Histopathological changes in the head kidney induced by cadmium in a neotropical fish Colossoma macropomum

R. Salazar-Lugo1*, A. Vargas1, L. Rojas2 and M. Lemus3

1Departamento de Bioanálisis, Laboratorio de Proteínas e Inmunotoxicidad, Postgrado de Biología Aplicada, Escuela de Ciencias, Núcleo de Sucre, Universidad de Oriente, Cumandá, Venezuela
2Laboratorio de Histología, Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas (IIBCA-UDO), Núcleo de Sucre, Universidad de Oriente, Cumandá, Venezuela
3Departamento de Biología, Laboratorio de Ecofisiología y Contaminación Ambiental, Escuela de Ciencias, Núcleo de Sucre, Universidad de Oriente, Cumandá, Venezuela

Abstract
We evaluated the effect of cadmium (Cd) on the structure and function of the head kidney in the freshwater fish Colossoma macropomum (C. macropomum). Juveniles were exposed to 0.1 mg/L CdCl2 for 31 days. Blood samples were examined using hematological tests and head kidney histology was determined by light microscopy. The concentration of Cd in the head and trunk kidneys was measured using an atomic absorption spectrophotometer. Cd produced histopathological changes in the head kidney, the most evident of these being: the thickening of the vein wall, an increase in the number of basophils/mast cells close to blood vessels and a severe depletion of hematopoietic precursors especially the granulopoietic series. In the blood, a decrease in the total leucocytes and hemoglobin concentration was observed. Cd-exposed fish showed higher Cd concentrations in the trunk kidney than the head kidney. In conclusion, exposure to Cd affected precursor hematopoietic cells in C. macropomum.

Keywords: Cadmium, Colossoma macropomum, Granulocytes, Head kidney, Histology.

Introduction
Cadmium (Cd) is a toxic heavy metal that has become increasingly important as an environmental hazard to both humans and wildlife. Cd can act as a mitogen, stimulates cell proliferation, inhibits apoptosis, inhibits DNA repair, and promotes cancer in a number of tissues (Satarug and Moore, 2012). In addition, it causes tissue damage, notably in the kidney, by inducing cell death. There is also evidence that environmental exposure to Cd affects renal function (Nordberg et al., 2012). In the fish, Dicentrarchus labrax and Centropomusundecimaries, Cd has been reported to induce histological and cytological changes to the kidneys (Giari et al., 2006). In Venezuela, moderate Cd concentrations in rivers and catchment basins have been reported (Vaquero et al., 2004), with mining activity and the petroleum industry being the primary source. Moderate Cd concentrations in fish, including Colossoma macropomum (C. macropomum) have also been registered (Salazar-Lugo, 2009). This species is widely distributed in South America and is abundant in the catchment areas of the Amazon and Orinoco rivers where it constitutes an important component of the local riverside economy. Moreover, the carchama presents a number of characteristics that may make it an appropriate model that can be used as indicator species in biomonitoring programs.

Chronic Cd exposure in C. macropomum produces a decrease in blood reticulocytes and total leukocytes (Salazar-Lugo et al., 2011) affecting its innate immune response. These observations suggest that Cd could be producing damage in leucopoietic organs such as the head kidney. This organ plays an important roles as main haematopoietic, immune and endocrine organ in many fish; because of this, Cd accumulation in head kidney can produce toxic effects on many important physiological processes such as changing hormonal level, immunological mechanism or hematological parameters (Lafuente et al., 2004; Dangre et al., 2010; Salazar-Lugo et al., 2011; Vargas et al., 2012; Kondera and Witeska, 2013) affecting the fish survival.

In this study we examined histologically the effect of chronic Cd exposure on the head kidney of C. macropomum as well as evaluating some blood parameters used as indicators of fish health.

