Effects of *Rhodococcus*-biosurfactants on the molybdenum ion phytotoxicity

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Abstract. The effect of molybdenum on the germination of seeds of agricultural plants common vetch, white mustard and oats in the presence of *Rhodococcus*-biosurfactants was studied. Molybdenum in concentrations from 10 MPC and above had a pronounced inhibitory effect on the germination of seeds. It has been found that pretreatment of seeds and their germination in the presence of biosurfactants contributed to a significant (up to 4.5 times) increase in germination, germinative energy and viability of *Avena sativa* L., *Sinapis alba* L. and *Vicia sativa* L.

1. Introduction

The environmental pollution by heavy metals (HMs) is one of the most common and environmentally hazardous for living organisms types of technogenic pollution. The industrial production is accompanied by uncontrolled entry of non-degradable and highly toxic waste containing high concentrations of HMs into natural ecosystems [1].

Molybdenum which is a HM, belongs to 4.1. Hazard Class. Long-term exposure of highly dispersed aerosols of molybdenum and its oxides on the human body causes atrophic catarrh, bronchitis, the initial forms of pneumoconiosis, gastritis, vegetovascular dystonia, and also disrupts metabolism. The effect of high concentrations of molybdenum on higher plants leads to oversaturation and unstable fixation of nitrogen, which in turn leads to the destruction of chlorophyll [2–4].

Traditional ways of soil remediation from HMs are based on the use of physicochemical methods. However, they are not environmentally friendly, since they do not ensure the complete removal of HMs ions from the soil. Modern methods involve the application of biological methods: leaching with the help of surfactants of biogenic origin [5, 6], phytoremediation [7–9]. However, each of these methods is individually ineffective in soil purification. The methods of phytoremediation at high (contamination index > 100 MPC) level of soil contamination with HMs are not used in connection with the death of plants. The use of biosurfactants is limited due to the impossibility of removing desorbed and mobilized forms of HMs from soil. Earlier in *in situ*, we demonstrated the effectiveness of the use of *Rhodococcus*-biosurfactants to enhance phytoremediation [10]. However, the effect of biosurfactants on plants has not been adequately studied at present.

The aim of this study is to evaluate the effect of molybdenum on germination of seeds and the development of seedlings of green manures in the presence of *Rhodococcus*-biosurfactants.
2. Materials and methods
The seeds of oat (Avena sativa L.), white mustard (Sinapis alba L.) and common vetch (Vicia sativa L.) were used. Molybdenum was used as the salt of ammonium molybdate ((NH₄)₆Mo₇O₂₄·4H₂O) in concentrations multiple of 1, 10, 50, and 100 maximum permissible concentrations (MPC), taking into account the Clark background in terms of pure metal [10]. The concentration of molybdenum corresponded to 10, 100, 500, and 1000 mg/kg of contaminated soil. The phytotoxicity level was determined in accordance with Standard Methodological Recommendations (Phytotest) [12]. Seeds were grown under illumination with white fluorescent lamps, the spectrum of which is as close as possible to daylight; temperature was 23–25°C, pH was 6.3–6.5.

The actinobacterial strain Rhodococcus ruber IEGM 231 is active producer of biosurfactants (http://www.iegmcol.ru/strains/rhodoc/ruber/r_ruber231.html). The bacterial culture was grown in RS medium (Rhodococcus Surfactant) on an orbital shaker for 7 days (160 rpm, 28°C). The RS medium contained (g/l) Na₂HPO₄ – 2.0; KH₂PO₄ – 2.0; KNO₃ – 1.0; (NH₄)₂SO₄ – 2.0; NaCl – 1.0; MgSO₄·7H₂O – 0.2; CaCl₂·2H₂O – 0.02; FeCl₃·7H₂O – 0.01. n-dodecane (C₁₂) or n-hexadecane (C₁₆) were used at a concentration of 3 vol.% as the only sources of carbon and energy. Crude Rhodococcus-biosurfactant complexes of glycolipid nature, produced by Rhodococcus, were obtained by the method previously described [14]. According this method, 5–7-day bacterial culture grown in RS medium was settled in a separatory funnel, after which the lower transparent aqueous layer was drained. The remaining uniform hydrophobic oily mass was subjected to ultrasonic sounding (30 min) under compulsory cooling conditions. To distinguish the biosurfactants produced by Rhodococcus in a liquid mineral medium with n-dodecane and n-hexadecane we used designations BS C₁₂ and BS C₁₆, respectively.

Experiments on the germination of seeds were carried out in triplicates. 25 dry and disease-free seeds were placed in Petri dish and 5 ml of emulsion of Rhodococcus-biosurfactants and ammonium molybdate was added. As a control, we used: (i) distilled water; (ii) aqueous solutions of biosurfactants in concentrations of 2.0, 4.0 and 8.0 g/l; (iii) aqueous solutions of ammonium molybdate. The germinative energy was determined on the third day, the germination and length of the seedlings were determined on the seventh day of the experiment. Statistical analysis of the obtained results was carried out by standard methods with calculation of the mean, standard deviation and confidence interval using the Microsoft Excel 2007 software package.

