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TRANSITION OF NANOPARTICLES Fe₃O₄ AND Al IN A SIMPLIFIED AQUATIC FOOD CHAIN

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ПЕРЕХОД НАНОЧАСТИЦ Fe₃O₄ и Al В ПРОСТОЙ ПИЩЕВОЙ ЦЕПИ В ВОДНОЙ ЭКОСИСТЕМЕ

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Abstract. This article presents the results of experiments on the transitions of Fe₃O₄ nanoparticles (20–30 nm) and Al (18 nm) from one organism to another, making up a simple food chain (plant–mollusk–fish). In experiments, mollusks (*Melonopsis praemorsa*) feed on the leaves of plant (*Elodea canadensis*) after being contaminated with Al or Fe₃O₄ nanoparticles. Nanoparticles were detected using TEM analysis in the cells of the mollusk's organs. Then the fish (*Oncorhynchus mykiss*) were fed with mollusks. The distribution and localization of nanoparticles in fish organs has been determined. Experimental results showed that nanoparticles can pass from one organism to another in the food chain. Nanoparticles accumulate mainly in the liver of mollusks and fish.

Аннотация. В статье представлены результаты экспериментов по переходам наночастиц Fe₃O₄ (20–30 нм) и Al (18 нм) от одного организма к другому, составляющие простую пищевую цепь (растение–моллюск–рыба). В экспериментах моллюски (*Melonopsis praemorsa*) питаются листьями растения (*Elodea canadensis*), загрязненными наночастицами Al или Fe₃O₄. Наночастицы были обнаружены с помощью ТЕМ-анализа в клетках органов моллюска. Затем рыбы (*Oncorhynchus mykiss*) были накормлены моллюсками. Определено распределение и локализация наночастиц в органах рыб. Результаты экспериментов показали, что наночастицы могут переходить от одного организма к другому в пищевой цепи. Наночастицы накапливаются в основном в печени моллюска и рыбы.

Keywords: nanoparticles, food chain, cells, organoids, distribution of nanoparticles.

Ключевые слова: наночастицы, пищевая цепь, клетки, органоиды, распределение наночастиц.

Introduction

The intensity of the use of nanotechnology products and the steady growth of the range of these products further diversifies the sources of their distribution in the environment [1]. Nanomaterials in most cases fall from the hydrophilic layers of the earth. The risk of nanoparticles spreading to water basins, rivers, seas and their impact on ecosystems is much higher here. It is connected with the nature of nanoparticles, as well as with the dynamics of aquatic ecosystems. The ecotoxicity of nanomaterials in water depends on, first of all, on which of the ecosystem components is affected and on the type and size of the nanoparticles. Nanomaterials, including nanoparticles, are adsorbed primarily on the surface of plant components, that is, on the

phytoplankton of aquatic ecosystems, and then spread to other elements of the ecosystem through the food chain. The movement of nanoparticles on the trophic levels of aquatic ecosystems has been investigated in a number of scientific studies [2–3].

The main goal of these studies was to find out how the nanoparticles pass from one organism to another in the food chain of aquatic ecosystems. The reason is that in aquatic ecosystems, living organisms are sensitive to nanoparticles accumulated in these organisms can move from one to another. One of such studies is the experience of McTeer and his collaborators. They investigated the movement of silver nanoparticles in the food chain, which are widely used in medicine, cosmetics, electronics. In these experiments, it is confirmed that Ag nanoparticles crossed with *Daphnia magna* fed by *Chlamydomonas reinhaedtii* algae. According to their experiments, it was found out that in the organism *Daphnia magna* fed with algae, which kept in silver salt AgNO_3 and Ag nanoparticles and two forms of silver were accumulated: both ionic and nanoparticles. At this time, Ag nanoparticles are less toxic in its ionic form [4]. The distribution and location of nanoparticles in aquatic environment also depends on the ionic content of water and their concentration. From the experiments made with graphene oxide nanoparticles, it is clear that the ions of the KCl and CaCl_2 salts accelerate of the graphene oxide nanoparticles transport in aqueous environment. The distribution and movement of nanoparticles depends on the temperature of water. So, during the temperature changes nanoparticles can agglomerate and deagglomerate. This depends on the changing temperature character of the water [5–9]. Thus, the distribution of nanoparticles in aquatic ecosystems and their transition from one living organism to another depending on their concentration, type, temperature of environment. The main purpose of experiments given in the presented article is to confirm the movement of nanoparticles in food chain depending on its type, size and concentration [10–18].