Materials and Methods
Fish maintenance
C. macropomum juveniles (87.69±34.23 g and 17.87±7.88 cm) were supplied by ALMACA Aquaculture (Punta de Mata, Venezuela) and maintained for 3 weeks in holding tanks with continuously aerated and flowing de-chlorinated tap water (pH 7.2 - 7.8) at a constant temperature of 28±1 °C. Fish were fed ad libitum with food specific for
**C. macropomum** (PURINA, Venezuela). Artificial illumination was reduced by surrounding the aquariums with transparent black bags so as not to disturb the natural condition of these fish because these fishes live in dark freshwater. Water, pH (7.3±0.05), temperatures (28±1 °C), oxygen level (3.8 mg/L), Total Hardness (106 mg/L) were controlled during experiments.

**LC₅₀ 96 h determination**

For the experimental LC₅₀ 96h protocol, ten (10) juvenile cachama were transferred to individual 40 L glass aquariums 48 h prior to the experiments. Five groups of fishes (with replicates) were exposed to 128, 64, 32, 16, 8, and 2 mg/L Cd for 96 h. Another group was kept in water free of Cd and was considered to be control fish. Mortality was determined at 12, 24, 36, and 96 h after exposure to Cd. The recorded data were analyzed using probit method (Stephan, 1977) with the help of EPA Probit Software 1.5 Version.

**Cd Bioassays**

Fish (n = 48) were divided in two experimental groups: Control and Cd-exposed groups (12 fish per group with replicates) and placed in 16 L glass aquariums (six fish /aquarium). A sub lethal concentration of 0.1 mg/L Cd from CdCl₂H₂O (Aldrich) was selected according to the LC₅₀ at 96 h (<0.1% LC₅₀ 96h). Fish were exposed for 31 days, using a semi-static test system.

Each aquarium was continuously aerated and the same physical and chemical characteristics of the water as those for the laboratory acclimation were maintained. The initial Cd concentration in the water was 0.85-0.95 mg/LCd; eighty percent (80%) of Cd aquarium water was renewed every day. Before water renewal, an aliquot was taken to measure the residual Cd concentration by spectrophotometer analysis (0.25 mg/LCd).

The fish were then fed and the remaining food was removed. Control fish were maintained under the same conditions in water devoid of detectable Cd. Fish were fed before adding Cd to the water (every 24 h). No fish mortality was observed during the experiment. Fish were sacrificed after 31 days of exposition and the head and trunk kidneys were dissected and weighed.

**Histological analyses**

The histological analyses of head kidney were done according to Rojas et al. (1997). Fish (n = 6, 3 fish per group) were sacrificed and the kidney samples dissected at 21°C. Each sample was cut into 2 mm² sections and placed in a fixative (3% glutaraldehyde pH 8.0) for 2 h. They were then washed in a 0.1 M phosphate buffer (15 min), post-fixed in 1% OsO₄ in a 0.1M phosphate buffer (1 h), rinsed in phosphate buffer followed by two baths in distilled water, dehydrated in a graded ethanol series and bathed in propylene oxide (10 min). The tissues were then successively infiltrated with a 2:1 mixture of Epon and propylene oxide for 6 h, and pure Epon-812 medium for another 2 h.

Finally, they were embedded in silicone rubber molds filled with Epon-812 and polymerized at 60°C for 48 h. Semi-thin (0.7 µm) sections were cut, mounted on glass slides and stained with toluidine blue. Observations and micrographics were made using a Zeiss photomicroscope, Axioscop FL40.

**Head kidney Imprints and Hematological analysis**

Head kidney imprints were obtained by pressing tissue on the slides. Blood smears were prepared from peripheral blood obtained by puncture of the caudal vein. Imprints were air-dried, fixed in absolute methanol for three min at room temperature and stained with 10% McGruwall-Wrigh-Giemsa. The slides were examined under a Zeiss photomicroscope, Axioscop FL40.

Approximately, 1.5 mL of venous blood was drawn from each subject. Hemoglobin was determined with Drabkin’s reagent and absorbance was measured at 540 nm. Hematocrit was determined by the microhematocrit technique (Blaxhall and Daisley, 1973). Blood smears were fixed with methanol and stained with 10% McGruwall-Wrigh-Giemsa. The slides were examined under a Zeiss photomicroscope, Axioscop FL40.

