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Research Trends of Ecotoxicity of Nanoparticles in Soil Environment

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We are consistently being exposed to nanomaterials in direct and/or indirect route as they are used in almost all the sectors in our life. Nations across the worlds are now trying to put global regulation policy on nanomaterials. Sometimes, they are reported to be more toxic than the corresponding ion and micro-materials. Therefore, safety research of nanoparticles has huge implications on a national economics. In this study, we evaluated and analyzed the research trend of ecotoxicity of nanoparticles in soil environment. Test species include terrestrial plants, earthworms, and soil nematode. Soil enzyme activities were also discussed. We found that the results of nanotoxicity studies were affected by many factors such as physicochemical properties, size, dispersion method and test medium of nanoparticle, which should be considered when conducting toxicity researches. In particular, more researches on the effect of physicochemical properties and fate of nanoparticles on toxicity effect should be conducted consistently.

Key words: Nanoparticle/Nanomaterials, Ecotoxicity, Earthworm, Plant, Soil nematode

INTRODUCTION

Nanotechnology has been applied to various fields such as medical science, industry, IT, energy, food, and environment. According to Global Environment Outlook (GEO) annual report by UN, nanotech-products will account for more than 14% of the total products or \$2.6 trillion by 2014, up from less than 0.1% in 2004 (UNEP, 2007). Nanomaterials are used in various products including appliances, electronics & computers, automotive, health & fitness, food & beverage, cross cutting, goods for children and home & garden, among which they are used most frequently in personal care, clothing and cosmetic sunscreen (http://www.nanotechproject.org/inventories/consumer/analysis_draft/). That means people are repetitively exposed to nanoparticles through various routes.

Nanoparticles exhibit greater reactivity as their sizes, less than 100nm size, are so small that their surface area is larger. Their transitional zone between atom or molecule and corresponding bulk material is more far from one another, which makes the original characteristics of the substance change (Hoet *et al*., 2004; Lin and Xing, 2007; Moore, 2006; Nel *et al*., 2006; Yang and Watts, 2005). It is also reported that such small size of substances can intrude into human body more easily and is more toxic than bulk material due to increased reactivity to cells in the body. International society is focusing on the toxicity of nanoparticle, and is hurriedly coming up with regulation on them. European Union gives six months prior report to the Commission before responsible person imports cosmetic product containing nanonmaterial into European nations under its cosmetics law (EC, 2009). According to the REACH regulation, manufacturers and importers are supposed to submit information about physicochemical properties and toxicity of nanomaterials (UBA, 2009). It recently passed an amendment in RoHS Directive (2002/95/EC), which restricts the use of silver nanoparticle and carbon nanotube in electronic goods (KORTRACT, 2010). US EPA recently defined silver nanoparticle as one of pesticides, and made the products go through mandatory approval process after studying their safety information under its Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

It is considered that nanotechnology will bring opportunity to improve national competitiveness and profits in several industrial fields. However, toxicity and risk of nanoparticles should be considered in advance. In this study, we investigated the overall trend of studies on nano-ecotoxicity in soil environment. We collected SCI(E) papers regarding nanotoxicity for plants, earthworms, soil nematode and soil enzyme activities, and thoroughly analyzed research trends of nano-ecotoxicty to get a better understanding how nanoparticles can be harmful in soil ecosystem.

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NANOTOXICITY IN SOIL ENVIRONMENT

This study collected the 41 SCI(E) papers on plants, earthworms, nematode, and soil enzyme activities, among which researches on plants accounted for the majority of them. Aquatic toxicity studies usually focused on carbon based nanoparticles such as fullerene (C60), single-wall carbon nanotubes (SWNTs), and multi-wall carbon nanotubes (MWNTs) while soil toxicity studies focused on metal (oxide) based nanoparticles.