3. Results and discussion
A positive correlation between the effect of seed treatment by Rhodococcus-biosurfactants on the decrease in phytotoxicity of ions molybdenum was revealed. The germinative energy of seeds of all plant species used in the work in the presence of Rhodococcus-biosurfactants is 2.0–4.5 times higher than that in conditions of contamination with molybdenum.

Table 1 shows the effect of Mo⁶⁺ ions on the length of shoots and roots of oat, mustard and vetch propagation in the presence of BS C₁₂. According to our data, pretreatment of seeds with this biosurfactant did not have a significant effect on seed growth.

While pretreatment of plant seeds with Rhodococcus-biosurfactants, produced in the presence of n-hexadecane, increases the shoots and roots of all the plant species studied more intensively (up to 2.5 times) (table 2).

After preliminary treatment of seeds with biosurfactants, their germination capacity increased to 4.5 times. As can be seen from figure 1, the highest germination (100%) of Avena sativa L. seeds was detected in the presence of molybdenum in concentrations up to 10 MPC. Apparently, molybdenum stimulates the germination of oat seeds, since it is a vital element. At higher concentrations (50 MPC and higher), molybdenum inhibited the germination process of seeds. Germination of seeds treated with BS C₁₆ was 1.2–1.6 times higher than that of BS C₁₂.
Table 1. Effect of molybdenum on the length of shoots and root system (mm) of oats, mustard and vetch sprouts in the presence of BS C_{12}.

| Variants            | Avena sativa L. | Sinapis alba L. | Vicia sativa L. |
|---------------------|-----------------|-----------------|-----------------|
|                     | Length of shoots| Length of roots | Length of shoots| Length of roots | Length of shoots| Length of roots |
| Control             | 49.1±8.3        | 40.8±5.2        | 25.2±9.9        | 22.5±4.8        | 27.3±6.0        | 19.3±9.8        |
| Mo^{6+} 1 MPC       | 44.2±15.5       | 55.5±19.3       | 23.3±11.3       | 33.0±28.1       | 8.4±4.2         |                |
| Mo^{6+} 10 MPC      | 47.2±15.1       | 24.5±11.3       | 11.0±2.7        | 10.2±3.8        | 15.0±2.6        |                |
| Mo^{6+} 50 MPC      | 37.9±12.6       | 6.9±3.4         |                |                |                |                |
| Mo^{6+} 100 MPC     | 24.8±11.6       | 8.4±3.5         |                |                |                |                |
| BS C_{12} 2 g/l     | 55.7±14.4       | 48.4±9.5        | 32.2±8.7        | 36.7±3.0        | 30.8±2.6        | 18.2±3.0        |
| BS C_{12} 4 g/l     | 67.4±5.7        | 53.0±6.1        | 39.7±6.7        | 37.0±3.8        | 32.5±1.8        | 19.3±3.4        |
| BS C_{12} 8 g/l     | 45.1±15.2       | 35.1±3.2        | 24.6±5.0        | 26.2±8.0        | 27.0±2.0        | 12.3±3.4        |
| 1 MPC / 2 g/l       | 60.6±25.9       | 58.0±24.2       | 23.3±9.6        | 18.4±18.8       |                |                |
| 1 MPC / 4 g/l       | 64.1±25.0       | 56.5±29.8       | 15.9±6.4        | 33.8±18.7       |                |                |
| 1 MPC / 8 g/l       | 51.8±23.1       | 46.7±24.8       | 16.7±9.3        | 21.1±10.7       |                |                |
| 10 MPC / 2 g/l      | 54.6±20.0       | 24.5±8.3        |                |                |                |                |
| 10 MPC / 4 g/l      | 44.3±16.2       | 28.3±7.0        |                |                |                |                |
| 10 MPC / 8 g/l      | 52.1±21.3       | 13.0±11.6       |                |                |                |                |

The solutions used in the work were presented as the concentrations of molybdenum ions / Rhodococcus-biosurfactants (MPC / g/l).

Table 2. Effect of molybdenum on the length of shoots and root system (mm) of oats, mustard and vetch sprouts in the presence of RB C_{16}.

