Polluted water from an urban reservoir (Madín dam, México) induces toxicity and oxidative stress in *Cyprinus carpio* embryos

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Abstract

The Madín Dam is a reservoir located in the municipalities of Naucalpan and Atizapán, in the metropolitan area adjacent to Mexico City. The reservoir supplies drinking water to nearby communities and provides an area for various recreational activities, including kayaking, sailing and carp fishing. Over time, the number of specimens of common carp has notably diminished in the reservoir, which receives direct domestic drainage from two towns as well as numerous neighborhoods along the Tlalnepantla River. Diverse studies have demonstrated that the pollutants in the water of the reservoir produce oxidative stress, genotoxicity and cytotoxicity in juvenile *Cyprinus carpio*, possibly explaining the reduction in the population of this species; however, it is necessary to assess whether these effects may also be occurring directly in the embryos. Hence, surface water samples were taken at five sites and pharmaceutical drugs, personal care products (especially sunscreens), organophosphate and organochlorine pesticides, and other persistent organic pollutants (e.g., polychlorinated biphenyls and polycyclic aromatic hydrocarbons) were identified. Embryos of *C. carpio* were exposed to the water samples to evaluate embryoletality, modifications in embryonic development, lipoperoxidation, the quantity of hydroperoxide and oxidized proteins, and antioxidant enzyme activity (superoxide dismutase, catalase and glutathione peroxidase). It was found that the polluted water of the Madín Dam gave rise to embryoletality, embryotoxicity, congenital abnormalities, and oxidative stress on the common carp embryos.

Capsule: The water of the Madín dam contains a complex mixture of pollutants, including hydrocarbons, which produce oxidative stress and embryotoxicity on *Cyprinus carpio*.

Keywords: *Cyprinus carpio*; embryoletality; embryotoxicity; teratogenicity; oxidative stress; organic pollutants; Madín reservoir.

1. Introduction

Freshwater bodies are frequently contaminated by anthropogenic chemical stressors, including metals, pesticides, emerging contaminants and others, resulting in significant modifications to the health of hydrobionts and ecosystems in general. This problem is of great importance for all aquatic ecosystems and for those close to urban localities that discharge directly into these reservoirs, such as the Madín Dam (MR) in Mexico.

The MR is located on the Tlalnepantla River between the municipalities of Naucalpan de Juárez and Atizapán de Zaragoza, in the State of Mexico. Drainage from different localities is dumped directly (without prior treatment) into the reservoir and the Tlalnepantla River. Nevertheless, MR is the source of drinking water for many neighborhoods in the surrounding municipalities (Pérez-Coyotl *et al.*, 2017). It is important to note that there has been evident environmental degradation of the reservoir, which is the habitat for diverse species of fish and birds.

Previous studies have shown that there are considerable concentrations of pollutants such as aluminum (Al, 6.04-24.45 mg L⁻¹) and iron (Fe, 1.51-5.10 mg L⁻¹), which exceed the maximum permissible concentration established to protect aquatic life (DOF, 1989), as well as nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac (0.20-0.31 µg L⁻¹), ibuprofen (3.61-4.51 µg L⁻¹) and naproxen (0.18 µg L⁻¹) (Galar-Martínez *et al.*, 2010; González-González *et al.*, 2014). However, given the urban and industrial characteristics of the areas surrounding the MR, it is necessary to explore the possible presence of other xenobiotics such as pharmaceuticals, personal care products (PCP), pesticides and other persistent organic pollutants (POPs).

Prior studies carried out by our work team have shown that contaminants present in MR water generate genotoxicity and cytotoxicity related to the production of oxidative stress in juveniles of *Cyprinus carpio* (González-González et al., 2014; Pérez-Coyotl et al., 2017). However, since the age of organisms is one of the most important factors modifying toxic response, it is very likely that individuals in early stages, and particularly on embryonic stage, are more susceptible to MR contaminants.

On the other hand, there is evidence that oxygen plays a key role in the metabolism of embryos and is critical in early embryonic development. Reactive oxygen species (ROS) have vital signaling functions and intervene in numerous physiological processes in the developing organism. During embryogenesis, ROS control cell division and the maturing of oocytes as well as the implantation and formation of blastocysts. When ROS are not duly regulated, they tend to generate oxidative stress and consequently embryotoxicity. Therefore, the antioxidant defense plays a vital role in the protection of organisms in formation, since pro-oxidant agents could elevate the concentration of ROS and induce oxidative damage in oocytes, mitochondrial alterations, depletion of the ATP content, DNA damage, lipoperoxidation (LPX), apoptosis, and/or a delay in the entire embryonic development process (Pašcová et al., 2011).

There are some reports on the role of metals as pro-oxidant agents and their effect on the embryonic development of hydrobionts. For instance, Al at a concentration of 1.87 mg L⁻¹ affected the embryonic development of a freshwater snail (*Radix quadrasi*), causing a delay in growth followed by edema and thinning of the shell, as well as a delay in hatching of the exposed organisms (Factor and de Chavez, 2012). Kong et al. (2012) demonstrated that in embryos exposed to 0.2, 1.5 and 10 µg L⁻¹ of mercury (Hg), the activity of catalase (CAT) decreased and the degree of LPX increased

in a concentration-dependent manner. After exposing *Pimephales promelas* to methylmercury, Devlin (2006) found a rapid accumulation of the compound and a relatively low concentration of proteins in the organisms.

Similarly, emerging contaminants such as NSAIDs, which have been shown to be embryotoxic and teratogenic in a wide range of aquatic species. This is the case of diclofenac which causes these effects on *Xenopus laevis*, *Lithobates catesbeianus* (Cardoso-Vera *et al.*, 2017) and *C. carpio* (Stepanova *et al.*, 2013). Prášková *et al.* (2013) determined that ketoprofen delays the hatching of embryos of the common carp. Besides, numerous other pollutants (e.g., PCPs, pesticides and POPs) are able to provoke oxidative stress and embryotoxicity in hydrobionts. Certain combinations of this chemical compounds likely generate interactions that intensify the toxic response, even when the exposure to individual pollutants is below the no-observed-adverse-effect level (NOAEL) (Beyer *et al.*, 2014).

One species commonly used in ecotoxicology studies is common carp (*C. carpio*). It is a very important organism from an ecological and economic point of view. In Mexico, this species inhabits 80% of the freshwater bodies and is consumed by a large number of people, occupying eighth place in world fish production (Nava-Álvarez *et al.*, 2014). However, it is endangered by the widespread contamination of its habitat in many locations such as the MR. Many reports have described a considerable reduction in the population of various species that inhabit different freshwater bodies.

The objective of the present work was to characterize the main organic contaminants present in MR, and to evaluate the toxicity produced by that polluted water on the embryonic development

of the common carp (*Cyprinus carpio*), correlating the morphological changes found with the production of oxidative stress.

2. Materials and methods

2.1 The study area and water sampling

The MR (at 19°31'37" N and 99°15'33" W) has a surface area of 190 hectares and a storage capacity of 16.6 million m³. It is bordered by the towns of Nuevo Madín and Viejo Madín, which discharge their waste directly into the reservoir. The dam was built to control the flow of the Tlalnepantla river and provide drinking water to nearby municipalities. The resulting water body is used for kayaking, sailing, carp fishing and other recreational activities (Pérez-Cóyotl *et al.*, 2017).

The water samples were collected in September 2016 during the rainy season (May-September), according to the procedure of the Mexican official norm on wastewater sampling (NMX-AA-003-1980) and treated as indicated by Pérez-Cóyotl *et al.*, (2017): Samples were taken at five sites of the reservoir: (1) the point of wastewater drainage into the reservoir from the town of Nuevo Madín, (2) the entry point of the Tlalnepantla River, (3) a side branch of the reservoir, (4) the curtain of the dam, and (5) the point of wastewater drainage into the reservoir from the town of Viejo Madín (Fig. 1). Water at each site was sampled by filling three 2-liter polyethylene bottles (previously rinsed with 3% nitric acid and fitted with an automatic sealing mechanism). The bottles were immediately transported to the laboratory and stored in the dark at 5 °C until being employed for chemical analysis and toxicity assays (in less than 7 days) (Pérez-Cóyotl *et al.*, 2017). For

chemical characterization of the samples, identification and quantification was carried out for pesticides, PCPs, drugs and POPs. Subsequently, embryotoxicity bioassays were conducted with each sample.

2.2. Quantification of pollutants

The samples from the five sampling sites were analyzed to detect and quantify pesticides, personal care products (PCPs), pharmaceuticals and persistent organic pollutants (POPs).

Pesticides in the water samples were evaluated by on-line solid phase extraction (SPE), high-performance liquid chromatography (HPLC) and tandem mass spectrometry (SPE-LC-MS/MS), as described in Köck-Schulmeyer *et al.* (2012, 2013).

Personal care products were evaluated by HPLC mass spectrometry analysis as described by Serra-Roig *et al.* (2016).

Sample extraction and ultra-high-performance liquid chromatography (UHPLC)-MS/MS analysis methods to quantify pharmaceutical products have been described elsewhere (López-Serna *et al.*, 2010).

Finally, chemical analysis of water samples to determine persistent organic pollutants was performed with a method based on SPE followed by gas chromatography-tandem mass spectrometry (GC-MS/MS), as described in Lacorte *et al.* (2000).

2.3 Embryo bioassays

C. carpio embryos were obtained by natural fertilization, carried out in the Carpícola Tiacaque Center (Mexico State). Four female and two male adults were placed in a fertilization tank. The ovideposit and fertilization of eggs were monitored. Eggs were selected 6 h post-fertilization and observed by stereomicroscopy to confirm fertilization, embryonic age stage (period of gastrulation) and viability (Hermsen *et al.*, 2011). Subsequently, they were exposed to water from each of the five sampling sites in order to explore possible oxidative stress and embryotoxicity (lethality and teratogenesis). Dissolved oxygen concentration at sampling sites ranged from 6.3 to 7.8 mg L⁻¹.

2.3.1 Evaluation of embryotoxicity and teratogenesis

For the examination of embryotoxicity, 60 embryos were selected at 6 h post-fertilization for each of the 5 sampling sites and placed on plates (1 embryo/well) for ELISA. Each well contained 300 µL of water from the sampling sites, and the plates were maintained at 24 ± 1 °C. The tests were carried out in a static environment without renewal. These embryos were observed under an inverted Optika XDS-2 microscope at 12, 24, 48, 72 and 96 h. The number of dead embryos (embryo lethality) was quantified, and the morphological development of each surviving embryo (embryotoxicity) was evaluated by utilizing the general morphological point system adapted by Kimmel *et al.* (1955) and Hermsen *et al.* (2011). This system is based on points assignment accordingly to the presence or absence of specific morphological characteristics, including the time of hatching, at specified testing times. The final scoring of the embryos exposed to water from the different sites of the MR was compared to the scoring of the control group. Dead embryos were not scored.

To determine the teratogenesis produced by the MR water samples, all organisms still surviving at 96 h of exposure were evaluated for pericardial edema, yolk sac edema, eye edema, tail, heart, head or sacculi/otolith malformation, modified chorda structure, scoliosis, rachischisis and yolk deformation. The results are expressed as the percentage of malformed embryos.

2.3.2 Determination of antioxidant defenses and oxidative stress parameters

Six lots were formed with 7 g of embryos each in aquariums of 1-L capacity, containing the water from the five sampling points of the MR and a control group, maintained in eggs water (60 ??g Instant Ocean salt mL⁻¹ of tridestilled water) (Westerfield, 2007). The systems were kept in rooms with controlled temperature at 24°C. At each of the exposure times (12, 24, 48, 72 and 96 h), 1 g of the embryos was homogenized in 1.5 mL of phosphate buffer solution (PBS, pH 7.4), and then the samples were centrifuged at 15,000 g for 15 min at 4 °C. The supernatant was used to determine the protein carbonyl content (PCC) and the activity of antioxidant enzymes: CAT, superoxide dismutase (SOD) and glutathione peroxidase (GPx). The homogenate was examined to establish the degree of LPX and the hydroperoxide content (HPC). The assay was performed in triplicate.

The degree of LPX was assessed with the method reported by Buege and Aust (1978). The results are expressed as nM of malondialdehyde, expressed as the measured environmental concentration of 1.56×10^5 /M/cm per mg of protein per g of tissue.

The HPC was quantified by the method of Jiang *et al.* (1992). Results were expressed as nanomoles (nM) of cumene per mg of protein per g of tissue.

The PCC was ascertained by the method described by Levine *et al.* (1994), with some modifications. The results are expressed as nM of reactive carbonyls (C=O), obtained by the measured environmental concentration of $21\,000\text{ M}^{-1}\text{ cm}^{-1}$ per mg of protein per g of tissue.

SOD activity was established with the commercial RANSOD kit (RANDOX). After following the kit instructions, the readings were obtained on an ELx800 reader (BioTek) and interpreted with a calibrating curve included in the kit. The data are expressed in U SOD per mg of protein per g of tissue.

CAT activity was estimated by the method of Radi *et al.* (1999). Results were expressed as mM of H_2O_2 per mg of protein per g of tissue.

GPx activity was determined with the RANSEL commercial kit (RANDOX). After following the instructions provided with the kit, the $\Delta A/\text{min}$ of each sample was multiplied by the factor of 8412 (provided by manufacturer) and the data are expressed as U/L of GPX per mg of protein per g of tissue.

The results of all the biomarkers of oxidative stress were normalized against total proteins, which were measured by the Bradford method (1976).

For more specifications on the methods described above, see the supplementary data.

2.4 Statistical Analysis

The values for biomarkers of oxidative stress were submitted to a bifactorial analysis of variance, considering time as factor A and the sampling sites as factor B (Two Way RM ANOVA, $p < 0.05$). The differences between the means were examined with the Student-Newman-Keuls method. The data from the scoring of embryonic development were subjected to two-way non-parametric analysis of variance (ANOVA) of repeated measurements, followed by the Tukey post hoc test for multiple measurements. A Pearson correlation was performed between contaminants found at all sampling sites (20 xenobiotics) and biomarkers of oxidative damage and embryotoxicity (Tables S1 in the supplementary section). The analyses were carried out using the software Sigma Plot 12.3.

3. Results and Discussion

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3.1 Quantification of pollutants

Among the pollutants identified in the MR (Table 1) were chlorfenvinphos, fenthion and diazinon. The latter showed the greatest concentration at all sampling sites. These pesticides have been detected in other water bodies in Europe and America. For example, Ccanccapa *et al.* (2016) found chlorfenvinphos, fenthion and diazinon in concentrations of 1.57-41.24, 0.33-2.64 and 0.12- 20.39 ng L⁻¹, respectively in the Ebro River (Spain). The concentrations observed in MR are lower. Since

no cropland exists near the MR, these pesticides are probably carried along the Tlalnepantla River from sites with nearby fields of crops, implying a dilution factor upon arriving to the reservoir.

Regarding PCPs, various compounds were detected (although only at sites SS5 and SS1), including 2,2',4,4'-tetrahydroxybenzophenone (BP2), OD-PABA, 4-methylbenzylidene camphor (4-MBC) and methyl benzotriazol (MeBZT). UV filters most frequently found in rivers are those derived from benzophenone, such as BP2 and the derivatives of camphor (e.g., 4-MBC) (Ramos *et al.*, 2015). For example, BP2 was found at 109 ng L⁻¹ in an urban basin (Singapur); OD-PABA at up to 748 ng L⁻¹ in the Adige river (Italy) and 4-MBC at 2,700 ng L⁻¹ in Switzerland lakes (Mao *et al.*, 2018; Mandaric *et al.*, 2017; Balmer *et al.*, 2005). Hence, the concentrations of PCPs determined herein are within the range observed in other water bodies around the world, and their prevalence at sampling sites SS1 and SS5 can be attributed to direct drainage of domestic waste from the surrounded towns.

Pharmaceutical products are a threat to aquatic ecosystems due to their inherent biological activity, and their resistance to inactivation after being excreted, (Santos *et al.*, 2010), which implies a potential risk for non-target organisms even when concentrations are low (Destrieux *et al.*, 2017). Among the most prevalent drugs in aquatic ecosystems are NSAIDs, antibiotics, antihypertensive agents and hypoglycemic drugs, which were represented at the sampling sites in the MR. The most prevalent were metformin, glibenclamide and acetaminophen. Diverse studies around the world have found pharmaceutical drugs in water bodies. Santos *et al.* (2010) described the levels of acetaminophen in some influents of Spain founding concentrations around 29,000-246,000 ng L⁻¹. Scheurer *et al.* (2012) reported that the level of metformin in some German rivers was around 130-1,700 ng L⁻¹. Estrada-Arriaga *et al.* (2016) found 66,000 ng L⁻¹ of acetaminophen and 94,600

ng L⁻¹ of metformin entering two treatment plants in Mexico. The concentrations of both in MR were much lower than in the cases previous mentioned.

The POPs detected at the greatest concentrations and at all the sampling sites were three PCBs (149, 118 and 101). PCBs are mainly of industrial origin and reach the environment through incineration plants for domestic residuals, garbage dumps, etc., (Gakuba *et al.*, 2015). PCBs 149, 118 and 101 were observed by Zhang *et al.* (2004) in the Tonghui River (China) at maximum concentrations of 12.71, 25.65 and 74.07 ng L⁻¹, respectively, constituting higher levels than those found in the MR.

Another group of POPs evaluated was PAHs. The ones found in the highest concentration and at all sampling sites were indene (1,2,3-c,d) pyrene, dibenz(a,h)anthracene and acenaphthylene. The sites with the greatest quantity of PAHs (considering all 16 together) were SS1 (24.66 ng L⁻¹) and SS2 (27.8 ng L⁻¹), corresponding to the drainage point from the town of Nuevo Madín and the side branch of the reservoir. Even so, the total concentrations were not above those reported for the Seine River (France, 20 ng L⁻¹) or Slave River (Canada, 29 ng L⁻¹) (Fernández *et al.*, 1997; McCarthy *et al.*, 1997). The source of PAHs is related to the activities in the nearby neighborhoods (to the reservoir) that could account for this type of pollutant: open garbage dumps, sporadic burning of garbage by inhabitants and domestic waste. Also, expanded plastic and polystyrene on the shores of the MR and in the water can break into fragments and release some PAHs upon exposure to UV radiation (Ríos *et al.*, 2007).

Concerning OCPs, DDT (dichloro-diphenyl-trichloroethane) and several of its metabolites were encountered in the MR. The OCP with the highest level was 4,4 DDE, followed by 2,4 DDE and

lindane. In the Niger River a maximum concentration of 421.3 ng L⁻¹ for DDT and 180.2 ng L⁻¹ for lindane were found (Unyimadu *et al.*, 2018). The concentrations in the MR are below those of the Niger River.

