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Copper intoxication in tropical freshwater prawn, Macrobrachium rosenbergii

H. A. Kaoud, Quratulan Ahmed
Department of Veterinary Hygiene and Environmental Pollution, Faculty of Veterinary Medicine, Cairo University, Egypt.
Department of Zoology, University of Karachi, Karachi, Pakistan

Abstract- The aim of this study was to investigate LC_{50} and toxic effect of Cu^{2+} on some defense functions of tropical freshwater prawn, Macrobrachium rosenbergii [including total hemocyte count (THC), hyaline cell count (HCC), and phagocytic activity] as well as survivability of the prawn. The experiments were conducted to determine LC_{50} and the toxic effect of copper sulphate (Cu^{2+}) on THC, HCC, phagocytic activity % and survival % for 24, 48, 72 and 96 hr exposure. The 24, 48, 72 and 96 hr LC_{50} (nominal calculation) were 0.60, 0.55, 0.45 and 0.35 mg L^{-1}, respectively. Survival of prawns exposed to more than 0.20 mgL^{-1} of Cu^{2+} was significantly ($P< 0.05$), reduced and resulted in great reduction in THC, HT, phagocytic activity %, and histopatho logical alterations in gills (hyper mucus, congestion, swelling , edema , hyperplasia, haemolymph cell infiltration as well as thickened and enlarged gill chambers & lamellar sinuses), hepatopancreas (dissolving of the hepatocytes, haemolysis, haemocytic infiltration in the interstitial sinuses, thickening and ruptures of the basal laminae) and skeletal muscles (degeneration in muscles with infiltration and aggregations of hemocytes between them and focal areas of necrosis). Caution should be exercised against water source contamination and exposure to fertilizer and industrial pollution.

Index—Copper; **Macrobrachium rosenbergii; Immunity; Toxicity**

I. INTRODUCTION

Crustaceans utilize hemocyanin as the oxygen-carrying pigment .This copper-containing pigment has analogous role to hemoglobin in red-blooded animals. In addition to the physiological functions of copper, high levels of environmental copper have been found to be toxic to a variety of aquatic species. Copper concentration far exceeds the background of copper level of water (0.5 μgL^{-1}). Due to toxic effects of dissolved copper, shrimp hatcheries routinely use water that treated with ethylenediaminetetraacetic acid to chelate free copper. In decapod crustaceans, 3 types of circulating hemocytes are recognized: hyaline, semi-granular and large granular cells [1]. They are involved in cellular immune responses that include phagocytosis and constitute the primary method of eliminating microorganisms or foreign particles [2]. In addition to phagocytosis, hemocytes are involved in coagulation and in the production of melanin via the prophenoloxidase system [3, 4]. Several physico-chemical parameters and environmental contaminants have been reported to affect the immune response in crustaceans and these have been reviewed by [5]. Copper (Cu^{2+}) toxicity for fish is primarily related to structural damage to the gills [6] but in crustaceans the physiological effects of copper toxicity are not as clearly understood. Circulating hemocytes can be affected by extrinsic factors in several species of decapods crustaceans [7, 8, 5, 9]. Environmental toxicants have been reported to cause a reduction in hemocyte count in the common shrimp *Crangon crangon* [10]. Copper salts (copper hydroxide, copper carbonate and copper sulphate) are widely used in agriculture as fungicide, algaecide and nutritional supplement in fertilizers. They are also used in veterinary practices and industrial applications. Copper sulphate is released to water as a result of natural weathering of soil and discharge from industries, sewage treatment plants and agricultural runoff. Copper sulphate is also intensively introduced in water reservoirs to kill algae. Thus excessive amount of copper accumulates in water bodies and cause toxicity to aquatic fauna and flora and ultimately to man. Cu^{2+} and its compounds have been designated as priority pollutants by [11]. Present study was carried out on the fresh water prawns *Macrobrachium rosenbergii* (Crustacean - Decapods) to evaluate the LC_{50} values of copper sulphate, its effect on immunity and survivability as well as the histopathological alterations in this tropical prawn.

