© by PSP Volume 25 – No.10/2016, pages 4052-4059 Fresenius Environmental Bulletin

RESPONSES OF MULTIPLE BIOMARKERS IN THE FISH *HOPLOSTERNUM LITTORALE* AFTER EXPOSURE TO CHROMIUM AND LEAD

Analía Ale[1], Carla Bacchetta[1], Jimena Cazenave[1,2*]

[1]Laboratorio de Ictiología, Instituto Nacional de Limnología (INALI-CONICET-UNL), Santa Fe, Argentina
[2]Facultad de Humanidades y Ciencias, Universidad Nacional del Litoral (FHUC-UNL), Santa Fe, Argentina

ABSTRACT

We studied the effect of chromium (Cr) and lead (Pb) and their combination using the freshwater fish *Hoplosternum littorale*. After 15 days, we analyzed the responses of multiple biomarkers, such as morphometric and hematological parameters, hepatic and renal enzymes activities (aspartate and alanine aminotransferases, alkaline phosphatase) and oxidative stress markers (antioxidant enzymes activities and lipid peroxidation). Cr caused a decrease in glucose levels and tissue damage in liver and kidney. Pb caused changes in hematological parameters, tissue damage in liver and kidney and induced liver lipid peroxidation. The metal combination modified hematological parameters and increased the hepatic injury biomarkers. According to multivariate statistical analysis, fish exposed to the mixture showed a differential physiologic profile to those exposed to individual metals. Thus, *H. littorale* has demonstrated to be sensitive for biomarkers response and suitable as potential test species. Our results suggest deleterious effects of sublethal concentrations of heavy metals and support the usefulness of a multi-biomarker approach for the characterization of toxicological mechanisms induced by the exposure to a combination of Cr and Pb.

KEYWORDS:
fish; heavy metals; hematology; oxidative stress; transaminases

INTRODUCTION

Heavy metals occur naturally in the environment and they are found in both ground and surface waters. Fish can absorb metals through the skin, the gill epithelium and the gastrointestinal tract. They are accumulated mainly in kidney, liver and gills, which anatomical and physiological features make them key target organs of toxic substances [1].

Chromium (Cr) and lead (Pb) have been found in water bodies of Argentina [2-4] and in native species tissues [1, 5]. Low concentrations of Cr can generate morphological abnormalities in fish liver and gills [6]. As regards Pb, it has caused cellular abnormalities by micronucleus formation [7] and inhibits the activity of the δ-aminolevulinic acid dehydratase enzyme in aquatic organisms [8].

Although toxicity of individual metals has been extensively studied in fish [9, 10], data about the effects of their mixtures on fish biomarkers are scarce. Some laboratory studies have investigated the effects of mixtures such as lead and cadmium [11], zinc and cadmium [12], chromium and nickel [13]. To our knowledge, this is the first report of a multi-biomarker assessment carried out on fish exposed to combined Cr + Pb.

The combined use of a set of complementary biomarkers can detect exposure to contaminants and quantify their impact on living organisms. Consequently, the multi-biomarker approach has gained considerable interest in ecotoxicological research, and has been recently applied in field and laboratory studies [14-16].

Hoplosternum littorale (Pisces, Callichthyidae) is a neotropical fish species widely distributed in South America. Because they are easy to collect and maintain in laboratory conditions [17-18], *H. littorale* is proposed as test species.

This study is aimed at evaluating multi-biomarker responses in *H. littorale*, after controlled exposure to an environmental concentration of Cr and Pb alone, and in combination. The multi-biomarker approach focuses on morphometric indexes, hematological responses, tissue damage markers and oxidative stress parameters.

MATERIALS AND METHODS

Fish. Adult *H. littorale* (n = 32; 9.38 ± 0.77 cm standard length; 21.95 ± 4.82 g) were obtained from an aquarium trade. For acclimation purpose, fish were held in 150-L tanks containing aerated dechlorinated water for two weeks, and fed once daily with dry commercial pellets. Both acclimation

and experimental periods were carried out in 12:12 h light–dark cycles and temperature was 25 ± 1 °C.

Experimental design. Based on previous reports of heavy metal concentrations in water bodies of Argentina [4, 19], *H. littorale* were exposed to relevant environmentally concentrations of Cr (100 µgCr·L^{-1}), Pb (100 µgPb·L^{-1}) and their combination (100 µgCr·L^{-1} + 100 µgPb·L^{-1}). According to previous studies, the mixture was defined as the addition of the two individual concentrations [9, 16, 20]. A group kept in tap water served as the control.

