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Evaluation of zinc oxide nanoparticle toxicity in sludge products applied to agricultural soil using multispecies soil systems

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HIGHLIGHTS

- Reductions on fresh biomass for plants were detected at the lowest application doses.
- Bioconcentration factors of Cd in the earthworm exposed to ZnO nanoparticles were >1.
- Soil phosphatase enzymatic activity decreased significantly in amended soils.
- Oxidative stress and redox status changes were detected in the RTG-2 fish cells.

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ABSTRACT

To study the environmental impact of nanoparticles, the sludges of wastewater (WWTS) and water treatment (WTS) plants enriched with ZnO nanoparticles were added to agricultural soil, and the toxic effects of the nanoparticles were studied using a microcosm system based on the soil. The WWTS treated soils were characterised by statistically significant decreases ($p < 0.05$) in *Vicia sativa* germination at the lowest (76.2%) and medium (95.2%) application rates, decreases in the fresh biomass for *Triticum aestivum* (19.5%), *Raphanus sativus* (64.1%), *V. sativa* (37.4%) and *Eisenia fetida* (33.6%) at the highest application rate and a dose-related significant increase ($p < 0.05$) in earthworm mortality. In WTS amended soils, significant reductions ($p < 0.05$) of the fresh biomass (17.2%) and the chlorophyll index (24.4%) for *T. aestivum* and the fresh biomass for *R. sativus* (31.4%) were only recorded at the highest application doses. In addition, the soil phosphatase enzymatic activity decreased significantly ($p < 0.05$) in both WWTS (dose related) and WTS treatments. For water organisms, a slight inhibition of the growth of *Chlorella vulgaris* was observed (WWTS treated soils), along with statistically significant dose-related inhibition responses on total glutathione cell content, and statistically significant dose-related induction responses on the glutathione S-transferase enzyme activity and the reactive oxygen species generation on the RTG-2 fish cell line.

1. Introduction

Nanotechnology-based products will constitute a $ 1 trillion market by the year 2015 (Kumar et al., 2012). The significant proliferation of nanotechnologies and their utilisation in large quantities may lead to a significant release of nanoparticles into the environment, which raises environmental interest regarding the unfavourable ecological effects of nanoparticles. Nanoparticle toxicity research indicated that substances previously considered biologically inactive may become toxic in their nanoparticulate forms, due to their greater reactivity and possibly elevated cellular uptake (Chithrani et al., 2006; Farré et al., 2009).

Because of the current lack of experimental data, the disposal of nanomaterials is not regulated. Application of sludge products to agricultural soils has many advantages, e.g., improving fertility by supplementing nutrients or improving the composition of the soil by adding organic matter. However, the use of heavy-metal polluted sludge can result in potentially toxic metal contents in soils and metal transference into freshwater and soil organisms. Water treatment plants occupy an intermediate position that limits the flux of nanoparticles from the anthropogenic environment to the ecological environment (Gottschalk et al., 2009). In the course of the sludge degradation procedure, nanoparticles could be combined with the sludge matrix and could be concentrated over time (Benn and Westerhoff, 2008). Insufficient information exists regarding the capability of removing nanoparticles in water treatment plants. Luo et al. (2014) and Sun et al. (2014) speculated that the regular water treatment procedure appears to be poorly adapted to the acquisition of nanoparticles. Whenever a sludge substance re-enters the scene by means of agronomical land use, pollutants in sludge can have effects not only on the populations of soil organisms, but also on aquatic populations (Parkpian

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et al., 2002). Consequently, the environmental effects of nanoparticles must be understood to address their effects when they re-enter the environment, e.g., via agronomic land application. The ecotoxicological estimations of the hazards of heterogeneous samples of amended soils suggest that a means of mitigating the exposure route should be developed, and that research of the hazards for a diversity of life organisms should be performed. In this respect, ecotoxicological tests establish an important option to the chemical analyses (Natal-da-luz et al., 2009; Oleszczuk, 2010; Ramirez et al., 2008). Ecotoxicological analyses of sludge provide knowledge not only about the appearance of the pollutants but also about the interactions between the pollutants present in the sludge (Domene et al., 2011). Regarding the proper function of soil microbial ecology, ground-dwelling invertebrates, particularly earthworms, and terrestrial plants are considered the most important organisms from the environmental and agronomical points of view. All of these receptors of contaminants can be assayed in a single experimental model using a multispecies soil system microcosm (MS-3) (Alonso et al., 2006; Fernández et al., 2004; Fernández et al., 2005), which is a suitable experimental design for studying the fate and effects of contaminants under conditions that resemble arable soils (Carbonell et al., 2009). To date, the estimation of the toxicity of sludges polluted with nanoparticles regarding their application as fertilisers, especially covering an extensive group of soil and water organisms has not been performed. We choose for this study zinc oxide nanoparticles (nZnO), because zinc oxide nanomaterials are the most used in terms of commercial production amounts and represent one of the main sources of nanoparticles introduced into consumer products available on the market (Lovestam et al., 2010). No field-scale studies about the environmental impact of nZnO and their characterisation in environmental matrices are available to date.

