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BIOSORPTION OF HEAVY METALS BY *PLEUROTUS OSTREATUS*

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The present study faces the problem of heavy metal absorption by mushrooms under two different point of view:

- 1) a physiological approach, evaluating mycelium tolerance to Cd and Pb within strains of a single species.

Four wild strains of the white rot fungus *Pleurotus ostreatus* picked up in different natural sites of Syria were compared with a control strain (N.684);

- 2) a technical approach, testing their biosorption ability for liquid waste detoxification.

Surveyed sites in Syria

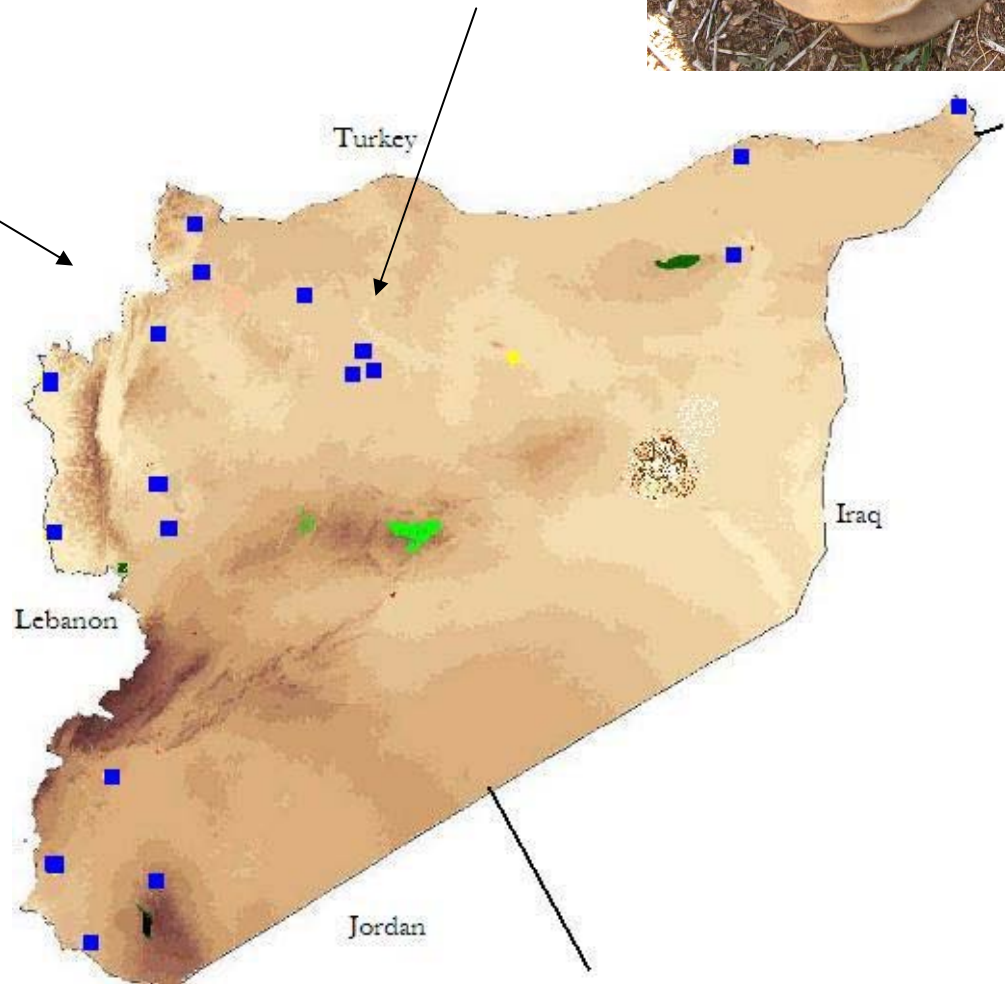
Harim 863,
864



Maskana, Aleppo 850



Mahajja 877



A complex interaction between cellular, physical and chemical phenomena influences the metal-fungal interaction.

