J. Mater. Environ. Sci. 6 (6) (2015) 1503-1509 *Bedioui et al.*
ISSN: 2028-2508
CODEN: JMESCN

Bioremediation of Mercury by Fungal Biomass

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Received 22 Nov 2014, Revised 22 Mar 2015, Accepted 22 Mar 2015
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Abstract
The environmental contamination by organic and inorganic pollutants is a real threat to water resources, because it disrupts ecosystems by introducing a large number of toxic substances such as mercury, which can be accumulated in the food chain .This accumulation can cause disturbances in the immune, kidney and neurological system; consequently it affects human and animal health. Molds have a cell wall which is rich of chitin and chitosan, capable of accumulating large amounts of heavy metals. These microorganisms have been used as a novel biological absorbent for the removal of mercury at low concentrations in the order of (10 µg / land 5×10^3µg/ l). In orders to study the kinetics of the bio-sorption, three parameters have been studied: the pH, the temperature and the initial concentration of mercury. The maximum bio-sorption was observed at pH7. A positive correlation was observed between the capacity of the bio-sorption and of the test medium temperature. The results of the adsorption temperature variation are in agreement with many works and all these parameters are related to the adsorption phenomenon. This experimental adsorption process has been proven in laboratory tests and deserves much more attention for its wide application.

Keywords: Bioremediation, Fungal Biomass, Biosorption, Mercury

1. Introduction
Pollution of protected wetlands zones specially Oubeira Lake which constitutes apart of El Kala National park, situated in east of Algeria with toxic metals such as mercury is mainly generated by human activities which represent more and more growing threat for humans and ecosystems. Industrial effluents and intensive use of agricultural fertilizers are the major causes of wide range symptoms affecting the top link organisms of the food chain. We can also cite as an example the methyl mercury which the body absorbs six times more easily than the inorganic mercury [14].The methyl mercury can cross the blood-brain and the placental barriers and react directly with embryonic and brain cells, by generating toxicological effects such as reproduction, growth , development and the learning neuroability reduction ,associated with behaviour al problems, potentially increasing the mortality rate and predation risk of certain wild species. The development of decontamination processes by fungi aims to reduce the toxic potency of pollutants in order to protecting the environment. Among the many methods, we can mention the reduction followed by chemical precipitation [18], ion exchange, reverse osmosis [19] and adsorption on activated carbon [17]. However, the use of these techniques has several draw backs particularly when they are applied to heavily contaminated effluents, their relatively high cost [10] and moreover the cost/devolution ratio is unjustified in most cases [23]. Thus, the search for biosorbents whose use has lower costs as commercial methods represents one of the main themes that have attracted the attention of researchers. Among the studied materials included mostly waste water and agro-industrial origin, such as marine algae [8], microbial biomass [8], pine bark [2], cotton [18] beet pulp [23], pine sawdust [23], the sugar cane bagasse [24] fibres, jute [4], the olive pit [13], the coconut and husks rice [7]. The water origin biosorbents mean a biomass constituted of filamentous fungal species. It is proved that the biomass (living or dead) has very good adsorption capacities particularly due to its physicochemical characteristics [7] However, it is preferable to use the dead biomass which could eliminate toxicity problems and culture medium maintenance [1]. The use of natural biomaterials is an interesting alternative related to their relative abundances and their low market values. Recently, many industrial, agricultural and forest wastes are used as biosorbents [30].This study focuses on the power of biosorbents which is an autochthon fungal biomass isolated and

identified (*Penicillium sp* and *Aspergillus sp*) from the Oubeira lake waters of El Kala region of Algeria. It also involves on the one hand a comparison between the two fungal species and on the other hand the influence of key parameters on their adsorption capacity to wards organic and inorganic pollutants (Hg), especially that there is actually no research on the ability of these species to eliminate mercury [9]. The effect of pH, biosorbents assay, the contact time, the initial metal concentration and temperature was investigated to understand the adsorption mechanism.

