24±0.55), and Cadmium (5.29±0.45) at pH 2. By the way the consortium showed the highest uptake ever for Iron (36.68±0.73 mg/g), Lead (31.55±0.67 mg/g) and Cadmium (26.31±0.70 mg/g) at pH 6 and the lowest uptake for Iron (8.12±0.50), Lead (7.57±0.52), and Cadmium (7.70±0.73) at pH 2. Thus, heavy metals ions (Iron, Lead, and Cadmium) were removed from their solution by tested separate or mixed biomasses of *M. hiemalis* and *Tr. Viride*. The maximum uptake of Fe, Pb and Cd by *M. hiemalis* occurs at pH 4 and the lowest uptake at pH 2 while for *Trichoderma viride* and mixed biomasses, the max. uptake occurs at pH 6 and the lowest one at pH 2. At last, we confirmed that biosorption of metal ions is pH depends, and the efficiency of biosorption can be elucidated on the basis of proton competitive adsorption reaction (**Nasri and Garima, 2004**).It is stated that at low pH values, biosorption capacity is very low as a result of the competition for binding sites between the cations and protons; where hydrogen ions compete with metal ions at adsorption sites (**Greene and Darnall, 1990; Deng and Wang 2012**). Alike, **Ali and Hashem (2007), El-Didamony (2014) and El-Morsy et al. (2013)** stated similar observations**.** As the pH increases, negatively charged cell surface, and metal uptake increases as such. Conversely, at high pH values (>7), some metals are precipitates, and thus inhibiting the contact of metal with the most fungal biomass **(Sun *et al*. 2012)**. In fact, pH is affecting the effectiveness of the biosorption processes by its strong effect on the chemistry of the metals, and cell wall (functional groups), as well as the competition of metallic ions for the binding site (**Abbas et al., 2014**).

Effect of contact time:

Contact time at which maximal biosorption of metal ions by dead biomasses of *M. hiemalis*, *Trichoderma viride*, and mixed of both species (*Mucor hiemalis + Trichoderma viride)* were determined after 5, 10, 15, 20, 30, 60, and 120 min **(Fig. 4 a, b, c).** For *M. hiemalis* the highest uptake of Iron, Lead and Cadmium were 37.08±0.57, 22.34±0.35 and 21.70±0.68 mg/g respectively at 15 min and the lowest uptake 15.33±0.58, 6.4±0.42, and 4.49±0.50 at 5 min. While for *Trichoderma viride* the highest uptake of Iron, Lead and Cadmium were 39.50±0.43, 29.41±0.66 and 25.74±0.66 mg/g respectively at 20 min and the lowest uptake 16.08±0.58, 7.52±0.49, and 6.28±0.55 at 5 min. A like, for mixed fungal, the highest uptake of Iron, Lead and Cadmium were 43.33±1.15, 32.87±0.46 at 20 min and 27.84±0.64 mg/g at 30 min respectively and the lowest uptake 19.15±0.53, 8.48±0.47 and 8.26±0.49 at 5 min. Therefore, heavy metals ions (Fe, Pb and Cd) were removed from their solution by separate or mixed biomasses experiments at variable contact times. For *M. hiemalis* the highest uptake occurs after 15 min of the contact between biosorbate and biosorbent that is reported earlier (**El-Didamony, 2014; El-Morsy, 2004; El-Morsy *et al*., 2013)**. For *Tr.*

viride the highest uptake occurs after 20 min. of contact time, similar to that reported by **Ali and Hashem (2007)**. In consortium experiments, the max. uptake values of Fe, and Pb occurs after 20 min. but for Cd occurs after 30 min. Generally, uptake of metal ions proceeds rapidly during the initial 15 minutes of contact and then proceeded very slowly with time and was stable after 30 and 60 min**.** Thus, biosorption mechanism occurs at a primary rapid and a secondary slow phase. The first one lasted for about 15 min and occupies the major biosorption section accounted. This is reefer to the rapid kinetics of metal adsorption during the early time of contact that is possibly due to electrostatic attraction (Tamjidi and Ameri, 2020; Tamjidi et al., 2021). Conversely, the second one is slow in uptake of metal ions that is may be owing to the reduced availability of active sites **(Sun *et al*. 2012)**.

