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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 embryolethality, 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 embryolethality, embryotoxicity, congenital abnormalities, and oxidative stress on the common carp embryos.

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

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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 critic 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 (Pašková 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 ha and a storage capacity of 16.6 million m^3. 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-Coyotl 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-Coyotl 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-L 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-Coyotl 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

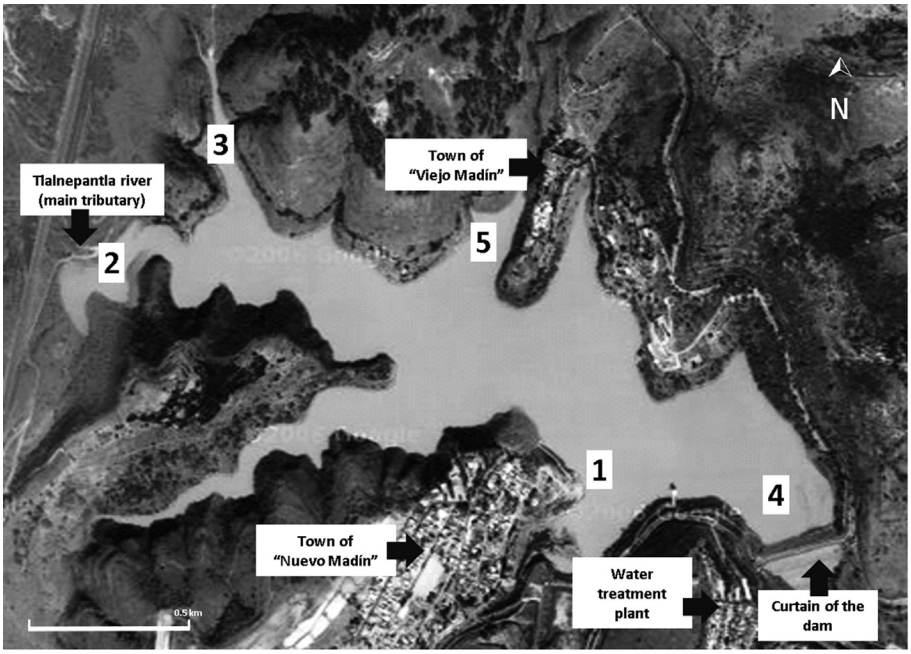


Fig. 1. Madín Reservoir, State of Mexico, Mexico. The location of sampling sites is shown: (1) the point of discharge from the town of Nuevo Madín, (2) the entry point of the Tlalnepantla River to the reservoir, (3) the side branch of the reservoir, (4) the curtain of the dam, and (5) the point of discharge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

et al., 2010).

Finally, chemical analysis of water samples to determine persistent organic pollutans 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 oviposit 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 (embryolethality) 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. (1995) and Hermsen et al. (2011). This system is based on points assignation 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 μg 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 M^{-1} 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. (1991). 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 ΔA/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 (Bradford, 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 (Table 3). The analyses were carried out using the software Sigma Plot 12.3.

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-methylbenzylidine 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 2700 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 arround 29,000–246,000 ng L^{-1}. Scheurer et al. (2012) reported that the level of metformin in some German rivers was around 130–1700 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}) (Fernándes 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 (Rios 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.

3.1.1. Determination of embryotoxicity and teratogenesis

Embryotoxicity was expressed as modifications in embryonic development (Fig. 2), embryolethality 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 (see Fig. 4).

The assessment of embryonic development was carried out with

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.

