Science of the Total Environment 644 (2018) 770–780

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Comparative study of the phytotoxicity of ZnO nanoparticles and Zn accumulation in nine crops grown in a calcareous soil and an acidic soil

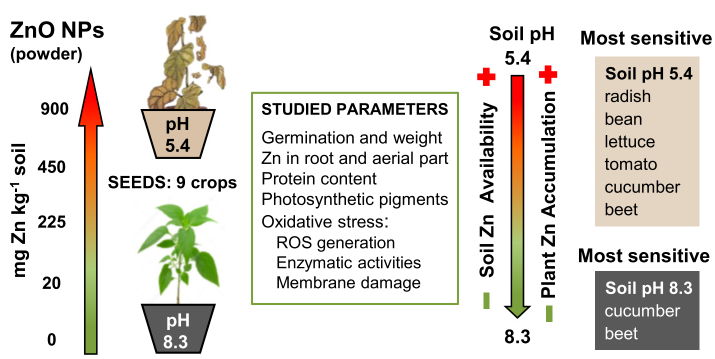
Concepción García-Gómez [a,*], Ana Obrador [b], Demetrio González [b], Mar Babín [a], María Dolores Fernández [a]

[a] Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Environment Department, Ctra. A Coruña, km 7.5, 28040 Madrid, Spain
[b] Technical University of Madrid (UPM), Chemical & Food Technology Department, Ciudad Universitaria. Avda. Complutense s/n, 28040 Madrid, Spain

HIGHLIGHTS

- Soil pH is crucial in Zn availability, Zn accumulation and phytotoxicity of ZnO NPs.
- Plant species is a key factor in ZnO NP toxicity and in Zn accumulation in tissues.
- Plant biomarker changes are not always associated with decreases in plant growth.
- Cucumber and beet were the most sensitive species to ZnO NP in the calcareous soil.
- Maize, wheat, and pea were the most tolerant crops to ZnO NP in the acidic soil.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 March 2018
Received in revised form 28 June 2018
Accepted 28 June 2018
Available online xxxx

Keywords:
ZnO nanoparticles
Plant oxidative stress
Zn uptake
Zn availability
Soil pH

ABSTRACT

The increasing use of zinc oxide nanoparticles (ZnO NPs) in agriculture and consumer products has created the need to evaluate their impact on crops. Nine crops were investigated: wheat, maize, radish, bean, lettuce, tomato, pea, cucumber, and beet. The toxic effects of ZnO NPs on seed germination, plant growth, and biochemical parameters, including photosynthetic pigments, protein and malondialdehyde (MDA) content, reactive oxygen species (ROS), enzymes of the antioxidant defence system, as well as the Zn translocation in the plants were investigated using pots containing non-contaminated or ZnO NP-contaminated soil at concentrations of 20, 225, 450, and 900 mg Zn kg^{-1}. Two soils with different physicochemical properties, namely a calcareous soil and an acidic soil, were used. The total and available Zn in the soils were correlated with the Zn in the plants (roots and shoots) and the observed effects. In the calcareous soil, the available Zn was very low and the phytotoxicity was limited to a slight reduction in the biomass for wheat, cucumber, and beet at the highest concentration. Only beet showed an increase in photosynthetic pigments. The parameters related to oxidative stress were affected to different degrees depending on the crop, with the exceptions of maize, lettuce, pea, and beet. In the acidic soil, the available Zn was high, and the germination of bean, tomato, lettuce, and beet, and the growth of most of the crops were seriously affected. The calculated EC50 values (growth) in the acidic soil ranged from 110 to 520 mg Zn kg^{-1}. The photosynthetic pigments and most of the markers of oxidative stress were negatively affected in maize, wheat, bean, and pea. However, these changes were not always associated with a decrease in plant weight. In summary, soil pH and plant species are key factors affecting the Zn availability and phytotoxicity of ZnO NPs.

