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ISOLATION AND IDENTIFICATION OF FUNGI AND YEAST RESISTANT TO LEAD (II)

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***Abstract*— Recently, has been studied the isolation and resistance to heavy metals of microorganisms, and his capacity of removal of different heavy metals in wastewater, such bacteria, yeast and fungi. Therefore, in this work, we isolate different fungal and yeast, which grow in presence of 200 ppm of lead (II), and we determined the tolerance to this metal by plate assays. Fungi found most frequently of wastewater were *Penicillium* sp., (44.5%), *Trichoderma* sp., (16.7%), and *Alternaria sp.,* (8.3%) and from sludge were obtained *Penicillium sp.,* (57.1%), and the yeast *Candida albicans* (42.8%). All species of isolated fungi and yeast were resistant to metal in the range of 500 to 2000 ppm. In addition, they have good capacity of removal this metal in solution: *Mucor* sp., (81%), *Paecilomyces* sp., I (66%), *Penicillium* sp., I, II, III, and IV (62%, 61%, 58%, and 55%). It was concluded that this fungi and yeast, could be used for decontamination of aquatic habitats polluted with lead (II).**

***Keywords*— Isolation, Fungi, Heavy Metals, Removal, Lead (II).**

I. INTRODUCTION

Heavy metals are potentially toxic elements whose presence in the environment has increased notably in recent decades, mainly by the action of man. Metal pollution poses an environmental threat important for living beings, since various metals that are essential micronutrients, such as copper and zinc, are toxic at high concentrations, while others, such as cadmium, lead mercury and arsenic, are toxic at minimal doses [1]. Lead and its derivatives are widely distributed in the environment: air, drinking water, rivers, lakes and oceans, soil, plants, animals, etc. This metal is used in the manufacture of pigments, coatings, containers, ointments, batteries electrical [2], and currently it has numerous applications in metallurgical industry: arms ammunition, metal bearings, cable cover, sheet lead, solders, pigments, and ceramic glaze. He occupational exposure limit (TLV-TWA) has been of 50-µg lead/m^3 [3]. In addition, the phenomenon known

as "pica" which it occurs in children who suck toys or objects paints or wrappers based lead salts is another source of pollution to consider [4]. Lead has the indirect effect of brain development strain [5], and more recently, it was detected in umbilical cord serum samples of mother-newborn pairs in Shengsi islands face the Yangtze River estuary and Hangzhou Bay in China [6].

On the other hand, different studies have been carried out on lead contamination in water, soil and air in the San Luis Potosí valley, for example: the quality of irrigation water on agricultural land crops, detecting that some samples of water exceeded the permissible limit for agricultural use in the concentration of sulfates (SO^{-2}_4) and the electrical conductivity (EC), which represents an excess of salts in the water [7]. The analysis of the atmospheric dust of the city of San Luis Potosí, obtained in the industrial zone, an average annual arithmetic of total suspended particles of 483 mg/m^3, considering the four seasons of sampling during the study period. These levels being very high in comparison with the maximum permissible limit of 90 mg/m^3, proposed by WHO, or 75 mg/m^3 according [8]. Also were obtained the levels of some heavy elements by absorption atomic, such Fe, Pb, Mn, Ni, Cu, Cd, and As for all sampling stations, resulting very high in relation to the limits recommended by the WHO. For example, in the case of Cu on the area Industrial, its concentration rises to 3.31- mg/m^3 while the permissible concentration is 0.6 mg/m^3; and the concentration is remarkable in the Tangamanga Park, which rises to 0.30 mg/m^3 while its limit permissible is 0.01 mg/m^3 [9]. The content of different heavy metals has been studied in samples of the mining waste of Villa de la Paz [10], finding contamination by As and Pb in soil and dust. Bioavailability was positive: 71% of the children analyzed had in urine levels of As above value normal. Toxicity studies showed hepatic damage and neurochemical alterations in rats treated with the residue, the analysis of blood Pb, urinary As, and genotoxic in blood cells in 5-11 years old living in contaminated areas in S.L.P., finding that almost half of the children, which living in these places, they present values above of CDC's mark of action As (50 m/g creatinine) and Pb (10 mg/dL in blood). In terms of DNA damage, 35% of children exceed the maximum value of damage found in the control population [11]. Too, has been studied, the capacity of tolerate and the biosorption of heavy metals by microorganisms [12]: The yeast *Pichia guillermonii* resistant to heavy metal [13], *Pichia anomala, Candida krusei* and *Cryptococcus laurentii* tolerated high concentrations of Zn (up to 20 mM) [14], the yeast *Saccharomyces cerevisiae* partly retain heavy metals (Cu, Fe, Pb, Zn, Ba) and As from soil extracts [15], hydroxyapatite/yeast biomass composites for the removal of Pb^{+2} [16], Pb-resistant microorganisms, isolated from industrial samples [17], the fungi *Aspergillus versicolor* [18], some mucorales [19], indigenous *Aspergillus niger* and *Penicillium* sp [20], the basidiomycete *Phanerochaete chrysosporium* ([21], the algae *Lobophora variegata* (Lamouroux) [22], and *Enteromorpha* [23]. The objective of this work was to isolate and identification of fungi resistant to lead (II) of industry waste lagoon, and his capacity of removal the same heavy metal.

