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Genotoxicity and oxidative stress induced by cadmium and zinc in the planarian, *Dugesia dorotocephala*

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This study sought to determine the DNA damage, and the lipoperoxidative effect, as well as changes in the superoxide dismutase (SOD) and catalase (CAT) activities induced by $CdSO_4$ (Cd), and $ZnSO_4$ (Zn), in addition to two different mixtures of the metals. Planarian *Dugesia dorotocephala* collected in the Ignacio Ramirez reservoir, México, adapted to laboratory conditions, and exposed to the metals in a controlled system was used. Initially, LC_{50} at 96 h of exposure was determined and the result obtained were 0.69 mg/L for Cd, 11.99 mg/L for Zn, 10.28 mg/L for mix 1, and 8.11 mg/L for mix 2. Then, the comet assay showed a DNA damage increase induced by Cd (0.13 and 0.2 mg/L) as high as 94% over the control level; the effect by Zn (from 0.2 to 2.7 mg/L) was clearly lower, although statistically significant with the high concentrations tested. As regards the two mixtures, we observed a concentration dependent increase. Similarly, in respect to lipoperoxidation, we found a strong effect by Cd, a slight effect by Zn, and a concentration dependent effect induced by the mixtures. Finally, the activity of the tested enzymes was modified by the metals in relation to the concentration applied.

Key words: Zinc, cadmium, planarian, DNA damage, oxidative stress.

INTRODUCTION

The level of contamination with heavy metals has been increasing in the last decades, partially because of increase in the use of such metals in a variety of agricultural and industrial processes. This has stimulated studies to identify the potential toxicity induced by such agents. In this regard, it has been reported that cadmium and zinc can exert toxicity at specific experimental conditions, which may also depend on the evaluated organism and the associated environmental factors (Kraemer et al., 2005; Aravind et al., 2009).

Cadmium is used in various industrial processes, and its exposure in humans has been suggested to be involved in the development of osteoporosis, anemia, emphysema, and kidney damage, besides being a potent carcinogen (Cuypers et al., 2010). On the other hand, zinc is also used for a number of industrial, medical, and cosmetic purposes. In animals, this metal plays a role regarding membrane integrity, regulation of insulin and glucose blood concentration, in the correct maintenance of the immune system, stabilization of transcription

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factors, and in cell signal transduction, moreover, zinc is a component of more than 70 enzymes (Valko et al., 2005). In higher amounts however, the chemical may affect the hematopoietic and endocrine systems (Valko et al., 2005; Johnson et al., 2011). Cadmium and zinc have some common characteristics, and interactions. For example, the presence of cadmium may aggravate a deficit of zinc due to its partial replacement in catalytic enzymes, and may inhibit the joining to DNA of compounds with zinc fingers in their structure, an effect that can be related with the similar ionic ratio of the two metals (Valko et al., 2005; Aravind et al., 2009; Hartwig, 2010; Nzengue et al., 2011). Besides, both metals may interact with phosphotidiletanolamine, phosphatidilserine, sulphydrils, and protein phosphate giving rise to different levels of their inactivation (Jihen et al., 2009; Rogalska et al., 2009).

Heavy metals reach aquatic systems through industrial or agricultural discharges, as well as by urban pluvial water (Vardanyan and Ingole, 2006; Dazy et al., 2009). Fishes exposed to cadmium have shown anemia, hyperexcitability, hypocalcemia, and muscular contractibility (Remyla et al., 2008; Pretto et al., 2010); besides, it is known that the agent may alter stationary cycles and spermatogenesis, provoke testicular necrosis and histological anomalies in other organs (Bonda et al., 2004; van Dyk et al., 2007; Dietrich et al., 2010; Garcia-Santos et al., 2013). With respect to the matter of our present report, cadmium is known to induce reactive oxygen species (ROS) in a number of aquatic organisms which, consequently, may be reflected in protein, lipid, or DNA damage (Jia et al., 2011; Dabas et al., 2012).

As regards the effect of zinc, embryological damage has been reported in *Pagrus major* including alterations in fecundity, larval size, as well as in embryos and larval survival (Huang et al., 2010). Toxicity in gills, hepatopancreas and muscle of several aquatic invertebrates has also been observed, as well as alterations in oxygen consumption, ammonia excretion, and osmotic pressure (Elumalai et al., 2007). Finally, zinc is known to alter oxidative processes in different aquatic organisms, for example, by modifying the activity of antioxidant compounds, as well as by increasing the level of lipoperoxidation (Geret and Bebianno, 2004; Franco et al., 2008).

In aquatic ecosystems, it is usual that more than one metal coexist. With reference to the presence of both cadmium and zinc, few studies have been published in aquatic organisms. However, reports have shown the induction of low osmotic pressure, increase of metallotioneins, and generation of ROS and antioxidant enzymes (Kraemer et al., 2005; Aravind et al., 2009).

Planarians are flatworms that live in the interphase of water and sediment layers, in contact with stones, roots and leaves of plants; therefore, they can be highly exposed to discharges of a variety of contaminants, and can be suitable organisms for studies of cyto/genotoxicity. Planarians have also been used as bio-indicators to assess toxic, biochemical, reproductive, carcinogenic, and teratogenic effects by potentially harmful substances (Guecheva et al., 2001; Horvat et al., 2005; Prá et al., 2005). Moreover, they have been suggested as an appropriate model for comparing field and laboratory studies (Guecheva et al., 2001; Zhang et al., 2012). The use of planarians in the genotoxicity field has also been increasing, including the evaluation of chromosome aberrations and micronuclei, as well as the DNA damage in a lesser extent (Lau et al., 2007; Knakievicz et al., 2008; Guecheva et al., 2001). Therefore, based on the above mentioned information, the present study was made to determine the genotoxic and oxidative damage induced by zinc and cadmium, separated or combined, on planarians exposed in laboratory conditions. This will contribute to the understanding of the toxicological significance of the studied metals in aquatic ecosystems.

