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AGRICULTURAL SOIL SCIENCE AND AGROECOLOGY

Growth and Mineral Nutrition of Spring Wheat Inoculated with Plant Growth Promoting Rhizobacterium in Soil Contaminated with Nickel

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Abstract—A greenhouse experiment was conducted to study the effect of the plant growth promoting rhizobacterium *Pseudomonas fluorescens* 20 on spring wheat productivity in agrogray soil artificially contaminated with a water-soluble nickel compound. Plants were grown until the booting stage; NPK fertilizers and $NiCl_2 \cdot 6H_2O$ were applied to soil. The contaminant application rate was 300 mg Ni/kg soil, which significantly exceeds its maximum permissible concentration in soils. The chemical composition of plants and the uptake of nickel and nutrients from soil by shoots and roots were examined. Concentrations of Ni and other ash elements were determined after wet combustion of plant samples using inductively coupled plasma optical emission spectrometry and flame photometry. The nitrogen content in plants was determined using the phenol method. Bacterial inoculation increases the resistance of plants to toxic effects of the heavy metal, which is expressed in the higher weight of their vegetative organs and roots in contaminated conditions. Simultaneously the mineral nutrition of spring wheat improves, and the from soil by the plants increases; apparently, this manifests their protective response to soil contamination with Ni. The general growth promotion observed in bacterially inoculated plants is not accompanied by significant changes in the content of elements in their shoots and roots. The application of the bacterium intensifies Ni phytoextraction from soil (i.e., soil purification from the heavy metal) and the Ni uptake by roots without significant changes in its concentration in shoots and roots. The amounts of metal accumulated in roots exceed those accumulated in shoots by an order of magnitude. Regardless of bacterial inoculation, soil contamination with Ni increases the content of Mg and some other nutrients in shoots and roots of plants.

Keywords: *Pseudomonas fluorescens* 20, *Triticum aestivum* L., $NiCl_2 \cdot 6H_2O$, agrogray soil, chemical composition of plants

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INTRODUCTION

The main anthropogenic sources of the biosphere pollution with Ni are fuel combustion, various industries, sewage sludge, and dump sites. In agriculture, mineral and organic fertilizers, as well as plant protection agents, can contaminate soils with Ni. Elevated Ni concentrations in soils inhibit plant growth and development; the chlorophyll content in leaves decreases [1]. Many researchers consider the use of plant growth promoting rhizobacteria (PGPR) an efficient remediation strategy for soils contaminated with heavy metals [2–4]. Special attention is paid to representatives of the genus *Pseudomonas* due to their widespread occurrence and a set of properties beneficial for plants [5]. Biopreparations that include bacteria of this genus enhance most efficiently such growth parameters of various crops as height, biomass, and ripening [6]. The application of PGPR [7], including the genus *Pseudomonas* [8, 9], significantly reduces the Ni phytotoxicity and increases plant resistance to toxic effects of this heavy metal. Inoculation with growth promoting bacteria of the genus *Pseudomonas* significantly reduces the uptake of lead and cadmium from contaminated agrogray soils by barley shoots at early ontogenesis stages, thus, increasing the plant resistance to toxic effects of heavy metals [10, 11]. Among other things, bacteria of the genus *Pseudomonas* stimulate the growth of agricultural crops by improving their mineral nutrition [12, 13]. Effects exercised by growth promoting rhizobacteria on the mineral nutrition of plants in soils contaminated with heavy metals, including Ni, remain understudied.

The purpose of this study was to determine the effect the plant growth promoting rhizobacterium *P. fluorescens* 20 on the growth of spring wheat and on the elemental chemical composition, including the Ni content, of plants cultivated in soil contaminated with this heavy metal.