II. MATERIALS AND METHODS

Experimental designs

In tests, freshwater was adjusted with the desired temperature of 20-28 °C. and the desired pH, freshwater was adjusted with 1 N HC1 or 1 N NaOH solutions (pH;7-7.8,dissolvedoxygen;5-8,salinity;12-15‰,hardness;10 0-150ppm $Ca(CO)_3$, total ammonia; less than 10 ppm, nitrate; 20 ppm, nitrite 1ppm).

Stock copper solution

Stock solution of copper sulphate ($CuSO_4.5H_2O$: AR grade: Elgomhoria laboratories, chemical division-Cairo, Egypt) was prepared by dissolving 100 mg of salt in 100 ml double distilled water. Two drops of glacial acetic acid was added to stock solution so as to prevent the precipitation [12].

Macrobrachium rosenbergii

Were obtained from a commercial farm in Egypt, and acclimated in the laboratory for 7 days before experimentation. For experiments, test and control groups comprised 10 prawns each in triplicate. After treatment, each group of 10 prawns was kept in a separate 30 L glass aquarium containing 25 L aerated water.

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Acute toxicity test

The acute toxicity test was performed according to the USEPA procedure for the static non-renewal technique [13]. After an acclimatization period, 7 days .Prawn [6.7 to 7.5 g, averaging 8.10 (± 0.15) g in weight] were transferred from the stock tank to the experimental aquaria. Ten prawns were randomly placed in each glass aquarium filled with 25 liter of water and were not fed for 48 hr before starting and for 96 hr during the experiment. The tests consisted of a control and at least five concentration groups (0.20, 0.40, 0.50, 0.60 and 0.80 mg L-1), five replicates per group, with ten prawns in each replicate. At the beginning of the test and every 24 hr, the symptoms and the number of dead prawn were recorded. The results of the median lethal concentration (LC50) at 24 hr, 48 hr, 72 hr and 96 hr were computed.

Immune activity

For immune activity assays, tests were carried out in triplicate or quadruplicate test groups consisting of 2 prawns each in separate 30 L glass aquaria containing 25 L aerated water. In all tests, prawns were fed twice daily with a formulated prawn diet. During experiments, water temperature was maintained at 20 -28 °C, pH;7-7.8,dissolved oxygen;5-8,salinity;12-15‰,total hardness;100-150 ppm Ca(CO)3,total ammonia; less than 10 ppm,nitrate;20 ppm,and nitrite 1ppm. The wet weight of prawn in the intermolt stage ranged from 6.7 to 7.5 g, averaging 8.10 (± 0.15) g (mean SD) with no significant difference among various treatments [14]. Immune activity assays were carried out in quadruplicate with test groups consisting of two prawns each in separate glass tanks (30 L) containing 25 L of aerated test solution- The prawns were exposed to each treatment for 96 hr.

Cells count

Hemolymph (100 µl) was sampled individually at the beginning of each test and at 96 h. It was withdrawn from the ventral sinus of each prawn into a 1 ml sterile syringe (25 gages) containing 0.9 ml anticoagulant solution (trisodium citrate 0.114 M, sodium chloride 0.1 M, pH 7.45, osmolality 490 mOsm kg–1). A drop of the anticoagulant-hemolymph mixture was placed on a hemocytometer to measure THC and HC using an inverted-phase contrast microscope

Culture of L. garvieae

The bacterial strain L. garvieae isolated from diseased (artificial infection) of Macrobrachium rosenbergii was used in this study. The bacterium was cultured on tryptic soy agar (TSA) for 24 h at 28. °C before being transferred to 10 ml of tryptic soy broth (TSB) for 24h at 28 °C as a stock culture. The stock cultures were then centrifuged at 7155 x g for I5 min at l4 °C. The supernatant fluid was removed and the bacterial pellet was resuspended in saline solution (0.85 NaCL) at 1010 cfu ml-1 as stock bacterial suspensions for testing.