**Cd Determination**

The concentration of Cd in tissues and water was analyzed using a Flame Atomic Absorption Spectrophotometric (Perkin Elmer 3110). The quality of the heavy metal measurements was assured by the use of DORM-2 dogfish muscle; Certified Reference Material for Trace Metals (National Research Council of Canada).

The average values of the heavy metals obtained by the analytical process agreed with the certified values 90-97% (Sardiñas and García, 1996).

**Statistics**

Results are expressed as mean ± standard error (SE) and the medians of the two groups (Cd-exposed and controls) were compared using the non-parametric Mann-Whitney (Wilcoxon) W test. Differences were considered significant at P<0.05. All statistical analysis was made with the program Stat graphics Plus Version 5.1 (Addlink Software Scientific, S.L.).

**Results**

Analysis of data collected on percent mortality at 96 h exposure to different concentrations of Cd revealed the LC₅₀ value to be at 38.47 mg/L. Fish showed dark skin and reduced movement. Nevertheless, the fish always stayed grouped.

Control head kidneys showed a compact homogeneous parenchyma. Large lymphocytes, little lymphocytes, plasma cells, monocytes, melano-
macrophages and red cells were observed. Granulocytes, lymphocytes and erythrocytes are 70±17 % of immature cells. The basal endothelial membrane was 1.87±0.32 µm wide and its integrity was maintained (Fig. 1A).

The most prominent features of the damage to the head kidneys of *C. macropomum* exposed to chronic levels of Cd were:

1. Depletion of the hematopoietic precursors (Table 1); 2. Thickening of the basal endothelial membrane, 35% thicker than that of the controls (Table 1); 3. The recruitment of basophils near blood vessels; 4. A disruption of tissue integrity: debris could be observed within cells and there were empty spaces between cells (Fig. 1B).

![Fig. 1. Cross-section of the head kidney of *C. macropomum*. A: head kidney from a control fish showing a characteristic parenchyma; vein wall (black arrow), vessel (V), erythrocytes inside vessel lumen (*), melano-macrophage (dotted arrow) (1000X). B: head kidney from a fish exposed to Cd showing an evident thickening of the vein wall (VW), the recruitment of basophils near blood vessels (black arrows) and the loss of the normal cytoarchitecture of the cellular parenchyma (1000X).](image)

Control head kidney imprints showed both granulocyte-aggregates and erythrocyte-aggregates. Immature granulocytes were large and contained eccentric nuclei with relaxed chromatin. Eosinophilic hyaline granules could be observed inside the cytoplasm, and immature erythrocytes showed a cytoplasm without granules or central nuclei (Fig. 2A).

Cd-exposed fish imprints showed hematopoietic precursors with different degrees of hypertrophy and vacuolization. Granulocytes were swollen and vacuolization and pinocytosis could be observed; many erythrocyte precursors had eccentric nuclei indicating an aging cell (Fig. 2B).

![Fig. 2. Head kidney imprints of *C. macropomum*. A: control imprint with immature granulocytes (*), immature erythrocytes (dotted arrows) and mature erythrocytes (continuous arrows). B: imprint of cadmium-exposed kidney showing precursor cells with evident swelling and eccentric nuclei (1000X).](image)

Cd accumulation in *C. macropomum* kidneys was lowest in the head kidney, where hematopoietic tissue dominates, and greatest in the posterior tail sections, where filtration tissues dominate (Table 2). As a consequence of the decrease in the number of precursor cells in the head kidney, the total blood leukocyte count and differential blood leukocyte count decreased. In contrast, the normal erythrocyte count found in the blood of Cd-exposed fish may be
explained by the fact that erythrocytes could be produced by other organs such as the trunk kidney and liver (Table 3).