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

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

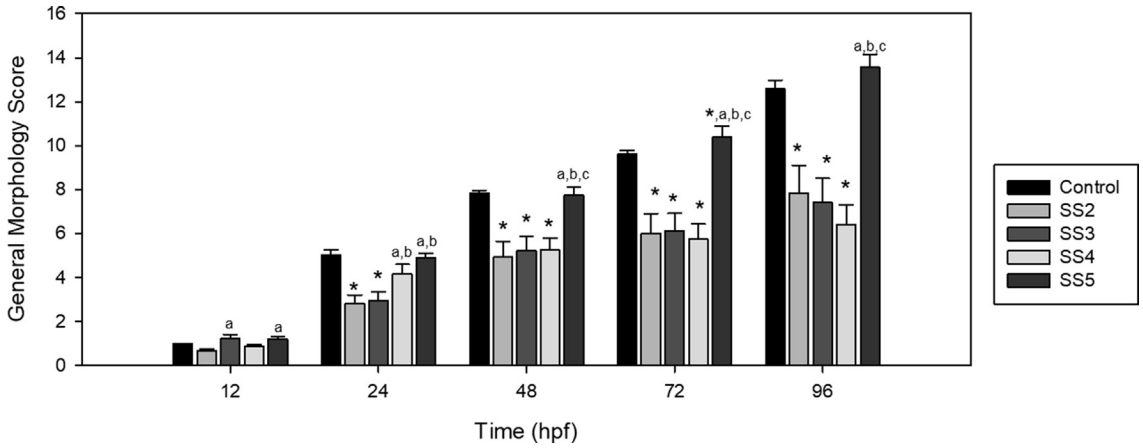


Fig. 2. Embryonic development of *Cyprinus carpio* exposed to water samples (for 12, 24, 48, 72 and 96 h) from five sites of the Madín Reservoir. Data are expressed as the mean of the morphological score ± SEM. Significantly different from: *the control group, [a] SS2, [b] SS3, [c] SS4, [d] SS5. Two-way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

Table 2
Results of embryolethality 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.

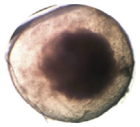
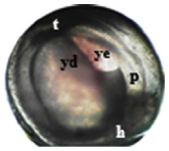
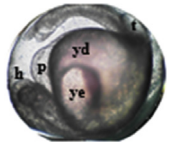
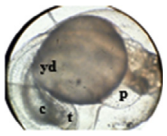
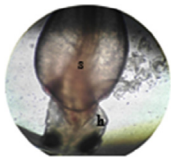
Group	Total number of embryos exposed	Total number of deaths	Total number of lives	% mortality	Total number of malformed embryos*	Type of malformations	% malformed embryos	Embryos at 96 hpf
Control	60	0	60	0.0	1	Pericardial edema	1.6	
SS1	60	60	0	100	–	–	–	
SS2	60	34	26	56.6	9	Pericardial edema, yolk 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 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.

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)

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 EC_{50} of 18.4 μg L^{-1} and an LC_{50} 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 et al., 2011) and in the production of oxidative stress.

3.1.2. 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. 3) 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

