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Accumulation of Copper in Gill, Liver, Spleen, Kidney and Muscle Tissues of *Clarias gariepinus* Exposed to the Metal Singly and in Mixture with Chitosan

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Abstract Accumulation of copper (Cu), applied singly and in mixture with chitosan (CT), in gill, liver, spleen, kidney and muscle tissues of *Clarias gariepinus* was studied after exposing the fish to no Cu (control), 5 ppm Cu, 5 ppm Cu + 75 ppm CT mixture over 1, 7 and 15 days. Metal levels in tissues were determined using an ICP-AES spectrophotometer. No mortality was observed during the experiments. Highest accumulation of Cu was observed in liver while lowest accumulation was observed in muscle tissue. Exposure to Cu–CT mixture decreased Cu accumulation in liver at all exposure periods and in kidney on the 15th day compared to Cu alone. Exposure to Cu alone and Cu–CT mixture had no effect on Cu accumulation in spleen or muscle tissue. Copper accumulation increased in gill tissue compared to control when exposed to Cu alone at all exposure periods, and exposure to the Cu–CT mixture significantly increased Cu accumulation in this tissue at all exposure periods compared to Cu alone.

Keywords Copper · Chitosan · Mixture · Accumulation · *Clarias gariepinus*

Heavy metal pollution is a serious environmental problem in the world, especially in developing countries. Copper is a basic element that is required for a number of metabolic functions (Cicik 2003; Tunçsoy et al. 2015). It is a structural component of various enzymes and plays a role in major processes, such as development, growth and reproduction. Exposure to this metal over certain concentrations, however, results in accumulation which may alter various physiological functions (Nussey et al. 1995). Fish are frequently used in metal accumulation studies since they can accumulate metals in excess amounts, resulting in potential health effects to human consumers. Some fish species are used as pollution indicators (Zhou et al. 2008).

Chitosan (CT) is produced by alkaline *N*-deacetylation of chitin, which is widely found in the exoskeletons of shellfish. CT has reactive amino groups which form complexes between metal ions and the polymer chain. McKay et al. (1989) reported strong adsorption capacities of CT for Hg, Cu, Ni, and Zn. Since CT possesses unique properties, including low-toxicity, biocompatibility, low-cost and good handling properties, it has attracted interest by the aquaculture industry (Samarakoon et al. 2013). In aquaculture, CT is found to have immune stimulatory activity in fish and shellfish (Sakai 1999).

Clarias gariepinus is a widely distributed species in inland waters and drainage channels of the Mediterranean region and is commonly consumed as a protein source. Due to its wide tolerance against pollutants and its habitat being under direct influence of agricultural and industrial activities, the species was chosen as the experimental fish.

Exposure to heavy metals results in the accumulation of the metals in tissues. CT is well known as an excellent biosorbent for metal cation removal from aqueous solution. Hence the aim of the present study was to determine metal accumulation in gill, liver, spleen, kidney and muscle tissues of *C. gariepinus* exposed to control water (no Cu or CT), 5 ppm Cu and 5 ppm Cu + 75 ppm CT over 1, 7 and 15 days.

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Materials and Methods

C. gariepinus was obtained from a private fish farm in Silifke–Mersin, TR. The mean length and weight of the animals were 21.9 ± 1.5 cm and 73 ± 3.11 g, respectively. Fish were adapted to laboratory conditions for 1 month in glass aquaria (40 × 120 × 40 cm in height). Experiments were run in triplicate, with 3 fish per replicate, hence 9 fish were placed into each aquarium. The same sized three aquaria were used in the experiments. The first two aquaria were filled with 120 L of 5.0 ppm Cu, 5.0 ppm Cu + 75 ppm CT, respectively while the third one was filled with the same amount of copper free tap water and used as control. Copper sulphate ($CuSO_4$:$5H_2O$) was used in the preparation of experimental solutions and trisodium citrate ($C_6H_5Na_3O_7 \cdot 5H_2O$) was used to prevent precipitation and adsorption of the metal. Mean copper levels in exposure media were determined as 4.95 ± 0.05 mg/L Cu. Experimental solutions were replaced daily, by serial dilutions of freshly prepared 1000 ppm stock solution of the metals. Acetic acid (1 %) was used to prepare CT stock solution (Aldrich, GR, Deacetylation ≥75 %). Some physical and chemical properties of experimental water are given in Table 1.