MATERIALS AND METHODS

Chemicals

The following chemicals were obtained from Sigma Chemicals (St Louis, MO, USA): $3CdSO_4•8H_2O$ (Cd), $ZnSO_4•7H_2O$ (Zn), phosphate buffer saline (calcium-and magnesium free, PBS), hidroxymethyl aminomethane hydrochloride (tris), sodium carbonate, disodium ethylenediamine-tetra-acetate (EDTA), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), dimethyl sulfoxide (DMSO), thiobarbituric acid (TBA), trichloroacetic acid (TA), Coomassie brilliant blue G, ethidium bromide (EB), bovine albumin, (-)-norepinephrine, and superoxide dismutase (SOD). Low melting point agarose (LMPA) and normal melting point agarose (NMPA) were purchased from GIBCO-BRL (Grand Island, NY, USA). Nitric acid and dibasic potassium phosphate were obtained from J.T. Baker (Phillisburg, NJ, USA).

Planarians

Dugesia dorotocephala specimens, (Platyhelmynthes, Turbellaria, Tricladida) were collected in the shore of the Ignacio Ramirez Reservoir, which is located 90 km south of Mexico City, and immediately transported to laboratory conditions. Planarians were cultivated in previously washed plastic containers where 5 L of reconstituted water [$NaHCO_3$ (174 mg/L), $MgSO_4$ (120 mg/L), KCl (6 mg/L), and $CaSO_4•2H_2O$ (120 mg/L), at pH 8] was added. The system was kept with constant air flow (6.4 to 6.6 mg/L of O_2) at room temperature, and the organisms were fed with chicken liver once a week. The organisms were cultivated for about a year (Amaya-Chávez et al., 2009); for the study, we used planarians 10 to 15 mm long corresponding to the second generation obtained by asexual reproduction.

Acute toxicity

Initially, we determined the acute toxicity level of both metals, as well as that of their mixture. In each case, ten animals per group were exposed to metals in recipients with 40 mL of reconstituted water (OECD, 2006; Greene et al., 1988). Feeding was not allowed during the assay and mortality was observed at 96 h of exposure (Knakievicz and Ferreira, 2008). The experiment was made in

Table 1. Concentrations tested for determining the LC_{50} of the studied metals.

Metal	Concentration (mg/L)
Cd	0.25, 0.39, 0.63, 1.00, 1.58
Zn	10.00, 10.96, 12.02, 13.18, 14.45
Mix 1	Cd 0.13 + Zn 6.01, 7.57, 9.53, 11.99, 15.09
Mix 2	Cd 0.2 + Zn 6.01, 7.57, 9.53, 11.99, 15.09

Observations were made at 96 h of exposure

triplicate and the LC_{50} calculation with 95% confidence limits was determined with the Probit method by using the EPA Probit Analysis Program, version 1.5. Table 1 shows the concentrations used for each metal in independent form, as well as those used for the two tested mixtures. In this last case, the variation was made in regard to the concentration of Zn, while the concentrations of Cd were constant: in mix 1 we used 0.13 mg/L of Cd, concentration at which no deaths occurred at 96 h (LC_0), plus 5 concentrations of Zn, and in mix 2 we applied 0.2 mg/L of Cd, its maximum permissible limit (NOM-001-1996) plus 5 concentrations of Zn.

Sublethal toxicity

Tested concentrations

To carry out the sublethal determinations of the present study, we initially obtained the LC_0 for mix 1 and mix 2. In the first case, such measurement was reached with 2.7 mg Zn/L + 0.13 mg/L of Cd. Then, the testing range of mix 1 was constituted by a constant amount of Cd (0.13 mg/L), concurrently with five different concentrations of Zn (0.7, 1.2, 1.7, 2.2, and 2.7 mg/L). These combinations corresponded approximately to 25, 44, 62, 81, and 100% of the LC_0 determined for the mix. Besides, in independent form, we tested the effect of 0.13 mg/L of Cd, as well as the same 5 concentrations of Zn included in the mixture. In regard to mix 2, its LC_0 was reached with 1.5 mg Zn/L + 0.2 mg Cd/L. In this case, the constant amount of Cd was 0.2 mg/L, concurrently with 5 different concentrations of Zn (0.50, 0.75, 1.0, 1.25, and 1.50 mg/L). These combinations corresponded approximately to 16, 50, 66, 83, and 100% of the LC_0 determined for the mix. In independent form, we also evaluated the effect of 0.2 mg/L of Cd, as well as the same 5 concentrations of Zn included in the mixture.

DNA damage

To determine the genotoxic potential of the evaluated metals, we applied the comet assay after 96 h of planarian exposure to the compounds. Such exposure was carried out under experimental conditions similar to those indicated for acute toxicity. Three replicates with 10 planarians each were exposed to the metals. Animals were placed in 500 µL of PBS and homogenized for 1 min; then, the degree of cellular viability was determined by means of the trypan blue exclusion technique (Ternjej et al., 2009). With this determination, we found 89% as the mean of viable cells.

