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Accumulation of Aluminium by Plants Exposed to Nano- and Microsized Particles of Al_2O_3

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ABSTRACT: Accumulation of aluminium by plants exposed to nano- and microsized particles of Al_2O_3 was investigated in terms of risk assessment and possible application in phytoremediation of contaminated sites. Four plant species (*Allium cepa* L., *Zea mays, Lepidium sativum* and *Kalanchoe daigremontiana)* were cultivated on media (soil or liquid medium) contaminated with nano- and microparticles of Al_2O_3. Bioavailability of aluminium in the soil was studied using water and EDTA extraction. Total amounts of aluminium in plants and soil extracts were determined by inductively coupled plasma mass spectrometry. All investigated plants accumulated aluminium and its concentration depended on the concentration of Al_2O_3 in the growth medium and the particle size. The most effective uptake and transport of aluminium was observed for Al_2O_3 nanoparticles. The highest content of aluminium was found in roots of plants. The extent of aluminium accumulation by plants was species-specific. The highest transfer factors were obtained for *Zea mays* cultivated on liquid medium supplemented with the lowest concentration of NPs. It was found that the nanoparticles sediment easily, but are still available for uptake by plants. Our studies give a perspective for future development of phytoremediation techniques of contaminated soils and waters.

Key words: Nanoparticles, Microparticles, Aluminium oxide, Bioacumulation

INTRODUCTION

A nanoparticle is a particle in which at least one of the dimensions does not exceed 100 nm. Physical and chemical properties of nano-sized materials and their interactions with media can differ considerably from those of the bulk ones (Aitken *et al.,* 2004) as a consequence of the high ratio of the surface area to volume or weight. Owing to the unique properties of nanomaterials, during the last two decades the implementation of nanotechnologies has increased rapidly, bringing a growing risk of creating a new generation of waste (nanowaste) and new potential threats to the environment (Bystrzejewska-Piotrowska *et al.,* 2009). All the steps of production, use and waste-disposal of nanoparticles may lead to their release into water, soil and air, so investigation of the uptake, bioaccumulation, biotranformation of and the risks posed by nanomaterials is urgently needed. There is also growing need to develop technologies for soil protection and remediation. Phytoremediation techniques, which are eco-friendly and less invasive, more cost effective and restorative compared to conventional methods (Kidd *et al.,* 2009; Ali *et al.,* 2013). A phytoremediation strategy which aims to remove environmental contaminants trough their uptake and accumulation by plants is called phytoextraction. Aluminum oxide nanoparticles (Al_2O_3-NPs) are among the most widely used nanosized materials (Stenger *et al.,* 2005; Schmid and Riediker, 2008, Wagner *et al.,* 2007; Chen *et al.,* 2008; Zhang et al. 2011; Kumar *et al.,* 2013;). Aluminum toxicity to plants is well known (Delhaize and Ryan, 1995; Poschenrieder *et al.,* 2008; Matsumoto and Motoda, 2012), while only a few studies have been conducted to investigate the phytotoxicity of Al_2O_3 nanoparticles (Yang and Watts, 2005; Lin and Xing, 2007; Lee *et al.,* 2010; Burklew *et al.,* 2012) Inhibition of root elongation of soybean, corn, carrot, cabbage and cucumber was reported by Yang and Watts, 2005; as an effect of nano-Al_2O_3 (13 nm) at a concentration of 2 g/L. As Murashov, 2006 observed, the question is whether that effect was really caused by Al_2O_3 nanoparticles or by aluminium ions present in aqueous solution. No phytotoxicity was observed for nanoparticles of Al_2O_3 (60 nm) at 2 g/L in the case of radish, rape, ryegrass, lettuce

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and cucumber, while the elongation of corn roots was reduced by 35 % (Lin and Xing, 2007). Lee *et al.*, 2010 have investigated phytotoxicity of Al_2O_3-NPs (~ 150 nm) to *Arabidopsis thaliana*. The applied concentration of NPs was 400, 2000 or 4000 mg/L and no toxic effects were observed. It should be noted that the sizes of nanoparticles used in those experiments were different, which could affect the obtained results. Several studies have been undertaken to investigate accumulation of nanoparticles by plants (Ma *et al.*, 2010; Jacob et al. 2013; Hawthorne *et al.*, 2012; Rico *et al.*, 2011; Bystrzejewska-Piotrowska *et al.*, 2012b), but the understanding of details of Al2O3-NPs accumulation by plants is still limited.