Metal stock solutions were prepared by dissolving appropriate amount of $K_2Cr_2O_7$ and $Pb(NO_3)_2$ (analytical grade, Merk) in distilled water, and the nominal test concentration of each metal or combination were obtained by adding appropriate aliquots of each stock solution to the aquaria. Levels of Cr and Pb in aquaria were measured by anatomic absorption spectrophotometer (Perkin Elmer Analyst).

Tests were conducted in glass aquaria containing 5 L of test solution and one fish. A control and three treatments were replicated eight times. The aquarium solutions were renewed every two days by transferring the fish to another aquarium.

After 15 days, fish were anesthetized and measured, weighted, sacrificed and dissected. Liver and kidney were immediately frozen in liquid nitrogen and stored at -80°C. All experiments were conducted in accordance with national and institutional guidelines for the protection of animal welfare.

Biomarkers. Morphometric and hematological parameters. The condition factor (CF) and the liver somatic index (LSI) were calculated according to Goede and Barton [21]. Blood was rapidly extracted from the caudal vessel by dissection of the caudal peduncle. Red blood cells (RBC), hemoglobin concentration (Hb) and hematocrit (Ht) were calculated according to Cazenave et al. [22]. Mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated from primary indices. Plasma was separated by centrifugation at 1409 *g* for 10 min. The glucose concentration (GL) and the total proteins (TP) were calculated employing commercial kits (Wiener Lab®). A drop of freshly collected blood was smeared on clean slides to estimate the total white blood cells (WBC) counts and for determination of leukocyte frequency according to Tavares-Dias and de Moraes [23].

Transaminases and alkaline phosphatase. Samples of liver and kidney were homogenized in phosphate buffer (pH 7.4). The homogenate was centrifuged at 25,000 *g* at 4 °C for 10 min, and supernatant was collected. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated according to Reitman and Frankel. [24]. Alkaline phosphatase (ALP) (Orthophosphoric monoester phosphohydrolase) activity was determined colorimetrically using a commercial kit (Wiener Lab®). Each sample was measured by triplicate and the enzymatic activity was calculated in terms of protein content [25].

Oxidative stress markers. Antioxidant enzyme extracts from liver and kidney were prepared according to Cazenave et al. [14]. The activity of glutathione S-transferase (GST) was determined according to Habig et al. [26]. Gluthation reductase activity (GR) was assayed according to Tanaka et al. [27]. The activity of glutathione peroxidase (GPx) was determined according to Drotar et al. [28]. Catalase activity (CAT) was determined according to Beutler. [29]. All enzymatic assays were carried out in triplicate and the enzymatic activity was calculated in terms of the sample protein content [25].

Liver lipid peroxidation (LPO) levels were determined according to Fatima et al. [30]. The LPO activity was expressed as nanomoles of TBARS formed per hour, per milligram of proteins (nmol TBARS· h^{-1}·mg prot^{-1}). Protein content of each sample was determined according to Bradford [30].

Statistical Analysis. All data are reported as mean ± standard error. Shapiro–Wilks test was applied to evaluate normality while Levene test was used to test the homogeneity of variance. Variables that had not a normal distribution and/or homogeny of variance were transformed using Log_{10} and tested again, prior to parametric analysis. For statistical comparisons of data among treatments, one way analysis of variance (ANOVA) followed by a Multiple Comparison Test (Tukey) were performed. *P*-values below 0.05 were regarded as significant. In addition, principal component analysis (PCA) was performed in order to get a comprehensive view of the results, and to define the most important parameters involved in metal toxicity. Multivariate analysis was carried out taking into account four cases (individuals with 30 variables measured). All statistical analysis was performed by the InfoStat software [31].

RESULTS AND DISCUSSION

Morphometric and hematological parameters. Morphometric and hematological biomarkers in control and exposed fish are summarized in Table 1. CF and LSI did not show

variation among treatments. These results are in agreement with Lombardi et al. [1], who observed no changes in *Prochilodus lineatus* collected from a river contaminated with Pb, Cd, Cu and Zn. *H. littorale* exposed to Pb and the mixture showed an increase in MCH (p=0.05) and MCHC (p=0.01). These results are in agreement with Ates et al. [32], who observed higher MCH levels in *Oncorhynchus mykiss* exposed to Pb and Cu individually for 72 hours.

