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Influence of silver and titanium nanoparticles on arbuscular mycorrhiza colonization and accumulation of radiocaesium in *Helianthus annuus*

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Abstract

The influence of arbuscular mycorrhizal fungus on ^{134}Cs uptake by *Helianthus annuus* was studied in a pilot study under growth chamber conditions. Mycorrhizal plants took up five times more ^{134}Cs (up to 250,000 Bq kg^{-1} dry weight) than non mycorrhizal plants. Silver and titanium nanoparticles, supplied into the surface soil layer decreased both the mycorrhizal colonization and Cs uptake by mycorrhizal plants. The application of activated carbon attenuated the effect of nanoparticles and increased ^{134}Cs uptake in the presence of mycorrhizal fungi (up to 400,000 Bq kg^{-1} dry weight). The results underline the possible application of phytoremediation techniques based on mycorrhiza assisted plants in decontamination of both radionuclides and nanoparticles.

Additional key words: ^{134}Cs, phytoextraction, phytoremediation, radioactivity, symbiosis.

Resumen

Influencia de las nanopartículas de plata y titanio en la colonización de micorrizas arbusculares y la acumulación de cesio radiactivo en *Helianthus annuus*

Se estudió mediante una prueba piloto en una cámara de crecimiento la influencia de hongos formadores de micorrizas arbusculares en la captación de ^{134}Cs por *Helianthus annuus.* Las plantas micorrizadas incrementaron hasta cinco veces la captación de ^{134}Cs (hasta 250.000 Bq kg^{-1} peso seco). La adición de nanopartículas de plata y titanio en la superficie del suelo disminuyó tanto la colonización del hongo como la captación de ^{134}Cs en las plantas micorrizadas. La aplicación de carbón activado atenuó el efecto de las nanopartículas e incrementó la captación de ^{134}Cs en presencia de hongos micorrízicos (hasta 400.000 Bq kg^{-1} peso seco). Los resultados sugieren una posible aplicación de plantas micorrizadas en técnicas de fitorremediación para la recuperación de suelos contaminados con radionucleotidos y nanopartículas.

Palabras clave adicionales: ^{134}Cs, fitoextracción, fitorremediación, radiactividad, simbiosis.

Introduction

Radionuclides and nanoparticles (NPs) that are found in the environment come from both natural and artificial sources, and both are of environmental concern. Radionuclides are released into the environment from aboveground nuclear tests, nuclear accidents and nuclear power generation plants (Zhu and Shaw, 2000). Contamination by radioactive isotopes is of great environmental concern as these elements can leak into the food chain and thus can cause health hazards (Howard *et al.*, 1991; Robison and Stone, 1992). As soil properties, especially soil texture and potassium status have a major effect on the soil-to-plant transfer of radiocaesium, heavy K fertilization, application of various mineral amendments, deep ploughing and mulching combined with selection of efficient plant cultivars are proposed to improve soil remediation (Zhu and Shaw, 2000; White *et al*., 2003). Recent studies also indicate that diverse microorganisms can significantly affect

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Abbreviations used: AM (arbuscular mycorrhiza), NP (nanoparticle), PEA (plant efficiency analyzer).

radionuclide uptake by plants (Dupre de Boulois *et al*., 2008).

Nanoparticles became of interest relatively recently, mostly because of their possible use in diverse technologies. They can be defined as objects ranging in size from 1-100 nm that due to their size may differ in the properties from the bulk material. This can result from the high surface to volume ratio that increases their reactivity, the ability to penetrate cell membranes and possible biochemical activity. There is an increasing interest in the use of nanoparticles for diverse purposes, starting from medical treatments, use in various branches of industry production such as solar and oxide fuel batteries for energy storage, wide incorporation into diverse materials of everyday use such as cosmetics or clothes (Rogers, 2005). Current annual global production of NPs was in the order of 103 tons in 2004, which is expected to increase further to 104-105 tons yr^{-1} after 2010 (The Royal Society & The Royal Academy of Engineering, 2004). It is expected that NPs will enter aquatic, terrestrial and atmospheric environments (Nowack and Bucheli, 2007), where their fate is largely unknown. The knowledge is especially limited in the case of plants. Some data on root phytotoxicity of ZnO and other nanoparticles are available (Lin and Xing, 2008; Yang and Watts, 2005). On the contrary, some plants, such as *Brassica juncea* and *Medicago sativa* were shown to be able to form and to take up Ag nanoparticles into their tissues (Harris *et al.*, 2008).