- **The environment can affect fungal growth, metabolism and differentiation and the availability and speciation of metal(s).**
- **Metals may affect fungal growth and alter the characteristics of an environment.**
- **Fungi may remove metals from the environment and alter it by metabolic activities**

Non essential metals are cotransported with Ca ions, normally present in high quantities in the fruit bodies of white-rot fungi, due to the low specificity of the transporters.

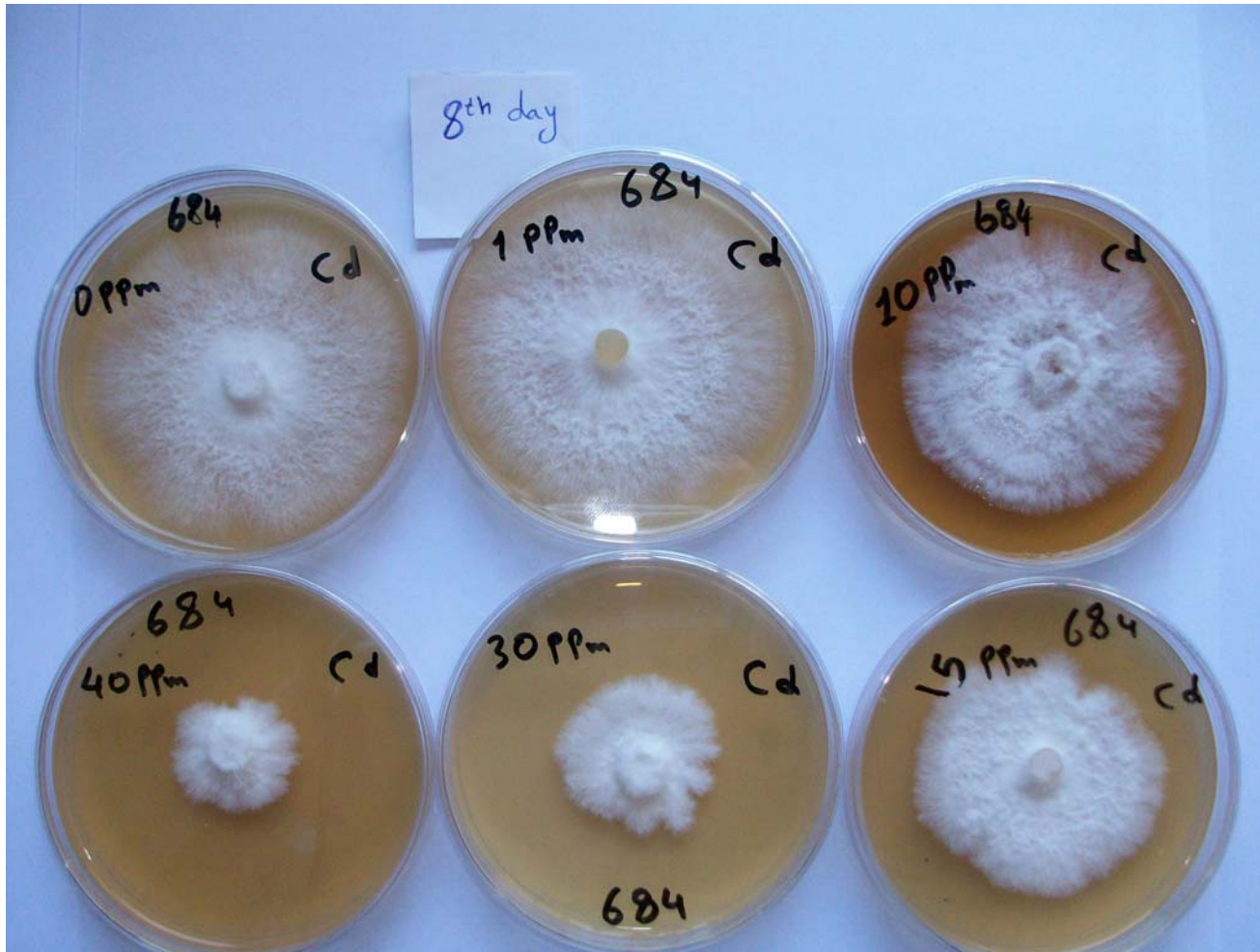
Experimental conditions

Sensitivity to heavy metals

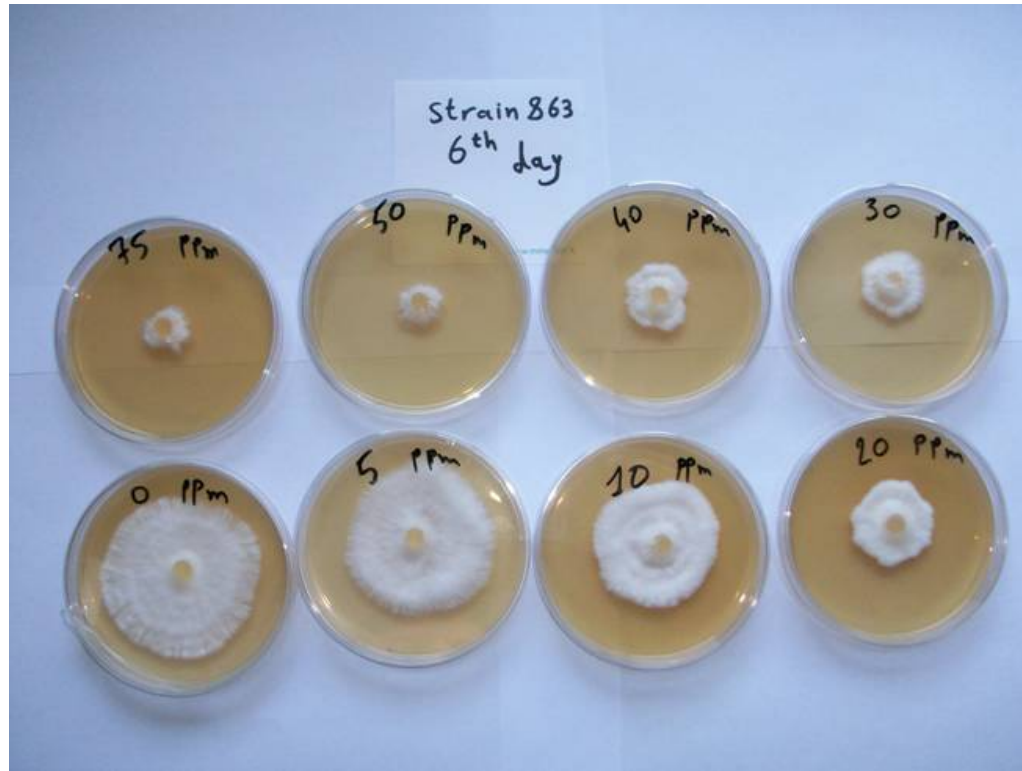
- ***Cadmium testing:*** when each mycelium reached an abundant biomass, it was cut into circles with 9 mm diameter. Each circle was placed in the centre of Petri dishes (85 mm diameter) containing malt extract agar and different concentrations of Cd (three replicates): 0 , 5, 10, 20, 30, 40, 50 and 75 ppm.
The solutions were prepared using a standard solution of Cadmium 1000 ppm (Cd in 2% HNO₃ - Exaxol Italia) and correcting the pH to a value 5.8-6.0. The mycelia were grown in a climatic chamber at T= 25°C and relative humidity =60%. Mycelium growth was tested by measuring the diameter of the growing colonies for 30 days.
- ***Lead testing:*** the same method was repeated with lead using a standard solution of lead 1005 ppm (Pb in 2% HNO₃- Exaxol Italia).

RESULTS

Strain 684 (control) – 8th day - Cd



Strain 863 – 6th day - Cd



With high concentrations of Cd an increase of hyphal density was evident, due to a decrease of distance between branch points.