* Corresponding author.
E-mail addresses: cgarcia@inia.es (C. García-Gómez), ana.obrador@upm.es (A. Obrador), demetrio.gonzalez@upm.es (D. González), babin@inia.es (M. Babín), mdfdez@inia.es (M.D. Fernández).

https://doi.org/10.1016/j.scitotenv.2018.06.356
0048-9697/

1. Introduction

Nanotechnology is a growing field of research. Over the last decades, there has been an extremely rapid expansion of engineered nanoparticles (ENPs) with a wide range of applications. ENPs are released unintentionally into the environment during their production, use, and disposal such as sewage treatment, recycling, waste incineration, and landfilling (Keller et al., 2013). However, ENPs can also be deliberately released into the environment. More specifically, farm applications of nanoparticles and nanocapsules containing agrochemicals provide an efficient means to distribute pesticides and fertilizers in a controlled procedure with high site specificity to reduce collateral damage (Khan et al., 2017; Wang et al., 2016).

Even though nanomaterials are used in agriculture to improve the efficiency and sustainability of agricultural practices by using less input and hence, generating less waste than conventional practices, the use of nano-agrochemicals is not without risk and this topic is getting increased attention (Coll et al., 2016). Zinc oxide nanoparticles (ZnO NPs) are of particular interest not only because of their extensive use in various consumer products but also because they are being increasingly incorporated into agricultural products (Rizwan et al., 2017). For example, ZnO NPs have been investigated as an active ingredient for insecticides (Salem et al., 2015), for their antifungal and antibacterial properties (Dimkpa et al., 2013), and as a coating for granular macronutrient fertilizers (Chhipa, 2017). Moreover, the direct addition of ZnO NPs to swine manure during its maturation process reduces H_2S and NH_3 emissions (Alvarado et al., 2015). These potential uses represent a new source of ZnO NPs entering the soil.

The soil is a structured, heterogeneous, and discontinuous system with a diverse biological population. Soil properties such as the content of natural organic substances, ionic strength, redox potential, pH, texture, and the edaphic biota may react with the medium to counteract the toxic effect of a given pollutant. The soil properties largely govern the behaviour of the ZnO NPs, the Zn speciation in the soil, as well as its bioavailability (Amde et al., 2017; Sheoran et al., 2016). Therefore, the use of artificial media does not allow for a correct evaluation of the ecotoxicity of ENPs. In spite of this, most ecotoxicological studies on ENPs have used non-natural (or soilless) media and this practice may result in significantly different toxicity effect than observed under natural environmental conditions (Servin and White, 2016).

Plants are important receptors of soil compartment and hence when ZnO NP enter the soil, plants are a potential pathway for the uptake, translocation, and bioaccumulation of nanoparticles in the food chain (Garcia-Gomez et al., 2015; Mukherjee et al., 2014). Zn is an essential metal for the normal development of plants and it is taken up as a soluble component or solubilised by root exudates (Dev et al., 2018).

However, excess Zn in the plant tissues has direct toxic impacts resulting in chlorosis or the generation of reactive oxygen species (ROS) due to the oxidative stress (Alonso-Blazquez et al., 2015) or indirect impacts by altering the nutritional value, which may affect the health of plants, humans, and animals (Asati et al., 2016; Peralta-Videa et al., 2014).

Considerable information is available on the effects of ZnO NPs on plants including alteration of biomass, root development, photosynthetic rate and ROS formation. Toxic effects were found on lettuce (Xu et al., 2018), on wheat (Tripathi et al., 2017), on maize or cucumber (Zhao et al., 2013, 2015; Zhang et al., 2015) or on green pea (Mukherjee et al., 2014). Furthermore, Manzo et al. (2011) identified genotoxicity in bean (*Vicia fava*). However, to the best of our knowledge, most studies were conducted in soilless media, limiting the reliability of the results, and/or different levels of contamination were not investigated to determine a threshold or dose-response effect; other studies only included one or a few plants species, which makes it difficult to compare the sensitivities to Zn under just exactly the same growth conditions. In summary, the information in the literature on the effects of ZnO NPs on plants is scarce and sometimes contradictory or lack of realism.