The aim of the present study was to investigate the effects of size of Al_2O_3 particles (nano or micro), their concentration in medium and type of medium (solid or liquid) on aluminium accumulation by different plant species and its distribution among major plant organs. The ability of four plant species to accumulate nanoparticles was investigated with an eye on possible application in phytoremediation of contaminated environment and the potential risk of nanoparticles entering the food chain.

MATERIAL & METHODS

Chemicals. Al_2O_3 microparticles (MPs) (aluminium oxide powder, < 10 μm), nanoparticles (NPs) (aluminium oxide nanopowder, < 50 nm) and nanowhiskers (NWs) (aluminium oxide nanopowder, whiskers, 2-4 nm × 200-400 nm) as well as aluminium nitrate nonahydrate were purchased from Sigma-Aldrich. Decomposition of plant material was carried out with 65 % HNO_3, 70 % $HClO_4$ and 37 % HCl (all Suprapur, Merck). Chemicals used for preparation of nutrient solutions were from POCh, Poland. For the preparation of all solutions 18 MΩ cm^{-1} Milli-Q water (Millipore, USA) was used.

Allium cepa, Onion – popular vegetable of well known biology, readily available and easy to cultivate under laboratory conditions. Healthy and equal-sized (diameter 1.6 – 1.8 cm) bulbs of *Allium cepa* from local market were selected for the studies. The scales of the bulbs were removed and the upper bulb portions were cut off gently. The bulbs were cultivated for 7 days in 120 mL containers (three bulbs per container) with distilled water (control) or with water supplemented with aluminium compounds (aluminium oxide NPs, NWs, MPs or aluminium salt). The pH of growth medium, controlled during cultivation, was 5.6 ± 0.3. Three concentrations of the aluminium compounds were used – 0.1, 1 and 10 g/L; the growth medium was stirred. For the concentration of 1 g/L cultivation on non-stirred medium was performed additionally.

Zea mays, Maize – widely cultivated throughout the world as a grain crop, adapts well to diverse environmental conditions. Maize seeds were left to germinate on moist perlite and seedlings at the cotyledon stage were placed in 5 L containers (25 plants in each) with a nutrient solution containing: $Ca(NO_3)_2$ - 1003 mg/L, KNO_3 - 583 mg/L, $MgSO_4$-513 mg/L, KH_2PO_4 - 263 mg/L, NH_4NO_3 - 488 mg/L, $MnSO_4$ - 6.1 mg/L, H_3BO_3 - 1.7 mg/L, $Na_2MnO_4 \cdot 2H_2O$- 0.37 mg/L, FeNa EDTA - 79.0 mg/L, $CuCl_2 \cdot 2H_2O$ - 0.39 mg/L, $ZnSO_4$ - 0.44 mg/L. To the nutrient solution, aluminium compounds were added to obtain the three concentrations as above. The pH of growth medium, controlled during cultivation, was 5.9 ± 0.4. Cultivation in medium without aluminium added was performed simultaneously. The medium was stirred and aerated for the whole time of cultivation. Plants were cultivated in a growth chamber for 14 days.

Lepidium sativum, Cress – edible plant of by well known biology, suitable for cultivation under laboratory conditions, accumulates many contaminants. Plant cultivation was performed in porcelain containers, each containing 7 g of soil. Four variants, differing in the amount of an Al compound added to the soil were used: control (soil without Al addition) and variants with Al_2O_3-NPs or Al_2O_3-MPs (concentrations of 1, 10 or 100 g/kg).

Seeds of *L. sativum*, were soaked for 1 h in deionised water, and than sown on the soil surface, 15 seeds per container. During the experiment equal and constant water volume was added to each container. Cultivation was conducted at room conditions for 7 days.

Kalanchoe daigremontiana, Alligator Plant or Mexican Hat Plant – tropical plant, rich in micro- and macro- elements, used in medicine, characterized by good resistance to adverse physical and chemical conditions.

