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Geno- and cytotoxicity induced on *Cyprinus carpio* by aluminum, iron, mercury and mixture thereof

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ABSTRACT

Metals such as Al, Fe and Hg are used in diverse anthropogenic activities. Their presence in water bodies is due mainly to domestic, agricultural and industrial wastewater discharges and constitutes a hazard for the organisms inhabiting these environments. The present study aimed to evaluate geno- and cytotoxicity induced by Al, Fe, Hg and the mixture of these metals on blood of the common carp *Cyprinus carpio*. Specimens were exposed to the permissible limits in water for human use and consumption according to the pertinent official Mexican norm [official Mexican norm NOM-127-SSA1-1994] Al (0.2 mg L^{-1}), Fe (0.3 mg L^{-1}), Hg (0.001 mg L^{-1}) and their mixture for 12, 24, 48, 72 and 96 h. Biomarkers of genotoxicity (comet assay and micronucleus test) and cytotoxicity (caspase-3 activity and TUNEL assay) were evaluated. Significant increases relative to the control group ($p < 0.05$) were observed in all biomarkers at all exposure times in all test systems; however, damage was greater when the metals were present as a mixture. Furthermore, correlations between metal concentrations and biomarkers of geno- and cytotoxicity were found only at certain exposure times. In conclusion, Al, Fe, Hg and the mixture of these metals induce geno- and cytotoxicity on blood of *C. carpio*.

1. Introduction

Chemical contaminants reach water bodies through diverse sources of exposure. The relevance of heavy metal studies resides in the characteristics of these elements: high toxicity and persistence, and rapid bioaccumulation by aquatic organisms (Cervantes and Moreno, 2010; Rosas, 2001).

A heavy metal is any metallic element having a relative high density and toxicity even at very low concentrations. Such metals include Al, Fe and Hg, among others (Lucho-Constantino et al., 2005). Aluminum is one of the most abundant metals in Earth's crust and is used in diverse anthropogenic activities (García-Medina et al., 2010). Iron is a micronutrient; it is essential for all living organisms and plays a major role in vital biochemical activities such as oxygen transport to tissue, electron transfer, and catalysis (Aisen et al., 2001; Pérez et al., 2005). It is also involved in DNA synthesis and is a component part of hemoglobin, myoglobin, cytochromes and diverse enzymes (Huang et al., 2015). Iron is naturally present in ground and surface water. In aquatic ecosystems, Hg presence results mainly from its atmospheric deposition due to anthropogenic activities (Chan et al., 2003; Morel et al.,

Abbreviations: AAS/AES, atomic absorption/emission spectrophotometry; ICP-MS, inductively coupled plasma-mass spectrometry; MeHg, methylmercury; MNi, micronuclei; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling; pNA, p-nitroaniline; ROS, reactive oxygen species; SEM, standard error of the mean

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1998; Pacyna and Pacyna, 2002). In water, inorganic Hg is transformed by bacterial action to methylmercury (MeHg), a highly toxic compound which accumulates in sediment (Chan et al., 2003; Driscoll et al., 2007; Morel et al., 1998; Ullrich et al., 2001).

Heavy metals enter the aquatic ecosystem through hospital, municipal and industrial residues which are discharged without prior treatment, resulting in the presence of high concentrations (Costa et al., 2015; Fernández-Dávila et al., 2012; García and Dorronsoro, 2005; Kim et al., 2013).

In Mexico, research on the environmental occurrence of this type of contaminants includes studies by Avila-Pérez et al. (1999), who detected Fe (880–33080 mg L^{-1}) and Hg (17–181 mg L^{-1}), among other metals, in surface water and the water column of José Antonio Alzate Reservoir, in the State of Mexico. In Aguascalientes, Al and Fe concentrations were measured in the Río San Pedro, concentrations of 0.06–62.60 and 1.04–22.60 mg L^{-1} respectively were detected (Guzmán et al., 2010). The presence of Fe (1.09–1.80 mg L^{-1}) has been reported in Río Chihuahua water (Gutiérrez et al., 2008), while in the State of Mexico Al (6.04–24.45 mg L^{-1}), Fe (1.37–5.10 mg L^{-1}) and Hg (<0.001 mg L^{-1}) were found in Madín Dam (González-González et al., 2014), and Hg (0.021 mg L^{-1}) was detected in wastewater from a public hospital (Neri-Cruz et al., 2015).

The constant release of contaminants into the aquatic ecosystem implies the presence of a mixture of these. Trace amounts or minimum quantities of metals can have positive or negative effects on aquatic organisms. Slight variations in their concentrations, decreases as well as increases, can have toxic consequences on aquatic organisms (Wittmann, 1981). In fish, Al is neurotoxic; it induces gill damage due to increased mucus production which affects osmoregulation and respiration (Exley et al., 1997; Ward et al., 2006), causing hypoxia, hypercapnia, metabolic acidosis and eventually respiratory insufficiency (Allin and Wilson, 2000; Røyset et al., 2005). Furthermore, it elicits diverse hematological changes (Bhagwant and Bhikajee, 2000; García-Medina et al., 2010). While Fe has diverse biological functions, at high concentrations this metal may induce DNA damage, hemochromatosis and carcinogenesis (Huang, 2004; Mello-Filho and Meneghini, 1991), the principal organs so damaged being the heart, liver and endocrine glands (Italia et al., 2015). In fish, the main damage induced by Hg is at the central nervous system level (Berntssen et al., 2003).

