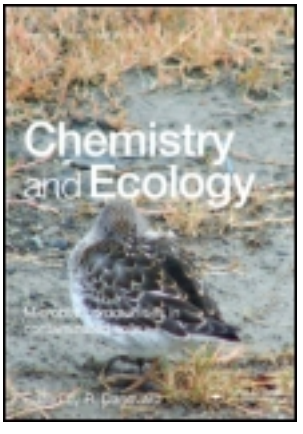

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gche20

Effects of Zn and ZnO nanoparticles and Zn^{2+} on soil enzyme activity and bioaccumulation of Zn in *Cucumis sativus*

Sunghyun Kim [a], Jungeun Kim [b] & Insook Lee [c]

[a] School of Civil and Environmental Engineering, Yonsei University, Seoul, South Korea

[b] Division of Life Science, Ewha Womans University, Seoul, South Korea

[c] Division of EcoScience, Ewha Womans University, Seoul, South Korea

Available online: 16 Feb 2011

To cite this article: Sunghyun Kim, Jungeun Kim & Insook Lee (2011): Effects of Zn and ZnO nanoparticles and Zn^{2+} on soil enzyme activity and bioaccumulation of Zn in *Cucumis sativus*, Chemistry and Ecology, 27:1, 49-55

To link to this article: http://dx.doi.org/10.1080/02757540.2010.529074

Chemistry and Ecology
Vol. 27, No. 1, February 2011, 49–55

Taylor & Francis
Taylor & Francis Group

Effects of Zn and ZnO nanoparticles and Zn^{2+} on soil enzyme activity and bioaccumulation of Zn in *Cucumis sativus*

Sunghyun Kim[a], Jungeun Kim[b] and Insook Lee[c]*

[a]*School of Civil and Environmental Engineering, Yonsei University, Seoul, South Korea;* [b]*Division of Life Science, Ewha Womans University, Seoul, South Korea;* [c]*Division of EcoScience, Ewha Womans University, Seoul, South Korea*

(*Received 26 April 2010; final version received 13 September 2010*)

The increased production and commercial use of nanoparticles (NPs), combined with a lack of regulation regarding their disposal, may result in the unwanted introduction of NPs to soils. In this study, the toxicity on soil enzyme activity and growth of *Cucumis sativus* treated with Zn or ZnO NPs was evaluated in pot soils. Specifically, *C. sativus* was cultivated in soils treated with Zn NPs, ZnO NPs or Zn^{2+} for eight weeks, after which the treatment effects on biomass and bioaccumulation were evaluated. In addition, the treatment effects on soil dehydrogenase, β-glucosidase and acid phosphatase were investigated. Soil enzyme activities were influenced by all treatments, with an especially large decrease in dehydrogenase activity in response to Zn^{2+} treatment. Biomass and root length also decreased in response to Zn^{2+} treatment. Finally, the Zn contents of *C. sativus* were much lower in the Zn NP and ZnO NP treatment groups than in the Zn^{2+} treatment group. Therefore, toxicity on soil microbial activity may have a greater influence than phytotoxicity due to immobilisation and aggregation of NPs in the soil.

Keywords: bioaccumulation; *Cucumis sativus*; soil enzyme activity; Zn nanoparticles; ZnO nanoparticles

1. Introduction

Nanoparticles (NPs) are introduced into the soil as a result of a number of human activities; however, the ecotoxicological properties and risks associated with NPs have not yet been fully characterised [1]. NPs with their high surface/volume ratio are highly reactive and have increased catalytic properties, which increases their potential to become toxic compared with their bulk counterparts [2,3]. In addition, ultrafine particles may be toxic and produce enhanced inflammatory responses compared with larger particles of the same chemical composition [4,5]. This observation indicates that NPs will have a longer residence time in the environment and may more easily damage plants, microorganisms and humans [2].

