2017-05

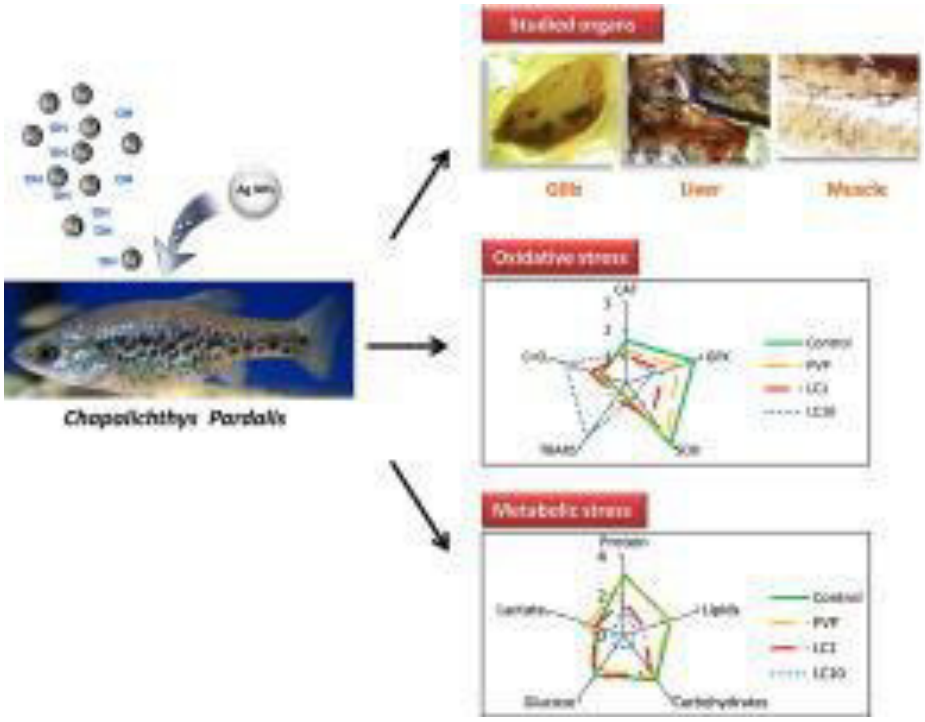
Science of the Total Environment: 583(2017): 308-318

Exposure to silver nanoparticles produces oxidative stress and affects macromolecular and metabolic biomarkers in the goodeid fish Chapalichthys pardalis

R.C. Valerio-Garcia, A.L. Carbajal-Hernandez, E. B. Martinez-Ruiz, V.H. Jarquin-Diaz, C. Haro-Perez, F. Martinez-Jeronimo

Roberto Carlos Valerio-Garcia, Ana Laura Carbajal-Hernandez, Erika Berenice Martinez-Ruíz, Víctor Hugo Jarquin-Diaz, Fernando Martinez-Jerónimo: Inst. Politécnico Nacional, Esc. Nacional de Ciencias Biológicas, Carpio y Plan de Ayala S/N, Col. Santo Tomas, Mexico City C.P. 11340, Mexico
Catalina Haro-Pérez: Univ. Autón. Metropolitana, Av. San Pablo No. 180, Col. Reynosa Tamaulipas, Azcapotzalco, Mexico City C.P. 02200, Mexico

Silver nanoparticles (AgNPs) are the most commercialized nanomaterial worldwide, mainly due to their microbicidal activity. Although, AgNPs have been shown to be toxic to aquatic species, their effect on endemic fish, like Goodeidae, has not been demonstrated. Endemic species are under strong pressures by anthropogenic contamination and destruction of their habitat; therefore, we studied adult *Chapalichthys pardalis*, an endemic fish of Mexico. We evaluated the toxic effect of AgNPs through oxidative stress, macromolecular and metabolic biomarkers. We determined the LC_{50} (96 h) and performed subchronic tests (21 days) using sublethal AgNPs concentrations (equivalent to CL_1 and CL_{10}). At the end of the bioassay, we quantified 10 stress biomarkers in the liver, gills, and muscle, including the antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT], and glutathione [GPx]), thiobarbituric acid reactive species (TBARS), protein oxidation (C═O), macromolecules (proteins, lipids, and carbohydrates), and metabolites (glucose and lactate). In addition, we determined the integrated biomarkers response (IBR). LC_{50} was of 10.32 mg L^{-1}. Results of subchronic exposure (21 days) revealed that AgNPs produce oxidative stress in *C. pardalis* adults, as evidenced by a diminution in antioxidant enzymes activity and an increase in TBARS and oxidized proteins. AgNPs also diminished levels of macromolecules and generated a high-energy consumption, reflected in the reduction of glucose levels, although lactate levels were not altered. The IBR analysis evidenced that the largest effect was produced in organisms exposed to LC_{10}, being the liver and gills the organs with the greatest damage. Results demonstrated that exposure to AgNPs induces acute and chronic toxic effects on *C. pardalis* and forewarns about the impact that these nanomaterials can exert on these ecologically relevant aquatic organisms.



Science of the Total Environment xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Exposure to silver nanoparticles produces oxidative stress and affects macromolecular and metabolic biomarkers in the goodeid fish *Chapalichthys pardalis*

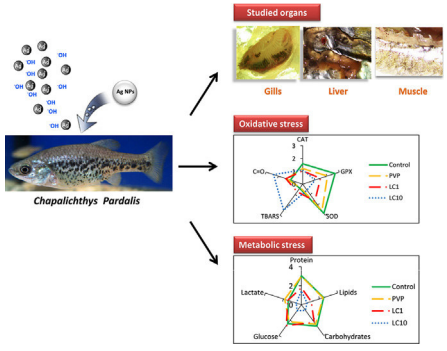
Roberto Carlos Valerio-García [a], Ana Laura Carbajal-Hernández [a], Erika Berenice Martínez-Ruíz [a], Víctor Hugo Jarquín-Díaz [a], Catalina Haro-Pérez [b], Fernando Martínez-Jerónimo [a,*]

[a] Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Carpio y Plan de Ayala S/N, Col. Santo Tomas, Mexico City C.P. 11340, Mexico
[b] Universidad Autónoma Metropolitana, Av. San Pablo No. 180, Col. Reynosa Tamaulipas, Azcapotzalco, Mexico City C.P. 02200, Mexico

HIGHLIGHTS

- The toxicity of Ag nanoparticles (AgNPs) was assessed in an endemic, livebearer fish.
- The antioxidant enzymatic response diminishes at sublethal AgNP's concentrations.
- Sublethal AgNP's concentrations produces oxidative damage in proteins and lipids.
- Macromolecules synthesis in *C. pardalis* adults is affected by AgNPs exposure.
- AgNPs sublethal exposure produced damage at the biochemical level in *C. pardalis*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 November 2016
Received in revised form 10 January 2017
Accepted 11 January 2017
Available online xxxx

Editor: D. Barcelo

Keywords:
Goodeidae
Chapalichthys pardalis
Oxidative stress
Biomarkers
Integrated biomarker response
Livebearer fish

ABSTRACT

Silver nanoparticles (AgNPs) are the most commercialized nanomaterial worldwide, mainly due to their microbicidal activity. Although, AgNPs have been shown to be toxic to aquatic species, their effect on endemic fish, like Goodeidae, has not been demonstrated. Endemic species are under strong pressures by anthropogenic contamination and destruction of their habitat; therefore, we studied adult *Chapalichthys pardalis*, an endemic fish of Mexico. We evaluated the toxic effect of AgNPs through oxidative stress, macromolecular and metabolic biomarkers. We determined the LC_{50} (96 h) and performed subchronic tests (21 days) using sublethal AgNPs concentrations (equivalent to CL_1 and CL_{10}). At the end of the bioassay, we quantified 10 stress biomarkers in the liver, gills, and muscle, including the antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT], and glutathione [GPx]), thiobarbituric acid reactive species (TBARS), protein oxidation (C═O), macromolecules (proteins, lipids, and carbohydrates), and metabolites (glucose and lactate). In addition, we determined the integrated biomarkers response (IBR). LC_{50} was of 10.32 mg L^{-1}. Results of subchronic exposure (21 days) revealed that AgNPs produce oxidative stress in *C. pardalis* adults, as evidenced by a diminution in antioxidant enzymes activity and an increase in TBARS and oxidized proteins. AgNPs also diminished levels of macromolecules and generated a high-energy consumption, reflected in the reduction of glucose levels, although lactate levels were not altered. The IBR analysis evidenced that the largest effect was produced in organisms exposed to

* Corresponding author.
E-mail addresses: ferjeronimo@hotmail.com, fjeroni@ipn.mx (F. Martínez-Jerónimo).

http://dx.doi.org/10.1016/j.scitotenv.2017.01.070

LC_{10}, being the liver and gills the organs with the greatest damage. Results demonstrated that exposure to AgNPs induces acute and chronic toxic effects on *C. pardalis* and forewarns about the impact that these nanomaterials can exert on these ecologically relevant aquatic organisms.

1. Introduction

An exponential increase in products containing nanomaterials has been recorded in the last years. Since 2005, The Project on Emerging Nanotechnologies (PEN) of the Woodrow Wilson International Center for Scholars has been performing an inventory on the products sold worldwide containing nanomaterials as one of their main components. According to PEN, until 2014, there were about 1885 products enlisted, pointing out those that employ silver nanoparticles (AgNPs) with 43.3%, followed by titanium dioxide with 19%, and carbon dioxide with 9.2%. Goreham et al. (2015) reported that 320 tons of AgNPs are produced yearly, and it is expected that the amount of these products will increase significantly in the next years (Woodrow Wilson Database, 2014).

AgNPs are currently one of the nanomaterials most used in frequently used products and in research laboratories (Seltenrich, 2013); these uses include nanomedical devices, environmental remediation, cosmetics, homecare products, *etc.* One of the important applications of AgNPs is due to their antimicrobial properties, for which they are included in a vast array of medical, household, and industrial products (Jung et al., 2007; Perelshtein et al., 2008; Arora et al., 2008; Li et al., 2008; Kumar et al., 2008; Schrand et al., 2008; Wang et al., 2008; Chen and Schluesener, 2008; Sun et al., 2008), which has given rise to great concerns from the environmental point of view (Paresh et al., 2009), because their high production increases the probability of AgNPs being discharged through effluents into the aquatic ecosystems (Monfared and Soltani, 2013; Saddick et al., 2015).

AgNPs concentrations in water bodies are not known, due to the lack of reliable methods for their quantification at low concentrations; however, Boxall et al. (2008) by using a predictive method were able to estimate a concentration of approximately 0.01 μg L^{-1} AgNPs in the aquatic environment (Tiede et al., 2009). Some studies have shown that washing of clothes and household devices containing AgNPs releases a considerable amount of silver (Ag). The amount of Ag released from clothes with AgNPs that contain up to 1360 μg of Ag g^{-1} is of about 650 μg in 500 mL of distilled water (Benn and Westerhoff, 2008), whereas a washing machine with a nano-washing system releases an average of 11 μg L^{-1} of Ag (Farkas et al., 2011). Estimations indicate that a household could potentially release 470 μg of Ag into the sewage every day, coming from products containing AgNPs (Benn et al., 2010); Ag can accumulate in the aquatic environment and produce toxic effects on the phytoplankton (Navarro et al., 2008; Oukarroum et al., 2012), zooplankton (Li et al., 2010; Blinova et al., 2013), and fish (Asharani et al., 2008; Cho et al., 2013; Rajkumar et al., 2016).

Fish are an important group of aquatic biota, as they are the most diverse among vertebrates (Nelson, 1994), and are also the most threatened by pollutants (Duncan and Lockwood, 2001). Fish are distributed among different trophic levels (Trujillo-Jiménez and Espinosa de los Monteros-Viveros, 2006), hence, the toxic effects exerted upon this group will affect importantly the whole structure of the community. AgNPs can produce teratogenesis in fish (Lee et al., 2007; Asharani et al., 2008; Bar-llan et al., 2009; Kannan et al., 2011), oxidative stress by the production of reactive oxygen species (ROS) (Wu and Zhou, 2012; Massarsky et al., 2013), damage to proteins, lipids, and DNA (Choi et al., 2010), diminish the content of proteins (Monfared and Soltani, 2013), and alter the levels of ammonium, ureic nitrogen (Lee et al., 2012), sodium (Na^+), potassium (K^+), chloride (Cl^-) ions, and glucose (Johari et al., 2013).