2. Materials and methods

2.1. Description of sampling site.

The Oubeira Lake is an aquatic ecosystem that belongs to a biogeography unity characterized by its exceptional biological diversity because it shelters many populations of plant and animal species, including several rare ones (water chestnut *Trapa natans*).These waters lay in El Kala region. Founded in 1983, the National Park covers an area of 76438 ha. Located in north-eastern of Algeria, it is bounded on the east by the Algerian-Tunisian border, on the north by the sea, on the west by Cape Rosa, and on the south by the foothills of El Ghorra Mountain. The park is composed of a mosaic of forest, lakes, dunes and sea ecosystems, giving it a high biological and ecological value in the Mediterranean basin. Its flora, fauna and its cultural heritage have earned it a place as a Biosphere Reserved by UNESCO in1990 [23].These wetlands (Oubeira Lake) have an international importance; it is the only Algerian site which contains the yellow water lily *Nuphar luteum*. The Bulrush gives the lake a great socio-economic interest through to fish production, but also to the use of water for irrigation. However, these waters are now threatened by uncontrolled pumping for speculative crops and wastewater discharge from neighbouring villages is also a significant threat whose effects are not yet visible [21]. The choice of sampling sites were established due to their accessible establishments for sampling, crossed by some cities with high density (population) as well as waterways along some farmlands.

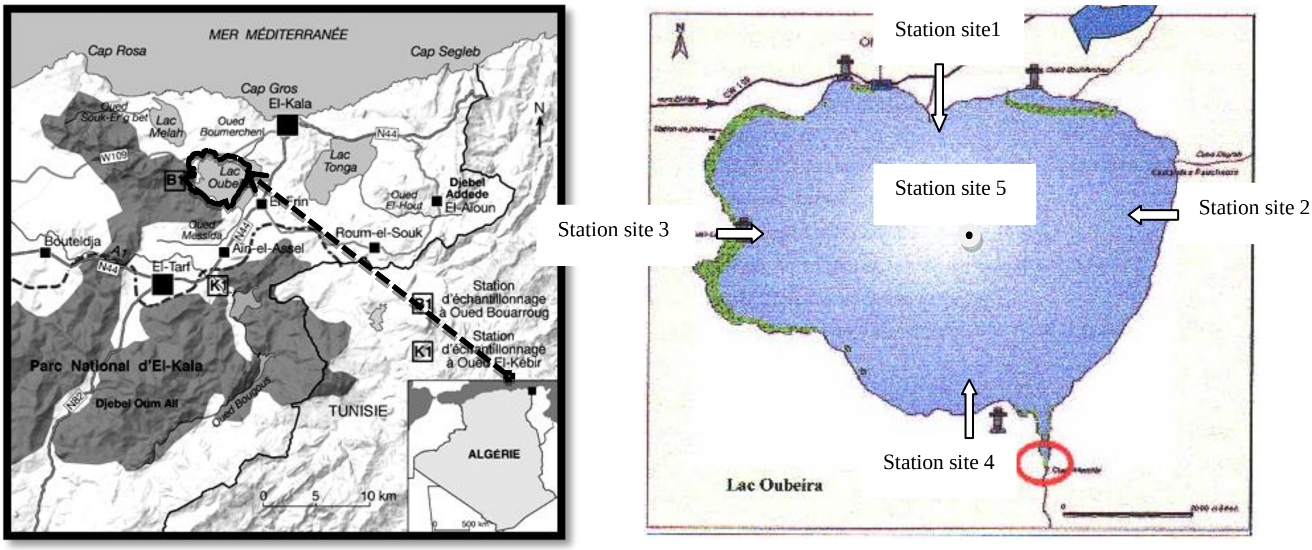

Figure 1: Study and location of sampling stations Site. Map prepared by the Directorate of Forestry El Kala El-Tarf Wilaya (2010)**.**

2.2 . Isolation and identification of fungi

The mold isolation has been effected by conventional techniques using Czapek medium. The identification was performed using an identification Mycology guide and based on the macroscopic and microscopic characters examination [28].

2.3. Sample preparation

Mercury analysis is performed according to the method approved by the AFNOR NFT 90-113[8] using an atomic adsorption spectrophotometer using an analyzer Hg Masson Perkin Elmer50LTD. In conical flasks of 500 ml, mercury was added at different concentrations in the presence of the fungus. This method is approved by the AFNOR NFT90 whose steps are:

•The sample mineralization.

•Injection of the sample into the atomic absorption spectrophotometer equipped with a reduction by sodium borohydride without enrichment.