MATERIALS AND METHODS

The works were performed in 2021; the greenhouse experiment involved spring wheat (*T. aestivum* L.), Zlata variety (Moscow Scientific Research Institute of Agriculture Nemchinovka) cultivated in soil artificially contaminated with a water-soluble Ni compound. Cultivation pots 10 cm in diameter and 11 cm in height were filled with 800 g of soil; ten plants were cultivated in each pot for 26 days until the booting stage. The effect of strain 20 of *P. fluorescens* bacterium on the growth and elemental chemical composition of plants, including the Ni concentration in them, was studied. The experiment included the following variants: no soil contamination with Ni and no bacterial inoculation (control); soil contamination with Ni without bacterial inoculation; and soil contamination with Ni in combination with bacterial inoculation. Spring wheat plants were cultivated in arable medium loamy agrogray soil (Luvisol) taken from a layer of 0–20 cm and used in the previous year for barley cultivation. Chemically pure salt $NiCl_2·6H_2O$ was applied to the soil 10 days before sowing the seeds at a rate of 300 mg Ni/kg in the form of a solution evenly mixed with the entire soil volume contained in cultivation pots. This Ni dose exceeds its maximum permissible concentration in soils in the total form by more than 3.5 times; in the mobile form (whose content in the original soil was at a trace level), it was by 75 times. Mineral fertilizers were applied at a rate of 100 mg of each active ingredient (nitrogen, phosphorus, and potassium) per 1 kg of soil in the form of ammonium nitrate, dibasic potassium phosphate, and potassium sulfate, respectively. Prior to the sowing, germinated seeds were inoculated with the aqueous bacterial suspension at a rate of 10^8 cells per plant. In the variant without inoculation, the seeds were treated in a similar way with an adequate amount of autoclaved bacterial suspension. During the growing season, the soil moisture content in the cultivation pots was maintained by watering at a level not lower than 60% of the water holding capacity.

After the end of cultivation (at the booting stage), the shoots-green matter (leaves and stems) and plant roots were dried at 70°C and weighed. The roots were washed from soil using tap water and then distilled water. After combusting the plant materials (0.5 g) in a mixture of concentrated acids $HNO_3 : HClO_4$ (2 : 1), they were analyzed to determine the content of Ni and other ash elements. The total nitrogen content was determined using the phenol method after combusting the plant materials (0.1 g) in dilute sulfuric acid (1 : 2) with a catalyst ($K_2SO_4 : Zn : Se : CuSO_4·4H_2O = 100 : 24 : 2 : 0.2$). Concentrations of Ni and other ash elements, except for potassium, in the solutions were determined using an ICP OES Optima 5300 DV spectrometer (United States) (inductively coupled plasma optical emission spectrometry). The potassium content was determined using a BWB XP flame photometer (United Kingdom) (flame photometry). Statistical data processing was performed using MS Excel 2010. Pearson's test was used to determine normality of the distributions. Standard deviations from the mean were computed to estimate the error; Student's *t*-test was used to assess the significance of differences in concentrations of elements in different specimens.

RESULTS AND DISCUSSION

Soil contamination with Ni significantly inhibited the growth of spring wheat plants (Table 1). The mass of vegetative organs and whole plants at the booting stage was two times less in comparison with the control (i.e., the variant without soil contamination with Ni and without bacterial inoculation). The root mass under the Ni stress conditions also decreased by more than half. The application of the bacterium *P. fluorescens* 20 significantly reduced the toxic effect of the heavy metal on plants. The shoot weight in plants exposed to Ni stress and inoculated with the bacteria was 1.5 times larger than in the variant involving soil contamination without inoculation. The root system growth improved as well. In the variant involving inoculation with *P. fluorescens* 20, the weight of plant roots in soil contaminated with the heavy metal increased by 70%. The application of rhizobacteria increased the shoot weight to 82% of the control level; while the weight of the roots increased to 68% of the control level.

The inoculation with *P. fluorescens* 20 did not significantly affect the Ni content in shoots and roots (Table 1). In roots, its concentration was tens of times higher than in aboveground parts of the plants. Bacterial inoculation increased the Ni uptake (μg/pot) by shoots and roots from soil contaminated with the heavy metal by 1.7–1.8 times (Table 2). The share of Ni in the total mass of plants in different experimental variants was 1.9–3.3% of the applied amount and increased in plants inoculated with the bacterium.

In the variant involving soil contamination with Ni, bacterial inoculation did not have a significant effect on the content of all studied macro- and microelements in vegetative organs and roots of the plants in comparison with the variant involving contamination without the application of bacterium (Table 3). As a result of soil contamination with Ni, the content of Mg in shoots was increased by 3.2–3.3 times, Zn by 2.1 times, Cu by 1.8–1.9 times, P by 1.5 times, and Mn by 1.3–1.4 times in comparison with the control variant, while the amount of Ca in shoots in contaminated conditions decreased by 2.4–2.5 times.

