STUDY ON BIOREMEDIATION OF HEAVY METAL-CONTAMINATED SOILS USING THE BACTERIA-PLANTS SYNERGY

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Abstract

It is well known that different plant species can be associated with microbial communities with unique characteristics. Various groups of viable microorganisms from soils can improve the plant growth mechanism, having the role of hiperaccumulators and mitigate the toxic effects of heavy metals on plants. Moreover, the bioavailability and retention of metals can be changed and improved as a result of microbial activity in soil. In order to survive and grow in soils contaminated by heavy metals, certain species of plants develop a synergistic mechanism with the rizo-associated bacteria, which can immobilize, mobilize or transform metals, making them inactive, thus allowing plants to tolerate them in the absorption process. This paper proposes a management strategy for the microbial populations in the rizosphere, by the application of microbial inoculations, consisting in a consortium of plant growth and promoting the rhizo-and nitrogen-fixing bacteria to act as allies of plants and biofertilizers, which could provide beneficial systems for ecosystem restoration.

Key words: *bioremediation, heavy metals, contaminated soil*

Rizosphere is the soil-plants interface and plays a significant role in the phito remedy of heavy-metal contaminated soils where microbial population can influence the mobility of heavy metals and the bioavailability thereof through the release of complexing, acidifying agents, phosphate solubility, change of the redox potential. This way, the microorganisms in the soil can intensify the phito remedy potential (Glick, 2010; Jing şi colab., 2007).

Rizosphere – the volume of soil influenced by plant roots – is the environment where interactions between three categories of units take place: plants, soil and microorganisms. Microorganisms can be associated with roots in the rizosphere, (mainly, rizobacterias which improve the plant growth, ***PGPR***) can increase the capacity of plants to absorb the nutrients directly or indirectly (Amora-Lazcano and collab., 2010; Glick and collab., 2007).

The application of **PGPR** as biofertilizers and fixing agents of the nitrogen from atmosphere has a critical contribution to the intensification of the phito extraction process. *Azotobacter* species is considered an oxygen fixing agent which can independently develop in aerobe environment (Saharan and Nehra, 2011). The studies showed that, in the presence of *Azotobacter*, the output of wheat crops can increase by up to 30% (Ghalomi and collab., 2009). Also the bioavailability of pollutants in the rizosphere is critical for the design of phito remedy technologies with predictable success (Khan, 2004; Wenzel, 2009).

The absorption of metals by plants through phito extraction has as triggering force the different concentration of soil metal and of plants, which is conditional on the transfer speed of the metal from the solid state and the soil solution towards the plants. PGPR microorganisms can represent an intermediate that ensures the "continuous supply" of metallic species from the solid state, in order to maintain the phito remedy process (fig. 5.1).

Generally, PGPR, namely *Azotobacter,* acts in three different ways (Glick, 2003):

- by synthesizing agents especially for plants;
- by facilitating the assimilation of certain nutrients from the environment;
- by protecting the plants against diseases.

In this work, we have assessed the synergy between *Lepdium sativum* and *Azotobacter sp.* with respect to tolerance to the metallic ion Cd(II).

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MATERIAL AND METHODS

The seeds of *Lepidium Sativum* plant were cropped based on concentrations of Cd(II) solutions, namely 2, 4, 6, 8 mg/L, located in the vicinity of the inhibition dose of Azotobacter sp. enzymatic system, in 1.5 mL bacterial suspension. The batch of seeds was acquired from a business center. The seeds were examined and selected, namely the uncolored, damaged or abnormally small seeds were removed. Three replicates have been used for each concentration.

Each sample included 20 seeds of *Lepidium sativum*, distributed on the filter paper of the Petri dishes. Afterwards, the solutions, at the above mentioned concentrations, were added. Also, there have been three replicates for the witness, where the filter paper has been soaked in distilled water, at pH 6-6.5. The Petri dishes were covered with a lid and left at the environment temperature for three days, in light/darkness alternation conditions (14 hours/10 hours) in order to initiate the germination process.

At the end of the three days of exposure for the *L. sativum* seeds, the lids have been removed and the general status of the plants has been observed for the first time, as well as certain fading, drying symptoms of the roots and discoloration of the leaves.

Further on, the plants were recovered from the Petri dishes, placed on a clean surface and measured. The measurements required in order to determine the growth consist of the **root length, the strain length, the mass of moist material of the strain and the dry mass**. The inhibiting effect of contaminants is determined by comparing the tested groups with the control (witness) groups.