The presence, concentration and spatial distribution of pollutants in a water body depends on the frequency and amount used, their physicochemical characteristics and abiotic components of the exposed ecosystem (water and sediment), among others. In the MR we find not only organic pollutants, but a complex mixture of xenobiotics that can damage the hydrobions that inhabit it, such as the common carp, being the early stages of development the most susceptible to damage.

3.2.1 Determination of embryotoxicity and teratogenesis

Embryotoxicity was expressed as modifications in embryonic development (Fig. 2), embryoletality and teratogenicity (Table 2). Whereas the water sample from SS1 caused 100% mortality in *C. carpio* embryos, water from SS2 resulted in 56.6% mortality, SS3 51.6%, SS4 46.6% and SS5 43.3%. Unlike the other sites, CFP, BP2, OD-PABA and MeBZT were found in the SS1 site. In addition, there were quantified the highest levels of metformin, penicillin V, lindane, fluorene, phenanthrene, fluoranthene and pyrene.

The assessment of embryonic development was carried out with the surviving embryos. Compared to the control embryos, exposure to the water samples from sites SS2, SS3 and SS4 led to a significant decrease in the score. The embryonic development of the embryos exposed to water from the SS5 site was significantly higher at both 72 and 96 h compared to the score of the other sites. However, all the embryos exposed to MR water presented a delay in the time of hatching and

a delay in the pigmentation of the eyes, head and body. Water samples from all sites induced teratogenicity in *C. carpio* (Table 2). SS3 and SS4 produced the greatest percentage of embryonic malformations (55.1 and 53.1 %, respectively), mainly yolk sac edema and pericardial edema. Despite the same type of malformations was found in the group of embryos exposed to water from the SS5 site, the percentage was close to half of that produced by water from sites SS3 and SS4. Although the contaminants found in the sites SS2, SS3, SS4 and SS5 were quite similar, lower concentrations of PCB, phenanthrene and metformin were found.

Several authors have studied the embryotoxicity and teratogenicity of organophosphate pesticides in aquatic species such as diazinon found in MR. Hamm and Hinton (2000) showed that the most common response in *Oryzias latipes* fish exposed to diazinon is the appearance of yolk sac and pericardial edema, malformations related to the development of the endothelium and the pericardial cavity. These effects were found in common carp embryos exposed to water at all MR sampling sites.

PCPs produced neurotoxicity and endocrine disruption, and thus a negative effect on development and reproduction (Araújo *et al.*, 2018). BP2 (at 9.85 mg L⁻¹) promotes the accumulation of lipids in the yolk sac, facial malformations, and harmful effects on the process of segmentation and blood circulation of *Danio rerio* (Fong *et al.*, 2016). At concentrations of 0.447-599 mg L⁻¹, 4-MBC reduces the rate of hatching of *Solea senegalensis* as well as mortality, malformations and diminished growth (Araújo *et al.*, 2018).

Regarding pharmaceutical drugs, it has been reported that hypoglycemic agents like metformin and glibenclamide cause only slight embryotoxicity in mammals, but the effect on aquatic species has

not been evaluated. On the other hand, acetaminophen at a concentration of 1-100 mg L⁻¹ has been associated with embryotoxicity in *Danio rerio*, in which deterioration of early development, hatching, organogenesis, larval growth, tail formation and pigmentation was found (David & Pancharatna, 2009). Pérez-Álvarez *et al.* (2018) discovered that a hospital effluent contained glibenclamide (1.92 µg L⁻¹), metformin (1.31 µg L⁻¹), penicillin G (3.77 µg L⁻¹) and acetaminophen (2.66 µg L⁻¹), inducing growth inhibition, microcephalia, facial and pericardial edema, eye malformations, and damage to the notochord, tail, fin and intestine of *Xenopus laevis* and *Lithobates catesbeianus*.

Finally, several POPs were found in MR, including some organochlorine insecticides such as hexachlorobenzene, lindane, DDT and its metabolites. Oliva *et al.* (2008) found that lindane produces myoskeletal defects, skin opacity, exophthalmia, and depigmentation in *S. aurata*. Pawar and Katdare (1984) demonstrated that hexachlorobenzene provokes distention of body cavities, curvature of the body axis, poor blood circulation, retarded growth, and poor pigmentation for *M. ornate*, evidencing its embryotoxicity and teratogenicity for aquatic invertebrates (Pašková *et al.*, 2011).

PCBs were another type of POP detected in the MR. After Blanc *et al.* (2017) exposed *Danio rerio* embryos to 7.5 µg L⁻¹ of PCB126, there was a boost in the expression of genes related to oxidative stress (*gpx1a* and *tp53*) and the biotransformation of xenobiotics (*ahr2* and *cyp1a*). The toxicity of this compound increased when the embryos were exposed to a mixture of perfluorooctane sulfonic acid and perfluorohexanoic acid, demonstrating the importance of the interactions between POPs. Because of containing a complex mixture of pollutants, the water of the MR probably produces

interactions that substantially modify the toxic response, particularly in organisms at early stages of development.

A wide range of PAHs were detected in the MR samples. These compounds have been well studied. For instance, Xie *et al.* (2017) exposed *Crassostrea gigas* larvae to benzo(a)pyrene, determining the effect on embryogenesis and survival with an EC₅₀ of 18.4 µg L⁻¹ and an LC₅₀ of 26.8 µg L⁻¹. They reported that this xenobiotic induces the breakage of DNA chains, leading to abnormalities and in some cases death. Le Bihanic *et al.* (2014) assessed the effect of a complex mixture of PAHs, extracted from the sediment of the Seine estuary (France) on embryos of Medaka, showing that PAHs produce a delay in hatching, developmental abnormalities and DNA damage. They pointed out the potential risk of exposure during the early stages of development to mixtures of PAHs. Although the mechanisms that affect development are still not completely clear, they include the inhibition of enzymes, damage to proteins, lipids and genetic material, as well as alterations in membranes, cell energy supply and signaling of retinoic acid. ROS play an important role in these alterations due to their participation in signaling processes (Pašková, 2011) and in the production of oxidative stress.

3.2.2 Determination of antioxidant defenses and oxidative stress parameters

Antioxidant defenses and oxidative stress resulted from tests with embryos exposed to water from all sites except SS1, as the organisms exposed to water from the latter site died in less than 12 h.

Data related to antioxidant defense (Fig. 4) demonstrate important modifications in the response of the embryos exposed to water samples from the four sites and at almost all times of exposure

(versus the control, $p \leq 0.05$). Regarding SOD, the increase in its activity was greater at sites SS2 and SS3 at 12 and 24 h. The activity of CAT was raised practically in all the groups and exposure times. Unlike CAT and SOD, the activity of GPx presented a similar behavior to the control group during the exposure times up to 72 h, but at 96 h of exposure to water from SS4 and SS5 the relative value of this parameter increased. Each of the samples generated a different level of antioxidant response, probably because of the distinct type and concentration of pollutants.

Oxidative damage is observed in figure 5. All parameters (LPX, PCC and HPC) were notably higher for the embryos exposed to the water from SS2, SS3, SS4 and SS5 (at all exposure times) compared to those of the control group ($p \leq 0.05$). The greatest damage to biomolecules was generated by the SS3 water sample to lipids (LPX: 6416.12 % at 48 h, HPC: 22293.1 % at 72 h) and the SS2 sample to proteins (8642.38% at 24 h).

When the production of ROS is increased, the activity of antioxidant enzymes is intensified in order to reestablish redox balance and health of the individual. The failure to reestablish this balance causes oxidative damage to biomolecules, compromising the ability of the organism to survive (Valavanidis et al; 2006). In this case, despite the increase in the activity of antioxidant enzymes in specific groups and times, oxidative damage occurred in all embryos exposed to MR water.

A capacity to promote oxidative stress has been shown by many environmental pollutants, including metals, pesticides, emerging pollutants (pharmaceutical drugs and PCPs) and POPs (Lushchak, 2011), including those detected in MR. Diazinon produce oxidative stress in *O. niloticus* (Toledo-Ibarra *et al.*, 2016) and *C. carpio* (Oru $\square$ & Usta, 2007). The rupture in neuronal and endocrine signaling, produced because of the inhibition of acetylcholinesterase, leads to an

intracellular flow of Ca^{2+} , triggering the activation of proteolytic enzymes and nitric oxide synthase as well as the generation of free radicals, thus promoting oxidative stress (Lushchak, 2011).

The PCPs identified in the MR (BP2, OD-PABA, 4-MBC and MeBZT) are also capable of stimulating oxidative stress. Gao *et al.* (2013) demonstrated that $1\ \mu\text{g L}^{-1}$ of BP3 and 4-MBC increased the activity of CAT in *T. thermophila*, while Liu *et al.* (2015) established that 0.48 and $4.78\ \text{mg L}^{-1}$ of BP2 produced a similar result in the liver of *Carassius auratus*. Ma *et al.* (2017) revealed that OD-PABA enhanced the activity of SOD and CAT in *C. auratus*. PCPs are biotransformed by phase I reactions (Le Fol *et al.*, 2015) or undergo reactions by means of abiotic transformations like photolysis (Calza *et al.*, 2016), giving rise to reactive metabolites and ROS, and consequently an imbalance in redox.

Many pharmaceutical products are able to cause oxidative stress through their respective processes of biotransformation, which can afford reactive metabolites and ROS. Such is the case of acetaminophen found in MR in *C. carpio* (Nava-Álvarez *et al.*, 2014). Other drugs detected in the MR are glibenclamide and metformin. Martínez-Viveros *et al.* (2018) demonstrated that glibenclamide (at 10, 100 and $1000\ \text{ng L}^{-1}$) promotes oxidative stress in *C. carpio*, increasing SOD and CAT activity, protein oxidation and LPX activity. The overproduction of ROS is related to its biotransformation process, which furnishes oxy-cytochrome P450 ($\text{O}_2\text{-P450-Fe}^{2+}\text{-GLD}$) by means of phase I reactions. The resulting rapid decomposition of glibenclamide leads to the release of the superoxide radical (Krest *et al.*, 2013). In the case of metformin, Adaramoye *et al.* (2012) found that this biguanide stimulates oxidative stress, necrosis and degeneration of the seminiferous tubules in Wistar rats, particularly when administered along with glibenclamide. Hence, it possibly brings about a redox imbalance in aquatic species (e.g., *C. carpio*). Even considering that

metformin is very stable and is practically eliminated in an unaltered form by the renal pathway, a part of it is biotransformed by aerobic bacteria to guanylurea (Kosma *et al.*, 2015) and nitrogenated compounds that can trigger oxidative stress (Nimptsch & Pflugmacher, 2007). The latter compounds may then exist in the MR. Also observed in the MR samples were two β -lactam antibiotics, penicillin V and G. There is no direct evidence that penicillin V and G promote oxidative stress in an organism. However, Elizalde-Velázquez *et al.* (2017) reported that another β -lactam antibiotic (amoxicillin) give rise to oxidative stress in *C. carpio*. On the other hand, Havelkova *et al.* (2016) showed low toxicity of penicillin G for *Daphnia magna* (EC_{50} = 1496.9 mg L⁻¹) and *Pseudokirchneriella subcapitata* (EC_{50} = 7114 mg L⁻¹).

The POPs found in the MR are organochlorine insecticides such as hexachlorobenzene, DDT (and its metabolites) and lindane, as well as PCBs and PAHs. POPs generate ROS during their biotransformation by cytochrome P450, and therefore induce oxidative stress (Lushchak, 2011). After exposing mussels (*Perna viridis*) to 10 μ g L⁻¹ DDT, Song *et al.* (2016) and Jiang *et al.* (2017) detected an overexpression of genes related to the synthesis of proteins associated with oxidative stress and detoxification. Di *et al.* (2016) examined oxidative stress produced by isomers of hexachlorocyclohexane on *Tubifex*, discovering that they bioaccumulate in the organism and increase SOD activity and LPX.

According to Leitão *et al.* (2003), PCBs trigger oxidative stress in the marine dinoflagellate *Lingulodinium polyedra*. They showed that arochlor 1254 (a mixture of approximately 70 different PCBs) at concentrations of 120 μ g L⁻¹ brings about a boost of 121% in protein oxidation and 146% in SOD activity. This is attributed to an adaptation mechanism to compensate for damage to proteins.

Among the PAHs occurring in the MR were acenaphthylene, dibenz (a,h) anthracene and indene (1,2,3-c,d) pyrene. Song *et al.* (2016) evaluated the effect of benzo(a)pyrene, on *Perna viridis*, reporting that induces cellular apoptosis and changes in energetic metabolism. When in the presence of DDT, it also affects proteins associated with oxidative stress. Guo *et al.* (2017) discovered that benzo(a)pyrene in a mixture with chrysene (0.1 and 1 $\mu\text{g L}^{-1}$) increased the levels of SOD, CAT and GPx, as well as protein oxidation and LPX activity in *Chlamys farreri*.

All POPs identified in the MR have the proven capacity to promote oxidative stress in diverse aquatic species. Thus, they may contribute to the effect observed in the embryos of common carp exposed to MR water. Additionally, they are known to be hormone disruptors, giving them the capacity to alter endocrine and reproductive functions in aquatic organisms and reduce reproduction capability of adults and limit the growth and survival of offspring (El-Shahawi *et al.*, 2010).

Table 3 presents the positive correlations observed at 96 h between concentrations of contaminants found at most sampling sites and biomarkers of oxidative stress and embryotoxicity. It can be seen that oxidative stress biomarkers have a strong association with different types of contaminants, e.g. metformin, PCB 170, phenanthrene and benz(a)anthracene. For IT contaminants such as diazinon, glibenclamine, benz(a)anthracene and dibenz(a,h) anthracene showed a high correlation coefficient. This result highlights the strong association of toxicity with the presence of important environmental pollutants.

4. Conclusions

The organic compounds herein detected (pesticides, PCPs, pharmaceutical products and POPs), did not exceed those found in other water bodies in the world. These xenobiotics, as well as other pollutants in the MR water, such as metals previously found (González-González *et al.*, 2014), undergo biotic and abiotic transformations or act as oxidizing agents, promoting reactive metabolites that trigger a redox imbalance (Beyer et al., 2014), and consequently oxidative damage and the embryotoxicity observed in *C. carpio*. Thus, probably the survival of the species is compromised in the reservoir, and with it, the balance of the ecosystem.

Conflict of interest

The authors declare that they have no current or potential competing financial interests.

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5. References

Adaramoye O., Akanni O., Adesanoye O., Labo-Popoola O., Olaremi O. (2012). Evaluation of

529 toxic effects of metformin hydrochloride and glibenclamide on some organs of male rats. *Niger J.*
530 *Physiol. Sci.*, 27: 137-144.

531 Araújo M.J., Rocha R.J.M., Soares A.M.V.M., Benedé J.L., Chisvert A., Monteiro M.S. (2018).
532 Effects of UV filter 4-methylbenzylidene camphor during early development of *Solea senegalensis*
533 Kaup, 1858. *Sci. Total Environ.*, 628-629: 1395-1404.
534 <https://doi.org/10.1016/j.scitotenv.2018.02.112>.

535 Beyer J., Petersen K., Song Y., Ruus A., Grung M., Bakke T., Tollefsen K.E., (2014).
536 Environmental risk assessment of combined effects in aquatic ecotoxicology: A discussion paper.
537 *Mar. Environ. Res.*, 96: 81-91. <https://doi.org/10.1016/j.marenvres.2013.10.008>.

538 Blanc M., Kärman A., Kukucka P., Scherbak N., Keiter S., (2017). Mixture-specific gene
539 expression in zebrafish (*Danio rerio*) embryos exposed to perfluorooctane sulfonic acid (PFOS),
540 perfluorohexanoic acid (PFHxA) and 3,3', 4,4'- pentachlorobiphenyl (PCB126). *Sci. Total*
541 *Environ.*, 590-591: 249-257. <http://dx.doi.org/10.1016/j.scitotenv.2017.02.232>.

542 Bradford M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities
543 of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254.
544 [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).

545 Buege, J.A., Aust, S.D., (1978). Microsomal lipid peroxidation. *Methods Enzymol.*, 52: 302–10.
546 [https://doi.org/10.1016/S0076-6879\(78\)52032-6](https://doi.org/10.1016/S0076-6879(78)52032-6).

547 Calza P., Vione D., Galli F., Fabbri D., Del Bello F., Medana C., (2016). Study of the
548 photochemical transformation of 2-ethylhexyl 4-(dimethylamino) benzoate (OD-PABA) under
549 conditions relevant to surface waters. *Water Res.*, 88: 235-244.
550 <http://dx.doi.org/10.1016/j.watres.2015.10.015>.

551 Cardoso-Vera D.J., Islas-Flores H., SanJuan-Reyes N., Montero-Castro E. I., Galar-Martínez M.,
552 García-Medina S., Elizalde-Velázquez A., Dublán-García O., Gómez-Oliván L.M. (2017).

553 Comparative study of diclofenac-induced embryotoxicity and teratogenesis in *Xenopus*
554 *laevis* and *Lithobates catesbeianus*, using the frog embryo teratogenesis assay: *Xenopus* (FETAX)
555 *Sci. Total Environ.*, 574: 467-475. <https://doi.org/10.1016/j.scitotenv.2016.09.095>.

556 Ccanccapa A., Masiá A., Navarro-Ortega A., Picó Y., Barceló D., (2016). Pesticides in the Ebro
557 River basin: Occurrence and risk assessment. *Environ. Pollut.*, 211; 414-424.
558 <http://dx.doi.org/10.1016/j.envpol.2015.12.059>.

559 David A., Pancharatna K., (2009). Effects of acetaminophen (paracetamol) in the embryonic
560 development of zebrafish, *Danio rerio*. *J. Appl. Toxicol.*, 29: 597-602. DOI.10.1002/jat.1446.

561 Destrieux D., Laurent F., Budzinski H., Pedelucq J., Vervier P., Gerino M., (2017). Drug residues
562 in urban water: A database for ecotoxicological risk management. *Sci. Total Environ.* 609: 927-
563 941. <http://dx.doi.org/10.1016/j.scitotenv.2017.07.043>.

564 Devlin E., (2006). Acute toxicity, uptake and histopathology of aqueous methyl mercury to fethead
565 minnow embryos. *Ecotoxicology*, 15: 97-110.

566 Di S., Zhang W., Chen L., Zhou Z., Diao J., (2016). Toxokinetics and oxidative stress in *Tubifex*
567 *tubifex* exposed to hexachlorocyclohexane isomers. *RSC Adv.*, 6: 19016-19024.
568 DOI:10.1039/c5ra26207k.

