**Application of neutron activation for investigation of Fe$_3$O$_4$ 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$_3$O$_4$-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$_3$O$_4$-NPs. For the identification of the presence of Fe$_3$O$_4$-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$_3$O$_4$-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$_3$O$_4$-NPs), magnetite. The synthesis of monodisperse nanocrystals of Fe$_3$O$_4$ has long been of scientific and technological interest. The compound exhibits unique electric and magnetic properties based on the transfer of electrons between Fe$^{3+}$ and Fe$^{2+}$ 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...
Materials and methods

Iron (II,III) oxide nanoparticles (Fe$_3$O$_4$, 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·L$^{-1}$ water suspensions.

For the irradiation, samples of Fe$_3$O$_4$ nanoparticles (80–100 mg) were weighed directly into HDPE snap-cap capsules (Faculteit Biologie, Vrije Universiteit, Amsterdam), wrapped in aluminum foil and irradiated in the nuclear reactor MARIÁ (Świerk, Poland), for 10 min at a thermal neutron flux of 10$^{14}$ cm$^{-2}$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 spectrometry System 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 $^{57}$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·L$^{-1}$. Lepidium sativum L was cultivated in 400 mL containers (4 plants/container). Concentration of NPs was 4 and 10 mol·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$_3$O$_4$ nanoparticles powder. Activity of Fe-59 was calculated with use of GENIE 2000 software and its 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$_3$O$_4$ 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 Fe$_3$O$_4$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$_3$O$_4$ 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$_3$O$_4$-NPs. Nanoparticles of iron (II,III) oxide were irradiated in nuclear reactor MARIÁ. During irradiation $^{59}$Fe ($T = 44.5$ days, $E_{\gamma \max} = 1099.2, 1291.6$ 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...
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roots and shoots of plants cultivated in the presence of Fe$_3$O$_4$-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 ($A_0$) taking into consideration the half-life of the iron isotope $^{59}$Fe (T) and the total time of the experiments ($\Delta$), according to the equation: 

$$A_0 = A^e(–0.693·\Delta)/T$$

($A_0$ – calculated activity of the samples). On the basis of the activities recalculated for the samples ($A_0$), the amount of Fe$_3$O$_4$ nanoparticles in plants ($C_{Fe_3O_4-NPs}$) was calculated, using equation:

$$C_{Fe_3O_4-NPs} [mg_{Fe_3O_4-NPs}/kg_{SAMPLE}] = A_0 [kBq/kg_{SAMPLE}] \times 1000/21.74 [kBq/g_{Fe_3O_4-NPs}]$$

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$_3$O$_4$-NPs in growth medium 4 mmol·L$^{-1}$ was more than 13 times lower than for *Lepidium sativum*. For the highest concentration of Fe$_3$O$_4$-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$_3$O$_4$-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$_3$O$_4$-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$_3$O$_4$-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$_3$O$_4$-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$_3$O$_4$-NPs are accumulated in the roots and transport to above-ground organs is inhibited. Second, relatively low accumulation of Fe$_3$O$_4$-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$_3$O$_4$-NPs accumulated by *Lepidium sativum* was found in the roots.

### Table 1. Fe$_3$O$_4$-NPs content [mg·kg$^{-1}$ dry weight] and transfer factors for *Lepidium sativum* and *Pisum sativum* L. (Results are presented as means ± standard deviations, $n = 3$)

<table>
<thead>
<tr>
<th>Concentration of Fe$_3$O$_4$ in growth medium (mmol·L$^{-1}$)</th>
<th>Concentration of Fe$_3$O$_4$-NPs in plants (mg·kg$^{-1}$ d.w.)</th>
<th>Transfer Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>roots</td>
<td>shoots</td>
</tr>
<tr>
<td><strong>Lepidium sativum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>0.01</td>
<td>323 ± 14</td>
<td>2.44 ± 0.26</td>
</tr>
<tr>
<td>0.10</td>
<td>1 685 ± 59</td>
<td>3.20 ± 0.67</td>
</tr>
<tr>
<td>0.50</td>
<td>2 033 ± 71</td>
<td>17.1 ± 1.4</td>
</tr>
<tr>
<td>4.0</td>
<td>7 805 ± 254</td>
<td>40.3 ± 2.8</td>
</tr>
<tr>
<td><strong>Pisum sativum L.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4.0</td>
<td>604 ± 25</td>
<td>20.1 ± 1.7</td>
</tr>
<tr>
<td>10</td>
<td>39 533 ± 1 243</td>
<td>4.59 ± 0.21</td>
</tr>
</tbody>
</table>
The suspensions was determined. It was found that the were filtered, ultracentrifuged and the amount of iron in under experimental conditions with and without plants) medium was tested. For this purpose growth media (kept plants. During studies the presence of iron ions in growth medium was significant, our assumption that Pisum sativum of TF and nanoparticle content in growing medium for perform a more detailed discussion of the relationship only for two concentration variants, it is impossible to make the assumption that Fe₃O₄-NPs are accumulated by plants and that way enter the trophic chain. Both investigated species (Lepidium sativum and Pisum sativum L.) accumulated Fe₃O₄-NPs, mainly in roots (> 90%). Accumulation of Fe₃O₄-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.

**Conclusions**

Rapid development of the nanotechnology and increasing application of nanoparticles 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 (Fe₃O₄-NPs) can be taken up by plants and that way enter the trophic chain. Both investigated species (Lepidium sativum and Pisum sativum L.) accumulated Fe₃O₄-NPs, mainly in roots (> 90%). Accumulation of Fe₃O₄-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**