Effect of platinum nanoparticles on morphological parameters of spring wheat seedlings in a substrate-plant system

T Astafurova, A Zotikova, Yu Morgalev, G Verkhoturova, V Postovalova, S Kulizhskiy, S Mikhailova
Tomsk State University, 36, Lenina ave., Tomsk, 634050, Russia, tel./fax: +7(3822) 534519

E-mail: yu.morgalev@gmail.com

Abstract. When wheat is cultivated in the media contaminated with platinum nanoparticles, the change in the morphological and physiological indexes of wheat seedlings depends on the physico-chemical parameters of the germination substrate. The changes become less pronounced with the decreasing bioaccessability of the nanomaterial in the following order: water suspension – luvisols – phaeozems. Contamination with nanoparticles affects the height parameters and activates the mechanisms protecting the plant from stress. When using wheat seedlings as test organisms for biotesting the environmental safety of NPs, it is advisable to use the following parameters: weight of roots, weight of aerial part, leaf area, and flavonoid content.

1. Introduction

With nanomaterial production on the steady increase, nanoparticles (NPs) are being released into the environment at different stages of their life cycle, which makes it necessary to research possible consequences of their interaction with elements of the biota and ecosystems. Recent research has shown that NPs that penetrate into biological tissue cause various bioeffects, both positive and negative [1-3]. Interacting with various cell structures, NPs can be catalysts in different reactions producing not only growth promoters but also inhibitors [4,5]. The effect of NPs on plants may differ from that of the salts of the same metals. Even in very low dosage, they can change physiological and biochemical processes, while maintaining prolonged action [6]. The discovered bioactivity of technogenous NPs confirms the necessity to research their migration and accumulation in plants growing on the territories subject to contamination with finely-dispersed materials.

The aforementioned papers contain little data on the research into platinum NPs phytotoxicity [7-9]. We know, however, that they show a strong regenerative ability related to their high antioxidant activity as well as unique catalytic propertie [10].

Phytotoxicity is difficult to assess, because toxicity does not only depend on the NP physical nature, production method, size and structure, but also on features of the biological models and, quite possibly, their growing technique.

The purpose of this paper was to compare the effect of platinum NPs 4 nm in size on the morpho-physiological traits of wheat seedlings, when growing them in water culture as well as on different soil types.

2. Materials and methods

The object of the study was water and soil cultures of 10-day plants of soft spring wheat (Triticum aestivum L.), variety Novosibirskaya - 29, which were grown in a laboratory setting in a temperature chamber at a constant temperature of 22 °C and light intensity of 150 W/m2 (photoperiod 12/12 hours). One set of experiments compared water and soil cultures. In water cultures, we grew the plants in a fine medium containing platinum NPs with the initial concentration of 10 mg/l and used distilled water as a control. In soil cultures, we grew the seedlings in containers with 200 g of Garant soil containing, per 100 grams of soil, 30-50 mg of nitrogen, 70 mg of phosphorus, 80-100 mg of potassium, pH 6.5, micronutrients, and humic growth promoters. Before seeding, the substrate was irrigated by a suspension of NPs until their concentration reached 10 mg/kg of soil. Another set of experiments used two types of soils, luvisol and phaeozem, through which we had poured a 10 mg/l suspension of platinum NPs in a filtration column. Luvisol retained 13.4 % (C = 1 mg/kg) of the platinum NPs introduced and phaeozem, 22.5 % (1.34 mg/kg). Uncontaminated soil served as a control.

Platinum nanoparticles (nPt) were obtained by laser ablation in distilled water from high-purity (99.97%) platinum bars [Morgalev et al., 2012]. The characteristics of NPs were verified by TEM (Phillips CM-12, France), dynamic light scattering (Zetasizer Nano ZS, USA), and BET (TriStar 3000, USA). Particle size Δ50=5 nm and specific surface S =36 m2/g.

Platinum nanoparticles bioaccumulation in the plants from the substrates was determined by ICP-MS (ELAN DRC-e, USA) in homogenates from the plant roots and aerial parts (leaves + stem). The morphometric changes were evaluated by the length of the root system, height of the seedlings and weight of the organs. The content of chlorophylls and flavonoids in the leaves was measured by a Dualex-4 sensor (France). The nitrogen index was calculated as a ratio of the sum of chlorophylls to that of flavonoids.

The agrochemical soil composition was studied according to the following GOSTs: pH of the salt extract, GOST 26483-85; labile phosphorus (P2 O5) and labile potassium (K2O) fractions, GOST R 54650-11; nitrate nitrogen, GOST 26951-86; exchangeable ammonium, GOST 26489-85; humus, GOST 26213-91; exchangeable calcium and exchangeable magnesium, GOST 26487-85; hydrolytic acidity, GOST 26212-91.

