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Full Research Article

Response of Rice Plant to Application of Nanoparticles of Fe and Zn at Elevated CO_2: A Hydroponic Experiment under Phytotron

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Abstract

Hydroponic experiments were conducted inside the phytotron growth chambers for investigating the response of rice plant to nanoparticles of Fe and Zn under ambient (390±10 ppm) and elevated (610±10 ppm) atmospheric CO_2 concentration. The 100, 500, 1000 ppm n-Fe_2O_3 or n-ZnO and $FeSO_4$ or $ZnSO_4$ were supplied. At 500 ppm concentration of both n-Fe_2O_3 and n-ZnO particles, highest accumulation of Fe and Zn to the extent of 1471 mg kg^{-1} and 1158 mg kg^{-1} respectively, was recorded. The trend in accumulating Fe or Zn among the treatment was 500 ppm>1000, ppm>100, ppm>$FeSO_4$ or $ZnSO_4$>control. Irrespective of treatments concentration of Fe and Zn was highest in root followed by stem and leaf. At elevated CO_2 concentration the accumulation of Fe or Zn was increased in all the plant parts.

1. Introduction

Rice is one of the major staple foods in South East Asian countries but it is not a good source of micronutrients like Zn and Fe needed by human and animals. Among rice consuming population a wide range of Zn and Fe deficiency was reported (Quijan et al., 2002). There were various breeding approaches and agronomic practices advocated to increase the concentration and uptake of Fe and Zn in rice plant. Still the micronutrient use efficiency is in the range of 5-10% in spite of all possible effort of the scientific communities (Prasad et al., 1989). Present day, an alternative approach is being considered to supply micronutrients in soil i.e., application of metal oxide nanoparticles, though some of the recent studies exposed the harmful toxic effect of nanoparticles in soil and plant. In these studies the application rate was quite high being in the range of 2000-4000 ppm (Lin and Xing, 2008; Stampoulis et al., 2009).

In recent years, several workers have reported about absorption and uptake of nanoparticles by plants, but mainly focused on their adverse effects. Nevertheless, in order to use nanoparticles as potential source of nutrients more systematic studies are needed to reveal the transport routes, and its interaction with different plant organs and tissues. Such studies are important not only for the application of nanoparticles in plant nutrition, but also for understanding their effects on plants. Plants are essential base component of all ecosystems and play a critical role in controlling the fate and transport of engineered nanoparticles (ENPs) in the environment through uptake and bioaccumulation (Monica and Cremonini, 2009). The impact of nanoparticles on different plant species can vary greatly being positive and negative. Lu et al. (2002) found positive effects on germination and growth of soybean by a mixture of nano-sized silicon dioxide (n-SiO_2) and nano-titanium dioxide (n-TiO_2) at low concentrations. The impacts of nanoparticles on plant depend on both the properties of nanoparticles like type, composition, concentration, size etc. and the plant properties like type and species etc. Nanoparticles could be potentially taken up by plant roots and transported to shoots through vascular systems depending upon the composition, shape, size of ENPs and plant anatomy. Even though scientific investigation on plant uptake and accumulation of nanoparticles is still in its infancy, there were some reports about the effect of nanoparticles on plant. Lin et al. (2009) investigated the

uptake and translocation of carbon nanomaterials by rice plants (*Oryza sativa*) and they found that fullerene C_{70} could be easily taken up by roots and transported to shoots. It has been also observed that plant growth rates are usually enhanced by atmospheric CO_2 enrichment (Ziska et al., 2003). At elevated CO_2, rhizosphere activities are expected to be changed with larger root exudates and soil organism. Presence of metal nano particles carrying important micronutrients such as Fe and Zn may be subjected to change and respond differently than ambient CO_2 concentration. There are thus still many unresolved issues and challenges concerning the biological effects of nanoparticles on terrestrial organisms, and particularly plants. There are thus still many unresolved issues and challenges concerning the biological effects of nanoparticles on terrestrial organisms, and particularly plants. The objective of this experiment was to was to provide more information on i) the uptake and translocation of engineered nanoparticles (n-Fe_2O_3 and n-ZnO) in different parts of rice plant ii) impact of elevated CO_2 on the uptake and translocation of nanoparticles indifferent parts of plants.