Goodeids are ecologically relevant fish due to their high endemicity; most of these species are threatened or endangered due to environmental degradation, chemical contamination of water bodies, and the introduction of exotic fish (Duncan and Lockwood, 2001; Domínguez-Domínguez et al., 2005; Dzul-Caamal et al., 2012). The study of this group of fish could provide information for their conservation, particularly if the destruction of their habitat represents a risk for their persistence and survival in nature (Olivares-Rubio et al., 2014; Goodeid Working Group).

Chapalichthys pardalis is a microendemic goodeid, its habitat is restricted to a water spring in the municipal park "Ojo de Agua" of Tocumbo, Michoacán, Mexico (19° 42′N, 102° 32′W) (Miller, 2009), and does not currently present any conservation issue according to either the Red List of IUNC or the Mexican environmental standard (NOM-059-SEMARNAT-2010). It is an omnivore fish, with an average size of 8.5 cm in length and several reproductive cycles per year (Álvarez del Villar, 1963); in captivity a female produces from 20 to 30 offspring.

Currently, no information exists on the toxic effect of AgNPs on native fish species, hence, the objective of this study was to evaluate the toxic response of adult *C. pardalis* exposed to a commercially available suspension of AgNPs. We performed acute toxicity tests and analyzed their subchronic effects by assessing antioxidant defense, oxidative damage, and macromolecular biomarkers, as well as metabolic parameters, in three organs (live, gills, and muscle) to be able to generate information on the damage caused by these nanomaterials on this species and the possible consequences that could impact this endemic ichthyofauna.

2. Materials and methods

2.1. Silver nanoparticles (AgNPs)

We used a commercial suspension of AgNPs, produced by a Mexican company (ID-NANO S.A. de C.V.), which are applied in industry. According to manufacturer's specifications, the product is a colloid of high AgNPs concentration (6000 mg L^{-1}), with an average diameter of 20 nm, dispersed in polyvinylpyrrolidone (PVP), amber in color, highly soluble in water, and a pH of 12.68.

2.2. Characterization of AgNPs

Physicochemical properties of nanomaterials are related to their toxicity, and can be modified by the dilution medium, as this can foster aggregation and precipitation of nanoparticles (Carlson et al., 2008; Choi and Hu, 2008; Horie et al., 2012). For this reason, it was necessary to characterize the AgNPs sample before performing the toxicity assays. This characterization included determining the distribution of sizes, shape, chemical composition, surface properties, and aggregation state (Oberdörster et al., 2005; Carlson et al., 2008; El Badawy et al., 2011).

The analysis of the size of AgNPs was performed with a high-resolution scanning electron microscope (SEM) JEOL (mod. JSM7800F). A 1:100 dilution of the concentrated AgNPs suspension was prepared with ultrapure water, 10 mL of the sample were placed on a copper grid coated with Formvar and strengthened with carbon (SPI, mod. 3420C-FA), which was placed in the vacuum chamber of the SEM; observation was done at 15.0 kV and micrographs were taken at 150,000×. We measured 1246 NPs to determine the distribution of

Please cite this article as: Valerio-García, R.C., et al., Exposure to silver nanoparticles produces oxidative stress and affects macromolecular and metabolic biomarkers in the goodeid fish..., *Sci Total Environ (2017)*, http://dx.doi.org/10.1016/j.scitotenv.2017.01.070

sizes, using the ImageJ 1.47 analyzer, and determining the coefficient of variation (CV). The chemical composition of the sample was determined in an X-rays energy dispersion spectroscopy (EDS) equipment coupled to the SEM.

The hydrodynamic diameter, which allows assessing the degree of AgNPs aggregation, was determined by the Dynamic Light Scattering (DLS) technique with the Zetasizer ZS90 equipment (Malvern). The hydrodynamic diameter value is obtained from a second-order cumulant fit of the correlation function of the light scattering intensity at 90°, as described by Berne and Pecora (2000). The zeta potential, related with the electrostatic surface charge of AgNPs, was obtained from measurements of the electrophoretic mobility, using the Smoluchowski model (Delgado et al., 2005), available with the equipment's software.

For these analyses, we prepared two AgNPs solutions: one with ultrapure water and the other with ISO medium (dilution water for the toxicity assays) (USEPA, 2002), at a concentration of 300 mg L^{-1} of AgNPs. Measurements were performed in triplicate at different times (0, 24, and 48 h) to observe the temporal evolution of the AgNPs and discard the possible destabilization of the suspension through aggregation of the nanoparticles. Finally, the obtained data served to establish the periods for the exchanges during toxicity evaluations, to maintain the same AgNPs dispersion conditions during the whole time of the bioassays.

2.3. Test organisms

Adult specimens of *C. pardalis* were kindly donated by the *Laboratorio de Biología Acuática* of the *Universidad Michoacana de San Nicolás de Hidalgo* in Morelia, Michoacán, Mexico, and transported to the *Laboratorio de Hidrobiología Experimental de la Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional*, in Mexico City. Fish were acclimated for 15 days in 40-L aquaria with dechlorinated tap water and constant aeration, at a temperature of 26 ± 1 °C and a 14:10 h photoperiod (light:darkness). Afterwards, fish were placed in a water recirculation system provided with a biological filter and UV light. During acclimation and maintenance, fish were fed twice daily with commercial micropellets (41.6% crude protein, AZOO®). Water conditions in the recirculation system were: temperature, 25.9 ± 0.16 °C; dissolved oxygen, 5.8 ± 0.3 mg L^{-1}; pH, 7.9 ± 0.2; conductivity, 686 ± 7.7 μs cm^{-1}; salinity, 0.36 ± 0.05 psu; hardness, 156.8 ± 3.3 mg L^{-1} of $CaCO_3$; ammonium, 0.13 ± 0.3 mg L^{-1} of NH_3.

2.4. Acute toxicity

This test followed the OECD protocol 203 (1992). This is a static test with a total renewal of the test medium every 24 h and without food supply. We selected 48 healthy adults of either sex, with an average size of 10 cm. We prepared 5 concentrations of the AgNPs sample (2, 4, 8, 16, and 32 mg L^{-1}) and a control (ISO medium) in 6-L aquaria, with a test volume of 4 L. For each concentration and control, two replicates were made and 4 adult organisms were placed in each; assays were incubated in a bioclimatic chamber during 96 h at 25 ± 1 °C, and a 14:10 photoperiod (light: darkness). Physicochemical parameters (dissolved oxygen, pH, salinity, and conductivity) were recorded at the beginning and end of the test. The criterion to accept the test was survival >90% in controls at 96 h. Mortality results were processed to determine the mean lethal concentration (LC_{50}), with the Probit method.

2.5. Subchronic toxicity

Adult *C. pardalis* were exposed for 21 days to two concentrations of AgNPs previously chosen (LC_1 = 1.93 mg L^{-1} and LC_{10} = 4.08 mg L^{-1}), in the ISO medium (control), and to the concentration of PVP (dispersing agent of AgNPs) that contained the LC_{10}, aimed at confirming the innocuity of PVP on fish. The bioassay was prepared in 6-L aquaria with a test volume of 5 L, and 6 organisms per treatment were placed in each aquarium. Test solutions were fully renewed each 24 h. In this assay the test organisms were fed *ad libitum* with balanced food (AZOO® micropellets) during 5–10 min at the time of solution renewal. The assay was incubated in a bioclimatic chamber for 21 days at 25 ± 1 °C, with a 14:10 h photoperiod (light:darkness).

At the end of the test, all organisms were euthanized by fast freezing; the liver, gills, and a muscle sample were extracted. Samples were macerated with a tissue homogenizer in 2 mL of 10 mM PBS, pH 7.4, and separated in two 1-mL fractions; one fraction was centrifuged at 9000*g* for 20 min at 4 °C, the supernatant was recovered (containing the protein fraction) and was used for enzymatic determinations, total protein content, as well as glucose and lactate content. The second, not centrifuged fraction, was used to determine TBARS, protein oxidation, and carbohydrates and lipids concentration.

2.6. Antioxidant enzymes

2.6.1. Catalase (CAT) activity

CAT activity was determined by means of the Radi et al. (1991) technique, modified by Vega-López et al. (2007). The method assesses the change in absorbance produced by the degradation of hydrogen peroxide (H_2O_2). The reaction mixtures consisted of 800 μL of buffer (0.3 M saccharose, 1 mM EDTA, 5 mM HEPES, 5.0 mM KH_2PO_4; pH adjusted to 7.4), 200 μL of H_2O_2 (20 mM) and 50 μL of the supernatant. Absorbance was measured immediately at 240 nm. Enzyme activity was estimated using the molar extinction coefficient of H_2O_2 (0.043 $mmol^{-1}$ cm^{-1}) and results were expressed as micromoles (μM) of H_2O_2 consumed per min per mg of protein.

2.6.2. Superoxide dismutase (SOD) activity

SOD activity was determined using the Crapo et al. (1978) method, which assesses the change in absorbance per minute of cytochrome C at 550 nm. The reaction mixture was supplemented with 20 μL of the supernatant and 700 μL of the substrate (1 mM EDTA, 10 mM reduced cytochrome C, 20 mM sodium azide, 10 μM xanthine); solubilized in carbonates buffer containing 10 mM sodium carbonate and 0.02% Triton X-100. The reaction was started by adding 50 μL of xanthine oxidase (0.34%). Results are expressed as units of SOD per mg of protein.

2.6.3. Glutathione peroxidase (GPx) activity

GPx activity was quantified with the Lei et al. (1995) method, using H_2O_2 as substrate of the selenium-dependent enzyme and measuring the decrement in the concentration of nicotinamide adenine dinucleotide phosphate (NADPH) in an assay coupled to glutathione reductase (GR), which catalyzed NADPH oxidation at 340 nm. The reaction mixture was supplemented with 800 μL of buffer (50 mM K_2PO_4, 3.5 mM GSH, $NaNO_3$), 20 μL of the supernatant, 100 μL of 40 mM glutathione reductase, and 10 μL of a25 mM NADH, and finally, 40 μL of 40 mM H_2O_2 to start the reaction. The activity of the enzyme was estimated using the molar extinction coefficient of NADPH (6.22 $mmol^{-1}$ cm^{-1}). Results were expressed as millimoles NADPH min^{-1} mg^{-1} of protein.

2.7. Oxidative damage biomarkers

2.7.1. Thiobarbituric acid reactive substances (TBARS)

The technique of Buege and Aust (1978) was used to quantify the final products of the peroxidation of lipids, such as malondialdehyde (MDA) and 4-hydroxyhexenal (4-HHE), which react with TBARS. Briefly, 200 μL of the crude extract were supplemented with 500 μL Tris-HCL, pH 7.4 (150 mM), incubated at 37 °C for 30 min and adding 1 mL of 0.375% thiobarbituric acid prepared in 15% trichloroacetic acid; the mixture was boiled for 45 min and then cooled in an ice bath; finally it was centrifuged at 2500*g* for 5 min, and read at 532 nm. TBARS quantification was estimated using the molar extinction coefficient of malondialdehyde (1.56×10^5 mol^{-1} cm^{-1}) and results were expressed per gram of tissue.