Phagocytic activity of M. rosenbergii to L. garvieae

After exposure in each treatment, prawns were injected in the cephalothoraxes with 20 µL of the bacteria suspension (1010 cfu mL-1 in 0.85% NaCI) resulting in 2 x 108 ' cfu prawnl-1 .After injection the prawns were held in their respective solutions for 3h. Hemolymph (200 µl) was collected from the ventral sinus and mixed with 200 µl of sterile anticoagulant containing sodium citrate, 0.8 g; EDTA, 0.34 g; Tween 80, 10 µl; distilled water, 100 ml; pH, 7.45; 490 mOsM kg-1.This mixture was used to measure phagocytic activity. With two prawns in each of three replicates, a total of 6 measurements parameter-1 existed for each treatment. Phagocytic activity was measured using the method described by [15]. Where 200 µl of diluted hemolymph sample was mixed with 0.2 ml of 0.1% Para formaldehyde for 30 min at 4 °C to fix the hemocytes. They were then centrifuged at 800x g at 4 °C, washed and resuspended in 0.4 ml of sterile phosphate buffer solution. The suspension (50 µl) was spread onto a slide glass and air-dried and stained with Diff-Quick stain. 200 hemocytes were counted using light microscope and the phagocytic rate was estimated as follows: PR = [(phagocytic hemocytes) / (total hemocytes)] x□ 100.

Cu^{2+} residue

Cu^{2+} residues were measured in water, liver and muscles according to method of [16]. The water samples were preserved by the addition of one mL of concentrated nitric acid per liter until the time of analysis. The water samples were filtered through 0.45µl membrane filter. The required volume (100 ml) of the filtrate was collected to measure Cu^{2+} levels in water samples by using Air/Acetylene Flame Atomic Absorption Spectrophotometer (UNICAM 696 AA Spectrometer). The analysis of tissue sample was represented by 0.5 gram of tissues dissected from the liver and muscles, then placed in a clean screw-capped tube and digested according to the method described by [17]. The obtained solutions were then analyzed by using Air/ Acetylene Flame Atomic Absorption Spectrophotometer (UNICAM 696 AA Spectrometer).

Histopathological examination

Tissue specimens from fresh *Macrobrachium rosenbergii* were taken (gill, hepatopancreas, Muscles) and fixed in 15 % buffered neutral formalin. They were processed to obtain five micron thick paraffin sections then stained with Hematoxylin and Eosin [18] and examined under light microscope.

Statistical analysis

Data were analyzed by analysis of variance and Pearson's correlation, which calculated the relationships between metal concentration and survival rate of prawn.

III. RESULTS

Acute toxicity

(LC_{50})

The 24 hr, 48 hr, 72 hr and 96 hr LC50 values for $CuSO_4.5H_2O$ in *Macrobrachium rosenbergii* were 0.60, 0.55, 0.45 and 0.35 mg L^{-1}, respectively (Fig.1). A regression analysis of prawn survival (%) on Cu^{2+} concentration was highly significant ($P < 0.001$; $r^2 = 0.979$). Using the resulting regression equation, the 72-hr LC_{50} for Cu^{2+} was calculated to be 0.45 mgL^{-1}, while, it was 0.35 mgL^{-1} for 96-hr.

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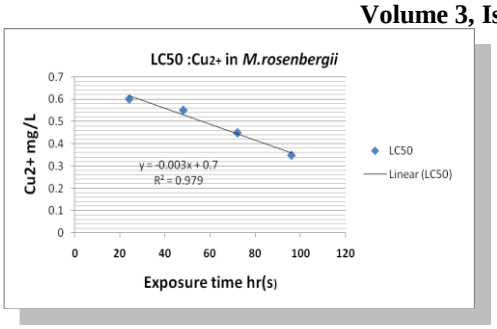

Fig.1: The 24 hr, 48 hr, 72 hr and 96 hr LC50 values for Cu^{2+} (nominal concentration).

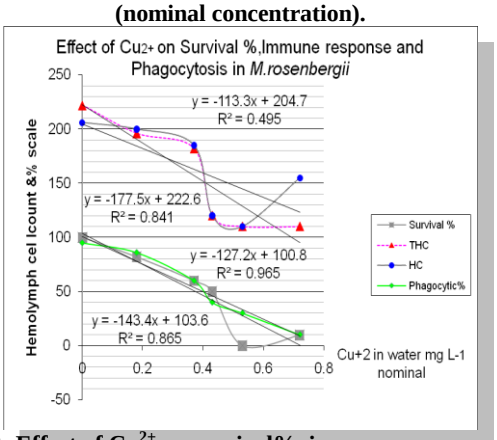

Fig.2: Effect of Cu^{2+} on survival%, immune-response and phagocytosis % in *M.rosenbergii*.