2. Materials and Methods

2.1. Experiment in phytotron chambers

A pot culture experiment was conducted by growing rice (*Oryza sativa*), [variety Pusa Rice Hybrid (PRH-10)] in phytotron at the National Phytotron Facility, Indian Agricultural Research Institute, New Delhi between august-2012 to march-2013. The test crop was grown under two levels of atmospheric CO_2 (390±10 µmol mol^{-1}) and (610±10 µmol mol^{-1}) in 1.5 kg capacity pots (15 cm diameter and 14 cm height), each containing three plants. The fluctuations of CO_2 concentration in the phytotron growth chamber was (±10 µmol mol^{-1}). The narrow range of such fluctuation has also been reported elsewhere (12, 13). In these chambers growth conditions were maintained as follows: Temperature: 32°C/26°C day/night, photoperiod 18 h, photon flux density 450 µmol m^{-2} s^{-1} (PAR), Relative humidity (RH) 90%. Equal amount of solution was added to each flask regularly to keep the roots moist until sampling. The temperature, humidity, light and CO_2 concentrations in the phytotron chamber were controlled using computer-based programmes. Natural day light hours and dark hours were simulated with the help of incandescent lamps. The crop was allowed to grow till panicle initiation stage. Plant parameters were recorded through sampling at two critical physiological stages of rice, viz. tillering stage and panicle initiation stage.

2.2. Hydroponic culture

The concentration of macro- and micro-nutrients used in the solution culture were as follows: 0.5 M potassium sulphate (K_2SO_4); 0.5 M calcium nitrate [$Ca(NO_3)_2 4H_2O$]; 0.1 M potassium dihydrogen phosphate (KH_2PO_4); 0.5 M magnesium sulphate ($MgSO_4 \cdot 7H_2O$); 0.1 M potassium chloride (KCl); 0.01 M boric acid (H_3BO_3); 0.001 M iron-sodium ethylene diamine tetra acetic acid (Fe-Na-EDTA); 0.006 M manganese sulphate ($MnSO_4 \cdot H_2O$); 0.001M copper sulphate ($CuSO_4.5H_2O$) and 0.0001M molybdic acid ($H_2MoO_4 \cdot H_2O$). The growing medium was continuously aerated and solution pH was maintained between 6.2-6.8 throughout the course of the experiment. The nutrient solution was replaced every 3 days. The experiment was triplicated and only three plants per conical flask were maintained.

2.3. Preparation of nanoparticles suspensions and application

Commercially produced metal oxide nanoparticles namely Iron (III) oxide (nano-Fe_2O_3) and zinc oxide (nano-ZnO) were used (Sigma Aldrich Co., St. Louis, USA). As per the specifications on the bottles, the sizes of nanoparticles were less than 100 nm. Nanoparticles stock suspensions of the desired concentration (1000 mg L^{-1}), was prepared by mixing pre-weighed nano-ZnO and nano-Fe_2O_3 particles in double distilled water. The dispersed nanoparticles were ultrasonicated using a 1000 mL glass conical flask containing 250 mL of suspension placing on an ultrasonic bath and treated for 30 min at maximum power (FS 30H, Fisher Scientific, 100 W, 42 kHz). To further stabilize this nanoparticle suspension, 10% (v/v) polyethylene glycol (PEG-400), was added as per the method described by Zhang et al. (2007). The suspensions were sonicated again for at least 1 min before use.

2.4. Quantification of Fe and Zn in plant biomass

For metal quantification, all plants were washed with 0.01M HNO_3 and Milli Pore Water to remove the nanoparticles stuck on root surface. Subsequently, the plants were cut in roots, stems, and leaves and dried at 60 ◦C for 4 days. The soil samples were digested using a CEM microwave (CEM Marsx, Mathews, NC). Samples treated with ZnO nanoparticles were digested using 3mL plasma pure HNO_3 and diluted to 25mL using MPW. Concentrations in plant parts were determined using ICP-MS (Nex ION 300X, Perkin Elmer, USA). Certified standard multielement calibration standard in 5% HNO_3 (Perkin Elmer, USA) of metals and metalloids were used for calibration and quality assurance/quality control.