Please cite this article as: Valerio-García, R.C., et al., Exposure to silver nanoparticles produces oxidative stress and affects macromolecular and metabolic biomarkers in the goodeid fish..., *Sci Total Environ (2017)*, http://dx.doi.org/10.1016/j.scitotenv.2017.01.070

2.7.2. Carbonylated proteins

To quantify the oxidative damage induced in proteins, the Levine et al. (1994) method was used, based on the formation of 2,4-dinitrophenylhydrazone from the reaction among the carbonyl groups of oxidized proteins (RC═O) and the reagent 2,4-dinitrophenylhydrazine (2,4-DNPH) in acid medium. Aliquots of 500 μL were taken from the homogenate and precipitated with 100 μL of 30% trichloroacetic acid (TCA); then, they were agitated for 10 s, centrifuged at 3000*g* for 5 min, and the supernatant was discarded. To this, 500 μL of DNPH (20 mM in 2 N HCl) were added, agitated for 30 s, and immediately incubated in darkness for 1 h at room temperature, shaking every 10 min. After this time, the mixture was precipitated again with 30% TCA and centrifuged at 3000*g* for 5 min; the supernatant was discarded and three washes with 500 μL of ethanol-ethyl acetate (1:1 v/v) were made to eliminate the excess DNPH. The precipitate was dissolved in 1 mL of 6 mM urea (dissolved in 20 mM potassium phosphate, pH 2.5); lastly, samples were incubated for 1 h at 37 °C and centrifuged at 3000*g* for 5 min. Samples were read at 366 nm. Concentration of carbonylated proteins was calculated using the molar extinction coefficient (22 mM^{-1} cm^{-1}) and expressed per gram of tissue.

2.8. Macromolecules

2.8.1. Proteins

The Lowry et al. (1951) colorimetric method was used to quantify proteins, which is based on two reactions: 1) previous reaction of proteins in an alkaline medium with Cu^{2+} ions in the presence of tartrate to avoid precipitation, forming a coordination complex between Cu^{2+} and the peptide nitrogen; 2) reaction of the Folin-Ciocalteau reagent with amino acids and phenolic groups (tyrosine, phenylalanine, and tryptophan), giving rise to an intense blue color. Absorbance was measured at 590 nm, and protein concentration was determined by interpolating a standard bovine serum albumin (BSA) curve.

2.8.2. Lipids

Lipids were determined according to the sulfo-phospho-vanillin method (Zöllner and Kirsch, 1962), a photometric procedure based on the reaction of non-saturated fatty acids with concentrated sulfuric acid (H_2SO_4) and a mixture of vanillin-phosphoric acid, which develops a pink color. To 100 μL of the homogenate, 200 μL of methanol/chloroform (2:1, v/v) was added and agitated in a vortex; then, 500 μL of deionized water was added to form two phases; the organic phase was desiccated at 80 °C, to which 50 μL of H_2SO_4 was added and heated for 2 min at 70 °C. Finally, 500 μL of phospho-vanillin was added, cooled on ice, and absorbance was read at 525 nm. The concentration of lipids was obtained using a standard cholesterol curve.

2.8.3. Carbohydrates

Carbohydrates content was determined according to Dubois et al. (1951), in which the soluble carbohydrates react with phenol and concentrated sulfuric acid yielding an orange color. To 100 μL of the tissue homogenate, 100 μL of 5% phenol was added and agitated for 10 s, then 500 μL of sulfuric acid was added and left to rest for 10 min; the mixture was agitated again for 10 s and left to rest for 30 min; finally, absorbance was read at 490 nm. The amount of carbohydrates was obtained by interpolating a standard dextrose curve.

2.9. Metabolic parameters

2.9.1. Glucose

Glucose levels were determined through a colorimetric technique, using the commercial kit GLU CAL (RANDOX®), using the microplate adaptation developed by Hernández-López (2001). Microplates of 96 wells with a volume of 0.4 mL were used, these were supplemented with 10 μL of the protein fraction and 200 μL of the reactive solution for glucose, incubating at 25–28 °C during 30 min. Absorbance was read at 490 nm. Glucose concentration was determined with the kit's standard curve.

2.9.2. Lactate

Lactate levels were determined through a colorimetric technique, using the commercial kit LAC (RANDOX®), adapted to microplates by Hernández-López (2001). Microplates of 96 wells were supplemented with 10 μL of protein fraction and 200 μL of the reactive solution for lactate, incubating at 25–28 °C during 30 min. Absorbance was read at 540 nm and lactate concentration was quantified with the kit's standard curve.

2.10. Integrated biomarkers response (IBR)

Results of the assessed biomarkers were analyzed through the integrated biomarkers response (IBR) (Beliaeff and Burgeot, 2002), following the modification by Devin et al. (2014); this tool allows establishing the global response of adult *C. pardalis* exposed to the different treatments (PVP, Lc_1 and Lc_{10} of AgNPs). Briefly, data from the response of biomarkers were standardized using the formula $Y_i = (X_i - m)/S$, where Y_i is the response of the standardized biomarker, X_i is the value of the response of each biomarker, m and S are the mean and standard deviation, respectively. Afterwards, the Z_i value was calculated for each biomarker, using the formula $Z_i = Y_i$ or $Z_i = -Y_i$ according to the observed biological effect (activation or inhibition, respectively). The value of S or scores were calculated with $S = Z + |\mathrm{Min}|$, where Min is the minimum value observed for all treatments of each biomarker; lastly, all S_i values were plotted in a star graph. The IBR was calculated as the total as shown by the star graph; to obtain the area, the equation proposed by Devin et al. (2014) was used:

$$A_i = S_i \times S_{i+1} \times \sin\left(\frac{2\pi}{k}\right)/2$$

The IBR value was calculated as follows:

$$IBR = \sum_{i=1}^{k} A_i$$

In this study, we evaluated the IBR for two groups, one that included the antioxidant enzymes and oxidative damage biomarkers (TBARS and RC═O), and the other comprised the macromolecules and metabolites.

2.11. Statistical analysis

Average values and standard error (SE) limits were determined for all the biomarkers. Treatment data *versus* control were assessed through a one-way variance analysis (ANOVA) and Dunnett's comparison tests. IBR values were compared using ANOVA. When significance was detected, Tukey's pairwise comparison test was applied. Before applying ANOVA, a Levene test was performed to prove homoscedasticity of variances.

3. Results

3.1. AgNPs characterization

3.1.1. Scanning electron microscopy (SEM)

The SEM micrograph (Fig. 1a) reveals the presence of NPs of diverse sizes, spherical in shape, without aggregates. According to the graph of size distribution, the average diameter of NPs was lower than that specified by the manufacturer (11.95 ± 5.3 nm), with CV of 48.6%. AgNPs of this sample depicted a high polydispersion (NPs of different sizes) as shown in Fig. 1b. The analysis of chemical elements performed with the EDS technique revealed that silver predominated in the sample (Fig. 2).

Please cite this article as: Valerio-García, R.C., et al., Exposure to silver nanoparticles produces oxidative stress and affects macromolecular and metabolic biomarkers in the goodeid fish..., *Sci Total Environ (2017)*, http://dx.doi.org/10.1016/j.scitotenv.2017.01.070

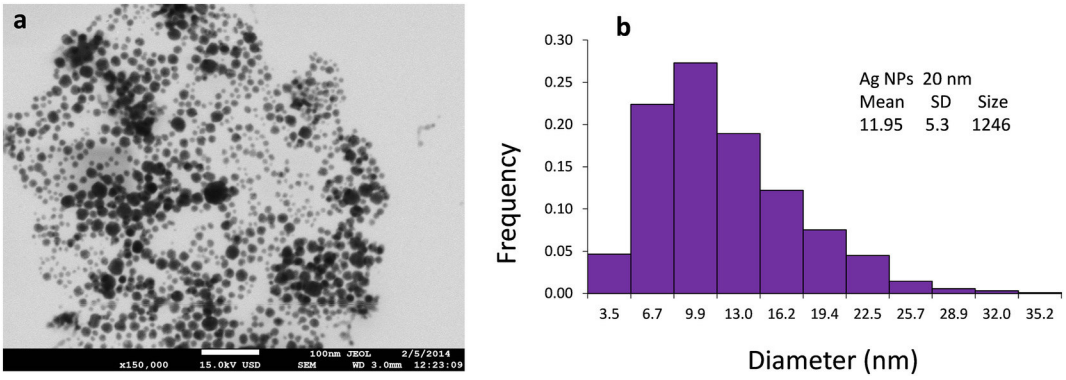


Fig. 1. a) SEM, micrograph of silver nanoparticles (AgNPs). b) Histogram of the size distribution of AgNPs in the analyzed commercial sample.

The hydrodynamic diameter (HD) and zeta potential behaviors of AgNPs with respect to time is shown in Fig. 3(a) and (b), respectively. The average HD of AgNPs in ultrapure water at time zero was of 31.35 ± 1.7 nm with a zeta potential of −39.85 ± 1.8 mV, and these values did not vary significantly at 24 and 48 h. For the ISO medium, at time zero, AgNPs presented an HD of 44.85 ± 1.65 nm and zeta potential of −16.1 ± 0.8 mV; at 24 h, the HD increased (71.3 ± 0.9 nm) and the zeta potential diminished slightly (−15.2 ± 0.5), these values were maintained after 48 h.

These results confirm that the used product corresponds to AgNPs in an adequate size and within the convenient limits for assays with NPs, and that they remained stable during the exposure time of the test organisms.

3.2. Mean lethal concentration (LC_{50})

After 96 h no mortality was recorded in controls. Mortality induced by treatments was proportional to the increase in AgNPs concentrations. The highest concentration (18 mg L^{-1}) produced 100% mortality at 72 h and the LC_{50} 96-h was 10.32 mg L^{-1} (6.03–14.54 mg L^{-1}, $P = 0.05$).

3.3. Response of biomarkers

Table 1 shows the average values and standard error of the biomarkers assessed in each organ and tissue of fish. In general, after 21 days of AgNPs exposure, the highest biomarker response was observed in fish exposed to the highest concentration (LC_{10}); no significant changes were observed in the values of organisms exposed to PVP.

3.3.1. Antioxidant enzymes

The activity of SOD diminished significantly in the liver of fish exposed to either AgNPs concentration (LC_1, $P < 0.05$ and LC_{10}, $P < 0.001$), as compared to the control. CAT activity diminished significantly in gills ($P < 0.001$) and muscle ($p < 0.05$) of organisms exposed to LC_1 and LC_{10}. Lastly, the enzymatic activity of GPx was significantly reduced ($P < 0.05$) in the liver of fish exposed to LC_1 and Lc_{10}, according to the Dunnett's test (Table 1).

3.3.2. Oxidative damage biomarkers

As compared to the control, TBARS levels increased significantly ($P < 0.001$) in the liver and gills of fish exposed to the highest AgNPs concentration (LC_{10}), whereas the levels of carbonylated proteins

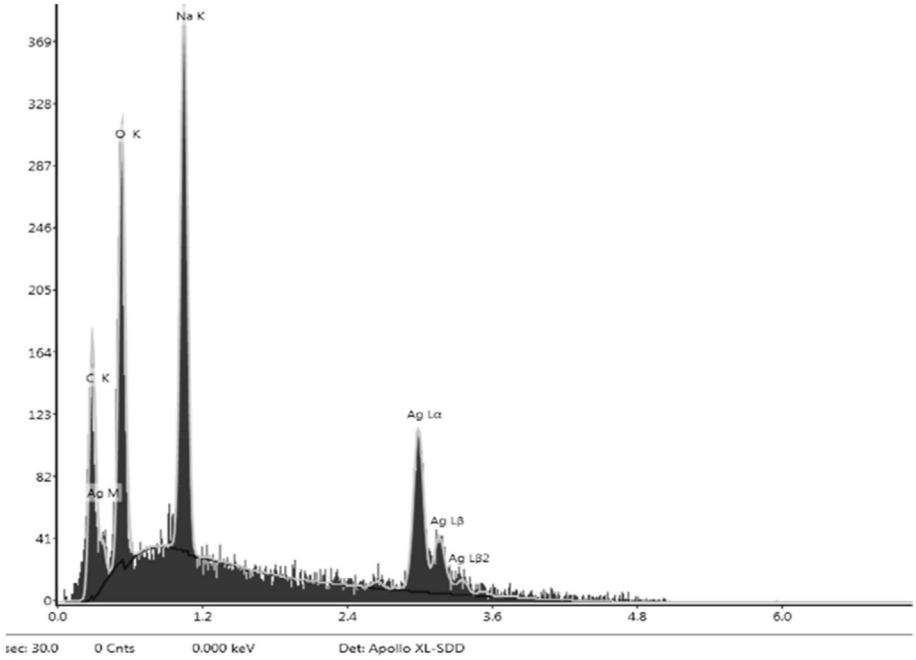


Fig. 2. Elemental analysis of AgNPs by X-rays dispersion spectroscopy.