Table 1. Effect of Cu^{2+}on survival, THC (total hemocyte count), HC (hyaline cell count) and phagocytic % of freshwater prawns, *Macrobrachium rosenbergii*, exposed to Cu^{2+} at different concentrations for 96 hr post-treatment. Values are means± SD (n = 4 prawns in each case).

Cu^{2+} & Survival %.		Immune réponses.		
In water		T H C²	H C²	Phagocyic %
0	100	222±72	206±38	95±1.20
0.18	82±1.62	196±19	200±22	86±1.20
0.37	60± 2.70*	182±25*	185±20*	60±0.60*
0.43	50±3.00*	120±12*	120±12*	40±0.10*
0.53	30 ±0.10*	110±7.0*	110±11*	30±0.12*
0.72	10±0.10*	110±8.0*	115±22	10±1.20

Survival percent

There were significant differences ($P < 0.05$) in the survival among different treatments. After 72 hr, mean *(± SD)* survival of prawns in control tanks (0 Cu^{2+}) was 100 % and significantly higher ($P < 0.05$) than that of prawns in all other treatments (Table 1). At 72 hr, survival of prawns exposed to 0.20 mgL^{-1} and 0.40 mgL^{-1} concentrations of Cu^{2+} were significantly greater ($P < 0.05$) than for prawns exposed to higher doses, but were not significantly different from each other ($P < 0.05$). Survival of prawns exposed to 0.6. , and 0.8 mgL^{-1} of copper were significantly lower ($P < 0.05$), with means *of (± SD)* 40 ±3.0, and 30 ± 0.10 %, respectively, but at concentration 1, 0 mgL^{-1} it was 0 % (Table 1). After 96 hr, mean (± SD) survival of prawns in control tanks (0 Cu^{2+}**)** was 100 % and significantly higher ($P < 0.05$) than that of prawns in all other treatments (Table 1). At 96 hr, survival of prawns exposed to 0.20 mgL^{-1} (92 ±1.62) was significantly greater ($P < 0.05$) than for prawns exposed to higher doses. Prawns exposed to 0.40, 0.50, 0.60 and 0.80 mgL^{-1} showed significantly reduction of survival rate *(P< 0.05)*, with means of (± SD) 60 ± 2.70, 50 ± 3.0, 30 ± 0.10 and 10 ± 0.2 %, respectively, while at concentration 1, 0 mgL^{-1} it was 0 % (Fig. 2).

Immune activity

72-hr Cu-exposure, of 0.40, 0.5, 0.6 and 0.8 mgL^{-1} concentrations were significantly ($P < 0.05$), had greater reduction in THC, HC and Phagocytic activity % than for prawns exposed to lower concentrations (0.20 mgL^{-1}). Concerning 96-hr exposure to 0.20 mgL^{-1} Cu^{2+}, THC, HC and phagocytic activity % were showed no significant reduction ($P < 0.05$), but, at concentrations 0.40, 0.5, 0.6 and 0.8 mgL^{-1} they were showed great significant ($P < 0.05$) reduction .

Histopathological alterations

Gills: showed hyper mucus, mild congestion, swelling and edema at low doses of Cu^{2+} intoxication. Severe edema, hyperplasia, haemolymph cell infiltration as well as thickened and enlarged gill chambers & lamellar sinuses at highest doses were observed (Fig.3).

Hepatopancreas: showed dissolving of the hepatocytes, haemolysis, haemocytic infiltration in the interstitial sinuses, thickening and ruptures of the basal laminae (Fig.4).

Muscles: Muscular tissues showed pathological alterations; included degeneration in muscles with infiltration and aggregations of hemocytes between them and focal areas of necrosis. Also, atrophy of muscle bundles, edema, hyaline degeneration and splitting of muscle fibers. A; splitting of muscle fibers, B: hyaline degeneration, C: infiltration of hemocytes, D: focal areas of necrosis, E: atrophy of muscles bundles and edema (Fig.5).

Table 2: Semiquantitative scoring of gill and hepatopancreas in freshwater prawn Macrobrachium rosenbergii during acute Cu^{2+} exposure.