**Table 1.** Thickness of basal membrane of vessel and cell count in head kidney of C. macropomum Cd-exposed.

| Parameters               | Control     | Cd exposed |
|--------------------------|-------------|------------|
| Thickness of basal membrane (µm) | 1.87±0.32 | 5.35±0.61*** |
| Precursor cells (%)      | 70±17.04 | 29±4.36* |

*** (P<0.001); * (P<0.05).

**Table 2.** Cadmium concentrations in the head kidney and trunk kidney of C. macropomum.

| Tissues             | Cd concentration (µg.g-1) |
|---------------------|--------------------------|
|                     | Control                  | Cd-exposed              |
| Trunk kidney        | 0.027±0.0274             | 5.39±1.8755***          |
| Head kidney         | 0.004±0.0015             | 0.018±0.0004***         |

*** (P<0.001).

**Table 3.** Hematological parameters of Cd-exposed fish C. macropomum.

| Parameters                   | Control       | Cd-exposed |
|------------------------------|---------------|------------|
| Hb (g/dl)                    | 7.31±1.79     | 5.40±1.32* |
| Hto (%)                      | 21.03±3.17    | 18.86±6.30 ns |
| Erythrocytes (x10⁶/mm³)     | 3.54±1.11     | 3.23±3.95 ns |
| MCV (fl)                     | 531±150       | 370±125ns |
| CHCM (%)                     | 33.86±2       | 28.08±1.9ns |
| Leukocytes (x10⁶/mm³)        | 3.36±1.81     | 1.54±0.60** |
| Granulocytes (Cel/mm³)       | 992.00±63.68  | 465.00±24.40*** |
| Lymphocytes (Cel/mm³)        | 2353.00±48.70 | 1065.00±21.62*** |

ns= no significant ** (P<0.01); *** (P<0.001)

**Discussion**

The median lethal concentration (LC₅₀ 96 hours) can be considered very high if we take into account that this metal has been reported as highly toxic and non-bio essential (Sadiq, 1992).

Unlike here, reports in other species of freshwater fish, such as Oreochromis mossambicus, has determined that the Cd 96-h LC₅₀ was 11.99 ppm (James, 2000) and Cd CL₅₀-96h to Cichlasoma dimerus was 44 mg/L (Hirt and Domitrovic, 2002).

The results regarding 96 hour LC₅₀ in cachama suggest that these fish are able to tolerate high concentrations of Cd. In natural environments, cachamas from apparently uncontaminated, moderate concentrations of Cd are reported (Salazar-Lugo, 2009).

We did not observe a nephron in the parenchyma, although lymphocyte-aggregate and granulocyte-aggregate regions were found, suggesting that the head kidney in C. macropomum is an important organ for hematopoiesis.

The observed features of the damage to the head kidneys suggest the involvement of the organ in an inflammatory process. Studies have shown that Cd, at relatively low sub lethal concentrations, can target vascular endothelial cells at a variety of molecular levels, including cell adhesion molecules, metal ion transporters and protein kinase signaling (Woods et al., 2008).

Cell loss has been reported as a typical characteristic of the chronic effects of Cd on the kidneys in animals (Wang et al., 2009; Sinha et al., 2009). On the other hand, the increase in the number of mast cells in various tissues and organs of teleosts seems to be linked to a wide range of stressful conditions, such as exposure to heavy metals (Cd, copper, lead and mercury) and exposure to herbicides and parasitic infections (Manera et al., 2011). In Sparus aurata, prolonged exposure to Polychlorinated Biphenyls (PCBs) caused a significant increase in the number of mast cells in connective tissue close to blood vessels of the muscularis layers of the intestine and in regions of active inflammatory responses (Lauriano et al., 2012).

The pattern of Cd accumulation in C. macropomum kidneys is similar to that reported by Woodling et al. (2001) in wild brown trout Salmo trutta and by Kondera et al. (2013) in Cyprinus carpio. Cd accumulation in Trunk kidney is well-known in fish (Singh et al., 2008, Rauf et al., 2009, Dugmonits et al., 2013). Cd is delivered to kidney by Cd-Metallothionein (Cd-Mt) complexes to eliminate in the urine, however, these complexes Cd-Mt can be retained in this organ for a long period of time (Tang et al., 1998).