The Mg content in roots and vegetative organs of plants cultivated in soil contaminated with Ni was significantly (by 2.1 times) higher in comparison with the control. Herewith, unlike aboveground parts of the plants, the Ca concentration in roots increased by more than three times, including the variant with the application of bacterium. In addition, the content of Fe in roots increased under the Ni pollution conditions more significantly (1.7–1.8 times) than in vege-

Table 1. Plant weight and Ni content in plants in variants involving soil contamination and bacterial inoculation

Variant	Plant weight (dry matter)			Ni content	
	shoots	roots	whole plant	shoots	roots, %
	g/pot			mg/kg	
No Ni, no bacterial inoculation (control)	2.44	1.08	3.52	8a*	0.02[a]
Ni without bacterial inoculation	1.20	0.43	1.63	254[b]	1.21[b]
Ni + *P. fluorescens* 20	2.00	0.73	2.73	265[b]	1.27[b]
LSD_{05}	0.37	0.12	0.60		

* values marked by different letters differ from each other at the 5% significance level.

Table 2. Ni uptake by plants in variants involving soil contamination and bacterial inoculation

Variant	Shoots (μg/pot)	Roots (μg/pot)	Whole plant	
			μg/pot	% of applied dose
No Ni, no bacterial inoculation (control)	20	216	236	–
Ni without bacterial inoculation	305	5203	5508	1.9
Ni + *P. fluorescens* 20	530	9271	9801	3.3
LSD_{05}	60	1711	1805	

Table 3. Content of nutrients in plants in variants involving soil contamination with Ni and bacterial inoculation

Variant	N	P	K	Ca	Mg	Fe*	Mn	Zn	Cu
	%				mg/kg				
Shoots									
No Ni, no bacterial inoculation (control)	3 9[a]**	0.4[a]	0.4[a]	0.5[a]	215[a]	159[a]	42[a]	22[a]	12[a]
Ni without bacterial inoculation	4.1[a]	0.6[b]	0.4[a]	0.2[b]	685[b]	180[b]	54[b]	45[b]	22[b]
Ni + *P. fluorescens* 20	4.1[a]	0.6[b]	0.4[a]	0.2[b]	710[b]	179[b]	57[b]	45[b]	23[b]
Roots									
No Ni, no bacterial inoculation (control)	3.1[a]	0.7[a]	2.1[a]	0.5[a]	663[a]	0.2[a]	277[a]	89[a]	144[a]
Ni without bacterial inoculation	3.1[a]	0.7[a]	2.1[a]	1.7[b]	1380°	0.4[b]	333[b]	103[b]	144[a]
Ni + *P. fluorescens* 20	2.6[a]	0.6[a]	2.0[a]	1.7[b]	1380°	0.4[b]	420[b]	99[b]	162[b]

* Fe content in roots is expressed in %.
** Values marked by different letters differ from each other at the 5% significance level.

tative organs; P, Zn, and Cu concentrations changed less significantly, while the Mn content increased approximately to the same extent as in vegetative organs. Under the Ni contamination conditions, the K concentration changed insignificantly in all experimental variants, both in roots and shoots. Regardless of bacterial inoculation, only a slight change in the nitrogen content in aboveground parts of the plants was noted under the Ni contamination conditions; in roots, the value of this parameters slightly decreased after the inoculation in comparison with the control variant.

Under the Ni stress conditions, the *P. fluorescens* 20 application increased the uptake of all studied nutrients from soil in comparison with the variant without inoculation: by 1.7–2.0 times for vegetative organs of plants and by 1.3–2.2 times for roots (Table 4). By contrast, without bacterial inoculation, soil contamination with Ni significantly reduced the uptake of most elements, except for Mg, Zn, and Cu, by shoots of the plants. Herewith, the Mg content in plants increased by some 1.5 times, while the amounts of Zn and Cu did not change. The uptake of all elements (except for Ca) by roots decreased.

Table 4. Nutrient uptake by plants in variants involving soil contamination with Ni and bacterial inoculation

Variant	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	mg/pot				μg/pot				
Shoots									
No Ni, no bacterial inoculation (control)	95	10	10	12	525	388	103	54	29
Ni without bacterial inoculation	49	7	4	2	822	216	65	54	26
Ni + *P. fluorescens* 20	82	12	8	4	1420	358	114	90	46
LSD_{05}	8	2	2	1	132	66	32	11	3
Roots									
No Ni, no bacterial inoculation (control)	34	6	23	6	716	2497	299	96	155
Ni without bacterial inoculation	13	3	12	7	593	1690	143	44	62
Ni + *P. fluorescens* 20	19	4	15	12	1007	3635	268	72	118
LSD_{05}	5	1	3	1	103	624	90	10	36

The *P. fluorescens* 20 application to the agrogray soil treated with a water-soluble Ni compound significantly reduces the toxic effect of the heavy metal on spring wheat plants at the booting stage. The bacterium simulate their growth, and the weight of vegetative organs and roots in contaminated conditions increases.