Seeds of *Kalanchoe daigremontiana* were left for 2 weeks to germinate on moist perlite. After that time the plants were transferred to the porcelain containers, each containing 50 g of soil (three plants per container) supplemented with Al_2O_3-NPs nanoparticles at concentrations 2, 5 or 10 g/kg or medium without Al_2O_3-NPs addition as a control. Cultivation was carried out for the next 3 months.

All cultivations were performed in a in greenhouse in terms 16 h light and 8 h night by relative humidity of air 50%. The soil, used for *L. sativum* and *K. daigremontiana* cultivation was characterized elsewhere (Bystrzejewska-Piotrowska, 2012a). Following cultivation the plants were gently removed from the containers and roots were rinsed with distilled water. Plants were then divided into roots, bulbs and assimilation leaves (*A. cepa*); roots and shoots (*Zea mays* and *L. sativum*) and roots, stems and leaves (*K. daigremontiana*). The number and the length of the

roots were measured. The plant material was dried for 48 h at 60°C. The dry plant material was ground in a mortar before further analysis. The soil samples were likewise dried and ground.

Determination of total aluminium content. About 250 mg of dried plant material was digested with a mixture of 2.5 mL HNO_3 and 0.5 mL $HClO_4$ using microwave laboratory system ETHOS 1 with ATC-400-CE automatic temperature control (Milestone, Italy). After digestion samples were quantitatively transferred into volumetric flasks (25 mL). Samples were analyzed by ICP MS (ELAN 6000 ICP mass spectrometer (PE-SCIEX, Concord, Canada)). Before analysis samples were diluted with water and acidified with nitric acid to obtain an approx. 2 % concentration of the acid.

Transmission electron microscopy analysis. NP and -MP solutions at the Al_2O_3 concentration of 10 g/L were used for microscopic characterization in a LEO 912AB transmission electron microscope equipped with a Proscan High Speed Slow Scan CCD camera. One drop of suspension was placed on a formvar coated grid and dried. To obtain images a was used. Plants from soil spiked with Al_2O_3-NPs at a aluminium dose of 10 g/kg were chosen for electron microscope analysis. Root samples of seven-day-old plants were fixed with 3% glutaraldehyde in 0.1 mol L^{-1} cacodylate buffer (pH 7.2) for 24 h at 4ºC, rinsed five times with the cacodylate buffer, then dehydrated stepwise in an ethanol solutions series of 30, 50, 70, 90, 96 and 100 %, 15 min per step. Finally, the samples were dehydrated twice for 5 minutes in acetone, embedded in epoxy resin, polymerized and hardened at 60ºC. Ultrathin sections (70 nm) were obtained with MTX ultramicrotome (RMC, Japan), placed on a copper grid and viewed in the electron microscope specified above.

Extraction. Aluminium was extracted from soil samples by elution with water or 0.05 mol/L EDTA. For this purpose, 1.0 g of soil was mixed with 10 mL of extracting agent and shaken on a reciprocating shaker for 1 h at room temperature. Afterwards, the suspension was filtered and ultracentrifuged. Three replicates per each extraction variant were prepared. The aluminium concentration in the extracts was measured using ICP-MS spectrometer specified above.