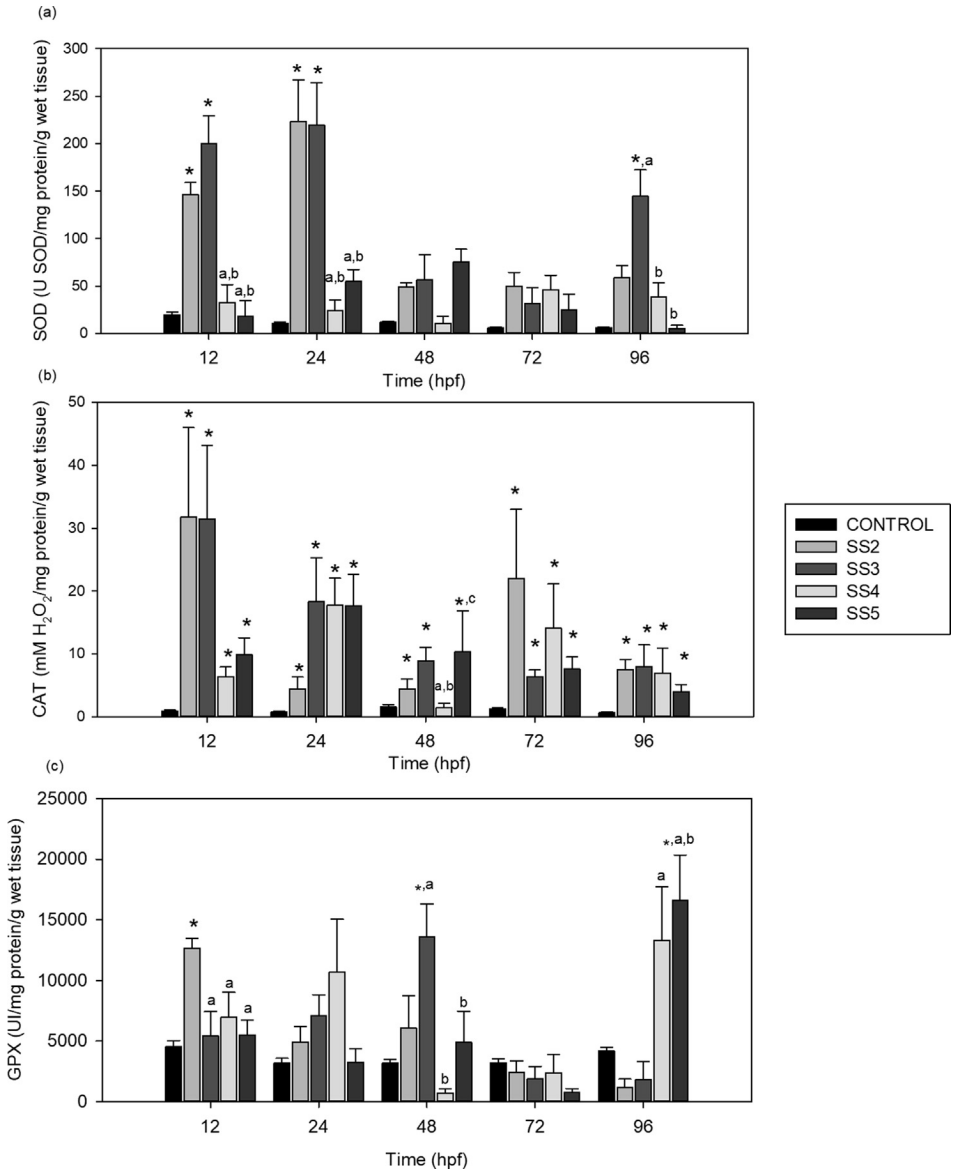


Fig. 3. Activity of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), in *Cyprinus carpio* embryos exposed to water samples (for 12, 24, 48, 72 and 96 h) from five sites of the Madín Reservoir. Data are expressed as the mean of three replicates ± SEM. Significantly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