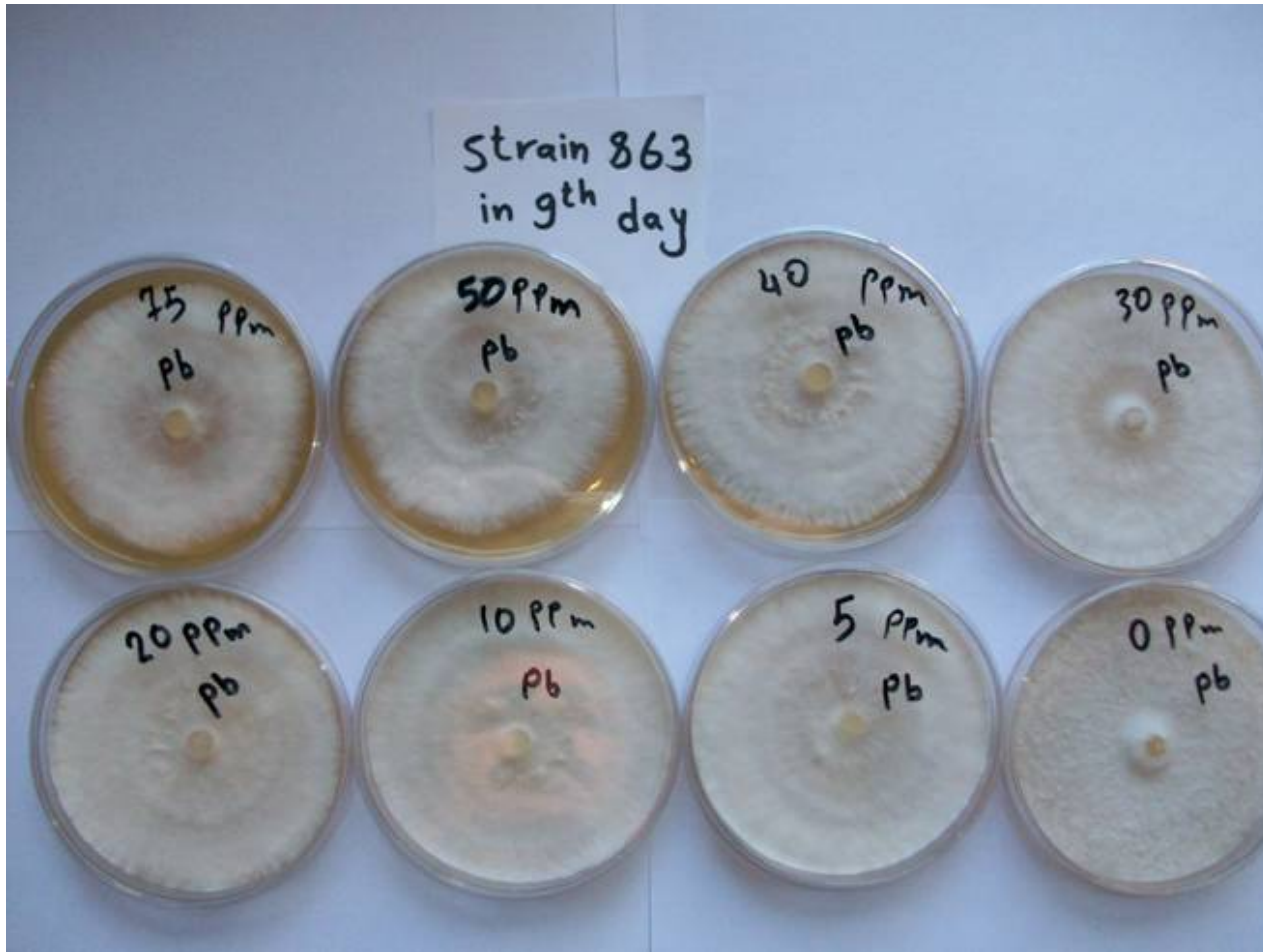
**Table 1. Growth of several strains in different concentrations of cadmium expressed as numbers of days to reach the maximum diameter (85 mm).
n.r. = never reached.**

Strain Cd (ppm)	684	850	863	864	877
0 (control)	8	8	8	7	8
5	10	12	8	8	12
10	11	17	10	11	16
20	17	n.r.	18	18	20
30	18	n.r.	21	22	23
40	19	n.r.	24	22	n.r.
50	21	n.r.	n.r.	n.r.	n.r.
75	26	n.r.	n.r.	n.r.	n.r.

Table 2. Growth of several strains in different concentrations of lead expressed as numbers of days to reach the maximum diameter (85 mm).

