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Application of neutron activation for investigation of Fe_3O_4 nanoparticles accumulation by plants

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Abstract. As a result of the rapid development of nanotechnology and increasing application of nanoproducts in many areas of everyday life, there is a growing risk of production of nanowastes potentially dangerous for the environment. This makes it necessary to investigate the accumulation and toxicity of nanoparticles (NPs) at different trophic levels. In the studies neutron activation was applied for the investigation of iron (II,III) oxide nanoparticle (Fe_3O_4-NPs) accumulation by *Lepidium sativum* and *Pisum sativum* L. Plants were cultivated on growth medium contaminated with different concentrations (0.01–10 mmol·L^{-1}) of Fe_3O_4-NPs. For the identification of the presence of Fe_3O_4-NPs in plant tissues gamma spectrometry following iron oxide (II,III) nanoparticles irradiation was applied. Both plant species were found to accumulate iron (II,III) oxide nanoparticles. The highest content of NPs was found in plant roots, reaching 40 g/kg for *Pisum sativum* L. More than 90% of accumulated NPs were found in roots. Accumulation of Fe_3O_4-NPs was found to depend on the concentration of nanostructures in the growth medium. The transfer factor for *Lepidium sativum* roots and shoots and *Pisum sativum* L. shoots decreased with increasing NP concentration in the medium; for *Pisum sativum* L. roots the tendency was reversed. Neutron activation of nanoparticles was shown to be a powerful tool for tracing the environmental fate of NPs and their uptake and accumulation in organisms.

Key words: iron (II,III) oxide • nanoparticles • neutron activation • plants

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Introduction

Since particles with dimensions in the nanoscale (nanoparticles, NPs) (ISO/TS 27687:2008), were found to exhibit novel physical, chemical and biological properties, different from their larger counterparts, nanotechnology became rapidly expanding field of technology and nanoproducts are used in many areas of everyday life [6]. One of the consequences of increasing implementation of nanotechnologies is the risk of creating a new generation of waste (nanowaste) and new potential threats to the environment [2], so there is a growing need for the investigation of the accumulation and toxicity of NPs at different trophic levels.

One of the most useful and interesting are magnetic nanoparticles, in particular iron (II,III) oxide nanoparticles (Fe_3O_4-NPs), magnetite. The synthesis of monodisperse nanocrystals of Fe_3O_4 has long been of scientific and technological interest. The compound exhibits unique electric and magnetic properties based on the transfer of electrons between Fe^{2+} and Fe^{3+} in the octahedral sites. Interest in magnetite has focused on applications such as magnetic storage devices, ferrofluids, sensors, spintronics, separation processes, MRI (magnetic resonance imaging) contrast enhancement agents, biomedical fields and especially in environmental remediation [1, 3, 4]. Properly coated or surface-modified magnetite nanoparticles can be applied in

clinical diagnosis and as pharmaceutical transporters i.e. in the field of medicine [5].

The knowledge about the accumulation and effects of iron (II,III) oxide nanoparticles on plants is very small and only a few studies have been undertaken in this field [8, 9]. Accumulation of magnetite (Fe_3O_4) nanoparticles by pumpkin plants (*Cucurbita maxima*) [8, 9] and ryegrass (*Lolium perenne* L.) [8] was investigated using hydroponics cultivation. Contradictory results were obtained in the case of pumpkin plants for similar iron (II,III) oxide nanoparticle concentrations in the growth medium, using similar detection methods (magnetometry measurements). Zhu *et al*. [9] have shown that Fe_3O_4-NPs are accumulated and transported to above-ground organs while Whang *et al*. [8] demonstrated that magnetite is not accumulated by pumpkin plants as well as by ryegrass.

In our work accumulation of Fe_3O_4-NPs by *Lepidium sativum* and *Pisum sativum* L., growing on aqueous medium containing iron (II,III) oxide nanoparticles was investigated. For the identification of the presence of Fe_3O_4-NPs in plant tissues gamma spectrometry following iron irradiation was applied. One of the main problems of investigation of NP uptake in plants is the lack of methods allowing confirmation of the presence of NPs or ions originating from NPs in plant tissues. Determination of the total amount of an element in plants does not give information about the source of the investigated element. The proposed method of neutron activation of nanoparticles, which does provide this information, is an excellent tool for tracing the environmental fate of NPs and their uptake and accumulation in organisms [7]. The main goal of the work was determination of Fe nanoparticles accumulation ratio for the investigated plants (Transfer Factor).

