



# Silver nanoparticle accumulation by aquatic organisms – neutron activation as a tool for the environmental fate of nanoparticles tracing

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**Abstract.** Water environments are noted as being some of the most exposed to the influence of toxic nanoparticles (NPs). Therefore, there is a growing need for the investigation of the accumulation and toxicity of NPs to aquatic organisms. In our studies neutron activation followed by gamma spectrometry and liquid scintillation counting were used for studying the accumulation of silver nanoparticles (AgNPs) by freshwater larvae of *Chironomus* and fish *Danio rerio*. The influence of exposition time, concentration and the source of nanoparticles on the efficiency of AgNP accumulation were studied. It was found that AgNPs are efficiently accumulated by *Chironomid* larvae for the first 30 hours of exposition; then, the amount of silver nanoparticles decreases. The silver content in larvae increases together with the NP concentration in water. Larvae which have accumulated AgNPs can be a source of nanoparticles for fish and certainly higher levels of Ag in the trophic chain. In comparison with water contamination, silver nanoparticles are more efficiently accumulated if fish are fed with AgNP-contaminated food. Finally, it was concluded that the applied study strategy, including neutron activation of nanoparticles, is very useful technique for tracing the uptake and accumulation of NPs in organisms.

**Key words:** silver • nanoparticles • neutron activation • *Chironomid* larvae • *Danio rerio*

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## Introduction

Silver nanoparticles (AgNPs) are one of the fastest growing classes of metal-NPs that are applied in various product applications [1]. The exceptional broad-spectrum bactericidal activity of silver and the relatively low cost of manufacturing AgNP have made them extremely popular in medicine and a broad range of consumer materials, including plastics, soaps, pastes, metals and textiles [2–4]. Silver nanoparticles exhibit a wide range of unique properties, i.e. high electrical and thermal conductivity, scattering, chemical stability, and catalytic activity, resulting in many current and future applications [5–7].

The consequences of the increasing use of nanoparticles is the risk of creating a new generation of waste (called nanowaste) and new potential threats to the environment [8]. So, there is the need to investigate the accumulation and toxicity of NPs at different trophic levels of food chain.

Due to the fact that a large part of industrial waste waters are dumped into rivers and seas, the water environment has been identified as one of the most exposed to the influence of toxic nanoparticles. A number of studies on the toxicity of silver nanoparticles towards diverse aquatic vertebrates, invertebrates, algae and bacteria have been reported [9, 10]. However, our understanding of silver nanoparticle accumulation by different species is

very narrow and is mainly related to biomedical applications of AgNPs [11].

*Chironomid* larvae are one of the most ubiquitous, ecologically diverse and abundant freshwater benthic invertebrates [12]. They represent a good study model because they can be easily cultured in the laboratory and are commonly used in toxicity tests [13]. Larval stages of *Chironomidae* can be found in widespread aquatic habitats and are important food items for fish, and so represent significant trophic levels of the food chain. The crucial questions are: i) can *Chironomid* larvae accumulate nanoparticles; and ii) is the accumulation of nanoparticles in higher levels of the trophic chain (i.e. fish) more efficient when effected directly from the water or indirectly from contaminated food (larvae)? It has been reported that freshwater larvae of the *Chironomus* accumulate heavy metals [14–16]; however, there is no information about the accumulation of nanoparticles.

To answer these questions, in the present studies, we undertook research aimed at:

- i) the investigation of the accumulation of AgNPs by freshwater larvae of the insect *Chironomus* (Diptera: Chironomidae),
- ii) the investigation of the possible inclusion of AgNPs into the trophic chain via a comparison of AgNP accumulation by fish *Danio rerio* exposed to AgNPs in water or in food.

For these studies, technique of neutron activation of silver was applied. Additionally, gamma spectrometry and liquid scintillation counting were employed for determination of AgNPs in the larvae/fish and solutions, respectively. The proposed analytical strategy allowed the confirmation of the presence of NPs or ions originating from NPs in studied samples and provided information about the source of the investigated element. This information is usually missing when determining the total amount of an element using common analytical methods, such as ICP, MS, and ASA. Neutron activation is a powerful method for tracing the environmental fate of NPs and their accumulation in organisms [17] and has previously been applied for the analysis of Fe<sub>3</sub>O<sub>4</sub>-NPs accumulation by plants [18].