Please cite this article as: Valerio-García, R.C., et al., Exposure to silver nanoparticles produces oxidative stress and affects macromolecular and metabolic biomarkers in the goodeid fish..., *Sci Total Environ (2017)*, http://dx.doi.org/10.1016/j.scitotenv.2017.01.070

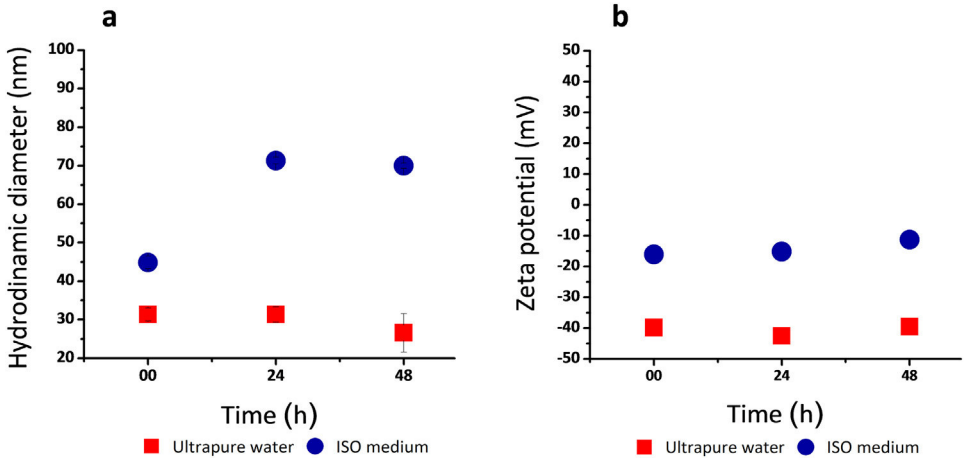


Fig. 3. Behavior of the hydrodynamic diameter (a) and zeta potential (b) of AgNPs respect to time, in two different dilution media (ultrapure water and ISO medium).

(RC═O) increased significantly ($P < 0.001$) only in the gills of fish exposed to LC_{10} (Table 1).

3.3.3. Macromolecules

Proteins concentration in liver and gills of fish exposed to either AgNPs concentration (LC_1 or LC_{10}) was significantly lower ($P < 0.05$) than in the control, whereas the concentration of lipids was significantly lower ($P < 0.05$) only in the liver of fish exposed to both concentrations. On the other side, carbohydrates diminished significantly ($P < 0.05$) in gills and muscle of organisms exposed to the highest AgNPs concentration (LC_{10}) (Table 1).

3.3.4. Metabolic biomarkers

Glucose concentration in the liver and gills diminished significantly ($P < 0.05$) in organisms exposed to the highest AgNPs concentration (LC_{10}). Regarding lactate, no significant alterations were recorded with any of the treatments (Table 1).

3.3.5. Integrated biomarkers response (IBR)

Fig. 4 shows the star graphs with the scores of the biomarkers of antioxidant enzymes and those of oxidative damage. As shown, in both the liver and gills of controls and fish exposed to LC_{10} the magnitude of the area is greater, with a clear difference between both; the area in controls is determined by the high scores of the antioxidant enzymes and a low value in the oxidative damage biomarkers. In contrast, in fish exposed to LC_{10}, the area is favored by a high score of oxidative damage biomarkers and a low score of the antioxidant enzymes activity (Fig. 4a and b). Regarding the muscle, the area of the polygons diminished with respect to concentration, particularly by a reduction of CAT and GPx activities (Fig. 4c).

Table 1
Average values and standard error (SE) for antioxidant enzymes, macromolecules, and metabolites assessed in different organs and tissues of adult *C. pardalis* exposed to AgNPs during 21 days. Results of ANOVA and Dunnett's *post hoc* test. $^{*}P < 0.05$, $^{***}P < 0.001$.

Biomarker	Organ/tissue	Control	PVP	LC_1	LC_{10}
SOD	Liver	32.7167 ± 3.2435	21.9106 ± 1.8134	21.3863 ± 2.0818*	10.4108 ± 2.4329***
(U mg^{-1} protein)	Gills	43.1562 ± 2.7409	36.4005 ± 4.4433	36.2713 ± 0.8632	30.0819 ± 6.6030
	Muscle	20.7835 ± 1.2895	19.4580 ± 2.1881	20.4300 ± 3.4806	21.6564 ± 0.6734
CAT	Liver	13.2768 ± 1.7052	11.9500 ± 1.6117	12.3421 ± 1.4201	11.5960 ± 1.4713
(mM min^{-1} mg^{-1} protein)	Gills	6.8536 ± 0.7449	4.3785 ± 0.4273	3.4331 ± 0.2800***	2.7019 ± 0.4879***
	Muscle	6.0454 ± 0.9328	5.2289 ± 0.4069	2.6507 ± 0.2596*	2.3036 ± 0.3400*
GPx	Liver	0.2956 ± 0.0204	0.2625 ± 0.0154	0.2315 ± 0.0183*	0.1913 ± 0.0205*
(mM min^{-1} mg^{-1} protein)	Gills	0.3676 ± 0.0279	0.3862 ± 0.0378	0.3196 ± 0.0175	0.3508 ± 0.0204
	Muscle	0.1089 ± 0.0055	0.1177 ± 0.0065	0.0896 ± 0.0050	0.0838 ± 0.0031*
TBARS	Liver	0.1151 ± 0.0166	0.1043 ± 0.0094	0.1635 ± 0.0484	0.4209 ± 0.0702***
(nM mg^{-1} protein)	Gills	0.9972 ± 0.0632	0.7820 ± 0.0542	0.8583 ± 0.0306	2.3457 ± 0.2187***
	Muscle	0.5203 ± 0.0318	0.6029 ± 0.0565	0.6183 ± 0.1706	0.4339 ± 0.0301
RC═O	Liver	0.0137 ± 0.0012	0.0147 ± 0.0019	0.0149 ± 0.0010	0.0178 ± 0.0003
(mM mg^{-1} protein)	Gills	0.0377 ± 0.0031	0.0353 ± 0.0019	0.0436 ± 0.0024	0.0608 ± 0.0010***
	Muscle	0.0184 ± 0.0009	0.0161 ± 0.0011	0.0182 ± 0.0013	0.0152 ± 0.0008
Proteins	Liver	62.9428 ± 4.5113	62.3181 ± 1.8497	49.1146 ± 1.8683*	45.5376 ± 6.4529*
(μg mg^{-1} tissue)	Gills	38.4011 ± 2.0524	39.5861 ± 1.7735	36.8962 ± 2.6278	29.9954 ± 1.9299*
	Muscle	43.4949 ± 2.0139	44.6472 ± 2.0136	45.9132 ± 1.7875	55.1696 ± 3.8859
Lipids	Liver	53.4766 ± 2.4721	52.1264 ± 4.9593	33.8310 ± 3.7180*	27.2978 ± 4.2571*
(μg mg^{-1} tissue)	Gills	30.1562 ± 1.5709	31.8387 ± 2.0259	28.7452 ± 1.4686	25.7170 ± 2.5531
	Muscle	14.6021 ± 1.1342	15.4048 ± 1.8749	12.7834 ± 1.3795	20.0505 ± 1.4083
Carbohydrates	Liver	40.0340 ± 3.2176	43.8226 ± 5.1184	46.6594 ± 6.7728	28.4974 ± 0.8665
(μg mg^{-1} tissue)	Gills	16.6628 ± 1.0175	21.1793 ± 1.2051	21.0168 ± 0.7747	15.7757 ± 0.4658*
	Muscle	9.4760 ± 0.3686	10.7274 ± 0.6353	9.3358 ± 0.4829	7.1389 ± 0.4572*
Glucose	Liver	34.3770 ± 0.9798	32.7928 ± 3.1061	36.6902 ± 2.5313	21.8162 ± 3.4045*
(μg mg^{-1} tissue)	Gills	2.7518 ± 0.4036	2.3067 ± 0.4339	2.4810 ± 0.3369	1.2904 ± 0.0773*
	Muscle	1.5516 ± 0.2223	1.7342 ± 0.2049	1.9372 ± 0.1125	1.4569 ± 0.1310
Lactate	Liver	3.5460 ± 0.2990	3.8153 ± 0.3690	3.5799 ± 0.2140	1.7289 ± 0.1301
(μg mg^{-1} tissue)	Gills	3.3781 ± 0.3074	3.4411 ± 0.1189	2.7130 ± 0.1214	2.8646 ± 0.0128
	Muscle	5.9124 ± 0.4524	6.7577 ± 0.4219	5.3527 ± 0.0557	4.8553 ± 0.2066

Please cite this article as: Valerio-García, R.C., et al., Exposure to silver nanoparticles produces oxidative stress and affects macromolecular and metabolic biomarkers in the goodeid fish..., *Sci Total Environ (2017)*, http://dx.doi.org/10.1016/j.scitotenv.2017.01.070

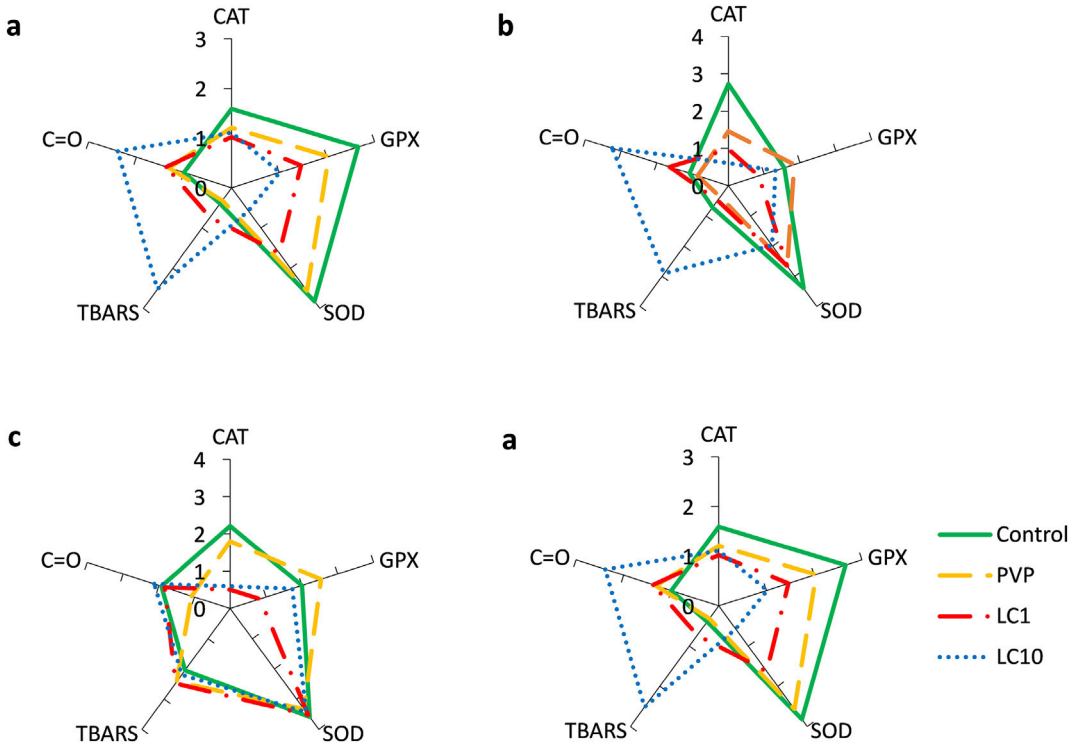


Fig. 4. Star graphs of the scores determined for biomarkers of antioxidant enzymes and oxidative damage in the liver (a), gills (b), and muscle (c) of *C. pardalis* exposed to PVP and to the equivalent of LC_1 and LC_{10} of AgNPs after 21 days of exposure.

