

Tolerance and accumulation of some heavy metals by microorganisms isolated from mining residues in Marrakech, Morocco

El Baz Soraia^{1,2}, El Moussouli Hind¹, El Gharmali Abdelhay², Imzilin Boujamâa¹

¹ University Cadi Ayyad, Faculty of Sciences Semlalia Marrakech, Laboratory of Biology and Biotechnology for Microorganisms, Environmental Microbiology and Toxicology Team, PO. Box 2390, Marrakech 40000, MOROCCO.

² University Cadi Ayyad, Faculty of Sciences Semlalia Marrakech, Laboratory of Hydrobiology, Ecotoxicology and Assainissement

Email: soraiaelbaz@yahoo.ca

Abstract

Industrial activities produce large amount of wastewater polluted by metals. Higher concentrations of toxic metal species are leading to serious ecological and health problems. Actually, bioremediation and use of microorganisms constitutes a veritable opportunity to reduce heavy metals pollution. In this work, we are interested to evaluate tolerance and accumulation capacities of some heavy metals by microorganisms isolated from mining residues in Marrakech region of Morocco. Tolerance test was made on fungi isolates with different concentrations of heavy metals (Pb, Cu, Cd, Zn, and Cr). For accumulation experiments, isolates of both fungi and actinobacteria were put in contact with metal solution (0.5mg/ml) during 3h. Quantities of metal removed by isolate's biomass were then calculated. Results of metals analysis showed that all of mining residues studied contain very high concentrations of metallic elements: 2.94g/kg, 10.18g/kg, 18.87g/kg, 0.07g/kg and 0.03g/kg respectively for copper, lead, zinc, cadmium, and chromium. pH values varied from 2 to 7, and conductivity from 1 to 10 mS/cm. Tolerance test showed that a higher metal concentration reduce the tolerance index, the percentage inhibition of growth can reach up to 80% at 1mg/ml of Cr for the isolate GA2. Paradoxically, the presence of metal can stimulate the growth (1% for the isolate GT16 at 0,05mg/ml of Cr). Quantitative accumulation results depend on the isolate, for example GT16, GT17, and GT23 can accumulate 25.25; 9.74; and 42.87 mg Cu/g of biomass respectively. In conclusion, these microorganisms can be potential candidates for further investigations regarding their ability to remove toxic metals from contaminated wastewaters.

Keywords: Heavy metals, accumulation, tolerance, fungi, actinobacteria, mining residues

Introduction

Generally, introduction of heavy metal into the environment induces to serious ecological and health problems (Cerbasi and Yetis, 2001). Therefore, there is a need to develop rapid, economical and environmentally benign technology for the removal of metals from industrial effluents. There are certain microorganisms, which can survive in high concentrations of metals and have the potential to

accumulate different metals. Indeed, the contaminated sites are the sources of metal resistant microorganisms. This is achieved by the virtue of covalent interaction of metal at cell surface or within the cell by different processes. These microbes can be of immense significance in the clean up of heavy metals from the environment.

Methods

Residues mining sampling and analyses

Mining residues samples were collected from various abandoned mining area (contaminated area) of Marrakech city in Morocco. The mining areas were: Bir nhass, Sidi Bouatman, Kettara, and Goundafa. The mining residues were analyzed for their total content of heavy metals (Pb, Cu, Zn, Cr and Cd). Conductivity and pH were also determinate for each mining residues sample.

Tolerance test

A disk of fungi was inoculated aseptically on modified Duxbury agar medium plates supplemented individually with different concentrations of heavy metal (Pb^{2+} , Cu^{2+} , Cd^{2+} , Zn^{2+} and Cr^{6+}). The effect of the heavy metal on the growth of the isolates tested was estimated by measuring the radius of the colony extension (mm) against the control (medium without metal) and the determination of the index of tolerance. The index is defined as the ratio of the extension radius of the treated colony to that of the untreated colony.

Determination of metal accumulation by microorganisms isolates

Microorganisms were cultured in medium agar plates without metals (Bennet for actinobacteria and Sabouraud for fungi). Microorganisms were washed once in phosphate-buffered saline (PBS) and pelleted by centrifugation. The remaining samples were resuspended in tubes containing 2 ml of metal solution (0.5 mg/ml). The samples were incubated at room temperature in a roller mixer for 3 h. The amount of residual metal present in the supernatant was measured by atomic absorption spectrophotometer. Quantities of metal removed by isolate's biomass were then calculated.

Results and discussion

Various microorganisms were isolated from mining residues in various mining area in Marrakesh city, Morocco, where the concentration of heavy metals is very high (Tab.1). 19 fungi belonged to the genera *Aspergillus*, *Penicillium* and *Cladosporium*, and 27 actinobacteria showed the high capacities to resist of heavy metal. Generally, pollution of soil and water by heavy metals may lead to a decrease in microbial diversity (Ezzouhri et al., 2009). Tolerance test showed that a higher metal concentration in the medium reduce the tolerance index, the percentage inhibition of growth can reach up to 80% at 1mg/ml of Cr for the isolate GA2 (*Aspergillus*). Paradoxically, the presence of metal can stimulate the

growth (1% for the isolate GT16 "*Penicillium*" at 0,05mg/ml of Cr) (Fig.2), other metals will be developed at the poster. The variation in the metal tolerance might be due to the presence of one or more types of tolerance strategies or resistance mechanisms exhibited by different fungi. Roane and Pepper (2000) reported that the differences in resistance levels were probably due to the potential variation in the mechanism of resistance.