Material and methods

To study the movement of nanoparticles in the food chain under laboratory conditions in the aquarium, a simple food chain was created from higher aquatic plants of plant–mollusks–fish [16]. The experimental food chain used the higher aquatic plants *Elodea canadensis* from the Hydrocharitaceae family, the mollusk *Melonopsis praemorsa* from the Gastropoda type and the fish *Oncorhynchus mykiss* from the Actinopterygii subclass (Figure 1).

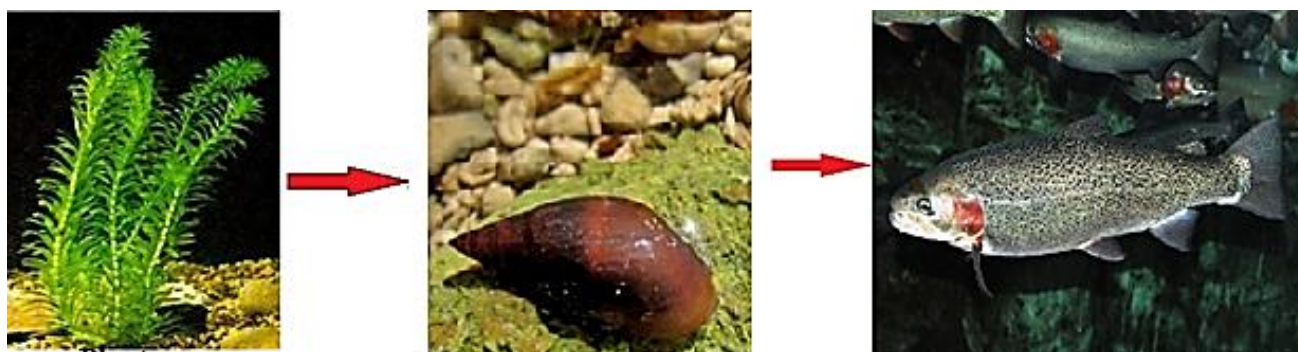


Figure 1. *Elodea canadensis* (plant), *Melonopsis praemorsa* (mollusk), *Oncorhynchus mykiss* (fish)

In this food chain, transitions of nanoparticles from plant to mollusk and from mollusk to fish are studied. *Elodea canadensis* has long been used as a model plant for physiological, biophysical and biochemical experiments [18]. To date, many electrophysiological studies are conducted using *Elodea*. The relatively large cell size of the *Elodea*, rapid reproduction and accessibility made it a good experimental component in the food chain. The second component of the food chain, *Melonopsis praemorsa*, is very diverse in the mollusk population. These mollusks are 10–20 mm in

the length and 6–10 mm in diameter. Mollusks feed mainly on diatoms and algae. They are sensitive to organic pollution. The third element of the food chain is rainbow trout. *Oncorhynchus mykiss* is a freshwater species of fish form belonging to the family Salmonidae. It is a species of redfish and grows well in freshwater. The optimum temperature for growing whitebait is about 16–19 degrees. *O. mykiss* is very demanding on the amount of oxygen dissolved in water. The minimum allowable value of this indicator for rainbow trout is 7 mg/litre. Its decrease will affect the activity of the fish, its appetite, and therefore with its growth. Unexposed species of salmon belong to the waterfall of the Pacific Ocean, Asia and North America. Food for *O. mykiss* is larvae, insects, butterflies, snails, fish eggs and even small fish, crustaceans.