Fig. 5 shows the star graph for macromolecules and metabolites. In the liver, gills, and muscle, the areas of the polygons showed a diminution that was proportional to the AgNPs concentration, with a marked reduction with the highest concentration (LC_{10}), reflecting the levels of all the biomarkers (Fig. 5a, b, c).

Fig. 6 shows the IBR values for each group. ANOVA applied to the antioxidant enzymes and oxidative stress biomarkers revealed significant differences ($P < 0.05$) in the liver and gills. According to the Tukey's test, IBR of organisms exposed to LC_1 diminished significantly with respect to the other treatments (Fig. 6a). On the other side, analysis of macromolecules and metabolites indicated significant differences ($P < 0.05$) in the liver, gills, and muscle; the Tukey's test revealed that in both the liver and gills, the IBR diminished significantly ($P < 0.05$) with respect to the other treatments; whereas, in the muscle, values were significantly

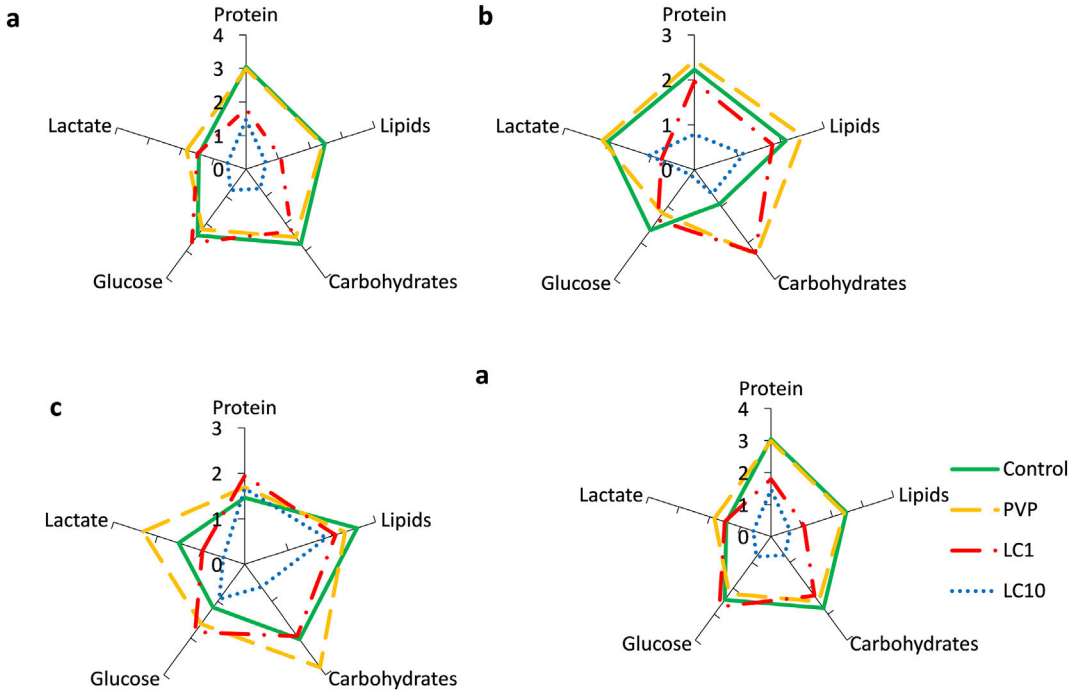


Fig. 5. Star graphs of the scores determined for macromolecules and metabolites in the liver (a), gills (b), and muscle (c) of *C. pardalis* exposed to PVP and to the equivalent of LC_1 and Lc_{10} of AgNPs after 21 days of exposure.

Please cite this article as: Valerio-García, R.C., et al., Exposure to silver nanoparticles produces oxidative stress and affects macromolecular and metabolic biomarkers in the goodeid fish..., *Sci Total Environ (2017)*, http://dx.doi.org/10.1016/j.scitotenv.2017.01.070

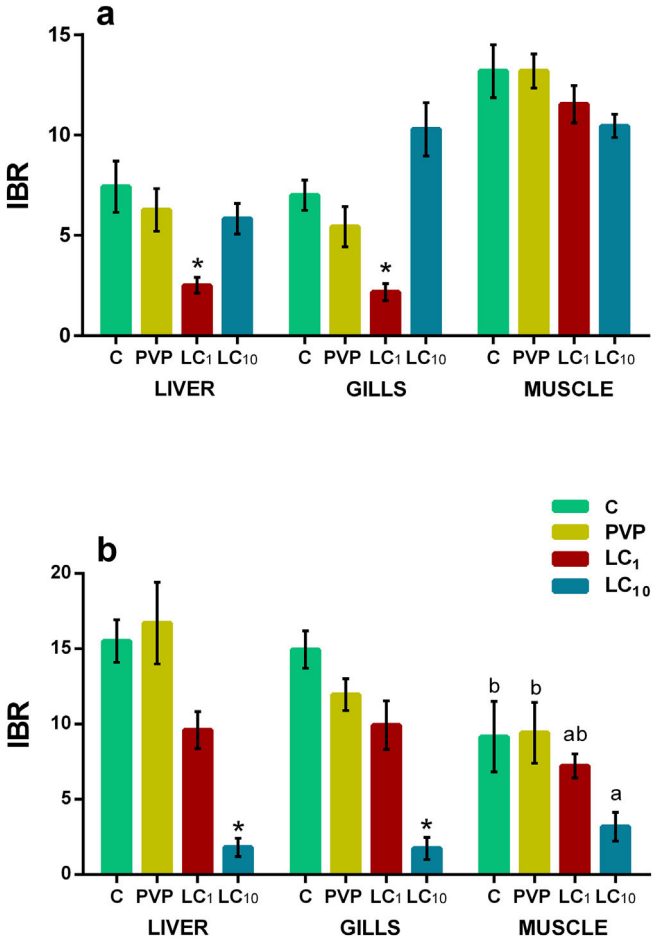


Fig. 6. Integrated biomarkers response (IBR) determined for biomarkers of antioxidant enzymes and oxidative damage (a) and for macromolecules and metabolites (b), determined after 21 days of exposure. Average values of six replicates and the standard error bars are shown. * indicates significant difference with respect to the other treatments ($P < 0.05$) and letters indicate significant differences between pairs as assessed by Tukey's *post hoc* test.

lower than in the organisms exposed to LC_{10}, as compared to control and PVP-exposed fish (Fig. 6b).

4. Discussion

According to the zeta potential, AgNPs possess negative surface charge, which is caused by the PVP coating; this charge diminished in the solution prepared with the ISO medium, without reaching the isoelectric point (net zero charge), at which aggregation and precipitation of AgNPs would have been observed (Salopek et al., 1992). DLS measurements revealed that AgNPs in ultrapure water did not present changes in their hydrodynamic diameter during the 48 h of sample measuring; however, in the sample prepared with the ISO medium, the hydrodynamic diameter increased reaching up to 89 nm at 48 h. According to results, the ISO medium caused diminution of the surface charge of AgNPs and increased the hydrodynamic diameter, inducing the formation of some aggregates without precipitation of NPs and without loosing their condition of nanoparticles.

No transformation of AgNPs was observed under the test conditions we used, but a slight, reduced aggregation was detected after 48 h; this effect was negligible because the test solutions were fully renewed every 24 h. Nevertheless, it is possible that, under natural conditions, transformation could happen, including sulfidation, solubilization, aggregation, and interaction with other environmental factors, which could modify the AgNPs' toxicity.

AgNPs were toxic for adult *C. pardalis* after 96 h of exposure, with an $LC_{50} = 10.23$ mg L^{-1} (6.03–14.54 mg L^{-1}). Table 2 shows the LC_{50} values obtained with AgNPs in different teleost fish; it is noteworthy that the acute toxicity of AgNPs induced in fish is variable, with LC_{50} values ranging from 0.0346 to 250 mg L^{-1}. The difference in sensitivity could be related mainly to factors like species-specific susceptibility, age, developmental stage, exposure time, and the size of the nanoparticles (Cho et al., 2013), but the effect of the dispersant could also influence results. Notwithstanding, adult *C. pardalis* depicted a very similar susceptibility to that observed in embryos of *Pimephales promelas* ($LC_{50} = 10.6$ mg L^{-1}, 96 h of exposure), which is considered a reference species (Laban et al., 2010).

According to the classification system of toxic substances by the USEPA (1975), and based on the determined LC_{50} value, the assessed AgNPs sample can be classified as slightly toxic (acute toxicity criterion) to adult *C. pardalis*; however, this classification must be taken carefully because the subchronic assay (21 days) showed that sublethal AgNPs concentrations, equivalent to LC_1 and LC_{10}, induced important biochemical alterations in diverse biomarkers and organs of the studied species.

Exposure to PVP did not produce significant effect on any of the stress biomarkers assessed; this polymer is considered an innocuous agent, widely used in medicine and processed food (Folttmann and Quadir, 2008; Rangel-Vázquez and Rodríguez, 2014). Other studies have confirmed the innocuity of PVP on aquatic organisms, and it has been reported that it could reduce the toxicity of NPs by hindering, through a steric effect, the direct contact of the NPs with the cells (Bilberg et al., 2012; Ahn et al., 2014).

Organisms exposed to either AgNPs concentration showed significant reduction in the activity of antioxidant enzymes, which was proportional to the AgNPs concentration. SOD presented diminution of activity in the liver, GPX in the liver and gills, and CAT in gills and muscle. SOD catalyzed the dismutation of the superoxide anion into oxygen and hydrogen peroxide; the latter is, in turn, metabolized to H_2O by enzymes CAT and GPx through GSH. It is probable that, in this study, exposure to AgNPs produced an excessive formation of the superoxide anion, hydrogen peroxide, and other oxygen radicals, which could have hindered the action of the first line antioxidant defense in *C. pardalis*. A similar response has been observed in *Oreochromis mossambicus* exposed to AgNPs by Govindasamy and Rahuman (2012), who reported a diminution of the activity of antioxidant enzymes in gills and liver.

TBARS (indirect marker of lipids oxidation) showed a significant increase in liver and gills of fish exposed to the highest AgNPs concentration (LC_{10}). The oxidative damage to lipids has also been reported in adult *O. mossambicus*, which presented increased levels of MDA in the liver, gills, and skin after 8 days of exposure to 25 and 50 mg L^{-1} of AgNPs (Wu and Zhou, 2012). Increased MDA has also been observed in the liver of adult zebrafish (*Danio rerio*) at AgNPs concentrations of 60 and 120 mg L^{-1} (Choi et al., 2010). Results show the ability of AgNPs to cause lipoperoxidation, particularly, in the liver, which due to its constitution and activity could be more prone to oxidative damage (Repetto et al., 2012).

Carbonylated proteins (RC═O) increased significantly in gills in those fish exposed to LC_{10}. AgNPs can release Ag+ ions when interacting with cells (Asharani et al., 2009), and both AgNPs and Ag+ ions can induce high ROS concentrations intracellularly, specifically hydroxyl radicals (OH^-) (He et al., 2012), which can react immediately with proteins, oxidizing them and producing carbonylation (Stadtman and Oliver, 1991; Nyström, 2005). Although, in this study we observed a significant damage to proteins oxidation only in gills, it has been reported that concentrations of 0.5 and 1 mg L^{-1} of AgNPs can promote oxidation of proteins in the liver, gonads, gills, and brain of adult *D. rerio*; damage to proteins by oxidation has also been documented in human hepatic cells exposed to AgNPs (Piao et al., 2011).

Proteins are important molecules involved mainly in structural, enzymatic, and cell transport functions (Cooper, 2000). In our study, the concentration of proteins diminished significantly in the liver of those

Please cite this article as: Valerio-García, R.C., et al., Exposure to silver nanoparticles produces oxidative stress and affects macromolecular and metabolic biomarkers in the goodeid fish..., *Sci Total Environ (2017)*, http://dx.doi.org/10.1016/j.scitotenv.2017.01.070

Table 2
Acute toxicity of AgNPs in adults and embryos of freshwater fish. When available, the confidence interval ($P = 0.05$) is shown.