Plant toxicity studies. Table 1 lists the studies on nanotoxicity of terrestrial plants. Researches on the effect of nanotoxicity on plants often used aqueous solution, filter paper, and agar media. Nanotoxicity studies on plant have been conducted with various species and nanoparticles since the first report of Yang and Watts (2005). They conducted a research on the effects of Al_2O_3 nanoparticles

Table 1. Nanotoxicity studies for terrestrial plants

Test NP	Test species	Note	Country	Author/Year
Al_2O_3	*Zea mays* (maize), *Cucumis sativus* (cucumber), *Glycine max* (soybean), *Brassica oleracea* (wild cabbage), *Daucus carota* (carrot)	Phytotoxicity of Al nanoparticles loaded with and without phenanthrene	USA	Yang and Watts, 2005
MWNT, Al, ZnO, Zn, Al_2O_3	*Brassica napus* (rape), *Raphanus sativus* (radish), *Lolium perenne* (ryegrass), *Lactuca sativa* (lettuce), *Zea mays*, *Cucumis sativus*	Phytotoxicity of five species nanoparticles	China&USA	Lin and Xing, 2007
TiO_2	*Spinacia oleracea* (spinach)	N_2 fixation in plant by TiO_2	China	Yang *et al.*, 2007
SWNTs	*Brassica oleracea* (cabbage), *Daucus carota, Cucumis sativus, Allium cepa* (onion), *Lycopersicon esculentum* (tomato), *Lactuca sativa*	Phytotoxicity of functionalized and nonfunctionalized SWNT on plant	USA	Cañas *et al*., 2008
Fe_3O_4	*Cucurbita maxima* (Pumpkin), *Phaseolus limensis* (lima bean)	Uptake, translocation and accumulation of Fe_2O_3	USA	Zhu *et al*., 2008
Carbon coated-Fe	*Cucurbita pepo* (pumpkin)	NP transformaton in plant	Spain	Gonzalez-Melendi *et al*., 2008
Pd	*Hordeum vulgare L. cv. Barke* (barely)	Bioaccumlation and growth by Pb	Germany	Battke *et al*., 2008
ZnO	*Lolium perenne*	Uptake and toxicity of ZnO	China&USA	Lin and Xing, 2008
Cu	*Phaseolus radiatus* (mung bean), *Triticum aestivum* (wheat)	Phytotoxicity of CuNP using plant agar test	Korea	Lee *et al*., 2008
Al	*Phaseolus vulgaris* (California red kidney bean), *Lolium perenne*	Effect of AlNP to plant	USA	Doshi *et al*., 2008
Ag	*Allium cepa*	Cytotoxic and genotoxic impact of plant	India	Kumari *et al.*, 2009
Ag, MWCNT, Cu, ZnO, Si	*Cucurbita pepo*	NP and BP effect to seed germination, root elongation, and biomass	USA	Stampoulis *et al*., 2009
Au, Ag, Fe_3O_4	*Lactuca sativa, Cucumis sativus*	Several nanoparticles toxicity effect to plant and microbial	Spain	Barrena *et al*., 2009
Si, Pd, Au, Cu	*Lactuca sativa*	4 metal nanoparticles toxicity effect to seed germination	USA	Shah and Belozerova, 2009
SWNT	*Nicotiana tobacum* L.cv. Bright Yellow (BY-2) cells	SWNT transports in plant cells	China	Lin *et al*., 2009
MWNT, C_{70}	Rice	Uptake and bioaccumulation of CNT in plant	USA	Liu *et al*.. 2009
MWNT	Rice cell	Effect of MWNT to rice cell	Japan	Tan *et al*., 2009
MWCT	Tomato seeds	Effect of MWNT to tomato seed germination and growth rate	USA	Khodakovskaya *et al*., 2009
MWNT, TiO_2, CeO_2	*Triticum aestivum*	Proposed investigation method of NP in plants cell	UK	Wild and Jones, 2009
TiO_2	Willow tree	Acute toxicity test of TiO_2	Denmark	Seeger *et al*., 2009
TiO_2	*Zea mays* L.	Effect of TiO_2 on root water transport	Israel	Asli and Neumann, 2009