	Exposure Time (hr)			
Histopathology	24	48	72	96
Gill				
Hypermucus	++	+++	+++	+++
Swelling and edema	+	++	+++	+++
Hyperplasia	-	+	++	+++
Thickened and enlarged	-	+	++	+++
Hepatopancreas				
Haemocytic infiltration	+	++	++	+++
Hepatocytes Degeneration	-	+	++	+++
Rupture basal laminae	-	-	+	+++
Muscles				
Necrosis	-	-	++	+++
Atrophy	-	-	+	++
Hyaline degeneration	-	-	+	++

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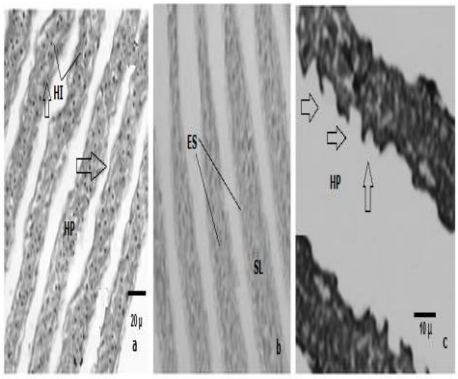

Fig.3: Exposed prawn shown hemocytic infiltration (HI), had swollen (SL) and enlargement of the lamellar sinuses (ES) and hyper-mucus (HM) in the interlamellar spaces, necrosis and hyperplasia (HP) of lamellae. H&E stain, (x200),c :(x400).

Bioaccumulation of Cu^{2+} in different tissues

The bioaccumulation of Cu^{2+} in different tissues of *M. rosenbergii*. The highest bioaccumulation of Cu^{2+} was observed in the organs mainly implicated in metal intoxication Cu^{2+} in tissues was high in the gills > hepatopancreas > muscles ,as shown in Table 3.

Table 3: Copper residue in water (µg L^{-1}), gills, hepatopancreas and muscles (µg Cu g^{-1} dry weigh) of Giant prawn *Macrobrachium rosenbergii*) exposed to Cu^{2+} (96 h LC_{50}) Values are means ± SE.

Cu^{2+} conc. (Cu^{2+} µgL^{-1})	Water†	Accumulation in tissues Cu^{2+} µg g^{-1}(dry weight) Gills	Hepatopancreas	Muscles
0	10 ± 0.11	-	-	-
200	179±10	0.50 ±0.008	0.20 ±0.006	0.05 ±0.001
400	340 ± 28	0.60 ±0.018	0.30 ±0.012	0.02 ±0.003
500	475 ± 30	0.65 ±0.021	0.40 ±0.009	0.02 ±0.005
600	555 ± 38	0.80 ±0.022	0.60 ±0.011	0.03 ±0.01
800	722 ± 44	10.0 ±0.011	0.80 ±0.012	0.55 ±0.01

Gills: The rate of accumulation of Cu^{2+} was maximum in gills of exposed prawns and no detectable amount of Cu^{2+} was observed in the gills of control prawns, as well as at the lowest concentration of Cu^{2+} (0.20 mg L^{-1}). The rate of accumulation increased along with the increasing of Cu^{2+} concentration, reaching 10 µg $.g^{-1}$ at 0.80 mg L^{-1}, Table 3. *Hepatopancreas:* As with the gills, Cu^{2+} could not be traced in the hepatopancreas of the control fish, as well as at the lowest concentration (.020 mg L^{-1}). Even though the quantity of accumulated Cu^{2+} was less in the case of hepatopancreas when compared to gills, the pattern of accumulation showed a more or less continuous increasing trend, Table 3.

Muscles: The rate of accumulation of Cu^{2+} in muscle increased along with the exposure to high concentrations. The mean rate of accumulation at 0.80 mg L^{-1} was 55 µg $.g^{-1}$, this rate of accumulation was less as compared with other tissues, Table 3.