Statistica 7 software package was used for statistical data processing. All the indicators were analyzed for normality of distribution. The significance threshold was set at $p<0.05$. The tables and figures show the mean data and SEM.

3. Results and discussion

The nPt introduced into the cultivation medium triggered changes in the height and weight parameters of the leaf and root system in wheat seedlings when those were cultivated in a water dispersion medium and Garant substrate. In water cultures, the length and weight of the root increased significantly, which indicates that platinum NPs actively interfere with the complicated mechanism of plant growth regulation. Soil cultures were superior to their water counterparts in height and weight parameters and the effects of NP introduction were less prominent (see Table 1).

One of the reasons for such differences is the NP lifetime in the nanosized state, which depends on the properties of the dispersion medium [11]. In water solutions with natural stabilizers, it ranges from several hours to 40 days and sometimes even exceeds a year [12, 13]. As to soil, there are very little data showing that NP inactivation in soils may be long-term as indicated for ultradispersed metal powders: they gradually oxidize in soils and serve as micronutrients for plants during their growth and development [14].

Among other things, NP aggregation may reduce their bioaccessibility, which must affect the bioaccumulation processes in the plant tissues. Our studies have shown that platinum NPs are massively accumulated in the root system of wheat seedlings at their early ontogenetic stages, if they are grown in water culture (see Table 2).

Table 1. Morphometric parameters of wheat seedlings grown in an aqueous dispersion medium or soil comprising nPt a concentration of 10 mg /l and 10 mg / kg.

Variant	Root		Aboveground part	
	Length (cm)	Weight (mg)	Height (cm)	Weight (mg)
		Water culture		
Control	6.67 ± 2.80	31±3	16.33 ± 0.85	93±2
Experiment	9.22 ± 0.72*	38±1*	17.93 ± 0,87	100±2*
		Soil culture		
Control	13.78 ± 1.98	57±1	24.4 ± 3.11	133±3
Experiment	14.12 ± 2.34	59±2	25.01 ± 2.49	145±5*

Note: the asterisk hereinafter marks significant differences of parameters as compared to the control with the confidence figure of p<0.05.

Table 2. nPt content in water and soil cultures of wheat .

Variant	nPt content (mkg/g wet weight)			
	Water culture		Soil culture	
	Root	Aboveground part	Root	Aboveground part
Control	0.0012 ± 0.0001	0.00062 ± 0.00010	0.0016 ± 0.0005	≤ 0.0001
Experiment	42.96 ± 8.69	0.44 ± 0.08	0.81 ± 1.16	0.10 ± 0.02

Water culture is an effective tool to evaluate not only the adaptative reserves of a growing organism but also the unique role played by the tillering node in the initial growth and development of plants. Located at the stem-root junction, it functions as a powerful physiological barrier to substance transport, which, along with other possible mechanisms, helps restrict the access of NPs into the vegetative part of plants at the early stage of their development. Therefore, fewer NPs entered the aerial part, yet even in this case, the differences between test and control samples were tremendous. It is noteworthy that NPs can not only migrate through xylem vessels from the root to leaves but also move in the opposite direction with the help of a phloem, which is known to transport photosynthates from leaves to where they are consumed [13].

In soil cultures, on the contrary, the nPt accumulation is much lower; nonetheless, the difference between the test and control samples of the root system was still considerable (more than 50 times). Crystalline nanostructures have much higher surface energy than macroforms, so their dissolution in the soil moisture and transition to the germination zone must be significantly easier [15]. In our research, however, the leading role in reducing NP bioaccumulation seems to belong to their adsorption by soil elements, since both the oxidative modification and dissolution are negligible for nPt in this time interval.

The other set of experiments studied the influence of the soil type on the display of biological effects of nanomaterials. We used typical soils of the Siberian region: luvisol and phaeozem.

The phaeozem had somewhat higher fertility level vs. luvisol due to a greater content of humus (6.4±0.6 vs. 5.4±0.5)%, labile phosphorus (175±35 vs.75±15) mg/kg, labile potassium (155±23 vs.100±15) mg/kg, exchangeable calcium and magnesium (20.4±1.5 vs.15.5±1.2 and 2.7±0.2 vs.4.9±0.59 respectively) mmol/100g. Both the soil types are favorable for grain farming.

Soil contamination with nPt led to a significant ($p<0.05$) increase in the content of exchangeable ammonium (from 4.5±0.7 to 9.0±1.4) mg/kg in the luvisol and (from 15.1±3.0 to 4.7±1.4) mg/kg in the phaeozem and hydrolytic acidity (from 4.9±0.59 to 6.66±0.8) mmol/100g in the luvisol and (from 5.01±0.6 to 6.8±0.8) mmol/100g in the phaeozem. At the same time, the content of nitrate nitrogen dropped (from 16.6±3.3 to 10.5±2.1) mg/kg in the luvisol and (from 15.1±3.0 to 4.7±1.4) mg/kg in the phaeozem. Thus, we can see the effect of nPt on the agrochemical properties of the soils under study and, therefore, on their fertility level.

