Lead Influence on the Main Properties of *Bradyrhizobium Japonicum*

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Abstract


The possibility to increase soil nitrogen as a result of biological nitrogen fixation, to obtain a profitable and ecologically clean production determines the increased interest to the factors that limit the trend of the process. This especially applies to the symbiotic system "Rhizobium - legumes" that has priority in the process of nitrogen fixation. The effect of lead in concentration two times Permissible Level Content on the basic properties of *B. japonicum* was studied in pot experiment with two soils (Leached Smolnitsa and Alluvial-Meadow soil) and soybean variety "Daniela". The virulence of the nodule bacteria (nodule number, nodule dry weight, nodule volume), fixed nitrogen amount and grain yield were determined. For each soil the variant without seed inoculation and unpolluted soil was included as a control. The obtained results showed, that lead in amount two times the Permissible Level Content decreased the virulence, symbiotically fixed nitrogen and the efficiency of the *Bradyrhizobium japonicum* strain 646. In polluted soils, the studied properties of *Bradyrhizobium japonicum* are decreased depending on the soil properties. The negative influence was more clearly pronounced in the Alluvial - Meadow soil. The different degree of the lead influence can be explained with the higher cation exchange capacity of the Haplic Vertisol. As a result the bigger part of the lead is bound in the soil absorption complex, and thus has lower toxicity.  

Key words: heavy metals, nitrogen fixation, *B. japonicum*, virulence

Introduction

The possibility to increase soil nitrogen as a result of biological nitrogen fixation, to receive a profitable and ecologically clean production determines the increased interest to the factors that limit the trend of the process. This especially applies to "Rhizobium-legumes" symbiotic system that has priority in the process of nitrogen fixation. The symbiotic nitrogen fixation is exceptionally complex biological process, in which macro, as well as micro-symbionts play specific roles. This process is influenced by many environmental factors: soil pH, temperature, soil moisture, the presence of heavy metals in the soil, etc (Sprent and Sprent, 1990). The
risk of heavy metals presence in soil is related to the fact that they can accumulate ever lastingly in toxic doses, and cannot be degraded. The most harmful are Cd, Pb, As, Cu, Ni, etc (McGrath, et al., 1995; Chen, 2000). Many authors report about the suppressing influence of the heavy metals pollution on the growth and activity of free living and symbiotic nitrogen fixing organisms. (Castro, 2000; Martyaniuk et al., 2003; Zviagintsev et al., 1997; Simon, 1999). This influence depends to a significant extend on the kind of the nitrogen fixing bacteria. Thus, Tong and Sadowsky (1994), announce, that the Bradyrhizobium strains are more stable to heavy metals pollution as compared to Rhizobium strains.

The aim of this study was to determine the influence of lead on the virulence, the amount of fixed nitrogen and the efficiency of Br. japonicum.

Material and Methods

The study was carried out on the basis of a pot experiment with two significantly different in CEC and humus content soils - Leached Smolnitsa (Haplic Vertisol) and Alluvial-Meadow soil (Fluvisol) (FAO-UNESCO,1997). The physicochemical characteristics of the used soils are presented on Table 1.

The lead (Pb) was applied as Pb(CH\textsubscript{3}COO\textsubscript{2}.3H\textsubscript{2}O a month before soybean sowing at a rate of 140 mg/kg soil which is equal to twice of the Permissible Level Content (PCL). The experiment was carried out by according to following scheme for each soil:

1. Unpolluted soil; uninoculated seeds (control);
2. Unpolluted soil + inoculated seeds;
3. Soil + 140 mg. kg\textsuperscript{-1} Pb + inoculated seeds.