II. EXPERIMENTAL

A. Obtaining the samples

In previously sterilized plastic containers, a sample of water he took (approximately 30 cm below of the surface) and one of sludge (this with a Gravity-type sampler), from each of the 4 zones near the farmland of the “Tanque Tenorio”, which is southeast of the city, in the municipality of Soledad de Graciano Sánchez, S.L.P., México, and is a catchment lagoon of wastewater, of which 60% is from urban origin and 40% of industrial origin, (it should be noted that the industrial zone of San Luis Potosí has more than 520 companies, among which are the mining-metallurgists, textiles and chemicals, [24].

B. Isolation of the yeast and fungi

1 mL of water and 1 g of sludge were taken and placed separately in test tubes containing 9 mL of sterile distilled water, shaking 1 min to homogenize. Dilutions (1:10, 1:100, and 1:1000) were then made and 100 µL aliquots of each of the samples were taken and seeded in Lee Minimal Medium (LMM) [25], [0.25% KH_2PO_4, 0.20% $MgSO_4$, 0.50% $(NH_4)_2SO_4$, 0.50% NaCl, 0.25% glucose] supplemented with 200 ppm of Lead (II) [$Pb(NO_3)_2$]; the pH of the medium was adjusted and maintained at 5.3 with 100 mMol/L citrate-phosphate buffer, and were incubated at 28°C for 7 days. The resultant colonies were purified by successive replicates in Malta Extract Agar (MEA) and Sabouraud Dextrose Agar (SDA). SDA and LMM culture medium, containing 200 ppm of Lead (II), were used to cultivate the fungi. Fungal strains were maintained in agar slant tubes, and spores were obtained after growth in SDA medium as described.

C. Identification of yeast and fungi

The yeast and fungal strain was identified based on their morphological structures such color, diameter of the mycelia, and microscopic observation of formation of spores, germ tube and chlamydoconidias [26].

C.1 Germ-tube induction

1 x 10^6 yeast/mL is taken, and seeded into LMM (added with proline and biotin, 0.5 and 0.001 g/L, respectively), and incubated at 37°C for 3 h. Subsequently, it is observed an aliquot in a microscopic, the formation of a germinal tube without constriction in its source of origin and with the characteristic shape of hand mirror [26].

C.2 Formation of Chlamydospores

Are grown 1 x 10^6 yeast/mL in corn flour agar medium and incubated at 48-72 h at 28°C, observing under microscope the formation of asexual, thick-walled and refringent spores, called chlamydospores,

which may be intercalated or in terminal position of the hyphae partitions or septate [26].

D. Plate resistant test

Petri dishes were prepared with 20 mL of LMM supplemented with 200-2000 ppm of lead (II) [$Pb(NO_3)_2$]. Afterwards, the plates were inoculated in the central part with 1 x 10^6 conidia and/or yeast/mL, of the corresponding fungus and/or yeast, and were incubated at 28°C for 7 days, comparing the growth with a control without metal. All experiments were performed at least 3 times by duplicate.

E. Biosorption of Lead (II) by using dry yeast and fungi

E.1 Obtaining cellular biomass

The yeast and fungi were grown at 28°C in an agitated and aerated liquid media containing thioglycolate broth, 8 g/L. After 7 days of incubation, the cells were centrifuged 3000 rpm for 10 min, washed twice with trideionized water and then dried at 80°C for 4 h in an oven. Finally, the yeast and fungal biomass was milled and stored in an amber bottle in the refrigerator until their use.

