ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 3 2011

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IMPACT OF SILVER CONTAINED IN THE NANO SILVER PREPARATION ON THE SURVIVAL OF BRINE SHRIMP (*Artemia salina* LEACH 1819) LARVAE

WPŁYW SREBRA ZAWARTEGO W PREPARACIE NANO SILVER NA PRZEŻYWALNOŚĆ LARW ARTEMII (*Artemia salina* LEACH 1819)

Abstract: At the Department of Limnology and Fisheries at Wroclaw University of Environmental and Life Sciences there was conducted an experiment aimed at examining the impact of silver from the Nano Silver preparation on the survivability of Brine Shrimp (*Artemia salina* Leach 1819).

The experiment lasted for six hours, as Brine Shrimp lives in fresh water for about eight hours. The mean survivability rate in three repetitions for all of the concentrations of Nano Silver amounted to 93 %, with no linear drop in Brine Shrimp's survivability as silver concentrations increased. It was shown that silver nanoparticles were only slightly toxic to aquatic crustaceans. This low toxicity may prove useful to cut losses as regards zooplankton when using the preparation in order to limit large-scale development of plants in ponds.

Keywords: silver, brine shrimp, survivability

Disinfection allows proper hygiene levels to be maintained and prevents illnesses in the breeding of all kinds of animals. Disinfection is achieved by means of various chemicals. These are characterized by a wide spectrum of operation, significant activity, and small toxicity, which ensures that their application is entirely safe. The ideal disinfectant should have an appropriate killing capacity in respect of a broad range of microorganisms and at the same time it should not result in the development of resistance. It should be non-toxic or only slightly toxic so that it can be used in the presence of animals. After a period of effective operation it should undergo fast biodegradation and should not leave any toxic remains in the environment. It should dissolve well in water and produce stable and long-lasting solutions, easy to keep for at

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least 24 hours, until they are used. It should not damage the surfaces subjected to disinfection. Unfortunately, so far no ideal preparation has been manufactured, as toxicity seems to grow in direct proportion to the efficiency of the chemical [1]. In the search for the ideal agent attention was drawn to silver, whose beneficial biocidal properties have been known for centuries. Silver nanoparticles offer very good value in terms of biocidal capacity. Nanosilver can be used on a mass scale, as it is ecologically pure and safe to the environment. Furthermore, fragmented nanoparticles have an incommesurately bigger active surface and so have an enormous biocidal potential. Nanosilver kills bacteria both under normal lighting conditions and in the dark. What is more, such properties are permanent – a silver nanoparticle kills bacteria until it is removed [2]. However it is important that nanosilver could be also risky for immune system.

Because of controversial reports regarding silver [3–6], a decision was made to check the influence of the preparation called Nano Silver made by Nanoco on the survivability of Brine Shrimp (*Artemia salina* Leach 1819).

Material and method

The study was carried out on Brine Shrimp (*Artemia salina* Leach 1819).

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Branchiopoda
Order: Anostraca
Family: Artemidae
Genus: Artemia
Species: *Artemia salina*

Silver for the study was sourced from the preparation called Nano Silver, made by Nanoco. The preparation contains 2000 mg Ag · dm^{-3} of silver in 1000 cm^3 of the product. The experiment was conducted at the laboratory of the Department of Limnology and Fisheries of Wroclaw University of Environmental and Life Sciences. The graphic presentation of the results of the study in the form of tables and graphs has been prepared using MS Excel 2002. The results were processed statistically by means of the Statistica 7 package.

Brine Shrimp (*Artemia salina* Leach 1819) is an aquatic crustacean and is the best known feed for fry [7]. Fully developed specimens (obtained after incubation in optimum conditions) were placed in ten Erlenmeyer flasks, ten specimens per flask. The flasks contained ISO standard water [8] and silver. Standard water was used because of its constant chemical composition, as there were reports about the influence of various compounds on the accumulation of silver in organisms [9]. Silver was obtained from Nano Silver made by Nanoco at concentrations of 5–100 mg Ag · dm^{-3} and from silver nitrate at concentrations of 5–100 mg Ag · dm^{-3}. The concentrations were many times higher than those normally present in water [10] or recommended by the WHO [11] and the EPA [12].

