Ecotoxicological effects of the application to soil of sewage sludge contaminated with ZnO nanoparticles

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Introduction

Zinc oxide nanoparticles (ZnO-NPs) are widespread and are increasingly applied in various commercial products, such as personal care products or pharmaceuticals. These products go to sewage waters and during wastewater treatment, nanoparticles may be integrated into the sewage sludge matrix. Consequently, land application of these residues may be an important pathway of ZnO-NPs into soils, with consequences to terrestrial and aquatic species.

Because the properties of the nanomaterial differ from those associated with bulk material, the potential toxicological effects of these products must be determined. Data concerning the potential impact of these NPs in the environment is still emerging. Specially, data on the ecotoxicity of metal oxide NPs remain scarce.

Ecotoxicological assessment of sewage sludges contaminated with NPs may provide a significant complement to chemical studies and may also permit the estimation of toxicity resulting from interactions among NPs and the particular contaminants present in the sludges. The present work investigates the effects on soil and aquatic organisms of ZnO-NPs contaminated sewage sludge, using a soil microcosm system, known as “Multispecies Soil System” (MS-3) [1].

Materials and Methods

Control soil, sludges and nanoparticles

Soil was collected from a superficial layer of a field located near Madrid (Spain). Soil was air dried and sieved (2-mm mesh). Main physicochemical characteristics of this soil were: clay 7.8%, silt 18.8%, sand 73.4 %; pH 7.27 and organic C 1.09%.

Sewage sludge samples (SS) were supplied from two municipal waste water treatment plants located in the north of Spain. Their characteristics were:

SS1: pH, 7.0; electric conductivity (EC), 1669 μS cm^{-1}; total organic matter (TOM), 28.29%; oxidizable organic matter (OOM), 20.43%; total nitrogen (TN), 3.09 %; ammonia-nitrogen (NH_4^+), 0.57%; Phosphorous (P_2O_5), 1.02%; potassium (K_2O), 1.13%; Zn, 868 mg kg^{-1} d.w. SS2: pH, 8.2; EC 187 μS cm^{-1}; TOM, 61.72%; OOM, 7.12%; TN, 0.76%; ammonia-nitrogen NH_4^+, 0.08%; P_2O_5, 0.59%; K_2O, 0.62%; Zn, 110.99 mg kg^{-1} d.w.

ZnO-NPs (<100 nm) were obtained from Sigma-Aldrich (Germany) with a nominal primary particle size of less than 100 nm (i.e., rp≤50 nm).

Treatments

Sludges were spiked with ZnO-NPs (nominal range 2500-20000 mg ZnO-NPs kg^{-1} sludge), thoroughly mixed to obtain homogeneity, and were then equilibrated for 7 d at 70% humidity. These sludges were mixed with the soil at a 5% w/w ratio to have 125, 250, 500, and 1000 mg Zn kg^{-1} soil.

Ecotoxicity assays

Sludge amended soils were assessed on the Multispecies Soil System MS-3 [1]. Briefly, samples were placed in 15 cm height x 15 cm diameter methacrylate columns and ten adult *Eisenia fetida* (Oligochaeta: Lumbricidae) were added on day 0 to each soil microcosm. Seven seeds of three plant species (i.e. wheat, *Triticum aestivum*; radish, *Rabanus sativus* and vetch, *Vicia sativa*) were sown on to the soil in each microcosm. Three replicates for each treatment were carried out. Columns were incubated in a climate room at controlled temperature (20±2ºC) and illumination (16:8 h; 3000-4000 lux). Columns were watered 5 days a week with 50 ml of dechlorinated water and the leachates were collected. After 21 days, MS-3 columns were opened and toxicity tests were performed to assess the effects of amended soils and their leachates to soil (earthworms, microorganisms and plants) and aquatic organisms (algae and Daphnia), respectively. Procedures were accomplished following principles of standardized methods (OECD). SPAD (soil-plant analysis development) index was measured to estimate the chlorophyll state in plants using a Minolta Chlorophyll Meter SPAD-502.

Enzymatic activities on microorganisms, earthworms and plants

In microorganisms dehydrogenase [2] and acidic phosphatase[3] activity were measured in soils.

In whole worms and plant leaves the following enzymatic activities were determined: Catalase (CAT) [4], Glutathione-S-transferase (GST) [5], Superoxide Dismutase (SOD) [6], Ascorbato peroxidasa (APX) [7], and Guaiacol peroxidase (POD) [8]. Total protein [9] and L-Glutathione reduced (GSH) contents [10] were also measured.

