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Histopathological changes in zebrafish exposed to sublethal concentrations of 89 nm silver nanoparticles for application in environmental diagnostics

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ABSTRACT

The use of biomarkers as indicators of environmental contamination has been shown to be an excellent indicator of changes in biota. Histopathological lesions are commonly used in biomonitoring studies as they provide information regarding both acute and chronic exposure. The use of nanoparticle materials has been widespread in recent years. However, not much is known about their ecological effects on the natural environment. Thus, the aim of this study was to assess the sublethal effects of silver nanoparticles (AgNP) with mean diameters of 89 nm in the zebrafish *Danio rerio* by the determination of the LC_{50}; 48 h and histopathological assays in gills. The obtained LC_{50}; 48 h was 8.18 μgL^{-1}. The histopathological gill assessment showed primary responses indicative of acute damage as aneurysms (32.76%), hyperplasia (20.69%) and partial (30.17%) and total lamellar fusion (6.9%) of secondary lamellae. No deposition of AgNP was observed in any tested sample gills, suggesting other organs target to absorption and detoxification. In fact, the AgNP causes sublethal damage in the gills of zebrafish but is not able to accumulate in this tissue. Finally, the data shown in this study contribute to the construction of a database on the AgNP exposure in aquatic organisms.

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biomarkers; Danio rerio; environmental health; gills; deposition

Introduction

Health is the complete homeostasis of the organism, independent of the taxon of the fauna and flora and their balanced relationship with the abiotic and biotic factors of the ecosystem (Aguirre *et al.* 2002). In recent years, the intrinsic relationship between the adverse environmental changes and their effects in the health is being increasingly perceived (Weihs and Mertens 2013). Intense anthropogenic activities have increased the level of chemical compounds in aquatic ecosystems, reducing considerably their environmental quality and, therefore, interfering in the healthiness of the resident organisms (Arias *et al.* 2007). The toxicity of organic and inorganic pollutants has been widely

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studied in aquatic environments, in particular their chemical characteristics and effect on the biota (Wood *et al.* 2011).

In many cases, these aquatic systems are the final destination of several xenobiotics, and in this way, the contaminants are subject to diverse interactions like antagonism, synergism, potentiation or addition (Manahan 1992). Therefore, the integrative study of the field (*ex situ*) with bioassay data (*in situ*) is extremely advantageous. On the other hand, the high concentrations to which test organisms are exposed in the controlled experimental conditions must be interpreted carefully and together with realistic data under natural conditions.

The use of fish as test organisms in bioassay is well established and has been broadly used as a biological model due to some of the biological characteristics of the organisms such as the body size, ease of handling and long life cycle when compared to microcrustacean species, which are also commonly used in ecotoxicological assay (Boettcher *et al.* 2010; Obiakor *et al.* 2014). Fish are also used for this purpose as they are vertebrate organisms and have mechanisms of response to chemical stress similar to that of mammals, in addition to being important as a link between trophic levels (Buss *et al.* 2003; Souza *et al.* 2013). Concerning their biological and metabolic features, fish have a high respiratory rate, large gaseous exchange area and a countercurrent system that increases the absorption of pollutants from the water into the bloodstream, allowing a better investigation of the potential interactions of xenobiotics with biota. Among the fish species with a good degree of knowledge, the zebrafish *Danio rerio* is widely used for this purpose because of its easy maintenance and cultivation in captivity (Meyer *et al.* 1993; Souza *et al.* 2013).

D. rerio zebrafish is a tropical fish originating in India and Pakistan and over time have been introduced to several parts of the world (Briggs 2002). This species is notorious in ecotoxicological studies and it is widely used in toxicity tests (Westerfield 2000; ABNT 2011; OECD Guideline 2013). Adult specimens of *D. rerio* reach a mean length of 4.5 cm and the sex of adults can be easily recognized by sexual dimorphism. Males are elongated, thin and lightly golden, especially in the abdomen and pectoral and caudal fins, with a complete abdominal stripe (Kullander 2015). On the other hand, females are robust, slightly larger than the males, silvery, usually have the abdomen very swollen due to the development of eggs and the abdominal stripe is incomplete (Kullander 2015). Zebrafish is dioecious, presents external fertilization and the first spawning can occur when the female reaches a length of 2.5 cm and the males 2.3 cm when kept at 25–26 °C (Westerfield 2000).

The use of biomarkers as tools of environmental monitoring and diagnostics concerning contamination processes are excellent indicators of changes in biota at the cellular and ecological levels (Van Der Oost et al. 2003).

Histopathological lesions are frequently used in biomonitoring studies as morphological biomarkers because they provide information on both acute and chronic exposure processes (Azevedo *et al.* 2013). Some authors consider that lesions in fish gills can be interpreted as a result of acute and chronic effects from different environmental stressors (Marques *et al.* 2009; Lukin *et al.* 2010). Branchial alterations such as lamellar fusions, hyperplasia, hypertrophy, mucosal cell proliferation and aneurysms are the most frequent effects associated with acute exposure of organisms to inorganic and organic compounds, for instance metals and hydrocarbons (Brunelli *et al.* 2011).