E.2 Lead (II) solutions

For analysis, were prepared a series of solutions of Lead (II) [$Pb(NO_3)_2$] of 100 mg/L, pH was adjusted with nitric acid and/o NaOH, and the quantity of biomass added to each flask was of 1 g/100 mL for the lead´s solution. It taken samples at 24 h, the biomass is removed for centrifugation (3000 rpm/5min) and the supernatant is analyzed to define the ion metal concentration.

E.3 Determination of Lead (II)

The concentration of lead ions in solution was determined by the colorimetric method of dithizone, with which a complex is formed dithizonate of lead cherry red color, which read at an absorbance of 510 nm with a minimum detectable concentration of lead solution of 1.0 µg/10 mL of dithizone [27].

III. Results and Discussion

A. Identified yeast and fungi of the analized samples

From the different samples analysed from four areas of "Tanque Tenorio" we isolated and identified of water supplemented with 200 ppm of Lead (II), 36 colonies of yeast and fungi, and they can grow in the presence of different concentrations of Lead (II) (Table No. 1), the most frequent was *Penicillium sp.,* (44.5%), followed by *Trichoderma sp.,* (16.7%), and not yeast were obtained. From sludge, we isolated 7 colonies, 4 of *Penicillium* sp., (57.14%), and 3 of *Candida albicans* (42.85%) (Table No. 2, Figures 1, 2, 3, 4, 5, 6, 7, and 8). It is indicating that isolated yeast and fungi developed resistance and perhaps, the degradation mechanism of Lead (II) in an environment contaminated with it, which coincides with a variety studies because from different sources, have been isolated different microorganisms with the capacity of resistance and lead (II) degradation [1, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 and 23].

TABLE 1. Identified Fungi of the analized water samples

Identified microorganism	***Total***	***Percentage (%)***	***Growth in Lead (II) (mg/L)***
Penicillium sp	16	44.44	1000-2000
Trichoderma sp	6	16.66	1000
Alternaria sp	3	8.33	500-1500
Acremonium sp	5	5.55	500
Aspergillus flavus	2	5.55	2000
Paecilomyces sp	2	5.55	1000
Scopulariopsis sp	2	5.55	1000
Cladosporium sp	1	2.77	2000
Fusarium sp	1	2.77	1000
Mucor sp	1	2.77	2000

TABLE 2. Identified Yeast and Fungi of the analized sludge samples

Identified microorganism	***Total***	***Percentage (%)***	***Growth in Lead (II) (mg/L)***
Penicillium sp	4	57.14	1000-2000
Candida albicans	3	42.85	1500-2000

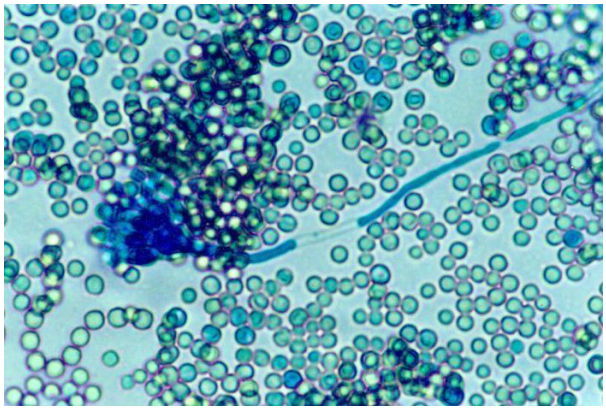

Figure 1: *Penicillium* sp., 100 x.

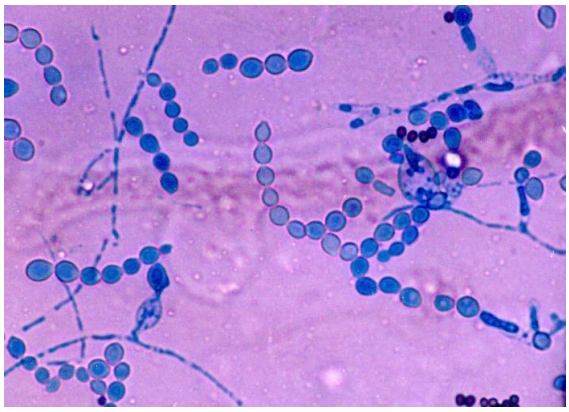

Figure 2*: Scopulariopsis* sp., 100 x.