Zn NPs are one of the most commonly used and widely applied types of nanomaterials. These compounds have been shown to have high anticorrosion properties [6]. In addition, they are

*Corresponding author. Email: islee@ewha.ac.kr

ISSN 0275-7540 print/ISSN 1029-0370 online

DOI: 10.1080/02757540.2010.529074
http://www.informaworld.com

known to have antibiotic properties, which has encouraged the pharmacological preparation of these compounds as well as assessment of their potential use in pharmaceuticals and cosmetology [2]. Moreover, ZnO NPs are now used in personal care products such as sunscreens, as well as in coatings and paints due to their high levels of UV absorption efficiency and transparency [7]. However, there is growing concern in the scientific community that the desirable technological characteristics of NPs may be offset by increased health and environmental risks, based on the increased potential for exposure as a result of their increased use [8].

To date, very little data exist regarding the toxicity of NPs on the soil microbial community. Enzyme activities, as excellent indicators of soil microbial function and of the components involved in nutrient cycling [9], have been applied in many studies to assess soil quality and management [10,11]. Soil acid phosphatase is an important enzyme involved in the catalysis of organic phosphatase esters into inorganic phosphate, which is important in phosphorus mineralisation and processing [12,13]. β-Glucosidase is an important factor in the first step of the transformation of complex forms of carbon [12,13]. Finally, dehydrogenase activity is commonly assessed as a general indicator of the oxidative capacity of soil microorganisms [14].

Phytotoxicity of NPs against higher plants has been reported. Specifically, Yang and Watts [15] reported that Al nanoparticles (13 nm) are potent root toxicants that inhibit the growth of the plant roots (corn, cucumber, soybean, cabbage and carrot) in soil systems. Lin and Xing [7] investigated the phytotoxicity of five types of NPs on the seed germination and root growth of six higher plant species. Moreover, Lin and Xing [16] reported that ZnO NPs greatly adhere to the root surface of rye grass seedlings, inhibiting germination and root growth. Lee et al. [17] found that Cu NPs dispersed in plant agar media are toxic to *Tritium aestivum* and *Phaseolus radiatus*, and were also bioavailable. Finally, TiO_2 NPs have been shown to have a positive effect on the growth of spinach [18,19].

Although the toxicity of NPs has been evaluated for a variety of application routes, their effects on phytotoxicity and microbial enzyme activities in soil ecosystems are still unknown. Therefore, the objectives of study were to investigate phytotoxicity and bioaccmulation in *Cucumis sativus* using Zn, ZnO NPs and Zn^{2+}. In this study, soil pot experiments were used to evaluate the effects of toxicity and accumulation of Zn and ZnO NPs toward *C. sativus* as well as soil enzyme activity.

2. Materials and methods

2.1. *Characterisation of Zn and ZnO Nanoparticles*

All chemicals and reagents used in this study were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA), stored according to the vendor's instructions and used as received. The size of the Zn and ZnO NPs was 50 nm. The surface area of the Zn NPs was 35–50 $m^2 \cdot g^{-1}$. $ZnCl_2$ was also purchased from Sigma Aldrich Co. and used as a reference. Transmission electron microscopy (TEM; LIBRA 120, Carl Zeiss, Germany) was used to visualise the particle size and shape in samples of NP suspensions prepared in an identical manner to that outlined for DLS. Samples were prepared by depositing one drop (6 μL) of the suspensions onto a carbon-coated copper specimen grid, followed by evaporation of the water under a laminar flow hood. TEM samples from aqueous medium blanks (no nanoparticles added) were included as controls.

The primary sizes of the particles were estimated from the TEM images (Figure 1). The Zn and ZnO NPs aggregated to a high extent, although individual particles with a nearly spherical shape were also observed. The size of the Zn and ZnO NPs was 50 nm.

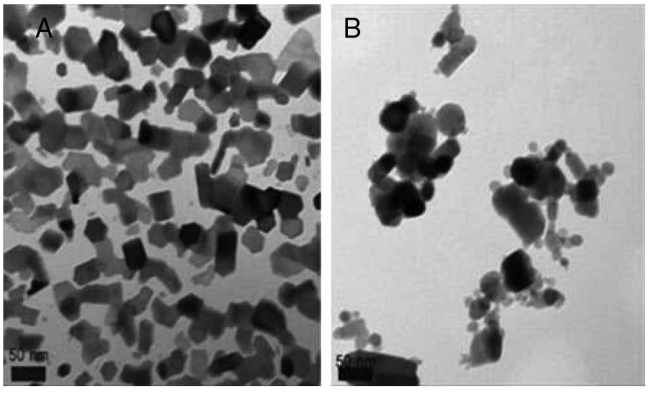

Figure 1. TEM analysis of NPs for primary size determination. Bars show the scale in nanometers (A, Zn NPs; B, ZnO NPs).