The use of nanoparticles has increased in recent years, mainly in the textile manufacturing, cosmetics and engineering industries. Due to their low size with a mean diameter $\leq$100 nm and high mobility, once in the aquatic environment these materials are available to the biota and can affect different levels of ecosystem organization (Bilberg *et al.* 2011). The silver nanoparticles (AgNP) have interesting properties such as good electrical conductivity, high catalytic effect and high surface area. AgNP are highly employed in healthcare because of their excellent antimicrobial action (Chen and Schluesener 2008; Antunes *et al.* 2013).

Although AgNP applications are increasing in several fields, their environmental and health impacts are still being studied. Tests with anti-fungal tissues like socks containing AgNP showed release of the ion Ag^{+}, indicating an input to the aquatic environment through domestic sewage. It is worth mentioning that the toxicity of nanoparticles is closely related to their mean diameter, concentration and rote of exposure, nature of the environment and the nanoparticle. In addition, nanoparticles of smaller diameter tend to initiate a higher proportion of deleterious cellular effects (Fabrega *et al.* 2011; Vale *et al.* 2016).

The aim of this study was to determine the LC50; 48 h in zebrafish *D. rerio* exposed to differential concentrations of AgNP 89 nm in the aquatic system in order to observe the development of sublethal effects performed by histopathological observation of the gills. In addition, the elemental composition of the branchial tissue was tested. Therefore, the principal goal is the integrative use of these tools in order to promote environmental diagnostics together the classic ecotoxicological assay to mortality evaluation by the LC_{50} of the AgNP 89 nm.

Material and methods

Chemicals and reagents

All materials used in the assays were washed in 10% HCL and 5% acetone. AgNP solution was gently provided by Khemia (22 mg L^{-1} stock solution) with mean size of 89 nm. For toxicants exposure, AgNPs were diluted in reconstituted water to 1 L (30 mg $CaSO_4.2H_2O$, 2 mg KCl, 48 mg $NaHCO_3$ and 61 mg $MgSO_4.7H_2O$). The control treatment contained only reconstituted water.

Zebrafish experimental design

Adult of male and female individuals of zebrafish (*D. rerio*) were cultured in the Ecotoxicology Laboratory at IPEN/CNEN-SP, Brazil. Individuals were placed in tanks with 45 L with a ratio of 1-g organisms/L. All abiotic parameters, such as temperature, pH and total hardness monitored in the tanks were in accordance with the established range for ecotoxicological assay by ABNT NBR 15088 (2011).

Zebrafish were submitted to acclimatization and kept in reconstituted water previously prepared and controlled for total hardness and pH for 7 days. The pH was maintained at 7.13 ± 0.24, total hardness ranging from 40 to 48 mg L^{-1} $CaCO_3$, 12:12 h light: dark cycle at 22 °C ± 0.67 in a re-circulation water system. The renewal of the culture water was carried out to maintain the adequate range of pH, low NH_3^{+} contents and

elimination of precipitated debris. This practice was performed once a week, with about 25% of the volume of the tanks being removed at each renewal. The organisms were fed 12:12 h with commercialized fish food, free of contaminants. The viability of the purchased fish lot was tested by the sensitivity assay after the acclimatization of the fish using NaCl as reference solution with 1.3 dilution factor.

Toxicity assay

Tests to determine the sublethal concentrations (LC_{50}) were carried out according to ABNT NBR 15088 (2011). Mortality was used as the endpoint to determine the acute toxicity by LC50. Six different concentrations of AgNP suspension (1.5; 2.3; 3.4; 5.1; 7.6 and 11.4 μg L^{-1}; 1.5 dilution factor) were prepared prior to the use without addition of salts or stabilizing. Ten zebrafish were randomly placed in each experimental group and exposed to the concentrations for 48 h in a 15-L tank containing 10 L of the AgNP test solution with temperature and pH controlled about 22 °C and 7.1, respectively. One group was the control, therefore without AgNP solution. Each treatment was made in triplicate under the same conditions. The zebrafish feed was interrupted 24 h prior to the experiments and during the experiments in order to avoid the absorption of NPs in food. The number of dead zebrafish was monitored at 24 and 48 h and they were removed from the tanks immediately to avoid contamination. After 48 h the assay were stopped, the remaining surviving fish were anesthetized with 1% benzocaine hydrochloride, measured, weighed, blood collected and decapitated to dissection of the gills. Temperature, pH and total hardness were monitored before and after the experiments.

Histophatology of gills

Fish were placed in Petri dishes containing 10 mM, pH 7.4 sodium phosphate buffer and submitted to dissection to obtain the intermediate branchial arches. These samples were placed into vials containing ALFAC fixative solution (ethanol 80%, formaldehyde 37–40% and glacial acetic acid) for 16 h, dehydrated in a graded series of ethanol baths, and embedded in Paraplast-Plus resin (Sigma©). Five-micrometer-thick sections were obtained, stained with hematoxilin, counterstained with eosin and observed under light microscope. The lesion index was determined following Bernet *et al.* (1999). After dissection, fish were euthanized by decapitation in accordance with Brazilian law 11.794/2008, packed in plastic zipper bags and kept at −20 °C for subsequent incineration.