The alkaline comet assay was performed as previously described (García-Medina et al., 2011). Each sample of cell suspension (10 µL) was mixed with 75 µL of LMPA 0.75%, and placed on a pre-coated microscope slide with 100 µl of NMPA (1%) for 5 min at 4°C, to allow solidification. Slides were immersed for 1 h at 4°C in the lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM trisma base, 10% DMSO, and 1% triton X-100, at pH 10), and then placed in a horizontal electrophoresis chamber, where cells were exposed to an alkaline solution (NaOH 300 mM, and EDTA 1 mM, at pH>13) to allow DNA unwinding and the expression of alkali-labile sites. In the next step, cells were electrophoresed at 300 mA, 25 v, and pH>13 for 20 min; the process was neutralized by rinsing the slides in trisma base 0.4 M at pH 7.4, and the nucleoids of each slide were stained with 50 µL of EB (20 µg/mL). The analysis was made at 400X magnification by means of an epifluorescent microscope (Axioscope, Carl Zeiss) equipped with a digital camera and software for the capture, processing and image analysis (Zeiss KS400 version 3.01). In 100 nucleoids per sample, the length to width index was determined by measuring the image length (T) and dividing the result by the nuclear diameter (N) so as to determine the T/N index (García-Medina et al., 2011).

Oxidative stress

To determine the level of oxidative stress, we exposed planarians to the metals for 96 h, and afterward, we assayed three replicates of 0.03 g of planarian. For this purpose, flatworms were homogenized for 1 min in 1 mL of PBS at pH 7.4. Then, the suspension was divided in two parts: one was centrifuged at 16000 x *g* for 15 min at 4°C and the supernatant was used to determine the protein content, as well as the activity of antioxidant enzymes; the other part was used to determine the level of lipid peroxidation.

Initially, we determined the protein content by using the method of Bradford (Noble and Bailey, 2009) to 25 µL of each evaluated supernatant. To evaluate lipoperoxidation in the organisms we quantified the level of malondialdehyde (MDA) according to the method described by Buege and Aust (1978) with slight modifications. Finally, to determine the activities of superoxide dismutase and catalase, we applied the methods described by Misra and Fridovich (1972) and Radi et al. (1991) with slight modifications.

Statistical analysis

In the case of the oxidative stress biomarkers, we applied a parametric ANOVA test followed by a Tukey multiple comparison test. In regard to the results obtained with the comet assay, we applied a non-parametric Kruskal–Wallis test, as well as the Dunn multiple comparison test. The analysis was carried out by using the Sigmastat Program, version 2.03.

RESULTS

Acute toxicity

The results obtained in regard to this parameter are shown in Table 2. We found a highly significant toxicity of Cd, which corresponded to more than 15 times the effect produced with Zn. The values determined for mix 1 and 2 tended to follow the results obtained with Zn alone, although showing somewhat more toxicity. Besides mortality, the planarians' toxic effect was manifested by changes in the size and color of their ocular spots and the slowing of body movements, followed by complete immobilization, body rolling, and tissue loss.

DNA damage

Figure 1A shows that 0.13 mg/L of Cd induced an in-

Table 2. Acute toxicity induced in *Dugesia dorotocephala* by Cd, Zn, and their mixtures.

Metal	LC_{50} (mg/L)	LC_{95} (mg/L)	LC_0 (mg/L)	Slope	R^2
Zn	11.99 (11.67-12.33)	14.26 (13.66-15.26)	7.09	21.88	0.9872
Cd	0.69 (0.62-0.77)	1.18 (1.02-1.52)	0.13	7.03	0.9103
Mix 1	10.28 (9.61-11.02)	15.69 (14.07-18.65)	2.7	8.96	0.9596
Mix 2	8.116 (7.40-8.77)	13.77 (12.17-16.93)	1.5	7.16	0.931

Results were obtained in ten planarians per group with three replicates using the EPA Probit analysis program, version 1.5. Values in parenthesis correspond to 95% confidence intervals. Mix 1= five different concentrations of Zn (from 6.01 to 15.09 mg/L) plus 0.13 mg/L Cd. Mix 2= five different concentrations.

crease of 83% over the control T/N index, and that, with respect to Zn, the T/N index increase induced with the three high concentrations tested was no more than 60% over the control level. Such index represents the extent of the comet, and was obtained by dividing the image length (T) by the nuclear diameter (N). The combination of both metals induced a concentration dependent response, and showed that Cd plus the tested high concentration of Zn gave rise to DNA damage similar to that observed with Cd alone. In Figure 1B, we observed a stronger effect by Cd alone (94 % over the control level), no significant effect with all the tested concentrations of Zn, and a concentration dependent effect induced by mix 2, which, as in the previous combination, reached a T/N index similar to that determined for Cd alone.

Lipid peroxidation

Figure 2 presents results obtained in regard to this evaluation. Figure 2A shows an increase of about five times the level of lipoperoxidation induced by Cd with respect to the control value, and a lower elevation induced by the highest concentration of Zn (two times over the control level). We also found a concentration-dependent increase induced by mix 1. In this case, the increase obtained with the two high concentrations of Zn was clearly superior to the lipoperoxidation induced with Cd alone, being 20% higher than such level. Figure 2B shows a pattern similar to that previously described, but with data more clearly marked. Cd had an increase of 8 times over the control level, and the tested high concentration of mix 2 reached 30% more lipoperoxidative activity than the level found with Cd alone.