569 Elizalde-Velázquez, A., Martínez-Rodríguez, H., Galar-Martínez, M., Dublán-García, O., Islas-
570 Flores, H., Rodríguez-Flores, J., Castañeda-Peñalvo, G., Lizcano-Sanz, I. and Gómez-Oliván, L.
571 M., (2017). Effect of amoxicillin exposure on brain, gill, liver, and kidney of common carp
572 (*Cyprinus carpio*): The role of amoxicilloic acid. *Environ. Toxicol.*, 32: 1102–1120.
573 doi:10.1002/tox.22307.

574 El-Shahawi M.S., Hamza A., Bashammakh A.S., Al-Saggaf W.T., (2010). An overview on the
575 accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of

576 persistent organic pollutants. *Talanta*, 80: 1587-1597. doi:10.1016/j.talanta.2009.09.055.

577 Estrada-Arriaga E.B., Cortés-Muñoz J.E., González-Herrera A., Calderón-Mólgora C.G., Rivera-
578 Huerta M.L., Ramírez-Camperos E., Montellano-Palacios L., Gelover-Santiago S.L., Pérez-
579 Castrejón S., Cardoso-Vigueros L., Martín-Domínguez A., García-Sánchez L., (2016). Assessment
580 of full-scale biological nutrient removal systems upgraded with physico-chemical processes for the
581 removal of emerging pollutants present in wastewaters from Mexico. *Sci. Total Environ.*,
582 571:1172-1182. <http://dx.doi.org/10.1016/j.scitotenv.2016.07.118>.

583 Factor C., de Chavez E.R. (2012). Toxicity of Arsenic, Aluminum, Chromium and Nickel to the
584 Embryos of the Freshwater Snail, *Radix quadrasi* von Möellendorf 1898. *Philipp. J. Sci.*, 141: 207-
585 216.

586 Fernández M.B., Sicre M.A., Boireau A., (1997). Polyaromatic hydrocarbon (PAH) distributions
587 in the Siene River and its estuary. *Mar. Pollut. Bull.*, 34: 857-867. [https://doi.org/10.1016/S0025-](https://doi.org/10.1016/S0025-326X(97)00063-5)
588 326X(97)00063-5.

589 Fong H.C.H., Ho J.C.H., Cheung A.H.Y., Lai K.P., Tse W.K.F., (2016). Developmental toxicity
590 of the common UV filter, benzophenone-2 in zebrafish embryos. *Chemosphere*, 164: 413-420.
591 <http://dx.doi.org/10.1016/j.chemosphere.2016.08.073>.

592 Gago-Ferrero P., Mastroianni N., Díaz-Cruz S.M., Barceló D., (2013). Fully automated
593 determination of nine ultraviolet filters and transformation products in natural waters and
594 wastewaters by on-line solid phase extraction–liquid chromatography–tandem mass spectrometry.
595 *J. Chromatogr. A.*, 1294: 106-116. <https://doi.org/10.1016/j.chroma.2013.04.037>

596 Gakuba E., Moodley B., Ndungu P., Birungi G., (2015). Occurrence and significance of
597 polychlorinated biphenyls in water, sediment pore water and surface sediments of Umgeni River,
598 KwaZulu-Natal, South Africa. *Environ. Monit. Assess.*, 187:568. [http://doi.org/10.1007/s10661-](http://doi.org/10.1007/s10661-015-4790-1)
599 015-4790-1.

600 Galar-Martínez M., Gómez-Oliván L.M., Amaya-Chávez A., Razo-Estrada, C., García-Medina S.,
601 (2010). Oxidative stress induced on *Cyprinus carpio* by pollutants present in the water and
602 sediment of Madín reservoir. *J. Environ. Sci. Health. Part A.* 45, 155-160.

603 Gao L., Yuan T., Zhou C., Cheng P., Bai Q., Ao J., Wang W., Zhang H., (2013). Effects of four
604 commonly used UV filters on the growth, cell viability and oxidative stress responses of the
605 *Tetrahymena thermophile*. *Chemosphere*, 93:2507-2513.
606 <http://dx.doi.org/10.1016/j.chemosphere.2013.09.041>.

607 Gómez-Oliván L.M., Neri-Cruz N., Galar-Martínez M., Islas-Flores H., García-Medina S., (2014).
608 Binary mixtures of diclofenac with paracetamol, ibuprofen, naproxen and acetylsalicylic acid and
609 these pharmaceuticals in isolated form induce oxidative stress on *Hyalella azteca*. *Environ. Monit.*
610 *Assess.*, 186:7259-7271, DOI 10.1007/s10661-014-3925-0.

611 Gómez-Oliván L.M., Neri-Cruz N., Galar-Martínez M., Vieyra-Reyes P., García-Medina S.,
612 (2012). Assessing the oxidative stress induced by paracetamol spiked in artificial sediment on
613 *Hyalella azteca*. *Water Air Soil Pollut.*, 223(8): 5097-5104. [https://doi.org/10.1007/s11270-012-](https://doi.org/10.1007/s11270-012-1261-y)
614 [1261-y](https://doi.org/10.1007/s11270-012-1261-y).

615 González-González E.D., Gómez-Oliván L.M., Galar-Martínez M., Vieyra-Reyes P., Islas-Flores
616 H., García-Medina S., Jiménez-Vargas J.M, Razo-Estrada A.C., Pérez-Pasten B.R., (2014). Metals
617 and nonsteroidal anti-inflammatory pharmaceuticals drugs present in water from Madín Reservoir
618 (Mexico) induce oxidative stress in gill, blood and muscle of common carp (*Cyprinus*
619 *carpio*). *Arch. Environ. Contam. Toxicol.*, 67:281–295. [https://doi.org/10.1007/s00244-014-](https://doi.org/10.1007/s00244-014-0048-0)
620 [0048-0](https://doi.org/10.1007/s00244-014-0048-0).

621 Guo R., Pan L., Lin P., Zheng L., (2017). The detoxification responses, damage effects and
622 bioaccumulation in the scallop *Chlamys farreri* exposed to single and mixtures of benzo(a)pyrene
623 and chrysene. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, 191: 36-51.

624 <http://dx.doi.org/10.1016/j.cbpc.2016.09.004>.

625 Hamm J.T., Hinton D.E., (2000). The role of development and duration of exposure to the
 626 embryotoxicity of diazinon. *Aquat. Toxicol.*, 48: 403-418. [https://doi.org/10.1016/S0166-](https://doi.org/10.1016/S0166-445X(99)00065-X)
 627 445X(99)00065-X.

628 Havelkova B., Beklova M., Kovacova V., Hlavkova D., Pikula J., (2016). Ecotoxicity of selected
 629 antibiotics for organisms of aquatic and terrestrial ecosystems. *Neuro Endocrinol. Lett.*, 37: 38-44.
 630 <https://doi.org/10.1074/jbc.R113.473108>.

631 Hermesen S.A.B., van der Brandhof E.J., van der Ven L.T.M., Piersma A.H., (2011). Relative
 632 embryotoxicity of two classes of chemicals in a modified zebrafish embryotoxicity test and
 633 comparison with their *in vivo* potencies. *Toxicol. In Vitro*, 25: 745-753.
 634 [doi:10.1016/j.tiv.2011.01.005](https://doi.org/10.1016/j.tiv.2011.01.005).

635 Jiang Z.Y., Hunt J.V., Wolff S.P., (1992). Ferrous ion oxidation in the presence of xylenol orange
 636 for detection of lipid hydroperoxide in low density lipoprotein. *Anal. Biochem.*, 202: 384-389.
 637 [https://doi.org/10.1016/0003-2697\(92\)90122-N](https://doi.org/10.1016/0003-2697(92)90122-N).

638 Jiang X., Tang T., Zhao H., Song Q., Zhou H., Han Q., Diao X., (2017). Differential gene responses
 639 in the embryo of the green mussel *Perna viridis* exposed to dichlorodiphenyltrichloroethane
 640 (DDT). *Toxicol. Res.*, 6: 477-486. DOI:10.1039/c7tx00087a.

641 Kimmel B.C., Ballard W.W., Kimmel S.R., Ullmann B., Schilling F.T., (1995). Stages of
 642 embryonic development of the zebrafish. *Dev. Dyn.*, 203: 253-310.
 643 <https://doi.org/10.1002/aja.1002030302>

644 Köck-Schulmeyer M., Ginebreda A., González S., Cortina J. L., de Alda M. L., Barceló D., (2012).
 645 Analysis of the occurrence and risk assessment of polar pesticides in the Llobregat River Basin
 646 (NE Spain). *Chemosphere*, 86: 8-16. <https://doi.org/10.1016/j.chemosphere.2011.08.034>.

647 Köck-Schulmeyer M., Villagrasa M., de Alda M. L., Céspedes-Sánchez R., Ventura F., Barceló
648 D., (2013). Occurrence and behavior of pesticides in wastewater treatment plants and their
649 environmental impact. *Sci. Total Environ.*, 458:466-476.
650 <https://doi.org/10.1016/j.scitotenv.2013.04.010>.

651 Kong X., Wang S., Jiang H., Nie G., Li X. (2012). Responses of acid/alkaline phosphatase,
652 lysozyme, and catalase activities and lipid peroxidation to mercury exposure during the embryonic
653 development of goldfish *Carassius auratus*. *Aquat. Toxicol.*, 120-121: 119-125.
654 <https://doi.org/10.1016/j.aquatox.2012.05.005>

655 Kosma C.I., Lambropoulou A.D., Albanis T.A., (2015). Comprehensive study of the antidiabetic
656 drug metformin and its transformation product guanylurea in Greek wastewaters. *Water Res.*, 70:
657 436-448. <http://dx.doi.org/10.1016/j.watres.2014.12.010>.

658 Krest, C.M., Onderko, E.L., Yosca, T.H., Calixto, J.C., Karp, R.F., Livada, J., (2013). Reactive
659 intermediates in cytochrome P450 catalysis. *J. Biol. Chem.*, 288, 17074-170781. Doi:
660 10.1074/jbc.R113.473108.

661 Lacorte S., Guiffard I., Fraisse D., Damià Barceló D., (2000). Broad Spectrum Analysis of 109
662 Priority Compounds Listed in the 76/464/CEE Council Directive Using Solid-Phase Extraction and
663 GC/EI/MS. *Anal.Chem.*, 72: 1430-1440. DOI: 10.1021/ac991080w.

664 Le Bihanic F., Clérandeau C., Le Menach K., Morin B., Budzinski H., Cousin X., Cachot J., (2014).
665 Developmental toxicity of PAH mixtures in fish early stages. Part II: adverse effects in Japanese
666 medaka. *Environ. Sci. Pollut. Res. Int.*, 21: 13732-13743. [https://doi.org/10.1007/s11356-014-](https://doi.org/10.1007/s11356-014-2676-3)
667 2676-3.

668 Le Fol V., Aït-Aïssa S., Cabaton N., Dolo L., Grimaldi M., Balaguer P., Perdu E., Debrauwer L.,
669 Brion F., Zalko D. (2015). Cell-specific biotransformation of benzophenone-2 and bisphenol-S in

zebrafish and human in vitro models used for toxicity and estrogenicity screening. *Environ. Sci. Technol. Lett.*, 49: 3860-3868. DOI:10.1021/es505302c.

Leitão M.A. da S., Cardozo K.H.M., Pinto E., Colepicolo P., (2003). PCB-induced oxidative stress in the unicellular marine dinoflagellate *Lingulodinium polyedrum*. *Arch. Environ. Contam. Toxicol.*, 45: 59-65. DOI: 10.1007/s00244-002-0208-5.

Levine, R.L., Williams, J.A., Stadtman, E.R., Shacter, E., (1994). Carbonyl assays for determination of oxidatively modified proteins. *Methods Enzymol.* 233,346–57. [https://doi.org/10.1016/S0076-6879\(94\)33040-9](https://doi.org/10.1016/S0076-6879(94)33040-9).

Liu H., Sun P., Ki H., Yang S., Wang L., Wang Z., (2015). Hepatic oxidative stress biomarker responses in freshwater fish *Carassius auratus* exposed to four benzophenone UV filters. *Ecotoxicol. Environ. Saf.*, 119: 116-122. <http://dx.doi.org/10.1016/j.ecoenv.2015.05.017>.

López-Serna, R., Pérez, S., Ginebreda, A., Petrović, M., Barceló, D., (2010). Fully automated determination of 74 pharmaceuticals in environmental and waste waters by online solid phase extraction–liquid chromatography–electrospray–tandem mass spectrometry. *Talanta*, 83: 410-424. <https://doi.org/10.1016/j.talanta.2010.09.046>.

Ma B., Lu G., Liu J., Yan Z., Yang H., Pan T., (2017). Bioconcentration and multi-biomarkers of organic UV filters (BM-DBM and OD-PABA) in crucian carp. *Ecotoxicol. Environ. Saf.*, 141: 178-187. <http://dx.doi.org/10.1016/j.ecoenv.2017.03.034>.

Mandaric L., Diamantini E., Stella E., Cano-Paoli K., Valle-Sistac J., Molins-Delgado D., Bellin A., Chiogna G., Majone B., Díaz-Cruz M.S., Sabater S., Barcelo D., Petrovic M., (2017). Contamination sources and distribution patterns of pharmaceuticals and personal care products in Alpine rivers strongly affected by tourism. *Sci. Total Environ.*, 590-591: 484-494. <http://dx.doi.org/10.1016/j.scitotenv.2017.02.185>.

693 Mao F., You L., Reinhard M., He Y., Yew-Hoong Gin K., (2018). Occurrence and fate of
 694 benzophenone-type UV filters in a tropical urban watershed. *Environ. Sci. Technol. Lett.*, 52: 3960-
 695 3967. DOI:10.1021/acs.est.7b05634.

696 Martínez-Viveros E.M.G., Islas-Flores H., Dublán-García O., Galar-Martínez M., SanJuan-Reyes
 697 N., García-Medina S., Hernández-Navarro M.D., Gómez-Oliván L.M., (2018). Environmentally
 698 relevant concentrations of glibenclamide induce oxidative stress in common carp (*Cyprinus*
 699 *carpio*). *Chemosphere*, 197: 105-116. <https://doi.org/10.1016/j.chemosphere.2018.01.020>.

700 McCarthy L.H., Williams G.R., Stephens J., (1997). Baseline studies in the Slave river, NWR
 701 1990-1994: Part I. Evaluation of the chemical quality of water and suspended sediment from Slave
 702 river (NWT). *Sci. Total Environ.*, 197: 21-53.

703 Nava-Álvarez R., Razo-Estrada A. C., García-Medina S., Gómez-Oliván L. M., Galar-Martínez
 704 M., (2014). Oxidative stress induced by mixture of diclofenac and acetaminophen on common carp
 705 (*Cyprinus carpio*). *Water Air Soil Pollut.*, 225(2): DOI: 10.1007/s11270-014-1873-5.

706 Nimptsch J., Pflugmacher S., (2007). Ammonia triggers the promotion of oxidative stress in the
 707 aquatic macrophyte *Myriophyllum mattogrossense*. *Chemosphere*, 66: 708-714.
 708 doi:10.1016/j.chemosphere.2006.07.064.

709 Oliva M., Garrido C., Sales D., González de Canales M.L., (2008). Lindane toxicity on early life
 710 stages of gilthead seabream (*Sparus aurata*) with a note on its histopathological manifestations.
 711 *Environ. Toxicol. Pharmacol.*, 25(1):94–102. <https://doi.org/10.1016/j.etap.2007.09.005>.

712 Oru O.E., Usta E., (2007). Evaluation of oxidative stress responses and neurotoxicity potential
 713 of diazinon in different tissues of *Cyprinus carpio*. *Environ. Toxicol. Pharmacol.*, 23: 48-55.
 714 doi:10.1016/j.etap.2006.06.005.

715 Pašková V., Hilscherová K., Bláha L., (2011). Teratogenicity and Embryotoxicity in Aquatic
 716 Organisms After Pesticide Exposure and the Role of Oxidative Stress. In: Whitacre D. (eds)

717 Reviews of Environmental Contamination and Toxicology Volume 211. *Rev. Environ. Contam.*
718 *Toxicol.*, (Continuation of Residue Reviews), Springer, New York, NY.
719 https://doi.org/10.1007/978-1-4419-8011-3_2.

720 Prášková E., Štěpánová S., Chromcová L., Plhalová L., Voslářová E., Pištěková V., Prokeš M.,
721 Svobodová Z. (2013). The effects of subchronic exposure to ketoprofen on early developmental
722 stages of common carp. *Acta Vet. Brno.* 82: 343-347.
723 <https://doi.org/10.2754/avb201382030343>

724 Pawar K.R., Katdare M., (1984). Toxic and teratogenic effects of fenitrothion, BHC and
725 carbofuran on embryonic development of the frog *Microhyla ornata*. *Toxicol. Lett.* 22:7–13.
726 [https://doi.org/10.1016/0378-4274\(84\)90038-9](https://doi.org/10.1016/0378-4274(84)90038-9).

727 Pérez-Álvarez I., Islas-Flores H., Gómez-Oliván L.M., Barceló D., López De Alda M., Pérez
728 Solsona S., Sánchez-Aceves L., SanJuan-Reyes N., Galar-Martínez M., (2018). Determination of
729 metals and pharmaceutical compounds released in hospital wastewater from Toluca, México, and
730 evaluation of their toxic impact. *Environ. Pollut.*, 240: 330-341.
731 <http://doi.org/10.1016/j.envpol.2018.04.116>.

732 Pérez-Coyotl, I., Martínez-Vieyra, C., Galar-Martínez, M., Gómez-Oliván, L. M., García-Medina,
733 S., Islas-Flores, H., Pérez-Pasten Borja, R., Gasca-Pérez, E., Novoa-Luna, K. A., Dublán-García,
734 O., (2017). DNA damage and cytotoxicity induced on common carp by pollutants in water from
735 an urban reservoir. Madín reservoir, a case study. *Chemosphere*, 185: 789-797.
736 <http://dx.doi.org/10.1016/j.chemosphere.2017.07.072>.

737 Prášková E., Štěpánová S., Chromcová L., Plhalová L., Voslářová E., Pištěková V., Prokeš M.,
738 Svobodová Z., (2013). The effects of subchronic exposure to ketoprofen on early developmental
739 stages of common carp. *Acta Vet. Brno.*, 82: 343-347. <https://doi.org/10.2754/avb201382030343>.

740 Radi, R., Turrens, J.F., Chang, L.Y., Bush, K.M., Carpo, J.D., Freeman, B.A. (1991). Detection of
 741 catalase in rat heart mitochondria. *J. Biol. Chem.*, 266, 22028–34.

742 Ramírez-Bastida P. (2000). Aves de humedales en zonas urbanas del noroeste de la Ciudad de
 743 México. Tesis, Facultad de Ciencias, División de Posgrado, Universidad Nacional Autónoma de
 744 México, pp DOI: 10.13140/RG.2.2.13509.45289.