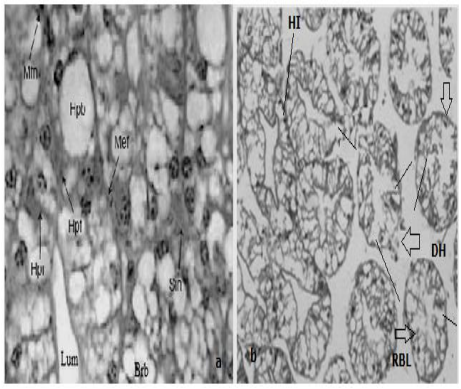

Fig.4-a: Cross sections of hepatopancreatic tubules: normal lumens (Lum), tubule tissues (Hpf: F-cell; Hpb: B-cell; Hpr: R-cell; Mfn: myoepithelial cell nuclei; Mef: myoepithelial layer; Brb: microvillus brush borders) and hemal sinuses (Sin) between tubules.

Fig.4-b: Cross sections of hepatopancreatic tubules: showed hemocytic infiltration (HI) in the interstitial sinuses, an increased number of hemocytes, thickening and ruptures of the basal laminae, and necrosis of the tubules (arrowheads). H&E stain, (x200).

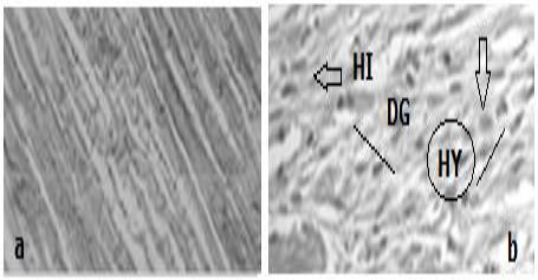

Fig.5-a: Longitudinal sections of muscle tissue, healthy prawn tissue showing normal muscle fibers. Fig4-b: Cross section showing degeneration (DG), focal areas of necrotic musculature infiltrated by hemocytes (HI) . Also, atrophy of muscle bundles, hyaline degeneration (HY) and splitting of muscle fibers were seen. H&E stain, (x200).

IV. DISCUSSION

Cu^{2+} toxicity for fish is primarily related to structural damage to the gills [6] but in crustaceans the physiological effects of copper toxicity are not as clearly understood. Circulating hemocytes can be affected by extrinsic factors in several species of decapods crustaceans [7, 8, 5, 9]. "Ref. [18] and [19]" found Cu^{2+} to be toxic to *P. aztecus* and *P. duorarum* larvae at 0.05 mgL^{-1} (50 ppb) while normal growth occurred at 0.025 mgl^{-1}. Inhibition of reproduction in the brine shrimp *Artemia salina* has also been shown after exposure to extremely low levels of CuSo4. While the 48-hour LD50 for this *Artemia* species was~25 ppt, adverse effects on reproduction were found at levels 24,000 to 156,000 times lower [20]. Cu^{2+} at 1.3 mgL^{-1} was lethal in 24 hr (s) to 100% of *P. stylirostris* larvae [21, 22] and was toxic at 0.1 mgL^{-1} to adult lobsters [23, 22]. Concentration of 0.14 mgl^{-1} Cu reduced survival of *Mysidopsis bahia* while 0.077 mgL^{-1} Cu^{2+} reduced reproduction in *M. bahia* [24,22] . Freshwater prawns appear to be more sensitive to copper than

