Volume 20 – No. 4a – 2011
REPRINT pp. 1089 – 1092
Short Communication

Fresenius
Environmental
Bulletin

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Nuray Çiftçi - Bedii Cicik - Cahit Erdem - Özcan Ay - Fahri Karayakar - Sahire Karaytuğ

PSP

Angerstr. 12
85354 Freising - Germany
Phone: ++49 – (0) 8161-48420
Fax: ++49 - (0) 8161-484248
Email: parlar@psp-parlar.de
www.psp-parlar.de

ISSN 1018 - 4619

Abstracted/ indexed in: Biology & Environmental Sciences, BIOSIS, C.A.B. International, Cambridge Scientific Abstracts, Chemical Abstracts, Current Awareness, Current Contents/ Agriculture, CSA Civil Engineering Abstracts, CSA Mechanical & Transportation Engineering, IBIDS database, Information Ventures, NISC, Research Alert, Science Citation Index (SCI), SciSearch, Selected Water Resources Abstracts

ACCUMULATION OF CHROMIUM IN HEPATOPANCREAS, GILL AND MUSCLE TISSUES OF *Callinectes sapidus*

Nuray Çiftçi[1], Bedii Cicik[1,*], Cahit Erdem[2], Özcan Ay[1], Fahri Karayakar[1] and Sahire Karaytuğ[1]

[1]University of Mersin, Faculty of Aquaculture, Yenişehir Kampüsü, C Blok, Kat 2, 33169 Mersin, Turkey
[2]University of Çukurova, Faculty of Art and Sciences, Biology Department, 01330 Balcalı, Adana, Turkey

ABSTRACT

Accumulation of chromium (VI) in hepatopancreas, gill and muscle tissues of *Callinectes sapidus* was studied after exposing the animals to 1.0, 2.0 and 4.0 ppm chromium (IV) over 96 hours. Tissue levels of the metal were determined using Atomic Absorbtion Spectrophotometric (AAS) methods. No mortality was observed in any concentration of chromium after 96 hours of exposure. Accumulation of chromium in hepatopancreas and gill tissues increased with increasing concentrations of the metal, whereas muscle accumulation was below detection limits of AAS at the concentrations tested. The following relationship was found among the tissues in accumulating chromium; Gill > Hepatopancreas > Muscle

KEYWORDS: Chromium, Accumulation, Gill, Hepatopancreas, Muscle, *Callinectes sapidus*

1. INTRODUCTION

Environmental pollution is one of the greatest global concerns in today's world. In addition to those coming from natural resources, human activities such as urbanization, industrialization and application of vast amounts of chemicals in agriculture increased the levels of inorganic and organic pollutants in water ecosystems [1].

Metals entering aquatic environments accumulate in various tissues of organisms which are then transferred to higher trophic levels through the food chain, resulting in important environmental and health problems.

Chromium is needed in trace amounts by organisms and although it has different chemical forms only its +3 and +6 valences are biologically active and is generally present at +3 valence in nature. Chromium (III) is a basic element in mammals functioning in carbohydrate, lipid and protein metabolisms [2-4]. The main source of chromium is the earth crust and has a natural recycling among lithosphere, hydrosphere and atmosphere. It is widely used in metallurgy and chemistry industries such as metal and electrode plating, leather tanning, textile, phosphate fertilizer, stainless steel, ferrochromium and pigment production [5].

High levels of chromium entering aquatic environments results in mortality, habitat changes, feeding disturbances, accumulation in tissues, inhibition of enzymatic activities, histopathologic changes and reduction in resistance against pathological organism in aquatic organisms [6-14].

Gills form the main bulk of visceral organs in *C. sapidus* and are the main intake route of heavy metals due to their large surface area interacting directly with the external media. Hepatopancreas is a metabolically active tissue which plays role in transformation of nutrients and in detoxification and storage of toxic substances such as heavy metals. Although the not metabolically active muscle tissue forms the main edible part for the organisms at higher trophic levels. Studies carried out with various aquatic invertebrates have shown that heavy metal accumulation is much higher in metabolically active tissues such as gills and hepatopancreas compared with muscle tissue [15-17].

Benthic animals are exposed to heavy metals more than the pelagic ones since metals tend to sink to bottom sediments due to their high density. Hence benthic species are widely used as bio-indicators in environmental pollution monitoring programs [18].

C. sapidus is a protein rich edible species which is common in Mediterranean coasts of Mersin. Since determination of heavy metal levels in aquatic animals reflects the pollution status of the environment, present study was undertaken to determine the accumulation of chromium in gill, hepatopancreas and muscle tissues of *C. sapidus* after exposing the animals to 1.0, 2.0 and 4.0 ppm concentrations of the metal over 96 hours.

2. MATERIALS AND METHODS

C. sapidus was obtained from the drainage channels of the Göksu delta, an environmental protection area around Silifke, Mersin. The weight and carapace length of *C.*

* Corresponding author

sapidus used in the experiments were 87.35 ± 5.40 g and 10.00 ± 0.85 cm respectively. Experiments were carried out in the culture laboratory of the Aquaculture Faculty, Mersin University which had a constant temperature of 24 ±1 °C. Twelve hours light/dark illumination regime was applied during the experiments. Animals were adopted to laboratory conditions in eight glass tanks, sized 40X 100X40 cm, over one month.

Experiments were run in four glass aquaria of the same size mentioned above. The first three aquaria were filled with 120 L of 1.0, 2.0 and 4.0 ppm chromium solutions and the fourth aquarium was filled with the same amount of chromium free tap water and used as control. Six specimens were placed in each aquaria and the levels of tissue chromium was measured after 96 hours.

Experimental aquaria were aerated with central aeration system and some physical and chemical properties of water are given in Table 1.