Species (age or stage)	Dispersant	Duration of exposure (h)	LC_{50} (95% CI) (mg L^{-1})	Reference
Chapalichthys pardalis (adults)	PVP	96	10.32 (6.03–14.54)	This study
Oryzia latipes (4-month-old)	Citrate	96	0.80 (0.65–0.96)	Cho et al. (2013)
O. latipez (adults)	PVP	48	1.03	Wu et al. (2010)
O. latipez (adults)	None	96	0.0346	Chae et al. (2009)
Danio rerio (adults)	PVP	48	0.084	Bilberg et al. (2012)
D. rerio (adults)	Citrate	48	7.07 (6.04–8.28)	Griffitt et al. (2008)
D. rerio (adults)	None	24	250	Choi et al. (2010)
Oncorhynchus mykiss (adults)	Not reported	96	2.3	Shahbazzadeh et al. (2009)
Hipophthalmichthys molitrix (adults)	Not reported	96	66.4	Jahanbakhshi et al. (2012)
Carassius auratus (adults)	Not reported	96	83.9	Jahanbakhshi et al. (2012)
Oreochromis mossambicus (adults)	Citrate	192	12.6	Govindasamy and Rahuman (2012)
O. latipes (embryos)	Citrate	96	0.84 (0.67–1.00)	Cho et al. (2013)
O. latipes (embryos)	None	96	1.39	Kashiwada et al. (2012)
Pimephales promelas	Citrate	96	10.6	Laban et al. (2010)

organisms exposed to either of the two AgNPs concentrations (LC_1 and LC_{10}) and in gills of those exposed to the higher concentration. Positively charged AgNPs can adhere to the cell membrane, modifying its permeability and thereby penetrating the cell (Raffi et al., 2008; Morones et al., 2005). Once inside the cell, AgNPs, in the presence of low concentrations of H_2O_2, can catalyze the release of silver ions ($Ag+$) and act synergistically on the thiol groups of proteins, interfering with their function (Morones et al., 2005; Zhang et al., 2014). It has also been reported the AgNPs cause depolarization of the mitochondrial membrane (Teodoro et al., 2011) and DNA damage (Massarsky et al., 2013) deteriorating cellular metabolism, which could lead to a diminution in the concentration of proteins (De Matteis et al., 2015).

Concentration of carbohydrates was significantly reduced in gills and muscle of those fish exposed to the higher AgNPs concentration (LC_{10}); these molecules are structurally relevant and an immediate source of energy for cells (Javed and Usmani, 2015). Diminution of carbohydrates has been related mainly to an increase in energetic demands for detoxification processes to cope with the effects of chemical contaminants (Arzate-Cárdenas and Martínez-Jerónimo, 2012), hence, their decrement could be related to the oxidative stress induced by the formation of ROS.

Lipids concentration was significantly lower in the liver of AgNPs-exposed fish. Lipids represent an important energetic reserve in cells (Birsoy et al., 2013) and their diminution, just as that of carbohydrates, has been related to an increase in energetic demands associated with toxic stress (Sancho et al., 1996); however, the reduction observed in the liver could have been induced not only by the large demand, but also by the metabolic deterioration of cells.

Glucose concentration diminished significantly in the liver and gills of fish exposed to LC_{10} of AgNPs. This metabolite is an important source of energy and can be used as a stress response parameter (Teles et al., 2004), because stress conditions induce an increase in glucose, which is initially generated by catecholamines-mediated glycogenolysis and, in later stages, by cortisol-mediated glyconeogenesis (Begg and Pankhurst, 2004). However, we observed a decrement in glucose concentration, which could also have been related to cellular metabolic deterioration, avoiding the synthesis of energetic reserves to produce glycogen. Decreased glucose levels have also been reported in the plasma of adult *Cyprinus carpio* exposed to sublethal AgNPs concentrations (Thangam et al., 2014).

No significant changes were observed in lactate levels in any of the assessed organs or tissues. An increase in the concentration of this metabolite would indicate a metabolic disorder and an acute respiratory tension (Begum and Vijayaraghavan, 1999). In our study, AgNPs did not induce a significant respiratory dysfunction in the organisms, despite having induced a high energetic consumption by the produced stress.

IBR is a useful tool for a global description of the health status of organisms under stress. This index provides both a graphical synthesis of the different biomarkers' responses and a numerical value that integrates all the responses at once (Devin et al., 2014). The star graphs allow visualizing the global tendency of the biomarkers in the different organs, showing that the liver and gills of the organisms exposed to the higher AgNPs concentration (LC_{10}) presented reduction in the activity of all antioxidant enzymes, diminution in the concentration of macromolecules and metabolites, as well as an increase in oxidative damage biomarkers.

The IBR corresponding to antioxidant enzymes and oxidative damage biomarkers reveals that in the liver and gills of control fish and those exposed to PVP the high values of their indices are related to an increased activity of the antioxidant enzymes and a low level of stress, as compared to those fish exposed to AgNPs. In organisms exposed to LC_1, the value of the index was significantly lower with respect to the other treatments in the same organs; this low value could have been related to the production of ROS and Ag^+, which caused a diminution in the antioxidant activity of enzymes without inducing a significant damage to lipids and proteins. On the other side, the high IBR value obtained in fish exposed to LC_{10} is related to the oxidative damage induced on lipids and proteins, caused by the high production of ROS and Ag^+.

The IBR of the group of macromolecules and metabolites reveals a reduction of the index in the liver, gills, and muscle, depending on the AgNPs concentration, which was significant in those fish exposed to the higher AgNPs concentration (LC_{10}); this response can be related to the high energetic expenditure and/or a greater damage to the metabolism of the cell due to ROS and $Ag+$, which could have promoted consumption and avoid the synthesis of energetic reserves.

Our results confirm that the IBR was useful for the integrated quantification of the stress responses induced by AgNPs in *C. pardalis*, similarly as observed by Xia et al. (2013) in *Carassius auratus* exposed to different nanoparticles of metal oxides (nCuO, nZnO, and $nCeO_2$).

In summary, adults of the endemic fish *C. pardalis* were affected significantly at the biochemical level when exposed subchronically to sublethal AgNPs concentrations. The aforementioned is forewarning on the possible damage that could be caused in some organisms that are under some status of protection or conservation, as occurs with the fish of the Goodeidae family.

5. Conclusions

This study shows the toxic effect of AgNPs in the endemic livebearing fish *C. pardalis*, with sensitivity values similar to those of reference species. In the subchronic test, AgNPs induced oxidative stress because of a diminished antioxidant enzymatic response and increased oxidation of lipids and proteins. AgNPs diminished glucose levels and altered mechanisms related to macromolecules synthesis. The most affected organs were the liver and gills. Production and use of AgNPs shows an increasing trend, hence, its release to the environment is expected also; therefore, it is essential to characterize their toxic effects

Please cite this article as: Valerio-García, R.C., et al., Exposure to silver nanoparticles produces oxidative stress and affects macromolecular and metabolic biomarkers in the goodeid fish..., *Sci Total Environ (2017)*, http://dx.doi.org/10.1016/j.scitotenv.2017.01.070

on diverse aquatic species to know the toxic concentrations and the response mechanisms of wild organisms. It is necessary to include in this research endemic species because of their great ecological relevance.

Acknowledgements

R. C. Valerio-García had a scholarship from *Consejo Nacional de Ciencia y Tecnología* (CONACYT) (No. 163156) and *Beca de Estímulo Institucional de Formación de Investigadores* of the *Instituto Politécnico Nacional* (BEIFI-IPN). Fernando Martínez-Jerónimo is fellow of the *Sistema de Estímulo al Desempeño de los Investigadores* (EDI) and the *Comisión de Operación y Fomento de Actividades Académicas* (COFAA) of the *Instituto Politécnico Nacional*. The authors are thankful to Dr. Omar Domínguez-Domínguez, *Laboratorio de Biología Acuática* of the *Universidad Michoacana de San Nicolás de Hidalgo*, for the kind donation of *C. pardalis*. We are also thankful to the *Centro de Nanociencias y Micro y Nanotecnologías*, *IPN*, for the important support in the processing of samples for SEM studies. Finally, we thank Ms. Ingrid Mascher for copyediting this manuscript.

References

Ahn, J.M., Eom, H.J., Yang, X., Meyer, J.N., Choi, J., 2014. Comparative toxicity of silver nanoparticles on oxidative stress and DNA damage in the nematode, *Caenorhabditis elegans*. Chemosphere 108:343–352. http://dx.doi.org/10.1016/j.chemosphere.2014.01.078.

Álvarez del Villar, J., 1963. Ictiología michoacana. 3. Los peces de San Juanico y de Tocumbo. Mich. Ann. Esc. Nac. Cienc. Biol. 12, 111–138.

Arora, S., Jain, J., Rajwade, J.M., Paknikar, K.M., 2008. Cellular responses induced by silver nanoparticles: in vitro studies. Toxicol. Lett. 179:93–100. http://dx.doi.org/10.1016/j.toxlet.2008.04.009.

Arzate-Cárdenas, M.A., Martínez-Jerónimo, F., 2012. Energy reserve modification in different age groups of *Daphnia schoedleri* (Anomopoda: Daphniidae) exposed to hexavalent chromium. Environ. Toxicol. Pharmacol. 34:106–116. http://dx.doi.org/10.1016/j.etap.2012.03.003.

Asharani, P.V., Wu, Y.L., Gong, Z., Valiyaveettil, S., 2008. Toxicity of silver nanoparticles in zebrafish models. Nanotechnology 19:2255102–2255107. http://dx.doi.org/10.1088/0957-4484/19/25/255102.

Asharani, P.V., Mun, G.L.K., Hande, M.P., Valiyaveettil, S., 2009. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano 3:279–290. http://dx.doi.org/10.1021/nn800596w.

Bar-Ilan, O., Albrecht, R.M., Fako, V.E., Furgeson, D.Y., 2009. Toxicity assessments of multi sized gold and silver nanoparticles in zebrafish embryos. Small 5:1897–1910. http://dx.doi.org/10.1002/smll.200801716.

Begg, K., Pankhurst, N.W., 2004. Endocrine and metabolic responses to stress in a laboratory population of the tropical damselfish *Acanthochromis polyacanthus*. J. Fish Biol. 64:133–145. http://dx.doi.org/10.1111/j.1095-8649.2004.00290.x.

Begum, G., Vijayaraghavan, S., 1999. Effect of acute exposure of the organophosphate insecticide rogor on some biochemical aspects of *Clarias batrachus* (Linnaeus). Environ. Res. 80:80–83. http://dx.doi.org/10.1006/enrs.1998.3871.

Beliaeff, B., Burgeot, T., 2002. Integrated biomarker response: a useful tool for ecological risk assessment. Environ. Toxicol. Chem. 21:316–1322. http://dx.doi.org/10.1002/etc.5620210629.

Benn, T.M., Westerhoff, P., 2008. Nanoparticle silver released into water from commercially available sock fabrics. Environ. Sci. Technol. 42:4133–4139. http://dx.doi.org/10.1021/es7032718.

Benn, T., Cavanagh, B., Hristovski, K., Posner, J.D., Westerhoff, P., 2010. The release of nanosilver from consumer products used in the home. J. Environ. Qual. 39: 1875–1882. http://dx.doi.org/10.2134/jeq2009.0363.

Berne, B.J., Pecora, R., 2000. Dynamic Light Scattering With Applications to Chemistry, Biology and Physics. Dover Publications, pp. 195–196.

Bilberg, K., Hovgaard, M.B., Besenbacher, F., Baatrup, E., 2012. In vivo toxicity of silver nanoparticles and silver ions in zebrafish (*Danio rerio*). J. Toxicol. 2012:9. http://dx.doi.org/10.1155/2012/293784.