2.5. Collection and processing of root samples

Root samples from selected portions of the plant were collected at 24 days after application of the nanoparticles. The 1 cm thin sections each of stem and root sample was cut out with the help of sharp blade. The samples were fixed in Karnovsky solution (4% paraformaldehyde+2.5% glutaraldehyde, in 0.1 M phosphate buffer, pH 7.4) for 24 h at 4°C. Three washing

of sample with phosphate buffer (0.1 M, pH 7.4) was given, first c at 4°C for overnight and second and third at 1 h each at 4 °C. Post fixation or secondary fixation was done in osmium tetra oxide (1% OsO_4 in 0.1 M phosphate buffer in a ratio of 1:1 for 2-4 hours). During this step sample color changed to jet black. Secondary washing of sample was done with phosphate buffer (0.1 M, pH 7.4), first change at 4°C for overnight and second and third change at 1 h each at 4°C. For small tissue two changes were for 2 hours each. The samples were dehydrated in series of acetone solution with different concentration (30, 50, 70, 90 and 95% for 1 h each). Dehydration with acetone was first done at 4°C and second dehydration was done at room temperature. Cleaning of sample was made using toluene for 2 hours at room temperature. First cleaning was done with 3 parts of toluene and 1 part of araldite mixture for overnight at room temperature and the second cleaning was done with 2 parts of toluene and 2 parts of araldite mixture for 2 hours at room temperature. The third infiltration change was done with1 part of toluene and 3 parts of araldite mixture for 2 hours at room temperature, second change was done overnight at room temperature. Pure araldite mixture was heated at 50°C for 2 hours. The temperature was maintained as it polymerises at the temperature of 51-52°C. Tissue embedding in flat or bean capsule with proper labels was done and placed at 50°C for overnight. The temperature was then increased to 60°C for 48 hours for drying the sample.

2.6. Microscopic analysis of root

For correlative microscopy, 1-2 µm sections were cut from the polymerised blocks, observed on a light photomicroscope (Leitz, Germany) under phase contrast, bright field and dark field to identify the presence of nanoparticle aggregates as previously described and photographed using an Olympus DC10 digital camera. The regions of interest were trimmed and 70-100 nm ultrathin sections were obtained. The sections were counterstained with 5% uranyl acetate for 30 min, rinsed in bi-distilled water, dried and observed on a scanning-transmission electron microscope (Zeiss EVOM A10) at 20 kV. Further magnification was done on a transmission microscope at 80 kV.

2.7. Statistical analysis

The experiment was conducted using a three-factor completely randomized design (CRD) with two levels of CO_2 (ambient and elevated). Data were statistically analyzed following standard statistical methods (Gomez and Gomez 1984). Analysis of variance pertaining to three-factor (atmospheric CO_2, treatment and stage of crop) CRD was performed using Microsoft Excel and MSTATC (version 1.41; Crop and Soil Department, East Lansing, Michigan State University, USA). Unless otherwise stated, the level of significance referred to in the results is $p<0.05$.

3. Results and Discussion

3.1. Uptake of Fe and Zn from nanoparticles and partitioning in different parts of rice plant

The concentration of Fe in different parts of rice plant during its growth under ambient and elevated CO_2 condition is presented in Table 1 and Figure 1. It can be noticed that application of different concentration of Fe nanoparticles as well as $FeSO_4$ had significant effect on the Fe concentrations in root, stem and leaf. Irrespective of sources, accumulation of Fe by rice plant was highest in root (973 mg Fe kg^{-1}) followed by stem (734 mg Fe kg^{-1}) and leaves (462 mg Fe kg^{-1}) under both ambient and elevated CO_2 (Table 2). Among the treatment, n-Fe_2O_3 at 500 ppm showed highest accumulation (1471mg Fe kg^{-1}) followed by 1000 ppm n-Fe_2O_3 (1270 mg Fe kg^{-1}), 100 ppm n-Fe_2O_3 (499 mg Fe kg^{-1}), $FeSO_4$ (358 mg Fe kg^{-1}) and control (15.7 mg Fe kg^{-1}) (Table 1).