Strain Pb (ppm)	684	850	863	864	877
0 (control)	8	8	9	8	8
5	8	8	9	9	8
10	8	8	9	9	9
20	9	8	9	9	9
30	9	8	9	9	10
40	9	8	11	10	10
50	10	9	12	11	11
75	11	12	13	12	11

Strain 863 – 9th day - Pb



- Mushroom mycelia can grow in the presence of high concentrations of heavy metal
- Strains 863 and 864, both picked up in the same region (Harim, in north west of Syria), show a similar growth in presence of both Cd and Pb.
- The greater differences are evident for those collected from Maskana (850), in the centre of Syria.

Our results show that the presence of Pb produced only a little delay in the growth, therefore if these mushroom should grow in lead polluted areas they could survive and probably produce fruit bodies.

The reproductive stages of development (spore formation and germination) are much more sensitive to heavy metals than mycelial growth.

The negative effect on the mycelium growth obtained with Cd let us to hope that the presence of high concentrations of the metal can inhibit growth and fructification.

Biosorption:

Biological method of metal removal which utilizes live or dead microorganisms, or their derivatives, to complex metal ions through the action of ligands or functional groups present in the cell membrane.

There is an increasing interest to take advantage of fungal ability in metal biosorption for their use as an efficient alternative to traditional methods of industrial wastewaters treatment when applied to waste containing low concentrations of metals

The process is relatively inexpensive, non-hazardous, and may permit the recovery of the metals from the absorbing biomass.

- Maintaining the survival of the fungal cells during biosorption process is difficult, because they require a continuous supply of nutrients and could suffer for metal toxicity.
- The use of lyophilized cells can eliminate these problems and permit to regenerate them and reuse for many cycles

Experimental conditions for biosorption

100 mg of lyophilized mycelium of each strain were put in batches containing 25 ml of different heavy metal (Cd and Pb) concentrations:

10 ppm, 20 ppm, 30 ppm.

Batches were stirred at 100 rpm for 30 min, then the solutions were filtered and heavy metal concentration remained in the filtrates was analyzed by an Atomic Absorption Spectrophotometer.

Table 3. Cadmium absorption by lyophilized mycelium.

Strain	Cd, initial concentration ppm	Cd absorbed by mycelium mg/g d.w. \pm S.D.	absorption %
684	10	0.60 \pm 0.06	24.0
	20	0.92 \pm 0.06	18.4
	30	1.47 \pm 0.24	19.6
863	10	0.21 \pm 0.01	8.4
	20	0.49 \pm 0.02	9.8
	30	1.13 \pm 0.07	15.1
864	10	0.25 \pm 0.03	10.0
	20	0.42 \pm 0.04	8.4
	30	0.78 \pm 0.15	10.4
850	10	0.16 \pm 0.07	6.5
	20	0.56 \pm 0.06	11.2
	30	1.08 \pm 0.34	14.4
877	10	0.21 \pm 0.03	8.4
	20	0.42 \pm 0.15	8.4
	30	0.78 \pm 0.01	10.4

Table 4. Lead absorption by lyophilized mycelium.

Strain	Pb, initial concentration ppm	Pb absorbed by mycelium mg/g d.w. \pm S.D.	Absorption %
684	10	1.33 \pm 0.36	53.4
	20	1.06 \pm 0.13	21.3
	30	1.41 \pm 0.40	18.8
863	10	1.20 \pm 0.00	47.8
	20	1.05 \pm 0.06	20.9
	30	1.12 \pm 0.03	15.0
864	10	1.24 \pm 0.23	49.9
	20	1.16 \pm 0.04	23.1
	30	1.29 \pm 0.08	17.2
850	10	1.53 \pm 0.02	61.6
	20	1.33 \pm 0.014	26.6
	30	1.55 \pm 0.026	20.7
877	10	1.08 \pm 0.182	43.3
	20	1.44 \pm 0.033	28.9
	30	1.53 \pm 0.187	20.4

Different results obtained could be linked to metal chemical characteristics

- different atomic number and/or
- different ionic ray and
- different affinity for the functional groups present in cell walls.