Several studies have shown that exposure to contaminants, including heavy metals, stimulates ROS (reactive oxygen species) production in the cell (Li et al., 2006; Sinha et al., 2007), contributing to oxidative stress generation and, consequently, DNA damage. Metallic ions interact with cellular components such as nuclear proteins and DNA, inducing DNA damage and conformational changes which may lead to cell cycle modulation, carcinogenesis or apoptosis (Beyersmann and Hartwig, 2008; Chang et al., 1996; Wang and Shi, 2001).

Fish are used as sensitive indicators of genotoxic and mutagenic changes since they bioaccumulate contaminants present in water (Yadav and Trivedi, 2009) and their response to chemical exposure is similar to response in higher vertebrates (Al-Sabti, 1991). The common carp *Cyprinus carpio* is a commercial species which, due to its economic importance and wide geographic distribution, has been proposed as a test aquatic organism in toxicological assays (De Boeck et al., 2007; Oruç and Usta, 2007). In Mexico, this species is consumed by humans and is frequently cultured in water bodies contaminated with diverse xenobiotics including heavy metals.

Therefore, the aim of the present study was to evaluate the geno- and cytotoxicity induced by Al, Fe, Hg and the mixture of these metals on blood of *C. carpio*.

2. Materials and methods

2.1. Specimen procurement and maintenance

Common carp (*Cyprinus carpio*) specimens were obtained from the aquaculture facility in Tiacaque (State of Mexico). Polyethylene bags containing water and oxygen were used to transport the fish to the Environmental Toxicology Laboratory at the Department of Chemistry (Universidad Autónoma del Estado de México). Carp were maintained for a 15-d acclimation period in 160-L fish tanks (a fish for each two liters of water was placed) with synthetic culture medium (pH 7.4), at room temperature, with constant aeration and a 12:12 h light/dark photoperiod, and were fed Pedregal Silver™ fish food.

2.2. Experimental design

Specimens 20.15 ± 0.28 cm long and weighing 55.67 ± 6.2 g were maintained under conditions similar to those used for acclimation. To test systems with six carp each was added Al (0.2 mg L^{-1}), Fe (0.3 mg L^{-1}) or Hg (0.001 mg L^{-1}) according to the permissible limits in water for human use and consumption in the official Mexican norm (NOM-127-SSA1-1994). The assay also included a test system to which was added the mixture of all three metals at the same concentrations, as well as a control system. Exposure times were 12, 24, 48, 72 and 96 h. Static systems were used without renewal of test solution. The assay was performed in triplicate, using a total of 450 fish. At the end of the exposure time, carp were removed from the systems and placed in a fish tank containing a xylocaine solution (0.02 mg mL^{-1}, AztraZeneca, Naucalpan, State of Mexico), to anesthetize them prior to collecting a blood sample (2 mL) from the caudal vein, using a heparinized 2-mL hypodermic syringe.

2.2.1. Comet assay

The procedure proposed by Tice et al. (2000) was used. Previously prepared microscope slides were used to obtain the sample. Frosted slides were covered with 200 μL of 1% agarose (Sigma-Aldrich, St Louis, MO) and maintained at room temperature until dry. Whole blood samples (10 μL) were mixed with 75 μL of 0.7% agarose (Sigma-Aldrich, St Louis), and 50 μL of this mixture was spread on the initial agarose layer and solidified on ice. The slides were placed inside a Coplin jar with lysis solution [2.5 M NaCl, 100 mM EDTA, 10 mM Tris (all Sigma-Aldrich, Toluca, Mexico), 10% dimethyl sulfoxide (DMSO, J.T. Baker, Center Valley, PA) and 1% Triton X-100 (Sigma-Aldrich, St Louis)] pH 10, for 1 h at 4 °C. The slides were then placed in the electrophoresis chamber with alkaline solution [300 mM NaOH and 1 mM EDTA (both Sigma-Aldrich, Toluca)] at pH 13 for 20 min. Electrophoresis was performed at 300 mA and 25 V (4 °C, 20 min, field strength: 0.8 V/cm) and was halted with a neutralization buffer [0.4 M Trizma base (Sigma-Aldrich, St Louis) pH 7.4]. The DNA was stained with 50 μL ethidium bromide (10 mg mL^{-1}; Sigma-Aldrich, St Louis) and examined in an epifluorescence microscope (Motic BA410) equipped with a Moticam Pro CCD digital camera.

2.2.2. Micronucleus test

Whole blood from each specimen was fixed with pure ethanol (Mallinckrodt Baker, State of Mexico) on a slide for 5 min, then stained with 10% Giemsa (Hycel, Mexico City, Mexico) for 9 min. A light microscope was used to examine a total of 1000 cells per sample. Results were expressed as the total number of micronucleated cells per 1000 cells (Çavaş and Ergene-Gözükara, 2005).

2.2.3. Caspase-3 activity

2.2.3.1. Cellular extract preparation. Jurkat cells (ATCC # TIB-152)