2.2. *Soil and experimental design*

Natural soil was collected from the campus of Ewha Womans University, Seoul, Korea. The physicochemical properties of the soil were as follows: soil texture, loamy sand (54.1% sand, 30.9% silt and 15.0% clay); total organic matter, 4%; total moisture content, 3%; cation-exchange capacity (CEC), 10.2 mmol·kg^{-1}; dehydrogenase activity, 27 μg^{-1}; pH, 5.5. The soil was passed through a 2-mm sieve and then air-dried. For each treatment, 27-kg soil aliquots were artificially contaminated with 2000 mg·kg^{-1} of Zn NPs, Zn oxide (ZnO) NPs and $ZnCl_2$. Soil was divided into three groups, a total of 27 pots, each of which included nine 1.5-L cylindrical plastic pots with diameters of 10 cm. *C. sativus* was selected as the model plant due to its common usage in phytotoxicity experiments by the U.S. Environmental Protection Agency [20] and its germination rate of >90%. Seeds of *C. sativus* were sown directly, after which the seedlings were thinned to a density of three per pot. The pots were then placed in a growth room controlled at 25 °C and subjected to a 16:8 h light/dark cycle. After 8 weeks, the plants were carefully harvested.

2.3. *Determination of soil enzyme activities*

The activities of β-glucosidase and acid phosphatase were measured using the MUF–substrate method [21]. The concentrations of the methylumbelliferone (MUF)–β-glucoside solutions were 400 μM (Sigma), whereas the concentration of the MUF–phosphate substrate solution was 800 μM (Sigma). The enzyme activities in soil and the substrate solution (1:5 w/v) were measured using a spectrophotometer. Dehydrogenase activity was measured by 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride (INT) assay [22]. Briefly, mixtures of soil (3 g fresh soil) and substrate solution were incubated for 24 h at 37 °C, after which the reaction products were detected based on the absorbance level measured at 485 nm using a spectrophotometer (DR/3000 Spectrophotometer, HACH).

2.4. *Determination of growth and Zn concentration*

The plants were gently harvested from the soil and then washed with water to remove soil deposits, after which the shoot and root lengths were measured. To determine the amount of Zn in the plant, roots and shoots were separated and oven-dried at 70 °C for 48 h. Plant samples were then digested in concentrated HNO_3 in a microwave digester (MDS-2000, CEM). Next, the Zn contents

were determined using an AAS (analysis 100, Perkin–Elmer) calibrated using certified reference materials (No. 10-c Rice Flour; National Institute for Environmental Studies in Japan).

To determine the total Zn concentration, 0.5 g of soil was extracted with 2.4 mL of aqua regia (35% HCl 1.8 mL + 65% HNO_3 0.6 mL) in an automatic microwave digester (MDS-2000, CEM). The extracts were then analysed using an atomic absorption spectrophotometer (AAS analysis 100, Perkin Elmer) calibrated using certified reference materials (MESS-2 Marine Sediment; National Research Council of Canada).

2.5. *Statistical analysis*

One-way analysis of variance (ANOVA) was used to determine differences in Zn accumulation and seedling growth among treatments. The data were further analysed by Tukey's post-hoc test when necessary. All analyses were conducted using SPSS 9.0 software.

3. Results

3.1. *Soil enzyme activities*

The enzyme activities of dehydrogenase, phosphatase and β-glucosidase in soil samples treated with Zn, ZnO NPs and Zn^{2+} were measured (Table 1). The activity of each enzyme decreased in response to all treatments when compared to control. The effects on β-glucosidase were similar in response to treatment with Zn NPs, ZnO NPs and Zn^{2+} treatment, whereas the greatest reduction in dehydrogenase activity was observed in response to Zn^{2+} treatment.