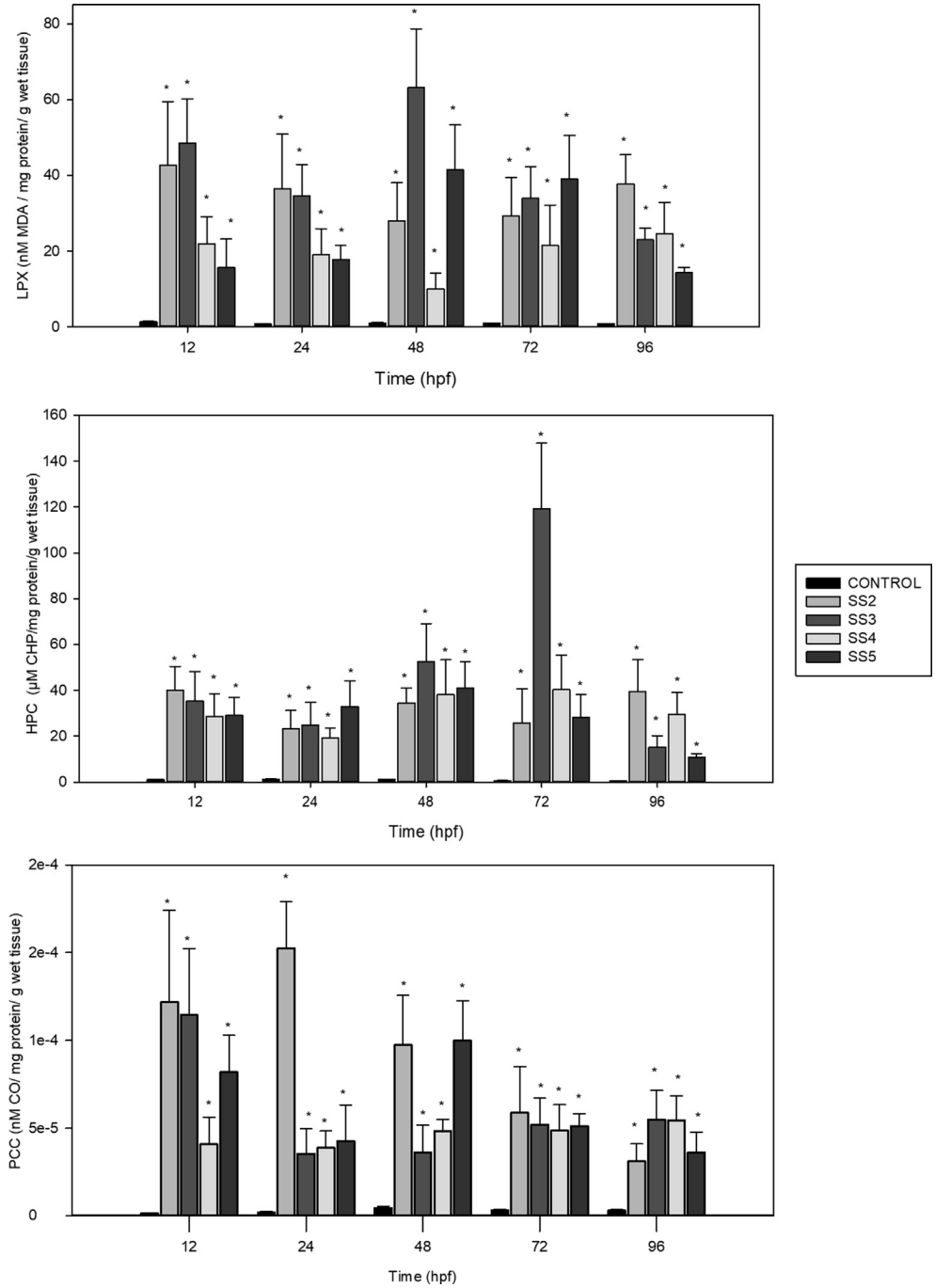


Fig. 4. Oxidative damage evaluated through various biomarkers. (1) Lipid peroxidation (LPX), (2) hydroperoxide content (HPC) and (3) protein carbonyl content (PCC) in *Cyprinus carpio* embryos exposed to water from each of the five sampling sites of the Madín Reservoir (MR) for 12, 24, 48, 72 and 96 h. Data are expressed as the mean of three replicates ± SEM. Significantly different from: *the control group. Two Way RM ANOVA and Student-Newman-Keuls multiple comparisons test $P < 0.05$.

different level of antioxidant response, probably because of the distinct type and concentration of pollutants.

Oxidative damage is observed in Fig. 4. 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*

(Oruç and 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 $\mu g\,L^{-1}$ 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 $mg\,L^{-1}$ 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 $ng\,L^{-1}$) 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 (O2-P450-Fe2+-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 $\mu g\,L^{-1}$ 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 $\mu g\,L^{-1}$ 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 $\mu g\,L^{-1}$) 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.

Conflicts of interest

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

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.04.095.

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.

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