## Materials and methods

Silver nanoparticles (Ag, nanopowder, <100 nm) were purchased from Sigma-Aldrich. The particle size and morphology were characterized using a TEM LEO 912AB transmission electron microscope (Zeiss) equipped with a Proscan High Speed Slow Scan CCD-camera. TEM analysis was performed using 1 mmol·L<sup>-1</sup> water suspensions. Silver nanoparticles were irradiated in a MARIA nuclear reactor (Świerk, Poland) according to the procedure described elsewhere [18].

Freshwater larvae of the insect *Chironomus* (1.2 cm, 50 adult individuals) were transferred to an aquaria containing water (control) and silver nanoparticles at a final concentration: 0.1, 1 and 10 mg·L<sup>-1</sup>. The cultivation volume was 100 mL. The incubation

was conducted under room conditions for 6, 24, 30 and 48 h. After finishing the cultivation, larvae were picked up, washed with deionized water, frozen and freeze-dried.

The exposition of *Danio rerio* fish (The International Institute of Molecular and Cell Biology, Warsaw, Poland) to nanoparticles was conducted in 25 L aquaria (8 adult, male fishes per aquarium) for 8 days in two variants. In the first experiment, fish were cultivated in water containing nanoparticles (0.05 mg·L<sup>-1</sup>). In the second, *Danio rerio* were fed with dried larvae of *Chironomus*, containing accumulated nanoparticles. The food portions were selected to obtain the final exposition concentration of AgNP 0.05 mg·L<sup>-1</sup>. After finishing the exposition, half of the fish in each variant were transferred to the water for 2 days to evacuate the gut content. Finally, tricaine (3-amino benzoic acidethylester) from Sigma was used for anesthetizing fishes. Next, samples were frozen and freeze-dried before further analysis. All exposition experiment were conducted in three replicates.

The level of <sup>110m</sup>Ag radionuclide in larvae and fish was determined by means of a gamma spectrometer with an HPGe detector (Canberra Packard). Silver radionuclide in solutions before and after cultivation was quantified with a Beckman LS 6000IC Liquid Scintillation Counter. For this purpose, samples of liquid media were mixed with Rotiszint (Roth) scintillation cocktail including 2 mL of sample and 10 mL of Rotiszint. Scintillations were counted within 1 min per sample in CPM mode.

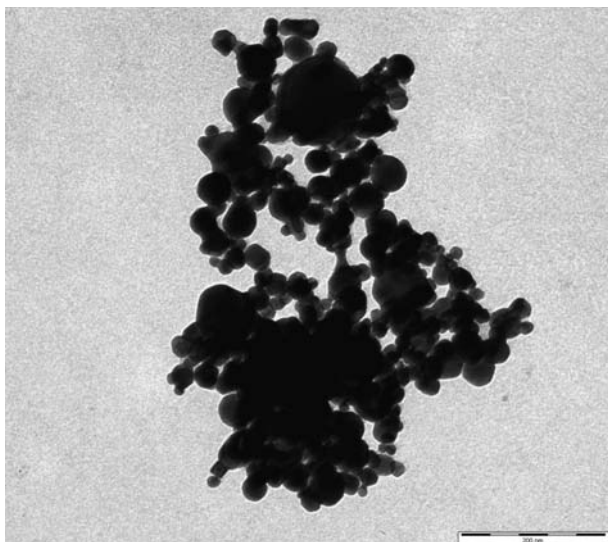
To test the presence of silver ions in the cultivation medium, the solutions (kept under experimental conditions with and without larvae/fishes) were filtered, ultracentrifuged and the amount of silver in the suspensions was determined.

## Results and discussion

Nanoparticles of Ag were characterized using transmission emission microscopy (Fig. 1). The major part of the particles is spherical and their size does not exceed 100 nm, which is in agreement with the declaration of the supplier.

During the studies no negative effects, i.e. reduced vitality, weight or enhanced mortality, were observed so it was concluded that larvae of *Chironomus* are tolerant to the applied, relatively high concentrations of AgNPs. However, detailed tests of the larval conditions were not the subject of the studies.