745 Ramos A.S., Correia A.T., Antunes S.C., Gonçalves F., Nunes B., (2014). Effect of
 746 acetaminophen exposure in *Oncorhynchus mykiss* gills and liver: Detoxification mechanisms,
 747 oxidative defence system and peroxidative damage. *Environ. Toxicol. Pharmacol.*, 37:1221-1228.
 748 <https://doi.org/10.1016/j.etap.2014.04.005>.

749 Rigaud C., Couillard C.M., Pellerin J., Légaré B., Gonzalez P., Hodson P.V. (2013). Relative
 750 potency of PCB126 to TCDD for sublethal embryotoxicity in the mummichog (*Fundulus*
 751 *heteroclitus*). *Aquat. Toxicol.*, 128-129: 203-214. <http://dx.doi.org/10.1016/j.aquatox.2012.12.013>.

752 Rios L.M., Moore Ch., Jones P.R., (2007). Persistent organic pollutants carried by synthetic
 753 polymers in the ocean environment. *Mar. Pollut. Bull.*, 54:1230-1237.
 754 [doi:10.1016/j.marpolbul.2007.03.022](http://dx.doi.org/10.1016/j.marpolbul.2007.03.022).

755 Santos L.H.M.L.M., Araújo A.N., Fachini A., Pena A., Delerue-Matos C., Montenegro
 756 M.C.B.S.M., (2010). Ecotoxicological aspects related to the presence of pharmaceuticals in the
 757 aquatic environment. *J. Hazard. Mater.*, 175: 45-95. [doi:10.1016/j.jhazmat.2009.10.100](http://dx.doi.org/10.1016/j.jhazmat.2009.10.100).

758 Scheurer M., Michel A., Brauch H.J., Ruck W., Sacher F., (2012). Occurrence and fate of the
 759 antidiabetic drug metformin and its metabolite guanyurea in the environment and during drinking
 760 water treatment. *Water Res.*, 46: 4790-4802. <http://dx.doi.org/10.1016/j.watres.2012.06.019>.

761 Serra-Roig M.P., Jurado A., Díaz-Cruz M.S., Vázquez-Suñé E., Pujades E., Barceló D. Occurrence,
 762 fate and risk assessment of personal care products in river-groundwater interface. *Sci. Total*

763 *Environ.* 568:829-837. doi: 10.1016/j.scitotenv.2016.06.006.

764 Smolowitz M.R., Schultz E.M., Stegeman J.J., (1992). Cytochrome P4501A induction in tissues,
 765 including olfactory epithelium, of topminnows (*Poeciliopsis* spp.) by waterborne benzo[a]pyrene.
 766 *Carcinogenesis*, 13: 2395–2402. <https://doi.org/10.1093/carcin/13.12.2395>

767 Song Q., Chen H., Li Y., Zhou H., Han Q., Diao X., (2016). Toxicological effects of
 768 benzo(a)pyrene, DDT and their mixture on the green mussel *Perna viridis* revealed by proteomic
 769 and metabolomics approaches. *Chemosphere*, 144: 214-224.
 770 <https://dx.doi.org/10.1016/j.chemosphere.2015.08.029>.

771 Stegeman J.J., (1989). Cytochrome P450 forms in fish: catalytic, immunological and sequence
 772 similarities. *Xenobiotica*, 19:1093–1110. DOI: 10.3109/00498258909043164

773 Stepanova S., Praskova E., Chromcova L., Plhalova L., Prokes M., Blahova J., Svobodova Z.,
 774 (2013). The effects of diclofenac on early life stages of common carp (*Cyprinus carpio*). *Environ.*
 775 *Toxicol. Pharmacol.*, 35:454-460. <https://doi.org/10.1016/j.etap.2012.09.011>.

776 Sylvie B.R., Pairault C., Vernet G., Boulekbache H., (1996). Effect of lindane on the
 777 ultrastructure of the liver of the rainbow trout, *Oncorhynchus mykiss*, sac-fry. *Chemosphere*,
 778 33(10):2065–2079. [https://doi.org/10.1016/0045-6535\(96\)00295-0](https://doi.org/10.1016/0045-6535(96)00295-0).

779 Toledo-Ibarra G.A., Díaz-Reséndiz K.J.G., Ventura-Ramón G.H., González-Jaime F., Vega-López
 780 A., Becerril-Villanueva E., Pavón L., Girón-Pérez M.I., (2016). Oxidative damage in gills and liver
 781 in Nile tilapia (*Oreochromis niloticus*) exposed to diazinon. *Comp. Biochem. Physiol. A*, 200: 3-8.
 782 <http://dx.doi.org/10.1016/j.cbpa.2016.05.007>.

783 Unyimadu J.P., Osibanjo O., Babayemi J.O., (2018). Selected persistent organic pollutants (POPs)
 784 in water of river Niger: occurrence and distribution. *Environ. Monit. Assess.*, 190:6.
 785 <http://doi.org/10.1007/s10661-017-6378-4>.

786 Valavanidis A., Vlahogianni T., Dassenakis M., Scoullos M., (2006). Molecular biomarkers of
 787 oxidative stress in aquatic organisms in relation to toxic environmental pollutants.
 788 *Ecotoxicol. Environ. Saf.*, 64: 178-189. doi:10.1016/j.ecoenv.2005.03.013.

789 Westerfield M., 2007. The zebrafish Book: A guide for the laboratory use of zebrafish (*Danio*
 790 *rerio*), 5th ed. University Oregon Press, Eugene, Oregon.

791 Wijnja H., Doherty J.J., Safie S.A., (2014). Changes in pesticide occurrence in suburban surface
 792 waters in Massachusetts, USA, 1999-2010. *Bull. Environ. Contam. Toxicol.*, 93: 228-232. DOI
 793 10.1007/s00128-014-1251-4.

794 Xie J., Yang D., Sun X., Cao R., Chen L., Wang Q., Li F., Wu H., Ji Ch. Cong M., Zhao J.,
 795 (2017). Individual and combined toxicities of benzo(a)pyrene and 2,2',4,4'-
 796 Tetrabromodiphenyl ether on early life stages of the pacific oyster, *Crassostrea gigas*. *Bull.*
 797 *Environ. Contam. Toxicol.*, 99: 582-588. DOI 10.1007/s00128-017-2164-9.

798 Zhang Z., Huang J., Yu G., Hong H., (2004). Occurrence of PAHs, PCBs and organochlorine
 799 pesticides in the Tonghui river of Beijing, China. *Environ. Pollut.*, 130: 249-261.
 800 doi:10.1016/j.envpol.2003.12.002.

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Highlights

1. The water of the Madin dam contains hydrocarbons of various types.
2. These hydrocarbons in combination with other contaminants from the site produce embryotoxicity in the common carp.
3. *Cyprinus carpio* is at risk in the Madín reservoir.

Madin reservoir

Polluted water from an urban reservoir (Madín dam, México) induces toxicity and oxidative stress in *Cyprinus carpio* embryos

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Abstract

The Madín Dam is a reservoir located in the municipalities of Naucalpan and Atizapán, in the metropolitan area adjacent to Mexico City. The reservoir supplies drinking water to nearby communities and provides an area for various recreational activities, including kayaking, sailing and carp fishing. Over time, the number of specimens of common carp has notably diminished in the reservoir, which receives direct domestic drainage from two towns as well as numerous neighborhoods along the Tlalnepantla River. Diverse studies have demonstrated that the pollutants in the water of the reservoir produce oxidative stress, genotoxicity and cytotoxicity in juvenile *Cyprinus carpio*, possibly explaining the reduction in the population of this species; however, it is necessary to assess whether these effects may also be occurring directly in the embryos. Hence, surface water samples were taken at five sites and pharmaceutical drugs, personal care products (especially sunscreens), organophosphate and organochlorine pesticides, and other persistent organic pollutants (e.g., polychlorinated biphenyls and polycyclic aromatic hydrocarbons) were identified. Embryos of *C. carpio* were exposed to the water samples to evaluate embryoletality, modifications in embryonic development, lipoperoxidation, the quantity of hydroperoxide and oxidized proteins, and antioxidant enzyme activity (superoxide dismutase, catalase and glutathione peroxidase). It was found that the polluted water of the Madín Dam gave rise to embryoletality, embryotoxicity, congenital abnormalities, and oxidative stress on the common carp embryos.

Capsule: The water of the Madín dam contains a complex mixture of pollutants, including hydrocarbons, which produce oxidative stress and embryotoxicity on *Cyprinus carpio*.

Keywords: *Cyprinus carpio*; embryoletality; embryotoxicity; teratogenicity; oxidative stress; organic pollutants; Madín reservoir.

1. Introduction

Freshwater bodies are frequently contaminated by anthropogenic chemical stressors, including metals, pesticides, emerging contaminants and others, resulting in significant modifications to the health of hydrobionts and ecosystems in general. This problem is of great importance for all aquatic ecosystems and for those close to urban localities that discharge directly into these reservoirs, such as the Madín Dam (MR) in Mexico.

The MR is located on the Tlalnepantla River between the municipalities of Naucalpan de Juárez and Atizapán de Zaragoza, in the State of Mexico. Drainage from different localities is dumped directly (without prior treatment) into the reservoir and the Tlalnepantla River. Nevertheless, MR is the source of drinking water for many neighborhoods in the surrounding municipalities (Pérez-Coyotl *et al.*, 2017). It is important to note that there has been evident environmental degradation of the reservoir, which is the habitat for diverse species of fish and birds.

Previous studies have shown that there are considerable concentrations of pollutants such as aluminum (Al, 6.04-24.45 mg L⁻¹) and iron (Fe, 1.51-5.10 mg L⁻¹), which exceed the maximum permissible concentration established to protect aquatic life (DOF, 1989), as well as nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac (0.20-0.31 µg L⁻¹), ibuprofen (3.61-4.51 µg L⁻¹) and naproxen (0.18 µg L⁻¹) (Galar-Martínez *et al.*, 2010; González-González *et al.*, 2014). However, given the urban and industrial characteristics of the areas surrounding the MR, it is necessary to explore the possible presence of other xenobiotics such as pharmaceuticals, personal care products (PCP), pesticides and other persistent organic pollutants (POPs).

Prior studies carried out by our work team have shown that contaminants present in MR water generate genotoxicity and cytotoxicity related to the production of oxidative stress in juveniles of *Cyprinus carpio* (González-González et al., 2014; Pérez-Coyotl et al., 2017). However, since the age of organisms is one of the most important factors modifying toxic response, it is very likely that individuals in early stages, and particularly on embryonic stage, are more susceptible to MR contaminants.

On the other hand, there is evidence that oxygen plays a key role in the metabolism of embryos and is critical in early embryonic development. Reactive oxygen species (ROS) have vital signaling functions and intervene in numerous physiological processes in the developing organism. During embryogenesis, ROS control cell division and the maturing of oocytes as well as the implantation and formation of blastocysts. When ROS are not duly regulated, they tend to generate oxidative stress and consequently embryotoxicity. Therefore, the antioxidant defense plays a vital role in the protection of organisms in formation, since pro-oxidant agents could elevate the concentration of ROS and induce oxidative damage in oocytes, mitochondrial alterations, depletion of the ATP content, DNA damage, lipoperoxidation (LPX), apoptosis, and/or a delay in the entire embryonic development process (Pašcová et al., 2011).

There are some reports on the role of metals as pro-oxidant agents and their effect on the embryonic development of hydrobionts. For instance, Al at a concentration of 1.87 mg L⁻¹ affected the embryonic development of a freshwater snail (*Radix quadrasi*), causing a delay in growth followed by edema and thinning of the shell, as well as a delay in hatching of the exposed organisms (Factor and de Chavez, 2012). Kong et al. (2012) demonstrated that in embryos exposed to 0.2, 1.5 and 10 µg L⁻¹ of mercury (Hg), the activity of catalase (CAT) decreased and the degree of LPX increased

in a concentration-dependent manner. After exposing *Pimephales promelas* to methylmercury, Devlin (2006) found a rapid accumulation of the compound and a relatively low concentration of proteins in the organisms.

Similarly, emerging contaminants such as NSAIDs, which have been shown to be embryotoxic and teratogenic in a wide range of aquatic species. This is the case of diclofenac which causes these effects on *Xenopus laevis*, *Lithobates catesbeianus* (Cardoso-Vera *et al.*, 2017) and *C. carpio* (Stepanova *et al.*, 2013). Prášková *et al.* (2013) determined that ketoprofen delays the hatching of embryos of the common carp. Besides, numerous other pollutants (e.g., PCPs, pesticides and POPs) are able to provoke oxidative stress and embryotoxicity in hydrobionts. Certain combinations of this chemical compounds likely generate interactions that intensify the toxic response, even when the exposure to individual pollutants is below the no-observed-adverse-effect level (NOAEL) (Beyer *et al.*, 2014).

One species commonly used in ecotoxicology studies is common carp (*C. carpio*). It is a very important organism from an ecological and economic point of view. In Mexico, this species inhabits 80% of the freshwater bodies and is consumed by a large number of people, occupying eighth place in world fish production (Nava-Álvarez *et al.*, 2014). However, it is endangered by the widespread contamination of its habitat in many locations such as the MR. Many reports have described a considerable reduction in the population of various species that inhabit different freshwater bodies.

The objective of the present work was to characterize the main organic contaminants present in MR, and to evaluate the toxicity produced by that polluted water on the embryonic development

of the common carp (*Cyprinus carpio*), correlating the morphological changes found with the production of oxidative stress.

2. Materials and methods

2.1 The study area and water sampling

The MR (at 19°31'37" N and 99°15'33" W) has a surface area of 190 hectares and a storage capacity of 16.6 million m³. It is bordered by the towns of Nuevo Madín and Viejo Madín, which discharge their waste directly into the reservoir. The dam was built to control the flow of the Tlalnepantla river and provide drinking water to nearby municipalities. The resulting water body is used for kayaking, sailing, carp fishing and other recreational activities (Pérez-Cóyotl *et al.*, 2017).

The water samples were collected in September 2016 during the rainy season (May-September), according to the procedure of the Mexican official norm on wastewater sampling (NMX-AA-003-1980) and treated as indicated by Pérez-Cóyotl *et al.*, (2017): Samples were taken at five sites of the reservoir: (1) the point of wastewater drainage into the reservoir from the town of Nuevo Madín, (2) the entry point of the Tlalnepantla River, (3) a side branch of the reservoir, (4) the curtain of the dam, and (5) the point of wastewater drainage into the reservoir from the town of Viejo Madín (Fig. 1). Water at each site was sampled by filling three 2-liter polyethylene bottles (previously rinsed with 3% nitric acid and fitted with an automatic sealing mechanism). The bottles were immediately transported to the laboratory and stored in the dark at 5 °C until being employed for chemical analysis and toxicity assays (in less than 7 days) (Pérez-Cóyotl *et al.*, 2017). For

chemical characterization of the samples, identification and quantification was carried out for pesticides, PCPs, drugs and POPs. Subsequently, embryotoxicity bioassays were conducted with each sample.

2.2. Quantification of pollutants

The samples from the five sampling sites were analyzed to detect and quantify pesticides, personal care products (PCPs), pharmaceuticals and persistent organic pollutants (POPs).

Pesticides in the water samples were evaluated by on-line solid phase extraction (SPE), high-performance liquid chromatography (HPLC) and tandem mass spectrometry (SPE-LC-MS/MS), as described in Köck-Schulmeyer *et al.* (2012, 2013).

Personal care products were evaluated by HPLC mass spectrometry analysis as described by Serra-Roig *et al.* (2016).

Sample extraction and ultra-high-performance liquid chromatography (UHPLC)-MS/MS analysis methods to quantify pharmaceutical products have been described elsewhere (López-Serna *et al.*, 2010).

Finally, chemical analysis of water samples to determine persistent organic pollutants was performed with a method based on SPE followed by gas chromatography-tandem mass spectrometry (GC-MS/MS), as described in Lacorte *et al.* (2000).

2.3 Embryo bioassays

C. carpio embryos were obtained by natural fertilization, carried out in the Carpícola Tiacaque Center (Mexico State). Four female and two male adults were placed in a fertilization tank. The ovideposit and fertilization of eggs were monitored. Eggs were selected 6 h post-fertilization and observed by stereomicroscopy to confirm fertilization, embryonic age stage (period of gastrulation) and viability (Hermsen *et al.*, 2011). Subsequently, they were exposed to water from each of the five sampling sites in order to explore possible oxidative stress and embryotoxicity (lethality and teratogenesis). Dissolved oxygen concentration at sampling sites ranged from 6.3 to 7.8 mg L⁻¹.

2.3.1 Evaluation of embryotoxicity and teratogenesis

For the examination of embryotoxicity, 60 embryos were selected at 6 h post-fertilization for each of the 5 sampling sites and placed on plates (1 embryo/well) for ELISA. Each well contained 300 µL of water from the sampling sites, and the plates were maintained at 24 ± 1 °C. The tests were carried out in a static environment without renewal. These embryos were observed under an inverted Optika XDS-2 microscope at 12, 24, 48, 72 and 96 h. The number of dead embryos (embryo lethality) was quantified, and the morphological development of each surviving embryo (embryotoxicity) was evaluated by utilizing the general morphological point system adapted by Kimmel *et al.* (1955) and Hermsen *et al.* (2011). This system is based on points assignment accordingly to the presence or absence of specific morphological characteristics, including the time of hatching, at specified testing times. The final scoring of the embryos exposed to water from the different sites of the MR was compared to the scoring of the control group. Dead embryos were not scored.

To determine the teratogenesis produced by the MR water samples, all organisms still surviving at 96 h of exposure were evaluated for pericardial edema, yolk sac edema, eye edema, tail, heart, head or sacculi/otolith malformation, modified chorda structure, scoliosis, rachischisis and yolk deformation. The results are expressed as the percentage of malformed embryos.

2.3.2 Determination of antioxidant defenses and oxidative stress parameters

Six lots were formed with 7 g of embryos each in aquariums of 1-L capacity, containing the water from the five sampling points of the MR and a control group, maintained in eggs water (60 ??g Instant Ocean salt mL⁻¹ of tridestilled water) (Westerfield, 2007). The systems were kept in rooms with controlled temperature at 24°C. At each of the exposure times (12, 24, 48, 72 and 96 h), 1 g of the embryos was homogenized in 1.5 mL of phosphate buffer solution (PBS, pH 7.4), and then the samples were centrifuged at 15,000 g for 15 min at 4 °C. The supernatant was used to determine the protein carbonyl content (PCC) and the activity of antioxidant enzymes: CAT, superoxide dismutase (SOD) and glutathione peroxidase (GPx). The homogenate was examined to establish the degree of LPX and the hydroperoxide content (HPC). The assay was performed in triplicate.