It is known that inoculants consisting of bacteria belonging to the genus *Pseudomonas* increase the weight of chickpea plants in greenhouse experiments at a Ni concentration of 2 mM [14] and significantly increase the mass of brown mustard (*Brassica juncea*) plants cultivated in soil contaminated with Ni [15]. Earlier greenhouse experiments have shown that the application of plant growth promoting bacteria of the genus *Pseudomonas* in agrogray soils contaminated with Pb and Cd compounds completely eliminates the toxic effect of heavy metals on barley plants [10, 11]. Furthermore, their weight is the same as in plants cultivated in soil not contaminated with heavy metals [10, 11]. In this experiment, bacterial inoculation of plants cultivated in agrogray soil contaminated with Ni did not completely eliminate the adverse effect of the heavy metal on spring wheat plants (despite its significant mitigation). Apparently, this is due to the heavy Ni application rate and a higher sensitivity of spring wheat to heavy metals in comparison with barley, which is known to be among the species resistant to the toxic effect of Cd [16].

The beneficial effect of the studied bacterium on plant growth under the Ni pollution conditions and the elevated resistance of inoculated spring wheat plants to the toxic effect of the heavy metal can be explained by the greater accumulation of nutrients in shoots and roots of inoculated plants resulting in their better mineral nutrition. Microorganisms associated with plants can stimulate their growth and exercise positive effects on their mineral nutrition even in the presence of heavy metals [4, 10, 11]. After the inoculation, the uptake of nutrients and Ni from contaminated soil by vegetative organs and the root system increased due to an increase in their weight (i.e., as a result of stimulation of growth processes) and without significant changes in the content of elements in vegetative organs and the root system. Our data indicate that, under the Ni stress conditions, the effects exercised by bacteria increase the nutrient uptake from contaminated soil by plants, including Mg that constitutes a part of chlorophyll and is directly involved in the photosynthesis process. Apparently, this is a protective response of inoculated plants to the presence of Ni. Without the application of bacterium, soil contamination with Ni inhibited the plant growth and reduced the plant weight. Herewith, elevated concentrations of Mg and a number of other elements were registered in shoots and roots of noninoculated plants.

The application of the bacterium *Pseudomonas* sp. to soils contaminated with Ni increased the biomass of brown mustard and had no effect on the heavy metal content in the plants [15]. Inoculation of Indian mustard with the Ni-resistant bacterium *Pseudomonas* Ps29C protected plants from the heavy metal applied to the soil at various concentrations and did not affect the Ni accumulation in their shoots and roots [17]. In our study, gains in plant weight were registered in the variant involving the application of *P. fluorescens* 20 in contaminated conditions; no significant changes in the Ni concentration were noted in vegetative organs and the root system. The Ni uptake is increased due to the increase in the plant weight. Herewith, the phytoextraction process was intensified, thus, contributing to soil purification from the heavy metal.

CONCLUSIONS

The application of the plant growth promoting rhizobacterium *P. fluorescens* 20 to agrogray soil artificially contaminated with Ni significantly reduces the heavy metal phytotoxicity, stimulates the plant growth, and increases the weight of vegetative organs and the root system in spring wheat plants at the booting stage; apparently, this manifests the protective response of inoculated plants to soil contamination with Ni. The bacterial inoculation does not completely eliminate toxic effects of the heavy metal applied at a rate of 300 mg/kg soil. The higher resistance of inoculated spring wheat plants to toxic effects of Ni is determined by their better mineral nutrition and accompanied by more intense uptake of macro- and microelements by shoots and the root system. Herewith, no significant changes are observed in the content of nutrients in shoots and roots. The application of the bacterium increases the removal of Ni from soil by vegetative organs and roots of the plants, thus, intensifying the phytoextraction process. This also occurs due to the increase in their weight without significant changes in the heavy metal content. Regardless of bacterial inoculation, soil contamination with Ni increases the content of Mg in shoots and roots of the plants.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interests. This article does not contain any studies involving animals or human participants performed by the authors.

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