RESULTS & DISCUSSION

Accumulation of aluminium by hydroponically cultivated onion and maize. The investigated plants accumulated aluminium originating from Al_2O_3 but the magnitude of the accumulation depended profoundly on the Al_2O_3 particle size and shapes and concentration (Table 1). In above-ground organs of the onion higher concentration of aluminium was found in bulbs in comparison with green leaves. In the variant with MPs the aluminium concentration in bulbs was similar as for control samples, indicating that MPs were in essence not accumulated in the bulbs. In plants cultivated with NPs the aluminium concentration was significantly higher than in the corresponding samples from plants cultivated with MPs. With an increasing concentration of NPs or MPs in the medium, the aluminum concentration in the green leaves also increased. The highest concentration of aluminum in the green leaves was observed for the nanowhiskers at the highest applied concentration. For Al_2O_3 particles at 10 g/L in the medium it was 9 and 3 times higher for nanowhiskers than for microparticles and nanoparticles, respectively. The Transfer Factor (TF; the ratio of concentration of Al in plants (mg/kg) to concentration of Al in growth medium (mg/L)) decreased with increasing concentration of aluminum oxide particles in growth medium. For the highest applied concentration of Al_2O_3 (10 g/L) the TF for MPs, NPs and NWs was respectively 0.008, 0.077 and 0.031 for bulbs and 0.005, 0.017 and 0.048 for green leaves. One can thus conclude that higher content of aluminum oxide in the medium inhibits accumulation of aluminum in above-ground organs of *Allium cepa* L. the higher values of TF for NPs than for MPs prove that the particle size affects accumulation of aluminum. Additionally, some effect of the particle shape on the aluminum accumulation and transport to above-ground organs was also observed. For the lower particle concentrations, the TF for *A. cepa* leaves was higher for NPs than for NWs. For the highest particle concentration investigated, the reverse was observed. The efficiency of aluminum transport to leaves was the highest for NWs at 10 g/L. The shape of the whiskers, much like nanotubes, allows their fairly easy transport to the leaves. Nanowhiskers, not only are transported to the leaves more easily than nanoplates, but are potentially more toxic, because they can pierce the cell membrane and damage the cells (Kirchner *et al.,* 2005).

Nanostructures easily sediment to the bottom of the container so the question arises as to whether they are still available to plants. A simple experiment was performed to answer that question, namely additional cultures were performed whiteout stirring of the medium. The sedimentation of NPs was observed within several hours. As expected, the aluminium concentration in plant tissues was substantially lower than that found were the medium was constantly stirred (Fig. 1). Stirring did not effect aluminium accumulation by plants exposed to aluminium salt. One can conclude that nanoparticles, deposited on the bottom of the reservoir, are still source of aluminium for plants.

A similar effect of particle concentration and size on aluminum accumulation was also observed for maize. Aluminum content in shoots of maize (Table

Table 1. Concentrations of Al and transfer factors for Allium cepa and Zea mays grown in liquid medium

	Al_2O_3 concentration in medium (g/L)	Concentrantion of Al (mg/kg) ***Transfer factor***			
		Allium cepa		*Zea mays*	
		leaves	bulbs	shoots	roots
control	0	5.2 ± 1.1	44.4 ± 5.5	22.2 ± 1.7	42.4 ± 14.3
Al_2O_3 MPs	0.1	7.0 ± 1.1	28.0 ± 2.9	22.8 ± 8.8	444.2 ± 139.9
		0.129	***0.510***	***0.422***	***8.226***
	1	24.9 ± 3.4	51.8 ± 3.3	24.9 ± 1.2	902.6 ± 44.2
		0.046	***0.096***	***0.046***	***1.671***
	10	28.7 ± 3.9	44.6 ± 5.8	40.8 ± 2.6	1244.5 ± 51.2
		0.005	***0.008***	***0.008***	***0.230***
Al_2O_3 NPs	0.1	20.5 ± 2.6	48.4 ± 7.9	106.4 ± 5.1	5798 ± 2648
		0.380	***0.896***	***1.970***	***107.4***
	1	89.5 ± 11.0	284.8 ± 31.3	186.0 ± 58.0	8554 ± 1739
		0.166	***0.527***	***0.344***	***15.84***
	10	89.4 ± 11.6	414.9 ± 57.2	1107 ± 656	25737 ± 1959
		0.017	***0.077***	***0.205***	***4.766***
Al_2O_3 NWs	0.1	6.4 ± 0.5	63.8 ± 6.4	ND	
		0.119	***1.181***		
	1	12.9 ± 1.4	54.4 ± 9.8	ND	
		0.024	***0.101***		
	10	256.7 ± 16.7	165.2 ± 27.8	ND	
		0.048	***0.031***		

ND – not done

1) grown on the suspension of MPs was, for low concentrations of Al_2O_3 (0.1 and 1 g/L), at the level found in control samples, and for the highest concentration of Al_2O_3 (10 g/L) it was 2 times higher, while for NPs, the aluminum content in shoots was definitely higher than for control and exceeded 1.1 g/kg.