In order to fill these gaps, in this study, we investigate the effects of ZnO NPs at four concentration levels on nine relevant crops that have differences in morphology, root and leaf density, and grow in two different soils, especially in pH values. The important objectives were i) to determine the potential availability of Zn in two agricultural soils treated with different doses of ZnO NPs and ascertain how the different physicochemical characteristics of the soils may govern the Zn availability, ii) to investigate the potential for the uptake and translocation of Zn from the ZnO NPs into the aerial parts of the plants, and iii) to determine the toxic effects of the ZnO NPs on germination, plant growth, and biochemical parameters of a broad variety of crops grown in two agricultural soils and correlate them with the Zn content in the soils. The results of this study will provide information on which plant species are most suitable for growing in soils with certain physicochemical characteristics, different levels of ZnO NP contamination, and with different soil pH values. In addition, we determine which plant species can be used to correct nutritional Zn deficiencies due to the potential for bioaccumulation in their edible parts (Horton, 2006).

2. Materials and methods

2.1. Chemicals, plants, and soils

Uncoated ZnO NPs with a nominal primary particle size of <100 nm (i.e., rp ≤ 50 nm) were obtained from Sigma-Aldrich (Germany). A transmission electron microscope (TEM; JEOL-1400 Plus) was used to determine the mean size of the nanoparticles and the particle size distribution.

Nine plant species were investigated, including maize (*Zea mays*), radish (*Raphanus sativus*), wheat (*Triticum aestivum* L.), bean (*Phaseolus vulgaris* L. *cv. Contender*), cherry tomato (*Solanum lycopersicum* L. *cv. cerasiforme*), lettuce (*Lactuca sativa* L.), green pea (*Pisum sativum* L.), cucumber (*Cucumis sativus* L.), and beet (*Beta vulgaris* L.). The seeds were provided by Semillas Battle, Barcelona, Spain.

The soils were collected from the surface layer (0–20 cm) of two agricultural fields located in Madrid (Spain); an acidic soil and a calcareous soil were used in the study. The soil samples were air-dried and sieved (2-mm mesh) prior to use in the plant growth experiment. The physicochemical characteristics and metal contents were determined previously by Garcia-Gomez et al. (2017). The values for the parameters of interest for the calcareous soil were: pH 8.3, organic matter (OM) 1.13%, clay 39%, sand 17.5%, silt 43.5%, electrical conductivity (EC) 225.9 dS cm^{-1}, total Zn 61.8 mg kg^{-1}; the values for the acidic soil were: pH 5.4, OM 1.69%, clay 18%, sand 25%, silt 57%, EC 145.9 dS cm^{-1}, total Zn 40.1 mg kg^{-1}.

The soil pH and EC were determined at the beginning of the experiments and after harvest (1 and 45 d post-Zn contamination) following the protocols of the Spanish Ministry of Agriculture (MAPA (Ministerio de Agricultura, Pesca y Alimentación), 1994) in soil:water (w/v) suspensions of 1:2.5 and 1:5, respectively.

2.2. Zinc exposure treatments

Two trials were performed with each plant species; the first trial was conducted with the calcareous soil and the second with the acidic soil. Each trial was comprised of four treatment groups (20, 225, 450, and 900 mg Zn kg^{-1} soil; Zn as ZnO NPs) in triplicate and one control group (natural Zn occurrence) with four replicates. The Zn concentrations were related to the mass of the dry soil (DW). The ZnO NP powder (0.075, 0.84, 1.68 or 3.36 g needed to get the soil Zn concentrations of 20, 225, 405 and 900 mg Zn Kg^{-1}, respectively) was directly dispersed in 3.0 kg of soil per treatment and intensively blended by hand for 10 min. Then, to ensure good homogenization of the mixture, the treated soils were 2-mm sieved three times as described in previous studies (Garcia-Gomez et al., 2014; Josko and Oleszczuk, 2013).