The transition of the nanoparticles to living organisms, which is a simple food chain, was studied using microscopic analysis using transmission electron microscopy (TEM). Initially, the Elodea plant was aged for 72 hours in a 0,1% suspension solution of Fe_3O_4 and Al nanoparticles, which is the first component of the food chain. Then for 7 days, the mollusks were fed with contaminated nanoparticles – containing Elodea, which is the second component of the food chain. After that, for 3 days, the fish were fed mollusks that ate contaminated elodea with nanoparticles. After completing the feeding process, samples were taken from elodea, mollusks and fish for analysis. After completing the feeding process, samples were taken from Elodea, mollusks and fish. Samples were prepared in 0.1 M phosphate buffer (pH 7.4) based on a 2.5% solution of glutaraldehyde, 2% paraformaldehyde, 4% sucrose, 0.1% picric acid. After storing for at least one day in the same fixative with pos phosphine in a 1% solution of osmium tetroxide, preparations were prepared in phosphate buffer (pH 7.4) for two hours. Araldite-Eponblocks with a size of 1–2 μm were prepared using a Leica EM UC7 ultramicrotome and were stained with Azur II methylene a button and a solid base. Photographs of samples required for a Promo Star (Zeiss) microscope were obtained using a Canon D650 digital camera. Ultrafine sections 50–70 nm thick taken from the same blocks were first stained with a 2% solution of uranium acetate, and then 0.6% pure lead citrate prepared in a solution of 0.1n NaOH. Prepared ultrathin sections were investigated in a transmission electron microscope (TEM) JEM — 1400 at a voltage of 80–120 kv.

Experiments and results

It is known that in aqueous environment initially nanoparticles are adsorbed onto phytoplankton (algae, higher aquatic plants, etc.) and after some time are absorbed by them. Therefore, at the beginning of the experiments, we investigated the absorption and localization of nanoparticles in the leaves of Elodea. For this, ultrathin sections were prepared for TEM analysis from different organs of the elodea. At the beginning of the experiments, TEM images of Elodea leaf cells were obtained, which were grown in normal solution. Figure 2 shows the TEM image of the Elodea cells. Figure 2 clearly shows the structure of the cell wall, intercellular space, plasma membranes, chloroplasts and mitochondria. TEM images thin structures of the cell, internal organoids, chloroplasts, mitochondria, plasma membranes and cell walls were also obtained in experimental preparations made from elodea leaves that were exposed in a nanoparticle solution.

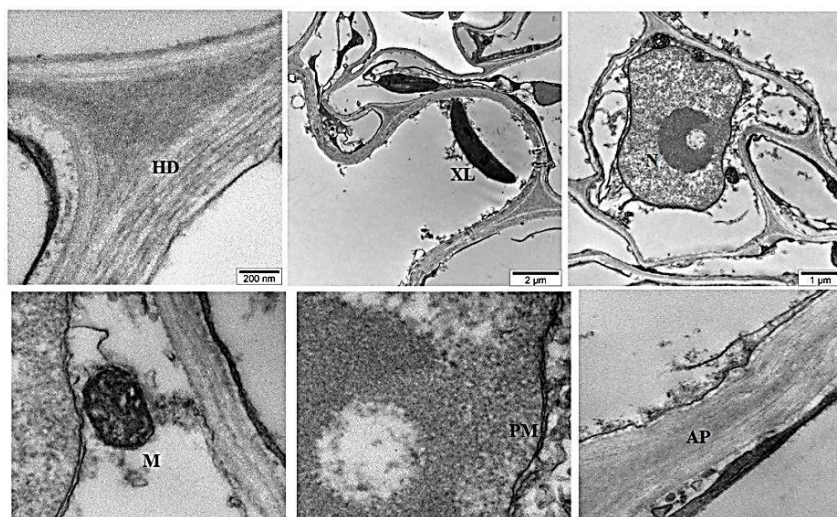


Figure 2. TEM images of a sample, made from leaves of an Elodea plant cultivated under normal conditions

Figure 3 shows TEM images of the cells of the leaf of the Elodea that were exposed in an Al nanoparticle solution. As can be seen from the figure, nanoparticles A are located on the surface of the mitochondria, and on the cell walls. Figure 4 shows the TEM image the leaf's cells of the elodea that were grown in a solution of Fe_3O_4 nanoparticle. As can be seen from the figure, Fe_3O_4 nanoparticles are located in the cytoplasm, intercellular space, surface of the mitochondria, and on the cell wall. TEM images obtained from the leaf's cells of the elodea show that Al and Fe_3O_4 nanoparticles can be localized to any organs. The presence and localization of nanoparticles in cell structures is confirmed by the elemental analysis diagram. Thus, TEM analyzes show that dissolved Al and Fe_3O_4 nanoparticles in water can penetrate through the cell walls into the cell and be adsorbed on the surface of cell organelles.