A decrease in the number of precursor cells in the head kidney suggests that Cd affects hematopoiesis, especially leucopoiesis, during the early stages of cellular differentiation. Exposure to Cd also reduced the hematopoietic potential of the common carp Cyprinus carpio, measured as the ratio of proliferating to apoptotic precursor cell frequency (Kondera and Witeska, 2013).

To summarize, chronic Cd exposure in the freshwater fish C. macropomum produced an anomalous head kidney structure, and induced an inflammatory process in this organ, affecting hematopoietic cell differentiation, especially with regard to granulocytes and perhaps affecting its function.

**Acknowledgements**

This study was supported by funding from FONACIT (Project No. G2005000775) and Consejo of
Investigación-Universidad de Oriente, Venezuela. We are grateful to Pisciculture ALMA C.A. for providing the fish used in this study.

References
Blaxhall, P. and Daisley, R. 1973. Routine haematological methods for use with fish blood. J. Fish. Biol. 5, 771-781.
Dangre, A.J., Manning, S. and Brouwer, M. 2010. Effects of cadmium on hypoxia-induced expression of hemoglobin and erythropoietin in larval sheeps head minnow, <i>Cynocinodon variegatus</i>. Aquat. Toxicol. 99, 168-175.
Dugmonits, K., Ferencz, A., Jancsó, Z., Juhász, R. and Heremész, E. 2013. Major distinctions in the antioxidant responses in liver and kidney of Cd2+-treated common carp (<i>Cyprinus carpio</i>). Comp. Biochem. Physiol.- Part C 158, 225-230.
Giari, L., Manera, M., Simoni, E. and Dezfuli, B. 2006. Cellular alterations in different organs of European sea bass <i>Dicentrarchus labrax</i> (L.) exposed to cadmium. Chemosphere 67, 1171-1181.
Hirt, M. and Domitrovic, H.A. 2002. Toxicidad y respuesta histopatológica en Cichlasoma dimerus (Pisces, Cichlidae) expuestos a cloruro de cadmio. En ensayos agudo (Pisces, Cichlidae). Biol. Fis. 17, 359-360.
Kondera, E., Lugowska, K. and Sarnowski, P. 2013. High affinity of cadmium and copper to head kidney of common carp (<i>Cyprinus carpio</i> L.). Fish Physiol. Biochem. DOI 10.1007/s10695-013-9819-1.
Kondera, E. and Witeska, M. 2013. Cadmium and copper reduce hematopoietic potential in common carp (<i>Cyprinus carpio</i> L.) head kidney. Fish Physiol. Biochem. 39(4), 755-764.
Lafuente, A., González-Caracedo, A. and Esquifino, A.I. 2004. Differential effects of cadmium on blood lymphocyte subsets. Biomarkers 17, 451-456.
Lauriano, E.R., Calò, M., Silvestri, G., Zaccone, D., Pergolizzi, S. and Cascio, P. 2012. Mast cells in the intestine and gills of the sea bream, <i>Sparus aurata</i>, exposed to a polychlorinated biphenyl, PCB 126. Acta Histochem. 114, 166-171.
Manera, M., Giannmarino, A., Borreca, C., Giari, L. and Dezfuli, B.S. 2011. Degranulation of mast cells due to compound 48/80 induces concentration-dependent intestinal contraction in rainbow trout (<i>Oncorhynchus mykiss</i> Walbaum) ex vivo. J. Exp. Zool. A. Ecol. Genet. Physiol. 315, 447-457.
Nordberg, G., Jin, T., Wu, X., Lu, J., Chen, L., Liang, Y., Lei, L., Hong, F., Bergdahl, I.A. and Nordberg, M. 2012. Kidney dysfunction and cadmium exposure factors influencing dose-response relationships. J. Trace Elem. Med. Biol. 26, 197-200.
Rauf, A., Javed, M. and Ubaidullah, M. 2009. Heavy metal levels in three major carps (<i>Calta calta</i>, <i>Labeo rohita</i> and <i>Cirrhina mirgila</i>) from the river Ravi, Pakistan. Pak. Vet. J. 29, 24-26.
Rojas, L., Mcneil, R., Cabana, T. and Lachapelle, P. 1997. Diurnal and nocturnal visual function in two tattle foraging waterbirds. The American white ibis and the black skimmer. The Condor 99, 191-200.
Sadiq, M. 1992. Toxic metals chemistry in marine environments. Editorial Marcel Dekker. New York, pp: 390.
Salazar-Lugo, R. 2009. Estado de conocimiento de las concentraciones de cadmio, mercurio y plomo en organismos acuáticos de Venezuela REDVET. Revista electrónica de Veterinaria. Vol. 10, Nº 11, http://www.veterinaria.org/revistas/redvet/n111109 .html <noviembre, 2009>
Salazar-Lugo, R., Blanco, Y., Centeno, L. and Lemus, C. 2011. Variaciones en los parámetros hematológicos y en la respuesta inmune inespecífica de la cachama negra <i>Colossoma macropomum</i> expuesta a cadmio. Saber 23, 28-35.
Sardiñas, O. and García, M. 1996. Método para el análisis de cadmio en tejidos biológicos por espectrometría de absorción atómica. Rev. Cubana de Higiene y Epidemiología 34, 2.
Satarug, S. and Moore, M.R. 2012. Emerging roles of cadmium and heme oxygenase in type-2 diabetes and cancer susceptibility. Tohoku. J. Exp. Med. 228, 267-288.
Singh, J., Kant, K., Sharma, H.B. and Rana, K.S. 2008. Bioaccumulation of cadmium in tissues of <i>Cirrhina mirgila</i> and <i>Calta catla</i>. Asian J. Exp. Sci. 22, 411-414.
Sinha, M., Manna, P. and Sil, P.C. 2009. Induction of necrosis in cadmium-induced hepatic oxidative stress and its prevention by the prophylactic properties of taurine. J. Trace Elem. Med. Biol. 23(4), 300-313.
Stephan, C. 1977. Methods for calculating of LC50. En: American Society for Testing and Materials (ASTM) Aquatic Toxicology and Hazard Evaluation. Mayer, F. & Hamelinck, E. (eds.), Philadelphia, Pensylvania, pp: 65-84.
Tang, W., Sadovic, S. and Shaikh, Z.A. 1998. Nephrotoxicity of cadmium-metallothionein:
protection by zinc and role of glutathione. Toxicol. Appl. Pharmacol. 151(2), 276-282.