Besides, fish exposed to Cr showed a decrease in glucose levels (p=0.03) (Table 1). This result coincides with Mehrim [33], who observed the same response in *Oreochromis niloticus* exposed to Cr for 12 weeks by diet treatment. The decrease of glucose caused by Cr may due to the utilization of glucose as an energetic substrate for repairing the damage caused by the metal, or homeostasis mechanisms related to glycogenolysis and glyconeogenesis that tend to keep glucose at normal levels [34].

An alteration in differential leucocytes count was showed in fish exposed to Pb compared with the control group; there was an increase in neutrophils (p=0.016) and a decrease in eosinophils (p=0.0232) (Table 1). Pb is involved in irreversible effects on cells due to their interaction with cellular macromolecules [35]. On the other hand, neutrophils function as phagocytes to salvage debris from injured tissues [36]. So, neutrophilia observed in our study could indicate an increased phagocytic action in order to eliminate cellular debris. As regards eosinophils, low concentrations have been widely reported in fish, but their function is not clear [37].

Transaminases and alkaline phosphatase. AST and ALT are enzymes involved in the metabolism of amino acids, and their alterations allow the identification of tissue damage in organs such as the liver and kidney [38]. Inductions of AST (p=0.0003) and ALT (p=0.0004) were observed in the liver of fish exposed to the mixture (Fig. 1A). In kidney, an induction of AST was observed in the Cr-exposed fish (p=0.0003) (Fig. 1B). These results agree with those found by other authors in the liver of fish exposed to heavy metals in field and laboratory studies [39, 40]. Thus, transaminases resulted in sensitive indicators of cellular damage, and an increase of their activity could be related to hepatic problems [38].

ALP activity was increased in the liver of fish exposed to the three treatments (p<0.0001) (Fig. 1A). This result correlates with other authors who exposed *O. niloticus* to Pb and Zn + Cd [20, 41]. However, in kidney decreased ALP activity was shown in fish exposed to all treatments (p=0.04) (Fig. 1B). ALP enzyme is very important for detoxification, and any alteration of its activity could cause biochemical deficiency, cellular dysfunction and tissue damage [20].

Oxidative stress biomarkers. Antioxidant enzymes activities in control and exposed fish are summarized in Table 2. CAT activity was decreased in the liver of fish exposed to Cr and Pb individually (p=0.0002). This coincides with Atli and Canli [42], who observed a decrease of CAT activity in liver of *O. niloticus* exposed to sublethal concentrations of Cr for 2 and 20 days. Other authors observed the same response in kidney of Cr-exposed fish [43, 44]. CAT inhibition may be

TABLE 1

Morphometric and hematological parameters of *H. littorale* exposed to chromium (100 µgCr ·L^{-1}) and lead (100 µgPb ·L^{-1}) individually, and in combination (Cr + Pb), for 15 days. The values are expressed as means ± SE. Means sharing the asterisk (*) are significantly different at p<0.05.

Parameter	Control	Cr	Pb	Cr + Pb
LSI	1.54 ± 0.07	1.52 ± 0.12	1.40 ± 0.19	1.59 ± 0.15
CF	1.27 ± 0.03	1.22 ± 0.04	1.26 ± 0.02	1.22 ± 0.02
RBC (10^6·µL^{-1})	1.80 ± 0.13	1.79 ± 0.37	1.81 ± 0.15	1.42 ± 0.15
Ht (%)	30.67 ± 2.03	28.97 ± 2.91	30.31 ± 2.41	26.51 ± 1.87
Hb (g·dL^{-1})	4.89 ± 0.44	4.56 ± 0.75	5.75 ± 0.69	5.54 ± 0.84
MCV (µm^3)	174.89 ± 12.53	180.43 ± 14.68	168.55 ± 7.53	191.50 ± 9.22
MCH (pg)	28.13 ± 2.97	27.33 ± 3.54	31.80 ± 2.82	39.71 ± 2.84 *
MCHC (%)	15.85 ± 0.66	15.19 ± 1.36	18.67 ± 1.06 *	21.18 ± 1.86 *
GL (g·L^{-1})	0.59 ± 0.04	0.37 ± 0.05 *	0.52 ± 0.07	0.68 ± 0.12
TP (g·dL^{-1})	3.00 ± 0.10	2.65 ± 0.30	3.23 ± 0.20	2.78 ± 0.27
WBC (µL)	10530 ± 1351	17449 ± 5062	11721 ± 2740	8650 ± 704
Lymphocytes (%)	65.02 ± 5.05	72.54 ± 4.44	64.23 ± 4.69	68.58 ± 2.99
Neutrophils (%)	7.45 ± 1.67	5.31 ± 0.96	16.53 ± 1.94 *	7.94 ± 1.01
Eosinophils (%)	20.56 ± 1.68	14.26 ± 2.64	5.80 ± 1.41*	16.66 ± 1.65
Monocytes (%)	4.88 ± 1.29	7.90 ± 2.50	7.31 ± 1.54	6.58 ± 0.89