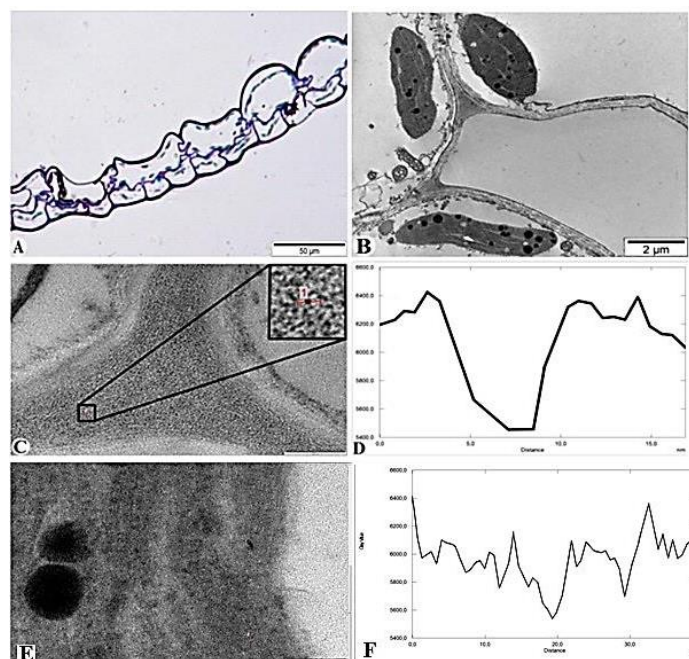


Figure 3. TEM is an image of the cells of the leaves of the Elodea grown in a 0.1% solution of Al nanoparticles: A — is an image of the cross section of the leaf, B — image of mitochondria, black dots on the surface Al nanoparticles, C — is an image of the cell wall, E — image of Al nanoparticles with high increase, D, F — elemental analysis chart showing the presence of nanoparticles in the scanned area

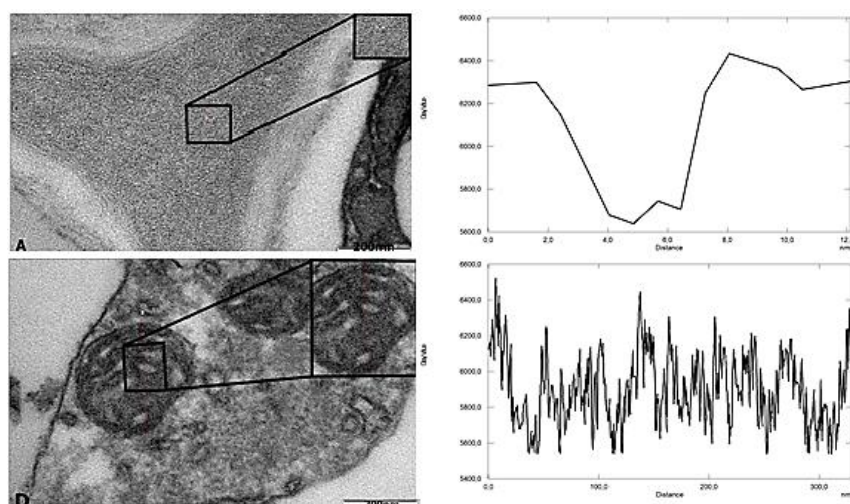


Figure 4. TEM is an image of the cells of the leaves of the Elodea grown in a 0.1% solution of Fe_3O_4 nanoparticles: A — nanoparticles in the cell wall; D — nanoparticles in the surfaces of the mitochondria. Elemental analysis charts show the presence of nanoparticles in the scanned area

To determine the transfer of nanoparticles from the plant to the mollusks, they were fed for 7 days from the leaves of the elodea contaminated with Fe_3O_4 nanoparticles. After feeding under a Stereomicroscope Zeiss Discovery V12 microscope, samples were taken from various mollusk organs and thin sections were made for the electron microscope. TEM images were obtained from these drugs. In Figure 5 shows the results of this experiment. As can be seen from the TEM images, Fe_3O_4 nanoparticles appear in the liver cells of mollusks. They are located in the lysosome (Figure 5A), in the mitochondria (Figure 5B) and in the cytoplasm of the cell (Figure 5C).