The experiment was conducted in pots containing 1.200 kg of air-dry soil collected from the plough layer 0-20 cm. "Daniela" soybean cultivar was tested. The soybean seeds were inoculated with Br. japonicum strain 646 suspension (108 cells.ml\textsuperscript{-1}). In each pot two plants were grown in five replications. During the vegetation, soil moisture was maintained at 60% FWC. The virulence of the nodule bacteria (number, volume and weight of the formed nodules) was determined in the bloom stage- the beginning of the pod formation (phase 1). The quan-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Physicochemical characteristics of the soils</th>
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<tbody>
<tr>
<td>Soil</td>
<td>pH\textsubscript{H\textsubscript{2}O}</td>
</tr>
<tr>
<td>Haplic Vertisol</td>
<td>7.6</td>
</tr>
<tr>
<td>Experimental station Bojurishte Fluvisol</td>
<td>6.6</td>
</tr>
<tr>
<td>Experimental station Tsalapitsa</td>
<td>6.6</td>
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</table>
quantity of symbiotically fixed nitrogen was determined on the basis of the difference in the total N uptake between plants inoculated and uni-noculated with the investigated strain, since in these soils there were no nodule bacteria of this species. The soybean was harvested at full maturity stage (phase 2). The strain effectiveness was assessed by grain yield and protein content. The total nitrogen content of grain was determined by the micro-Kjeldahl method. Data were processed using analysis of variance.

### Table 2

**Content of protein (%) in the soybean grains**

<table>
<thead>
<tr>
<th>Variant</th>
<th>Doze of Pb</th>
<th>Leached Smolnitsa</th>
<th>Alluvial-Meadow soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unpolluted soil</td>
<td>0</td>
<td>32.66</td>
<td>30.72</td>
</tr>
<tr>
<td>Polluted soil</td>
<td>2 PLC</td>
<td>30.95</td>
<td>30.95</td>
</tr>
</tbody>
</table>

**Fig. 1. Influence of lead (Pb) on the virulence of Br. japonicum (control = 0)**
Results and Discussion

Data about the virulence of Br. japonicum strain are represented in Figure 1. They show that there are no local races of nodule bacteria in the studied soil, as no nodules have been formed on the roots of the control plants. In polluted soils the virulence of the studied strain is decreased depending on the soil properties. In Leached Smolnitsa (Haplic Vertisol) on the roots of soybean plants 10% less nodules were formed which had less volume and weight compared to those formed on the roots of soybean plants of the unpolluted variants.

In Alluvial-Meadow soil (Fluvisol), which is characterized with lower CEC and humus content, the indexes of the strain virulence decreased with about 20%, in comparison to those of the unpolluted variant.

The different degree of the lead influence on the strain virulence in the two soils can be explained with the higher cation exchange capacity of the Haplic Vertisol. As a result the bigger part of the lead is bound in the soil absorption complex, and thus has lower toxicity.

Data on Figure 2 show, that in the phase 1 (the blooming - the beginning of the pod formation) the amount of the fixed nitrogen in the Haplic Vertisol is 25% less, than that in the unpolluted variants. At harvesting (phase 2) the deviations from the unpolluted variants are up to 10%, which is most probably due to the adaptation of the symbiotic system. In the Alluvial-Meadow soil these values are 35% less fixed nitrogen during the first phase, and 20% less at phase 2 respectively. Data for the fixed nitrogen correlate with the virulence of the strain. Nodules formed in polluted Leached Smolnitsa have larger volume and weight than in polluted Alluvial-Meadow soil which is considered by some authors as one of the criteria for more active symbiosis (Markova and Chanova, 1984).

The influence of Lead (Pb) on the virulence and nitrogen fixing capability of Br. japonicum strain 646 reflected on
its effectiveness as well. The yield of soybean grain, grown on polluted Haplic Vertisol decreased with 10% compared to that of the unpolluted variants, and at Fluvisol the decrease was 15% respectively (Figure 3).

The Lead did not influence significantly the content of protein in the grains of soybean sort "Daniela" grown on both of the studied soils (Table 2).

**Conclusion**

The obtained results show, that lead (Pb) in amount two times the Permissible Level Content decreases the nitrogen fixing activity, the virulence, and the efficiency of the strain 646 of Bradyrhizobium japonicum. The negative influence is more clearly pronounced in the Alluvial - Meadow soil.

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**References**


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