The aluminium content was also determined in maize roots and for all the variants its concentration was significantly higher than for the control plants (from 10 to 30-fold for cultivation with MPs and from 138 to 613-fold for NPs). For the variant with the highest concentration of aluminium oxide NPs, the aluminium content in maize roots reached 25 g/kg. That high content of aluminium determined in roots is a consequence of adsorption of nano- and microstructures on the root surface.

For maize, as for onion, the values of TF decreased with increasing Al_2O_3 concentration in the growth medium. The TFs for shoots and roots of maize were higher in plants cultivated with NPs in comparison with MPs. This confirms the influence of particle size on the efficiency of aluminium accumulation in plants. The concentration of aluminium in the investigated plants was relatively high and therefore some phytotoxic effects could be expected. As the best toxicity test for hydroponically cultivated plants in short-time experiments changes in root length were chosen (Poschenrieder *et al.*, 2008). The length of the roots in *A. cepa* increase insignificantly with increasing concentration of MPs of Al_2O_3 (from 2.5 ± 2.1cm to 6.8 ± 4.0 cm) and in the case of NPs it was virtually unaffected and averaged 2.8 ± 0.6 cm (for control - 2.8 ± 2.0 cm). Some differences between variants were observed for the length of roots in *Z. mays*. For the lowest concentration of MPs the length of roots was 33.7 ± 10.1 cm, while for control plants and other Al_2O_3 concentrations it was 21.1 ± 1.8 cm. In variants with NPs, plants cultivated with the lowest Al_2O_3 concentration (0.1 g/L) and control plants had similar root length (31.8 ± 14.9 and 35.9 ± 16.4 cm), while for the NP concentration of 1 g/L the average root length was higher (45.3 ± 4,0). NPs at 10 g/L caused inhibition of root elongation (root length averaged 20.0 ± 3.0 cm). The highest applied concentration of Al_2O_3 was about 5 times higher than that reported to be toxic for some plants by Yang and Watts (2005) and Lin and Xing, 2007 (2 g/L). The effect of Al_2O_3 on root elongation can be due to two factors. One is the toxic influence of aluminium ions. As the solubility of Al_2O_3 under the applied conditions is negligible, this seems unlikely. The second possibility is an effect caused by Al_2O_3 particles adsorbed on the root