3.2. *Toxicity of Zn and ZnO NPs on* C. sativus

The effects of Zn and ZnO NPs or Zn^{2+} treatment on the *C. sativus* shoot length, root length and biomass are shown in Table 2. Root elongation and biomass were inhibited only by Zn^{2+}

Table 1. Soil enzyme activity following treatment with Zn nanoparticles (NPs), ZnO NPs or Zn^{2+}.

	Dehydrogenase	Acid phosphatise ($\mu g \cdot g^{-1}$)	β-Glucosidase
Control	220.0 ± 36.0^{a}	6836.6 ± 949^{a}	1013.5 ± 161^{a}
Zn NPs	183.2 ± 21.4^{a}	4658.3 ± 382^{b}	762.6 ± 48^{b}
ZnO NPs	150.0 ± 9.4^{b}	5710.5 ± 671^{c}	835.0 ± 77^{b}
Zn^{2+}	46.4 ± 10.2^{c}	3801.3 ± 788^{d}	850.0 ± 58^{b}

Notes: Values are the means $\pm$ SD ($n = 9$); values within a row or a column followed by the same letter do not differ significantly at the 0.05 level according to Tukey's test.

Table 2. Effects of Zn nanoparticles (NPs), ZnO NPs or Zn^{2+}on shoot length, root length and biomass of *C. sativus*.

Treatment	Root length (cm)	Shoot length (cm)	Biomass (g dw)
Control	10.4 ± 1.5^{a}	3.8 ± 0.6^{a}	0.22 ± 0.04^{a}
Zn NPs	10.4 ± 1.5^{a}	4.2 ± 0.5^{a}	0.23 ± 0.06^{a}
ZnO NPs	9.1 ± 1.9^{a}	4.0 ± 0.4^{a}	0.23 ± 0.05^{a}
Zn^{2+}	5.7 ± 2.6^{b}	3.8 ± 0.6^{a}	0.08 ± 0.03^{b}

Notes: Values are given as means $\pm$ SD ($n = 9$); values within a row or column followed by the same letter do not differ significantly at the 0.01 level according to Tukey's test.

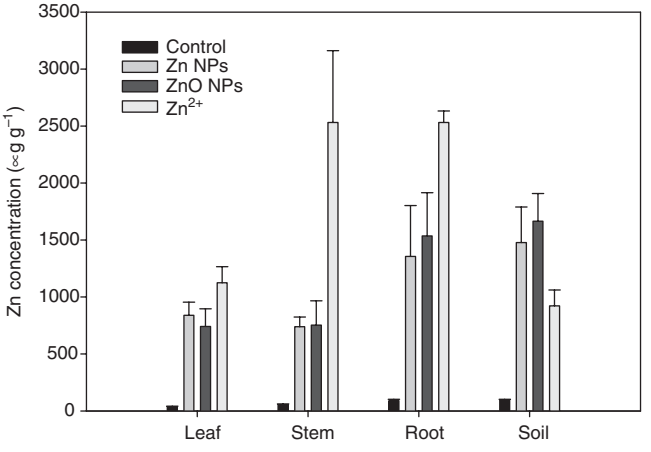

Figure 2. Zn concentration in *C. sativus* in different parts and soil following treatment with Zn NPs, ZnO NPs and Zn^{2+} (2000 mg·kg^{-1}).

treatment compared to control. Specifically, the shoots became yellow in the presence of only 2000 mg·kg^{-1} of Zn^{2+}, and the seedlings almost died in response to 2000 mg·L^{-1}.

3.3. *Bioaccumulation of Zn in* C. sativus *and total Zn concentration in soil*

Figure 2 shows the total Zn contents in *C. sativus* and soil in response to treatment with Zn and ZnO NPs and Zn^{2+}. There was no significant difference in the amount of Zn in plants or soil between the Zn and ZnO NP treatments. The concentration of Zn in plant organs increased in response to Zn^{2+} treatment, whereas the soil Zn concentration decreased. These results suggest that the concentrations of Zn in *C. sativus* and soil were highly correlated with the presence of Zn^{2+}.