Materials and methods

Iron (II,III) oxide nanoparticles (Fe_3O_4, nanopowder, spherical, particle size < 50 nm (TEM) surface area BET surf. area > 60 m^2/g) were purchased from Sigma-Aldrich. The particle size and morphology were assessed using transmission electron microscope TEM LEO 912AB (Zeiss) equipped with Proscan High Speed Slow Scan CCD-camera. TEM analysis was performed using 1 $mmol \cdot L^{-1}$ water suspensions.

For the irradiation, samples of Fe_3O_4 nanoparticles (80–100 mg) were weighed directly into HDPE snap-cap capsules (Faculteit Biologie, Vrije Universiteit, Amsterdam), wrapped in aluminium foil and irradiated in the nuclear reactor MARIA (Świerk, Poland), for 10 min at a thermal neutron flux of 10^{14} $cm^{-2} \cdot s^{-1}$. After 10 days of cooling the samples were unwrapped and used for plant cultivation. The purity of irradiated nanoparticles was confirmed using gamma-ray spectrometer GENIE-2000 Canberra Gamma Spectrometry System with HPGe detector (Canberra), active volume 255 cm^3, well type, well diameter 16 mm and depth 40 mm, resolution 2.4 keV for the 1332.4 keV peak of ^{60}Co, relative efficiency 24%.

The *Lepidium sativum* plants were cultivated in 300 mL containers (50 plants/container) with distilled water (control) or with water supplemented with iron (II,III) oxide nanoparticles. The NP concentrations were 0.01, 0.1, 0.5 and 4 $mmol \cdot L^{-1}$. *Pisum sativum* L. was cultivated in 400 mL containers (4 plants/container). Concentration of NPs was 4 and 10 $mol \cdot L^{-1}$. Three replicates per each variant were prepared. During the experiment equal and constant water volume was added to each container. Cultivations were conducted at room conditions.

After 7 days of cultivation the plants were harvested, roots were washed with deionised water and plants were divided into roots and shoots. The shoot and root lengths of particular plants were measured. Plants were oven dried at 60°C for 72 h and dry mass was estimated. The level of ^{59}Fe radionuclide was determined by means of a gamma spectrometer with HPGe detector (Canberra Packard). The calculation of the activity of radioisotope Fe-59 was based on energy calibration, and the efficiency calibration, which were made for summary characterization of HPGe coaxial detector. Both energy, and efficiency calibration curves are fixed in GENIE 2000 software, and used on demand whilst spectrum analysis. Energy calibration was made with use of multi gamma source (SZM-3, Certificate No JM/11/Z/00). This calibration determined the positions of Fe-59 lines on the spectrum. Efficiency calibration was made with use of IAEA reference material IAEA-375 (soil) with the density most similar to the Fe_3O_4 nanoparticles powder. Activity of Fe-59 was calculated with use of GENIE 2000 software and it's built-up libraries.

The vessels containing dry samples (of known mass and comparable density) were put directly on the detector surface. All the measurements were carried out in the comparable geometry. The results were corrected for half-time of iron radioisotope. Single point efficiency calibration, containing ^{59}Fe line, was involved to measure pure activated Fe_3O_4 nanoparticles activity (weights of the samples were ranging from 100 to 400 mg). On the basis of the obtained results, the mean activity 21.74 ± 1.00 $kB/g_{Fe3O4\text{-}NPs}$ was determined.

All results are expressed as means ± standard deviations, $n = 3$. Analysis of variance (ANOVA) was used to determine statistical significance of the differences between values. The level of significance was accepted at $P \leq 0.05$.

Results and discussion

Nanoparticles of Fe_3O_4 were characterized using transmission emission microscopy (Fig. 1). The major part of particles is spherical and their size does not exceed 50 nm, what is in agreement with the declaration of the supplier.