Table 1. Continued

Test NP	Test species	Note	Country	Author/Year
TiO_2	*Arabidopsis thaliana* (Arabidopsis)	Uptake and distribution of TiO_2in plant	USA	Kurepa *et al*., 2010
Al_2O_3, SiO_2, Fe_3O_4, ZnO	*Arabidopsis thaliana*	4 metal NP and bulk materials toxicity to plant	USA	Lee *et al*., 2010
La_2O_3, Gd_2O_3, CeO_2, Yb_2O_3	*Brassica napus, Raphanus sativus, Triticum aestivum, Lactuca sativa, Brassica oleracea, Lycopersicon esculentum, Cucumis sativus*	Two rare earth oxide nanoparticles effect to plant	China	Ma *et al*., 2010
ZnO, CeO_2	*Glycine max*	Random amplified polymorphic DNA (RAPD) assay	Puerto Rico	López-Moreno *et al*., 2010
Pb	Kiwi	Pb toxicity to kiwifruit pollen	Italy	Speranza *et al*., 2010
Fe_3O_4	*Lolium perenne* L., *Cucurbita mixta*	Physiological effect of magnetic NP on plant	USA&China	Wang *et al*., 2010

loaded with and without phenanthrene on root elongation of five plants (*Zea mays*, *Cucumis sativus*, *Glycine max*, *Brassica oleracea*, and *Daucus carota*). Lin and Xing (2007) performed a research on the effects of MWNTs, Al_2O_3, Al, Zn, and ZnO nanoparticles on root elongation of plants, and found that Zn and ZnO nanoparticles affected plant germination and had the largest effects in root elongation. Zn and ZnO nanoparticles were most common used in such researches, and many reported that they induced the decrease in biomass and change in root shape (Lin and Xing, 2008). Lee *et al*. (2010) found that ZnO nanoparticle was most toxic than Al_2O_3, SiO_2, and Fe_3O_4 nanoparticles. Whereas, Stampoulis *et al*. (2009) reported that they did not see any significant effects of ZnO on *Cucurbita pepo*. Aluminum did not accumulate in *Phaseolus vulgaris* when *P. vulgaris* and *Lolium perenne* were exposed to Aluminum, but did accumulate in *L. perenne* 2.5 times more than in control (Doshi *et al*., 2008). There is a report that TiO_2 reduced the use of water in *Z. may*, and change the path of apoplast (Asli and Neumann, 2009), while not affecting willow tree in such aspects as growth, production and water use efficiency (Seeger *et al*., 2009). Copper nanoparticle caused that growth inhibition of *Phaseolus radiates* and *Triticum aestivum* using plant agar test (Lee *et al*., 2008). Battke *et al*. (2008) observed the effect of the particle size of palladium (Pd) nanoparticle on barley, and found out that barely leaves accumulated palladium nanoparticle. In addition, it is reported that Ag nanoparticle reduced biomass and transpiration of *C. pepo*, and affected the germination of *C. sativus*, but did not have any effects on *Lactuca sativa* (Stampoulis *et al*., 2009). Zhu *et al*. (2008) evaluated uptake, translocation, and accumulation of Fe_2O_3 nanoparticle in pumpkin and lima bean. These results were varied depending on the types of media (growth liquid medium, sand, and soil) of test. They identified magnetic signal in pumpkin plant while the magnetic signal was not detected in lima bean. In liquid media, magnetic signal was detected in all pumpkin plant tissue. However, magnetic signal was not detected in soil test. Cañas *et al*. (2008) observed root elongation of functionalized and nonfunctionalized SWNTs on six crop plants species (*Brassica oleracea*, *D. carota*, *C. sativus*, *Allium cepa*, *Lycopersicon esculentum*, and *L. sativa*). They found that SWNTs with nonfunctionalized had more effects on plants than those functionalized, and the effect was clearer to see when the plants were exposed to them for 24 hours rather than 48 hours. In a research about the effect of C_{70} on rice, C_{70} was transmitted to the progeny through seeds (Lin *et al*., 2009). MWNTs increased oxidative stress and reduced the number of living cells in rice cells (Tan *et al*., 2009). Liu *et al*. (2009) found that SWNTs penetrate the plant cell and cell membrane. Kumari *et al*. (2009) performed an experiment cytotoxic and genotoxic of Ag nanoparticle using *A. cepa* roots cell. The toxicity effect evaluated using mitotic index (MI), distribution of cells in mitotic phase, different type of chromosomal aberrations, disturbed metaphase, sticky chromosome, cell wall disintegration, and breaks of root tip cell. They were found that chromosomal aberration was not observed and MI value was decreased with Ag nanoparticle concentration dependent. López-Moreno *et al*. (2010) performed biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles using *G. max*. They found that biotransformation of nanoparticles in plant and DNA damage and mutations by nanoparticles.