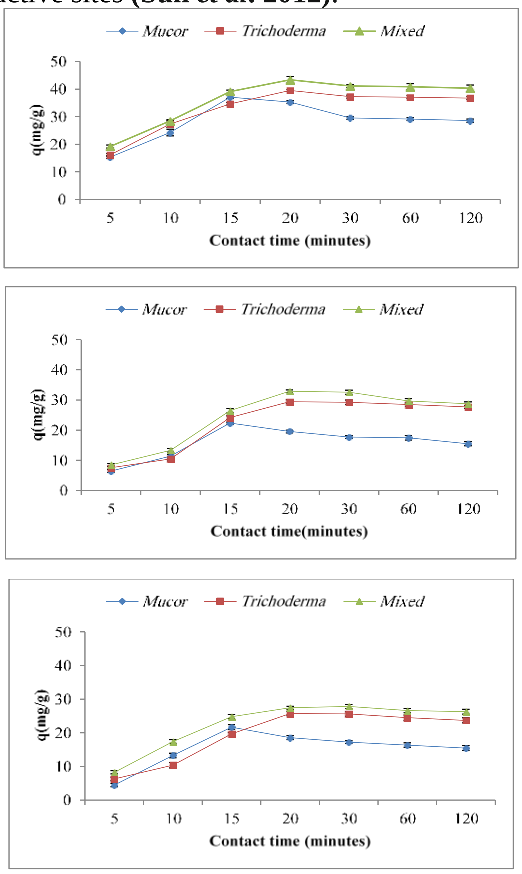

Figure 4. Effect of **contact time** concentration on biosorption of heavy metals by separate and mixed dead biomasses of *Mucor hiemalis* and *Trichoderma viride*. (a); Iron, b; Lead, c; Cadmium

Biosorption mechanism:

Biosorption occurs by the complexation mechanism (donor–acceptor) where atoms of the exposed functional group donate electrons to the biosorbate. Sorption isotherms represent the equilibrium distribution of metal ions between the aqueous and solid phases, when the concentration increases. Sorption isotherms of Fe, Pb and Cd by *M. himalis*, *Tr viride*, and their consortium are illustrated in **(Fig. 5, a,b,c).** It represents the distribution of metal ions between the two phases while the concentration of metal ions increases, the adsorption rate increases unless binding sites are saturated. From these isotherms the adsorption capacities and dissociation constants of metal ions can be calculated. They follow the typical nonlinear Langmuir adsorption model**.** The isotherm assumes monolayer adsorption of one molecule in thickness, with adsorption occurring at a limited number of specific restricted sites, which are identical and stoichiometric, with no lateral interaction and strict obstacle between adsorbed molecules. **(Foo and Hameed, 2010).** The relation appears nonlinear where the solute absorptivity and adsorptive energy were much less in contrast to the linear model **(López-Luna et al., 2019).**

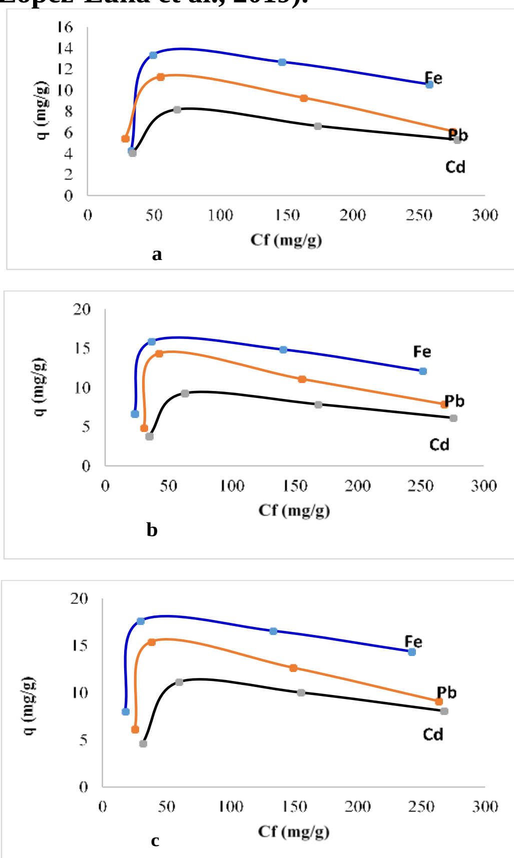

Figure 5. Adsorption isotherm (Langmiur) for Fe, Pb and Cd by dead biomasses of *Mucor hiemalis* (a), *Trichoderma viride* (b) and their consortium (c).

Generally, the level of uptake (Q_{max}) by *M. hiemalis* increased in the following sequence (Table 1): Fe (49.23 mg/g) <Pb (54.8 mg/g)< Cd (67.31 mg/g). For *Tr. Viride,* the sequence was Fe (36.61mg/g) <Pb (42.62 mg/g) <Cd (62.97mg/g). Likely, in the consortium experiment, the sequence is similar Fe (29.59mg/g)< Pb (38.31mg/g) <Cd (59.87mg/g).