Elementary composition

Branchial arches that were not analyzed for histopathological changes were analyzed for the Ag adsorption on fish gills. Samples were placed on glass slides and dehydrated at 30 °C for 7 days. For each sample, surfaces were mounted individually onto specimen stubs and held by double-sided conductive carbon tape. Digital images were acquired using a Hitachi Tabletop microscope model TM-3000 (High Technologies America, Schaumburg, IL) equipped with a Bulker Quantax 70 X-ray (Billerica, MA) microanalysis system. A scanning electron microscopy (SEM) coupled with energy dispersive X-ray

spectroscopy (SEM–EDS) was used as it provides qualitative and quantitative information concerning the elements present on the surface of a sample.

Statistical analysis

Data are showed as mean ± standard deviation and range values. The nonparametric Trimmed Spearman–Karber method was used to determine the CL_{50};48 h (Hamilton *et al.* 1977) since is used by the Brazilian Association for Technical Rules (ABNT) and provide a good accuracy, precision and robustness for real and hypothetical data (Hamilton *et al.* 1977). A p-value < 0.05 was considered for statistical significance.

Results

No signs of stress, such as abnormal behavior, excessive hemorrhage or mucus were observed in the cultures. The sensitivity test is performed in order to verify the lot's quality of the organism test in the experimental groups. When absence of lethality is observed on exposure to the reference substance, for instance NaCl, it is determined that these individuals are not sensitive and, therefore, the lot is discarded. Figure 1 shows results concerning the sensitivity of zebrafish with NaCl in concentrations ranging from 5.5 to 15.7 g L^{-1}. The LC_{50}; 48 h obtained was 9.81 g L^{-1} with upper (UL) and lower limits (LL) of 10.98 and 8.64 g L^{-1}, respectively.

With respect to the toxicity of the AgNP suspension in zebrafish, results concerning temperature, pH and hardness monitored in each tested tank are shown in Figure 2. A slight increase was found in these parameters between the beginning and end of the experiment. However, this variation is within the established criteria by ABNT NBR 15088 (2011) for zebrafish sublethal toxicity assay and thus is not responsive to lethality of the organisms.

Lethality data concerning sublethal toxicity of AgNP on zebrafish are presented in Figure 3. It is possible to observe a lower sensitivity of the organisms in the third assay. The mean obtained LC_{50} was 8.18 μg L^{-1}. The LL and UL of the second bioassay cannot be calculated as 50% of the lethality of the population occurred at the highest concentration tested.

In general, zebrafish of the third bioassay had lower mean values of total length (TL) and total weight (TW) (TL = 28.87 ± 2.62; TW = 0.23 ± 0.07) than individuals of the first (TL = 31.48 ± 2.19; TW = 0.29 ± 0.07) and second (TL = 31.34 ± 2.60; TW = 0.30 ± 0.08) bioassays. Mean values of TL and TW to each tested AgNP concentration are shown in Table 1. In fact, the lower biotic values were found in the third bioassay for most of the tested AgNP concentrations. However, the length–weight relationship obtained for fish from each bioassay showed a larger dispersion regarding the second bioassay, and a positive and significant correlation for the three bioassays was found. Therefore, all used fish were in the established conditions by ABNT NBR 15088 (2011).

The normal lamellar pattern and the observed branchial changes obtained in zebrafish exposed to differential AgNP concentrations are shown in Figure 4. Partial lamellar fusion and aneurisms were the most frequent alterations found in gills of zebrafish, with occurrence 30.17% and 32.76%, respectively. Hyperplasia, total lamellar fusion and