Birsoy, K., Festuccia, W.T., Laplante, M., 2013. A comparative perspective on lipid storage in animals. J. Cell Sci. 126:1541–1552. http://dx.doi.org/10.1242/jcs.104992.

Blinova, I., Niskanen, J., Kajankari, P., Kanarbik, 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. http://dx.doi.org/10.1007/s11356-012-1290-5.

Boxall, A.B.A., Chaudhry, Q., Jones, A., Jefferson, B., Watts, C.D., 2008. Current and Future Predicted Environmental Exposure to Engineered Nanoparticles. Central Science Laboratory, York, UK.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 52: 302–310. http://dx.doi.org/10.1016/S0076-6879(78)52032-6.

Carlson, C., Hussain, S.M., Schrand, A.M., Braydich-Stolle, L.K., Hess, K.L., Jones, R.L., Schlager, J.J., 2008. Unique celular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. J. Phys. Chem. B 112:13608–13619. http://dx.doi.org/10.1021/jp712087m.

Chae, Y.J., Pham, C.H., Lee, J., Bae, E., Yi, J., Gu, M.B., 2009. Evaluation of the toxic impact of silver nanoparticles on Japanese medaka (*Oryzias latipes*). Aquat. Toxicol. 94: 320–327. http://dx.doi.org/10.1016/j.aquatox.2009.07.019.

Chen, X., Schluesener, H.J., 2008. Nanosilver: a nanoproduct in medical application. Toxicol. Lett. 176:1–12. http://dx.doi.org/10.1016/j.toxlet.2007.10.004.

Cho, J.G., Kim, K.T., Ryu, T.K., Lee, J.W., Kim, J.E., Kim, J., Lee, B.C., Yoon, J., Eom, I.C., Choi, K., Kim, P., 2013. Stepwise embryonic toxicity of silver nanoparticles on *Oryzias latipes*. Biomed. Res. Int. 2013:494671. http://dx.doi.org/10.1155/2013/494671.

Choi, O., Hu, Z.Q., 2008. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. Environ. Sci. Technol. 42:4583–4588. http://dx.doi.org/10.1021/es703238h.

Choi, J.E., Kim, S., Ahn, J.H., Youn, P., Kang, J.S., Park, K., Yi, J., Ryu, D.Y., 2010. Induction of oxidative stress and apoptosis by silver nanoparticles in the liver of adult zebra fish. Aquat. Toxicol. 100:151–159. http://dx.doi.org/10.1016/j.aquatox.2009.12.012.

Cooper, G.M., 2000. The cell: a molecular approach. Cell Membranes, second ed. Sinauer Associates, Sunderland (MA) (Available from: https://www.ncbi.nlm.nih.gov/books/NBK9928/ Accessed 22 september 2016).

Crapo, J.D., MacCord, J.M., Fridovich, I., 1978. Preparation and assay of superoxide dismutases. Methods Enzymol. 53:382–393. http://dx.doi.org/10.1016/S0076-6879(78)53044-9.

De Matteis, V., Malvindi, M.A., Galeone, A., Brunetti, V., De Luca, E., Kote, S., Kshirsagar, P., Sabella, S., Bardi, G., Pompa, P.P., 2015. Negligible particle-specific toxicity mechanism of silver nanoparticles: the role of Ag+ ion release in the cytosol. Nanomedicine 11: 731–739. http://dx.doi.org/10.1016/j.nano.2014.11.002.

Delgado, A.V., Gonzalez-Caballero, F., Hunter, R.J., Koopal, L.K., Lyklema, J., 2005. Measurement and interpretation of electrokinetic phenomena (IUPAC technical report). Pure Appl. Med. 77:1753–1850. http://dx.doi.org/10.1351/pac200577101753.

Devin, S., Burgeot, T., Giambérini, L., Minguez, L., Pain-Devin, S., 2014. The integrated biomarker response revisited: optimization to avoid misuse. Environ. Sci. Pollut. Res. 21: 2448–2454. http://dx.doi.org/10.1007/s11356-013-2169-9.

Domínguez-Domínguez, O., Mercado-Silva, N., Lyons, J., 2005. Conservation status of Mexican goodeids: problems, perspectives, and solutions. In: Uribe-Aranzabal, M.C., Grier, H. (Eds.), Proceedings of the II International Symposium on Livebearing Fishes. New Life, Homestead, Florida, pp. 495–504.

Dubois, M., Gilles, K., Hamilton, J.K., Rebers, P.A., Smith, F., 1951. A colorimetric method for the determination of sugars. Nature 168 (4265):167. http://dx.doi.org/10.1038/168167a0.

Duncan, J.R., Lockwood, J.L., 2001. Extinction in a field of bullets: a search for causes in the decline of the world's freshwater fishes. Biol. Conserv. 102:97–105. http://dx.doi.org/10.1016/S0006-3207(01)00077-5.

Dzul-Caamal, R., Olivares-Rubio, H.F., Medina-Segura, C.G., Vega-López, A., 2012. Endangered Mexican fish special protection: diagnosis of habitat fragmentation, protection, and future—a review. In book. Endangered Species: Habitat, Protection and Ecological Significance 1. Nova Sci Publ, pp. 109–130.

El Badawy, A., Silva, R., Morris, B., Scheckel, K., Suidan, M., Tolaymat, T., 2011. Surface charge-dependent toxicity of silver nanoparticles. Environ. Sci. Technol. 4:283–287. http://dx.doi.org/10.1021/es1034188.

Farkas, J., Peter, H., Christian, P., Gallego-Urrea, J.A., Hassellöv, M., Tuoriniemi, J., Gustafsson, S., Olsson, E., Hylland, K., Thomas, K.V., 2011. Characterization of the effluent from a nanosilver producing washing machine. Environ. Int. 37:1057–1062. http://dx.doi.org/10.1016/j.envint.2011.03.006.

Folttmann, H., Quadir, A., 2008. Polyvinylpyrrolidone (PVP)—one of the most widely used excipients in pharmaceuticals: an overview. J. Drug Deliv. Technol. 8, 22–27.

Goreham, R.V., Thompson, V.C., Samura, Y., Gibson, C.T., Shapter, J.G., Köper, I., 2015. Interaction of silver nanoparticles with tethered bilayer lipid membranes. Langmuir 31: 868–5874. http://dx.doi.org/10.1021/acs.langmuir.5b0058.

Govindasamy, R., Rahuman, A.A., 2012. Histopathological studies and oxidative stress of synthesized silver nanoparticles in Mozambique tilapia (*Oreochromis mossambicus*). J. Environ. Sci. 24:1091–1098. http://dx.doi.org/10.1016/S1001-0742(11)60845-0.

Griffitt, R.J., Luo, J., Gao, J., Bonzongo, J.C., Barber, D.S., 2008. Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ. Toxicol. Chem. 27:1972–1978. http://dx.doi.org/10.1897/08-002.1.

He, W., Zhou, Y.T., Wamer, W.G., Boudreau, M.D., Yin, J.J., 2012. Mechanisms of the pH dependent generation of hydroxyl radicals and oxygen induced by Ag nanoparticles. Biomaterials 33:7547–7555. http://dx.doi.org/10.1016/j.biomaterials.2012.06.076.

Hernández-López, J., 2001. Diseño de técnicas para la cuantificación de moléculas plasmáticas de camarón. Tesis Doctoral en Ciencias. Centro de Investigación en Alimentación y Desarrollo, A.C., Hermosillo, Sonora, p. 104.

Horie, M., Kato, H., Fujita, K., Endoh, S., Iwahashi, H., 2012. In vitro evaluation of cellular response induced by manufactured nanoparticles. Chem. Res. Toxicol. 25:605–619. http://dx.doi.org/10.1021/tx200470e.

Jahanbakhshi, A., Shaluei, F., Hedayati, A., 2012. Detection of silver nanoparticles (Nanosil) LC_{50} in silver carp (*Hypophthalmichthys molitrix*) and goldfish (*Carassius auratus*). World J. Zool. 7:126–130. http://dx.doi.org/10.5829/idosi.wjz.2012.7.2.62129.

Javed, M., Usmani, N., 2015. Stress response of biomolecules (carbohydrate, protein and lipid profiles) in fish *Channa punctatus* inhabiting river polluted by Thermal Power Plant effluent. Saudi J. Biol. Sci. 22:237–242. http://dx.doi.org/10.1016/j.sjbs.2014.09.021.

Johari, S., Kalbassi, M., Soltani, M., Yu, I., 2013. Toxicity comparison of coloidal silver nanoparticles invarious life stages of rainbow trout (*Oncorhynchus mykiss*). Iran. J. Fish. Sci. 12, 76–95.

Jung, W.K., Kim, S.H., Koo, H.C., Shin, S., Kim, J.M., Park, Y.K., Hwang, S.Y., Yang, H., Park, Y.H., 2007. Antifungal activity of the silver ion against contaminated fabric. Mycoses 50:265–269. http://dx.doi.org/10.1111/j.1439-0507.2007.01372.x.

Please cite this article as: Valerio-García, R.C., et al., Exposure to silver nanoparticles produces oxidative stress and affects macromolecular and metabolic biomarkers in the goodeid fish..., *Sci Total Environ (2017)*, http://dx.doi.org/10.1016/j.scitotenv.2017.01.070

Kannan, R.R., Jerley, A.J.A., Ranjani, M., Prakash, V.S.G., 2011. Antimicrobial silver nanoparticle induces organ deformities in the developing Zebrafish (*Danio rerio*) embryos. J. Biomed. Sci. Eng. 4:246–252. http://dx.doi.org/10.4236/jbise.2011.44034.

Kashiwada, S., Ariza, M.E., Kawaguchi, T., Nakagame, Y., Jayasinghe, B.S., Gärtner, K., Nakamura, H., Kagami, Y., Sabo-Attwood, T., Ferguson, P.L., Chandler, G.T., 2012. Silver nanocolloids disrupt medaka embryogenesis through vital gene expressions. Environ. Sci. Technol. 46:6278–6287. http://dx.doi.org/10.1021/es2045647.

Kumar, P.S.S., Sivakumar, R., Anandan, S., Madhavan, J., Maruthamuthu, P., Ashokkumar, M., 2008. Photocatalytic degradation of Acid Red 88 using Au–TiO2 nanoparticles in aqueous solutions. Water Res. 42:4878–4884. http://dx.doi.org/10.1016/j.watres.2008.09.027.

Laban, G., Nies, L.F., Turco, R.F., Bickham, J.W., Sepúlveda, M.S., 2010. The effects of silver nanoparticles on fathead minnow (*Pimephales promelas*) embryos. Ecotoxicology 19:185–195. http://dx.doi.org/10.1007/s10646-009-0404-4.

Lee, K.J., Nallathamby, P.D., Browning, L.M., Osgood, C.J., Xu, N.X., 2007. In vivo imaging of transport and biocompatibility of single silver nanoparticles in early development of Zebrafish embryos. ACS Nano 1:133–143. http://dx.doi.org/10.1021/nn700048y.

Lee, B., Duong, C.N., Cho, J., Lee, J., Seo, K.Y., Kim, P., Choi, K., Yoon, J., 2012. Toxicity of citrate-capped silver nanoparticles in common carp (*Cyprinus carpio*). J. Biomed. Biotechnol. 2012:262670. http://dx.doi.org/10.1155/2012/262670.

Lei, X.G., Evenson, J.K., Thompson, K.M., Sunde, R.A., 1995. Glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase are differentially regulated in rats by dietary selenium. J. Nutr. 125, 1438–1446.

Levine, R.L., Williams, J.A., Stadtman, E.P., Shacter, E., 1994. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol. 233:346–357. http://dx.doi.org/10.1016/S0076-6879(94)33040-9.

Li, Q.L., Mahendra, S., Lyon, D.Y., Brunet, L., Liga, M.V., Li, D., Alvarez, P.J.J., 2008. Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. Water Res. 42:4591–4602. http://dx.doi.org/10.1016/j.watres.2008.08.015.