Fish were fed once a day with commercial fish food (Pınar, Pellet No: 2, İzmir, TR) at amounts of 2 % of total biomass. Three fish were removed from each aquaria at the end of each exposure period. Fish were anesthetized with MS222. They were washed, dried and dissected for their gill, liver, spleen, kidney and muscle tissues. Tissues were transferred to petri dishes and were placed in a drying oven set at 150°C for 48 h. Dried tissues were weighed, transferred to experimental tubes and digested in nitric acid (Merck, 65 %)/perchloric acid (Merck, 60 %)(Darmstadt, GR) mixture (2/1; v/v) at 120°C for 3 h (Muramoto 1983). Digested tissues were transferred to polyethylene tubes and their volumes were made up to 10 mL with ultra-pure water. Metal levels in tissues were determined using a Perkin Elmer 7000 DV optical emission spectrophotometer (Waltham, Massachusetts, USA). Quality assurance was checked using a standard reference material (lobster hepatopancreas) provided by the National Research Council, Canada-TORT II. Results were within the limits of 106.0 ± 10.0 µg Cu/g d.w. certified value. Statistical evaluation of the experimental data was carried out by a series of analysis of variance and Student Newman Keul's procedures (Sokal and Rohlf 1995).

Results and Discussion

There was no mortality in *C. gariepinus* exposed to 5.0 ppm Cu over 15 days. Previously observed behavioral differences such as food rejection and increase in operculum movements in *Oreochromis niloticus* exposed to Cu, Zn and Cd were also true for *C. gariepinus* exposed to copper at the beginning of the experiments (Duran and Erdem 2014; Tunçsoy and Erdem 2014).

Gills are the target organs in accumulating metals since they are in direct contact with the external media (Heath 1995). CT obtained from deacetylation of chitin is used as a flocculent for sewage and brewery wastes, and as a chelator of heavy metals. It has generally been considered to be nontoxic to animals.

However, CT has been shown to be toxic to rainbow trout *Oncorhynchus mykiss* (Bullock et al. 2000). Exposure to 0.038, 0.075 and 0.75 ppm CT resulted in mortality in less than a week exposure, while exposure to 0.019 ppm resulted in no mortality after 14 days of exposure. Histological examination of gills, skin, muscle, and internal organs indicated significant and consistent pathological changes only in gills. Lifting of lamellar epithelium, hypertrophy and hyperplasia of lamellar epithelial cells occurred in trout exposed to 0.019 and 0.038 ppm. Large areas of lamellar fusion were observed in trout exposed to 0.75 or 0.075 ppm CT (Bullock et al. 2000).

Higher concentrations of CT were acutely toxic with most fish dying within 24 h when exposed to 0.75 ppm, and within 72 h when exposed to 0.075 ppm. Epithelial lifting and cellular hypertrophy were observed in these fish. These lesions have been reported as acute responses to heavy metals such as cadmium, mercury, and copper (Ferguson 1989). The author noted that the glycoprotein in the mucous covering may be altered by the toxicant, which favors adhesion to adjacent lamellae by affecting a negative charge of the epithelium.

There are a number of studies on immune stimulatory activity of chitin and CT in a range of fish including *O. mykiss* (Anderson et al. 1995), *Sparus aurata* (Esteban et al. 2001), *Cyprinus carpio* (Gopalakannan and Arul 2006) and *Paralichthys olivaceus* (Cha et al. 2008). In our study, copper accumulation increased in gill tissue of *C. gariepinus* compared to control fish when exposed to Cu alone at all exposure periods (Fig. 1a). Exposure to the Cu–CT mixture significantly increased copper accumulation in this tissue at all exposure periods compared to Cu alone

Table 1 Some properties of the exposure water

Illumination regime: 12 h/12 h light/dark	Temperature: 22 ± 1°C
Total alkalinity: 305 ± 0.5 mg $CaCO_3$/L	Dissolved oxygen: 7.1 ± 0.5 mg/L
pH: 8.2 ± 0.5	