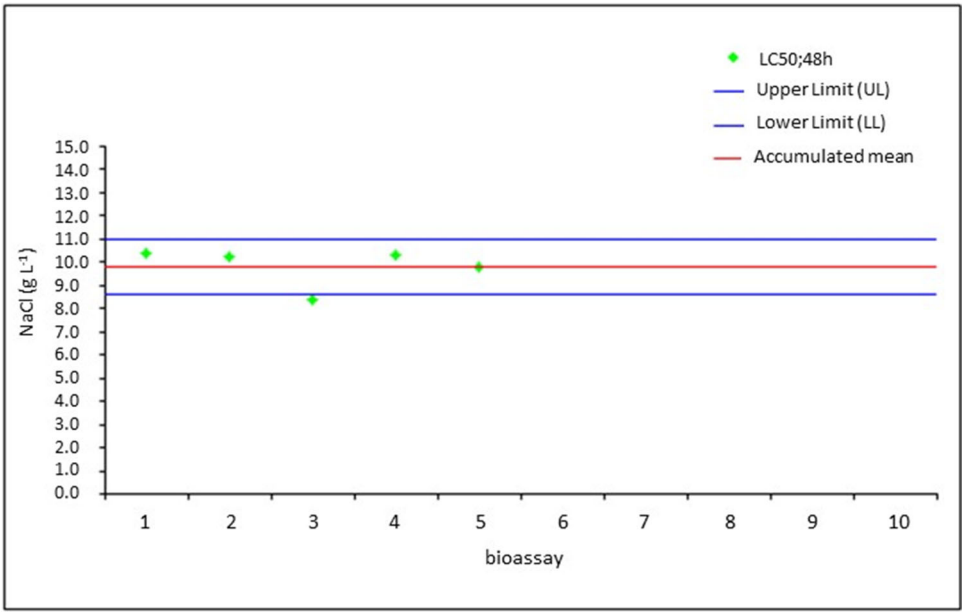


Figure 1. Sensitivity of zebrafish exposed to NaCl concentrations ranging from 5.5 to 15.7 g L^{-1}, with the LC50; 48 h value, upper (UL) and lower limits (LL). Data shown from five assays.

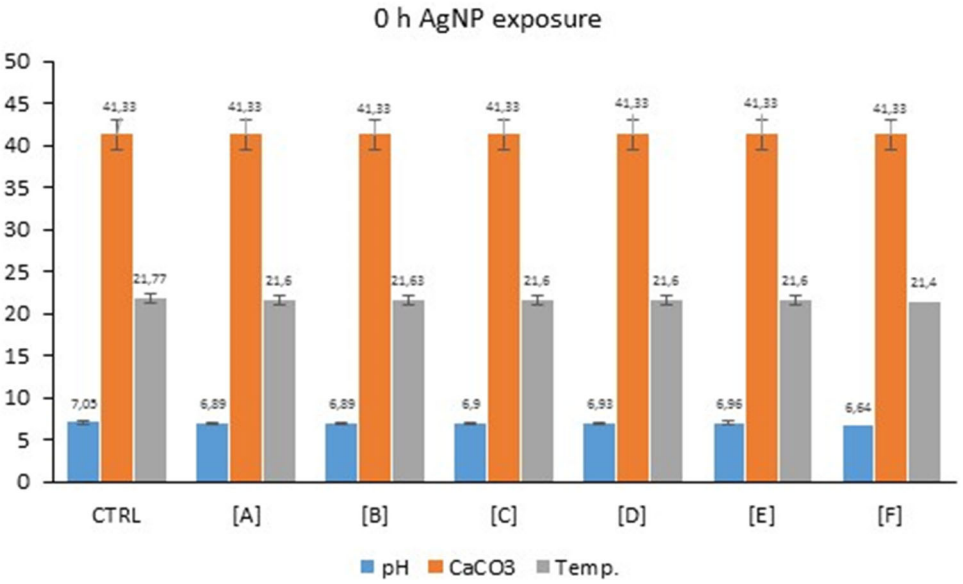
leukocytes infiltration were also observed but with occurrence of 20.69, 6.9 and 6.0%, respectively. In general, gills with aneurisms were more obvious in fish submitted to the higher AgNP concentrations. The index of branchial injuries (Ib) in fish exposed to each AgNP concentration showed primary alterations along all of the tested concentration (Figure 5).

In order to verify the Ag adsorption on fish gills, these samples were submitted to elemental microanalysis. The energy spectrum of the elements and the SEM images of the possible Ag particle are shown in Figure 6. The obtained data showed the presence of the elements: oxygen (O), carbon (C), phosphorus (P), sulfur (S), calcium (Ca), magnesium (Mg), aluminum (Al), silicon (Si), potassium (K), iron (Fe), nitrogen (N), titanium (Ti) and zirconium (Zr). Ag was not found in any of the analyzed gill samples, suggesting absence of deposition of this element in the gills.

Discussion

The use of different tools to environmental diagnose in aquatic ecosystems is important. Considering AgNP, some previous studies suggest that several factors should be considered, as exposure time, size of the particle and fish species.

Methods to determine the median lethal concentration (LC50) usually use the probit and logit models, where the relationship between mean mortality and concentration of toxicants is described. However, these models provide several anomalies when working with a large number of bioassays (Hamilton *et al.* 1977). For this reason, Hamilton *et al.* (1977) proposed another method to LC50 estimation. These authors to affirm that "Estimation of the LC50 by the Trimmer Spearman-Karber procedure has some advantages such as: (1) The calculations described can be programed for a computer and/or