•The absorbance measurement [25, 27].

2.4. Experimental procedure
Adsorption kinetic is the first indicator parameter of treatment performance of an adsorbent. It enables to estimate the amount of pollutants adsorbed over time. The experiments were performed by contacting the biomass of 8 to 1 µg/l with varying concentrations of mercury from 10 to 5.10^3µg/l and a pH (0-14). The tests were conducted in a water bath with stirring. For the adsorption kinetics, sampling was performed at predetermined time intervals; samples were centrifuged for 2 minutes at 5000 rounds/ min and then analyzed. After a contact time of 3 hours, the quantity of mercury absorbed in µg/l of *Penicillium sp* was calculated by the following formula:

$$ABS = 2\text{-}T.\log 10$$

A: absorbance
T: transmittance.
The temperature and pH of the test medium were measured.

2.5. Physicochemical parameters of Oubeira Lake water
In fact nine physicochemical parameters were measured in field and in laboratory. The pH, temperature, and dissolved oxygen were determined in situ using a pH-meter, thermo-meter and oximeter.
The other parameters (Nitrite, nitrate, chemical and Biochemical Oxygen Demand,) were measured in the laboratory by volumetric method [25].

3. Results and discussion

3.1. The parameters physic-chemical of Oubeira Lake
The change in physico-chemical parameters of the Oubeira Lake water are shown in Table 01.

Table 1: The Parameters Physic-Chemical of Oubeira Lake

pH	6,37±0,04
Nitrite(mg/l)	21±0,002
Nitrate(mg/l)	52±0,4
DBO_5/DCO	0,5025±0,03
Temperature	15,17 ±0,96
Mercury (µg/l)	10 ±0
Material suspension (mg/l)	43,5±15,68
DCO g/l	27±10,13
DBO_5 g/l	13,5± 4,79

The results of physico-chemical analysis indicate that during the study period of Oubeira Lake water, the temperature was around 15° C, which promotes the development of aquatic microorganism ms specifically fungi and a slightly acidic pH of order of6.37, which is an appropriate and an essential factor of fungal cells proliferation [29]. The ratio (BOD_5/COD) which is an indicator of water biodegradability was greater than 0,5.This allows us to note that the degradation of carbohydrate, lipid and protein compounds of organic matter sources of domestic waters coming from the surrounding coastal agglomerations in the lake expresses at first the presence of carbon chains decomposition [22] and an important mineralization of the ground which is also directly related to the Oubeira Lake[11].The very high amounts of nitrites and nitrates of industrial or agricultural origins explain an incomplete oxidation of ammonia .and nitrification is not brought to completion this is due, on the one hand to a nitrate reduction under the influence of a denitrifying action or that the nitrogen compounds and certain aromatic rings and aliphatic chains can escape to the oxidation [25,10]. On the other hand, the industries use these waters as wastewater treatment station for their discharge. So the pollution of these waters (Oubeira Lake) is the main cause of pollution, whose origin seems to be much more urban and industrial [12]. The wastewater contains a toxic material which is mercury exceeding the standards of the World Health Organization (WHO) (02 g/l) [25,31] (10 times more than the standards). Especially with large amounts of nutrients such as organic materials shall, through its discharge, the continued rapid growth of algae and aquatic plants and fungi [3].

3.2Influence of the Initial biomass concentration
Preliminary tests performed on several strains of fungi isolated from the lake water have given us more or less important results. This led us to use only strains that showed the best percentage adsorption. We have

conducted adsorption essays by varying two parameters, namely: the mercury concentration and the contact time. Figures 2 and 3 summarize the essence of our results.