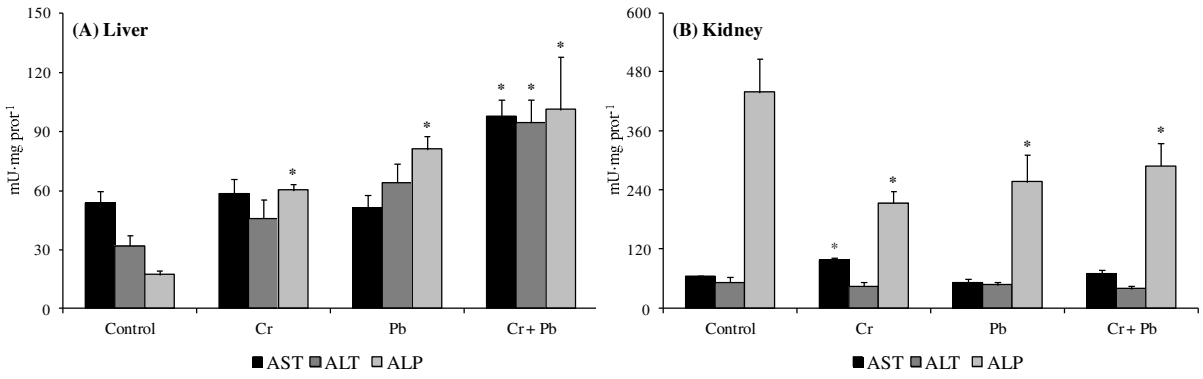

FIGURE 1

Aspartate aminotransferase (AST), alanine aminotranferase (ALT), and alkaline phosphatase (ALP) activities in liver and kidney of *H. littorale* exposed to chromium (100 µgCr ·L^{-1}) and lead (100 µgPb ·L^{-1}) individually, and in combination (Cr + Pb), for 15 days. Means sharing the asterisk (*) are significantly different at $p<0.05$.

related to the accompanied direct binding of metal ions to sulfhydryl groups on the enzyme molecule. Reduced CAT activity may increase cellular H_2O_2, and this molecule has the potential of inducing oxidative stress and starting the LPO process [45]. Such response was observed in the liver of *H. littorale* exposed to Pb individually, in which the inhibition of CAT was accompanied by an increased in LPO levels (p=0.0198) (Table 2).

Many authors have found a positive correlation between the increase of LPO levels and the inhibition of CAT activity in fish exposed to different xenobitics [46, 47]. Oliva et al. [48] found an increase in LPO levels in the liver of *Solea senegalensis* inhabiting a river contaminated with metals. However, Campana et al. [49] observed a decrease in LPO levels in the liver of *Halobratrachus didactylus* exposed to Pb for 7 days. Thus, the results observed in *H. littorale* exposed to Pb could indicate that the liver suffered an oxidative damage that the antioxidant defense system could not prevent.

Multi-biomarker approach. Many authors suggest that the selection of an appropriate battery of biomarkers and their integral analysis are important to determine deleterious effects caused by both complex mixtures of pollutants and chronic situation of contamination [15, 16, 48]. When a wide number of biomarkers are measured, many approaches are used to analyze their results. The principal component analysis (PCA) is one of the techniques usually employed [50]. In the present

TABLE 2

Oxidative stress markers in liver and kidney of *H. littorale* exposed to chromium (100 µgCr ·L^{-1}) and lead (100 µgPb ·L^{-1}) individually, and in combination (Cr + Pb), for 15 days. The values of antioxidant enzyme activities (nkat mg prot^{-1}) and lipid peroxidation (nmol TBARS· h^{-1}·mg prot^{-1}) are expressed as means ± SE. Means sharing the asterisk (*) are significantly different at $p<0.05$.