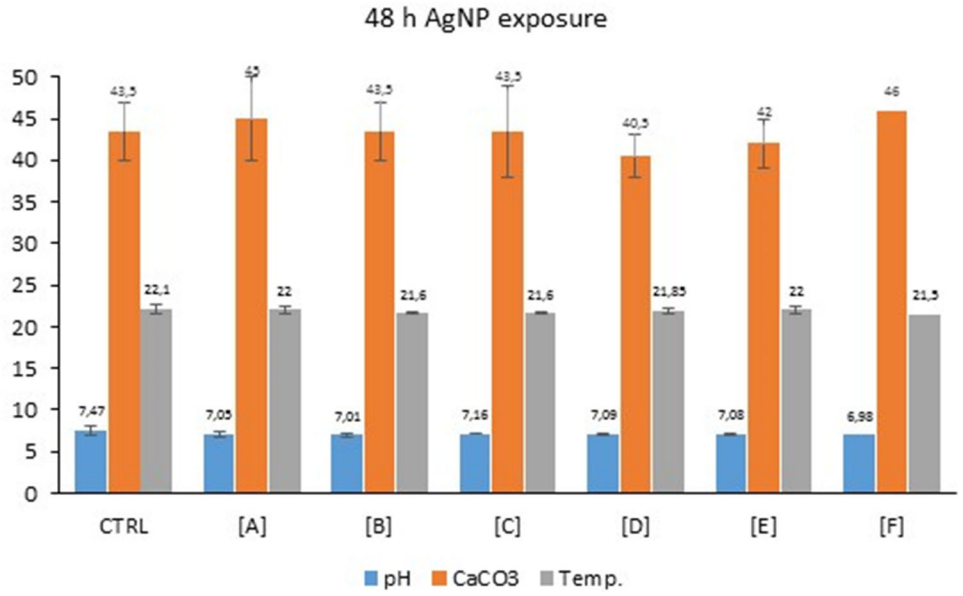


Figure 2. Physicochemical parameters as pH, total hardness (mg CaCO3) and temperature (°C) of the solutions before and after exposure to AgNP at 0 and 48 h in the assay. Data are showed as mean values ± standard deviation of three bioassays.

can be done on a desk calculator; (2) The method *never fails,* no matter which unusual mortality pattern is observed; and 3) always provides an estimate of μ if $p_1 \leq 0.5 \leq p_k$". Therefore, taking into account the real and hypothetical data, accuracy, precision, computability and robustness, this method is overall a better method than the methods based on the probit and logit models (Hamilton *et al.* 1977).