Brine Shrimp larvae were kept at 18 ^{o}C. Because under normal conditions Brine Shrimp lives in fresh water for about eight hours, the experiment was set to last six hours. After that time the number of surviving Brine Shrimp specimens were counted and then compared with that obtained in standard ecotoxicological tests, ie tests in which Brine Shrimp are subjected to an experimental factor in the salt water in which they developed [13]. In the study in question Brine Shrimp were tested in fresh water in order to obtain results applicable to pond management.The test was done three times, applying the same parameters.

Results and discussion

The impact of silver on the survivability of Brine Shrimp was tested in three repetitions.

A decision was made to conduct an additional study aimed at verifying whether the preparation Nano Silver has the same damaging effect on Brine Shrimp larvae (Fig. 1) as silver nitrate at similar concentrations (Fig. 2).

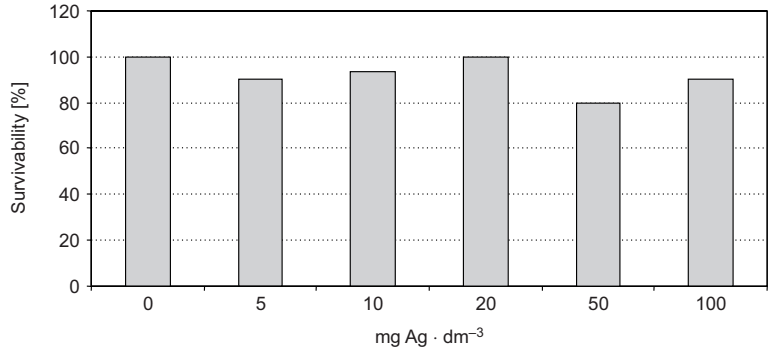

Fig. 1. Average survivability of Brine Shrimp at various concentrations of silver from the Nano Silver preparation (n = 3)

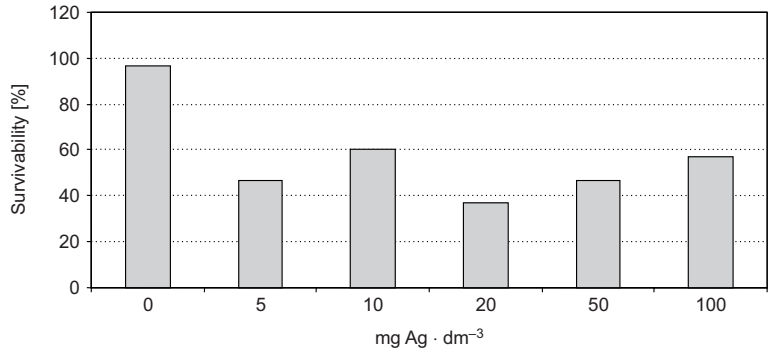

Fig. 2. Average survivability of Brine Shrimp at various concentrations of silver from silver nitrate (n = 3)

In the control sample there were no changes as to the number of surviving specimens. The average survivability, depending on the concentration, was as presented on the graph (Fig. 2). A comparison of the successive concentrations indicates that survivability is not directly proportional to the increase in silver concentration.

The correlation coefficient has been computed for silver nitrate and Brine Shrimp survivability at r = 0.30265. For Nano Silver r = –0.3432.

At the silver concentration of 36 μg · dm^{-3} the copepod *Acartia tonsa* dies in 96 h LC50 [12], whereas the Daphnia (*Daphnia magna*) shows LC50 96 h already at the concentration of 5 μg · dm^{-3} [14]. In the test with Nano Silver the value could not be calculated for Brine Shrimp, as its survivability was higher.

The study regarding Brine Shrimp was carried out using silver nanoparticles and, for comparison purposes, silver from silver nitrate. The study brief provided for checking the effect of Nano Silver only. The results obtained were so surprising that a decision was made to verify them with silver nitrate, which is frequently present in water.