Table 1: the heavy metals content for each mining residues sample

Mining area	Mining residues content						
	heavy metals content g/Kg						
	Pb	Cu	Zn	Cd	Cr	pH	Conductivité mS/cm
Kettara	0,028 ± 0,007	2,942 ± 0,134	0,685 ± 0,092	0 ± 0	0,017 ± 0,002	2,2	10,28
Bir Nhass	1,407 ± 0,341	0,095 ± 0,024	9,989 ± 1,223	0,075 ± 0,011	0,031 ± 0,005	6,52	2,21
Sidi Bouatman	10,189 ± 3,580	1,102 ± 0,029	18,870 ± 5,733	0,044 ± 0,017	0,035 ± 0,005	7,42	2,08
Mine Arbar	1,619 ± 0,432	1,243 ± 0,079	14,434 ± 0,928	0,070 ± 0,007	0,006 ± 0,001	7,17	2,27
Mine Tenfit	0,098 ± 0,002	0,498 ± 0,068	2,093 ± 1,020	0 ± 0	0,014 ± 0,001	7,52	1,46

Quantitative accumulation results depend on the isolate, for example the isolates from "*Penicillium*" GT16, GT17, and GT23 can accumulate 25.25; 9.74; and 42.87 mg Cu/g of biomass respectively (Fig.3).

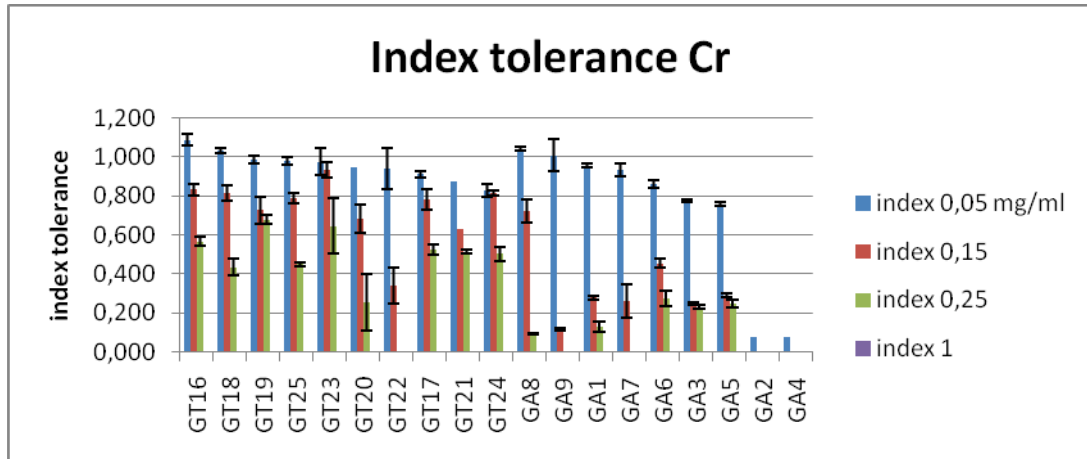


Figure2: Index tolerance for Cr⁶⁺ for each isolate at different concentrations

Metal accumulation or biotransformation is an alternative mechanism for metal detoxification in microorganisms (Gadd, 1992).

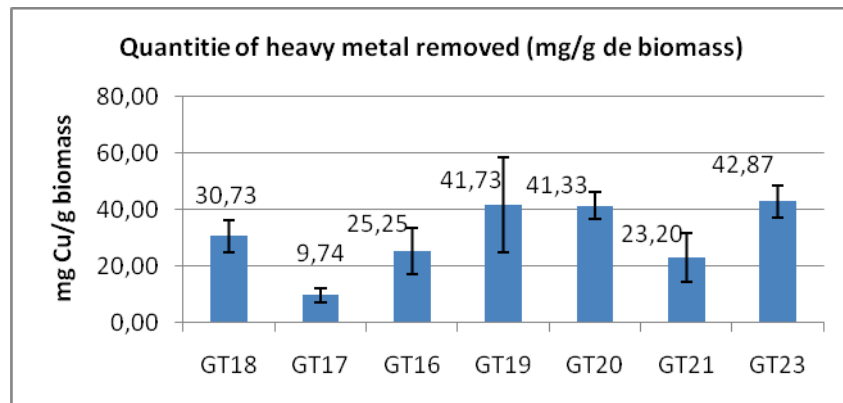


Figure3: Quantities of heavy metal removed by each isolate of fungi

Conclusion

The removal of toxic components from industrial effluents is of great importance, not only because of the decontamination effect but also because this removal protects the environment. The microorganisms isolates described here may be of use for removing heavy metals from contaminated effluents.

References

Roane TM, Pepper LL (2000). Microbial responses to environmentally toxic cadmium. *Microb. Ecol.* 38: 358–364.

Ezzouhri L., Castro E., Moya M., Espinola F. and Lairini K. (2009). Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco. *African Journal of Microbiology Research* Vol. 3 (2) pp. 035–048

Cerbasi, I.H., Yetis, U., (2001). Biosorption of Ni(ii) and Pb(ii) by *Phanerochaete chrysosporium* from binary metal system – Kinetics. *Water Res.* 27, 15–20.

Gadd, G. M. (1992). Metals and microorganisms: a problem of definition. *FEMS Microbiol. Lett.* 79:197–203