The aim of the present paper was to evaluate the effect of Ag and Ti nanoparticles on the development of mycorrhiza in *Helianthus annus* that was cultivated in the presence of radioactive ^{134}Cs. In addition to mycorrhizal colonization also the effect of radioactivity and nanoparticles on plants were assessed and possible way of attenuation of both by application of active carbon was tested.

Methods

Seeds of *Helianthus annuus* (PL814/32/220/1945/A) were germinated on wet filter paper in Petri dishes. Two weeks-old seedlings were transferred into 24 containers, each containing 1.3 kg of pasteurized substratum composed of sand and clay in proportion 3:1 (v:v) with addition of rock phosphate (50 g L^{-1}). Inoculum composed of *Glomus intraradices* UNIJAG PL24-1 propagules (ca. 50 propagules g^{-1} of substratum) was introduced into 12 containers. All mycorrhizal (N = 12) and separately nonmycorrhizal plants (N = 12) were grown on soil spiked with ^{134}Cs ("POLATOM" Radioisotope Centre, Świerk, Poland). $^{134}CsCl$ of initial activity 100,000 Bq mL^{-1} was diluted prior to the experiment in deionized water to achieve a concentration 1,000 Bq mL^{-1}. The initial activity of ^{134}Cs in the soil was 77,000 Bq kg^{-1} (100 kBq per container). Sunflowers (N = 3) were grown in soil: a) without any further additions; b) supplemented with nanoparticles [0.2 g of P&T-230Ag (NANOPAC, Poland) per pot]; c) supplemented with activated carbon (2 g per pot) and d) supplemented with nanoparticles and activated carbon (in amounts as in b and c) (Fig. 1). The added substances were mixed thoroughly in the upper 10 cm layer of substrata. The plants were cultivated in a growth chamber at 20°C, at 12 h of light and 12h darkness, at photosynthetic photon flux density 30 ± 6 $\mu mol \cdot (s \cdot m^2)^{-1}$; water was supplemented twice a week. Plant vitality was evaluated before harvesting using a Plant Efficiency Analyzer (PEA) fluorimeter (Hansatech Instruments, UK) estimating chlorophyll *a* fluorescence transients of intact leaves. The data were acquired as described by Strasser *et al.* (1995) and vitality index PI_{TOTAL} was calculated (Strasser *et al.*, 2000). Plants were harvested after 12 weeks of growth. Roots were used to estimate mycorrhizal colonization. They were carefully washed and cleared in 10% KOH for 24 h at room temperature. Subsequently, after careful washing in tap water, the roots were acidified for 1 h in 5% lactic acid and stained for 24 h at room temperature in 0.05% aniline blue in lactic acid, in order to visualize the fungal structures inside the roots. Material obtained in this way was cut into 1 cm pieces and mounted on slides in lactic acid. The following parameters were assessed: frequency of mycorrhiza (F%), mycorrhizal intensity relative (M%) and absolute (m%), arbuscular richness relative (A%) and absolute (a%) according to Trouvelot *et al*. (1986) (http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html) and the above mentioned mycorrhizal parameters were calculated.

For radioactivity estimation the plant shoots were washed in deionized water, dried at room temperature, crushed and homogenized. Afterwards they were mixed with 5-10 ml mixture of deionized water and 96% alcohol in proportion 1:1 (v:v) and trace amounts of sucrose. The suspension was evaporated to dryness in electric oven at 55°C for 12-15 h.

Samples were analysed for 10,000-250,000 s to minimize the counting error. Counting errors for the measurements of ^{134}Cs were always lower than 15% in both