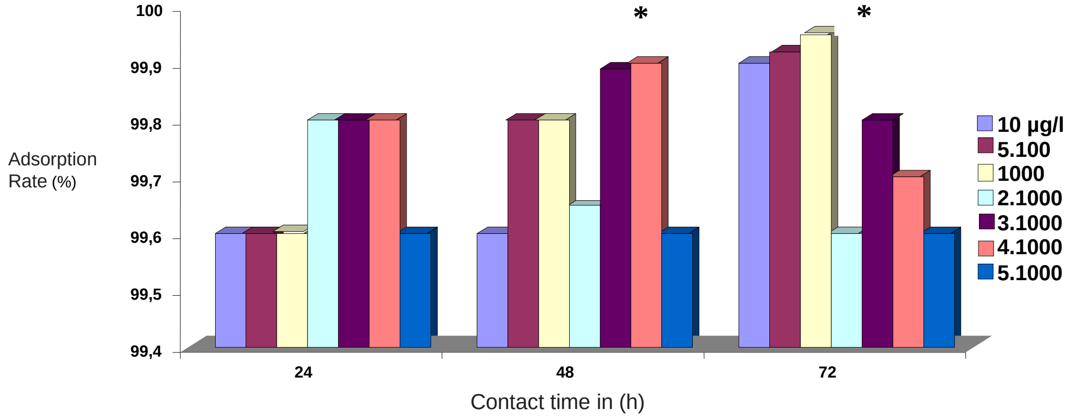

Figure 2: Change in the adsorption rate in the presence *of Penicillium sp* according the contact time

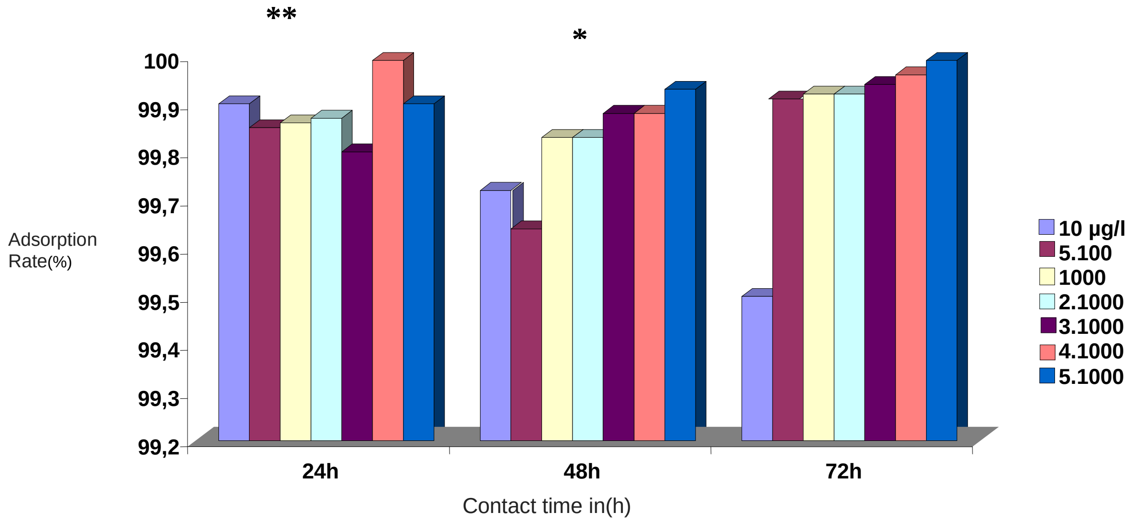

Figure 3: Change in the adsorption rate in the presence of *Aspergillus sp* according the Contact time
Significant deference are denoted by * ($p<0.05$) and ** ($p<0.01$).

•Adsorption after24 hours of contact

The adsorption rate of mercury in the presence of *Aspergillus sp* ranging from the lowest concentration (10 µg / l) up to the highest concentration ($5x10^3$ µg /l) is important up to 99.9%. Whereas *Penicillium sp* with lower concentrations the adsorption rate is 99.6% and for other concentrations, the rate is the same as for *Aspergillus*.

•Adsorption after48 hours of contact

Aspergillus reacts almost similarly with the same concentrations and the mercury adsorption rate is identical. *Penicillium sp* exhibits a decrease in the level of adsorption after 48h. This could be the result of a redesorption.

•Adsorption after72 hours of contact

The adsorbing rate reaches 100% in the presence of *Aspergillus* to the highest concentration. This could be the result of are desorption [16]. After comparison we found this generates (*Aspergillus*). Is the most reliable that's why we made them kinetic adsorption.

3.3. Influence of pH on adsorption (fungus-medium contact)

The initial pH of the solution is an important parameter which must be taken in consideration during the Adsorption. In fact, this factor affects the evolution of the adsorption capacity which was analyzed on a pH range from 0 to14. The results are shown in Figure 4.