Like Fe, maximum accumulation of Zn was observed in the case of n-ZnO at 500 ppm (1158 mg Zn kg^{-1}) and significantly decreased when the concentration was increased to 1000 ppm (982 mg Zn kg^{-1}) (Table 3). Reduction in the uptake Zn by rice plant at higher n-Fe_2O_3 or n-ZnO concentration might be due

Table 1: Iron concentration (mg Fe kg^{-1}) in roots, stems, and leaves of rice plants grown in hydroponics and treated with different concentration of n-Fe_2O_3 at ambient and elevated CO_2

	Ambient			Elevated			
Treatment	Root	Stem	Leaf	Root	Stem	Leaf	Mean
Control	22.8	15	6.9	26.2	14.5	8.43	15.7
n-Fe_2O_3 100 ppm	619	485	289	695	562	346	499
n-Fe_2O_3 500 ppm	1850	1319	858	2145	1585	1068	1471
n-Fe_2O_3 1000 ppm	1453	1188	678	1951	1424	924	1270
$FeSO_4$	443	346	222	530	397	212	358
Mean	877	670	411	1069	796	511	
LSD (p=0.05)	T=4.9	PP=6.03	C=7.78	T×PP=8.5	PP×C=13.5	T×C=11.1	T×PP×C=19

T: Treatment; PP: plant parts; C: CO_2 concentration

to aggregations of the nanoparticles in the nutrient solution as previously reported by Chen et al. (2006) and Franklin et al. (2007). Irrespective of source, accumulation of Zn was highest in root (841 mg Zn kg^{-1}) followed by stem (579 mg Zn kg^{-1}) and leaves (262 mg Zn kg^{-1}) under both ambient and elevated CO_2 (Table 6.4)

Effect of CO_2 levels also strongly influenced the Fe and Zn uptake by plants (Fig. 1, 2). The data also revealed that at elevated CO_2 the Fe and Zn concentration in each parts of rice plant was higher than that at ambient CO_2 level. A critical perusal of data indicates that nanoparticles here acted as source of Fe. This also conveyed that nanoparticles get destabilized under the plant roots activity presumably be due to exudation of different acids and siderophores.

3.2. Accumulation of nanoparticles in root tissue

The study clearly demonstrated the accumulation of Fe and Zn oxide nanoparticles in cells of plant root tissues. (Plate 1, 2) The areas with nanoparticle deposits were identified and were further selected for reconfirmation by correlative microscopy. When regions were observed under Transmission Electron Microscopy (TEM), the presences of nanoparticles were observed in the root cells and sometimes in aggregates (Plate 1, 2). However, at higher concentration of both the nanoparticles treatment (500 and 1000 ppm), dark minute spots were observed at low magnification (S-TEM, 20 kV accelerating voltage), indicating electron-dense accumulations along the plant cell walls. These were further analyzed in the TEM at higher magnification (80 kV accelerating voltage) where nanoparticles were clearly observed in root cells. The representative TEM image of the n-ZnO showed that most of the particles were spherical with sizes ranging between approximately 50 and 100 nm, and the particles were found to be clustered. The root cells of the control were free from nanoparticles accumulation, however, a higher accumulation of the n-Fe_2O_3 and n-ZnO into the root system was evident. This kind of coverage increased with the increasing concentration of the n-Fe_2O_3 and n-ZnO in nutrient culture solution. In our result, we noticed that accumulation of nanoparticles were high in root tissue at elevated CO_2. It has also shown that with increasing biomass at elevated CO_2 plant translocation of n-Fe_2O_3 and n-ZnO was more. Using Ag-nanoparticles in root tip cells of onion (*Allium cepa*), researchers have demonstrated that Ag-nanoparticles could disrupt cell division process causing chromatin bridge, stickiness and cell disintegration (Kumari et al., 2010). Lin and Xing, (2008) investigated the uptake and translocation of carbon nanomaterials by rice plants (Oryza sativa) and they found that fullerene C_{70} could be easily

Table 2: Iron concentration (mg Fe kg^{-1}) in roots, stems, and leaves of rice plants grown in hydroponics and treated with different concentration of n-Fe_2O_3