Superoxide dismutase activity

Figure 3A shows the results obtained for the metals and mix 1 concerning SOD activity. In comparison with the control level, we found a decrease of such activity by the exposure to Cd, a significant increase with the two low concentrations of Zn, as well as with the low concentration of the mixture. In Figure 3B, a pattern similar to the one mentioned above is observed, although with variations related to the differences in the used concentrations. For example, 0.2 mg/L of Cd originated a stronger depression of SOD activity, and the low concentration of Zn created a stronger increase in such activity. Also, the effect obtained with mix 2 was generally lower than that observed with mix 1.

Catalase activity

Results of this activity are presented in Figure 4. In comparison with the control organisms, we observed more than a 40% decrease of CAT activity induced by Cd (0.13 mg/L), and an increase of such activity with all tested concentrations of Zn, which reached a level as high as 300 % in regards to the control level. Besides, we determined that the exposure to mix 1 gave rise to CAT activities which were in the range of the control level (Figure 4A). Figure 4B shows a similar pattern to the one described above, although with a stronger decrease of CAT activity by Cd (0.2 mg/L), a result that was also observed in regard to the effect of mix 2.

DISCUSSION

This work for the first time studies the toxic effect of Cd and Zn by using planarians as the experimental model. With respect to acute toxicity, previous determinations with the selected metals had been made in other aquatic invertebrates (Dap, 1998; Vanegas et al., 1997; Wu and Chen, 2004; Barbieri, 2009). A comparison of our LC_{50} with the one determined in the mentioned organisms suggests that *D. dorotocephala* is more sensitive for evaluating the toxicity of the studied chemicals, and therefore, that planarian is a useful model to determine metal toxicity. This conclusion is also congruent with the strongest response found with respect to Cd, 17 times greater than that of Zn, which is an expected result, considering that heavy metal toxicity in aquatic organisms