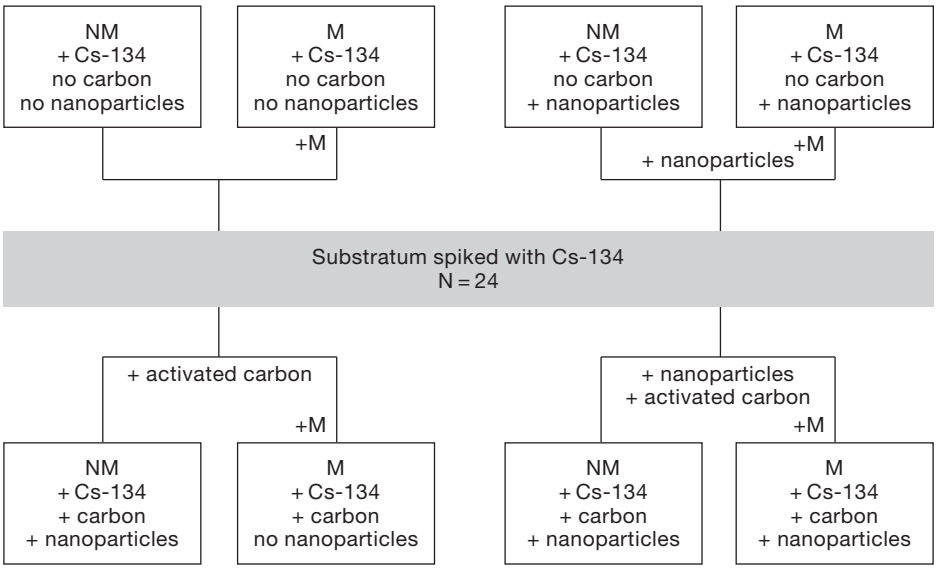

Figure 1. Schematic diagram of the different treatments in the experiment in which the substratum was treated with ^{134}Cs, activated carbon, and nanoparticles; mycorrhizal inoculum (+M) was added after mixing the substratum.

cases. The activity concentrations of ^{134}Cs were derived from their gamma lines at 605 and 796 keV, according to the following formula:

$$A\left[Bq \cdot kg^{-1}\right] = \frac{N_0 - N_b}{k_\gamma \cdot \varepsilon \cdot m} \qquad [1],$$

where N_0 = count rate in corresponding peak of radionuclide (counts per second – cps); N_b = background count rate (cps); k_γ = quantum yield of emission line; ε = efficiency of gamma-rays registration; m = mass of sample (kg).

The-distribution of ^{134}Cs activity concentration was determined using a gamma-spectrometer with semiconductor coaxial HPGe detector (efficiency 15%) shielded by 10 cm of lead.

Statistical analysis

Statistical analysis of data was carried out with the nonparametric Kruskal-Wallis test-[STATISTICA ver. 8.0 software (Statsoft, USA)].

Results

Growth and photosynthetic parameters

Three months old mycorrhizal plants produced longer shoots and a higher number of leaves in most cases, although statistically significant differences between nonmycorrhizal and mycorrhizal plants were found only in the case of control soil and soil supplemented with nanoparticles and activated carbon (not shown). The differences were more pronounced while dry shoot mass was compared. In plants treated with nanoparticles the mycorrhizal ones had lower dry weight as compared to nonmycorrhizal (Fig. 2). The plant vitality index PI_{TOTAL} (Fig. 3) was the highest in the case of mycorrhizal control plants and mycorrhizal plants cultivated on soil supplemented with activated carbon. Plants treated with nanoparticles did not differ regard-

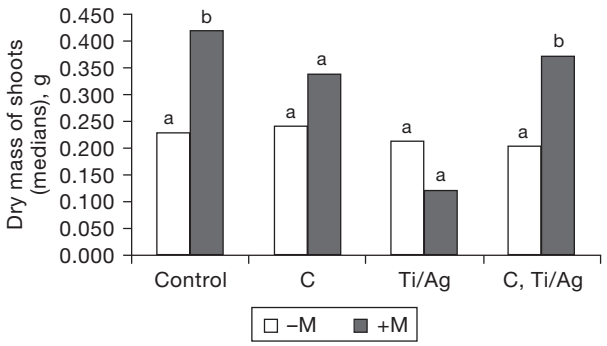

Figure 2. Dry mass (g) of mycorrhizal (+M) and nonmycorrhizal (–M) *Helianthus annuus* cultivated on soil spiked with ^{134}Cs: without any further additions (Control), supplemented with activated carbon (C), supplemented with nanoparticles (Ti/Ag); and supplemented with activated carbon and nanoparticles (C, Ti/Ag). The different letters above bars mean statistically significant differences.