In this paper, we study the fate of soil organisms and the toxicological effects on soil organisms associated with the use of sludge products (wastewater treatment sludge and water treatment sludge) contaminated with nZnO in soil, as well as the possible leaching of nanoparticles through the soil column.

2. Material & methods

2.1. Chemical properties of soil, sludges and nanoparticles

A typical agricultural soil (S) was collected from the top 5–10 cm layer of an untreated field where pesticides and fertilisers had not been applied for at least the last 10 years. The soil was air dried, sieved (2 mm mesh), homogenised and preserved at room temperature until use.

One sample of wastewater treatment sludge (wastewater sanitation) (WWTS) and one sample of water treatment sludge (drinking water sanitation) (WTS), were supplied from two municipal water treatment plants located in the north of Spain. The WWTS treatment was performed by thermophilic anaerobic stabilisation; the WTS treatment was performed by a conventional process using primary and secondary disinfections by the addition of Na hypochlorite. Table 1 presents the physico-chemical properties of both the soil and the amendments used in this study.

The electrical conductivity (EC) and pH were determined in the soil and sludge samples at 0 (beginning) and 21 (end) days of the experiment using a conductivity meter (Crison, Barcelona, Spain) and a pH-meter (Orion 520A, Boston, MA, USA), respectively.

Zinc oxide nanopowder (<100 nm) was obtained from Sigma-Aldrich (Germany) with a nominal primary particle size of less than 100 nm (i.e., $r_p \leq 50$ nm). The primary particle size and shape analyses were performed by transmission electron microscopy (TEM). The particle size distribution in 1 mg/ml water nZnO stock solution, after a 20-min dispersion using a homogeniser, was determined using a Nano-Zeta Sizer. TEM images indicated that nZnO sizes were between 20 nm and 100 nm (58.4 nm mean size) in diameter. Using DLS, the average hydrodynamic diameter was 167 nm (98.3% intensity) (results not shown). The zeta-potential of the nZnO in deionized water was −13.7 mV.

2.2. Multispecies soil system experimental protocol

The multispecies soil system MS-3 is composed of columns of strained soil containing plants and invertebrate organisms. The system permits a realistic integration of the chemicals that is similar to the ordinary agricultural usage for the inclusion of sludge. A mechanism for collecting the leachate (funnel and bottle) was added to the system. Columns were irrigated 5 days a week with 50 ml of dechlorinated water simulating 1000 mm rainfall/year, allowing the soils to drain to field capacity. Leachates were collected during 21 days, in association with watering events. Successive leachate fractions of each microcosm were mixed and kept refrigerated at 4 °C for further chemical analysis and used in toxicity tests with aquatic organisms.

It has been suggested that test organisms should be exposed to nanoparticles in an environmentally relevant way (Crane et al., 2008).

Table 1
Physical and chemical characteristics of the reference soil and the sludges used in this study. The ratio of soil to water (w:v) used in the measurements is included in parentheses.

Parameter	Unit	Soil (S)	Wastewater treatment sludge (WWTS)	Water treatment sludge (WTS)
pH (1:2.5)		7.8	7.0	8.2
EC (1:5)	(mS/cm)	0.39	1.67	0.19
Organic matter	%	ND	28.29	61.72
Oxidizable organic C	%	1.94	20.32	7.12
Total P	%	ND	1.02	0.59
Total K	%	ND	1.13	0.62
Total N	%	ND	3.09	0.76
N–NH3	(mg/kg)	ND	0.57	0.08
Cd	(mg/kg)	0.22	4.69	2.82
Cr	(mg/kg)	13.94	88.95	54.82
Cu	(mg/kg)	27.51	384.07	130.57
Ni	(mg/kg)	3.33	3.53	3.82
Pb	(mg/kg)	31.25	107.74	102.79
Zn	(mg/kg)	53.69	868.52	110.99
Texture				
Sand	%	73.4	ND	ND
Silt	%	18.8	ND	ND
Clay	%	7.8	ND	ND

ND, not determined.