When cultivated in the phaeozem, the control sample had a greater dry weight of its roots and aerial part as well as root length and leaf area than in the luvisol, which corresponds to the agrochemical evaluation (see Table 3).

However, although the agrochemical composition of both the soil types changed after nPt contamination, the height parameters and vegetative weight of the plants changed significantly only in the luvisol, and these are important indicators of cell division and elongation intensity.

Table 3. nPt impact on morphological parameters of 10-day wheat seedlings grown in different soils.

	Luvisols		Phaeozems	
Parameters	Control	Experiment	Control	Experiment
Leaf area (cm^2)	4.15±0.49	5.14±0.24*	6.57±0.54	5.20±0.65
Dry above-ground mass (mg)	34.5 ± 0.2	47.6±0.2*	53.5±0.0	49.1±0.4
Length of root (cm)	15.9±1.2	16.5±1.8	20.9±2.6	18.7±1.6
Dry weight of roots (mg)	25.1 ±0.2	26.2±0.0	30.2±0.2	27.2±0.3

Under nPt contamination of the luvisol, both the leaf area and the overall aerial weight of the wheat seedlings increased significantly, whereas the root system remained unchanged (see Table 3). According to the literature, the stimulating effect may be due to the increased activity of redox enzymes and photochemmical activity in chloroplasts as well as activating ATP synthesis and resynthesis as one of the major factors of metabolisn regulation [16].

The absence of pronounced effects of the phaeozem contamination may be due to NP adsorption by soil elements. As noted above, this soil retained more nanomaterial than the luvisol did, when we poured the suspension through it. This may have been caused by different content of exchangeable divalent cations promoting NP adsorption by pore walls. A more acidic reaction of the medium in the luvisol could have contributed as well, judging by the pH of the salt extract (5.1±0.1 vs. 5.3±0.1). Greater content of humus in the phaeozem could also have enhanced the protective function guarding the plant roots from NP penetration.

This fact requires a cautious attitude to evaluating the factor of NP bioaccumulation by plants from the soil. Its calculation as a ratio of the marker element concentration in a plant to that in a soil may be misleading, since the bioaccessability of NPs may be considerably lower due to adsorption and aggregation processes in the soil.

When exposed to heavy metals, plants usually accumulate secondary phenolic substances, which form complexes with heavy metals, thus inactivating them. Besides, flavonoids (vegetable polyphenols) have antioxidant properties and can neutralize free radicals produced by oxidative stress. NPs of some metals can induce active forms of oxygen, which cause significant damage to cell structures [17]. On the other hand, increasing content of flavonoids does not only indicate unfavorable processes going on but also the activation of mechanisms preventing destructive changes.

The experiments have shown that soil contamination with nPt leads to variation of flavonoids in seedlings grown in both the luvisol and phaeozem soils (see Figure 1). Especially striking is the flavonoid content increase in wheat in the luvisol: 40% after the introduction of nPt. Most probably, the growing amount of flavonoids in wheat seedlings is due to the defense mechanisms activated as a response to nPt impact. The efficiency of this mechanism reveals itself in the stable content of chlorophylls when exposed to nPt, since their amount is heavily influenced by the changes in the microelement soil composition.

The nitrogen index calculated as a ratio of chlorophylls to flavonoids was changing downwards, which indicated that the growth and development processes were intensified in the seedlings and they required additional nitrogen nutrition.