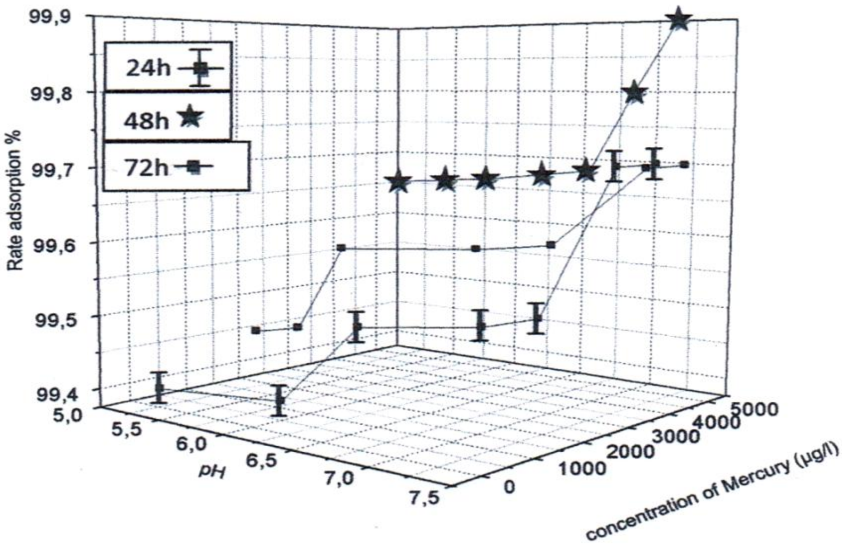

Figure 4: The change in pH according to adsorption rate and mercury dose**.**

During the adsorption kinetics we found that the pH evolved in parallel to the amount of the fixed metal (adsorption rate).The initial pH of the *Aspergillus sp suspension* is approximately 7.5. The addition of the metal leads initially to a slower evolution in the adsorption rate after it was getting faster, so we can see that the pH depends to the nature and amount of metal initially introduced because according to *Crist and al* the amount of released proton is lower than that of metal absorbed $10^5 \times 10^3$ µg/l [6,8]. This pH change is probably due to a complication of biomass functional groups by metal cations (mercury) [5]. We can think that this evolution of pH is consecutive to a proton release in the medium or to a hydroxide ion adsorption which constitutes a limiting process to the adsorption phenomena [8,17].

3.4. Effect of temperature on adsorption (fungus-water contact)

We have sought to evaluate the effect of temperature on the adsorptions rate of a selected metal (mercury) by fungal biomass (*Aspergillus sp)*. We observed that the temperature increases slightly with the adsorption rate and this last reaches its maximum value when the ambient temperature is at the maximum dose of mercury because the metal binding at this low concentration represents sigmoid shape which can be explained by several hypotheses [2]: effect of lateral interaction between adsorption sites influences the adsorption of non-mastered factor that causes a change in the adsorption mechanism. We will see later that the latter hypothesis is more likely [22, 29].

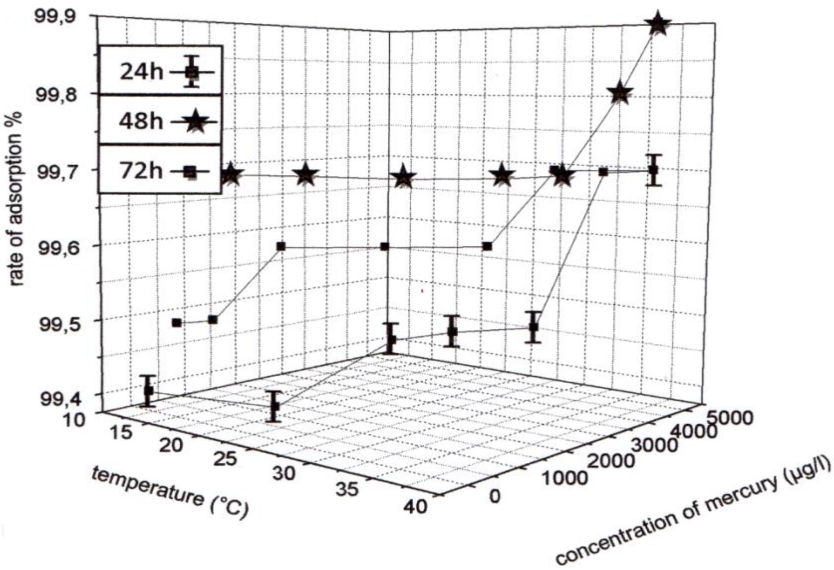

Figure 5: The temperature variation depending on the dose and rate adsorptions mercury

