Chemosphere 185 (2017) 789–797

Contents lists available at ScienceDirect

Chemosphere

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

DNA damage and cytotoxicity induced on common carp by pollutants in water from an urban reservoir. Madín reservoir, a case study

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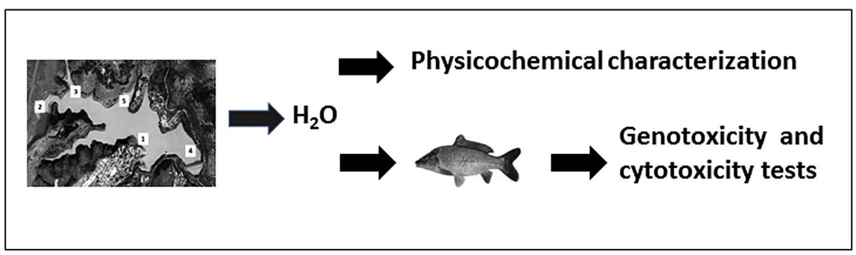
HIGHLIGHTS

- The Madín Dam is polluted by cytotoxic and genotoxic compounds, like heavy metals and pharmaceutical products.
- *Cyprinus carpio* is at risk in the Madín reservoir.
- Cytotoxicity and genotoxicity tests are a good tool to assess the risk of pollutants in water bodies.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 May 2017
Received in revised form
20 June 2017
Accepted 14 July 2017
Available online 18 July 2017

Handling Editor: Jim Lazorchak

Keywords:
Madín reservoir
Cyprinus carpio
Genotoxicity
Cytotoxicity

ABSTRACT

Madín Reservoir provides a substantial amount of drinking water to two municipalities close to Mexico City metropolitan area. However, it receives untreated wastewater discharges from domestic sources in the towns of Nuevo Madín and others, as well as diverse pollutants which are hauled by the Río Tlalnepantla from its upper reaches, so that the xenobiotics in the reservoir are highly diverse in terms of type and quantity. Previous studies showed that MR is contaminated with xenobiotics such as Al, Hg and Fe, as well as NSAIDs, at concentrations exceeding the limits established for aquatic life protection. These pollutants have been shown to induce oxidative stress on *Cyprinus carpio* and may therefore also damage the genetic material of exposed organisms, eliciting cytotoxicity as well. The present study aimed to determine the genotoxicity and cytotoxicity induced on blood, liver and gill of *C. carpio* by the pollutants present in MR water. Specimens were exposed to water from five sampling sites and the following biomarkers were evaluated: DNA damage by comet assay, frequency of micronuclei, apoptosis by TUNEL assay and caspase-3 activity. Significant increases relative to the control group ($P < 0.05$) were found with all biomarkers in all tissues evaluated, with the level of damage differing between sampling sites. In conclusion, pollutants present in MR water are genotoxic and cytotoxic to *C. carpio*, and this sentinel

Abbreviations: ANOVA, analysis of variance; BOD, biochemical oxygen demand; DCF, diclofenac; FBS, fetal bovine serum; IBP, ibuprofen; MNi, micronuclei; MR, Madín Reservoir; NPX, naproxen; NSAID, nonsteroidal anti-inflammatory drug; PAH, polynuclear aromatic hydrocarbon; PBS, phosphate buffered saline; pNA, *p*-nitroaniline; RNS, reactive nitrogen species; ROS, reactive oxygen species; SEM, standard error of the mean; SS, sampling site; TUNEL, TdT-mediated dUTP nick end labeling.

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http://dx.doi.org/10.1016/j.chemosphere.2017.07.072
0045-6535/

species, coupled with the biomarkers evaluated herein, is a reliable tool for assessing the health risk to wildlife posed by exposure to pollutants in freshwater bodies.

1. Introduction

Freshwater bodies around the world have been used as reservoirs for a large number of chemical stressors of anthropogenic origin such as heavy metals, pesticides and emerging pollutants among others, which, together with changes in the physicochemical properties of the water and sediments, habitat alteration and invasive/exotic species introduction increase the toxicological risk to hydrobionts (Colin et al., 2016; Holmstrup et al., 2010). This problem is particularly important in reservoirs located near large urban areas with high populations since, in developing countries, these areas discharge their waste directly into water bodies due to the lack of adequate treatment plants, as is the case of Madín Reservoir (MR) in Mexico.