		Control	Cr	Pb	Cr + Pb
Liver					
	GST	9.85 ± 0.81	8.06 ± 0.43	7.76 ± 1.04	9.13 ± 0.98
	GR	0.49 ± 0.05	0.51 ± 0.06	0.57 ± 0.06	0.53 ± 0.05
	GPx	3.95 ± 0.24	4.39 ± 0.48	4.12 ± 0.38	3.80 ± 0.34
	CAT	552.24 ± 34.76	338.88 ± 25.64 *	355.52 ± 35.33 *	657.13 ± 28.24
	LPO	0.06 ± 0.01	0.07 ± 0.01	0.12 ± 0.01*	0.04 ± 0.02
Kidney					
	GST	4.94 ± 0.36	4.27 ± 0.24	4.64 ± 0.37	3.55 ± 0.31
	GR	0.98 ± 0.11	0.74 ± 0.13	0.80 ± 0.07	0.68 ± 0.12
	GPx	6.58 ± 0.65	6.01 ± 0.27	6.28 ± 0.57	5.17 ± 0.66
	CAT	106.33 ± 18.87	87.73 ± 6.07	103.08 ± 6.53	104.72 ± 12.87

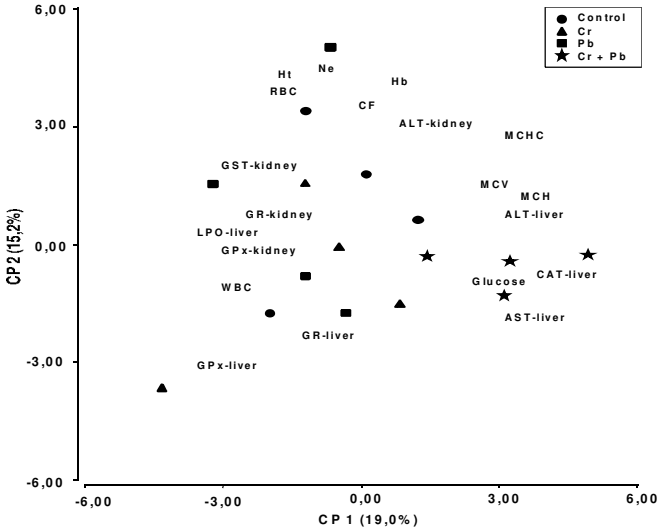

FIGURE 2
Representation of the biomarkers (in letters) and individuals (in forms, n = 4 per treatment) onto the first factorial plane of the principal component analysis (PCA). Biomarkers with correlation coefficients >0.5 were represented in the PCA. Biomarkers abbreviations are explained in the text with the exception: Ne (neutrophils).

study, two components were extracted by applying the PCA (Fig. 2). According to Legendre and Legendre [51], interpretation of principal components may be done for eigenvalues of the data matrix higher than 1. The PCA indicated that 10 eigenvalues were higher than 1; moreover correlation coefficients are significant when they are higher than $\sqrt{d/n}$, d being the number of principal components and n the number of variables. Therefore, correlation coefficients >0.5 were indicative of a good representation of the variables with principal component axes. The components accounted for 34.2% of the original dataset variance. The first principal component (PC1) explained 19.0% of the variance, and showed significant positive loadings for glucose, MCV, MCH, MCHC, AST, ALT and CAT in liver. On the contrary, a negative correlation was found in WBC, GST, RBC and GPx in kidney, and GPx and LPO in liver. The second principal component (PC2) explained 15.2% of the variance, and showed positive correlations for the CF, Ht, Hb, neutrophils and ALT in kidney, but negative correlations for GPx and RBC in liver. Thus, Figure 2 represents that PC1 separated clearly fish exposed to Cr + Pb from the other treatments and PC2 shows the great variability of individual physiologic responses (independently of the treatment).

The increase of both transaminases activities and oxidative damage found in our study suggests that the liver is a target organ under the action of the metal combination. These results are in agreement with many authors, who claimed that the liver is more sensitive to metal exposure than the kidney [42].

CONCLUSIONS

In summary, our results showed different effects on *H. littorale* exposed to individual metals and their combination. Cr produced nutrients mobilization by a decrease in plasma glucose, tissue damage in liver and kidney, and an inhibition of an antioxidant enzyme (CAT). On the other hand, hematological alterations, tissue damage in liver and kidney and evident hepatic oxidative stress were found in fish exposed to Pb. The combination of metals caused changes in hematological parameters and damages in tissues. The multi-biomarker approach based on a wide array of physiological parameters showed that fish exposed to Cr + Pb had a different physiologic profile to those exposed to individual metals. Thus, this work underscores the need for new studies to explore how complex mixtures affect aquatic organisms.

ACKNOWLEDGEMENTS

This work was supported by grants from CONICET and Universidad Nacional del Litoral (CAI+D).