During the studies it was found that *Lepidium sativum* and *Pisum sativum* L. are tolerant to the applied, relatively high concentrations of Fe_3O_4-NPs. Nanoparticles of iron (II,III) oxide were irradiated in nuclear reactor MARIA. During irradiation ^{59}Fe (T = 44.5 days, E_{gamma} = 1099.2, 1291.6 keV) is formed and applied for investigation. No negative effects were observed, however, detailed tests of the plant conditions were not the subject of the investigations. Both investigated plant species accumulated iron (II,III) oxide NPs. Significantly elevated activity was measured in

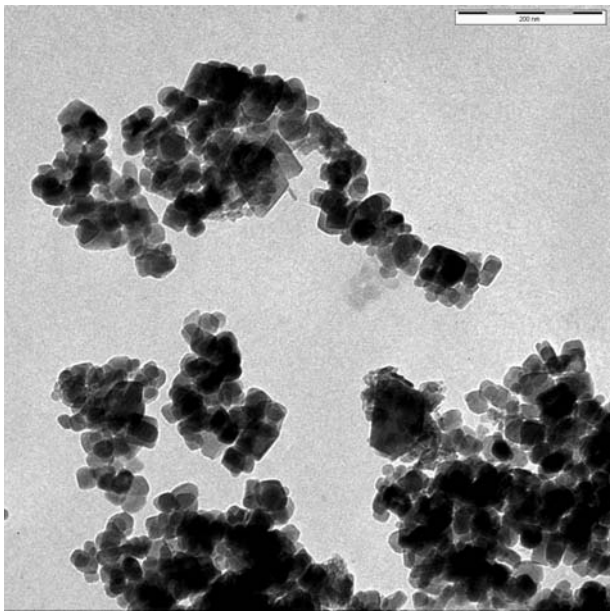

Fig. 1. Transmission-electron micrographs of Fe_3O_4 nanoparticles (1 mmol·L^{-1} suspension in water) used in neutron-activation experiments and plants cultivation.

roots and shoots of plants cultivated in the presence of Fe_3O_4-NPs, simultaneously no activity was detected in control samples. Measured activity was within the range 0.5 Bq/g of dry mass for the *Lepidium sativum* shoots (NP concentration in growth medium 0.01 mol·L^{-1}) to 8027 Bq/g for roots of *Pisum sativum* L. (NP concentration in growth medium 10 mol·L^{-1}). It was necessary to recalculate all measured activities (A_S) taking into consideration the half-life of the iron isotope ^{59}Fe (T) and the total time of the experiments (Δt), according to the equation: $A_{0S} = A/e^{(-0.693\cdot\Delta t)/T}$ (A_{0S} – calculated activity of the samples). On the basis of the activities recalculated for the samples (A_{0S}), the amount of Fe_3O_4 nanoparticles in plants ($C_{Fe_3O_4\text{-NPs}}$) was calculated, using equation:

$$C_{Fe_3O_4-NPs}\ [mg_{Fe_3O_4-NPs} / kg_{SAMPLE}] = A_{0S}\ [kBq / kg_{SAMPLE}] \times 1000 / 21.74\ [kBq / g_{Fe_3O_4-NPs}]$$

The highest concentration of iron (II,III) oxide nanoparticles was found in the plant roots (Table 1) and for *Lepidium sativum* it was from about 0.3 to nearly 8 g·kg^{-1}. In the case of *Pisum sativum* L. the amount of iron oxide taken up was much less and for the concentration of Fe_3O_4-NPs in growth medium 4 mmol·L^{-1} was more than 13 times lower than for *Lepidium sativum*. For the highest concentration of Fe_3O_4-NPs in growth medium (10 mmol·L^{-1}) the nanoparticle concentration in *Pisum sativum* L. roots reached the value of 40 g·kg^{-1}. Relatively high concentration of iron in roots was caused by adsorption of particles at the root surface. After finishing the cultivation it could be observed that particles are strongly adsorbed at the surface of the roots and the binding of NPs with the components of the roots is so strong that it was impossible to remove adsorbed particles by flushing the roots with water.

The concentration of Fe_3O_4-NPs in the plant shoots was much lower than in roots. For *Lepidium sativum* the amount of nanoparticles accumulated in shoots increased with concentration in growth medium (from 2.44 to 40.3 μg·kg^{-1}). An inverse tendency was observed for *Pisum sativum* L. – concentration of Fe_3O_4-NPs in shoots decreased from 20.1 to 4.59 μg·kg^{-1} (the NP concentration in growth medium was 4 and 10 mmol·L^{-1}, respectively).