MR was constructed on the Río Tlalnepantla in the State of Mexico at the three-way meeting point of the municipalities of Naucalpan de Juárez, Tlalnepantla de Baz and Atizapán de Zaragoza, within the Mexico City metropolitan area, for the purpose of controlling river flows and as a reservoir of drinking water. In addition to its being a source of drinking water, it is also used for recreational activities such as kayaking, sailing, and fishing of common carp. There are several urban communities located in its surroundings, such as Viejo Madín, Nuevo Madín and Zona Esmeralda, which discharge all or part of their waste into MR. Previous studies conducted in 2010 and 2013 showed that MR contains, among other pollutants, heavy metals and nonsteroidal anti-inflammatory drugs (NSAIDs) at concentrations exceeding permissible limits for aquatic life protection (Galar-Martínez et al., 2010; González-González et al., 2014), with the following having been detected at diverse sampling sites: Fe (1.51–5.10 mg L^{-1}), Hg (<0.001 mg L^{-1}), Al (6.04–24.45 mg L^{-1}), diclofenac (DCF, 0.20–0.31 μg L^{1}) ibuprofen (IBP, 3.61–4.51 μg L^{-1}) and naproxen (NPX, 0.18 μg L^{-1}). Nevertheless, the presence of xenobiotics in an aquatic ecosystem is not by itself indicative of the possible induction of deleterious effects. To ascertain this, the existence of a correlation between exposure levels and signs of early damage needs to be determined with the use of biomarkers (Colin et al., 2016; Van der Oost et al., 2003).

Diverse studies have shown that both heavy metals and NSAIDs induce increases in reactive oxygen and nitrogen species (ROS and RNS) production, which in the aquatic environment lead to the generation of oxidative stress that puts the hydrobionts at risk (Rimblas, 2004; Ward et al., 2010; Lushchak, 2011; Wang and Wang, 2012; Antunes et al., 2013; Oliveira et al., 2015). In this sense, several studies carried out by our research team, using oxidative stress biomarkers such as lipid peroxidation, protein carbonyl content and the activity of antioxidant enzymes, have shown that the pollutants present in MR, including the above-mentioned metals and pharmaceuticals, increase ROS production and elicit and/or contribute to oxidative stress induction (Galar-Martínez et al., 2010; González-González et al., 2014; Morachis-Valdez et al., 2015).

The involvement of ROS in cell death has been well documented, particularly in the various stages of the apoptotic process that have now been clearly established, such as induction of mitochondrial permeability transition, release of mitochondrial death amplifying factors, intracellular caspase activation, and damage to DNA (Forman and Torres, 2001; Le Bras et al., 2005). Therefore, the pollutants present in MR can potentially induce DNA damage, reduce DNA repair, and increase susceptibility to apoptosis, factors that can all lead to cytotoxicity and mutagenic and carcinogenic events (Baršiene et al., 2013; García-Medina et al., 2013). Biomarkers of genotoxicity and cytotoxicity are useful indicators of the impact of pollutants in aquatic ecosystems since this type of damage affects the fecundity, health and life cycle of the organisms involved (Theodorakis et al., 2000).

The common carp (*Cyprinus carpio*) is a cosmopolitan species living in diverse water bodies around the world, and MR is no exception. In Mexico, in addition to its ecological value, since it is present in 80% of water bodies, it is also economically relevant as a source of food for the human population. In 2012 alone, 7 metric tons of this species, with a commercial value of 6 million US dollars, were grown and consumed in the country (RNPA, 2013; Nava-Álvarez et al., 2014). Like other fishes, *C. carpio* can bioconcentrate and bioaccumulate the pollutants present in water and sediments, which makes it a reliable sentinel organism. It has been used as a bioindicator in toxicity assays and risk assessment due to its sensitivity and easy maintenance under laboratory conditions (Fent et al., 2006). The present study aimed to determine the genotoxicity and cytotoxicity induced on blood, liver and gill of *C. carpio* by pollutants present in water from the MD.

2. Materials and methods

2.1. Study area

MR is located on the Río Tlalnepantla at 19°31′37″ N and 99°15′33″ W. Its dam is flanked by the towns of Nuevo and Viejo Madín, within the municipalities of Naucalpan de Juárez, Atizapán de Zaragoza and Tlalnepantla de Baz (State of Mexico) (Fig. 1), and was built to control river flows and supply drinking water to these municipalities. The impounded lake is also used for recreational activities such as kayaking, sailing, and fishing of common carp. Several urban communities are located in its vicinity, including Nuevo Madín, Viejo Madín, Zona Esmeralda and Lomas Verdes section VI, some of which discharge their waste directly into the reservoir.

2.2. Collection of water samples from the reservoir

Water samples were collected during the wet season (September 2015) using the procedure in the official Mexican norm on wastewater sampling (NMX-AA-003-1980). Sampling was done at surface level with a bottle fitted with an automatic sealing mechanism, at five sampling sites corresponding to: (1) discharge from the town of Nuevo Madín, (2) entry point of the Río Tlalnepantla tributary, (3) side branch of the reservoir, (4) curtain of the dam, and (5) discharge from the town of Viejo Madín (Fig. 1). Samples were placed in plastic bottles previously rinsed with 3% nitric acid, and transported to the laboratory for later analysis and use in toxicity assays. These five sampling sites represent the most relevant entry points of pollutants: domestic wastewater discharges from adjacent towns (1 and 5), influx of the main tributary (2), curtain of the dam (4) and a comparatively uncontaminated site, since there are no adjacent sources of pollutants (3). Sixty liters