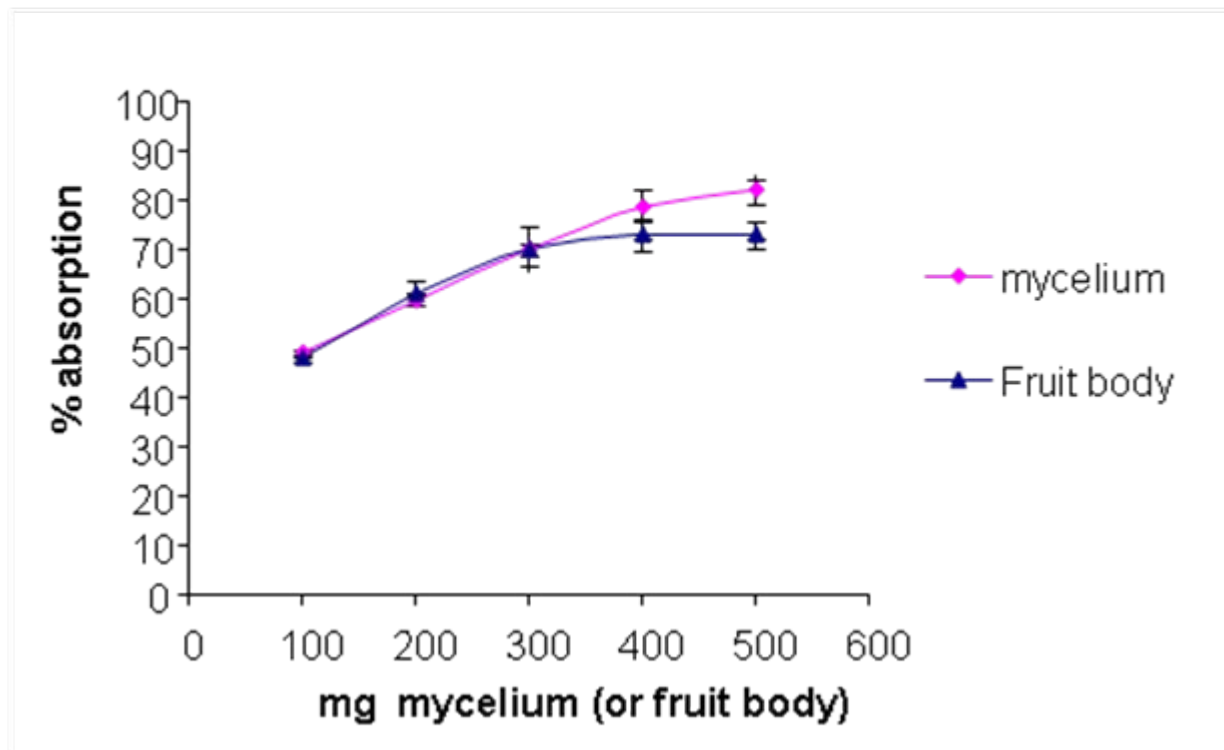
Cell immobilization with Ca-alginate, or carboxymethylcellulose or polyvinyl alcohol permits to obtain a material with the right size, mechanical strength, rigidity and porosity suitable for its use in remediation.

In a previous work, the lyophilized strain 684 immobilized on polyvinyl alcohol and packed into columns was used to remove heavy metals from a solution containing Cu, Zn and Ni, with an efficiency greater than 90%.

For its practical application the procurement of raw biomass seems the factor determining the overall cost of the system and the handling of pure mycelium can create some difficulties.

The use of mushroom fruit bodies could be of particular interest because their handling is easy and they could be obtained as a waste of mushroom production.

Comparison between mycelium or fruit body of *P. ostreatus* ability in the adsorption of Cd (10 mg L⁻¹).



CONCLUSIONS

- The knowledge of heavy metal contamination in growth environment for spontaneous and cultivated mushrooms is fundamental to ensure fruiting bodies safe for human health.
- For the biotechnological treatment of wastewaters and the recovery of metals, the facility of growth of *Pleurotus* spp., its availability and cheapness permit to consider it as an interesting fungal species for biosorption.