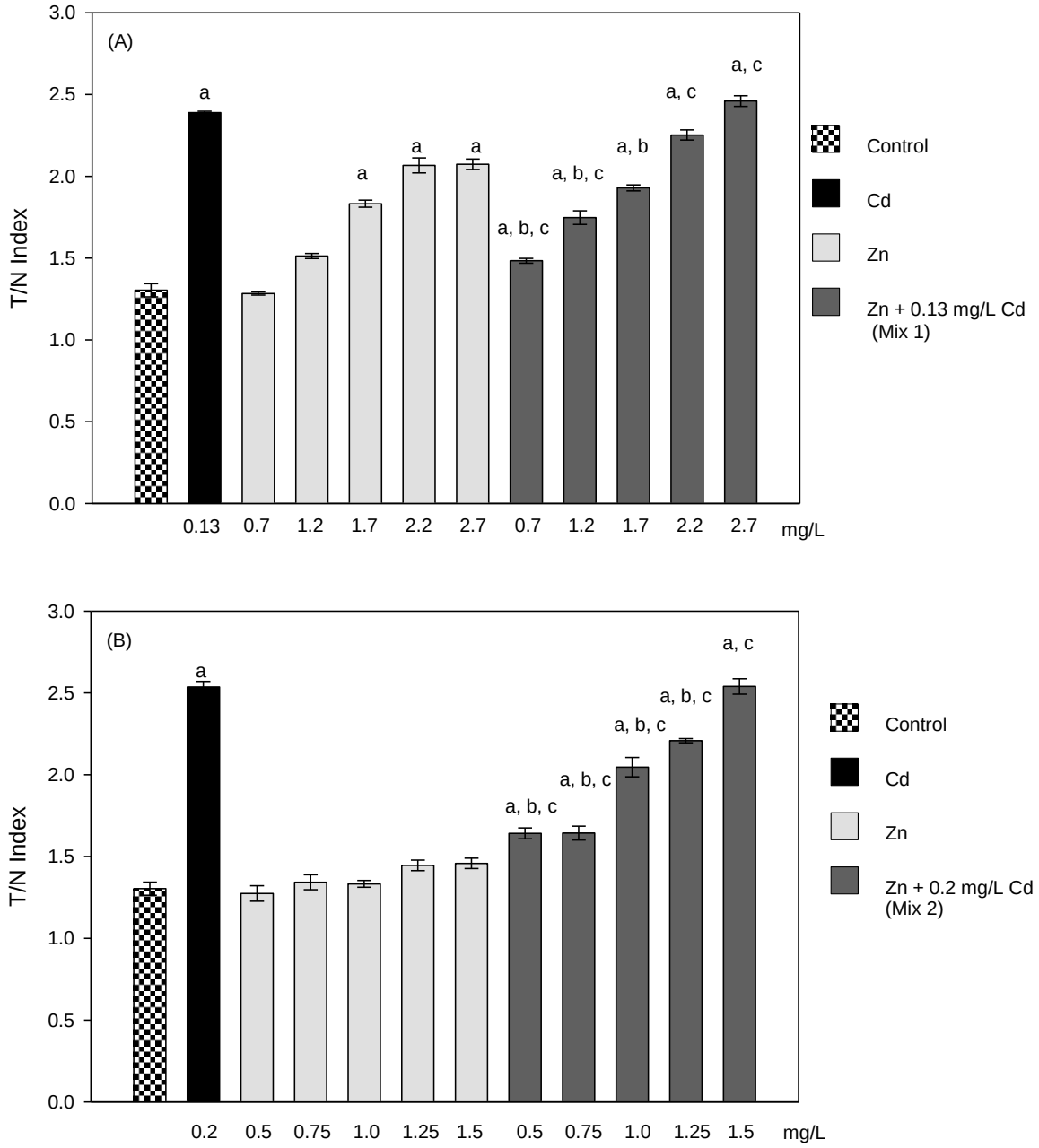

Figure 1. DNA damage induced in *Dugesia dorotocephala* by Cd, Zn, and their mixtures. The determinations were made with the comet assay at 96 h of exposure. Bars represent the mean ± SEM of three replicates with 5 individuals each. [a]Statistically significant difference with respect to the control value, [b]with respect to Cd value, and [c]with respect to the Zn value. Kruskal–Wallis and Dunn tests, $P < 0.05$. Figure 1A includes the effect of 0.13 mg/L of Cd, the effect of Zn (0.7 to 2.7 mg/L), and of mix 1 formed by 0.13 mg/L of Cd plus each of five different concentrations of Zn (0.7 to 2.7 mg/L). Figure 1B includes the effect of 0.2 mg/L of Cd, the effect of Zn (0.5 to 1.5 mg/L), and of mix 2 formed by 0.2 mg/L of Cd plus each of five different concentrations of Zn (0.5 to 1.5 mg/L).

is usually low for compounds with biological activity, such as Zn, in contrast with Cd that has no known function (Barbieri, 2009).

In regard to the acute toxicity induced by the mixtures, the two LC_{50} obtained suggest that Cd plays an important role in the observed toxicity. For example, when the exposure to Cd increased from 0.13 to 0.2 mg/l, the LC_{50} increased 21 % even when it was combined with the lower concentration of Zn (8.1 m/L). The interaction between the two involved compounds was related with the expression of similar mechanisms of action, as suggested by their similarity regarding the ionic ratio and