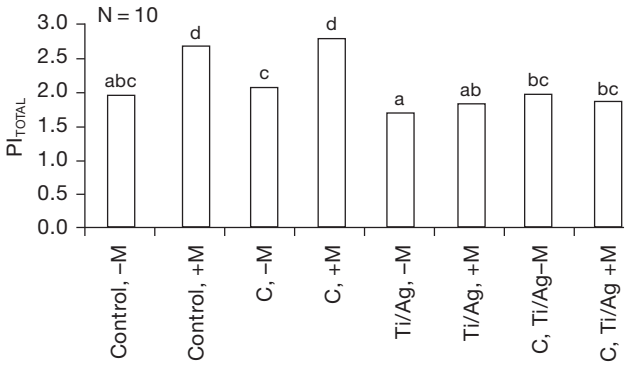

Figure 3. Performance indexes (PI_{TOTAL}) of mycorrhizal (+M) and nonmycorrhizal (–M) *Helianthus annuus* cultivated on soil spiked with ^{134}Cs: without any further additions (Control); supplemented with activated carbon (C); supplemented with nanoparticles (Ti/Ag); and supplemented with activated carbon and nanoparticles (C, Ti/Ag). The different letters above bars mean statistically significant differences.

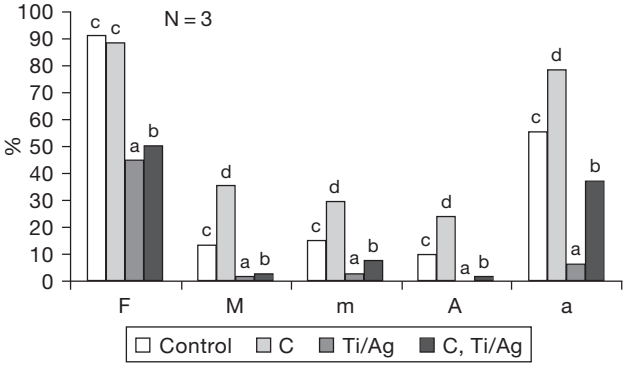

Figure 4. Arbuscular mycorrhizal colonization parameters of *Helianthus annuus* inoculated with *Glomus intraradices*: F(%), frequency of mycorrhiza; M(%), mycorrhizal colonization intensity for all roots; m(%), mycorrhizal colonization intensity within individual mycorrhizal roots; A(%), arbuscular richness for all roots; a(%), arbuscule richness in root fragments where the arbuscules were present. Plants were cultivated on soil spiked with ^{134}Cs: without any further additions (Control); supplemented with activated carbon (C); supplemented with nanoparticles (Ti/Ag); and supplemented with activated carbon and nanoparticles (C, Ti/Ag). The different letters above bars mean statistically significant differences.

less of the presence or absence of mycorrhiza. Significantly higher PI_{TOTAL} was observed for nonmycorrhizal and mycorrhizal plants supplemented with nanoparticles and activated carbon in comparison to nonmycorrhizal, nanoparticles treated plants.

Mycorrhizal colonization

All parameters describing mycorrhizal colonization of *H. annuus* were significantly lower in plants exposed to nanoparticles (Fig. 4). The addition of activated carbon to the soil treated with nanoparticles significantly increased all colonization parameters. Activated carbon also increased mycorrhizal colonization and arbuscule richness in control soil, while no differences were visible in the case of propagule frequency (F%).

Radioactivity of plants

Gamma-spectrometry revealed increased ^{134}Cs activity concentration in shoots of mycorrhizal plants compared to nonmycorrhizal ones, which were grown on a) soil treated only with ^{134}Cs, b) soil with activated carbon, and c) soil with titanium-silver nanoparticles mixed with activated carbon (Fig. 5). The addition of Ti/Ag nanoparticles to the soil had reduced ^{134}Cs content in shoots of mycorrhizal *H. annuus*. In that case ^{134}Cs levels in shoots of mycorrhizal and nonmycorrhizal plants were comparable. On the other hand, the simultaneous addition of Ti/Ag nanoparticles and activated charcoal to the soil had resulted to the considerable increase (up to 8 fold) of ^{134}Cs activity concentration in shoots of mycorrhizal plants.

Discussion

The role of arbuscular mycorrhizal (AM) fungi has been recently summarized by Dupré De Boulois *et al*.