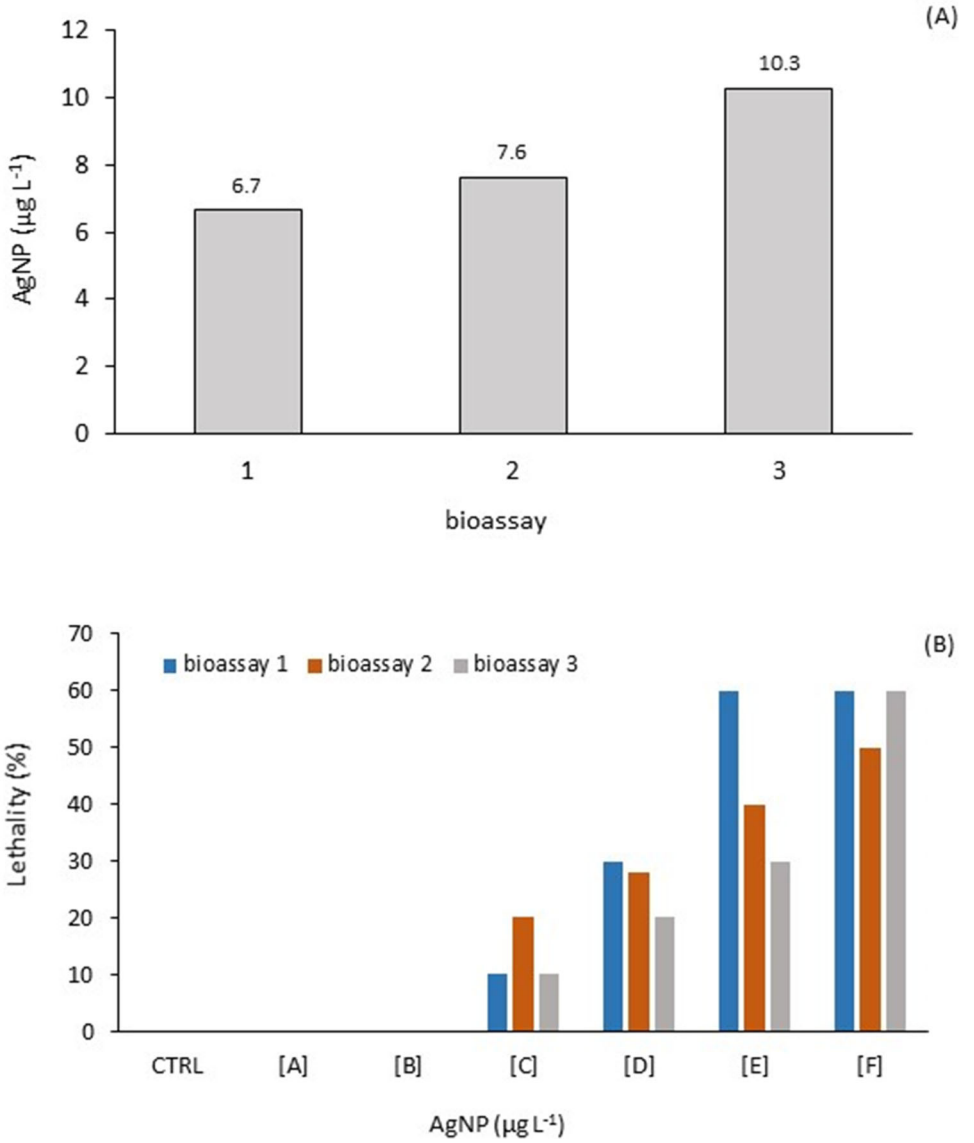


Figure 3. Bioassays concerning the LC50; 48 h to zebrafish *Danio rerio* exposed to AgNP suspension (A) and sublethal ecotoxicity assay in zebrafish expressed as lethality (%) to each tested AgNP concentration (B). [CTRL]: control; [A]: 1.5 µg L^{-1}; [B]: 2.3 µg L^{-1}; [C]: 3.4 µg L^{-1}; [D]: 5.1 µg L^{-1}; [E]: 7.6 µg L^{-1}; [F]: 11.4 µg L^{-1}.

Studies with adults of *Oryzias latipes* (Wu *et al.* 2010) obtained a LC_{50}; 24 h of 1.03 mg L^{-1} using AgNP with 25 nm of mean diameter. On the other hand, the LC_{50}; 96 h found values of 34.6 µg L^{-1} for adults of the same species but using 50 nm of AgNP (Chae *et al.* 2009). However, there are differences concerning the exposure time and the mean diameter of the AgNP; these fish species had similar length and husbandry conditions as the zebrafish tested in this work where a LC_{50}; 48 h of 8.18 µg L^{-1} was found using a AgNP with 89 nm mean diameter. It was observed, therefore, that the obtained lethal concentration varies according to the size of the nanoparticles and

Table 1. Biometric data as total length (TL–mm) and total weight (TW–g) of zebrafish exposed to different AgNP concentrations.

		AgNP Concentrations (μg L^{-1})						
Assay	Variable	CTRL	[A]	[B]	[C]	[D]	[E]	[F]
1	TW	0.29 ± 0.08	0.33 ± 0.12	0.29 ± 0.10	0.26 ± 0.09	0.32 ± 0.07	0.31 ± 0.09	0.29 ± 0.06
	TL	30.90 ± 4.04	32.50 ± 3.49	31.45 ± 2.55	30.30 ± 3.21	32.0 ± 2.12	31.70 ± 1.77	29.08 ± 2.56
2	TW	0.27 ± 0.10	0.24 ± 0.07	0.31 ± 0.08	0.31 ± 0.11	0.29 ± 0.12	0.36 ± 0.12	0.28 ± 0.09
	TL	31.63 ± 3.35	30.24 ± 2.31	32.73 ± 2.03	31.23 ± 4.06	31.0 ± 3.38	31.23 ± 4.06	30.85 ± 3.05
3	TW	0.21 ± 0.08	0.29 ± 0.13	0.20 ± 0.07	0.17 ± 0.08	0.20 ± 0.03	0.27 ± 0.10	0.31 ± 0.12
	TL	28.38 ± 3.01	31.47 ± 3.57	28.06 ± 2.96	26.67 ± 3.4	28.23 ± 1.49	30.13 ± 3.43	33.96 ± 5.52

*[CTRL]: control; [A]: 1.5 μg L^{-1}; [B]: 2.3 μg L^{-1}; [C]: 3.4 μg L^{-1}; [D]: 5.1μg L^{-1}; [E]: 7.6 μg L^{-1}; [F]:11.4 μg L^{-1}.
Data are presented as mean values ± standard deviation. $n = 10$/concentration.

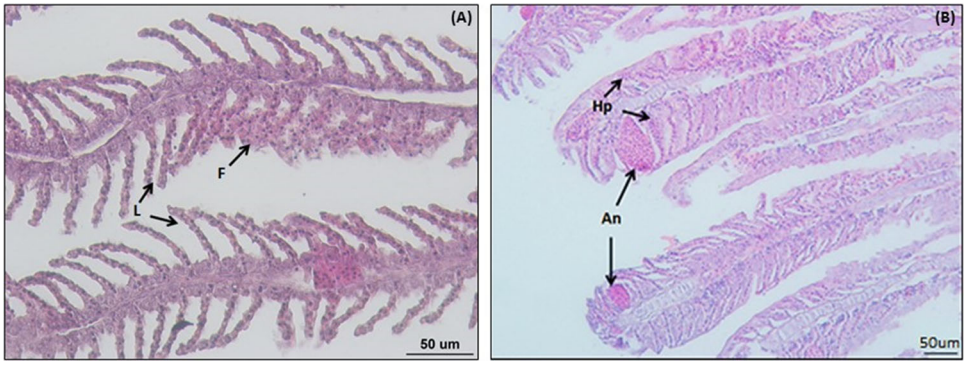


Figure 4. Cross-sections of gills of zebrafish *Danio rerio* exposed to different concentrations of AgNP. (A) Gill showing secondary lamellae without lesions (L) and lamellar fusion with cell proliferation among secondary lamellar (F); (B) Hyperplasia (Hp) and aneurysm (An). Bar scale = 50 μm.