Earthworm toxicity studies. Table 2 lists earthworm nanotoxicity studies. Petersen *et al*. (2008) exposed C-14 labeled MWNTs and SWNTs to *Eisenia foetida* in two different natural soils. They assessed uptake and depuration of earthworm exposed to MWNTs and SWNTs. Scott-Fordsmand *et al*. (2008) observed lethal and sub-lethal toxicity to *Eisenia veneta* while providing carbon nanotubes amended food. They provided food contaminated with double-walled carbon nanotubes (DWNTs) and C_{60} every 7 day to earthworms, and 28 days later, they

Table 2. Nanotoxicity studies for earthworms

Test NP	Test species	Note	Country	Author/ Year
MWNT, SWNT	*Eisenia foetida*	CNT toxicity and bioaccumlation to earthworm	USA	Petersen *et al*., 2008
DWNT, C_{60}	*Eisenia veneta*	CNT toxicity to earthworm	Danmark	Scott-Fordsmand *et al*., 2008
TiO_2, ZnO	*Eisenia foetida*	Acute and chronic toxicity test of ZnO and TiO_2 nanoparticles	USA	Qi, 2009
Al_2O_3	*Eisenia foetida*	Effect of NP and BP of aluminum in earthworm	USA	Coleman *et al*., 2010
TiO_2, ZnO	*Eisenia foetida*	Toxicity of TiO_2 and ZnO to earthworm	China	Hu *et al*., 2010

Table 3. Nanotoxicity studies for soil nematode

Test NP	Test species	Note	Country	Author/Year
Pt	*Caenorhabditis elegans*	Toxicity and antioxidant on *C. elegans* to PtNP	Japan	Kim *et al*., 2008
Ag	*Caenorhabditis elegans*	Gene expression analysis	Korea	Roh *et al*., 2009
CeO_2, TiO_2	*Caenorhabditis elegans*	Gene expression, growth, mortality of *C. elegans* exposed to CeO_2 and TiO_2	Korea	Roh *et al*., 2009
ZnO	*Caenorhabditis elegans*	Toxicity of ZnO in *C. elegans*	USA	Ma *et al*., 2009
ZnO, Al_2O_3, TiO_2	*Caenorhabditis elegans*	Toxicity of ZnO, Al_2O_3, and TiO_2 NP and BP in *C. elegans*	USA	Wang *et al*., 2009

observed that the nanoparticles did not affect on their hatchability, growth, and survival, but it caused toxic to earthworm reproduction (cocoon production). In particular, C_{60}, when given as food contaminated with 1000 mg/kg of C_{60}, reduced the reproduction rate to 78%. Hu *et al*. (2010) observed toxicity effect exposed TiO_2 and ZnO nanoparticles to earthworms. They found significant DNA damage as result of comet assay to earthworms when doses were greater than 1.0 g/kg. Bioaccumulation of Ti and Zn in earthworm was increased exposure nanoparticle concentration dependent. Qi (2009) performed acute (filter paper and sand test) and chronic (artificial soil and sand-mature) toxicity with TiO_2 and ZnO nanoparticles. Toxic effects were observed in filter paper (acute test) and artificial soil test (chronic test) with ZnO nanoparticles, but no toxicity was observed with TiO_2 nanoparticles. Neither of the ZnO and TiO_2 nanoparticles showed toxicity effect in sand (acute test), but chronic toxicity in sand-manure observed toxicity effect. Coleman *et al*. (2010) performed a chronic toxicity test using Al_2O_3 nanoparticle in different particle sizes, and found that no earthworm died when they gave them up to 10000 mg/kg of Al_2O_3 nanoparticle. Bioaccumulation increased when the particle size was smaller. In addition, they reported that earthworms avoid the NP amended soil at the exceeded 5000 mg/kg of Al_2O_3 nanoparticles.