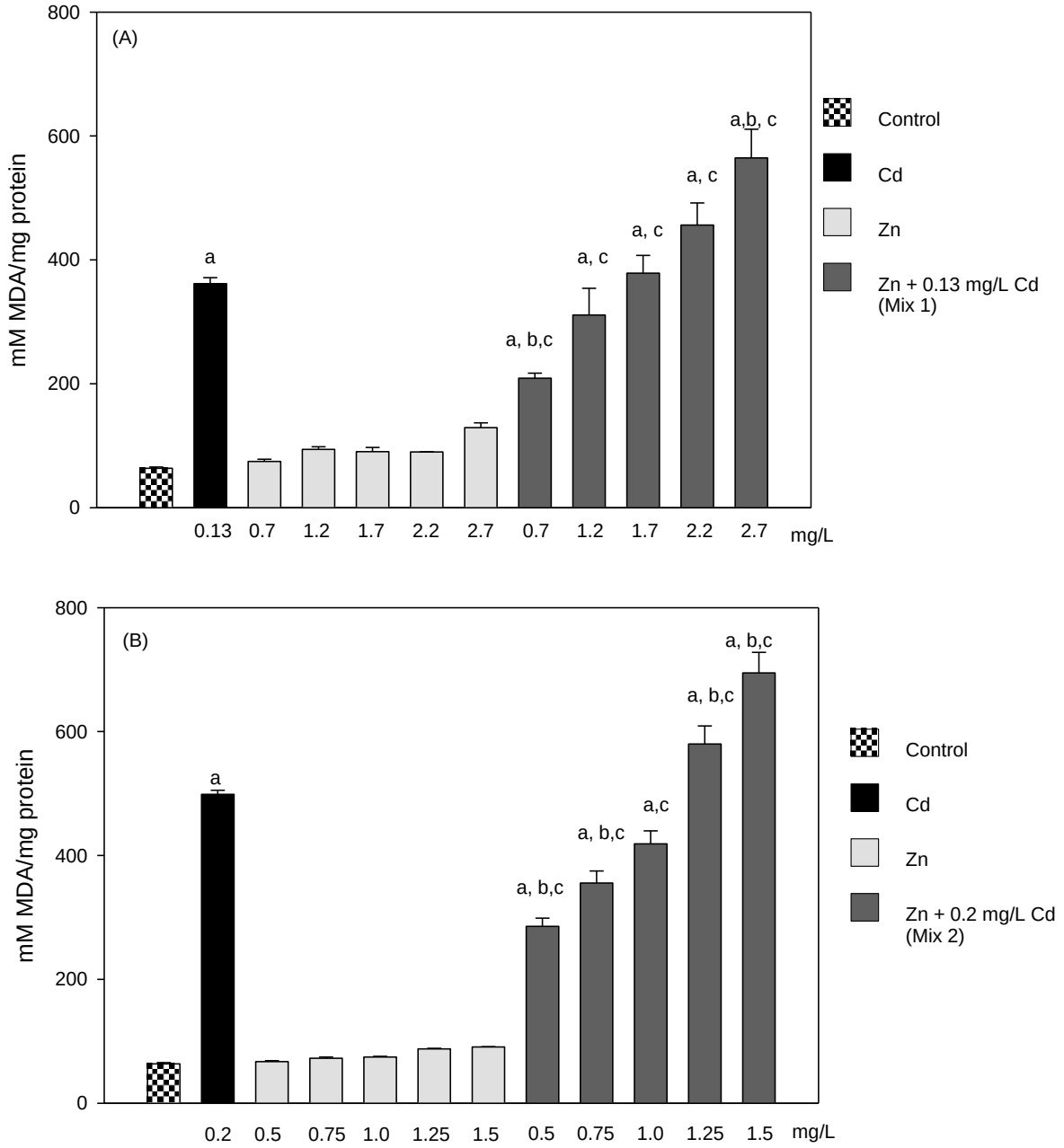

Figure 2. Lipid peroxidation induced in *Dugesia dorotocephala* by Cd, Zn, and their mixtures. The determinations were made at 96 h of exposure. Bars represent the mean ± SEM of three replicates with 0.03 g of planarians each. [a]Statistically significant difference with respect to the control value, [b]with respect to Cd value, and [c]with respect to the Zn value. ANOVA and Tuckey tests; $P < 0.05$. Figure 2A includes the effect of 0.13 mg/L of Cd, the effect of Zn (0.7 to 2.7 mg/L), and of mix 1 formed by 0.13 mg/L of Cd plus each of five different concentrations of Zn (0.7 to 2.7 mg/L). Figure 2B includes the effect of 0.2 mg/L of Cd, the effect of Zn (0.5 to 1.5 mg/L), and of mix 2 formed by 0.2 mg/L of Cd plus each of five different concentrations of Zn (0.5 to 1.5 mg/L).

oxidation number (Gallego et al., 2007).

The capacity of xenobiotics to affect the organisms' DNA is a useful marker to evaluate their genotoxicity, and the use of the comet assay has proved to be a sensitive, reliable and rapid method to determine DNA strand breaks, alkali-labile sites and repair. The effect of Zn and Cd had not been studied in planarians; however, various reports have suggested the usefulness of these organisms to determine DNA damage by environmental contaminants, such as with copper sulfate and the pesticide norflurazon (Guecheva et al., 2001; Horvat et al., 2005). Also, flatworms have been successfully used