The degree of LPX was assessed with the method reported by Buege and Aust (1978). The results are expressed as nM of malondialdehyde, expressed as the measured environmental concentration of 1.56×10^5 M/cm per mg of protein per g of tissue.

The HPC was quantified by the method of Jiang *et al.* (1992). Results were expressed as nanomoles (nM) of cumene per mg of protein per g of tissue.

The PCC was ascertained by the method described by Levine *et al.* (1994), with some modifications. The results are expressed as nM of reactive carbonyls (C=O), obtained by the measured environmental concentration of $21\,000\text{ M}^{-1}\text{ cm}^{-1}$ per mg of protein per g of tissue.

SOD activity was established with the commercial RANSOD kit (RANDOX). After following the kit instructions, the readings were obtained on an ELx800 reader (BioTek) and interpreted with a calibrating curve included in the kit. The data are expressed in U SOD per mg of protein per g of tissue.

CAT activity was estimated by the method of Radi *et al.* (1999). Results were expressed as mM of H_2O_2 per mg of protein per g of tissue.

GPx activity was determined with the RANSEL commercial kit (RANDOX). After following the instructions provided with the kit, the $\Delta A/\text{min}$ of each sample was multiplied by the factor of 8412 (provided by manufacturer) and the data are expressed as U/L of GPX per mg of protein per g of tissue.

The results of all the biomarkers of oxidative stress were normalized against total proteins, which were measured by the Bradford method (1976).

For more specifications on the methods described above, see the supplementary data.

2.4 Statistical Analysis

The values for biomarkers of oxidative stress were submitted to a bifactorial analysis of variance, considering time as factor A and the sampling sites as factor B (Two Way RM ANOVA, $p < 0.05$). The differences between the means were examined with the Student-Newman-Keuls method. The data from the scoring of embryonic development were subjected to two-way non-parametric analysis of variance (ANOVA) of repeated measurements, followed by the Tukey post hoc test for multiple measurements. A Pearson correlation was performed between contaminants found at all sampling sites (20 xenobiotics) and biomarkers of oxidative damage and embryotoxicity (Tables S1 in the supplementary section). The analyses were carried out using the software Sigma Plot 12.3.

3. Results and Discussion

3. Results and Discussion

3.1 Quantification of pollutants

Among the pollutants identified in the MR (Table 1) were chlorfenvinphos, fenthion and diazinon. The latter showed the greatest concentration at all sampling sites. These pesticides have been detected in other water bodies in Europe and America. For example, Ccanccapa *et al.* (2016) found chlorfenvinphos, fenthion and diazinon in concentrations of 1.57-41.24, 0.33-2.64 and 0.12- 20.39 ng L⁻¹, respectively in the Ebro River (Spain). The concentrations observed in MR are lower. Since

no cropland exists near the MR, these pesticides are probably carried along the Tlalnepantla River from sites with nearby fields of crops, implying a dilution factor upon arriving to the reservoir.

Regarding PCPs, various compounds were detected (although only at sites SS5 and SS1), including 2,2',4,4'-tetrahydroxybenzophenone (BP2), OD-PABA, 4-methylbenzylidene camphor (4-MBC) and methyl benzotriazol (MeBZT). UV filters most frequently found in rivers are those derived from benzophenone, such as BP2 and the derivatives of camphor (e.g., 4-MBC) (Ramos *et al.*, 2015). For example, BP2 was found at 109 ng L⁻¹ in an urban basin (Singapur); OD-PABA at up to 748 ng L⁻¹ in the Adige river (Italy) and 4-MBC at 2,700 ng L⁻¹ in Switzerland lakes (Mao *et al.*, 2018; Mandaric *et al.*, 2017; Balmer *et al.*, 2005). Hence, the concentrations of PCPs determined herein are within the range observed in other water bodies around the world, and their prevalence at sampling sites SS1 and SS5 can be attributed to direct drainage of domestic waste from the surrounded towns.

Pharmaceutical products are a threat to aquatic ecosystems due to their inherent biological activity, and their resistance to inactivation after being excreted, (Santos *et al.*, 2010), which implies a potential risk for non-target organisms even when concentrations are low (Destrieux *et al.*, 2017). Among the most prevalent drugs in aquatic ecosystems are NSAIDs, antibiotics, antihypertensive agents and hypoglycemic drugs, which were represented at the sampling sites in the MR. The most prevalent were metformin, glibenclamide and acetaminophen. Diverse studies around the world have found pharmaceutical drugs in water bodies. Santos *et al.* (2010) described the levels of acetaminophen in some influents of Spain founding concentrations around 29,000-246,000 ng L⁻¹. Scheurer *et al.* (2012) reported that the level of metformin in some German rivers was around 130-1,700 ng L⁻¹. Estrada-Arriaga *et al.* (2016) found 66,000 ng L⁻¹ of acetaminophen and 94,600

ng L⁻¹ of metformin entering two treatment plants in Mexico. The concentrations of both in MR were much lower than in the cases previous mentioned.

The POPs detected at the greatest concentrations and at all the sampling sites were three PCBs (149, 118 and 101). PCBs are mainly of industrial origin and reach the environment through incineration plants for domestic residuals, garbage dumps, etc., (Gakuba *et al.*, 2015). PCBs 149, 118 and 101 were observed by Zhang *et al.* (2004) in the Tonghui River (China) at maximum concentrations of 12.71, 25.65 and 74.07 ng L⁻¹, respectively, constituting higher levels than those found in the MR.

Another group of POPs evaluated was PAHs. The ones found in the highest concentration and at all sampling sites were indene (1,2,3-c,d) pyrene, dibenz(a,h)anthracene and acenaphthylene. The sites with the greatest quantity of PAHs (considering all 16 together) were SS1 (24.66 ng L⁻¹) and SS2 (27.8 ng L⁻¹), corresponding to the drainage point from the town of Nuevo Madín and the side branch of the reservoir. Even so, the total concentrations were not above those reported for the Seine River (France, 20 ng L⁻¹) or Slave River (Canada, 29 ng L⁻¹) (Fernández *et al.*, 1997; McCarthy *et al.*, 1997). The source of PAHs is related to the activities in the nearby neighborhoods (to the reservoir) that could account for this type of pollutant: open garbage dumps, sporadic burning of garbage by inhabitants and domestic waste. Also, expanded plastic and polystyrene on the shores of the MR and in the water can break into fragments and release some PAHs upon exposure to UV radiation (Ríos *et al.*, 2007).

Concerning OCPs, DDT (dichloro-diphenyl-trichloroethane) and several of its metabolites were encountered in the MR. The OCP with the highest level was 4,4 DDE, followed by 2,4 DDE and

lindane. In the Niger River a maximum concentration of 421.3 ng L⁻¹ for DDT and 180.2 ng L⁻¹ for lindane were found (Unyimadu *et al.*, 2018). The concentrations in the MR are below those of the Niger River.

The presence, concentration and spatial distribution of pollutants in a water body depends on the frequency and amount used, their physicochemical characteristics and abiotic components of the exposed ecosystem (water and sediment), among others. In the MR we find not only organic pollutants, but a complex mixture of xenobiotics that can damage the hydrobions that inhabit it, such as the common carp, being the early stages of development the most susceptible to damage.

3.2.1 Determination of embryotoxicity and teratogenesis

Embryotoxicity was expressed as modifications in embryonic development (Fig. 2), embryoletality and teratogenicity (Table 2). Whereas the water sample from SS1 caused 100% mortality in *C. carpio* embryos, water from SS2 resulted in 56.6% mortality, SS3 51.6%, SS4 46.6% and SS5 43.3%. Unlike the other sites, CFP, BP2, OD-PABA and MeBZT were found in the SS1 site. In addition, there were quantified the highest levels of metformin, penicillin V, lindane, fluorene, phenanthrene, fluoranthene and pyrene.

The assessment of embryonic development was carried out with the surviving embryos. Compared to the control embryos, exposure to the water samples from sites SS2, SS3 and SS4 led to a significant decrease in the score. The embryonic development of the embryos exposed to water from the SS5 site was significantly higher at both 72 and 96 h compared to the score of the other sites. However, all the embryos exposed to MR water presented a delay in the time of hatching and

a delay in the pigmentation of the eyes, head and body. Water samples from all sites induced teratogenicity in *C. carpio* (Table 2). SS3 and SS4 produced the greatest percentage of embryonic malformations (55.1 and 53.1 %, respectively), mainly yolk sac edema and pericardial edema. Despite the same type of malformations was found in the group of embryos exposed to water from the SS5 site, the percentage was close to half of that produced by water from sites SS3 and SS4. Although the contaminants found in the sites SS2, SS3, SS4 and SS5 were quite similar, lower concentrations of PCB, phenanthrene and metformin were found.

Several authors have studied the embryotoxicity and teratogenicity of organophosphate pesticides in aquatic species such as diazinon found in MR. Hamm and Hinton (2000) showed that the most common response in *Oryzias latipes* fish exposed to diazinon is the appearance of yolk sac and pericardial edema, malformations related to the development of the endothelium and the pericardial cavity. These effects were found in common carp embryos exposed to water at all MR sampling sites.

PCPs produced neurotoxicity and endocrine disruption, and thus a negative effect on development and reproduction (Araújo *et al.*, 2018). BP2 (at 9.85 mg L⁻¹) promotes the accumulation of lipids in the yolk sac, facial malformations, and harmful effects on the process of segmentation and blood circulation of *Danio rerio* (Fong *et al.*, 2016). At concentrations of 0.447-599 mg L⁻¹, 4-MBC reduces the rate of hatching of *Solea senegalensis* as well as mortality, malformations and diminished growth (Araújo *et al.*, 2018).

Regarding pharmaceutical drugs, it has been reported that hypoglycemic agents like metformin and glibenclamide cause only slight embryotoxicity in mammals, but the effect on aquatic species has

not been evaluated. On the other hand, acetaminophen at a concentration of 1-100 mg L⁻¹ has been associated with embryotoxicity in *Danio rerio*, in which deterioration of early development, hatching, organogenesis, larval growth, tail formation and pigmentation was found (David & Pancharatna, 2009). Pérez-Álvarez *et al.* (2018) discovered that a hospital effluent contained glibenclamide (1.92 µg L⁻¹), metformin (1.31 µg L⁻¹), penicillin G (3.77 µg L⁻¹) and acetaminophen (2.66 µg L⁻¹), inducing growth inhibition, microcephalia, facial and pericardial edema, eye malformations, and damage to the notochord, tail, fin and intestine of *Xenopus laevis* and *Lithobates catesbeianus*.

Finally, several POPs were found in MR, including some organochlorine insecticides such as hexachlorobenzene, lindane, DDT and its metabolites. Oliva *et al.* (2008) found that lindane produces myoskeletal defects, skin opacity, exophthalmia, and depigmentation in *S. aurata*. Pawar and Katdare (1984) demonstrated that hexachlorobenzene provokes distention of body cavities, curvature of the body axis, poor blood circulation, retarded growth, and poor pigmentation for *M. ornate*, evidencing its embryotoxicity and teratogenicity for aquatic invertebrates (Pašková *et al.*, 2011).

PCBs were another type of POP detected in the MR. After Blanc *et al.* (2017) exposed *Danio rerio* embryos to 7.5 µg L⁻¹ of PCB126, there was a boost in the expression of genes related to oxidative stress (*gpx1a* and *tp53*) and the biotransformation of xenobiotics (*ahr2* and *cyp1a*). The toxicity of this compound increased when the embryos were exposed to a mixture of perfluorooctane sulfonic acid and perfluorohexanoic acid, demonstrating the importance of the interactions between POPs. Because of containing a complex mixture of pollutants, the water of the MR probably produces

interactions that substantially modify the toxic response, particularly in organisms at early stages of development.

A wide range of PAHs were detected in the MR samples. These compounds have been well studied. For instance, Xie *et al.* (2017) exposed *Crassostrea gigas* larvae to benzo(a)pyrene, determining the effect on embryogenesis and survival with an EC₅₀ of 18.4 µg L⁻¹ and an LC₅₀ of 26.8 µg L⁻¹. They reported that this xenobiotic induces the breakage of DNA chains, leading to abnormalities and in some cases death. Le Bihanic *et al.* (2014) assessed the effect of a complex mixture of PAHs, extracted from the sediment of the Seine estuary (France) on embryos of Medaka, showing that PAHs produce a delay in hatching, developmental abnormalities and DNA damage. They pointed out the potential risk of exposure during the early stages of development to mixtures of PAHs. Although the mechanisms that affect development are still not completely clear, they include the inhibition of enzymes, damage to proteins, lipids and genetic material, as well as alterations in membranes, cell energy supply and signaling of retinoic acid. ROS play an important role in these alterations due to their participation in signaling processes (Pašková, 2011) and in the production of oxidative stress.

3.2.2 Determination of antioxidant defenses and oxidative stress parameters

Antioxidant defenses and oxidative stress resulted from tests with embryos exposed to water from all sites except SS1, as the organisms exposed to water from the latter site died in less than 12 h.

Data related to antioxidant defense (Fig. 4) demonstrate important modifications in the response of the embryos exposed to water samples from the four sites and at almost all times of exposure

(versus the control, $p \leq 0.05$). Regarding SOD, the increase in its activity was greater at sites SS2 and SS3 at 12 and 24 h. The activity of CAT was raised practically in all the groups and exposure times. Unlike CAT and SOD, the activity of GPx presented a similar behavior to the control group during the exposure times up to 72 h, but at 96 h of exposure to water from SS4 and SS5 the relative value of this parameter increased. Each of the samples generated a different level of antioxidant response, probably because of the distinct type and concentration of pollutants.

Oxidative damage is observed in figure 5. All parameters (LPX, PCC and HPC) were notably higher for the embryos exposed to the water from SS2, SS3, SS4 and SS5 (at all exposure times) compared to those of the control group ($p \leq 0.05$). The greatest damage to biomolecules was generated by the SS3 water sample to lipids (LPX: 6416.12 % at 48 h, HPC: 22293.1 % at 72 h) and the SS2 sample to proteins (8642.38% at 24 h).

When the production of ROS is increased, the activity of antioxidant enzymes is intensified in order to reestablish redox balance and health of the individual. The failure to reestablish this balance causes oxidative damage to biomolecules, compromising the ability of the organism to survive (Valavanidis et al; 2006). In this case, despite the increase in the activity of antioxidant enzymes in specific groups and times, oxidative damage occurred in all embryos exposed to MR water.

A capacity to promote oxidative stress has been shown by many environmental pollutants, including metals, pesticides, emerging pollutants (pharmaceutical drugs and PCPs) and POPs (Lushchak, 2011), including those detected in MR. Diazinon produce oxidative stress in *O. niloticus* (Toledo-Ibarra *et al.*, 2016) and *C. carpio* (Oru $\square$ & Usta, 2007). The rupture in neuronal and endocrine signaling, produced because of the inhibition of acetylcholinesterase, leads to an

intracellular flow of Ca^{2+} , triggering the activation of proteolytic enzymes and nitric oxide synthase as well as the generation of free radicals, thus promoting oxidative stress (Lushchak, 2011).

The PCPs identified in the MR (BP2, OD-PABA, 4-MBC and MeBZT) are also capable of stimulating oxidative stress. Gao *et al.* (2013) demonstrated that $1\ \mu\text{g L}^{-1}$ of BP3 and 4-MBC increased the activity of CAT in *T. thermophila*, while Liu *et al.* (2015) established that 0.48 and $4.78\ \text{mg L}^{-1}$ of BP2 produced a similar result in the liver of *Carassius auratus*. Ma *et al.* (2017) revealed that OD-PABA enhanced the activity of SOD and CAT in *C. auratus*. PCPs are biotransformed by phase I reactions (Le Fol *et al.*, 2015) or undergo reactions by means of abiotic transformations like photolysis (Calza *et al.*, 2016), giving rise to reactive metabolites and ROS, and consequently an imbalance in redox.

Many pharmaceutical products are able to cause oxidative stress through their respective processes of biotransformation, which can afford reactive metabolites and ROS. Such is the case of acetaminophen found in MR in *C. carpio* (Nava-Álvarez *et al.*, 2014). Other drugs detected in the MR are glibenclamide and metformin. Martínez-Viveros *et al.* (2018) demonstrated that glibenclamide (at 10, 100 and $1000\ \text{ng L}^{-1}$) promotes oxidative stress in *C. carpio*, increasing SOD and CAT activity, protein oxidation and LPX activity. The overproduction of ROS is related to its biotransformation process, which furnishes oxy-cytochrome P450 ($\text{O}_2\text{-P450-Fe}^{2+}\text{-GLD}$) by means of phase I reactions. The resulting rapid decomposition of glibenclamide leads to the release of the superoxide radical (Krest *et al.*, 2013). In the case of metformin, Adaramoye *et al.* (2012) found that this biguanide stimulates oxidative stress, necrosis and degeneration of the seminiferous tubules in Wistar rats, particularly when administered along with glibenclamide. Hence, it possibly brings about a redox imbalance in aquatic species (e.g., *C. carpio*). Even considering that

metformin is very stable and is practically eliminated in an unaltered form by the renal pathway, a part of it is biotransformed by aerobic bacteria to guanylsurea (Kosma *et al.*, 2015) and nitrogenated compounds that can trigger oxidative stress (Nimptsch & Pflugmacher, 2007). The latter compounds may then exist in the MR. Also observed in the MR samples were two β -lactam antibiotics, penicillin V and G. There is no direct evidence that penicillin V and G promote oxidative stress in an organism. However, Elizalde-Velázquez *et al.* (2017) reported that another β -lactam antibiotic (amoxicillin) give rise to oxidative stress in *C. carpio*. On the other hand, Havelkova *et al.* (2016) showed low toxicity of penicillin G for *Daphnia magna* (EC_{50} = 1496.9 mg L⁻¹) and *Pseudokirchneriella subcapitata* (EC_{50} = 7114 mg L⁻¹).

The POPs found in the MR are organochlorine insecticides such as hexachlorobenzene, DDT (and its metabolites) and lindane, as well as PCBs and PAHs. POPs generate ROS during their biotransformation by cytochrome P450, and therefore induce oxidative stress (Lushchak, 2011). After exposing mussels (*Perna viridis*) to 10 μ g L⁻¹ DDT, Song *et al.* (2016) and Jiang *et al.* (2017) detected an overexpression of genes related to the synthesis of proteins associated with oxidative stress and detoxification. Di *et al.* (2016) examined oxidative stress produced by isomers of hexachlorocyclohexane on *Tubifex*, discovering that they bioaccumulate in the organism and increase SOD activity and LPX.