Soil nematode toxicity studies. Table 3 shows the nanotoxicity studies lists of soil nematode. Most of researches have been performed using *C. elegans*, the most well-known soil nematode and evaluated the level of toxicity of nanoparticle in various test method based on the existing standard test methods. Roh *et al*. (2009) evaluated genotoxicity, survival, growth, and reproduction of *C. elegans* for Ag nanoparticles dispersed in K-medium. They found that Ag nanoparticles reduced reproduction of *C. elegans* supposedly due to oxidative stress. Wang *et al*. (2009) compared to toxicity of ZnO, Al_2O_3, and TiO_2 nanoparticles depending on their particle size (bulk and nano). Nanoparticle and bulk-particle significantly affected the growth and reproduction of *C. elegans*, and particularly, Al_2O_3 and TiO_2 nanoparticles affected them differently depending on their particle sizes. Ma *et al*. (2009) compared toxicity between of ZnO nanoparticle and $ZnCl_2$. They reported that there is no significant effect on lethality, behavior, reproduction, and transegene expression of *C. elegans*. Roh *et al*. (2010) investigated toxicity of CeO_2 and TiO_2 nanoparticles using gene expression, growth, fertility, and survivals as endpoint in *C. elegans*. They were found that fertility and survival were may be related with the *cyp35a2* gene. Kim *et al*. (2008) confirmed the toxicity of platinum nanparticle by observing oxidative stress levels in *C. elegans* using ROS effect and SOD activity observation.

Soil enzyme activity studies. Table 4 listed soil enzyme activity of nanoparticles studies. Hänsch and Emmerling (2010) were assessment of Ag nanoparticles toxicity using six soil enzymes assay (*eucine-aminopeptidase*, *β-cellobiohydrolase*, *acid phosphatase*, *β-Glucosidase, chitinase,* and *xylosidase*), and found no significant toxicity in them. Also, Tong *et al*. (2007) reported no significant effects of C_{60} on four soil enzymes assay (*β-Glucosidase*, *acid phosphatase*, *dehydrogenase*, and *Urease*).

TREND OF NANOTOXICITY IN SOIL ENVIRONMENT

Nanotoxicity studies on soil organisms such as plants,

Table 4. Nanotoxicity studies for soil enzyme activities

Test NP	Enzyme	Note	Country	Author/Year
C_{60}	eucine-aminopeptidase, β-cellobiohydrolase, acid phosphatase, β-Glucosidase, chitinase, xylosidase	Impact of C_{60} on soil microbial	USA	Tong *et al*., 2007
Ag	β-Glucosidase, acid phosphatase, dehydrogenase, Urease	Effect of AgNP to soil microbial activity	Germany	Hnsch and Emmerling, 2010

earthworms, nematode, and enzyme activity collected here can be sorted out as 29 on plants, 5 on earthworms, 5 on soil nematode, and 2 on soil enzyme activity in terms of subjects (Fig. 1(A)). Plants and earthworm were the representative soil organisms among them. And they can be sorted as 36 on metal oxide, 19 on metal, and 12 on carbon in terms of types of NP. In particular, plants were most frequently used for the toxicity test including rice, wheat, bean, lettuce and pumpkin. *E. feotida* and *E. veneta* in earthworm toxicity test and *C. elegans* in soil nematode were favorites for many researchers above. The 41 studies can be also sorted to be 1 in 2005, the first year of Al nanoparticle research on plants, 3 in 2007, 12 in 2008, 16 in 2009 and 9 in 2010 so far. In terms of nationality, 46% of the researches were conducted in the US after which China (17%), Korea (7%), Denmark (5%), Japan, Germany, Spain (each 5%), UK, Belgium, Italy, Israel, India, France and Puerto Rico (each 2%) were following (Fig. 1(B)). Researches on C_{60}, C_{70}, MWNTs, SWNTs and DWNTs in carbon based nanoparticles and TiO_2, ZnO, Ag, Al_2O_3 and Cu in metal (oxide) based nanoparticles were performed.