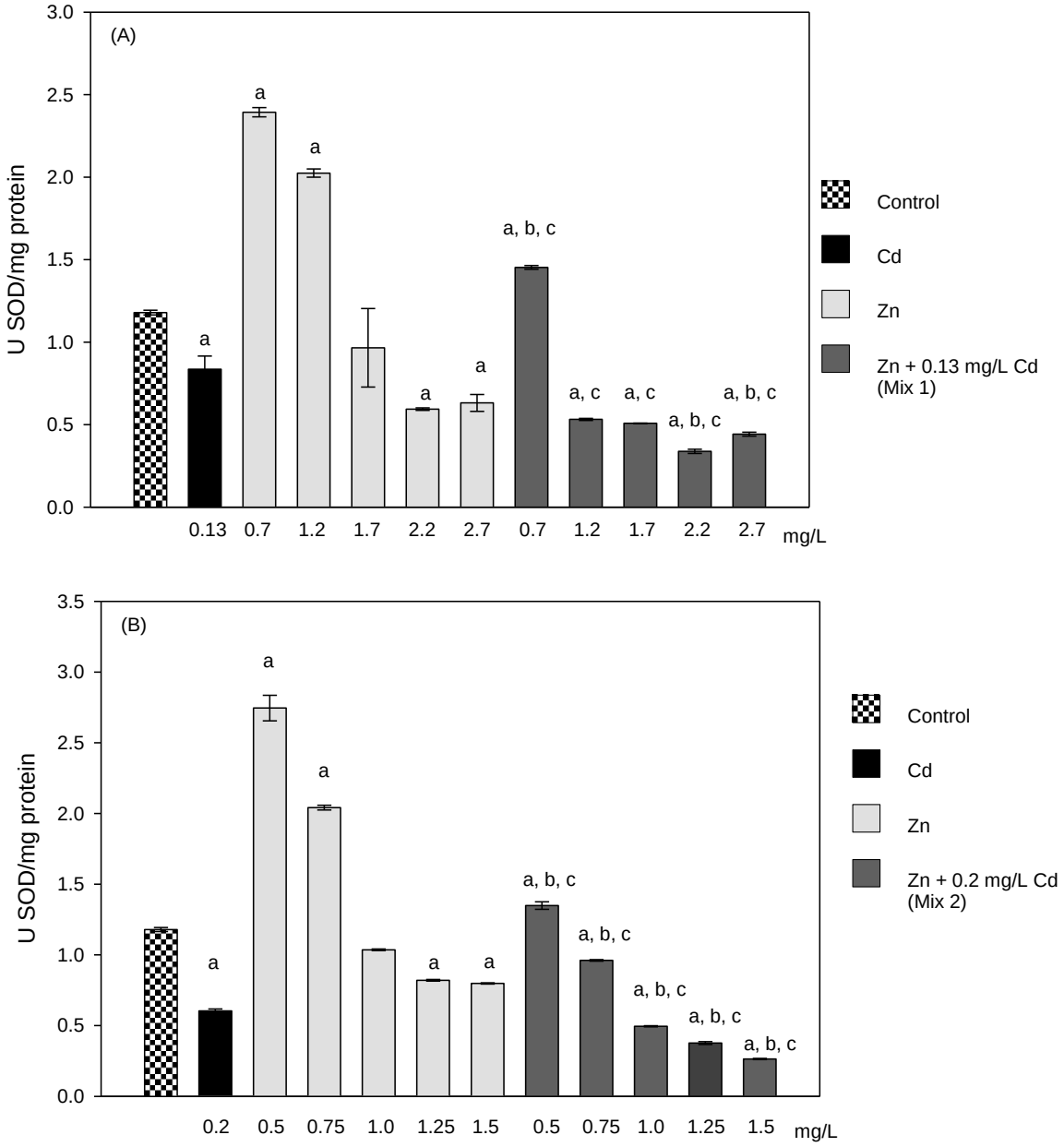

Figure 3. Effect of Cd, Zn, and their mixtures on the activity of superoxide dismutase (SOD) in *Dugesia dorotocephala*. The determinations were made at 96 h of exposure. Bars represent the mean ± SEM of three replicates with 0.03 g of planarians each. [a]Statistically significant difference with respect to the control value, [b]with respect to Cd value, and [c]with respect to the Zn value. ANOVA and Tuckey tests; $P < 0.05$.Figure 3A includes the effect of 0.13 mg/L of Cd, the effect of Zn (0.7 to 2.7 mg/L), and of mix 1 formed by 0.13 mg/L of Cd plus each of five different concentrations of Zn (0.7 to 2.7 mg/L). Figure 3B includes the effect of 0.2 mg/L of Cd, the effect of Zn (0.5 to 1.5 mg/L), and of mix 2 formed by 0.2 mg/L of Cd plus each of five different concentrations of Zn (0.5 to 1.5 mg/L).

to determine a correlation between the genetic damage and the level of urban stream water (Prá et al., 2005). Moreover, flatworms have been sensitive to the genotoxic effect of agents with different mechanism of action, such as methyl methanesulfonate, cyclophosphamide, and gamma radiation (Lau et al., 2007).

In the present assay, we clearly established the genotoxic effect by Cd, as well as a lower potential for Zn. Our results with Cd were congruent with previous studies made in fishes such as *Anabas testudineus,* and in rainbow trout (Ahmed et al., 2010). Such effect may be related with two different types of alterations, one of