Differences in accumulation of Fe_3O_4-NPs as a function of particle concentration in growth medium can be observed regarding Transfer Factors (TF). TF, here defined as the ratio of concentration of Fe_3O_4-NPs in plants (mg·kg^{-1}) to the concentration of Fe_3O_4-NPs in growth solution (mg·L^{-1}), describes the movement and distribution of particles from growth medium to roots or shoots of the plants. In the case of *Lepidium sativum* the strong, exponential correlation of the values of TF with concentration of particles in growth medium can be found (Fig. 2), both for roots and shoots. TFs decrease with increasing concentration of Fe_3O_4-NPs in medium. It is worth noticing that the TF values are relatively high for roots (Table 1) and for the lowest concentration of Fe_3O_4-NPs in the medium it reached 140. For shoots the Transfer Factor values are at least two orders of magnitude lower. This can be due to two factors. First, Fe_3O_4-NPs are accumulated in the roots and transport to above-ground organs is inhibited. Second, relatively low accumulation of Fe_3O_4-NPs in shoots can be caused by absorption of NPs on the root surface, making further uptake of particles impossible.

As a consequence, 98.5 ± 1.3% of Fe_3O_4-NPs accumulated by *Lepidium sativum* was found in the roots,

Table 1. Fe_3O_4-NPs content [mg·kg^{-1} dry weight] and transfer factors for *Lepidium sativum* and *Pisum sativum* L. (Results are presented as means ± standard deviations, n = 3)

Concentration of Fe_3O_4 in growth medium (mmol·L^{-1})	Concentration of Fe_3O_4-NPs in plants (mg·kg^{-1} d.w.)		Transfer Factor	
	roots	shoots	roots	shoots
Lepidum sativum				
0	–	–	–	–
0.01	323 ± 14	2.44 ± 0.26	139.2	1.054
0.10	1 685 ± 59	3.20 ± 0.67	72.6	0.138
0.50	2 033 ± 71	17.1 ± 1.4	17.5	0.148
4.0	7 805 ± 254	40.3 ± 2.8	8.4	0.043
Pisum sativum L.				
0	–	–	–	–
4.0	604 ± 25	20.1 ± 1.7	0.7	0.018
10	39 533 ± 1 243	4.59 ± 0.21	17.0	0.002

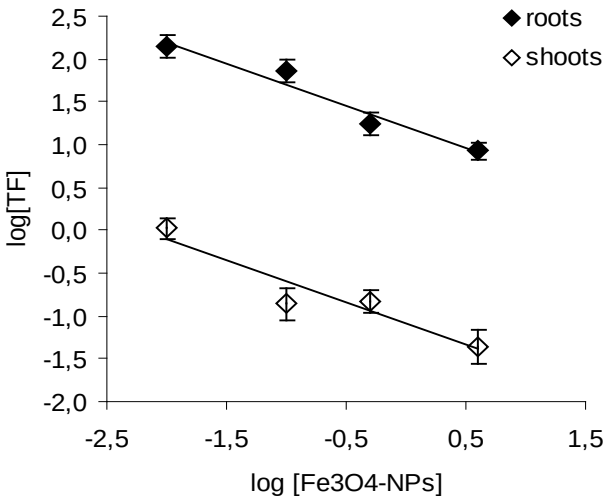

Fig. 2. Correlation of logarithms of Tranfer Factors (log[TF]) and concentration of Fe_3O_4 nanoparticles in growth medium (log[Fe_3O_4-NPs]) for roots and shoots of *Lepidium sativum.*

practically independently on the iron (II,III) oxide nanostructure concentration in the growth medium. Different results were obtained for *Pisum sativum* L. TF of Fe_3O_4-NPs was 12 times lower than for *Lepidium sativum*, comparing variants with corresponding concentration of nanostructures in the medium (4 mmol·L^{-1}). Unexpectedly TF calculated for roots of *Pisum sativum* L. growing on the solution with higher concentration of Fe_3O_4-NPs (10 mmol·L^{-1}) was 25 times lower. In contrast to *Lepidium sativum*, TF calculated for roots increased with increasing concentration of nanoobjects in the medium. For shoots TF was about 2 times lower than for corresponding samples of *Lepidium sativum* and decreased with concentration of Fe_3O_4-NPs in growth solution. Because the experiments were performed only for two concentration variants, it is impossible to perform a more detailed discussion of the relationship of TF and nanoparticle content in growing medium for *Pisum sativum* L.

