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The effect of inorganic nanoparticles (ZnO, Cr_2O_3, CuO and Ni) and their bulk counterparts on enzyme activities in different soils

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ABSTRACT

The objective of the study was to determine the effect of nanoparticles (NPs) and their bulk counterparts on the enzymatic activity of two soils. The study comprised analyses of the effect of four NPs (ZnO, CuO, Cr_2O_3, Ni) on the activity of dehydrogenase, urease, acidic and alkaline phosphatase in two soils with different physicochemical properties. The effect of the concentration of NPs (10, 100 and 1000 mg/kg), size of particles and contact time between NPs and soil (24 and 196 days) was studied. Depending on the enzyme and soil type tested, an inhibitory or a stimulating effect of NPs on the activity of the enzymes was observed. The absence of the dose–effect relationship made it difficult to compare the effects among the individual NPs. It could be clearly noted, however, that relatively the most frequent negative effect was observed for CuO NPs. The levels of inhibition/stimulation of the NPs varied notably also in relation to the soil type, duration of contact between NPs and the soil, and particle size (nano, bulk).

1. Introduction

The increasing production and use of nanoproducts result in the release of nanoparticles (NPs) from them, with their consequential spread in the environment (Gottschalk and Nowack, 2011). The properties of nanoparticles, that determine their applicability in various branches of industry, create a hazard for various elements of the environment and for living organisms (Klaine et al., 2008). Thanks to their "nano"-size, NPs can easily penetrate into organisms, where they can cause pathological changes already at the level of the DNA (Auffan et al., 2012). Moreover, the size of NPs causes that they are characterised by greater specific surface area, reactivity and solubility than their bulk counterparts (Nowack and Bucheli, 2007). While those "more effective" properties are desirable for the industry, in the case of environmental issues the contact of NPs with organisms may determine their greater toxicity.

The scale of production of NPs entails their unavoidable influx to various elements of the environment, including soils. NPs may get into soils as a result of intentional application for the purpose of soil remediation (e.g. nZVI), and they can also be introduced together with mineral fertilisers (like e.g. TiO_2) or organic fertilisers such as biosolids, especially sewage sludges (Ma et al., 2014). Nanoparticles can also migrate into soils with pesticides (Cu, CuO) (Gogos et al., 2012). Soil contamination with NPs may take place also as a result of the use of products containing NPs in their composition, such as vehicle tyres or fuels. Indirectly soil contamination with NPs may result from deposition of NPs from air or ground water (Pan and Xing, 2012).

In recent years the presence of NPs in the soil is more and more popular as an object of research. The studies are concerned both with the fate of NPs in soils (like e.g. speciation) and their effect on soil organisms (Du et al., 2011; Ge et al., 2012; Jośko and Oleszczuk, 2013). Studies on the effect of NPs on soil microorganisms are of particular importance. Microorganisms determine the biological status of soils, which is of fundamental importance for soil quality. Studies conducted so far on the effect of NPs on the soil microflora were focused primarily on such parameters as the species and quantitative composition of microorganisms and their biomass (Choi et al., 2008; Ge et al., 2011, 2012; Vittori Antisari et al., 2013). There have been relatively few studies on the effect of NPs on the activity of enzymes which may be indicators of the functioning of soil microorganisms. The need for such studies finds support in the biochemical and microbiological role of enzymes in soils, which makes them "sensors" of soil health (Caldwell, 2005). Dehydrogenase, urease and phosphatases are among the most frequently evaluated soil enzymes (Burns et al., 2013). Those enzymes are extremely important as they participate in microbial respiration (dehydrogenase) and in the cycle of elements such as nitrogen (urease) and phosphorus (phosphatases) (Aon and Colaneri, 2001). Dysfunction of the enzymatic activity of soils may disturb the biological equilibrium of soil, which may have ecological and economic consequences.

Research conducted so far on the effect of NPs (mainly multi-walled carbon nanotubes, nano-Ag, nano-ZnO and nano-TiO_2) on the enzymatic activity of soils has been relatively scarce and demonstrated their diverse effects (Du et al., 2011; Jin et al., 2013, 2014; Peyrot et al., 2014).

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Shrestha et al. (2013) did not observe any significant effect of multi-walled carbon nanotubes (MWCNTs) on the enzymatic activity (acid phosphatase, β-glucosidase, β-glucosaminidase, dehydrogenase) of soils even at their concentration at the level of 1000 mg/kg. In turn, studies involving the use of other NPs revealed a decrease in the enzymatic activity in the presence of single walled carbon nanotubes, nano-CuO, nano-ZnO (Kim et al., 2013) and nano-TiO_2 (Du et al., 2011). Although those studies provided a lot of valuable information, there are still many unknowns related with the problem of the effect of NPs on the enzymatic activity of soils. First of all there is no comparison of the effect of NPs on the enzymatic activity in various soils. As demonstrated earlier (Oleszczuk and Hollert, 2011), soil properties may determine significantly the toxicity of e.g. sewage sludge. Soil properties affect also the behaviour of NP (content of mineral components, natural organic matter, pH), which may determine diverse ecotoxicological effects (Dinesh et al., 2012; Jośko and Oleszczuk, 2013; Pan and Xing, 2012).

The objective of the study was the estimation of the activity of four enzymes (dehydrogenase, urease, acid and alkaline phosphatase) in two soils with different physicochemical properties under the effect of the following nanoparticles — nano-CuO, nano-ZnO, nano-Cr_2O_3 and nano-Ni and their bulk counterparts. The study included also an analysis of factors that may modify the effect of NPs on the enzymatic activity of soils, such as the concentration and aging of NPs.