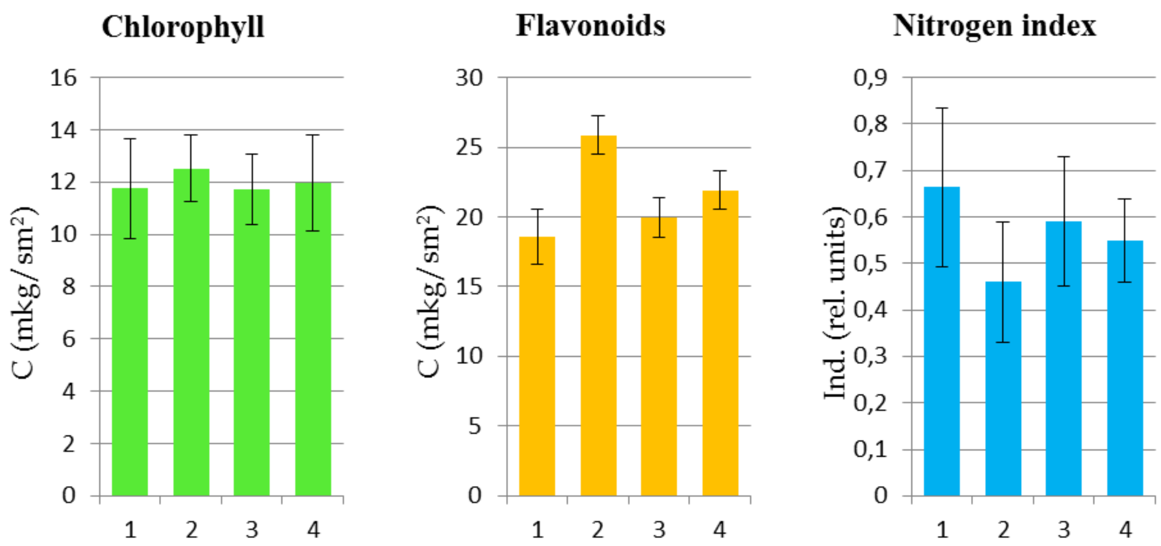

Figure 1. nPt impact on biochemical parameters of 10-day wheat seedlings grown in luvisol and phaeozem soils. Control and test samples, luvisol (1, 2); control and test samples, phaeozem (3, 4).

4. Conclusion

The research findings show that nPt penetrate plants better when they are cultivated in water media as compared to soil substrates. Pt nanoparticles change the agrochemical composition of both the luvisol and phaeozem soils. The morphological, physiological and biochemical changes in the plants under study, when introducing nPt into the cultivation medium, become less pronounced depending on the type of substrate and bioaccessibility of the nPt introduced in the following order: water suspension – luvisol – phaeozem. Here, nPt affect the height parameters and leave the chlorophyll content virtually unchanged, while activating protective mechanisms, which is confirmed by the increase in flavonoids.

When using wheat seedlings as test organisms for biotesting the environmental safety of NPs, it is advisable to use the following parameters: weight of roots, weight of aerial part, leaf area, and total flavonoid content.

Acknowledgments

The results obtained in the framework of the state task № 37.901.2014/K Ministry of education and science of Russia.

Work was conducted with the application of the Tomsk regional common use centre technical equipment acquired thanks to a grant of the Russian Ministry of the Agreement No.14.594.21.0001 (RFMEFI59414X0001).

References

[1] Adili A, Crowe S, Beaux M 2008 *Nanotoxicology* **2** 1–8
[2] Ma X, Geiser-Lee J, Deng Y and Kolmakov A *Sci. Total Environ* 2010 **408** 3053–61
[3] Salama H. *J. Biotechnology* 2012 **3** 190–7

[4] Prasad M 2003 *Plant physiology* **50** 764–80
[5] Lam C, James J, McClyskey R *Crit. Rev. Toxicology* 2006 **36** 189–217
[6] Churilov G and Ampleeva L 2010 (Rjazan: Rjazan State University Press) p 148
[7] Morgalev Yu, Astafurova T, Borovikova G, Zotikova A, Zaytseva T, Postovalova V, Verkhoturova G and Morgaleva T *Nanotechnic* 2012 **3** 81–6
[8] Zotikova A, Astafurova T, Mikhaylova C and Bender O. 2013 *Conf. Factors of plant resistance to extreme natural and man-made environment* (Tomsk) 94 –7
[9] Astafurova T, Morgalev Yu, Borovikova G, Zotikova A, Zaytseva T, Postovalova V, Verkhaturova G and Morgaleva T 2013 *Plant Physiology and Genetics* **45** 544–9
[10] Ershov B *Rus.Chen.J.* 2001 **45** 20–30
[11] Morgalev S, Morgaleva T, Morgalev Yu and Gosteva I 2015 *Adv. Mat. Res.* **1085** 424-30
[12] Svetlichnyi Vand Lapin I 2013 *Russian Physics Journal* **56** 581-7
[13] Wang Zhenyu and Xie Xiaoyan, Zhao Jian et al. 2012 *Environ. Sci. Technol*. **46** 4434–41
[14] Panitchkin L and Raikova A 2008 *Nanotechnology in agriculture* **3** 79–81
[15] Montaño M 2014 *Environmental Chemistry* **4** 351–66
[16] Davronov K, Usmanov R and Kutchkarov K 2008 *Agricultural biology* **1** 65–9
[17] Markovic Z, Todorovic-Markovic B, Kleut D, Nikolic N, Vranjes-Djuric S, Misirkic M, Vucicevic L, Janjetovic K, Isakovic A, Harhaji L, Babic-Stojic B, Dramicanin M and Trajkovic V 2007 *Biomaterials* **28** 5437–48