Li, T., Albee, B., Alemayehu, M., Diaz, R., Ingham, L., Kamal, S., Rodriguez, M., Bishnoi, S.W., 2010. Comparative toxicity study of Ag, Au, and Ag–Au bimetallic nanoparticles on *Daphnia magna*. Anal. Bioanal. Chem. 398:689–700. http://dx.doi.org/10.1007/s00216-010-3915-1.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Massarsky, A., Dupuis, L., Taylor, J., Eisa-Beygi, S., Strek, L., Trudeau, V.L., Moon, T.W., 2013. Assessment of nanosilver toxicity during zebrafish (*Danio rerio*) development. Chemosphere 92:59–66. http://dx.doi.org/10.1016/j.chemosphere.2013.02.060.

Miller, R.R., 2009. Peces dulceacuícolas de México. Comisión nacional para el conocimiento y uso de la biodiversidad. Sociedad ictiológica Mexicana A C. El Colegio de la Frontera Sur, México. Consejo de los Peces del Desierto, México-Estados Unidos. p. 559.

Monfared, A.L., Soltani, S., 2013. Effects of silver nanoparticles administration on the liver of rainbow trout (*Oncorhynchus mykiss*): histological and biochemical studies. Eur. J. Exp. Biol. 3, 285–289.

Morones, J.R., Elechiguerra, J.L., Camacho-Bragado, A., Holt, K., Kouri, J.B., Tapia-Ramírez, J., Yacaman, M.J., 2005. The bactericidal effect of silver nanoparticles. Nanotechnology 16:2346–2353. http://dx.doi.org/10.1088/0957-4484/16/10/059.

Navarro, E., Piccapietra, F., Wagner, B., Marconi, F., Kaegi, R., Odzak, N., Sigg, L., Behra, R., 2008. Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. Environ. Sci. Technol. 42:8959–8964. http://dx.doi.org/10.1021/es801785m.

Nelson, J.S., 1994. Fishes of the World. third ed. John Wiley and Sons, Inc., New York.

NOM-059-SEMARNAT-2010, 2010. Protección ambiental-especies nativas de México de flora y fauna silvestres-categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. Diario Oficial de la Federación, (30 de diciembre de 2010, Segunda Sección, México).

Nyström, T., 2005. Role of oxidative carbonylation in protein quality control and senescence. EMBO J. 24:1311–1317. http://dx.doi.org/10.1038/sj.emboj.7600599.

Oberdörster, G., Oberdörster, E., Oberdörster, J., 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect. 113: 823–839. http://dx.doi.org/10.1289/ehp.7339.

Olivares-Rubio, H.F., Martínez-Torres, M., Najera-Martínez, M., Dzul-Caamal, R., Domínguez-López, M., García-Latorre, E., Vega-López, A., 2014. Biomarkers involved in energy metabolism and oxidative stress response in the liver of *Goodea gracilis* Hubbs and Turner, 1939 exposed to the microcystin-producing *Microcystis aeruginosa* LB85 strain. Environ. Toxicol. 30:1113–1124. http://dx.doi.org/10.1002/tox.21984.

Oukarroum, A., Bras, S., Perreault, F., Popovic, R., 2012. Inhibitory effects of silver nanoparticles in two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*. Ecotoxicol. Environ. Saf. 78:80–85. http://dx.doi.org/10.1016/j.ecoenv.2011.11.012.

Paresh, C.R., Hongtao, Y., Peter, P.F., 2009. Toxicity and environmental risks of nanomaterials: challenges and future needs. J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 27:1–35. http://dx.doi.org/10.1080/10590500802708267.

Perelshtein, I., Applero, G., Perkas, N., Guibert, G., Mikhailov, S., Gedanken, A., 2008. Sonochemical coating of silver nanoparticles on textile fabrics (nylon, polyester and cotton) and their antibacterial activity. Nanotechnology 19:245705. http://dx.doi.org/10.1088/0957-4484/19/24/245705.

Piao, M.J., Kang, K.A., Lee, I.K., Kim, H.S., Kim, S., Choi, J.Y., Choi, J., Hyun, J.W., 2011. Silver nanoparticles induce oxidative cell damage in human liver cells through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis. Toxicol. Lett. 201:92–100. http://dx.doi.org/10.1016/j.toxlet.2010.12.010.

Radi, R., Turrens, J.F., Chang, L.Y., Bush, K.M., Crapo, J.D., Freeman, B.A., 1991. Detection of catalase in rat heart mitochondria. J. Biol. Chem. 266, 22028–22034.

Raffi, M., Hussain, F., Bhatti, T.M., Akhter, J.I., Hameed, A., Hasan, M.M., 2008. Antibacterial characterization of silver nanoparticles against *E. coli* ATCC-15224. J. Mater. Sci. Technol. 24, 192–196.

Rajkumar, S.K., Kanipandian, N., Thirumurugan, R., 2016. Toxicity assessment on haemotology, biochemical and histopathological alterations of silver nanoparticles-exposed freshwater fish *Labeo rohita*. Appl. Nanosci. 6:19–29. http://dx.doi.org/10.1007/s13204-015-0417-7.

Rangel-Vázquez, N.A., Rodríguez, F.F., 2014. Computational Chemistry Applied in the Analyses of Chitosan/Polyvinylpyrrolidone/Mimosa Tenuiflora, Chapter 4 Polyvinylpyrrolidone (PVP). Science Publishing Group, New York.

Repetto, M., Boveris, A., Semprine, J., 2012. Lipid Peroxidation: Chemical Mechanism, Biological Implications and Analytical Determination. INTECH Open Access Publisher Book:pp. 1–30 http://dx.doi.org/10.5772/45943.

Saddick, S., Afifi, M., Abu-Zinada, O.A., 2015. Effect of zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of *Oreochromis niloticus* and *Tilapia zillii*. Saudi J. Biol. Sci. http://dx.doi.org/10.1016/j.sjbs.2015.10.021.

Salopek, B., Krasic, D., Filipovic, S., 1992. Measurement and application of zeta-potential. Rudarsko-geoloiko-naftni zbornik 4, 147–151.

Sancho, E., Ferrando, M.D., Andreu, E., 1996. Physiological stress responses of *Anguilla anguilla* to Fenitrothion. J. Environ. Sci. Health B 31:87–98. http://dx.doi.org/10.1080/03601239609372976.

Schrand, A.M., Braydich-Stolle, L.K., Schlager, J.J., Dai, L., Hussain, S.M., 2008. Can silver nanoparticles be useful as potential biological labels? Nanotechnology 19:235104. http://dx.doi.org/10.1088/0957-4484/19/23/235104.

Seltenrich, N., 2013. Nanosilver: weighing the risks and benefits. Environ. Health Perspect. 121:A220–A225. http://dx.doi.org/10.1289/ehp.121-a220.

Shahbazzadeh, D., Ahari, H., Rahimi, N.M., Dastmalchi, F., Soltani, M., Fotovat, M., Rahmannya, J., Khorasani, N., 2009. The effects of nanosilver (Nanocid) on survival percentage of rainbow trout (*Oncorhynchus mykiss*). Pak. J. Nutr. 8:1178–1179. http://dx.doi.org/10.3923/pjn.2009.1178.1179.

Stadtman, E.R., Oliver, C.N., 1991. Metal-catalyzed oxidation of proteins. Physiological consequences. J. Biol. Chem. 266, 2005–2008.

Sun, L., Singh, A.K., Vig, K., Pillai, S.R., Singh, S.R., 2008. Silver nanoparticles inhibit replication of respiratory syncytial virus. J. Biomed. Nanotechnol. 4:149–158. http://dx.doi.org/10.1166/jbn.2008.012.

Teles, M., Maria, V.L., Pacheco, M., Santos, M.A., 2004. *Anguilla anguilla* L. plasma cortisol lactate and glucose responses to abietic acid dehydroabietic acid and retene. Environ. Int. 29:995–1000. http://dx.doi.org/10.1016/S0160-4120(03)00093-X.

Teodoro, J.S., Simões, A.M., Duarte, F.V., Rolo, A.P., Murdoch, R.C., Hussain, S.M., Palmeira, C.M., 2011. Assessment of the toxicity of silver nanoparticles in vitro: a mitochondrial perspective. Toxicol. In Vitro 25:664–670. http://dx.doi.org/10.1016/j.tiv.2011.01.004.

Thangam, Y., Umavathi, S., Revathi, S., Kalamani, P., 2014. Toxic effect of silver and silver nanoparticle in glucose level to freshwater fish "*Cyprinus carpio*". MCAS J. Res. 1l.

Tiede, K., Hassellöv, M., Breitbarth, E., Chaudhry, Q., Boxall, A.B.A., 2009. Considerations for environmental fate and ecotoxicity testing to support environmental risk assessments for engineered nanoparticles. J. Chromatogr. A 1216:503–509. http://dx.doi.org/10.1016/j.chroma.2008.09.008.

Trujillo-Jiménez, P., Espinosa de los Monteros-Viveros, E., 2006. La ecología alimentaria del pez endémico *Girardinichthys multiradiatus* (Cyprinidontiformes: Goodeidae), en el parque nacional lagunas de Zempoala, México. Rev. Biol. Trop. 54:1247–1255. http://dx.doi.org/10.15517/rbt.v54i4.3102.

USEPA, 1975. Summary of Hazardous Substance Classification Systems: a Current Report on Solid Waste Management. p. 59.

USEPA, 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Quinta ed. U.S. EPA Office of Water, Washington, D.C., p. 266 EPA-821-R-02-012.

Vega-López, A., Galar-Martínez, M., Jiménez-Orozco, F.A., García-Latorre, E., Domínguez-López, M.L., 2007. Gender related differences in the oxidative stress response to PCBs in an endangered goodeid fish (*Girardinichthys viviparus*). Comp. Biochem. Physiol. Part A. 146:672–678. http://dx.doi.org/10.1016/j.cbpa.2006.04.022.

Wang, H.T., Nafday, O.A., Haamheim, J.R., Tevaarwerk, E., Amro, N.A., Sanedrin, R.G., Chang, C.Y., Ren, F., Pearton, S.J., 2008. Toward conductive traces: dip pen nanolithography of silver nanoparticle-based inks. Appl. Phys. Lett. 93:143105. http://dx.doi.org/10.1063/1.2995859.

Woodrow Wilson Database, 2014. Consumer Products Inventory. The Project on Emerging Nanotechnologies. (Web site: http://www.nanotechproject.org/cpi/browse/nanomaterials/).

Wu, Y., Zhou, Q., 2012. Dose-and time-related changes in aerobic metabolism, chorionic disruption, and oxidative stress in embryonic medaka (*Oryzias latipes*): underlying mechanisms for silver nanoparticle developmental toxicity. Aquat. Toxicol. 124: 238–246. http://dx.doi.org/10.1016/j.aquatox.2012.08.009.

Wu, Y., Zhou, Q., Li, H., Liu, W., Wang, T., Jiang, G., 2010. Effects of silver nanoparticles on the development and histopathology biomarkers of Japanese medaka (*Oryzias latipes*) using the partial-life test. Aquat. Toxicol. 100:160–167. http://dx.doi.org/10.1016/j.aquatox.2009.11.014.

Xia, J., Zhao, H.Z., Lu, G.H., 2013. Effects of selected metal oxide nanoparticles on multiple biomarkers in *Carassius auratus*. Biomed. Environ. Sci. 26:742–749. http://dx.doi.org/10.3967/0895-3988.2013.09.005.

Zhang, T., Wang, L., Chen, Q., Chen, C., 2014. Cytotoxic potential of silver nanoparticles. Yonsei Med. J. 55:283–291. http://dx.doi.org/10.3349/ymj.2014.55.2.283.

Zöllner, N., Kirsch, K., 1962. Microdetermination of lipids by the sulphophosphovanillin reaction. Zeitschrift fur die Gesamte Experimentelle Medizin einschliesslich Experimenteller Chirurgie 135, 545–561.

Please cite this article as: Valerio-García, R.C., et al., Exposure to silver nanoparticles produces oxidative stress and affects macromolecular and metabolic biomarkers in the goodeid fish..., *Sci Total Environ (2017)*, http://dx.doi.org/10.1016/j.scitotenv.2017.01.070