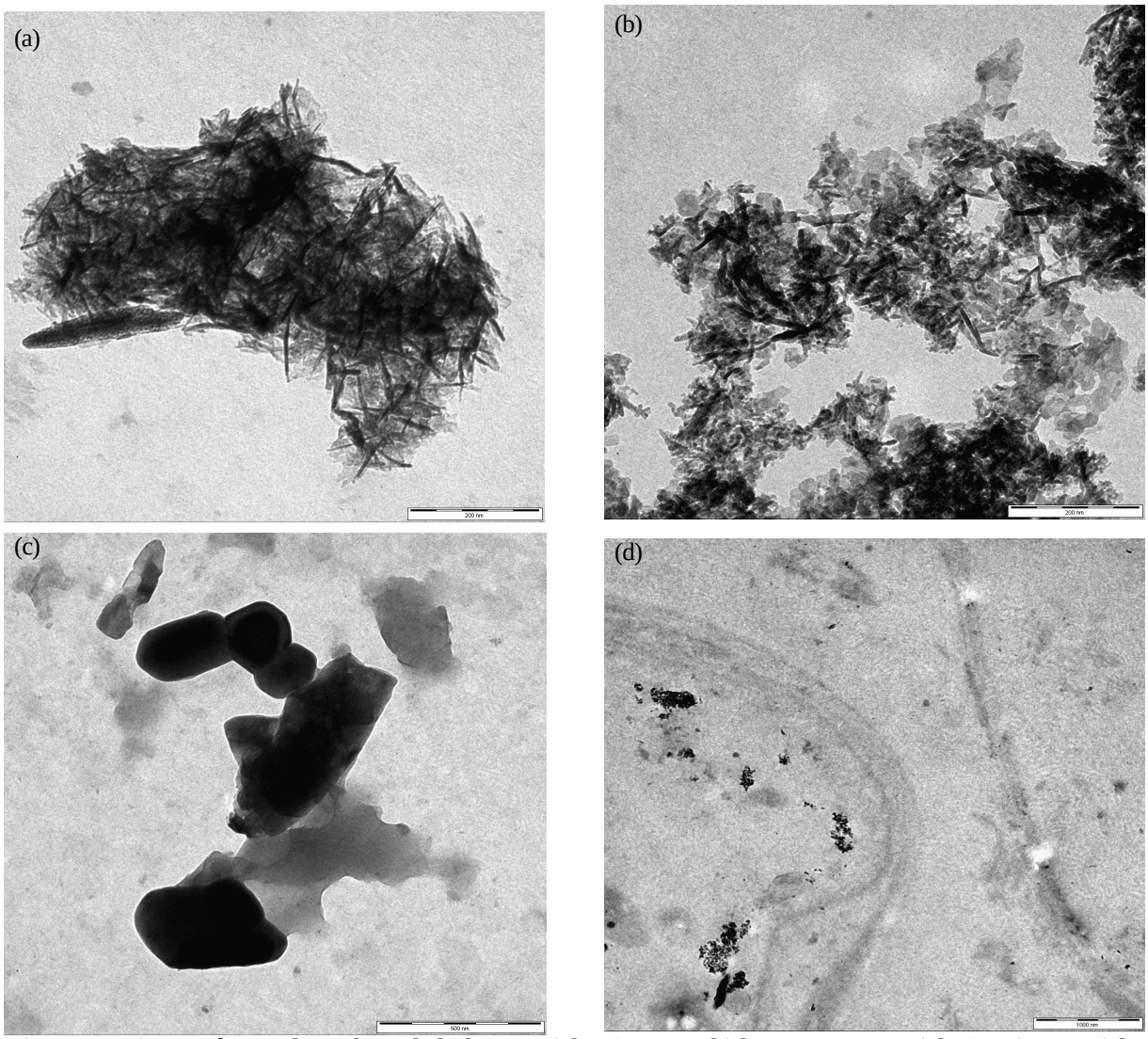

Fig. 1. TEM images free and root-bounded Al_2O_3 particles. A - nanowhisker, B – nanoparticle C - microparticle suspensions in water; D - agglomerates of nanoparticles Al_2O_3 located close to the epidermis of Lepidium sativum root cells. Scale bar is 200 nm (A, B); 500 nm (C) and 1000 nm (D)

surface, which could disturb the root functions. The relatively high concentrations of aluminium found in plants grown in the presence of MPs, NWs and NPs of Al_2O_3 combined with the negligible solubility of aluminium oxide strongly suggests accumulation and transport of intact particles of Al_2O_3. The lower size of NPs and NWs compared with MPs explains their more effective uptake and higher levels of aluminium in plants tissues.

Water extractability of Al from soil studied after completion of *L. sativum* cultivation, in the case of both MPs and NPs, did not depend on the Al_2O_3 concentration in the soil, was relatively low (8.87 ± 0.87 and 8.80 ± 6.08 mg/kg, respectively) and did not differ significantly from that for control samples (8.0 ± 1.4 mg/kg). The amount of aluminium in the bioavailable (EDTA-extractable) form was higher in comparison with the water-soluble fraction. It amounted to 150 ± 12 mg/kg for control soil and 143 ± 6 for soil contaminated with MPs, independently of Al_2O_3-MP concentration. In soil contaminated with nanoparticles 134.3 ± 40.4, 158.1 ± 18,5 and 540.1 ± 72.7 mg/kg aluminium was determined in EDTA-extractable fraction for 1, 10 and 100 g/kg Al_2O_3 NP concentration, respectively. Thus, the bioavailability of Al was significantly higher (~3.6 fold) only in the case of Al_2O_3-NPs at 100 g/kg. It was at the control level in the case of the lower NP concentrations and all MP concentrations. This leads to the conclusion that in those variants soil itself rather than the Al_2O_3 added was the main source of Al in the bioavailable and water-extractable fraction.