It was found that the studied species accumulated AgNPs. Significantly elevated activity was measured in invertebrates and fish exposed to AgNPs. Simultaneously, no activity was detected in control samples. The measured activity was within the range 0.4 Bq for the larvae exposed for 6 h to NPs in concentration 0.1 mg·L<sup>-1</sup> in growth medium and 9000 Bq for invertebrates exposed to NPs for 30 h in concentration 10 mg·L<sup>-1</sup> in growth medium. All the measured activities were recalculated, taking into consideration the half-life of the silver isotope <sup>110m</sup>Ag (250 days), and the total time of all experiments. Based on the activities measured for the



**Fig. 1.** Transmission-electron micrographs of Ag nanoparticles ( $1 \text{ mmol}\cdot\text{L}^{-1}$  suspension in water) used in neutron-activation experiments and cultivation.

samples, the amount of silver nanoparticles ( $\text{mg}\cdot\text{kg}^{-1}$  d.w.) in larvae and fish was calculated.

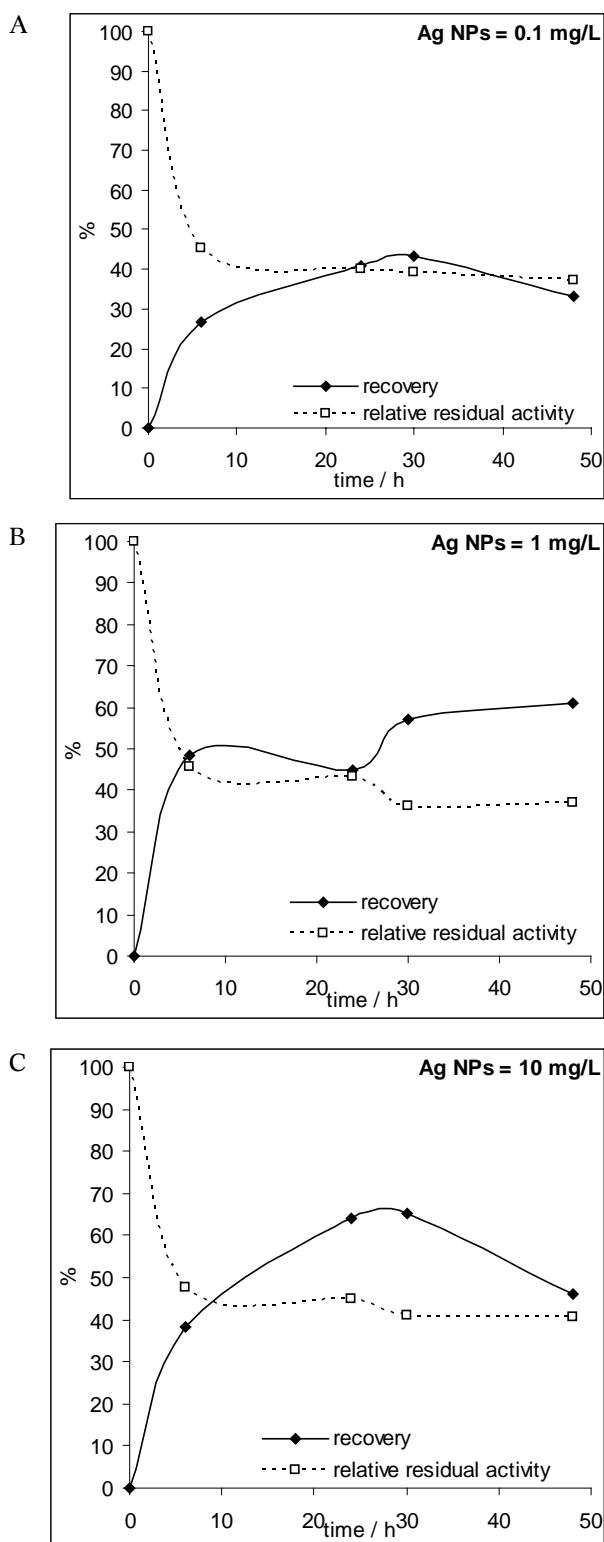
The accumulation of silver nanoparticles in larvae depended on the time of exposition and AgNP concentration in medium (Table 1). For the lowest ( $0.1 \text{ mg}\cdot\text{L}^{-1}$ ) and highest ( $10 \text{ mg}\cdot\text{L}^{-1}$ ) AgNP concentrations in the medium, the silver content in larvae increased with the exposition time (6 to 30 h) and for the longest experiment time (48 h) it decreased. For the nanoparticles concentration in growth medium  $1 \text{ mg}\cdot\text{L}^{-1}$  the amount of accumulated silver was stable through the whole study. The amount of accumulated AgNPs also increased together with increasing exposition concentration; however, the relationship is not directly proportional. The concentration of silver nanoparticles in growth medium increased 10 and 100 times in comparison with the lowest exposition, while the average AgNP content in larvae increased 17 and 152 times, respectively.