Results

The toxicity of ZnO-NPs to soil and aquatic organisms was influenced by the sludge´s chemical characteristics. In general, the toxicity of soil amended with SS1 spiked with ZnO-NPs was higher than the observed in soil amended with SS2 spiked with same concentration of NPs.

SS1-soil caused a 43±4% of mortality on earthworms exposed to the highest dose (1000 mg kg^{-1} soil d.w.) whereas effects on weight were not detected. Regarding biochemical parameters, the only significant effect was a enhancement of GSH content from 1.9±0.1 in untreated SS1-soil to 2.3±0.1 µmol g^{-1} worm in SS1-soil spiked with 1000 mg Zn kg^{-1} soil. By contrast, in SS2-soil no effects were observed in any lethal or sublethal parameters at all doses tested.

ZnO-NPs did not affect seedling emergence. However, samples exposed to treated SS1-soil exhibited a inhibition of growth. Radish was the most sensible species showing a dose dependent decrease of wet weight with inhibitions of 47±10 and 54±12% at 500 and 1000 mg Zn kg^{-1} soil, respectively. SS2-soil did not produce adverse effects in seedling germination or plant growth in any of the three plant species. Effects on chlorophyll content measured as SPAD index were observed only for wheat exposed to ZnO-NPs in both sludges. Effects on enzymatic activities, and GSH and protein content varied between plant species and sludges (See figure).

For microorganism inhibition of carbon mineralization (34±3%) and dehydrogenase activity (56±6%) of ZnO-NPs were observed only for SS1-soils, at 1000 mg Zn kg^{-1} soil. However, both sludges inhibited acidic phosphatase activity in a dose dependent manner with an EC50=984(702-2244) for SS1-soil and EC20=817(631-1135) mg Zn kg^{-1} soil d.w. for SS2-soil.

For aquatic organisms, leachates from both sludges-soil mixes did not produce effect on algae growth. However, inhibition of daphnia mortality was observed for ZnO-NPs spiked SS1-soil with EC50=477(416-559) mg Zn kg^{-1} soil d.w.

In summary, the addition to soil of sewage sludges contaminated with ZnO-NPs can cause effects in soil and aquatic organisms which depend on the physicochemical characteristics of the sludges. These findings are important with respect to the consideration of the environmental risk of routine additions of sewage sludge amendments to soil. However, the levels of ZnO-NPs causing toxicity were very elevated compared to expected sludge concentrations of NPs and consequently, immediate effects of sewage sludges for ecosystems should not be expected.

Acknowledgments

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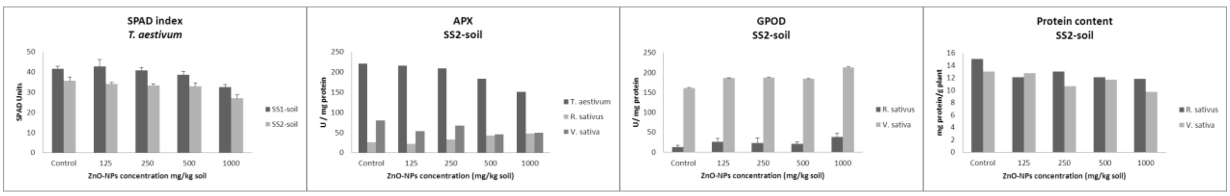
References

[1] Fernández, M.D. Cagigal, E. Vega, M.M. Urzelai, A. et al. Environ. Ecotox. Saf. 62 (2005) 174-184.
[2] Carbonell G, Pablos MV, Garcia P, Ramos C, Sanchez P, et al.Sci Total Environ 247 (2000) 143-50.
[3] Freeman C, Liska G, Ostle NJ, Jones SE, Lock MA. Plant Soil 175 (1995) 147-52.
[4] Aebi H. In Methods in enzymology. Vol 105. Ed. Academic Press, Inc. New York 1984.
[5] Habig W., Pabst M., Jakoby W. The Journal of Biological Chemistry vol. 249, 22 (1974) 7130-9.
[6] Peskin AV, Winterbourn CC. Clinica Chimica Acta 293 (2000) 157-66.
[7] Gupta, AM., Ahmad M. Toxicol Environ Chem, 93 (2011) 1166-1179.
[8] El-Shabrawi, H, Kumar B, Kaul, T, Reddy, MK, et al. Protoplasma, 245 (2010) 85–96.
[9] Bradford MM. Analytical Biochemistry 72 (1976) 248-54.
[10] Hissin PJ, Hilf RA Anal Biochem 74 (1976) 214–226.

Figures



Effects on plants of sewage sludges contaminated with ZnO-NPs (only parameters significantly differences from control at p<0.05 by the LSD procedure are shown).