Table 1. Uptake capacities of various heavy metals by *Mucor himalis, Trichoderma viride* and Consortium experiments derived from the Langmuir equation ($C_f/q=b/Q + C_f/Q$).

	Mucor himalis			***Trichoderma viride***			**Consortium**		
Parameters	Fe	Pb	Cd	Fe	Pb	Cd	Fe	Pb	Cd
A. W (g)	55.84	207.19	112.41	55.84	207.19	112.41	55.84	207.19	112.41
Q(mg/g)	49.23	54.8	67.31	36.61	42.62	62.97	29.59	38.31	59.87
Q(mmole/g)	0.88	0.26	0.59	0.655	0.206	0.56	0.53	0.184	0.53
b(mM)	0.243	0.2488	0.2595	0.2488	0.2463	0.250	0.249	0.253	0.250
Affinity (1/b)	4.10	4.02	3.85	4.02	4.1	4.0	4.01	3.95	3.99

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

In conclusion *M. himalis, Tr. viride,* and their consortium are effective biosorbents and alternative ecofriendly method for heavy metals bioremoval. It is one of the promising green technique for bioremoval of heavy metals from polluted water.

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الملخص العربي

عنوان البحث: الامتصاص الحيوي للعناصر الثقيلة باستخدام الكتلة الحيوية الميتة لفطرتي *Mucor Hiemalis Wehmer* and *Trichoderma viride Pers* منفصلتين او في مزارع مشتركة

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تم تقيم مقدرة والكتل الحيوية لفطرتي Mucor hiemalis وTrichoderma viride منفصلين وفي مزرعة مشتركة كمواد ماصة للمعادن الثقيلة في السوائل. تم فحص تأثير تركيز الأولي للمعادن المختبرة وتركيز الكتلة الحيوية ودرجة الحموضة ووقت التلامس على قدرتهما على الامتصاص الحيوي للحديد والرصاص والكادميوم في مزارع منفصلة ومشتركة. ووجد ان الزيادة في معدل الامتصاص الحيوي تزداد مع زيادة تركيز أيون المعدن الأولي، طالما أن مواقع الارتباط غير مشبعة. تتبع Sorption isotherms الامتصاص مبدأ الامتزاز للانجمير Langmuir غير الخطي. و كان الحد الأقصى لامتصاص Fe و Pb و Cd (١٠٠ جزء في المليون) كانت ٥٠,٧٧ ± ٠,٧٨ و ٤٥,٢٠ ± ٠,٥٠ و ٣٢,٦٩ ± ٠,٥٥ مجم / جم ؛ ٦٣,٣٩ ± ١,٧٤ و ٥٧,٣٨ ± ٠,٤٠ و٣٧,٠٣ ± ٠,٥٦ مجم / جم و ٧٠,٤١ ± ٠,٤٣ و ٦١,٦٩ ± ٠,٤٦ و ٤٠,١٣ ± ٠,٥٩ مجم / جم بالنسبة لـ ميكور هيمالايس، تريكوديرما فيرييد منفصلين وفي مزرعة مشتركة على التوالي. تحدث أقصى كفاءة للامتصاص الحيوي biosorption عند ٢٠٠ مجم. أعلى امتصاص لـ Fe و Pb و Cd بواسطة ميكورهيماليس كان عند الرقم الهيدروجيني ٤، بينما كان في تريكوديرما فيرييد واتحادهم حدث أعلى امتصاص عند الرقم الهيدروجيني ٦. وكان لكل فطر وقت تلامس contact time محدد للامتصاص الحيوي Fe و Pb و Cd max. حدث أعلى امتصاص في ١٥ دقيقة لـ Mucor hiemalis. ولكن بالنسبة تريكوديرما فيرييد يحدث أعلى امتصاص عند ٢٠ دقيقة. في تجربة المزرعة المشتركة consortium ، كان أعلى امتصاص للحديد والرصاص عند ٢٠ دقيقة ولكن للكاد عند ٣٠ دقيقة. بشكل عام، يستمر الامتصاص الحيوي لأيونات المعادن بسرعة خلال الـ ١٥ دقيقة الأولى. من التلامس ثم تبطئ مع الوقت وتصبح مستقرة بعد ٣٠ و٦٠ دقيقة. بناءً على قيم Qmax ، تعتبر تريكوديرما فيرييد وميكور هيمالايس منفصلين و مشتركين مواد ماصة حيوية جيدة للحديد والرصاص والكادميوم.