The weight of the moist biomass has been determined immediately after the measurements of the plants, thus impeding the loss of water content from the leaves.

The dry substance has been determined by placing the moist biomass into a drying chamber at 105^0C, for 24 hours. Porcelain capsules previously weighted through the analytic balance were used in this respect. The weight of the capsule plus the moist biomass were written down, and after the drying process the capsules were weighted once again. The difference between the initial and the final weight represents the quantity of dry substance.

The germination process of the control seeds should range between 90 and 100%, and the inhibition noticed at these control groups control should be low, compared to the testing groups.

The germination capacity of the seeds and strain length of *Lepidium sativum* in distilled water (the witness sample) were 91 ± 5% and 5.9 ± 0.97 cm. Also, **the roots length was 5.5 ± 0.8 cm, and the dry biomass 0.190 ± 0.051** (table 5.4)

The inhibition percentage of the germination is also expressed based on the total seeds, irrespective of the replicate, and is expressed as follows:

$$\text{Rata de germinare} = \frac{\%\text{germinare martor} - \%\text{germinare test}}{\%\text{germinare martor}}$$

No value has been assigned to the non-germinated seeds for the length and weight growing measurements. The average length of the strain and root of germinated seeds in the witness group and the testing concentrations are determined based on cumulated data (group of 20, irrespective of the replicate) and are used in order to express the inhibition percentage compared to the witness group. The results were analyzed through the unidimensional variation analysis (ANOVA). The standard error of estimates did not exceed 8% (Montvydiene and Marciulioniene, 2004).

Further on, the plants were recovered from the Petri dishes, placed on a clean surface and measured. The measurements required in order to determine the growth consist of the root length and the strain length.

The tolerance index (TI) was also determined, expressed as the ratio between the strain length, or of the root of cropped plants, respectively, and the lack of metallic ion (Burd and collab., 2000). All results have been statistically interpreted by means of ANOVA.

RESULTS AND DISCUSSIONS

The witness sample showed that *Azotobacter sp*. bacteria can stimulate te growth of *Lepidium sativum* plant, by improving the average root length by 20%, and the strain length by 30% (fig. 1). The response of *Lepidium sativum* inoculated with Azotobacter sp. and exposed to various concentrations of Cd shows that this species is sensitive to all concentrations of analyzed metallic ion, and the influence of *Azotobacter sp.* presence depends on the concentration of the metallic ion (fig. 2). The results of measurements performed on the root length of *L. sativum* plants imoculated with Azotobacter sp and exposed to cadmium (fig. 5.27) showed that the rizobacteria does not foster the growth of roots at the higher values of heavy metal concentration (more than 6 mg/L Cd(II)).

The result is similar in case of strains: the rizobacteria does not foster the growth of strains at the higher values of heavy metal concentration (more than 6 mg/L Cd(II)) (fig. 3).

The obtained results, which imply the exploration and use of these natural mechanisms for the detoxification and accumulation of metals represent the scientific grounds for the wide scale

implementation of soil phito remedy strategies by means of hyper-accumulating plants, bioabsorption – bioaccumulation and by means of the rizobacterias-hyper-accumulating plants synergy.

The study consisted of the monitored growth of *Lepidium sativus* plant, cropped by means of Cd(II) solutions with concentrations between 2- 8 mg/L, located in the vicinity of the inhibition dose of the enzymatic system of *Azotobacter sp.* and bacterial suspension. The witness sample showed that *Azotobacter sp.* bacteria can stimulate the growth of *Lepidus sativus* plant, by improving the average root length by 20%, and the strain length by 30%. The response of *Lepidus sativus* inoculated with *Azotobacter sp.* exposed to various concentrations of Cd shows that this species is sensitive to all analyzed concentrations of metallic ion, and that the influence of *Azotobacter sp.* presence depends on the concentration of the metallic ion.