| Variants            | Avena sativa L. | Sinapis alba L. | Vicia sativa L. |
|---------------------|-----------------|-----------------|-----------------|
|                     | Length of shoots| Length of roots | Length of shoots| Length of roots | Length of shoots| Length of roots |
| RB C_{16} 2 g/l     | 39.5±26.0       | 41.8±21.3       | 30.5±11.1       | 33.6±26.3       | 29.2±2.8        | 21.3±1.6        |
| RB C_{16} 4 g/l     | 31.9±19.3       | 35.3±18.3       | 27.9±8.7        | 33.8±20.1       | 31.8±1.7        | 19.8±0.9        |
| RB C_{16} 8 g/l     | 23.5±7.0        | 23.9±13.6       | 20.5±7.4        | 40.2±29.7       | 30.8±1.8        | 25.4±2.2        |
| 1 MPC / 2 g/l       | 69.2±24.3       | 72.8±30.1       | 24.2±8.1        | 39.9±20.8       | 17.4±3.6        | 10.3±4.4        |
| 1 MPC / 4 g/l       | 57.5±31.4       | 56.6±27.3       | 25.7±8.3        | 39.5±21.0       | 17.0±0.8        | 12.0±3.2        |
| 1 MPC / 8 g/l       | 47.3±36.5       | 61.5±28.5       | 33.9±6.8        | 34.2±11.6       | 14.7±2.1        | 13.0±3.6        |
| 10 MPC / 2 g/l      | 55.3±22.0       | 29.5±17.3       | 16.7±6.4        | 12.8±3.8        | 24.5±0.8        | 8.6±2.3         |
| 10 MPC / 4 g/l      | 56.0±17.2       | 23.5±13.5       | 17.2±7.1        | 15.7±4.7        | 18.0±1.0        | 9.2±0.8         |
| 10 MPC / 8 g/l      | 51.5±27.2       | 34.5±15.9       | 20.1±6.3        | 21.4±12.3       | 18.0±1.0        | 10.0±0.2        |
| 50 MPC / 2 g/l      | 48.1±5.6        | 17.6±1.8        | 6.3±2.0         |                | 9.3±2.5         | 9.6±5.8         |
| 50 MPC / 4 g/l      | 40.8±14.6       | 14.1±8.3        | 6.3±1.2         |                | 8.4±3.6         | 17.0±2.6        |
| 50 MPC / 8 g/l      | 42.7±3.5        | 12.4±2.6        |                |                | 8.0±2.2         | 4.5±0.7         |
| 100 MPC / 2 g/l     | 29.8±5.9        | 15.0±1.4        |                |                | 5.5±2.1         | 9.0±0          |
| 100 MPC / 4 g/l     | 34.0±11.8       |                |                |                | 3.5±1.2         | 6.4±0.5         |
| 100 MPC / 8 g/l     | 28.1±4.6        |                |                |                | 7.6±1.5         | 5.4±2.3         |

The solutions used in the work were presented as the concentrations of molybdenum ions / Rhodococcus-biosurfactants (MPC / g/l).
Figure 1. Influence of biosurfactants on the germination capacity of *Avena sativa* L. seeds in the presence of molybdenum ions.
A BS C\textsubscript{12}, B – BS C\textsubscript{16}; 1, 10, 50, 100 – concentration of molybdenum 1, 10, 50, 100 MPC; 1/2, 1/4, 1/8 – concentration of molybdenum, MPC / concentration of biosurfactants, g/l.

The revealed regularity was also maintained when germination of mustard seeds: the amount of germinating seeds when processing them BS C\textsubscript{16} to 3.5 was higher, compared with that after treatment with BS C\textsubscript{12}. Molybdenum at a concentration of 50 MPC inhibited the germination of mustard seeds, whereas after processing with *Rhodococcus*-biosurfactants, up to 15% of mustard seeds germinated in the presence of this level of environmental contamination with molybdenum (see figure 2).

Figure 2. Influence of biosurfactants on the germination capacity of *Sinapis alba* L. seeds in the presence of molybdenum ions.
A BS C\textsubscript{12}, B – BS C\textsubscript{16}; 1, 10, 50 – concentration of molybdenum 1, 10, 50 MPC; 1/2, 1/4, 1/8 – concentration of molybdenum, MPC / concentration of biosurfactants, g/l.
A similar dependence was observed in experiments with *Vicia sativa* L. As can be seen in figure 3, the seed germination after treatment with BS C$_{16}$ was repeatedly increased, the vetch seeds germinated even at a molybdenum concentration of up to 100 MPC in the germination medium, whereas BS C$_{12}$ did not significantly affect the germination of the vetch seeds.

![Figure 3](image)

**Figure 3.** Influence of biosurfactants on the germination capacity of *Vicia sativa* L. seeds in the presence of molybdenum ions.

A BS C$_{12}$, B – BS C$_{16}$; 1, 10, 50, 100 – concentration of molybdenum 1, 10, 50, 100 MPC; 1/2, 1/4, 1/8 – concentration of molybdenum, MPC / concentration of biosurfactants, g/l.

4. Conclusion

As a result of the conducted studies it was established that the most stable plant to the action of molybdenum was white mustard, less stable plant was oats. In terms of the degree of resistance to molybdenum, the plants used in the work could be distributed in a series: *Sinapis alba* L. > *Vicia sativa* L. > *Avena sativa* L. The dependence of the decrease in phytotoxicity of molybdenum after treatment of BS C$_{16}$ seeds was revealed. Thus, after preliminary treatment of the seeds with an aqueous emulsion of *Rhodococcus*-biosurfactants, the germination and germinative power of the plants studied increased to 4.5 times and the growth rate of the roots and shoots of the seeds under study in conditions of pollution with molybdenum increased up to 2.5 times.

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