REFERENCES

[1] Lombardi, P.E.; Peri, S.I.; Verrengia Guerrero, N.R. (2010) Trace metal levels in *Prochilodus lineatus* collected from the La Plata River, Argentina. Water Air Soil Pollut. 160, 47-59.

[2] Gagneten, A.M.; Gervasio, S.; Paggi, J.C. (2007) Heavy metal pollution and eutrophication in the Lower Salado River Basin (Argentina). Water Air Soil Pollut. 178, 335-349.

[3] Marchese, M.; Rodriguez, A.R.; Pave, P.J.; Carignano, M.R. (2008) Benthic invertebrates structure in wetlands of a tributary of the middle Parana River (Argentina) affected by hydrologic and anthropogenic disturbances. J. Environ. Biol. 29, 343-348.

[4] Peluso, L.; Abelando, M.; Apartín, C.D.; Almada, P.; Ronco, A.E. (2013) Integrated ecotoxicological assessment of bottom sediments from the Paraná basin, Argentina. Ecotox. Environ. Safe. 198, 179-186.

[5] Giarratano, E.; Duarte, C.A.; Amin, O.A. (2010) Biomarkers and heavy metal bioaccumulation in mussels transplanted to coastal waters of the Beagle Channel. Ecotox. Environ, Safe. 73, 270-279.

[6] Antón, A.; Serrano, T.; Angulo, E.; Ferrero, G.; Rallo, A. (2000) The use of two species of crayfish as environmental quality sentinels: the relationship between heavy metal content, cell and tissue biomarkers and physic-chemical characteristics of the environment. Sci. Total Environ. 247, 239-251.

[7] Ferraro, M.V.M.; Fenocchiol, A.S.; Mantovani, M.S.; Oliveira Ribeiro, C.; Cestari, M.M. (2004) Mutagenic effects of tributyltin and inorganic lead (Pb II) on the fish *H. malabaricus* as evaluated using the comet assay and the piscine micronucleus and chromosome aberration tests. Genet. Mol. Biol. 27(1), 103-107.

[8] Lombardi, P.E.; Peri, S.I.; Verrengia Guerrero, N.R. (2010) ALA-D and ALA-D reactivated as biomarkers of lead contamination in the fish *Prochilodus lineatus*. Ecotox. Environ. Safe. 73, 1704-1711.

[9] Firat, Ö.; Cogun, H.Y.; Aslanyavrusu, S.; Kargin, F. (2008) Antioxidant responses and metal accumulation in tissues of Nile Tilapia *Oreochromis niloticus* under Zn, Cd and Zn plus Cd exposures. J. Appl. Toxicol. 29, 295-301.

[10] Pandey, S.; Parvez, S.; Ahamd Ansari, R.; Ali, M.; Kaur, M.; Hayat, F.; Ahmad, F.; Raisuddin, S. (2008) Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctata* Bloch. Chem. Biol. Interac. 174, 183-192.

[11] Birceanu, O.; Chowdhury, M.J.; Gillis, P.L.; McGeer, J.C.; Wood, C.M.; Wilkie, M.P. (2008) Modes of metal toxicity and impaired branchial ion regulation in rainbow trout exposed to mixtures of Pb and Cd in soft water. Aquat. Toxicol. 89, 222-231.

[12] Firat, Ö. and Kargin, F. (2009) Biochemical alterations induced by Zn and Cd individually or in combination in the serum of *Oreochromis niloticus*. Fish Physiol. Biochem. 36(3), 647-653.

[13] Palaniappan, P.L.R. and Karthikeyan, S. (2009) Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of freshwater fish *Cirrhinus mrigala* individually and in binary solutions with nickel. J. Environ. Sci. 21, 229-236.

[14] Cazenave, J.; Bacchetta, C.; Parma, M.J.; Scarabotti, P.A.; Wunderlin, D.A. (2009) Multiple biomarkers responses in *Prochilodus lineatus* allowed assessing changes in the water quality of Salado River basin (Santa Fe, Argentina). Environ. Pollut. 157, 3025-3033.

[15] Cazenave, J.; Bacchetta, C.; Rossi, A.; Ale, A.; Campana, M.; Parma, M.J. (2014) Deleterious effects of wastewater on the health status of fish: A field caging study. Ecol. Ind. 38, 104-112.

[16] Bacchetta, C.; Rossi, A.; Ale, A.; Campana, M.; Parma, M.J.; Cazenave, J. (2014) Combined toxicological effects of pesticides: A fish multi-biomarker approach. Ecol. Ind. 36, 532-538.