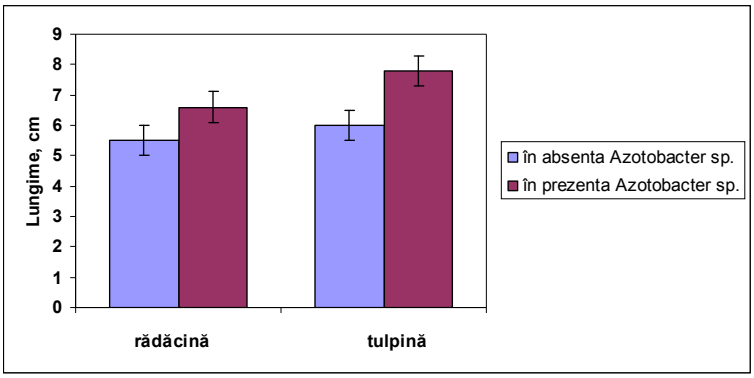

Figure 1. Root and strain length of L. sativus not inoculated and inoculated with Azotobacter sp. (average values ±SD of the three replicates; no significant differences between the experiments, P <0,05)

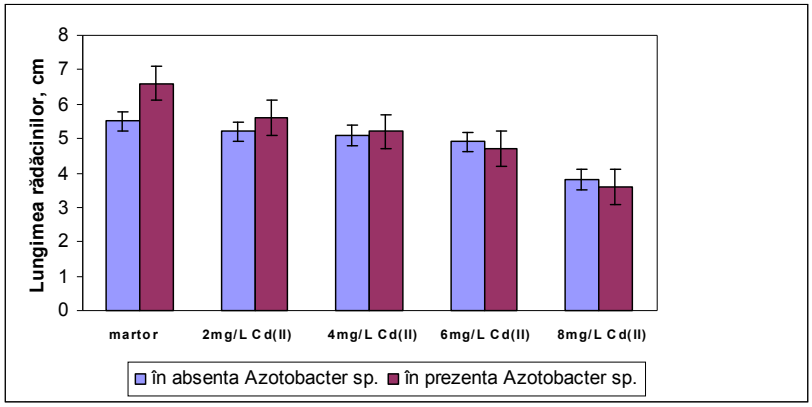

Figure 2. Root length of L. sativus not inoculated and inoculated with Azotobacter sp. at various concentrations of Cd(II) in the crop environment (average values ±SD of the three replicates; no significant differences between the experiments, P <0,05).

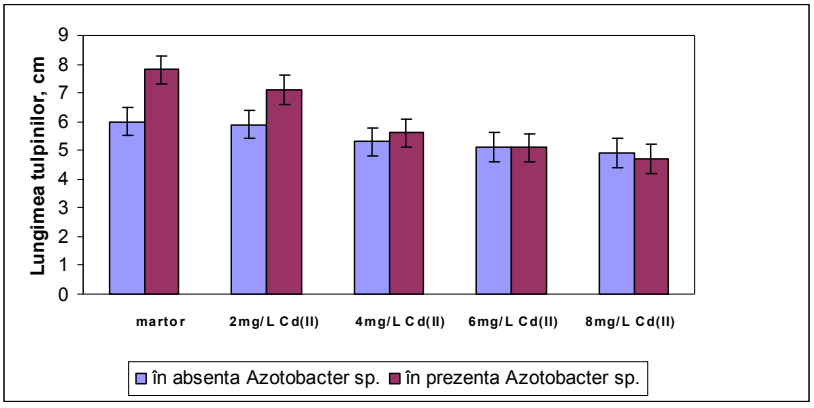

Figure 3. Strain length of *L.* sativus plants inoculated and inoculated with *Azotobacter sp.* at various concentrations of Cd(II) in the crop environment (average values ±SD of the three replicates; no significant differences between the experiments, P <0,05).

CONCLUSIONS

1. The study concerning the intensifying potential off bio remedy by means of bacteria-plant synergy takes into consideration the fact that certain plant species promote a synergy mechanism with the associated rizobacterias, through which these can immobilize, mobilize or transform metals, by making such inactive, and thus enabling the plants to tolerate them during the absorption process.

2. In this context, the paper reviews, based on experimental bases, at laboratory scale, a management strategy for microbial populations in the rizosphere, by applying a microbial inoculation, which consists of a consortium of plant growing and promotion of nitrogen fixing rizobacterias, with the role of allies of plants and bio-fertilizers, which might ensure benefic systems for the recovery of ecosystems.

3. The exploration and use of these natural mechanisms for the detoxification and accumulation of heavy metals from soil can be considered a bio remedy strategy through phito remedy, based on hyper-accumulating plants, or bio absorption and bio accumulation, by means of microorganisms, of heavy metal polluted soils.

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