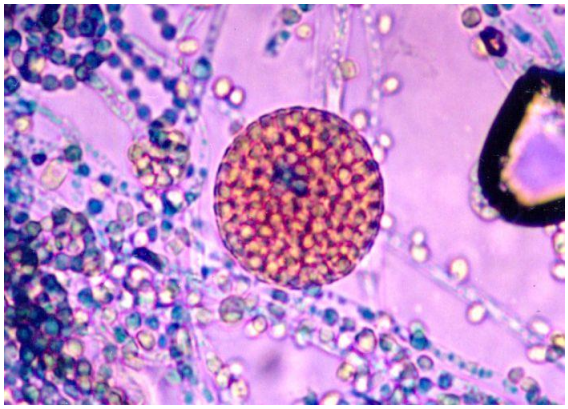

Figure 3*: Mucor* sp., 100 x.

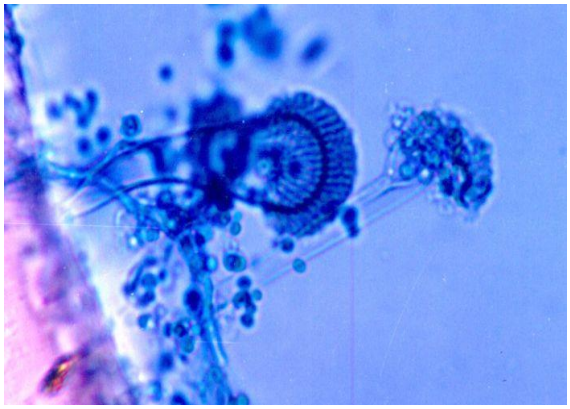

Figure 4*: Aspergillus fumigatus.* 100 x

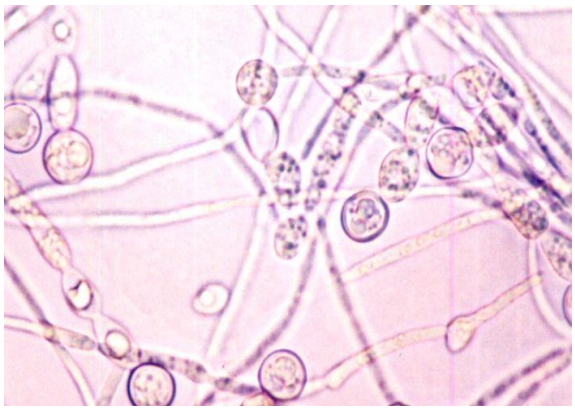

Figure 5*:* Chlamydoconidios of *Candida albicans.* Grown in corn flour agar medium and incubated 72 h at 28ºC,

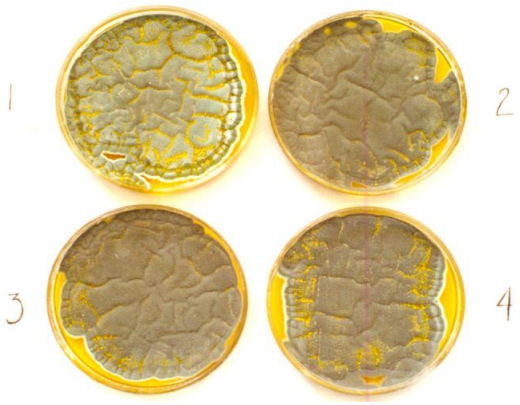

Figure 6*:* Macroscopic characteristics of *Penicillium* sp. I. AEM.120 h. 28ºC.
1. - Control without Lead (II)
2. - Fungi with 500 mg/L of Lead (II)
3. - Fungi with 100 mg/L of Lead (II)
4. - Fungi with 2000 mg/L of Lead (II)

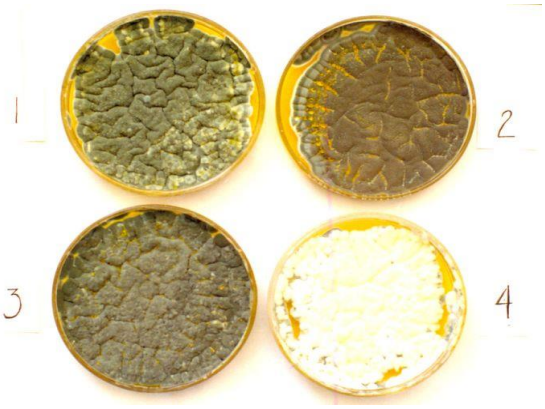

Figure 6*:* Macroscopic characteristics of *Penicillium* sp. II. AEM.120 h. 28ºC.
1. - Control without Lead (II)
2. - Fungi with 500 mg/L of Lead (II)
3. - Fungi with 100 mg/L of Lead (II)
4. - Fungi with 2000 mg/L of Lead (II)