Treatment	Root	Stem	Leaf	Mean
Control	24.6	14.8	7.7	15.7
n-Fe_2O_3 100 ppm	657	524	318	499
n-Fe_2O_3 500 ppm	1997	524	963	1471
n-Fe_2O_3 1000 ppm	1702	1306	801	1270
$FeSO_4$	487	371	217	358
Mean	973	734	462	
LSD (p=0.05)	T×PP=8.5			

T: Treatment; PP: plant parts

Table 4: Zinc concentration (mg Zn kg^{-1}) in roots, stems, and leaves of rice plants grown in hydroponics and treated with different concentration of n-ZnO

Treatment	Root	Stem	Leaf	Mean
Control	18.2	12.1	8.4	12.9
n-ZnO 100 ppm	531	365	278	391
n-ZnO 500 ppm	1634	1146	694	1158
n-ZnO 1000 ppm	1358	961	629	982
$ZnSO_4$	668	411	262	447
Mean	841	579	374	
LSD (p=0.05)	T×PP=13.3			

T: Treatment; PP: plant parts

Table 3: Zinc concentration (mg Zn kg^{-1}) in roots, stems, and leaves of rice plants grown in hydroponics and treated with different concentration of n-ZnO at ambient and elevated CO_2

	Ambient			Elevated			
Treatment	Root	Stem	Leaf	Root	Stem	Leaf	Mean
Control	17.5	9.767	8.433	18.8	14.5	8.4	12.9
n-ZnO 100 ppm	478	319.67	238	583	410	318	391
n-ZnO 500 ppm	1538	1214	739	1729	1079	648	1158
n-ZnO 1000 ppm	1279	845	479	1437	1077	779	982
$ZnSO_4$	651	408	249	685	412	276	447
Mean	792	559	343	890	598	406	
LSD (p=0.05)	T=7.7	PP=9.4	C=12.2	T×P=13.3	PP×C=17.3	T×C=21.2	T×PP×C=29

T:Treatment; PP: Plant parts; C: CO_2 concentration

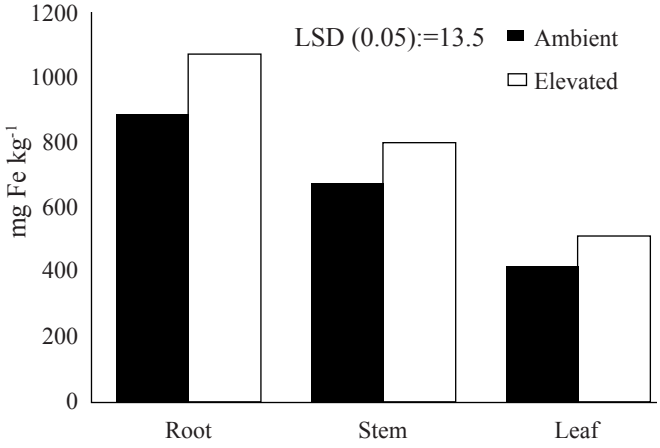


Figure 1: Partitioning of total Fe uptake (mg Fe kg^{-1}) in different parts of rice plants grown in hydroponics under ambient and elevated CO_2