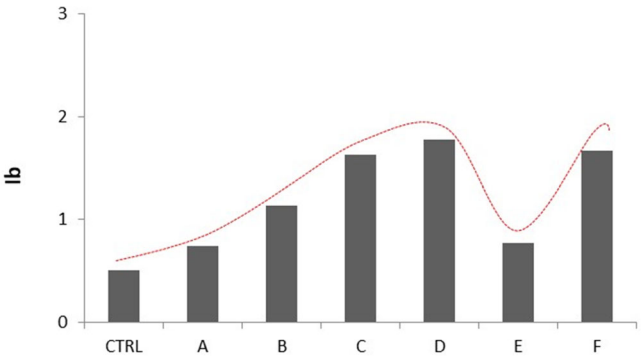


Figure 5. Lesion index in gill (Ib) of zebrafish *Danio rerio* exposed to different concentrations of AgNP. The results are expressed as mean values. [CTRL]: control; [A]: 1.5 μg L^{-1}; [B]: 2.3 μg L^{-1}; [C]: 3.4 μg L^{-1}; [D]: 5.1 μg L^{-1}; [E]: 7.6 μg L^{-1}; [F]: 11.4 μg L^{-1}.

the time of exposure. In fact, exposures with smaller particles and smaller exposure time give higher concentration values for the LC_{50}.

Another important point to consider, beyond the exposure time and the concentrations of the nanoparticles, is the selection of the fish species. Govindasamy and

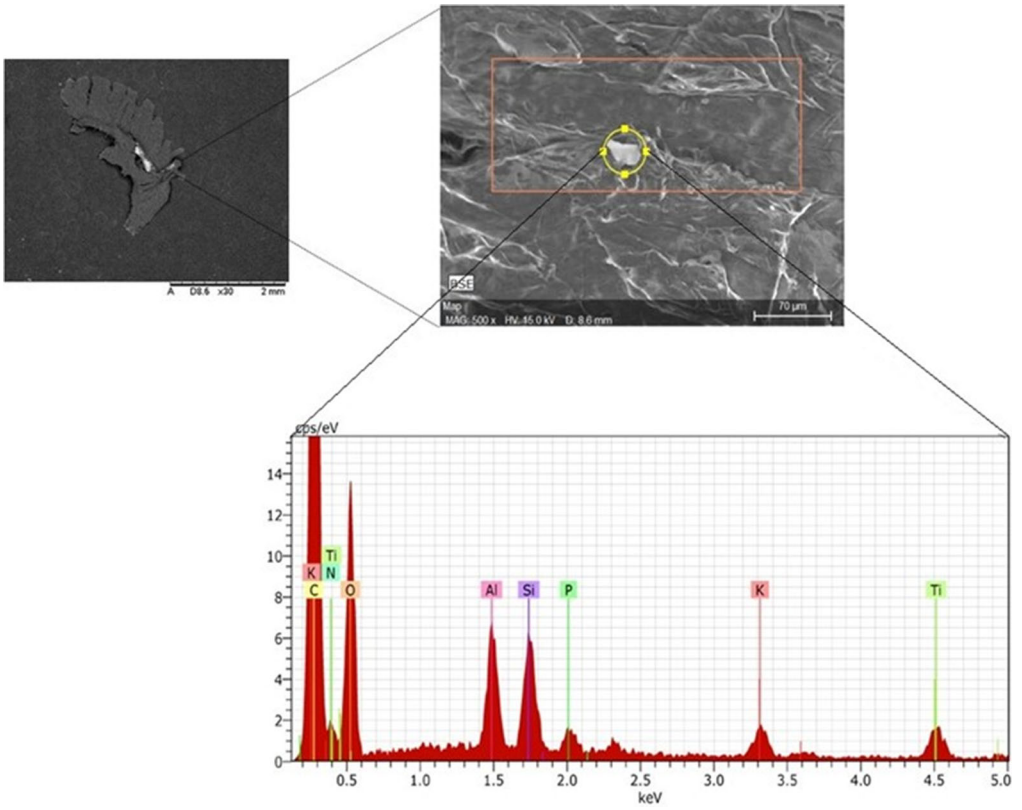


Figure 6. Scanning electron micrographs of gill of zebrafish *Danio rerio* exposed to different concentrations of AgNP (89 nm) showing the total gill, specific area analyzed concerning elemental composition and the obtained energy spectrum.