TABLE 1 - Some physical and chemical properties of experimental water.

Temperature (°C)	22 ±1
Total Hardness (ppm $CaCO_3$)	246,4 ± 3,28
Total Alkalinity (ppm $CaCO_3$)	409 ± 0,63
pH	8,02 ± 0,06
Dissolved Oxygen (mg/L)	7,42 ± 0,47

Experimental solutions were changed daily by serial dilution of newly prepared stock solution ($K_2Cr_2O_7$, Merck) against possible alterations due to adsorption, evaporation and precipitation.

All experimental and control animals were removed from aquaria at the end of 96 hours and their gill, hepatopancreas and muscle tissues were dissected separately for chromium analysis. Dissected tissues were dried to a constant weight at 105°C for 72 hours, their dry weights were determined and then they were transferred to the experimental tubes. There they were digested in nitric acid / perchloric acid mixture (2/1; v/v) at 120°C for four hours. Digested tissues were transferred to polyethylene tubes and their volumes were made up to 10 ml with distilled water.

Chromium analyses of the tissue samples were made using a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer (AAS). Student Newman Keul's procedure was applied in statistical evaluation of data [19].

3. RESULTS AND DISCUSSION

No mortality was observed in *C. sapidus* exposed to the tested concentrations of chromium over 96 hours. The levels of chromium in muscle tissue were below detection limits of the AAS at all the concentrations tested. The means of chromium accumulation in gill and hepatopancreas tissues of the species after exposure to the metal are given in Table 2.

TABLE 2 - Accumulation of chromium (µg Cr / g d.w.) in gill and hepatopancreas tissues of *C. sapidus*

Cr (VI) Concentration (ppm)	Hepatopancreas $\overline{X} \pm S\overline{x}$ *	Gill $\overline{X} \pm S\overline{x}$ *
0.0	0,95 ± 0,25 ax	1,07 ± 0,11 ax
1.0	5,94 ± 0,72 bx	18,93 ± 0,98 by
2.0	13,89 ± 1,08 cx	33,01 ± 1,17 cy
4.0	36,56 ± 1,57 dx	67,50 ± 3,61 dy

*SNK; Data shown with different letters indicate significant difference at P<0.05 level. Letters x and y are used to show differences between tissues and a, b, c, d among concentrations.
X ± Sx = Mean ± Standard error

Metal accumulation was the highest in gill tissue followed by hepatopancreas at all chromium concentration tested (P<0.05) while muscle levels were below the detection limits of AAS [Table 2]. Tissue accumulation of chromium in gill and hepatopancreas increased with the concentration of metal in the medium.

Effects of heavy metals on mortality in aquatic organisms depend on the species. However mortality rate rapidly increases above a given concentration of the metal in all species [20]. In the study of Ma et al. [21] it was shown that mortality rates of *Sinopotamon henanense* exposed to 5.8, 11.6 and 23.2 ppm Cd were 0%, 6% and 16% under laboratory conditions [21]. In present study no mortality was observed in *C. sapidus* exposed to 1.0, 2.0 and 4.0 ppm chromium which were possibly were sublethal for this species over 96 hours.

Accumulation and toxic effects of chromium are dependent not only upon the chemical form, concentration and exposure period but also upon the biotic factors such as species, developmental period and sex and upon abiotic factors such as water pH, dissolved oxygen, temperature, hardness, salinity and dissolved materials [22].

Metal accumulation was higher in gill tissue followed by hepatopancreas and muscle tissues exposed to sublethal concentrations of chromium in *Cancer irroratus* and *Cancer magister* [23, 24] and in *S. henanense* exposed to sublethal concentrations of cadmium [21].

Highest accumulation of Cd and Zn was found to be in hepatopancreas in *Mytilus galloprovincialis* [15] whereas highest accumulation was in gill tissue in *Vesicomya gigas* [16]. Accumulation of Cd and Cu was higher in hepatopancreas tissue while Pb accumulation was higher in gill tissue of *Ruditapes philippinarum* exposed to sublethal concentrations of Cd, Pb and Cu [25].

Brachidontes pharaonis exposed to 1.0 ppm copper [26] and to 0.1, 0.2 and 0.4 ppm lead over 96 hours [27] accumulated the metals in their gill, hepatopancreas and muscle tissues in decreasing order.

C. sapidus exposed to 1.0, 2.0 and 4.0 ppm concentrations of Cr (VI) over 96 hours accumulated highest levels of metal in its gill tissue followed by hepatopancreas, whereas accumulation in muscle tissue was undetectable.

Heavy metal accumulation in aquatic organisms does not only depend on differences among the tissues but also on anatomical and morphological differences between the species and on the metal uptake routes [28]. Studies carried out with decapods species both in field [29, 30] and under laboratory conditions [21, 26, 27, 31] have shown that muscle is not an active tissue in accumulating metals which might be due to the carapace coverage of the tissue and its interaction with the metals is only via hemolymph. It has also shown that metabolically active tissues such as gills and hepatopancreas accumulate metals to rather high levels. Since gills have large surface area and is in direct contact with the external media and the hepatopancreas is the main site in synthesizing metal binding proteins and is the detoxification center of the organism.

C. sapidus also accumulated high levels of Cr (VI) in its gill and hepatopancreas tissues after being exposed to 1.0, 2.0 and 4.0 ppm chromium over 96 hours which revealed the metabolic activeness of these tissues.

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Received: October 25, 2010
Accepted: December 08, 2010

CORRESPONDING AUTHOR

Bedii Cicik
University of Mersin
Faculty of Aquaculture
Yenişehir Kampüsü, C Blok, Kat 2
33169 Mersin
TURKEY

E-mail: bcicik@mersin.edu.tr