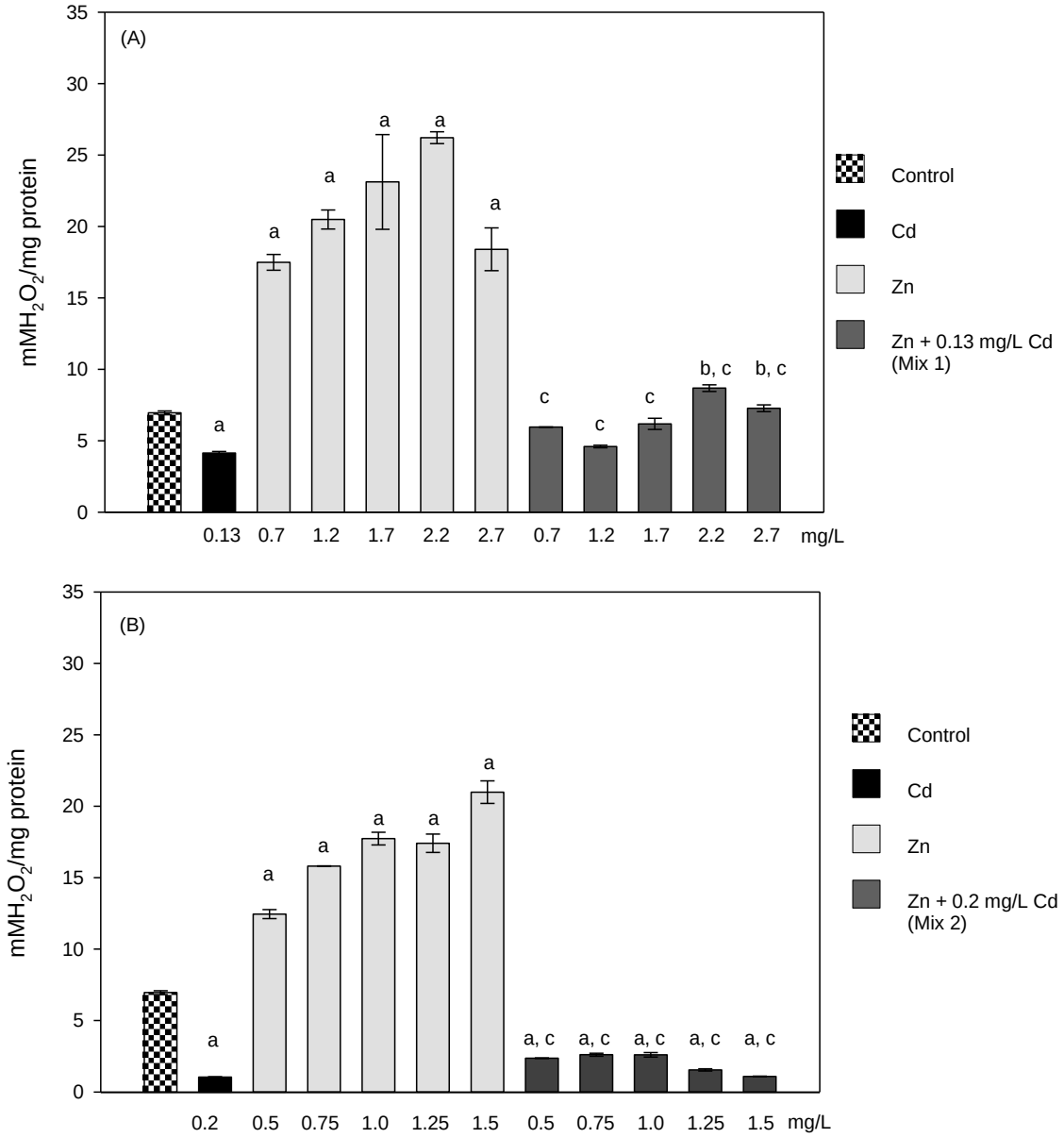

Figure 4. Effect of Cd, Zn, and their mixtures on the activity of catalase (CAT) in *Dugesia dorotocephala.* The determinations were made at 96 h of exposure. Bars represent the mean ± SEM of three replicates with 0.03 g planarians each. [a]Statistically significant difference with respect to the control value, [b]with respect to Cd value, and [c]with respect to the Zn value. ANOVA and Tuckey tests, $P < 0.05$. Figure 4A includes the effect of 0.13 mg/L of Cd, the effect of Zn (from 0.7 to 2.7 mg/L), and of mix 1 formed by 0.13 mg/L of Cd plus each of five different concentrations of Zn (from 0.7 to 2.7 mg/L). Figure 4B includes the effect of 0.2 mg/L of Cd, the effect of Zn (from 0.5 to 1.5 mg/L), and of mix 2 formed by 0.2 mg/L of Cd plus each of five different concentrations of Zn (from 0.5 to 1.5 mg/L).

these refers to different disturbances of DNA repair (Hartwig, 2010), and the other, which has been more deeply studied, refers to the induction of ROS. In this last case, disruption of cellular homeostasis by the metal can lead to oxidative stress, a state where increased formation of ROS overwhelms body antioxidant protection and subsequently induces DNA damage, lipid peroxidation, protein modification and other effects. In this case, the metal may form superoxide, hydroxyl and other radicals, which in turn may give rise to the mutagenic/carcinogenic MDA and other exocyclic DNA adducts, which may affect a number of cellular processes such as proliferation, differentiation, apoptosis, and the inactivation of the tumor suppressor genes (Filipič et al., 2006; Hartwig, 2010; Jomova and Valko, 2011). Our results show a significant elevation in the level of lipid

peroxidation, and decreases in the activity of antioxidant enzymes related with the efforts to cope with ROS. These alterations suggest then that the induction of free radicals might have been a factor in the observed DNA damage. This agrees with observations in other aquatic animals, where the metal has been reported to induce DNA damage, alterations in gene expression and/or elevation in oxidant biomarkers (Schröder et al., 2005; Planelló et al., 2007; Jia et al., 2011). On the contrary, our results regarding DNA damage by Zn showed a lower effect than that determined with Cd. The result agrees with the moderate but significant level of lipoperoxidation detected with the metal, and, interestingly, with an increase in the activity of SOD and CAT with lower concentrations as a probable response to counteract the excess of ROS, and their decrease with higher concentrations of Zn, which is also an observed response of antioxidant enzymes against oxidative damage (Jia et al., 2011).

Zinc is an essential component of proteins which, in fact, may be involved in the defense against oxidative stress. Its depletion is what can more often be related with DNA damage. Nevertheless, depending on the amount of the metal, and on the examined organism or tissue, various studies have shown that it can also be involved in the production of ROS; for example, in mouse TS/A adenocarcinoma cells (Provinciali et al., 2002), and in the cancer HEP-2 cell line (Rudolf et al., 2005). Also, in aquatic organisms, such as the clam *Ruditapes decussatus* and the brown mussel *Perna perna*, high concentrations of Zn are known to modify the level of antioxidant enzymes and to enhance lipoperoxidation (Geret and Bebiano, 2004; Franco et al., 2008).

Knowledge regarding the characteristics and consequences of compound interaction, particularly in regard to metals, is an incipient and complex area of research, where a number of conditions may modify the observed effect, including the involved tissue/organ, metal ratios, concentration tested and duration of the exposure (Kamunde and MacPhail, 2011). Data of our study in regard to lipoperoxidation suggest a synergistic effect by the two metals at high doses, at low doses however, our results suggest an antagonist effect. Such results are congruent with those obtained by Cherif et al. (2011) in *Solanum lycopersicum*. The authors found that supplementation of low concentrations of zinc to cadmium treated plants caused a significant decrease in TBARS content, suggesting a role of zinc in the stabilization of biomembranes from oxidative damage. In the case of the antioxidant enzymes, the metals gave rise to a modification in their activity as a response to the oxidative damage. In this context, a high degree of correlation between Cd and Zn was found in *Daphnia magna*, where the authors also suggest that such interactions may affect the bioavailability of the involved metals (Komjarova and Blust, 2008).

The effects induced in planarian by the tested mixtures may be related with the binding capacity that Cd has on various biomolecules and transition metals, which are particularly sensitive because they enclose cationic sites to which the toxic metal can bind (Moulis, 2010). However, the understanding of such an interaction seems far more complex given that, for example, both metals are able to induce metallotioneins, which are oxidative stress inhibitors, and therefore, their combination may modify cellular activities depending on the specific rates involved (Rana and Kumar, 2000). Interestingly, the interaction between the metals studied in this report could be of relevance for human health because a high Cd:Zn ratio can be a useful biomarker for prostate cancer in smokers, in view that it seems a central element associated with oxidative stress, DNA damage, mutation, and other effects related with carcinogenic risk (Anetor et al., 2008).

In summary, the present assay confirmed the importance of planarians to evaluate xenobiotic's toxicity, a fact that can be partially related with their regeneration capacity, easy reproduction, and their habitat, which may be in contact with a variety of contaminants. Besides, we determined that Cd is a stronger DNA damage and oxidative inducer than Zn, and that, when combined, the metals show an evident interaction that affects the measurement of DNA damage, lipoperoxidation, and the studied antioxidant enzymes; therefore, our study suggests being careful with aquatic metal contamination.

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