The water solubility and bioavailability of aluminium from soil assayed following *K.*

daigremontiana cultivation was in general similar to therefore *L. sativum* soil. The water soluble fraction was 3.88 ± 0.32 and 14.3 ± 2.6 mg/kg for MP and NP contaminated soil, respectively. Aluminium content in the EDTA-extractable fraction was independent of particle concentration for soil contaminated with MPs and averaged 143 ± 6 mg/kg. In soil with NPs, the amount of aluminium in the bioavailable fraction depended slightly on the Al_2O_3 concentration (173.6 ± 9.1, 250.1 ± 16.4 and 369.1 ± 19.3 mg/kg for 1, 10 and 100 g/kg^{-1} Al_2O_3-NP in soil, respectively). The extractability of aluminium in control samples was close to level of MP contaminated soils.

In the case of EDTA-extractability was higher than in control samples, suggesting that Al_2O_3 NPs are a better source of aluminium in comparison with MPs. Additionally, some influence of cultivation duration and plant species on aluminium bioavailability can be observed. As it was shown in our previous studies, bioavailability of metals originating from NPs depends on the soil-particles interaction time and the presence of earthworm *Dendrobeana veneta* (Bystrzejewska-Piotrowska, 2012a).

The aluminium content in roots of *L. sativum* cultivated in soil spiked with MPs was about two times higher than in the case of control plants and was concentration-independent (Table 2). In plants from soil with the lower Al_2O_3-NP concentrations, Al content in the roots increased with the NP concentration. In shoots, significantly higher Al concentrations were observed only in plants cultivated in soil spiked with NPs. The corresponding concentrations were 4.9, 6.2 and 7.3 times lower than in roots of plants from soil with NPs at 1, 10 and 100 g/kg, respectively. These results show that roots are the main plant organ where Al is accumulated.The transfer factors (TF) calculated as the ratio of Al concentration in roots to the concentration of bioavailable Al (EDTA-extractable) are presented in Table 2. In the case of MPs, the TF did not depend on the particle soil concentration. This means that the Al concentration in roots of plants from soil with MPs depends only on the EDTA-extractable Al concentration. In the case of plants from soil spiked with NPs, there is a positive correlation between the TF values and particle concentration.

The results confirm the hypothesis that plants are able to take up NPs from soil by roots and translocate them to shoots. It was also shown that root accumulation of NPs increased with increasing concentration of NPs in the soil. The presence of nanoparticles in roots was confirmed using transmission electron microscopy. The picture of an *L. sativum* root (Fig. 2) clearly shows associated agglomerates of Al_2O_3-NPs, with a structure similar to the one found for a suspension of Al_2O_3-NPs. The same shape, size and constitution of Al_2O_3-NP agglomerates can be seen. The TEM analysis of suspensions of Al_2O_3 (Fig. 1) shows evident aggregation of NPs and NWs outside the nanoscale range, which does not, however, preclude their uptake by plants and transport to above-ground organs more effective than for MPs.

Although the nanoparticle concentrations used here were higher than those described in the literature (2 g/L, Yang and Watts (2005) Lin and Xing, (2007) or 4 g/L, Lee *et al.,* 2010), they did not affect root

Table 2. Concentration of Al and transfer factors* for L. sativum growing on the soil

	Al_2O_3 concentration in medium (g/kg)	Concentrantion of Al (mg/kg) ***Transfer factor****	
		stems	roots
control	0	4.5 ± 0.5	17.6 ± 5.7
Al_2O_3 MPs	1	2.4 ± 0.3 ***0.016***	32.6 ± 2.7 ***0.223***
	10	4.9 ± 0.4 ***0.036***	27.6 ± 2.8 ***0.203***
	100	4.3 ± 0.6 ***0.029***	27.8 ± 2.3 ***0.183***
Al_2O_3NPs	1	11.6 ± 1.5 ***0.086***	56.5 ± 5.7 ***0.421***
	10	49.2 ± 6.6 ***0.311***	306.1 ± 37.7 ***1.936***
	100	561 ± 72.5 ***1.038***	4077 ± 285 ***7.549***

* Transfer factor defined as ratio of aluminum content in plants (mg/kg dry weight) to bioavailable (EDTA-extractable) aluminum content in soil (mg/kg dry weight)