According to Leitão *et al.* (2003), PCBs trigger oxidative stress in the marine dinoflagellate *Lingulodinium polyedra*. They showed that arochlor 1254 (a mixture of approximately 70 different PCBs) at concentrations of 120 μ g L⁻¹ brings about a boost of 121% in protein oxidation and 146% in SOD activity. This is attributed to an adaptation mechanism to compensate for damage to proteins.

Among the PAHs occurring in the MR were acenaphthylene, dibenz (a,h) anthracene and indene (1,2,3-c,d) pyrene. Song *et al.* (2016) evaluated the effect of benzo(a)pyrene, on *Perna viridis*, reporting that induces cellular apoptosis and changes in energetic metabolism. When in the presence of DDT, it also affects proteins associated with oxidative stress. Guo *et al.* (2017) discovered that benzo(a)pyrene in a mixture with chrysene (0.1 and 1 $\mu\text{g L}^{-1}$) increased the levels of SOD, CAT and GPx, as well as protein oxidation and LPX activity in *Chlamys farreri*.

All POPs identified in the MR have the proven capacity to promote oxidative stress in diverse aquatic species. Thus, they may contribute to the effect observed in the embryos of common carp exposed to MR water. Additionally, they are known to be hormone disruptors, giving them the capacity to alter endocrine and reproductive functions in aquatic organisms and reduce reproduction capability of adults and limit the growth and survival of offspring (El-Shahawi *et al.*, 2010).

Table 3 presents the positive correlations observed at 96 h between concentrations of contaminants found at most sampling sites and biomarkers of oxidative stress and embryotoxicity. It can be seen that oxidative stress biomarkers have a strong association with different types of contaminants, e.g. metformin, PCB 170, phenanthrene and benz(a)anthracene. For IT contaminants such as diazinon, glibenclamine, benz(a)anthracene and dibenz(a,h) anthracene showed a high correlation coefficient. This result highlights the strong association of toxicity with the presence of important environmental pollutants.

4. Conclusions

The organic compounds herein detected (pesticides, PCPs, pharmaceutical products and POPs), did not exceed those found in other water bodies in the world. These xenobiotics, as well as other pollutants in the MR water, such as metals previously found (González-González *et al.*, 2014), undergo biotic and abiotic transformations or act as oxidizing agents, promoting reactive metabolites that trigger a redox imbalance (Beyer et al., 2014), and consequently oxidative damage and the embryotoxicity observed in *C. carpio*. Thus, probably the survival of the species is compromised in the reservoir, and with it, the balance of the ecosystem.

Conflict of interest

The authors declare that they have no current or potential competing financial interests.

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5. References

Adaramoye O., Akanni O., Adesanoye O., Labo-Popoola O., Olaremi O. (2012). Evaluation of

529 toxic effects of metformin hydrochloride and glibenclamide on some organs of male rats. *Niger J.*
530 *Physiol. Sci.*, 27: 137-144.

531 Araújo M.J., Rocha R.J.M., Soares A.M.V.M., Benedé J.L., Chisvert A., Monteiro M.S. (2018).
532 Effects of UV filter 4-methylbenzylidene camphor during early development of *Solea senegalensis*
533 Kaup, 1858. *Sci. Total Environ.*, 628-629: 1395-1404.
534 <https://doi.org/10.1016/j.scitotenv.2018.02.112>.

535 Beyer J., Petersen K., Song Y., Ruus A., Grung M., Bakke T., Tollefsen K.E., (2014).
536 Environmental risk assessment of combined effects in aquatic ecotoxicology: A discussion paper.
537 *Mar. Environ. Res.*, 96: 81-91. <https://doi.org/10.1016/j.marenvres.2013.10.008>.

538 Blanc M., Kärman A., Kukucka P., Scherbak N., Keiter S., (2017). Mixture-specific gene
539 expression in zebrafish (*Danio rerio*) embryos exposed to perfluorooctane sulfonic acid (PFOS),
540 perfluorohexanoic acid (PFHxA) and 3,3', 4,4'- pentachlorobiphenyl (PCB126). *Sci. Total*
541 *Environ.*, 590-591: 249-257. <http://dx.doi.org/10.1016/j.scitotenv.2017.02.232>.

542 Bradford M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities
543 of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254.
544 [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).

545 Buege, J.A., Aust, S.D., (1978). Microsomal lipid peroxidation. *Methods Enzymol.*, 52: 302–10.
546 [https://doi.org/10.1016/S0076-6879\(78\)52032-6](https://doi.org/10.1016/S0076-6879(78)52032-6).

547 Calza P., Vione D., Galli F., Fabbri D., Del Bello F., Medana C., (2016). Study of the
548 photochemical transformation of 2-ethylhexyl 4-(dimethylamino) benzoate (OD-PABA) under
549 conditions relevant to surface waters. *Water Res.*, 88: 235-244.
550 <http://dx.doi.org/10.1016/j.watres.2015.10.015>.

551 Cardoso-Vera D.J., Islas-Flores H., SanJuan-Reyes N., Montero-Castro E. I., Galar-Martínez M.,
552 García-Medina S., Elizalde-Velázquez A., Dublán-García O., Gómez-Oliván L.M. (2017).

553 Comparative study of diclofenac-induced embryotoxicity and teratogenesis in *Xenopus*
554 *laevis* and *Lithobates catesbeianus*, using the frog embryo teratogenesis assay: *Xenopus* (FETAX)
555 *Sci. Total Environ.*, 574: 467-475. <https://doi.org/10.1016/j.scitotenv.2016.09.095>.

556 Ccanccapa A., Masiá A., Navarro-Ortega A., Picó Y., Barceló D., (2016). Pesticides in the Ebro
557 River basin: Occurrence and risk assessment. *Environ. Pollut.*, 211; 414-424.
558 <http://dx.doi.org/10.1016/j.envpol.2015.12.059>.

559 David A., Pancharatna K., (2009). Effects of acetaminophen (paracetamol) in the embryonic
560 development of zebrafish, *Danio rerio*. *J. Appl. Toxicol.*, 29: 597-602. DOI.10.1002/jat.1446.

561 Destrieux D., Laurent F., Budzinski H., Pedelucq J., Vervier P., Gerino M., (2017). Drug residues
562 in urban water: A database for ecotoxicological risk management. *Sci. Total Environ.* 609: 927-
563 941. <http://dx.doi.org/10.1016/j.scitotenv.2017.07.043>.

564 Devlin E., (2006). Acute toxicity, uptake and histopathology of aqueous methyl mercury to fethead
565 minnow embryos. *Ecotoxicology*, 15: 97-110.

566 Di S., Zhang W., Chen L., Zhou Z., Diao J., (2016). Toxokinetics and oxidative stress in *Tubifex*
567 *tubifex* exposed to hexachlorocyclohexane isomers. *RSC Adv.*, 6: 19016-19024.
568 DOI:10.1039/c5ra26207k.

569 Elizalde-Velázquez, A., Martínez-Rodríguez, H., Galar-Martínez, M., Dublán-García, O., Islas-
570 Flores, H., Rodríguez-Flores, J., Castañeda-Peñalvo, G., Lizcano-Sanz, I. and Gómez-Oliván, L.
571 M., (2017). Effect of amoxicillin exposure on brain, gill, liver, and kidney of common carp
572 (*Cyprinus carpio*): The role of amoxicilloic acid. *Environ. Toxicol.*, 32: 1102–1120.
573 doi:10.1002/tox.22307.

574 El-Shahawi M.S., Hamza A., Bashammakh A.S., Al-Saggaf W.T., (2010). An overview on the
575 accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of

576 persistent organic pollutants. *Talanta*, 80: 1587-1597. doi:10.1016/j.talanta.2009.09.055.

577 Estrada-Arriaga E.B., Cortés-Muñoz J.E., González-Herrera A., Calderón-Mólgora C.G., Rivera-
578 Huerta M.L., Ramírez-Camperos E., Montellano-Palacios L., Gelover-Santiago S.L., Pérez-
579 Castrejón S., Cardoso-Vigueros L., Martín-Domínguez A., García-Sánchez L., (2016). Assessment
580 of full-scale biological nutrient removal systems upgraded with physico-chemical processes for the
581 removal of emerging pollutants present in wastewaters from Mexico. *Sci. Total Environ.*,
582 571:1172-1182. <http://dx.doi.org/10.1016/j.scitotenv.2016.07.118>.

583 Factor C., de Chavez E.R. (2012). Toxicity of Arsenic, Aluminum, Chromium and Nickel to the
584 Embryos of the Freshwater Snail, *Radix quadrasi* von Möellendorf 1898. *Philipp. J. Sci.*, 141: 207-
585 216.

586 Fernández M.B., Sicre M.A., Boireau A., (1997). Polyaromatic hydrocarbon (PAH) distributions
587 in the Siene River and its estuary. *Mar. Pollut. Bull.*, 34: 857-867. [https://doi.org/10.1016/S0025-](https://doi.org/10.1016/S0025-326X(97)00063-5)
588 326X(97)00063-5.

589 Fong H.C.H., Ho J.C.H., Cheung A.H.Y., Lai K.P., Tse W.K.F., (2016). Developmental toxicity
590 of the common UV filter, benzophenone-2 in zebrafish embryos. *Chemosphere*, 164: 413-420.
591 <http://dx.doi.org/10.1016/j.chemosphere.2016.08.073>.

592 Gago-Ferrero P., Mastroianni N., Díaz-Cruz S.M., Barceló D., (2013). Fully automated
593 determination of nine ultraviolet filters and transformation products in natural waters and
594 wastewaters by on-line solid phase extraction–liquid chromatography–tandem mass spectrometry.
595 *J. Chromatogr. A.*, 1294: 106-116. <https://doi.org/10.1016/j.chroma.2013.04.037>

596 Gakuba E., Moodley B., Ndungu P., Birungi G., (2015). Occurrence and significance of
597 polychlorinated biphenyls in water, sediment pore water and surface sediments of Umgeni River,
598 KwaZulu-Natal, South Africa. *Environ. Monit. Assess.*, 187:568. [http://doi.org/10.1007/s10661-](http://doi.org/10.1007/s10661-015-4790-1)
599 015-4790-1.

600 Galar-Martínez M., Gómez-Oliván L.M., Amaya-Chávez A., Razo-Estrada, C., García-Medina S.,
601 (2010). Oxidative stress induced on *Cyprinus carpio* by pollutants present in the water and
602 sediment of Madín reservoir. *J. Environ. Sci. Health. Part A.* 45, 155-160.

603 Gao L., Yuan T., Zhou C., Cheng P., Bai Q., Ao J., Wang W., Zhang H., (2013). Effects of four
604 commonly used UV filters on the growth, cell viability and oxidative stress responses of the
605 *Tetrahymena thermophile*. *Chemosphere*, 93:2507-2513.
606 <http://dx.doi.org/10.1016/j.chemosphere.2013.09.041>.

607 Gómez-Oliván L.M., Neri-Cruz N., Galar-Martínez M., Islas-Flores H., García-Medina S., (2014).
608 Binary mixtures of diclofenac with paracetamol, ibuprofen, naproxen and acetylsalicylic acid and
609 these pharmaceuticals in isolated form induce oxidative stress on *Hyalella azteca*. *Environ. Monit.*
610 *Assess.*, 186:7259-7271, DOI 10.1007/s10661-014-3925-0.

611 Gómez-Oliván L.M., Neri-Cruz N., Galar-Martínez M., Vieyra-Reyes P., García-Medina S.,
612 (2012). Assessing the oxidative stress induced by paracetamol spiked in artificial sediment on
613 *Hyalella azteca*. *Water Air Soil Pollut.*, 223(8): 5097-5104. [https://doi.org/10.1007/s11270-012-](https://doi.org/10.1007/s11270-012-1261-y)
614 [1261-y](https://doi.org/10.1007/s11270-012-1261-y).

615 González-González E.D., Gómez-Oliván L.M., Galar-Martínez M., Vieyra-Reyes P., Islas-Flores
616 H., García-Medina S., Jiménez-Vargas J.M, Razo-Estrada A.C., Pérez-Pasten B.R., (2014). Metals
617 and nonsteroidal anti-inflammatory pharmaceuticals drugs present in water from Madín Reservoir
618 (Mexico) induce oxidative stress in gill, blood and muscle of common carp (*Cyprinus*
619 *carpio*). *Arch. Environ. Contam. Toxicol.*, 67:281–295. [https://doi.org/10.1007/s00244-014-](https://doi.org/10.1007/s00244-014-0048-0)
620 [0048-0](https://doi.org/10.1007/s00244-014-0048-0).

621 Guo R., Pan L., Lin P., Zheng L., (2017). The detoxification responses, damage effects and
622 bioaccumulation in the scallop *Chlamys farreri* exposed to single and mixtures of benzo(a)pyrene
623 and chrysene. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, 191: 36-51.

624 <http://dx.doi.org/10.1016/j.cbpc.2016.09.004>.

625 Hamm J.T., Hinton D.E., (2000). The role of development and duration of exposure to the
 626 embryotoxicity of diazinon. *Aquat. Toxicol.*, 48: 403-418. <https://doi.org/10.1016/S0166->
 627 445X(99)00065-X.

628 Havelkova B., Beklova M., Kovacova V., Hlavkova D., Pikula J., (2016). Ecotoxicity of selected
 629 antibiotics for organisms of aquatic and terrestrial ecosystems. *Neuro Endocrinol. Lett.*, 37: 38-44.
 630 <https://doi.org/10.1074/jbc.R113.473108>.

631 Hermesen S.A.B., van der Brandhof E.J., van der Ven L.T.M., Piersma A.H., (2011). Relative
 632 embryotoxicity of two classes of chemicals in a modified zebrafish embryotoxicity test and
 633 comparison with their *in vivo* potencies. *Toxicol. In Vitro*, 25: 745-753.
 634 [doi:10.1016/j.tiv.2011.01.005](https://doi.org/10.1016/j.tiv.2011.01.005).

635 Jiang Z.Y., Hunt J.V., Wolff S.P., (1992). Ferrous ion oxidation in the presence of xylenol orange
 636 for detection of lipid hydroperoxide in low density lipoprotein. *Anal. Biochem.*, 202: 384-389.
 637 [https://doi.org/10.1016/0003-2697\(92\)90122-N](https://doi.org/10.1016/0003-2697(92)90122-N).

638 Jiang X., Tang T., Zhao H., Song Q., Zhou H., Han Q., Diao X., (2017). Differential gene responses
 639 in the embryo of the green mussel *Perna viridis* exposed to dichlorodiphenyltrichloroethane
 640 (DDT). *Toxicol. Res.*, 6: 477-486. DOI:10.1039/c7tx00087a.

641 Kimmel B.C., Ballard W.W., Kimmel S.R., Ullmann B., Schilling F.T., (1995). Stages of
 642 embryonic development of the zebrafish. *Dev. Dyn.*, 203: 253-310.
 643 <https://doi.org/10.1002/aja.1002030302>

644 Köck-Schulmeyer M., Ginebreda A., González S., Cortina J. L., de Alda M. L., Barceló D., (2012).
 645 Analysis of the occurrence and risk assessment of polar pesticides in the Llobregat River Basin
 646 (NE Spain). *Chemosphere*, 86: 8-16. <https://doi.org/10.1016/j.chemosphere.2011.08.034>.

647 Köck-Schulmeyer M., Villagrasa M., de Alda M. L., Céspedes-Sánchez R., Ventura F., Barceló
 648 D., (2013). Occurrence and behavior of pesticides in wastewater treatment plants and their
 649 environmental impact. *Sci. Total Environ.*, 458:466-476.
 650 <https://doi.org/10.1016/j.scitotenv.2013.04.010>.

651 Kong X., Wang S., Jiang H., Nie G., Li X. (2012). Responses of acid/alkaline phosphatase,
 652 lysozyme, and catalase activities and lipid peroxidation to mercury exposure during the embryonic
 653 development of goldfish *Carassius auratus*. *Aquat. Toxicol.*, 120-121: 119-125.
 654 <https://doi.org/10.1016/j.aquatox.2012.05.005>

655 Kosma C.I., Lambropoulou A.D., Albanis T.A., (2015). Comprehensive study of the antidiabetic
 656 drug metformin and its transformation product guanyurea in Greek wastewaters. *Water Res.*, 70:
 657 436-448. <http://dx.doi.org/10.1016/j.watres.2014.12.010>.

658 Krest, C.M., Onderko, E.L., Yosca, T.H., Calixto, J.C., Karp, R.F., Livada, J., (2013). Reactive
 659 intermediates in cytochrome P450 catalysis. *J. Biol. Chem.*, 288, 17074-170781. Doi:
 660 10.1074/jbc.R113.473108.

661 Lacorte S., Guiffard I., Fraisse D., Damià Barceló D., (2000). Broad Spectrum Analysis of 109
 662 Priority Compounds Listed in the 76/464/CEE Council Directive Using Solid-Phase Extraction and
 663 GC/EI/MS. *Anal. Chem.*, 72: 1430-1440. DOI: 10.1021/ac991080w.

664 Le Bihanic F., Clérandeau C., Le Menach K., Morin B., Budzinski H., Cousin X., Cachot J., (2014).
 665 Developmental toxicity of PAH mixtures in fish early stages. Part II: adverse effects in Japanese
 666 medaka. *Environ. Sci. Pollut. Res. Int.*, 21: 13732-13743. [https://doi.org/10.1007/s11356-014-](https://doi.org/10.1007/s11356-014-2676-3)
 667 2676-3.

668 Le Fol V., Aït-Aïssa S., Cabaton N., Dolo L., Grimaldi M., Balaguer P., Perdu E., Debrauwer L.,
 669 Brion F., Zalko D. (2015). Cell-specific biotransformation of benzophenone-2 and bisphenol-S in

zebrafish and human in vitro models used for toxicity and estrogenicity screening. *Environ. Sci. Technol. Lett.*, 49: 3860-3868. DOI:10.1021/es505302c.

Leitão M.A. da S., Cardozo K.H.M., Pinto E., Colepicolo P., (2003). PCB-induced oxidative stress in the unicelular marine dinoflagellate *Lingulodinium polyedrum*. *Arch. Environ. Contam. Toxicol.*, 45: 59-65. DOI: 10.1007/s00244-002-0208-5.

Levine, R.L., Williams, J.A., Stadtman, E.R., Shacter, E., (1994). Carbonyl assays for determination of oxidatively modified proteins. *Methods Enzymol.* 233,346–57. [https://doi.org/10.1016/S0076-6879\(94\)33040-9](https://doi.org/10.1016/S0076-6879(94)33040-9).

Liu H., Sun P., Ki H., Yang S., Wang L., Wang Z., (2015). Hepatic oxidative stress biomarker responses in freshwater fish *Carassius auratus* exposed to four benzophenone UV filters. *Ecotoxicol. Environ. Saf.*, 119: 116-122. <http://dx.doi.org/10.1016/j.ecoenv.2015.05.017>.