Differences in the accumulation of AgNPs as a function of exposition time and nanoparticle concentration can be observed regarding the recovery of AgNPs. The recovery was calculated according to the equation:

$$\text{Ag}_{\text{larvae}} \times M \times 100\% / \text{Ag}_{\text{medium}}$$

where:  $\text{Ag}_{\text{larvae}}$  – Ag content in larvae [ $\text{mg}\cdot\text{g}^{-1}$ ]; M – weight of larvae [g],  $\text{Ag}_{\text{medium}}$  – Ag content in the medium [mg].

It was found that the recovery increased with the exposition time (6 to 30 h), while for the longest experiment time (48 h) it decreased (Fig. 2). In the



**Fig. 2.** Recovery of AgNPs and relative residual activity as a function of exposition time (RSD of the results  $< 5\%$ ).

**Table 1.** Silver content [ $\text{mg}\cdot\text{kg}^{-1}$  d.w.] in *Chironomus* larvae exposed to AgNPs. (Results are presented as means  $\pm$  standard deviations; three repetitions, 50 individuals per repetition)

AgNPs concentration [mg/L]	Ag content [ $\text{mg}\cdot\text{kg}^{-1}$ d.w.]			
	6 h	24 h	30 h	48 h
0.1	$7.84 \pm 0.26$	$9.12 \pm 0.37$	$11.6 \pm 0.5$	$7.44 \pm 0.18$
1	$139.6 \pm 6.2$	$136.0 \pm 6.8$	$155.3 \pm 6.2$	$152.4 \pm 5.2$
10	$945 \pm 41$	$1609 \pm 60$	$1671 \pm 59$	$1249 \pm 33$

**Table 2.** Silver content [ $\text{mg}\cdot\text{kg}^{-1}$  d.w.] in *Danio rerio* exposed to various sources of AgNPs. (Results are presented as means  $\pm$  standard deviations; three repetitions, 8 individuals per repetition)

Source of contamination	With the gut content	After evacuation of the gut content
Water	$57.7 \pm 3.6$	$0.43 \pm 0.08$
Food (larvae)	$6477 \pm 149$	$0.36 \pm 0.09$

case of an exposition concentration of  $1 \text{ mg}\cdot\text{L}^{-1}$  the recovery increased constantly. The average recovery was the lowest for the lowest AgNP concentration in growth medium and for the rest the exposition variants stayed at the similar level.

According to the Table of Radioactive Isotopes (<http://ie.lbl.gov/databases/databases.html#TORI>), there are two types of  $^{110\text{m}}\text{Ag}$  decay: isomeric transition and  $\beta$ -decay which produce a wide spectrum of  $\gamma$ -,  $\beta$ -, and x-ray radiation. The largest portion of energy is emitted from  $\beta$ -decay with  $\gamma$  emission 657.76 KeV, where 94.0% of total energy is emitted. This line was investigated with an HPGe spectrometer for fish and larvae samples. Additionally, total  $\beta$ -radiation was investigated using liquid scintillation counting. To complement the information obtained during analysis of larvae, the activity of water solutions before and after larvae cultivation was measured. The relative residual activity ( $R_A$ ) was calculated according to the equation:

$$R_A = A_0 \times 100\% / A_t$$

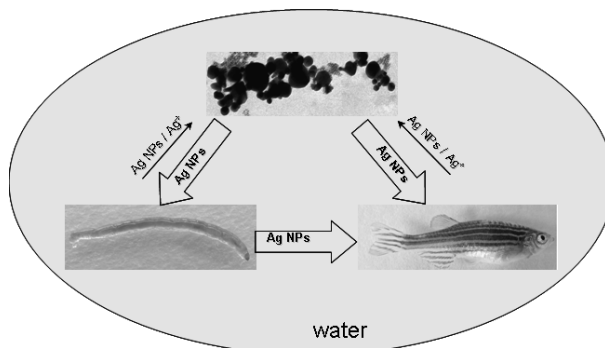
where:  $A_0$  – activity at the beginning of the experiments,  $A_t$  – activity after the exposition time ( $t$ ).