4. Discussion

Early assessment of the impact of NPs on soils should be conducted before significant releases occur [23]. The measurement of enzyme activities can provide a relatively inexpensive and rapid assessment of environmental samples [24]. In this experiment, the enzyme activities of dehydrogenase, phosphatase and β-glucosidase were measured in soils treated with Zn, ZnO NPs or Zn^{2+}. Our results show that enzymes were decreased in activity in response to all treatments. Assessment of the enzyme activity of dehydrogenase, an indicator of the oxidase capacity of soil microorganisms, was most sensitive to Zn^{2+}. β-Glucosidase activity was not significantly changed in response to treatment with Zn NPs, ZnO NPs or Zn^{2+}. Reduction in enzyme activity is the expected response to an acutely toxic chemical.

The concentration of Zn^{2+} was significantly decreased in the root as well as in the overall biomass, although Zn and ZnO NPs had no effect on plant biomass. The reason the NPs had no effect could be due to their immobilisation in the soil [25]. The transport of NP colloids to the soil indicates that the surface coatings of the NPs are important in determining the characteristics and solid phase of the soil [26]. The toxicity of Zn is related to its solubility because the aggregation of NPs may influence bioavailibility [27].

The bioaccumulation of Zn and ZnO NPs was generally correlated with the size of the particles. Nanoparticles greatly adhered to the root surface and made it difficult to uptake Zn. The literature

regarding the accumulation of NPs is very limited, so our results may prove useful in predicting the bioaccumulation of NPs [7].

Although NPs have been reported to have negative and positive phytotoxic effects on higher plants, there is very little literature regarding the toxicity of NPs on soil organisms. NPs can adhere to the surfaces of root cells or enter the root cell membrane, depending on the identity of the metal NPs, the uptake of ions, particle aggregation and the presence of hydrophobic NPs [7]. Therefore, further research is needed to better understand the ecological effects of NPs and to determine whether exposure of agricultural soil to various NPs would have any long- or short-term effects on the natural environment.

Acknowledgements

This work was supported by a National Research Foundation of Korea Grant funded by the Korean Government (2009-0073454), URP 2010 from the Korea Foundation for the Advancement of Science and Creativity (KOFAC, 2010-10-043).