López-Serna, R., Pérez, S., Ginebreda, A., Petrović, M., Barceló, D., (2010). Fully automated determination of 74 pharmaceuticals in environmental and waste waters by online solid phase extraction–liquid chromatography–electrospray–tandem mass spectrometry. *Talanta*, 83: 410-424. <https://doi.org/10.1016/j.talanta.2010.09.046>.

Ma B., Lu G., Liu J., Yan Z., Yang H., Pan T., (2017). Bioconcentration and multi-biomarkers of organic UV filters (BM-DBM and OD-PABA) in crucian carp. *Ecotoxicol. Environ. Saf.*, 141: 178-187. <http://dx.doi.org/10.1016/j.ecoenv.2017.03.034>.

Mandaric L., Diamantini E., Stella E., Cano-Paoli K., Valle-Sistac J., Molins-Delgado D., Bellin A., Chiogna G., Majone B., Díaz-Cruz M.S., Sabater S., Barcelo D., Petrovic M., (2017). Contamination sources and distribution patterns of pharmaceuticals and personal care products in Alpine rivers strongly affected by tourism. *Sci. Total Environ.*, 590-591: 484-494. <http://dx.doi.org/10.1016/j.scitotenv.2017.02.185>.

693 Mao F., You L., Reinhard M., He Y., Yew-Hoong Gin K., (2018). Occurrence and fate of
 694 benzophenone-type UV filters in a tropical urban watershed. *Environ. Sci. Technol. Lett.*, 52: 3960-
 695 3967. DOI:10.1021/acs.est.7b05634.

696 Martínez-Viveros E.M.G., Islas-Flores H., Dublán-García O., Galar-Martínez M., SanJuan-Reyes
 697 N., García-Medina S., Hernández-Navarro M.D., Gómez-Oliván L.M., (2018). Environmentally
 698 relevant concentrations of glibenclamide induce oxidative stress in common carp (*Cyprinus*
 699 *carpio*). *Chemosphere*, 197: 105-116. <https://doi.org/10.1016/j.chemosphere.2018.01.020>.

700 McCarthy L.H., Williams G.R., Stephens J., (1997). Baseline studies in the Slave river, NWR
 701 1990-1994: Part I. Evaluation of the chemical quality of water and suspended sediment from Slave
 702 river (NWT). *Sci. Total Environ.*, 197: 21-53.

703 Nava-Álvarez R., Razo-Estrada A. C., García-Medina S., Gómez-Oliván L. M., Galar-Martínez
 704 M., (2014). Oxidative stress induced by mixture of diclofenac and acetaminophen on common carp
 705 (*Cyprinus carpio*). *Water Air Soil Pollut.*, 225(2): DOI: 10.1007/s11270-014-1873-5.

706 Nimptsch J., Pflugmacher S., (2007). Ammonia triggers the promotion of oxidative stress in the
 707 aquatic macrophyte *Myriophyllum mattogrossense*. *Chemosphere*, 66: 708-714.
 708 doi:10.1016/j.chemosphere.2006.07.064.

709 Oliva M., Garrido C., Sales D., González de Canales M.L., (2008). Lindane toxicity on early life
 710 stages of gilthead seabream (*Sparus aurata*) with a note on its histopathological manifestations.
 711 *Environ. Toxicol. Pharmacol.*, 25(1):94–102. <https://doi.org/10.1016/j.etap.2007.09.005>.

712 Oru O.E., Usta E., (2007). Evaluation of oxidative stress responses and neurotoxicity potential
 713 of diazinon in different tissues of *Cyprinus carpio*. *Environ. Toxicol. Pharmacol.*, 23: 48-55.
 714 doi:10.1016/j.etap.2006.06.005.

715 Pašková V., Hilscherová K., Bláha L., (2011). Teratogenicity and Embryotoxicity in Aquatic
 716 Organisms After Pesticide Exposure and the Role of Oxidative Stress. In: Whitacre D. (eds)

717 Reviews of Environmental Contamination and Toxicology Volume 211. *Rev. Environ. Contam.*
718 *Toxicol.*, (Continuation of Residue Reviews), Springer, New York, NY.
719 https://doi.org/10.1007/978-1-4419-8011-3_2.

720 Prášková E., Štěpánová S., Chromcová L., Plhalová L., Voslářová E., Pištěková V., Prokeš M.,
721 Svobodová Z. (2013). The effects of subchronic exposure to ketoprofen on early developmental
722 stages of common carp. *Acta Vet. Brno.* 82: 343-347.
723 <https://doi.org/10.2754/avb201382030343>

724 Pawar K.R., Katdare M., (1984). Toxic and teratogenic effects of fenitrothion, BHC and
725 carbofuran on embryonic development of the frog *Microhyla ornata*. *Toxicol. Lett.* 22:7–13.
726 [https://doi.org/10.1016/0378-4274\(84\)90038-9](https://doi.org/10.1016/0378-4274(84)90038-9).

727 Pérez-Álvarez I., Islas-Flores H., Gómez-Oliván L.M., Barceló D., López De Alda M., Pérez
728 Solsona S., Sánchez-Aceves L., SanJuan-Reyes N., Galar-Martínez M., (2018). Determination of
729 metals and pharmaceutical compounds released in hospital wastewater from Toluca, México, and
730 evaluation of their toxic impact. *Environ. Pollut.*, 240: 330-341.
731 <http://doi.org/10.1016/j.envpol.2018.04.116>.

732 Pérez-Coyotl, I., Martínez-Vieyra, C., Galar-Martínez, M., Gómez-Oliván, L. M., García-Medina,
733 S., Islas-Flores, H., Pérez-Pasten Borja, R., Gasca-Pérez, E., Novoa-Luna, K. A., Dublán-García,
734 O., (2017). DNA damage and cytotoxicity induced on common carp by pollutants in water from
735 an urban reservoir. Madín reservoir, a case study. *Chemosphere*, 185: 789-797.
736 <http://dx.doi.org/10.1016/j.chemosphere.2017.07.072>.

737 Prášková E., Štěpánová S., Chromcová L., Plhalová L., Voslářová E., Pištěková V., Prokeš M.,
738 Svobodová Z., (2013). The effects of subchronic exposure to ketoprofen on early developmental
739 stages of common carp. *Acta Vet. Brno.*, 82: 343-347. <https://doi.org/10.2754/avb201382030343>.

740 Radi, R., Turrens, J.F., Chang, L.Y., Bush, K.M., Carpo, J.D., Freeman, B.A. (1991). Detection of
 741 catalase in rat heart mitochondria. *J. Biol. Chem.*, 266, 22028–34.

742 Ramírez-Bastida P. (2000). Aves de humedales en zonas urbanas del noroeste de la Ciudad de
 743 México. Tesis, Facultad de Ciencias, División de Posgrado, Universidad Nacional Autónoma de
 744 México, pp DOI: 10.13140/RG.2.2.13509.45289.

745 Ramos A.S., Correia A.T., Antunes S.C., Gonçalves F., Nunes B., (2014). Effect of
 746 acetaminophen exposure in *Oncorhynchus mykiss* gills and liver: Detoxification mechanisms,
 747 oxidative defence system and peroxidative damage. *Environ. Toxicol. Pharmacol.*, 37:1221-1228.
 748 <https://doi.org/10.1016/j.etap.2014.04.005>.

749 Rigaud C., Couillard C.M., Pellerin J., Légaré B., Gonzalez P., Hodson P.V. (2013). Relative
 750 potency of PCB126 to TCDD for sublethal embryotoxicity in the mummichog (*Fundulus*
 751 *heteroclitus*). *Aquat. Toxicol.*, 128-129: 203-214. <http://dx.doi.org/10.1016/j.aquatox.2012.12.013>.

752 Rios L.M., Moore Ch., Jones P.R., (2007). Persistent organic pollutants carried by synthetic
 753 polymers in the ocean environment. *Mar. Pollut. Bull.*, 54:1230-1237.
 754 [doi:10.1016/j.marpolbul.2007.03.022](http://dx.doi.org/10.1016/j.marpolbul.2007.03.022).

755 Santos L.H.M.L.M., Araújo A.N., Fachini A., Pena A., Delerue-Matos C., Montenegro
 756 M.C.B.S.M., (2010). Ecotoxicological aspects related to the presence of pharmaceuticals in the
 757 aquatic environment. *J. Hazard. Mater.*, 175: 45-95. [doi:10.1016/j.jhazmat.2009.10.100](http://dx.doi.org/10.1016/j.jhazmat.2009.10.100).

758 Scheurer M., Michel A., Brauch H.J., Ruck W., Sacher F., (2012). Occurrence and fate of the
 759 antidiabetic drug metformin and its metabolite guanyurea in the environment and during drinking
 760 water treatment. *Water Res.*, 46: 4790-4802. <http://dx.doi.org/10.1016/j.watres.2012.06.019>.

761 Serra-Roig M.P., Jurado A., Díaz-Cruz M.S., Vázquez-Suñé E., Pujades E., Barceló D. Occurrence,
 762 fate and risk assessment of personal care products in river-groundwater interface. *Sci. Total*

763 *Environ.* 568:829-837. doi: 10.1016/j.scitotenv.2016.06.006.

764 Smolowitz M.R., Schultz E.M., Stegeman J.J., (1992). Cytochrome P4501A induction in tissues,
 765 including olfactory epithelium, of topminnows (*Poeciliopsis* spp.) by waterborne benzo[a]pyrene.
 766 *Carcinogenesis*, 13: 2395–2402. <https://doi.org/10.1093/carcin/13.12.2395>

767 Song Q., Chen H., Li Y., Zhou H., Han Q., Diao X., (2016). Toxicological effects of
 768 benzo(a)pyrene, DDT and their mixture on the green mussel *Perna viridis* revealed by proteomic
 769 and metabolomics approaches. *Chemosphere*, 144: 214-224.
 770 <https://dx.doi.org/10.1016/j.chemosphere.2015.08.029>.

771 Stegeman J.J., (1989). Cytochrome P450 forms in fish: catalytic, immunological and sequence
 772 similarities. *Xenobiotica*, 19:1093–1110. DOI: 10.3109/00498258909043164

773 Stepanova S., Praskova E., Chromcova L., Plhalova L., Prokes M., Blahova J., Svobodova Z.,
 774 (2013). The effects of diclofenac on early life stages of common carp (*Cyprinus carpio*). *Environ.*
 775 *Toxicol. Pharmacol.*, 35:454-460. <https://doi.org/10.1016/j.etap.2012.09.011>.

776 Sylvie B.R., Pairault C., Vernet G., Boulekbache H., (1996). Effect of lindane on the
 777 ultrastructure of the liver of the rainbow trout, *Oncorhynchus mykiss*, sac-fry. *Chemosphere*,
 778 33(10):2065–2079. [https://doi.org/10.1016/0045-6535\(96\)00295-0](https://doi.org/10.1016/0045-6535(96)00295-0).

779 Toledo-Ibarra G.A., Díaz-Reséndiz K.J.G., Ventura-Ramón G.H., González-Jaime F., Vega-López
 780 A., Becerril-Villanueva E., Pavón L., Girón-Pérez M.I., (2016). Oxidative damage in gills and liver
 781 in Nile tilapia (*Oreochromis niloticus*) exposed to diazinon. *Comp. Biochem. Physiol. A*, 200: 3-8.
 782 <http://dx.doi.org/10.1016/j.cbpa.2016.05.007>.

783 Unyimadu J.P., Osibanjo O., Babayemi J.O., (2018). Selected persistent organic pollutants (POPs)
 784 in water of river Niger: occurrence and distribution. *Environ. Monit. Assess.*, 190:6.
 785 <http://doi.org/10.1007/s10661-017-6378-4>.

786 Valavanidis A., Vlahogianni T., Dassenakis M., Scoullou M., (2006). Molecular biomarkers of
787 oxidative stress in aquatic organisms in relation to toxic environmental pollutants.
788 *Ecotoxicol. Environ. Saf.*, 64: 178-189. doi:10.1016/j.ecoenv.2005.03.013.

789 Westerfield M., 2007. The zebrafish Book: A guide for the laboratory use of zebrafish (*Danio*
790 *rerio*), 5th ed. University Oregon Press, Eugene, Oregon.

791 Wijnja H., Doherty J.J., Safie S.A., (2014). Changes in pesticide occurrence in suburban surface
792 waters in Massachusetts, USA, 1999-2010. *Bull. Environ. Contam. Toxicol.*, 93: 228-232. DOI
793 10.1007/s00128-014-1251-4.

794 Xie J., Yang D., Sun X., Cao R., Chen L., Wang Q., Li F., Wu H., Ji Ch. Cong M., Zhao J.,
795 (2017). Individual and combined toxicities of benzo(a)pyrene and 2,2',4,4'-
796 Tetrabromodiphenyl ether on early life stages of the pacific oyster, *Crassostrea gigas*. *Bull.*
797 *Environ. Contam. Toxicol.*, 99: 582-588. DOI 10.1007/s00128-017-2164-9.

798 Zhang Z., Huang J., Yu G., Hong H., (2004). Occurrence of PAHs, PCBs and organochlorine
799 pesticides in the Tonghui river of Beijing, China. *Environ. Pollut.*, 130: 249-261.
800 doi:10.1016/j.envpol.2003.12.002.

801

PESTICIDES	units	LOD	LOQ	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5
Diazinon	ng L ⁻¹	0.07	0.23	7.437	7.007	12.592	5.926	7.224
CFP	ng L ⁻¹	0.31	1.05	1.204	nd	nd	nd	nd
Fenthion sulfoxide	ng L ⁻¹	0.68	2.28	nd	nd	2.465	nd	nq
PERSONAL CARE PRODUCTS								
BP2	ng L ⁻¹	13	44	13.7	nd	nd	nd	nd
4HB	ng L ⁻¹	7	25	nd	nd	nd	nd	nd
4DHB	ng L ⁻¹	6	21	nd	<LOQ	nd	nd	nd
DHMB	ng L ⁻¹	5	17	nd	nd	<LOQ	nd	nd
Et-PABA	ng L ⁻¹	11	38	nd	nd	nd	<LOQ	nd
4MBC	ng L ⁻¹	0.8	2.6	nd	nd	nd	nd	134
OD-PABA	ng L ⁻¹			29	nd	nd	nd	nd
MeBZT	ng L ⁻¹	0.5	1.8	250	nd	nd	nd	nd
DMBZT	ng L ⁻¹	3.5	11.8	<LOQ	nd	nd	nd	nd
PHARMACEUTICAL PRODUCTS								
Glibenclamide	ng L ⁻¹	13.54	45.13	nd	353	3449	386	2148
Metformine	ng L ⁻¹	0.05	0.17	9557	11694	nd	4912	378
Penicillin G	ng L ⁻¹	0.04	0.13	295	280	257	249	279
Penicillin V	ng L ⁻¹	1.22	4.06	11.49	14.34	nd	nd	nd
Naproxen	ng L ⁻¹			nd	nd	nd	8.5	nd
Acetaminophen	ng L ⁻¹	241	805	2810	1124	1141	1352	9156
PERSISTENT ORGANIC POLLUTANTS								
Hexachlorbenzene	ng L ⁻¹	0.04	0.12	0.20	1.24	1.37	0.76	1.50
2,4 DDE	ng L ⁻¹	0.01	0.03	1.89	3.15	0.94	3.78	1.89
2,4 DDD	ng L ⁻¹	0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01
4,4 DDE	ng L ⁻¹	0.01	0.03	2.80	4.67	1.40	5.60	2.80
2,4 DDD	ng L ⁻¹	0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01
4,4 DDD + 2,4 DDT	ng L ⁻¹	0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01
4,4 DDT	ng L ⁻¹	0.03	0.09	0.30	0.26	0.00	0.46	0.25
γ - HCH (Lindane)	ng L ⁻¹	0.03	0.09	0.25	<0.03	1.82	0.12	<0.09
PCB18	ng L ⁻¹	0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01
PCB 28+31	ng L ⁻¹	0.02	0.06	<0.02	<0.02	<0.02	<0.02	<0.02
PCB 52	ng L ⁻¹	0.02	0.06	<0.02	<0.02	<0.02	<0.02	<0.02
PERSISTENT ORGANIC POLLUTANTS	units	LOD	LOQ	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5
PCB 44	ng L ⁻¹	0.01	0.03	<0.01	<0.01	<0.01	1.73	<0.01

PCB101	ng L ⁻¹	0.02	0.06	3.59	5.98	1.79	7.18	3.59
PCB 149	ng L ⁻¹	0.03	0.09	6.81	11.36	3.41	13.63	6.81
PCB 118	ng L ⁻¹	0.02	0.06	3.56	5.93	1.78	7.12	3.56
PCB 153	ng L ⁻¹	0.01	0.03	1.60	2.65	1.03	0.83	<0.03
PCB 138	ng L ⁻¹	0.02	0.06	<0.02	<0.02	<0.02	<0.02	0.31
PCB 180	ng L ⁻¹	0.04	0.12	<0.04	<0.04	<0.04	<0.04	0.84
PCB 170	ng L ⁻¹	0.03	0.09	<0.03	0.47	0.92	0.29	<0.03
PCB 194	ng L ⁻¹	0.05	0.15	<0.15	<0.05	<0.05	<0.05	<0.05
Naphthalene	ng L ⁻¹	0.03	0.09	1.94	1.60	2.37	1.41	2.83
Acenaphthylene	ng L ⁻¹	0.02	0.06	2.18	5.15	5.80	6.28	6.84
Acenaphthene	ng L ⁻¹	0.02	0.06	0.38	0.38	0.38	0.38	1.95
Fluorene	ng L ⁻¹	0.01	0.03	1.53	0.93	0.73	<0.01	0.32
Phenanthrene	ng L ⁻¹	0.01	0.03	3.51	1.01	2.02	1.45	<0.03
Fluoranthene	ng L ⁻¹	0.01	0.03	0.23	<0.01	<0.01	<0.01	<0.01
Anthracene	ng L ⁻¹	0.01	0.03	0.06	<0.01	<0.01	<0.01	<0.01
Pyrene	ng L ⁻¹	0.01	0.03	0.42	<0.01	<0.01	<0.03	<0.01
Benz(a)Anthracene	ng L ⁻¹	0.01	0.03	0.79	0.78	0.86	0.82	0.78
Chrysene	ng L ⁻¹	0.01	0.03	0.59	0.61	0.57	0.62	0.61
Benzo (b) fluoranthene	ng L ⁻¹	0.02	0.06	<0.02	<0.02	<0.02	<0.02	<0.02
Benzo (k) fluoranthene	ng L ⁻¹	0.02	0.06	<0.02	<0.02	<0.02	<0.02	<0.02
Benzo (a) pyrene	ng L ⁻¹	0.02	0.06	<0.02	<0.02	<0.02	<0.02	<0.02
Indene(1,2,3-c,d) pyrene	ng L ⁻¹	0.02	0.06	6.05	5.73	6.12	6.14	5.98
Dibenz (a,h) anthracene	ng L ⁻¹	0.02	0.06	5.67	3.51	7.80	3.83	3.13
Benzo (g,h,i) perylene	ng L ⁻¹	0.02	0.06	1.25	1.16	1.06	1.05	1.09

Table 1. Compounds and concentrations found in each sampling site of the Madin reservoir. 35 POP's, 12 pharmaceuticals, 9 personal care products and 17 pesticides were searched, but only the data of those compounds that were found in at least one of the sampling sites are shown. LOD: Limit of detection; LOQ: Limit of quantification.