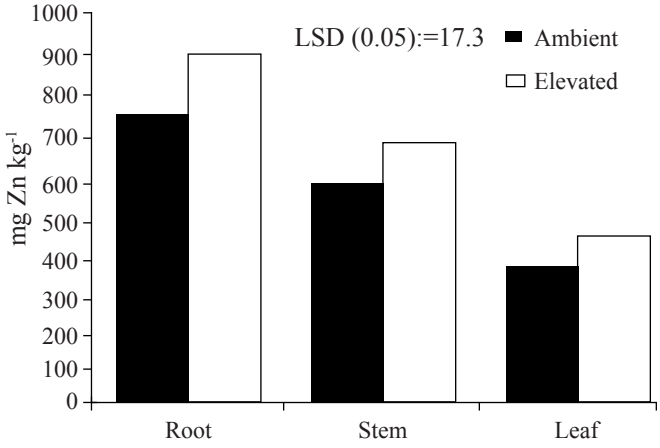
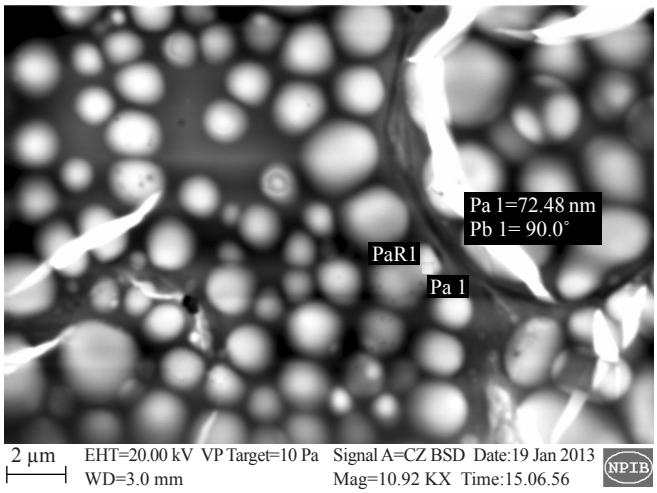


Figure 2: Partitioning of total Zn uptake (mg Zn kg^{-1}) in different parts of rice plants grown in hydroponics under ambient and elevated CO_2



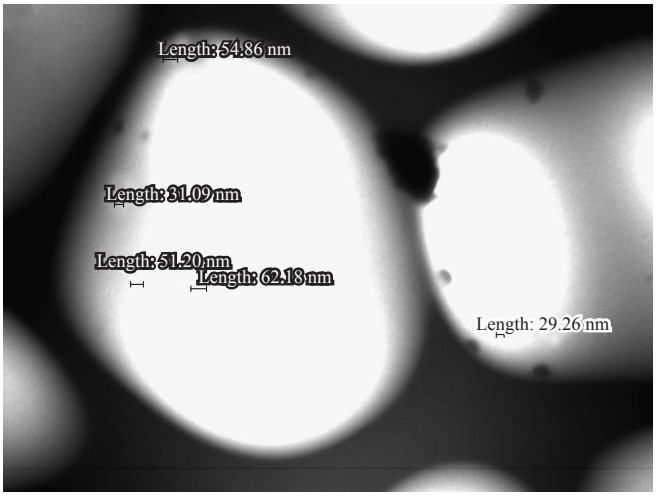
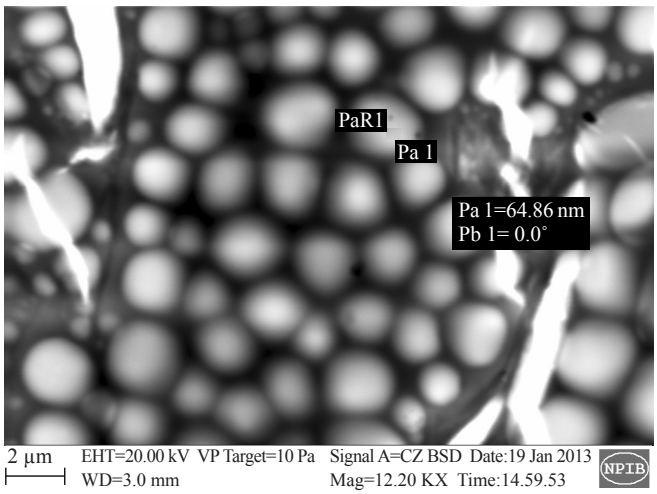


Plate 1: Accumulation of n-Fe_2O_3 at 1000 ppm concentration in root cells of rice plant