References

[1] R. Cai, G.M. Van, P.K. Aw, and K. Itoh, *Solar-driven self-cleaning coating for a painted surface*, Chemosphere 9 (2006), pp. 829–835.
[2] A. Nel, T. Xia, L. Moedler, and N. Li, *Toxic potential of materials at nanolevel*, Science 311 (2006), pp. 622–627.
[3] G. Oberdorster, E. Oberdorster, and J. Oberdorster, *Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles*, Environ. Health Perspect. 113 (2005), pp. 823–839.
[4] B. Mowack and T.D. Bucheli, *Occurrence, behavior and effects of nanoparticles in the environment*, Environ. Pollut. 150 (2007), pp. 5–22.
[5] P. Oleszczuk, B. Pan, and B. Xing, *Adsorption and desorption of oxytetracycline and carbamazepine by multiwalled carbon nanotubes*, Environ. Sci. Technol. 43 (2009), pp. 9167–9173.
[6] M. Heinlaan, A. Ivask, I. Blinova, H. Dubourguier, and A. Kahru, *Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria* Vilbrio fischeri *and crustaceans* Daphnia magna *and* Thamnocephalus platyurus, Chemosphere 71 (2008), pp. 1308–1316.
[7] D. Lin and B. Xing, *Phytotoxicity of nanoparticles: inhibition of seed germination and root growth*, Environ. Pollut. 150 (2007), pp. 243–250.
[8] N. Serpone, D. Dondi, and A. Albini, *Inorganic and organic UV filters: their role and efficacy in sunscreens and suncare products*, Inorg. Chim. Acta 360 (2007), pp. 794–802.
[9] W. Shi, J. Becker, M. Bischoff, R. F. Turco, and A.E. Konopka, *Association of microbial community composition and activity with lead, chromium, and hydrocarbon contamination*, App. Environ. Microbiol. 68 (2002), pp. 3859–3866.
[10] R.P. Dick, D.P. Breakwell, and R.F. Turco, *Methods for Assessing Soil Quality*, Soil Science Society American, Madison, WI, 1996, pp. 247–271.
[11] L.V. Verchot and T. Borelli, *Application of* para-*nitrophenol (pNP) enzyme assays in degraded tropical soils*, Soil Biol. Biochem. 37 (2005), pp. 625–633.
[12] O.N. Belyaeva, R.J. Haynes, and O.A. Birukova, *Barely yield and soil microbial and enzyme activities as affected by contamination of two soils with lead, zinc, or copper*, Biol. Fertil. Soils 41 (2005), pp. 85–94.
[13] R. Margesin, B.A. Zimmer, and F. Schinner, *Monitoring of bioremediation by soil biological activities*, Chemosphere 40 (2000), pp. 339–346.
[14] P. Sukul, *Enzymatic activities and microbial biomass in soil as influenced by metalaxyl residues*, Soil Biol. Biochem. 38 (2006), pp. 320–326.
[15] L. Yang and D.J. Watts, *Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles*, Toxicol. Lett. 158 (2005), pp 122–132.
[16] D. Lin and B. Xing, *Root uptake and phytotoxicity of ZnO nanoparticles*, Environ. Sci. Technol. 42 (2008), pp. 5580–5585.
[17] W.M. Lee, Y.J. An, H. Yoon, and H.K. Kweon, *Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean* (Phaseolus radiatus) *and wheat* (Tritium aestivum): *plant agar test for water-insoluble nanoparticles*, Environ. Toxicol. Chem. 27 (2008), pp. 1915–1921.
[18] F.Q. Gao, F.H. Hong, C. Liu, L. Zheng, M.Y. Su, X. Wu, F. Yang, C. Wu, and P. Yang, *Mechanism of nano-anatase TiO_2 on promoting photosynthetic carbon reaction of spinach-inducing complex of Rubisco-Rubisco activase*, Biol. Trace Element Res. 111 (2006), pp. 239–253.
[19] F. Yang, F.S. Hong, W.J. You, C. Liu, F.Q. Gao, C. Wu, and P. Yang, *Influence of nano-anatase TiO_2 on the nitrogen metabolism of growing spinach*, Biol. Trace Element Res. 110 (2006), pp. 179–190.
[20] US Environmental Protection Agency, *Ecological effects test guidelines, OPPTS 850. 4200. Seed germination/root elongation toxicity test*, US Environmental Protection Agency, Washington, DC, 1996.

[21] C. Freeman, G. Liska, N.J. Ostle, M.A. Lock, B. Reynolds, and J. Hudson, *Microbial activity and enzymic decomposition processes following peatland water table drawdown*, Plant Soil. 180 (1996), pp. 121–127.
[22] M.A. Tabatabai, *Soil enzymes*, in *Methods of Soil Analysis, Part 2, Agronomy Monograph*, A.L. Page, ed., American Society of Agronomy, Madison, WI, 1982, pp. 903–904.
[23] L. Zhang, Y. Jiang, Y. Ding, Y. Povey, and D. York, *Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids)*, J. Nanoparticle. Res. 9 (2007), pp. 479–489.
[24] T. Yuranova, D. Laub, and J. Kiwi, *Synthesis, activity and characterization of textiles showing self-cleaning activity under daylight irradiation*, Catal. Today 122 (2007), pp. 109–117.
[25] C.M. Goodman, C.D. McCusker, T. Yilmaz, and V.M. Rotello, *Toxicity of gold nanoparticles functionalized with cationic and anionic side chains*, Bioconjug. Chem. 15 (2004), pp. 897–900.
[26] E.J. Wolfrum, J. Huang, D.M. Blake, P.C. Maness, Z. Huang, J. Fiest, and M.A. Jacoby, *Photocatalytic oxidation of bacteria, bacterial and fungal spores, and model biofilm components to carbon dioxide on titanium dioxide-coated surfaces*, Environ. Sci. Technol. 36 (2002), pp. 3412–3419.
[27] N.M. Franklin, N.J. Rogers, S.C. Apte, G. Batley, G.E. Gadd, and P.S. Casey, *Comparative toxicity of nanoparticulate ZnO, bulk ZnO and $ZnCl_2$ to a freshwater microalga* (Pseudokirchneriella subcapitata): *the importance of particle solubility*, Environ. Sci. Technol. 41 (2007), pp. 8484–8490.