Group	Total number of embryos exposed	Total number of deaths	Total number of lives	% mortality	Total number of malformed embryos*	Type of malformations	% malformed embryos	Embryos at 96 hpf
Control	60	0	60	0.0	1	Pericardial edema	1.6
SS1	60	60	0	100	-	-	-
SS2	60	34	26	56.6	9	Pericardial edema, yolk sac edema, malformation of the head, malformation of tail and yolk deformation	34.6
SS3	60	31	29	51.6	16	Pericardial edema, yolk sac edema, malformation of the head, malformation of tail, scoliosis and yolk deformation	55.1
SS4	60	28	32	46.6	17	Pericardial edema, yolk sac edema, malformation of heart, malformation of tail, modified chorda structure and yolk deformation	53.1
SS5	60	26	34	43.3	8	Pericardial edema, malformation of the head, malformation of tail, scoliosis and yolk deformation	23.5

Table 2. Results of embryoletality and teratogenesis in *Cyprinus carpio* embryos exposed for 96 h to MR water. Observed malformations, p: pericardial edema; yd: yolk sac deformation; t: tail malformation; ye: yolk sac edema; h: head malformation; c: modified chorda structure; s: scoliosis. * Teratogenesis test was done with the surviving embryos.

Compounds found		Biomarkers of oxidative stress						Biomarkers of embryotoxicity	
		SOD	CAT	GPx	LPx	HPx	PCC	General Morphology Score	IT
Pesticides	Diazinon	0.892 (0.108)	0.428 (0.572)	-0.572 (0.428)	-0.147 (0.853)	-0.482 (0.518)	0.419 (0.581)	-0.0566 (0.943)	0.830 (0.170)
Pharmaceutical products	Glibenclamide	0.578 (0.422)	-0.0437 (0.956)	-0.142 (0.858)	-0.575 (0.425)	-0.836 (0.164)	0.342 (0.658)	-0.305 (0.695)	0.914 (0.0863)
	Metformine	-0.178 (0.822)	0.38 (0.620)	-0.404 (0.596)	0.915 (0.0851)	0.964 (0.0360)	-0.521 (0.479)	0.118 (0.882)	-0.879 (0.121)
	Penicillin G	-0.405 (0.595)	-0.462 (0.538)	-0.0179 (0.982)	0.140 (0.860)	0.0529 (0.947)	-0.966 (0.033)	-0.903 (0.097)	-0.482 (0.518)
	Acetaminophen	-0.644 (0.356)	-0.974 (0.0261)	0.74 (0.260)	-0.739 (0.261)	-0.651 (0.349)	-0.417 (0.583)	-0.735 (0.265)	0.0484 (0.952)
Persistent organic pollutants	Hexachlorbenzene	0.126 (0.874)	-0.37 (0.630)	-0.127 (0.873)	-0.307 (0.693)	-0.566 (0.434)	-0.476 (0.524)	-0.900 (0.100)	0.305 (0.695)
	2,4 DDE	-0.559 (0.441)	0.0578 (0.942)	0.231 (0.769)	0.473 (0.527)	0.773 (0.227)	-0.146 (0.854)	0.466 (0.534)	-0.808 (0.192)
	4,4 DDE	-0.558 (0.442)	0.0591 (0.941)	0.230 (0.770)	0.474 (0.526)	0.773 (0.227)	-0.146 (0.854)	0.467 (0.533)	-0.808 (0.192)
	4,4 DDT	-0.763 (0.237)	-0.272 (0.728)	0.586 (0.414)	0.101 (0.899)	0.471 (0.529)	-0.0858 (0.914)	0.375 (0.625)	-0.673 (0.327)
	PCB 170	0.989 (0.011)	0.866 (0.134)	-0.864 (0.136)	0.373 (0.627)	0.0761 (0.924)	0.466 (0.534)	0.339 (0.661)	0.505 (0.495)
	Naphthalene	-0.00987 (0.990)	-0.613 (0.387)	0.285 (0.715)	-0.752 (0.248)	-0.898 (0.102)	-0.146 (0.854)	-0.74 (0.260)	0.566 (0.434)
	Acenaphthylene	-0.529 (0.471)	-0.817 (0.183)	0.920 (0.079)	-0.936 (0.063)	-0.736 (0.264)	0.197 (0.803)	-0.165 (0.835)	0.329 (0.671)
	Acenaphthene	-0.633 (0.367)	-0.969 (0.031)	0.722 (0.278)	-0.731 (0.269)	-0.652 (0.348)	-0.43 (0.570)	-0.752 (0.248)	0.0504 (0.950)
	Fluorene	0.531 (0.469)	0.459 (0.541)	-0.853 (0.147)	0.589 (0.411)	0.258 (0.742)	-0.452 (0.548)	-0.418 (0.582)	-0.0739 (0.926)
	Phenanthrene	0.857 (0.143)	0.909 (0.091)	-0.653 (0.347)	0.349 (0.651)	0.203 (0.797)	0.740 (0.260)	0.738 (0.262)	0.439 (0.561)
	Benz(a)Anthracene	0.835 (0.165)	0.600 (0.400)	-0.371 (0.629)	-0.149 (0.851)	-0.303 (0.697)	0.894 (0.106)	0.575 (0.425)	0.817 (0.183)
	Chrysene	-0.897 (0.103)	-0.442 (0.558)	0.599 (0.401)	0.116 (0.884)	0.458 (0.542)	-0.391 (0.609)	0.0711 (0.929)	-0.808 (0.192)
	Indene(1,2,3-c,d) pyrene	0.258 (0.742)	0.0168 (0.983)	0.345 (0.655)	-0.642 (0.358)	-0.565 (0.435)	0.920 (0.080)	0.548 (0.452)	0.754 (0.246)
	Dibenz (a,h) anthracene	0.953 (0.046)	0.604 (0.396)	-0.586 (0.414)	-0.0689 (0.931)	-0.351 (0.649)	0.646 (0.354)	0.257 (0.743)	0.831 (0.169)
	Benzo (g,h,i) perylene	-0.201 (0.799)	0.0356 (0.964)	-0.400 (0.600)	0.671 (0.329)	0.576 (0.424)	-0.899 (0.101)	-0.531 (0.469)	-0.731 (0.269)

Table 3. Pearson correlation between the concentrations of the compounds found and the biomarkers of oxidative stress, morphological score and teratogenesis. Correlation coefficients are shown and in parentheses the value of p. Correlation values > 0.8 and probability values < 0.05 are marked in bold.

Novelty Statement

The Madin Dam is an important water reservoir but impacted by multiple pollutants that are already severely damaging hydrobionts. In previous works it has been demonstrated that the toxics in the reservoir generate oxidative stress in juvenile common carp. The present work tries to relate this type of damage with the embryotoxic effect, to give answer to the decrease of the populations of this organism in the dam. The results now shown will demonstrate that pollutants discharged from both domestic and industrial discharges are damaging hydrobionts and could serve as a basis for promoting their clean-up and restoration.

Figure captions

Figure 1. Madín Reservoir, State of Mexico, Mexico. The location of sampling sites is shown: (1) the point of discharge from the town of Nuevo Madín, (2) the entry point of the Tlalnepantla River to the reservoir, (3) the side branch of the reservoir, (4) the curtain of the dam, and (5) the point of discharge from the town of Viejo Madín.

Figure 2. Embryonic development of *Cyprinus carpio* exposed to water samples (for 12, 24, 48, 72 and 96 h) from five sites of the Madín Reservoir. Data are expressed as the mean of the morphological score $\pm$ SEM. Significantly different from: *the control group, ^a SS2, ^b SS3, ^c SS4, ^d SS5. Two-way non-parametric analysis of variance (ANOVA) of repeated measurements, and Tukey multiple comparisons test ($P<0.05$).

Figure 3. Activity of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), in *Cyprinus carpio* embryos exposed to water samples (for 12, 24, 48, 72 and 96 h) from five sites of the Madín Reservoir. Data are expressed as the mean of three replicates $\pm$ SEM. Significantly different from: *the control group, ^a SS2, ^b SS3, ^c SS4, ^d SS5. Two Way RM ANOVA and Student-Newman-Keuls multiple comparisons test $P<0.05$.

Figure 4. Oxidative damage evaluated through various biomarkers. (1) Lipid peroxidation (LPX), (2) hydroperoxide content (HPC) and (3) protein carbonyl content (PCC) in *Cyprinus carpio* embryos exposed to water from each of the five sampling sites of the Madín Reservoir (MR) for 12, 24, 48, 72 and 96 h. Data are expressed as the mean of three replicates $\pm$ SEM. Significantly

24 different from: *the control group. Two Way RM ANOVA and Student-Newman-Keuls multiple
25 comparisons test $P < 0.05$.

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Supplementary data

S. Methods

S.1. Quantification of pollutants

S.1.1. Pesticides quantification

In accordance with Köck-Schulmeyer *et al.* (2012, 2013), LC-MS/MS system consisted of a binary HPLC pump (model 1525) connected to a TDQ mass spectrometer equipped with an electrospray ionization (ESI) interface (Waters Corporation, Milford, MA, USA). A reversed-phase Purospher STAR-RP-18e column preceded by a guard column (4×4 mm, $5 \mu\text{m}$) were used (Merck; Darmstadt, Germany). The pesticides evaluated were: diazinon, dimethoate, malathion, fenitrothion oxon, malaoxon (MOX), chlorpyrifos, chlorfenvinphos, dichlorvos, azinphos-ethyl, azinphos-methyl, azinphos-methyl oxon, fenthion oxon, fenthion oxon sulfone, fenthion sulfone, fenthion sulfoxide, fenthion oxon sulfoxide and fenitrothion (Sigma-Aldrich; Steinheim, Germany).

S.1.2. Personal care products determination

Based on Serra-Roig *et al.* (2016), in a first step, an automated on-line SPE-HPLC instrument (Symbiosis TM Pico from Spark Holland, Emmen, Netherlands) was used to extract, purify and pre-concentrate the samples. Later, chromatographic separation and mass spectrometry analysis was performed on a 4000 Q TRAP TM MS/MS system (Applied Biosystems Sciex, Foster City, CA, USA) coupled a Hibar Purosher [®] STAR [®] HR R-18 (50 mm X 2.0 mm, $5 \mu\text{m}$; Merck). The PI analysis was done according to Gago-Ferrero *et al.* (2013), under the following conditions: a source temperature of 700°C , 30 psi curtain gas, 5000 V voltage, 150 psi ion source gas, 260 psi ion source gas, and 10 V of entrance potential.

Benzophenone-3 (BP3), BP1, 4-hydroxybenzophenone (4HB), BP4, 4DHB and Et-PABA were purchased from Sigma-Aldrich (Steinheim, Germany), while 4-MBC. BP2 and 2,2'-dihydroxy-4-methoxybenzophenone (DHMB) were acquired from Merck (Darmstadt, Germany). The isotopically

labeled compounds 2-hydroxy-4-methoxy-2',3',4',5',6'-d5 (BP3-d5) and 3-(4-methylbenzylidene-d4) camphor, used as internal standards, were obtained from CDN isotopes (Quebec, Canada). All these standards were of >99% purity. MeOH, CAN, HPLC-grade water and formic acid (98% purity) were provided by Merck. N₂ and Ar purchased from Air Liquide (Barcelona, Spain) were of 99.995% purity. Glass fiber filters and nylon membrane filters were acquired from Whatman International Ltd. (Maidstone, England).

S.1.3. Pharmaceutical products quantification

According to López-Serna *et al.* (2010), the chromatographic separation was carried out on an Acquity UHPLC and a reversed-phase column BEHC18 (2.1 mm x 100 mm, 1.7 µm particle size, Waters Corporation). 200 µL of internal standards (0.05 ng µL⁻¹) were added (glibenclamide-d3, carbamazepine-d10, atenolol -d7, metoprolol-d7, naproxeno-d3, diclofenac-d3, ibuprofen-d3, 17 B-estradiol -d2, ofloxacin-d8, acetaminophen-d4, gemfibrozil-d6) before SPE for both positive ion (PI) and negative ion (NI) analysis.

To examine compounds in the PI mode, the elution started at 10% of ACN, was linearly increased 50% to 7.5 min in 6.2 min, then to 100% in 0.35 min, and finally kept isocratic for 0.5 min. The chromatographic analysis took a total of 8 min. For the NI analysis, the compounds were eluted by ACN: MeOH (1:1, v/v) and 10 mM ammonium acetate. The elution gradient was linearly increased from 20% ACN to 80% in 1.3 min, then to 90% in 0.7 min, and finally kept isocratic for 2 min, with a total running time of 5 min. The flow rate for both PI and NI was 0.4 mL min⁻¹ with an injection volume of 20 µL. Samples were preserved at 15 °C in the injection station and at 30 °C in the column.

For most compounds, two SRM transitions between the precursor ion and the most abundant fragment ions were monitored. The limit of detection, calculated as the concentration of analyte producing a

signal-to-noise ratio of 3, corresponds to the average value for each analyte. The limit of quantification was calculated as the concentration of analyte generating a signal-to-noise ratio of 10.

All pharmaceutical standards were of high purity (>90%). Naproxen (as sodium salt), metoprolol, glibenclamide (dehydrated) and carbamazepine were purchased from Sigma-Aldrich (Steinheim, Germany), diclofenac (hydrochloride) from LGC Promochem (London, UK), and atenolol (as calcium salt), acetaminophen and ibuprofen from Cerilliant (TX, USA). Regarding the isotopically-labeled compounds used as surrogates, metoprolol-d7 and naproxen-d3 were acquired from Toronto Research Chemicals (Toronto, Canada), while atenolol-d7, carbamazepine-d10, ibuprofen-d3 and diclofenac-d4 were purchased from CDN Isotopes (Quebec, Canada).

S.1.4. Persistent organic pollutants quantification

As described by Lacorte *et al.* (2000), GC-MS/MS analysis was performed on a gas chromatograph HP6890 coupled to a mass spectrometer HP 5973 (Hewlett-Packard, Waldbronn, Germany). The mass spectrometer was operated in the electron impact ionization mode (ionizing energy 70 eV, emission current 300 μ A, ion source temperature was 230 °C). A HP-5MS (30 m $\times$ 0.25 mm id, 0.25- μ m film thickness) containing 5% phenyl methyl siloxane was programmed (HP 19091S-433) from 40 °C (keeping this temperature for 2 min) to 175 °C at 3 °C/min (keeping this temperature for 4 min), then from 175 to 240 °C at 3 °C/min, and finally to 320 °C at 7 °C/min (keeping this temperature for 10 min). The total analysis time was 93 min and the equilibration time 3 min. Helium served as the carrier gas at 7 psi. The injection volume was 1 μ L, applied with a splitless injector. All standard chemicals were of high purity (99.9%), purchased from Promochem (Wesel, Germany). Standard solutions were prepared by weight in dichloromethane (SDS, Peypin, France) at a concentration of 1000 μ g mL⁻¹.

A working solution containing all compounds, prepared in ACN at a concentration of 5 ng/μL, was used for spiking water samples. Chloridic acid was procured from Prolabo. Oasis 60 mg SPE cartridges were from Waters Corporation.

S.2. Oxidative Stress Biomarkers

S.2.1. Determination of antioxidant defenses and oxidative damage parameters

Degree of LPX (Büege and Aust, 1978). To 200 μL of homogenate was added 1 mL of TBA-TCA [thiobarbituric acid 0.375 % (TBA, Sigma-Aldrich) in trichloroacetic acid at 15 % (TCA, Sigma-Aldrich)] reagent. The mix was vortex agitated and water bathed until ebullition for 15 min. Once tempered, the samples were centrifuged at 900 g for 5 min. The supernatant was read on a spectrophotometer (Metash) at 535 nm with respect to the reagent blank.

Hydroperoxide content (Jiang *et al.* 1992). To 400 μL of the homogenate were added 400 μL of TCA at 15%. After 5 min at room temperature, the mix was centrifuged at 9600 g for 10 min. To 200 μL of supernatant were added 800 μL of reagent solution [hydroxytoluene butyrate 4 mM, xylenol orange 0.01 mM in methanol at 90% and Fe₂SO₄ 0.25 mM in HCl 250 mM (Sigma)]. The mix was then brought for 60 min incubation at room temperature. Absorbance was measured against reagent blank, while the concentration was determined by data interpolation on a cumene pattern curve (cumene hydroperoxide, Sigma).

Protein carbonyl content (Levine *et al.* 1994), with some modifications. To 200 μL of supernatant were added 300 μL of dinitrophenylhydrazine at 10 mM in HCl (2M, Sigma) prior to incubation in the dark for 1 h. Subsequently, 1 mL trichloroacetic acid was added and left to stand for 15 min before being centrifuged at 10,000 g for 10 min. The precipitate was washed three times with an ethanol/ethyl

acetate mix in 1:1 proportion, dissolved in 1mL of guanidine 6M (pH 2.3). Absorbance was evaluated on a spectrophotometer at 366 nm.

CAT activity (Radi *et al.* 1999). To 40 μ L of supernatant were added 960 μ L of isolation buffer solution pH 7.4 (sucrose 0.3 M, EDTA 1 mM, HEPES 5 mM and KH_2PO_4 5mM) and 200 μ L of H_2O_2 20 mM. Absorbance was measured on a spectrophotometer (Metash) at 240 nm at 0 s and 60 s. The following equation was used to get the results: $\text{CAT activity} = (A_0 - A_{60}) / \text{CEM} (0.043 \text{ nM cm}^{-1})$.

The results of all the biomarkers of oxidative stress were normalized against total proteins, which were measured by the Bradford method (1976). To 2.5 μ L of supernatant were added 7.5 μ L deionized H_2O and 250 μ L of the Bradford reactive (0.05 g Coomassie blue, 25 mL of ethanol at 96%, and 50 mL H_3PO_4 , in 500 mL of deionized water), and the mixture was left in the dark for 5 min. The samples were read on a spectrophotometer at 595 nm on an ELISA reader (ELx800, BioTek).