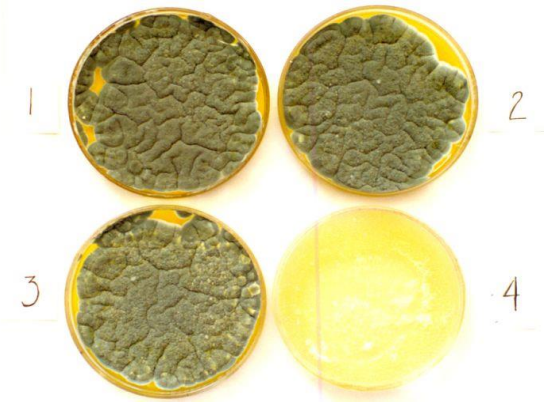

Figure 7*:* Macroscopic characteristics of *Penicillium* sp. III. AEM.120 h. 28ºC.
1. - Control without Lead (II)
2. - Fungi with 500 mg/L of Lead (II)
3. - Fungi with 100 mg/L of Lead (II)
4. - Fungi with 2000 mg/L of Lead (II)

Figure 8*:* Macroscopic characteristics of *Candida albicans* 1. AEM.120 h. 28ºC.
1. – *C. albicans* with 2000 mg/L of Lead (II)
2. – *C. albicans* with 1000 mg/L of Lead (II)
3. – *C. albicans* with 500 mg/L of Lead (II)
4. – *C. albicans* without Lead (II)

B. Removal of Lead (II) by yeast and fungal biomass

We also analysed the capacity of Lead (II) removal by some of the yeast and fungal biomasses isolated. The results are shown in Table 3. The biomasses of *Mucor* sp., (81%, *Paecilomyces* sp., I, (665), *Penicillium* sp., III (62%), and *Penicillium* sp. I, (61%), removal efficiently the metal in solution, while *Alternaria* sp. I (31%), *Alternaria* sp. III 28%), and *Acremonium* sp. (21%), were the less efficient. The dead yeast and fungal cells can be effective metal accumulators and there is evidence that some biomass-based clean-up processes are economically viable [15].

The tolerance of some microorganisms to heavy metals, as well as the physiological response to them, have been also determined [1]. The removal of heavy metal ions, using yeast as biosorbents, was previously investigated [1, 12, 15, 16, 17, and 19]. Our results confirm the capacity of this yeast and fungal biomass, for the removal of heavy metals with different effectivity, like removal of lead ions from industrial wastewater [12], yeast-based microbiological decontamination of heavy metals contaminated soils of Tarnita [15], adsorption of Lead (II) by hydroxyapatite/yeast biomass composites [16], the bioremediation of lead by lead-resistant microorganisms, isolated from industrial samples [17], removal of Lead (II) of contaminated sites [19], treatment of wastewate by *Phanerochaete chrysosporium* [21], the biosorption of Cd(II) and Pb(II) by *Lobophora variegate* and *Enteromorpha Algae* [22, 23],

TABLE 3. Removal of 100 mg/L of Lead (II) by yeast and fungal biomass. 28ºC, 100 rpm. pH 4.0. 1 g of biomass. 24 h.

Yeast or fungal biomass	***Percentage of Removal (%)***
Mucor sp	81
Paecilomyces sp. 1	66
Penicillium sp. 3	62
Penicillium sp. 1	61
Penicillium sp. 2	58
Penicillium sp. 4	55
Aspergillus flavus 1	51
Fusarium sp.	43
Candida albicans 1	43
Cladosporium sp.	40
Candida albicans 2	40
Trichoderma sp. 1	38
Alternaria sp. 1	31
Alternaria sp. 3	28
Acremonium sp. 1	21

IV. CONCLUSIONS

We isolated different strains of yeast and fungi, resistant to different concentrations of Lead (II), with the potential for removal this heavy metal of wastewater.

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