[17] Affonso, E.D.; Polez, V.L.P.; Correa, C.; Mazon, A.F.; Araujo, M.R.R.; Moraes, G.; Rantin, F.T. (2004) Physiological responses to sulfide toxicity by the air-breathing catfish, *Hoplosternum littorale* (Siluriformes, Callichthyidae). Comp. Biochem. Physiol. 139(4), 251-257.

[18] Rossi A., Cazenave J., Bacchetta C., Campana M., Parma M.J. (2015) Physiological and metabolic adjustments of *Hoplosternum littorale* (Teleostei, Callichthyidae) during starvation. Ecol. Ind. 56: 161-170.

[19] Topalián, M.L.; Castañé, P.M.; Rovedatti, M.G.; Salibián, A. (1999) Principal component analysis of dissolved heavy metals in water of the Reconquista river (Buenos Aires, Argentina). Bull. Environ. Contam. Toxicol. 63, 484-490.

[20] Firat, Ö. and Kargin, F. (2010) Individual and combined effects of heavy metals on serum biochemistry of Nile Tilapia *Oreochromis niloticus*. Arch. Environ. Contam. Toxicol. 58, 151-157.

[21] Goede, R.W. and Barton, B.A. (1999) Organismic indices and autopsy-based

assessment as indicators of health and condition of fish. Am. Fish Soc. Symp. 8, 93-108.

[22] Cazenave, J.; Wunderlin, D.A.; Hued, A.C.; Bistoni, M,Á. (2005) Haematological parameters in a neotropical fish, *Corydoras paleatus* (Jenyns, 1842) (Pisces, Callichthyidae) captured from pristine and polluted water. Hydrobiologia. 53, 25-33.

[23] Tavares-Dias, M. and Ruas de Moraes, F. (2007) Leukocyte and thrombocyte reference values for channel catfish (*Ictalurus punctatus* Raf), with an assessment of morphhologic, cytochemical, and ultrastructural features. Vet. Clin. Path. 36, 49-54.

[24] Reitman, S. and Frankel, F. (1957) A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic acid transaminase. Am. J. Clin. Path. 28, 56-63.

[25] Bradford, M.M. (1976) A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

[26] Habig, W.H.; Pabst, M.J.; Jakoby, W.B. (1974) Glutathione S-transferases. The first step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139.

[27] Tanaka, K.; Sano, T.; Ishizuka, K.; Kitta, K.; Kawamura, Y. (1994) Comparison of properties of leaf and root glutathione reductases from spinach. Physiol. Plant. 91, 353-358.

[28] Drotar, A.; Phelps, P.; Fall, R. (1985) Evidence for glutathione peroxidase activities in cultured plant cells. Plant Sci. 42, 35-40.

[29] Beutler, E. (1982) Catalase. In Red Cell Metabolism, a Manual of Biochemical Methods, 3rd Ed.; Grune and Stratton Inc.: New York, 105-106 pp.

[30] Fatima, M.; Ahmad, I.; Sayeed, I.; Athar, M.; Raisuddin, S. (2000) Pollutant-induced over-activation of phagocytes is concomitantly associated with peroxidative damage in fish tissues. Aquat. Toxicol. 49, 243-250.

[31] Di Rienzo, J.A.; Casanoves, F.; Balzarini, M.G.; Gonzalez, L.; Tablada, M.; Robledo, C.W. InfoStat versión 2012. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar (accessed Oct 2013)

[32] Ates, B.; Orun, I.; Talas, Z.S.; Durmaz, G.; Yilmaz, I. (2008) Effects of sodium selenite on some biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) exposed to Pb^{2+} and Cu^{2+}. Fish Physiol. Biochem. 34, 53-59.

[33] Mehrim, A.I.M. (2014) Physiological, biochemical and histometric responses of Nile tilapia (*Oreochromis niloticus* L.) by dietary organic chromium (chromium picolinate) supplementation. J. Adv. Res. 5(3), 303-310.

[34] Ricard, A.C.; Daniel, C.; Anderson, P.; Hontela, A. (1998) Effects of subchronic exposure to cadmium chloride on endocrine and metabolic functions in rainbow trout *Oncorhynchus mykiss*. Arch. Environ. Contam. Toxicol. 34, 377-381.

[35] Heath, A. G. (1995) In Water pollution and fish physiology, 2nd Ed. CRC Press Inc.: Boca Raton, Florida, 373 pp.