When the preparation was used, Brine Shrimp's survivability was high, as it amounted to 93.33 % for all repetitions. At the highest concentrations Brine Shrimp's survivability increased. Only in one case – at 50 mg Ag · dm^{-3} – the value fell to 60 %, albeit in one repetition only. In the case of the other repetitions with the same concentration the survivability was higher, at 80 %. Only at these two highest concentrations (50 and 100 mg Ag · dm^{-3}) did some specimen die in every repetition. In other cases there was always one concentration at which all specimens survived, which meant that silver had no effect on Brine Shrimp's survivability. It should be pointed out that the silver concentrations used in the experiment may only occur in heavily polluted water reservoirs [15]. At the concentration of 20 m gAg · dm^{-3} the obtained result was similar to that found for the control sample, ie all specimens survived in all repetitions. This is interesting, as such a result was not obtained even at the lowest concentration. The highest mortality rate, observed at 50 mg Ag · dm^{-3}, amounted to 20 %, and the average for all repetitions – only to 7.5 %. The result may indicate low toxicity of silver nanoparticles in respect of this aquatic crustacean. The survivability did not fall linearly with increasing silver concentrations. It seems that silver nanoparticles can be safely used in waters which are home not only to Brine Shrimp but also to other aquatic crustaceans.

The test with silver nitrate was done after Brine Shrimp's very high survivability rate was obtained in the experiment with Nano Silver. The average survivability of Brine Shrimp when silver nitrate was used amounted to as little as 49.33 %, and was much lower than when nanoparticles were applied. This proves that silver may have a different impact on zooplankton, such as Brine Shrimp, depending on its source. At the lowest concentration used, 5 mg Ag · dm^{-3}, 50 % of the specimens died. This was five times more than when Nano Silver was used (10 %). At all of the remaining concentrations a similar result was obtained. In one repetition with 20 mg Ag · dm^{-3} of silver nitrate only 20 % of the specimens survived in one flask. The highest survivability, at 70 %, was determined for 100 mg Ag · dm^{-3}. All of the obtained results point to the advantage of the Nano Silver preparation. However, in one of the control samples one specimen

died. A certain natural mortality rate should always be expected, even in the case of a population not subjected to any toxic substance [16].

In their study concerning *Daphnia magna*, Gloger and Wood observed a very desirable phenomenon – silver was removed by Daphnia after a 24-hour period of its accumulation. Already after 60–70 hours silver concentration was down to the level before the accumulation [17].

The use of the Nano Silver preparation may be very advantageous in pond management.

Use of silver in the form of Nano Silver enables low doses of silver to be used and so limits bioaccumulation and biomagnification of the metal in aquatic environments.

The study indicates that Nano Silver is less toxic to Brine Shrimp than silver nitrate.

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WPŁYW SREBRA ZAWARTEGO W PREPARACIE NANO SILVER NA PRZEŻYWALNOŚĆ LARW ARTEMII (*Artemia salina* LEACH 1819)

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Abstrakt: W Zakładzie Limnologii i Rybactwa na Uniwersytecie Przyrodniczym we Wrocławiu przeprowadzono doświadczenie mające na celu wykazanie wpływu srebra pochodzącego z preparatu Nano Silver na przeżywalność artemii (*Artemia salina*). Czas trwania doświadczenia wyniósł sześć godzin, ponieważ artemia przeżywalność wodzie słodkiej żyje około ośmiu godzin. Średnia przeżywalność w trzech powtórzeniach dla

wszystkich stężeń dla preparatu Nano Silver wyniosła 93 %, przy czym nie wykazano liniowego spadku żywotności artemii wraz ze wzrastającym stężeniem srebra. Wykazano, że nanocząstki srebra są mało toksyczne dla skorupiaka wodnego. Niska toksyczność może się okazać pomocna przy zmniejszeniu strat w zooplanktonie podczas stosowania preparatu w celu ograniczenia masowego rozwoju roślin w stawach.

Słowa kluczowe: srebro, artemia, przeżywalność