More effective transport of Fe_3O_4-NPs to above-ground organs of *Pisum sativum* L. and its relation with particle concentration can be seen looking at the percentage of nanoparticles accumulated in roots; it is 90.2 ± 1.1 and 99.8 ± 0.3% for concentration of NPs 4 and 10 mM, respectively.

During the experiment and discussion of results, we make the assumption that Fe_3O_4-NPs are accumulated by plants. During studies the presence of iron ions in growth medium was tested. For this purpose growth media (kept under experimental conditions with and without plants) were filtered, ultracentrifuged and the amount of iron in the suspensions was determined. It was found that the activity of the ^{59}Fe in the suspensions is below detection limit, so the iron ions originating from nanoparticles are not present in the solution. As the activity of ^{59}Fe in plant samples was significant, our assumption that Fe_3O_4-nanoparticles are present in plants is reasonable and correct. It is worth noticing that the applied method allows the identification of the source of the element present in plants. Using a method, commonly used for determination of the total amount of an element i.e. inductively coupled plasma mass spectrometry (ICP-MS), we can only obtain information about the total amount of the element in the samples. Another advantage of the proposed method is avoiding complicated sample preparation procedures. The proposed method seems to be very useful for tracing the uptake and accumulation of iron (II,III) oxide nanoparticles in organisms.

Conclusions

Rapid development of the nanotechnology and increasing application of nanoproducts causes a growing risk of production of nanowastes dangerous for the environment, causing a need for the investigation of the accumulation and toxicity of NPs at different trophic levels. During the studies it was shown that neutron activation of nanoparticles is a powerful tool for tracing the environmental fate of NPs and their uptake and accumulation in organisms. The applied method allowed the conclusion to be drawn that iron (II,III) oxide nanoparticles (Fe_3O_4-NPs) can be taken up by plants and that way enter the trophic chain. Both investigated species (*Lepidium sativum* and *Pisum sativum* L.) accumulated Fe_3O_4-NPs, mainly in roots (> 90%). Accumulation of Fe_3O_4-NPs clearly depends on the concentration of nanostructures in growth medium. Transfer factor for *Lepidium sativum* roots and shoots and *Pisum sativum* L. shoots decreased with increasing NP concentration in medium while for *Pisum sativum* L. roots the tendency was reversed.

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References

1. Abhilash KR, Pandey BD (2011) Microbial synthesis of iron-based nanomaterials – a review. Bull Mater Sci 34;2:191–198
2. Bystrzejewska-Piotrowska G, Golimowski J, Urban L (2009) Nanoparticles: Their potential toxicity, waste and environmental management. Waste Manage 29;9:2587–2595
3. Fu L, Dravid VP, Johnson DL (2001) Self-assembled (SA) bilayer molecular coating on magnetic nanoparticles. Appl Surf Sci 181:173–178
4. Kim DK, Zhang Y, Kehr J, Klason T, Bjelke B, Muhammed M (2001) Superparamagnetic iron oxide nanoparticles for bio-medical application. J Magn Magn Mater 225:256–261
5. Kim DK, Zhang Y, Voit W *et al.* (2001) Superparamagnetic iron oxide nanoparticles for bio-medical applications. Scripta Mater 44:1713–1717
6. Nanonet website, http://www.nanonet.pl
7. Oughton DH, Hertel-AAS T, Pollicer E, Mendoza E, Joner EJ (2008) Neutron activation of engineered nanoparticles as a tool for tracing their environmental fate and uptake in organisms. Environ Toxic Chem 27;9:1883–1887
8. Wang H, Kou X, Pei Z, Xiao JQ, Shan X, Xing B (2011) Physiological effects of magnetite (Fe_3O_4) nanoparticles on perennial ryegrass (*Lolium perenne* L.) and pumpkin (*Cucurbita mixta*) plants. Nanotoxicology 5;1:30–42
9. Zhu H, Han J, Xiao JQ, Jin Y (2008) Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. J Environ Monit 10:713–717