The obtained relative residual activities present contrasting changes in comparison with the recoveries calculated for larvae (Fig. 2). An increase in silver accumulation in larvae results in a decrease in the activity of the solutions. It confirms the efficient accumulation of AgNPs in larvae.

On the basis of the obtained results, some conclusions can be drawn about the mechanism of nanoparticle accumulation. We can suppose that during the first 30 hours larvae accumulate and metabolize AgNPs and after 48 hours nanoparticles are excreted. It is proven by the increasing accumulation of nanoparticles until a dynamic equilibrium is reached (0–30 h). The subsequent decrease in AgNP accumulation indicates the possible removal of accumulated silver as nanoparticles or as ions.

Besides the active accumulation, the relatively high concentration of silver in larvae was partly caused by the adsorption of particles at the invertebrate surface. It was clearly visible that after finishing the cultivation particles are strongly adsorbed at the surface of the larvae. The binding of AgNPs was so strong that it was difficult to remove adsorbed particles by flushing the samples with water.

The final step of the study was to investigate the possible migration of the AgNPs in the small ecosystem. For this purpose *Danio rerio* fish were exposed to AgNPs. Two sources of AgNPs were investigated: water solution and food-dried larvae contaminated with AgNPs. It was found (Table 2) that the silver content in fish living in water containing NPs is 110-fold lower than that in organisms fed with con-

**Fig. 3.** Scheme illustrating the possible migration of AgNPs in a small aquatic ecosystem.

taminated larvae. After evacuation of the gut content the amount of silver in *Danio rerio* was only slightly higher than the background. This proves that under the applied exposition time and concentration Ag is not accumulated in the fish bodies, but remained only in the digestive system.

As presented in the scheme (Fig. 3), silver nanoparticles can be accumulated directly from the water environment both by larvae and fish. Additionally, AgNPs can be accumulated by larvae and then be transported to the fish and higher elements of the trophic chain. Accumulation of AgNPs from food is much more efficient than that from water. The question about the chemical form of silver is after its passing the digestive system is still open and needs additional studies.

During the discussion of results and drawing conclusions, the assumption was made that in the cultivation medium AgNPs, and not silver ions, are present. The presence of silver ions in cultivation medium was tested and it was found that the activity of the  $^{110\text{m}}\text{Ag}$  in the solution is below the detection limit, while the activity of silver radioisotope in larvae and fish samples is significant. Based on the obtained results, our assumption that in the solution Ag ions originating from nanoparticles are not present seems to be reasonable and correct.

## Summary

Water environments have been identified as one of the most exposed to the influence of toxic nanoparticles, so there is a growing need for the investigation of the accumulation and toxicity of NPs to aquatic organisms. In our studies, neutron activation followed by gamma spectrometry and liquid scintillation counting were employed for studying the accumulation of silver nanoparticles by freshwater larvae of *Chironomus* and fish *Danio rerio*. Based on the results obtained during our studies we can draw the following conclusions: i) silver nanoparticles (AgNPs) can be taken up by

- Chironomid* larvae and that way enter the trophic chain;
- ii) accumulation of AgNPs clearly depends on the concentration of nanostructures and exposition time;
  - iii) larvae which have accumulated AgNPs can be a source of nanoparticles in fish and certainly higher organisms; accumulation of silver nanoparticles from food is much more efficient than that from water;
  - iv) freshwater larvae of the insect *Chironomus* can be used for biomonitoring of the nanoparticle contamination of the aquatic environment.

Finally, it should be noted that the applied analytical strategy, including neutron activation of nanoparticles, is very useful for tracing the uptake and accumulation of nanoparticles in organisms. The main advantage of the proposed method was that it enabled us to avoid complicated sample preparation procedures, while simultaneously giving us important information concerning the source of the element present in samples. This is information which is missing if common techniques used for the determination of the total amount of an element are applied.