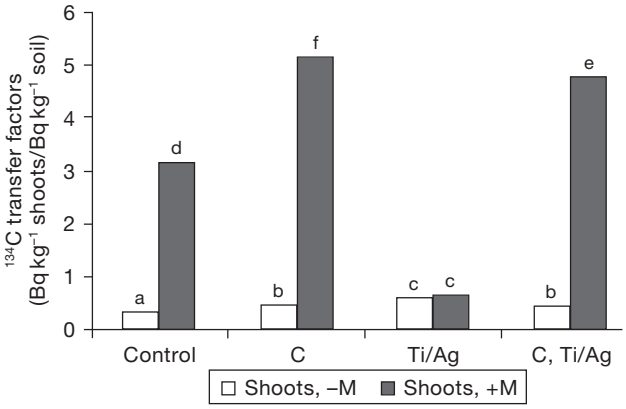

Figure 5. ^{134}Cs transfer factors in shoots of mycorrhizal (+M) and nonmycorrhizal (–M) *Helianthus annuus* cultivated on soil spiked with ^{134}Cs: without any further additions (Control); supplemented with activated carbon (C); supplemented with nanoparticles (Ti/Ag); and supplemented with activated carbon and nanoparticles (C, Ti/Ag). The different letters above bars mean statistically significant differences.

(2008), including various mechanisms that could be potentially involved in the transport and immobilization of radiocaesium. According to literature, depending on the plant, AM fungal species and on the experimental conditions, the accumulation of radiocaesium in mycorrhizal plants can be lower (Dighton and Terry, 1996; Berreck and Haselwandter, 2001), similar (Rogers and Williams, 1986; Roseen *et al*., 2005) or higher (McGraw *et al*., 1979; Entry *et al*., 1996; Roseen *et al*., 2005) than in nonmycorrhizal plants. In the present study *Helianthus annuus* was used. The species was previously shown to be an effective hyperaccumulator of As and Cd under hydroponic conditions (January *et al*., 2008) and bioaccumulator of ^{137}Cs and ^{60}Co (Hornik *et al*., 2005), although the ability of this plant to form mycorrhiza has not been studied. In the present paper we were used soil substratum spiked with ^{134}Cs. The inoculation with *G. intraradices* visibly increased the uptake of ^{134}Cs and this phenomenon could be of potentially economic value, although this result should be treated as a pilot study. In this particular case the increased radioactivity of the mycorrhizal plants cannot be explained by increased biomass, as the biomass of individual mycorrhizal plants was only two times higher than the mass of nonmycorrhizal ones, while the radioactivity increased over 10 times.

The main aim of the present study was to see the effect of nanoparticles on the formation of mycorrhiza and the uptake of Cs by sunflower. So far, the knowledge of the effect of nanoparticles on mycorrhizal fungi is very limited, only one study was carried out, where it was suggested that mycorrhizal fungi assisting plants can alleviate the toxicity of Cu by the formation of Cu nanoparticles and thus limiting its toxicity (Manceau *et al*., 2008). In the present paper nanoparticles were used according to manufacturer's instructions. The product is sold as a disinfecting spray. However, research carried out on the influence of nanoproducts on fungi showed that these products were actually inefficient in inhibition of most saprobic fungi tested (Ogar, 2009). On the contrary, AM fungus used in the present paper was sensitive to those nanoparticles. The mycorrhizal colonization was seriously decreased and all the positive effects of mycorrhiza, such as enhanced growth and photosynthesis of mycorrhizal plants grown in control soil, were decreased to the level of nonmycorrhizal plants. This result implies that we should use the nanoparticles included in a wide range of modern products with more care and see their transfer into groundwater as a possible risk for the environment. The negative effect was partly alleviated by the use of activated carbon, that works as an adsorbent. The use of this adsorbent was shown previously to accelerate 2-4-fold radioisotope uptake by maize, rape, sunflower, lupin and potatoes in a field experiment in the Chernobyl zone (Polesye and Chernobyl districts) in 1997-2000 (Mikhalovsky and Nikolaev, 2006). The mycorrhizal status of plants used in the field experiment was unknown, but sunflower and maize are almost always mycorrhizal. The data reported in the present paper confirm the acceleration of the radionuclide uptake by activated carbon and the possible use of carbon for cost-effective decontamination of soil, not only from radionuclides but also from nanoparticles that could be in the future a common pollutant of terrestrial habitats. As the price of activated carbon are continuously growing there could be the possibility to substitute it with other substances such as biochar (Steinbeiss *et al*., 2009). Future research will focus on the impact of diverse doses of nanoparticles, the use of different strains of mycorrhizal fungi, elucidating the mechanisms of toxicity. An interesting issue will be to monitor the fate of mycorrhiza while nanoparticles are being produced within the plant tissue (Harris *et al*., 2006; Leela and Vivekanandan, 2008) what is a relatively novel approach.

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