Rahuman (2012) found a LC_{50}; 8 days of 12.6 mg L^{-1} testing AgNP with 60–80 nm in the fish *Oreochromis mossambicus*. In order to evaluate the acute effect of AgNP between 20 and 30 nm in different trophic levels, Griffitt *et al.* (2008) found a LC_{50}; 48 h of 0.04 mg L^{-1}, 7.07 mg L^{-1} and 7.2 mg L^{-1} respectively for *Daphinia pulex*, adults of zebrafish and juveniles of zebrafish. The dispersion of the NP and the concentrations of the Ag ions in solution was not determined in this work. In fact, silver (Ag) in the nanoparticulate form is less toxic than in soluble forms (Griffitt *et al.* 2008). Similarly, in addition to the factors already mentioned, differences between the LC_{50} values obtained in this study and those observed in the literature may be a consequence of the final composition of the tested AgNP solution, as the lethality on the fish can be caused by alterations in the osmoregulation process of the gills when they react with the AgNP and the silver ions of the solution to which they are exposed (Govindasamy and Rahuman 2012).

In fact, the choice of range concerning the tested AgNP concentrations is in accordance with that observed in the natural environment.

The initial defense mechanism of vertebrates through cells is to interfere with the access of the pollutant on the bloodstream. In this way, they reduce gas exchange and induce vasodilation, such as the aneurysms and hyperplasias observed in the bioassays performed with zebrafish in this study. These changes are considered primary responses, which may

be caused by other stress factors such as temperature and oxygen variations in the environment (Bernet *et al.* 1999) and have a low exposure time to expression. However, in the present study, there were no variations regarding these abiotic parameters. The secondary responses generally are formed with a medium to long exposure time as a longer exposure time of the organism to sublethal stressors is required. These responses are considered good biomarkers of chronic exposure, rather than the primary responses that are indicative of acute exposure (Bernet *et al.* 1999; Azevedo *et al.* 2013). The secondary responses, such as necrosis, were not observed in this study, probably due to the short time of exposure.

According to Govindasamy and Rahuman (2012), branchial damages as constrictions, fusion and hyperplasia of the primary lamellae were observed in *Oreochromis mossambicus* exposed to 50 mg L^{-1} AgNP with 60–80 nm mean diameters. Similar to the histopathological data found in this study, these branchial changes occurred in all tested AgNP concentrations, including the control fish. These results were also influenced by the sample number observed at each tested concentration, as the sampling was reduced by the occurrence of lethality at the highest concentrations. In addition, the presence of primary changes in control fish may be associated with the quality of the obtained batch.

Furthermore, in the fish species *Eurasian perch,* it was observed that the production of mucus in the tanks when fish were exposed to higher concentrations of NPAg (81 nm), possibly arose from the gills' protection mechanisms to xenobiotic exposure (Bilberg *et al.* 2011). However, the lack of achievement to perform the histological evaluation in this assay makes it difficult to compare the organ damage in different species. In histological studies of gills performed with other types of nanoparticles, for instance CuNP, Al-Bairuty *et al.* (2013) also observed the development of hyperplasias, aneurysms, lamellar fusion and edemas in the fish *Oncorhynchus mykiss* exposed to 100 μg L^{-1} CuNP with 50 nm of mean diameters during 10 days.

Finally, histopathological evaluation is an important tool used as a biomarker as it allows for the predicting of the induction of primary and secondary responses, possible loss of organ function and different types of interaction that may occur in the tissue according to the concentration, time of exposure and chemical characteristics of the tested substance, and biotic aspects as taxon, age and sex. The highest Ib found in zebrafish from the control tanks suggests a previous stress prior to the acclimatization and the bioassay of acute toxicity with AgNP. This stress is probably related to transport and/or quality of the acquired zebrafish batch.

When the contaminant is found in the aquatic environment, it can behave in different ways and interact with the organisms in different ways, for instance being absorbed, accumulated, biotransformed or excreted. The gills, feeding, water uptake and the epithelial tissue are the most common input route of the xenobiotic in the fish. Thus, the elemental analysis of the gill tissue by SEM as proposed in this study was performed to verify the possible deposition of AgNP in the gills of zebrafish when exposed to AgNP concentrations similar to observed in the aquatic system by domestic and industrial discharge, as the gills have the highest surface area of contact with the AgNP contained in the water.

No deposition of AgNP was observed in the gills at any tested sample. This achievement is in accordance with the obtained microanalysis by SEM performed in the fish species *Eurasian perch* exposed to AgNP with similar mean diameters as in this study

(Bilberg *et al.* 2011). According to Fabrega *et al.* (2011) AgNP of 10 nm are more likely to associate with gills and/or accumulate in the liver than nanoparticles of higher diameters. This profile may be due to competition for the gill surface binding sites between the AgNP and the Ag^{+} ions in the solution (Griffitt *et al.* 2013). In this way, AgNP can cause histological changes in the gills but do not necessarily tend to accumulate in this tissue.

Most of the observed elements in the gills of zebrafish by SEM have great biological functions in the metabolic processes for the organism, such as oxygen, carbon, nitrogen, iron, phosphorus, sulfur, calcium and magnesium. These elements are important, for example in the breathing process, acting as enzymatic cofactors, composition of organic molecules such as proteins and maintenance of bone structures (Barbosa 2010). Others elements, such as aluminum and silicon, are also found in the food fish used to feed the test organisms, which justifies their presence in the microanalysis of the gill performed by SEM.

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Compliance with ethical standard

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