



# *SUMMARY*





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The objective of the present investigation was to isolate some metal resistant fungal strains from soil and evaluated their bioremediation abilities of  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{As}^{5+}$  ions and then studied the suitable conditions under which they could remove these metal ions more efficiently. A comparison between the bioaccumulation and biosorption of these metal ions by the tested fungi was achieved along the thesis. Also, the physiological effects of these heavy metals on the fungal cell (represented by *S. cerevisiae* cell) were studied. This can be summarized as follow:

1. Twelve fungal species were isolated from soil as well as the strain *S. cerevisiae* was obtained from the effluent of food factory (Holw Elsham Company). Then the effect of different metal concentrations on the viability and growth of these isolates were tested.
2. These isolates were classified into four groups according to their viability: Group A represents the completely inhibited isolates while group D represent the high resistance isolates. In between, (group B and C) represent the isolates that depend on metal type and its concentration.
3. The eight isolates (groups: B, C, and D) were then tested for their ability to remove of  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{As}^{5+}$  either from liquid growth media (bioaccumulation) or from aqueous metal solution (biosorption).

4. In bioaccumulation study; All tested fungi able to grow and tolerate 100 mg/l  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{As}^{5+}$  ions in growth media except *S. brevicaulis* that was inhibited by 100 mg/l  $\text{Pb}^{2+}$  and  $\text{As}^{5+}$ . This tolerance was varied among tested fungi and this reflected on their bioaccumulation capacity, where the high tolerance accompanied by high bioaccumulation capacity.
5. Better  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$  bioaccumulation was done by *A. niger* while lead and arsenate showed high bioaccumulation results by *S. cerevisiae* and *P. chrysogenum*, respectively. The bioaccumulation of metals reached 70.74%, 84.3%, 73.98% and 61.89% removal for  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{As}^{5+}$ , respectively at fourteen days of incubation (saturation done during the first four days).
6. In biosorption study; dried and killed biomasses were used. *A. niger* and *A. terreus* were the best microorganisms in removal of all metals except  $\text{Pb}^{2+}$  that was removed better by dried form of *S. cerevisiae*; also  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  were removed better by killed form of *P. chrysogenum*. The highest metal uptake values by dried biomasses were 6.49, 7.17, 7.43 and 5.58 while by killed biomasses were 6.4, 7.51, 6.2 and 4.67 mg/g at pH 5 for  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{As}^{5+}$ , respectively during the 96 h.
7. The factor of time was not essential with the same degree as in the bioaccumulation process for most tested fungi because this

process is non-metabolism dependant, and faster than bioaccumulation process. Saturation of fungi with metals varied according to the individual fungus and the type of metal ions. For most fungi, saturation not requires long time but it takes place during the first 4-6 hours.

8. All tested fungi able to adsorb an amount of  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{As}^{5+}$  ions. This amount (q) was varied among tested fungi according to the type of metal ions, the main composition of cell wall.
9. *S. cerevisiae* was selected among other tested fungi for further studies as it performed well in both bioaccumulation and biosorption study. In addition, it is easy to cultivate at large scale, its biomass can be obtained from various food and beverage industries and it was accepted by the public when applied practically. Finally it is an ideal model organism to identify the mechanism of biosorption in metal ion removal, especially to investigate the interactions of metal–microbe at molecular level for future studies.
10. In bioaccumulation study; the type of nutrient medium, pH, incubation periods and initial metal concentration were the culture conditions that affected on the *S. cerevisiae* growth as well as its metal bioaccumulation capacity. Maximum growth of *S. cerevisiae* was achieved when the organism was cultivated on glucose-peptone broth, at pH 5 for four days incubation at

27 °C in absence or presence of metal ions. On the other hand, by increasing metal ion concentration, the growth and also bioaccumulation capacity of *S. cerevisiae* decrease.

11. In biosorption study; The yeast cells were treated physically and chemically for increasing their biosorption capacity, since the highest metal uptake values (6.78, 8, 7.54 and 6.12 mg/g for  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{As}^{5+}$  respectively at pH 5) were obtained by ethanol treated yeast cells for  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{As}^{5+}$ , but NaOH treated yeast cells for  $\text{Cu}^{2+}$ . The optimum pH value that achieves best biosorption capacity was 5 for all metal ions except  $\text{Cd}^{2+}$ , where its uptake value increased at pH 6 and become 7.87 mg/g.
12. Increasing of  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{As}^{5+}$  concentrations in their aqueous solutions containing suitable form of *S. cerevisiae*, was accompanied by increasing their biosorption process. We noted that the biosorption was very fast for all metal ions during the first 2 h but, in the remaining period, final metal concentrations in the liquid reached an equilibrium concentration value.
13. In addition, metal ions adsorbed by the biomass could be eluted effectively with  $\text{HNO}_3$ . Regeneration of the biomass with NaOH enhanced the biosorption capacity even after five cycles of adsorption–elution–regeneration.

14. The effect of  $\text{Cu}^{2+}$ ,  $\text{As}^{5+}$ ,  $\text{Pb}^{2+}$  and  $\text{Cd}^{2+}$  ions with different concentrations on growth phases of *S. cerevisiae* showed inhibition of the yeast cell reproduction. This inhibition differs from metal to another according to the degree of metal toxicity. With increasing metals concentration, the metal uptake gradually decreased and the curves were flatter and flatter.
15. By increasing the concentration of  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  or  $\text{As}^{5+}$  ions, the total *S. cerevisiae* DNA, protein, carbohydrates and lipids decreased. The degree of these negative effects was varied according to the type of metal species and the target metabolite.