2. Materials and methods

2.1. Nanoparticles

The study was conducted with the use of four NPs, containing in their composition heavy metals commonly considered as toxic: Zn, Cu, Cr, Ni. The selection of the NPs was based on their common application in consumer products (cosmetics, paints, catalysts, pesticides, batteries, dyes) (Horie et al., 2013; Nowack and Bucheli, 2007), the use of which may lead to the transfer of those compounds to the environment. Nanoparticles ZnO (nano-ZnO), CuO (nano-CuO), Cr_2O_3 (nano-Cr_2O_3), Ni (nano-Ni) and their bulk counterparts (bulk-ZnO, bulk-CuO, bulk-Cr_2O_3 and bulk-Ni) were purchased from Sigma-Aldrich (USA). The primary particle size of nanoparticles was as follows: nano-ZnO < 100 nm; nano-CuO < 50 nm; nano-Cr_2O_3 < 100 nm; nano-Ni < 100 nm. The size of nanoparticles was determined by transmission electron microscope (JEM-3010 TEM JEOL, Ltd., Japan).

2.2. Soil characterisation

Two different soils with different physico-chemical properties were selected for the presented study (Table 1): Haplic Podzol originating from sand (SL1) and Haplic Luvisol originating from silt (SL2). Soil samples were taken from the surface horizon (0–20 cm), from locations in the arable areas of south-eastern Poland (the area is not exposed to industrial and urban contamination). Fresh soil samples were mixed to obtain representative sample and were stored at 4 °C. For chemical and enzymatic analysis soils were first air-dried and then sieved through 2 mm sieve. The chemical properties of soils studied were determined by standard methods. The particle size distribution of the soils was assayed with the areometric method. The dry weight of soils was determined by drying in an oven at 105 °C. The pH was measured potentiometrically in 1 M KCl after 24 h in the liquid/soil ratio of 2.5, the total of the exchangeable bases (TEB) were determined in the 0.1 N HCl extract. The cation exchange capacity (CEC) and concentrations of P_2O_5, K_2O, Mg, Ca, and Na were determined according to "Procedures for Soil Analysis" (van Reeuwijk, 1993). Total organic carbon (TOC) was determined by TOC-VCSH (SHIMADZU) with Solid Sample Module SSM-5000. The total nitrogen (N_t) was determined by the Kjeldahl's method without the application of Devarda's alloy (Cu–Al–Zn alloy-reducer of nitrites and nitrates) (Bremner, 1996). The heavy metal total content was measured by ICP-OES (Leeman Labs (PS 950) apparatus with ICP induction in argon). The composition of organic matter was determined in accordance with Schnitzer's method (Griffith and Schnitzer, 1975). The water holding capacity (WHC) of soils were measured as stored water by percolation test.

Table 1
Physico-chemical properties of soils used in the experiment.

	SL1	SL2
Sand	61	14
Silt	36	76
Clay	3	10
pH	4.7	6.7
CEC	52.7	99.1
TEB	19.2	86.6
K_2O	1.5	2.6
P_2O_5	0.7	5.5
Mg	0.3	1.1
Na	0.2	0.8
Ca	5.5	27.9
N_t	1.1	1.4
TOC	0.6	1.2
Heavy metals		
Cd	0.4	0.6
Zn	26.2	38.9
Pb	25	30.7
Cr	11.9	21.6
Cu	–	–
Ni	6.7	11.4
Composition of organic matter		
Humic acids	11.3	25.9
Fulvic acids	2.8	15.2
Cellulose	3.7	3.8
Residual fraction	82.2	55.1

Sand, clay, silt contribution (%). pH — reactivity in KCl. CEC — cation exchange capacity (mmol/kg). TEB — the total of the exchangeable bases (mmol/kg). K_2O, P_2O_5, and Mg — available forms of phosphorous, potassium and magnesium (mg/kg). Na and Ca — content (mg/kg). N_t — total nitrogen content (g/kg). TOC — total organic carbon content (g/kg). Heavy metals content (mg/kg). The composition of organic matter-fraction (%).

The soils, used in the experiment, differed in the properties (Table 1). Soil SL1 had lower pH, CEC and TOC values than soil SL2. The N_t content was at the similar level in soils SL1 and SL2. Soils varied significantly in the nutrients content, soil SL2 characterised higher content of microelements in comparison to soil SL1. Soil SL2 contained of more heavy metals than soil SL1 (Table 1).

The soils used in the study differed also in the content of the particular fractions of organic matter; only the cellulose fraction was at a similar level in both soils. The organic matter of soil SL2 was characterised by higher levels of humic and fulvic acids (Table 1) compared to soil SL1.

2.3. Sample preparation

The NPs (ZnO, CuO, Cr_2O_3, Ni) were introduced into the two soils in the following concentrations: 10, 100 and 1000 mg/kg (experiment with the effect of NP concentration). The NPs were applied to the soils in the form of powder. To achieve homogeneous distribution of the NPs in the soils the samples were mixed (in a mixer, Rotax 6.8, VELP–OMC, ENVAG) for 1 h. Next the soil samples were hydrated with redistilled water (60% of WHC). The test material prepared as above was then incubated for 24 days in darkness, at room temperature of 22 °C ± 2 °C.

In the experiment aimed at the comparison of the effect of particle size on the enzymatic activity of soils (experiment with the comparison between NPs and their bulk counterparts), the NPs and their bulk counterparts were added to the soils at concentration of 100 mg/kg. These samples were also incubated for 24 days under identical conditions to those in the experiment described above.