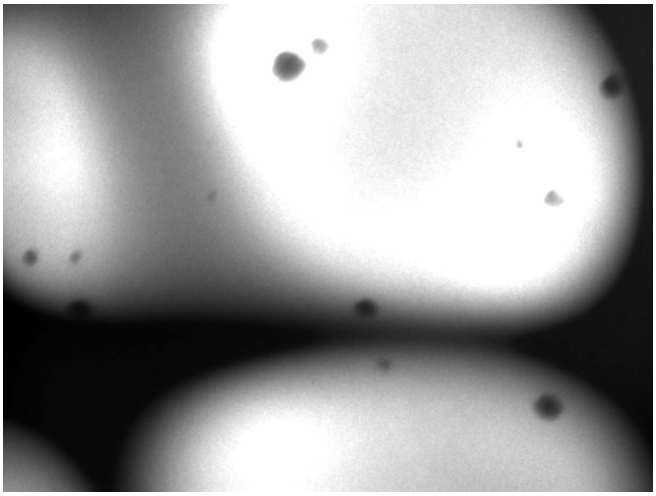


Plate 2: Accumulation of n-ZnO at 1000 ppm concentration in root cells of rice plant

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taken up by roots and transported to shoots (Lin and Xing, 2007). Most of these studies focused on the potential toxicity of nanoparticles to plants and both positive and negative or inconsequential effects have been reported. A latest work showed that the inhibition of leaf growth and transpiration of maize seedlings (*Zea mays* L.) by bentonite and TiO_2 nanoparticles is primarily due to the reduction of hydraulic conductivities (Asli and Neumann, 2009).

4. Conclusion

n- Fe_2O_3 and n-ZnO acted as potential carriers of Fe and Zn, respectively. Release of both Fe and Zn were evident at all the concentrations in the hydroponic and pot culture experiment. At elevated CO_2, Fe and Zn content in plants were higher than ambient CO_2 concentration and root biomass showed highest amount of Fe and Zn irrespective of sources. Accumulation of n-Fe_2O_3 and n-ZnO in root cell of rice was evident when nanoparticles were applied at higher concentration.

5. References

Asli, S., Neumann, P.M., 2009. Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. Plant Cell and Environment 32(5), 577-584.

Chen, K.L., Elimelech, M., 2006. Aggregation and deposition kinetics of fullerene (C60) nanoparticles. Langmuir 22(26), 10994-11001.

Franklin, N.M., Rogers, N.J., Apte, S.C., Batley, G.E., Gadd, G.E., Casey, P.S., 2007. Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and $ZnCl_2$ to a freshwater microalga (*Pseudokirchneriella subcapitata*): the importance of particle solubility. Environmental Science and Technology 41(24), 8484-8490.

Gomez, K.A., Gomez, A.A., 1984. Statistical procedures for agricultural research. John Wiley and Sons, New York.

Kumari, A., Yadav, S.K., Yadav, S.C., 2010. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids and Surfaces B: Biointerfaces 75(1), 1-18.

Lin, D., Xing, B., 2008. Root uptake and phytotoxicity of ZnO nanoparticles. Environmental Science and Technology 42(15), 5580-5585.

Lin, H., Wang, R., Qian, Q., Yan, M., Meng, X., Fu, Z., Wang, Y., 2009. DWARF27, an iron-containing protein required for the biosynthesis of strigolactones regulates rice tiller bud outgrowth. The Plant Cell 21(5), 1512-1525.

Lu, C.M., Zhang, C.Y., Wen, J.Q., Wu, G.R., Tao, M.X., 2002. Research of the effect of nanometermaterals on germination and growth enhancement of Glycine max and its mechanism. Soybean Science 21, 168-172.

Monica, R.C., Cremonini, R., 2009. Nanoparticles and higher plants. Caryologia 62, 161-165.

Prasad, R., Kumar, A., Prasad, J., 1989. Indian Journal of agricultural science 59, 300-305.

Quijano, G.C., Kirk, G.J.D., Portugal, A.M., Bartolome, V.I., McLaren, G.C., 2002. Tolerance of rice germplasm to zinc deficiency. Field Crops Research 76, 123-130.

Stampoulis, D., Sinha, S.K., White, J.C., 2009. Assay-dependent phytotoxicity of nanoparticles to plants. Environmental Science &Technology 43(24), 9473-9479.

Zhang, L., Jiang, Y., Ding, Y., Povey, M., York, D., 2007. Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnOnanofluids). Journal of Nanoparticles Research 9, 479-489.

Ziska, L.H., 2003. Evaluation of the growth response of six invasive species to past, present and future atmospheric carbon dioxide. Journal of Experimental Botany 54(381), 395-404.