As for dispersion methods within the test medium, sonication or mix were used in most cases except using solvent such as THF (Tong *et al*., 2007) and acetic acid (Ma *et al*., 2009). The researchers performed their studies on aquatic organism with usually carbon based nanoparticles, but more used metal (oxide) based nanoparticles for soil organisms. Carbon based nanoparticles were mostly used in early toxicity researches, but later, metal (oxide) based nanoparticles were vastly used. Soil toxicity studies, which came after aquatic toxicity researches, adopted much more metal (oxide) based nanoparticles as their materials. They were performed ion and micro toxicity test as controls against nanotoxicity. As for test medium, filter paper test, agar medium, and aqueous solutions for plants were used in the past, but currently various methods of mixing nanoparticles with soil or feed are being used.

CONCLUSION

To understand the current research trends of nanoparticle ecotoxicity in soil environment, research papers on nanotoxicity for soil biota including plant, earthworm, soil nematode were thoroughly analyzed and discussed in terms of the kinds of nanoparticles, test species, and exposure medium. We also included soil enzyme activity. The researches demonstrated the a wide range of results from one another even when using the same a nanoparticles because the particle size, surface coating, dispersion methods, and test medium supposedly made difference in the results. Therefore, future researches should be conducted by considering these factors. In particular, as soil media clearly affect their physico-

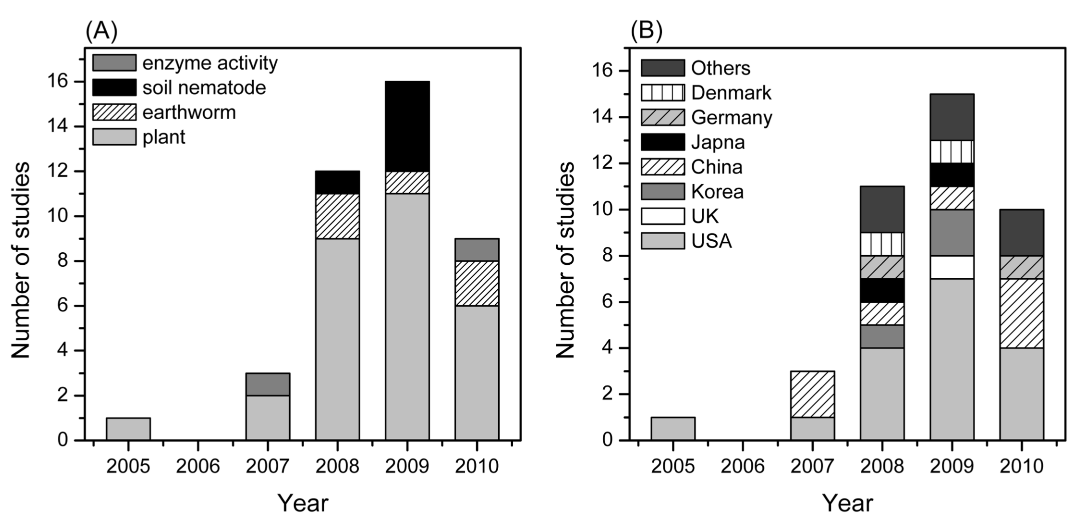

Fig. 1. Studies for nanotoxicity as related with (A) test species and (B) countries.

chemical properties and fate of nanoparticles, future researches needs to be done in this regard.

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