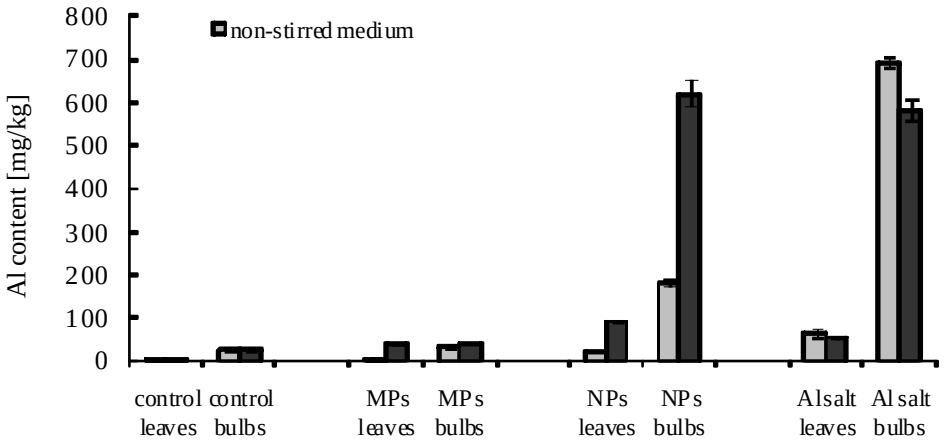

Fig. 2. Concentration of Al in the leaves and bulbs of Alium cepa grown in liquid medium

Table 3. Concentrations of Al and transfer factors for K. daigremontiana growing on the soil

	Al_2O_3 concentration in medium (g/kg)	Concentrantion of Al (mg/kg) ***Transfer factor****		
		leaves	stems	roots
control	0	9.32 ± 0.72	12.4 ± 1.6	190.0 ± 9.3
Al_2O_3 MPs	2	19.3 ± 1.6	31.4 ± 1.9	302.0 ± 31.7
		0.1608	**0.2595**	**2.4959**
	5	16.2 ± 1.2	16.0 ± 1.6	242.2 ± 10.9
		0.1339	**0.1322**	2.0016
	10	14.0 ± 1.2	14.9 ± 1.3	279.9 ± 28.0
		0.1094	**0.1164**	**2.1867**
Al_2O_3 NPs	2	10.9 ± 1.0	13.0 ± 1.0	353.8 ± 30.4
		0.0626	**0.0747**	**2.0333**
	5	12.3 ± 1.0	33.6 ± 3.8	506.1 ± 50.6
		0.0492	**0.1344**	**2.024**
	10	11.6 ± 0.9	22.0 ± 2.0	754.9 ± 36.2
		0.0314	**0.0596**	**2.0458**

* Transfer factor is defined as ratio of aluminum content in plants (mg/kg dry weight) to bioavailable (EDTA-extractable) aluminum content in soil (mg/kg dry weight)

elongation. No effects of the particles on root and shoot dry masses could be observed for the lower concentrations, only at the highest Al_2O_3-NP concentration (100 g/kg), a 33% decrease of root dry mass was noticed. This indicates that *L. sativum* plants are tolerant to a wide range of Al_2O_3-NPs soil concentrations.

Kalanchoe daigremontiana was found to be tolerant to Al_2O_3 presence in the soil - for all applied concentrations no toxic effects were observed. The mode and efficiency of aluminum accumulation were different than in the case of *L. sativum*. Aluminium content in leaves and stems of *K. daigremontiana* was independent of the Al_2O_3 concentration in soil or the particle size and averaged 14 and 22 mg/kg, respectively (Table 3). For roots of plants cultivated in soil contaminated with MPs of Al_2O_3, aluminium content was ca. 270 mg/kg, independently of the soil MPs content. The mean values were higher than those obtained for control samples. In contrast for *K. daigremontiana* cultivated in soil contaminated with NPs, aluminum concentration in roots depended significantly on the aluminum content in the EDTA-extractable (bioavailable) fraction. The transfer factor was in this case constant and amounted to 2. This suggests that *K. daigremontiana* does not accumulate nanoparticles, but only the aluminum present in a bioavailable form.

CONCLUSION

Presented results show that plants are able to accumulate NPs from water and soil and the process of NP uptake depends greatly on the plant species and the size and shape of the NPs. Future developments of phytoremediation techniques of contaminated soils and waters should be conducted with those specificities in mind.

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