Vaquero, J., Quilarte, L., López, J., Williams, V., Rojas, L., Bonilla, J. and Ramírez, A. 2004. Evaluación de la concentración por metales de la cuenca del río tigre. Acta Científica Venezolana 55, 81-90.

Vargas, A., Blanco, Y. and Salazar-Lugo, R. 2012. Efecto del cobre y del cadmio sobre la respuesta inmune innata del pez Colossoma macropomum. Rev. Toxicol. 29, 10-14.

Wang, L., Cao, J., Chen, D., Liu, X., Lu, H. and Liu, Z. 2009. Role of oxidative stress, apoptosis, and intracellular homeostasis in primary cultures of rat proximal tubular cells exposed to cadmium. Biol. Trace Elem. Res. 127, 53-68.

Woodling, J.D., Brinkman, S.F. and Horn, B.J. 2001. Nonuniform accumulation of cadmium and copper in kidneys of wild brown trout (Salmo trutta) populations. Arch. Environ. Contam. Toxicol. 40, 381-385.

Woods, J.M., Leone, M., Klosowska, K., Lamar, P.C., Shaknovsky, T.J. and Prozialeck, W.C. 2008. Direct Antiangiogenic Actions of Cadmium on Human Vascular Endothelial Cells. Toxicol. In Vitro 22, 643-651.