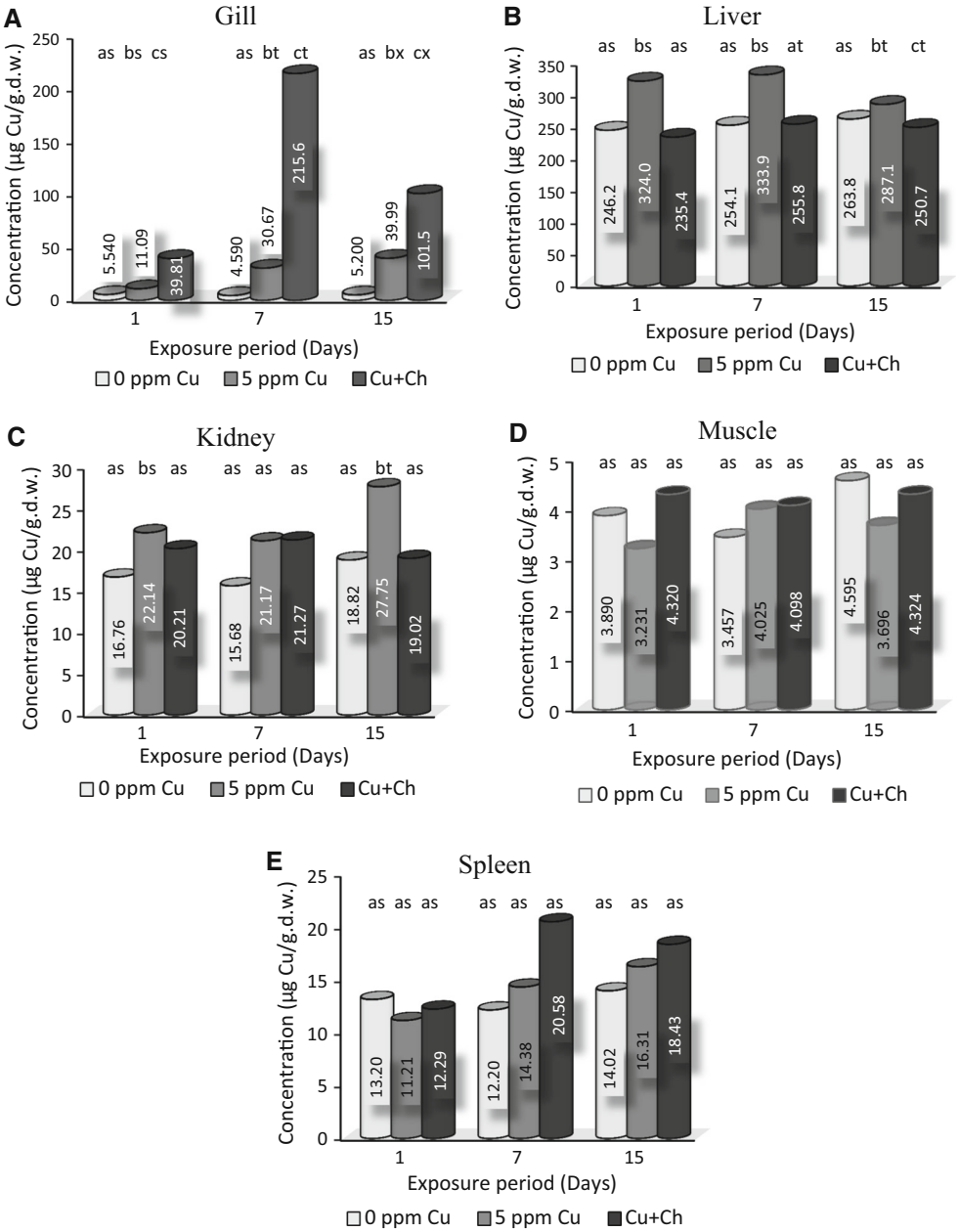

Fig. 1 Copper accumulation in various tissues of *Clarias gariepinus* exposed to Cu and Cu–CT mixture over 1, 7 and 15 days (µg Cu/g.d.w.). *Letters a*, *b*, *c* and *s*, *t*, *x* show differences among concentrations and exposure periods at a given tissue, respectively ($p < 0.05$)

(Fig. 1a; $p < 0.05$). Toxic effects of CT on gill tissue might result in increased mucus secretion and elevate its metal binding capacity as a protective immunostimulant response.

Metals entering from gills are first transferred to liver, by the circulatory system, and when the carrying capacity of liver is exceeded, they are transferred and stored in metabolically active tissues, especially in kidney (Heath 1995). The Cu and CT complex decreased oxidative stress in *C. carpio* (Dautremepuits et al. 2004). Copper accumulation increased in liver tissue of *C. gariepinus* compared to control when exposed to Cu alone at all periods (Fig. 1b; $p < 0.05$) while kidney accumulation increased only on the 15th day. Exposure to the Cu–CT mixture decreased copper accumulation in liver at all exposure periods (Fig. 1b; $p < 0.05$) and in kidney on 15th day compared to Cu alone (Fig. 1c; $p < 0.05$). This might result from differences in metabolic activities of these tissues and/or reactive amino groups of CT forming complexes with Cu ions in liver.

Although muscle is not an effective tissue in binding metals, it is important to know metal levels in this tissue as far as food chain transfer and human health are concerned. Exposure to Cu alone and the Cu–CT mixture had no effect on Cu accumulation in muscle tissue (Fig. 1d). Likewise, Cu accumulation in the spleen was not affected by the addition of CT in the exposure water (Fig. 1e).

It was concluded that CT was shown to bind copper effectively in gill tissue of *C. gariepinus*. CT seemed to have a synergistic effect on Cu accumulation in this tissue as a result of elevated metal binding capacity due to increased mucus secretion as a protective immunostimulant response. This in turn might be the reason for the decrease in liver copper accumulation at the exposure periods tested.

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