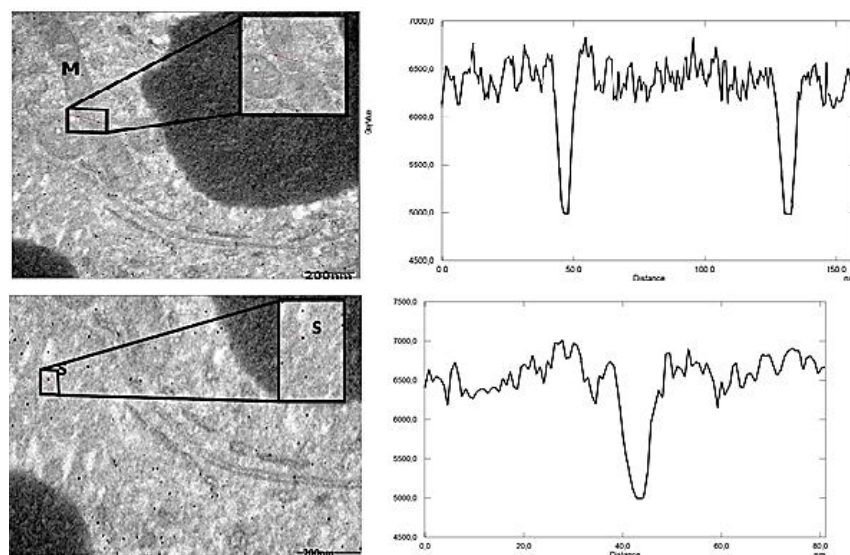


Figure 5. TEM is an image of the liver cells of the mollusk *Melonopsis praemorsa* fed in a 0.1% solution of Fe_3O_4 nanoparticles: M — nanoparticles in mitochondria, S — nanoparticles in the cytoplasm. Elemental analysis charts show the presence of nanoparticles in the scanned area

A similar experiment was conducted with Al nanoparticles of mollusks feeding for 7 days from Elodea leaves contaminated with Al nanoparticles. After feeding under a Stereomicroscope Zeiss Discovery V12 microscope, samples were taken from different mollusk organs and thin sections were made for the electron microscope. TEM images were obtained from these drugs. In

figure 6 shows the results of this experiment. As can be seen from the TEM image, Al nanoparticles are found in the intestinal walls (Figure 6B) and in the liver cells (Figure 6D) of mollusks.

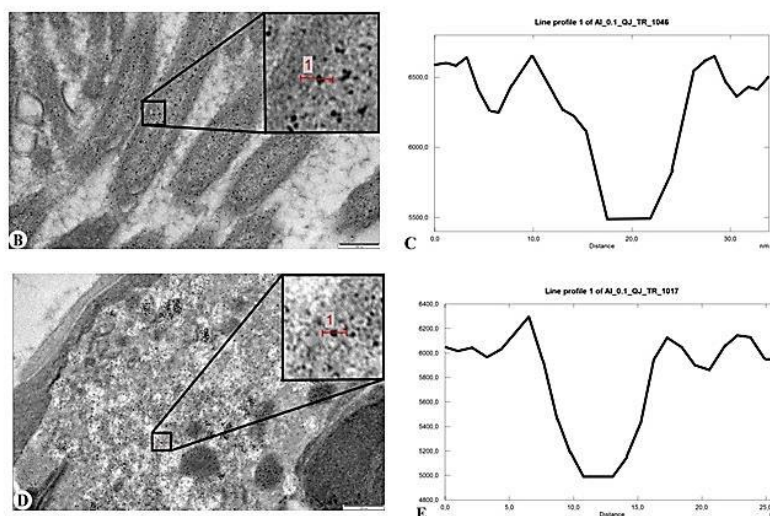


Figure 6. TEM image of intestinal walls (B) and liver cells of mollusks *Melonopsis praemorsa* fed in a 0.1% solution of Al nanoparticles: Elemental analysis charts show the presence of nanoparticles in the scanned area

In order to determine the transport of nanoparticles to fish, which are the third trophic level in the selected food chain, rainbow trout fed on mollusks that were fed with Elodea leaves for 3 days. Elodea was also contaminated with nanoparticles. Figure 7 shows the results of experiments with Al nanoparticles, and Figure 8 with Fe_3O_4 nanoparticles. TEM analyzes showed that in Al nanoparticles, as well as Fe_3O_4 , they can appear in fish organs if they fed on mollusks that are contaminated with nanoparticles. As can be seen from Figure 7, Al nanoparticles are located in the granular endoplasmic reticulum (Figure 7A) and in the mitochondria of liver cells (Figure 7B). Of these images, which are shown in Fig. 8, it can be found in the hepatocyte nanoparticles (Figure 8A) and the mitochondria of the liver cells (Figure 8B).