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## References

1. Ahamed, M., AlSalhi, M. S., & Siddiqui, M. K. J. (2010). Silver nanoparticle applications and human health. *Clin. Chim. Acta*, *411*, 1841–1848. DOI: 10.1016/j.cca.2010.08.016.
2. Capek, I. (2004). Preparation of metal nanoparticles in water-in-oil (w/o) microemulsions. *Adv. Colloid Interface Sci.*, *110*, 49–74. DOI: 10.1016/j.cis.2004.02.005.
3. Mahendra, R., Alka, Y., & Aniket, G. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv.*, *27*(1), 76–85. DOI: 10.1016/j.biotechadv.2008.09.002.
4. Frattini, A., Pellegrini, N., Nicastro, D., & Sanctis, O. D. (2005). Effect of amine groups in the synthesis of Ag nanoparticles using aminosilanes. *Mater. Chem. Phys.*, *94*, 148–152. DOI: 10.1016/j.matchemphys.2005.04.023
5. Rand, B. P., Peumans, P., & Forrest, S. R. (2004). Long-range absorption enhancement in organic tandem thin-film solar cells containing silver nanoclusters. *J. Appl. Phys.*, *96*, 7519–7526. DOI: 10.1063/1.1812589.
6. Zhai, H. J., Sun, D. W., & Wang, H. S. (2006). Catalytic properties of silica/silver nanocomposites. *J. Nanosci. Nanotechnol.*, *6*, 1968–1972. DOI: 10.1166/jnn.2006.320.
7. Yamamoto, S., & Watarai, H. (2006). Surface-enhanced Raman spectroscopy of dodecanethiol-bound silver nanoparticles at the liquid/liquid interface. *Langmuir*, *22*, 6562–6569. DOI: 10.1021/la0603119.
8. Bystrzejewska-Piotrowska, G., Golimowski, J., & Urban, L. (2009). Nanoparticles: Their potential toxicity, waste and environmental management. *Waste Manage.*, *29*(9), 2587–2595. DOI: 10.1016/j.wasman.2009.04.001.
9. Fabrega, J., Luoma, S. N., Tyler, C. R., Galloway, T. S., & Lead, J. R. (2011). Silver nanoparticles: Behaviour and effects in the aquatic environment. *Environ. Int.*, *37*, 517–531. DOI: 10.1016/j.envint.2010.10.012.
10. Blinova, I., Niskanen, J., Kajankari, P., Kanaribik, L., Käkinen, A., Tenhu, H., Penttinen, O. P., & Kahru, A. (2013). Toxicity of two types of silver nanoparticles to aquatic crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. *Environ. Sci. Pollut. Res.*, *20*, 3456–3463. DOI: 10.1007/s11356-012-1290-5.
11. Tang, J., Xiong, L., Wang, S., Wang, J., Liu, L., Li, J., Yuan, F., & Xi, T. (2009). Distribution, translocation and accumulation of silver nanoparticles in rats. *J. Nanosci. Nanotechnol.*, *9*(8), 4924–4932. DOI: 10.1166/jnn.2009.1269.
12. Pinder, L. C. V. (1986). Biology of freshwater chironomidae. *Annu. Rev. Entomol.*, *31*, 1–23. DOI: 10.1146/annurev.en.31.010186.000245.
13. OECD. (2004). Test guideline 218 sediment-water chironomid toxicity test using spiked sediment.
14. Krantzberg, G. (1989). Metal accumulation by chironomid larvae: the effects of age and body weight on metal body burdens. *Hydrobiologia*, *188/189*, 497–506. DOI: 10.1007/BF00027817.
15. Goodyear, K. L., & McNeill, S. (1999). Bioaccumulation of heavy metals by aquatic macro-invertebrates of different feeding guilds: a review. *Sci. Total Environ.*, *229*, 1–19. DOI: 10.1016/S0048-9697(99)00051-0.
16. Azevedo-Pereira, H. M. V. S., Abreu, S. N., Lemos, M. F. L., & Soares, A. M. V. M. (2012). Bioaccumulation and elimination of waterborne mercury in the midge larvae, *Chironomus riparius* Meigen (Diptera: Chironomidae). *Bull. Environ. Contam. Toxicol.*, *89*, 245–250. DOI: 10.1007/s00128-012-0674-z.
17. Oughton, D. H., Hertel-AAS, T., Pollicer, E., Mendoza, E., & Jøner, E. J. (2008). Neutron activation of engineered nanoparticles as a tool for tracing their environmental fate and uptake in organisms. *Environ. Toxicol. Chem.*, *27*(9), 1883–1887. DOI: 10.1897/07-578.1.
18. Bystrzejewska-Piotrowska, G., Asztemborska, M., Steborowski, R., Ryniewicz, J., Polkowska-Motrenko, H., & Danko, B. (2012). Application of neutron activation for investigation of Fe<sub>3</sub>O<sub>4</sub> nanoparticles accumulation by plants. *Nukleonika*, *57*(3), 427–430.