[36] Witko-Sarsat, V.; Rieu, P.; Descamps-Latsha, B.; Lesavre, P.; Halbwachs-Mecarel, L. (2000) Neutrophils: molecules, functions and pathophysiological aspects. Lab. Invest. 80, 617-653.

[37] Takashima, F. and Hibiya, T. (1995) In: An atlas of fish histology. Normal and pathological features. Kodansha Ltd Inc., Tokio, 192 pp.

[38] Ramaiah, S.K. (2007) A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food Chem. Toxicol. 45, 1551-1557.

[39] Levesque, H.M.; Moon, T.W.; Campbell, P.G.C.; Hontela, A. (2002) Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in field. Aquat. Toxicol. 60, 257-267.

[40] Ibrahim, S.A. and Mahmoud, S.A. (2005) Effect of heavy metals accumulation on enzyme activity and histology in liver on some nile fish in Egypt. Egypt. Aquat. Biol. Fish. 9, 203-219.

[41] Atli, G. and Canli, M. (2007) Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*. Comp. Biochem. Physiol. 145(C), 282-287.

[42] Atli, G. and Canli, M. (2010) Response of antioxidant system of freshwater fish *Oreochromis niloticus* to acute and chronic metal (Cd, Cu, Cr, Zn, Fe) exposures. Ecotox. Environ. Safe. 73, 1884-1889.

[43] Atli, G.; Alptekin, O.; Tükel, S.; Canli, M. (2006) Response of catalase activity to Ag^{+}, $Cd^{2+,}$ Cr^{6+}, Cu^{2+} and Zn^{2+} in five tissues of freshwater fish *Oreochromis niloticus*. Comp. Biochem. Physiol. 143(C), 218-224.

[44] Lushchak, O.V.; Kubrak, O.I.; Lozinsky, O.V.; Storey, J.; Storey, K.B.; Lushchak, V.I. (2009) Chromium (III) induces oxidative stress in goldfish liver and kidney. Aquat. Toxicol. 93, 45-52.

[45] Kong, X.; Wang, S.; Jiang, H.; Nie, G.; Li, X. (2010) Responses of acid/alkaline phosphatase, lysozyme, and catalase activities and lipid peroxidation to mercury exposure

during the embryonic development of goldfish *Carassius auratus*. Aquat. Toxicol. 120-121, 119-125.
[46] Atif, F.; Ali, M.; Kaur, M.; Rehman, H.; Raisuddin, S. (2005) Modulatory effect of cadmium injection on endosulfan-induced oxidative stress in the freshwater fish *Channa punctata* Bloch. Bull. Environ. Contam. Toxicol. 74, 777-784.
[47] Ferreira, D.; Costa da Motta, A.; Kreutz, L.C.; Toni, C.; Loro, V.L.; Barcellos, L.J.G. (2010) Assessment of oxidative stress in *Rhamdia quelen* exposed to agrochemicals. Chemosphere. 79, 914-921.
[48] Oliva, M.; Vicente, J.J.; Gravato, C.; Guilhermino, L.; Galindo-Riano, M.D. (2010) Oxidative stress biomarkers in Senegalsole, *Solea senegalensis*, to assess the impact of heavy metal pollution in a Huelva estuary (SW Spain): Seasonal and spatial variation. Ecotox. Environ. Safe. 75, 151-162.
[49] Campana, O.; Sarasquete, C.; Blasco, J. (2003) Effect of lead on ALA-D activity, metallothinein levels, and lipid peroxidation in blood, kidney and liver of the toadfish *Halobatrachus didactylus*. Ecotox. Environ. Safe. 55, 116-125.
[50] Wepener, V. (2008) Application of active biomonitoring within an integrated water resources management framework in South Africa. S. Afr. J. Chem. 104, 367-373.
[51] Legendre, L. and Legendre, P. (1979) Ecologie numérique. 2-la structure desdonnées écologiques. Chapitre 8. In: *L'ordination en espace r duit.* Les Presses de l'Universit du Qu bec, Masson, Paris, New York, 101-146 pp.

Received: **26.10.2015**
Accepted: **19.02.2016**

CORRESPONDING AUTHOR

Jimena Cazenave
Laboratorio de Ictiología, Instituto Nacional de Limnología (INALI-CONICET-UNL), Paraje El Pozo, Ciudad Universitaria UNL, 3000 Santa Fe, Argentina

e-mail: jcazenave@inali.unl.edu.ar