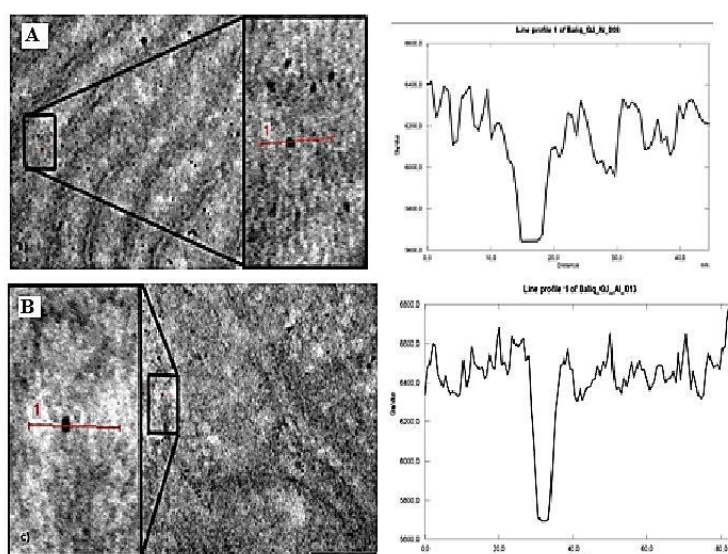


Figure 7. TEM is an image of the granular endoplasmic reticulum (A) and mitochondria (B) of rainbow trout cells fed by the mollusks *Melonopsis praemorsa* (C) Al nanoparticles. Elemental analysis charts show the presence of nanoparticles in the scanned area

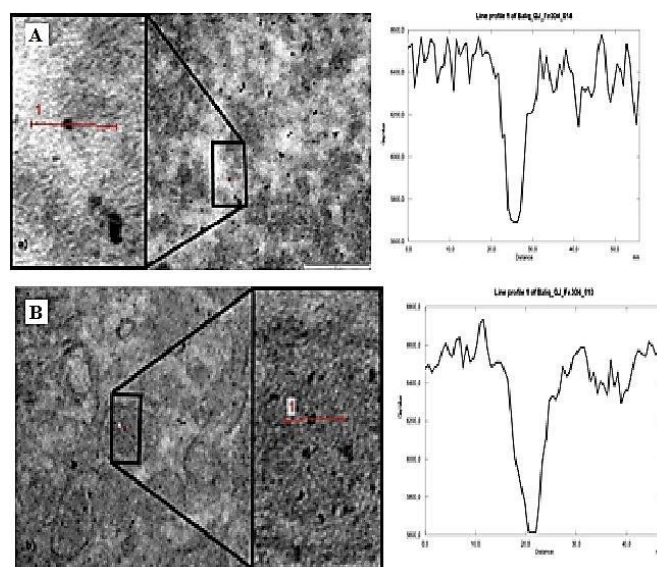


Figure 8. TEM is an image of the nucleus of hepatocyte (A) and mitochondria (B) of *Oncorhynchus mykiss* cells fed by mollusks *Melonopsis praemorsa* (C) Fe_3O_4 nanoparticles. Elemental analysis charts show the presence of nanoparticles in the scanned area

Conclusion

Nanoparticles are mostly adsorbed by phytoplankton in aquatic ecosystems for a number of reasons. In the course of the experiments, it was found that the plant with high moisture is an absorbing element of the elongate cells in the alphabets of the algeneous cells in the nanoparticles Al and Fe_3O_4 . When the elodea nanoparticles is contaminated with nanoparticles Al or Fe_3O_4 , these nanoparticles can penetrate into the body of the mollusks and accumulate in the cells of the body. Nanoparticles have been found in rainbow trout that feed on these mollusks. The analysis shows that the nanoparticles collect on the cell wall and mitochondria of the plant — Elodea. Nanoparticles accumulate in mollusks and larvae, mainly in the liver.

Thus, it can be concluded from experiments that nanoparticles dissolved in aquatic ecosystems can move in the food chain depending on the type, concentration, size and accumulate in its elements.

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