3.5. The combination with multiple types of similar species

To evaluate the adsorption capacity of such biosorbents, we used several similar experiments by varying the contact time and the mercury concentration. The obtained results show proportional relationship between the mercury concentration and the contact time after the addition of these species [20]. Indeed in effluents, mercury is under the ionic or neutral form that can interact with these species by altering their affinity towards these biosorbents [26] on the one hand and on the other hand these species have a significant exchange capacity through the existence of COOH groups that play a big role in mercury fixation for each biomass [15,18].

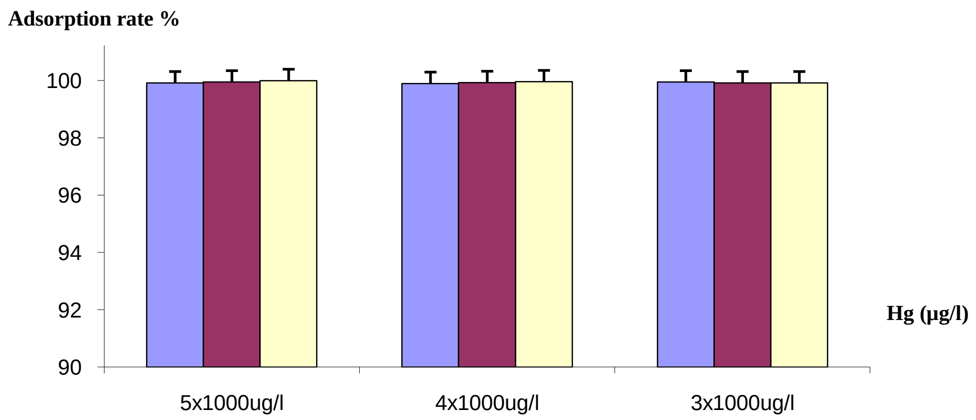

Figure 6: Changes in adsorptions rate according to mercury concentration with presence of *Aspergillus sp* and other similar species

Conclusion

Pollution represents a serious environmental problem because of dumped discharges in the Oubeira Lake waters and excessive use of fertilizers in agriculture. The contamination degree varies from one zone to another with contents which sometimes exceed those recommended by WHO. Water, by its high solvency power, dissolves substances released by human activity. There are many Chemical pollutants which have various origins and the most harmful are nitrogen compounds such as nitrite and mercury. Once absorbed, mercury salts may be accumulated in certain organs: liver, red blood cells, bone marrow, kidneys, intestines, lungs, skin and central nervous system and they quickly become very harmful, and cause irritation of aerial ways in the lungs, stomatitis after ingestion, and nervous troubles causing severe disorders in young vertebrates by degradation of haemoglobin in the blood and the production of toxic methemo-globin (methemoglobinemia in infants). They can cause hypertension and are the precursors of carcinogenic nitrosamines.

The biosorbents are materials with a storage capacity against the toxic pollutants (mercury). Their maximum adsorption capacity is quite competitive when they are put with other similar species. Their adsorption kinetics allows quick attachment with mercury mainly metals ions.

The aim of this study was to look for high adsorption capacity (biosorbents) to mercury by fungal biomass (*Penicillium sp* and *Aspergillus sp*) isolated and identified from the Oubeira lake waters located in North East of El-Kala National Park. The adsorption capacity can be optimized by increasing the amount of biomass and the temperature by increasing the initial mercury concentration. The adsorption analysis shows that these species could be considered as organic materials enough promoters to be used as an effective absorbent for the removal of mercury from liquid effluents.

Acknowledgements-This research was conducted on laboratory from petrochemical CP1KP SONATRACH Skikda Algeria under the direction of Ms Gesmi kirane djamila

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ISSN: 2028-2508
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