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most other species of crustaceans that have been studied. [25] studied the toxic effects of water-borne copper on the giant freshwater prawn *Macrobrachium rosenbergii*, they recorded that, exposure to elevated copper levels might damage the ultrastructure of the gills and hepatopancreas of *M. rosenbergii* and might further weaken their normal physical activities The LC_{50} values obtained in present study (Fig,1) are mainly closure to the findings of [26,27] (*Macrobrachium lamarrei* and *Macrobrachium dayanum* exposed to Cu^{2+} (the 24, 48, 72 and 96 hr LC_{50} values of copper sulphate for *M. lamarrei* were 0.38, 0.361, 0.343 and 0.300 mgL^{-1} and for *M. dayanum* were 1.634, 0.988, 0.532 and 0.418 mg l^{-1} ,respectively) and [28] (LC_{50} 96 hr of Cu^{2+} value for *Macrobrachium lanchesteri* was 32.3 μgL^{-1}). The structural alterations observed in gills in the present study were similar to those of *Macrobrachium lamarrei* and *Macrobrachium dayanum* exposed to Cu^{2+} [27] *Macrobrachium kistenensis and Caridina sp.* [29], profused secretion of mucous on whole body parts and more pronounced in gill region, *Puntius conchonius* [30] and *S. gairdneri* [31] after exposure to copper and in the reviews of [32, 33]. Furthermore, the hisological structures of gill and hepatopancreas in the present study were similar to those of *Charybdis japonica* [34] ,filaments of *Charybdis japonica* exposed to 2 mgl^{-1} Cu^{2+}were thickened irregularly and enlarged gill chambers in which haemolymph cells appeared much more, hepatopancreas dissolved and only an envelope of collected tissue was left around the hepatopancreas duct. “Ref. [35]” studied the effect of three concentrations of Cu^{2+} (3.512, 1.756 and 0.877 mg L^{-1}) on juvenile *Litopenaeus vannamei* and found that there were severe time- and dose-dependent structural damages, such as necrosis, loss of regular structure and infiltration of haemocytes in the gill tissues, as well as atrophy, necrosis and irregular tubular structure in the hepatopancreas. All these lesions may impair respiratory function. Hyperplasia of epithelium increased the diffusion distance thus affecting the exchange of gases, and the fusion of lamellae causes a decrease in the total respiratory area of the gills, resulting in a decreased oxygen-uptake capacity of fish gills [36]. Fish fail to get adequate oxygen for total metabolic activities. Increased thickness of the epithelial layers has been reported to result from hyperplasia following experimental exposure to pesticides [36]. Inflammatory changes, such as swelling and lifting of lamellar epithelium and hyperplasia have also been noted in the gill lamellae of various species of fish following exposure to insecticides [37, 38, 36]. “Ref. [38]” reported that copper was accumulated and regulated in the hepatopancreas of the Semaphore crab, *Heloecius cordiformis.* “Ref. [39]” demonstrated the ability of white shrimp to detoxify copper by granule formation in the hepatopancreas tubules and excretion through the feces. “Ref. [10]” indicated that hymolymph protein and hemocyanin levels were lower during the post-molt than during the pre-molt stage in prawns due to water and Ca^{2+} uptake during the molt. Crustaceans that have recently molted may be more sensitive to copper due to changes in hemolymph osmolality. According to “Ref. [40]” copper sulfate concentrations of 1.0 mgL^{-1} or more are needed to kill most algae in water with alkalinities higher than 100 mgL^{-1}. In the study of [41] the 0.03 mgL^{-1} copper sulfate treatment in water resulted in 100 % juvenile prawn mortality. According to our data, the Copper sulfate is not a suitable compound for use as an algaecide in prawn-production ponds unless lower than 0.20 mgL^{-1}. Copper sulfate is also commonly used to control species of blue-green algae that are responsible for off-flavor in fish and marine shrimp [42]. “Ref. [43]” reported that copper sulfate is effective at a rate of 0.084 mgL^{-1} for use in controlling blooms of *Microcystis* and other blue-green algae responsible for "off-flavor" in ponds. Toxicity of copper sulfate on advanced juvenile sizes and adult freshwater prawns needs to be determined so that the potential for using copper sulfate for controlling blue green algae in ponds can be established. “Ref. [44]” tested Cu^{2+} in *P. monodon* and found that Cu^{2+} was toxic. [45] , suspected that Cd^{+2} and Cu^{2+} were the cause of mortalities in hatchery farms in Taiwan in 1980-1981, with the heavy metals coming from the waste water discharged by nearby industries. The highest bioaccumulation of Cu^{2+} was observed in the organs mainly implicated in metal intoxication. Cu^{2+} in tissues was high in the gills > hepatopancreas > muscles. The highest Cu^{2+} concentration in gills might be related to the important quantity of this metal in the haemolymph and or the necrosed tissues, or these organs might constitute the entry sites of the metal and act as a transient store for accumulated Cu^{2+} [46] . The relatively higher Cu^{2+} concentration in gills than the hepatopancreas could originate from a progressive transfer of Cu^{2+} from gills to the hepatopancreas via the haemolymph [47], and/or from a process of differentiation of hepato-pancreatic epithelium as observed by [48] in the isopod *Porcellio spinicornis*, leading to transfer of the metal into the intestinal lumen and from this site to the exterior as observed by [49], in crayfish. However, the higher Cu^{2+} concentration in the hepatopancreas suggested that this organ plays a role in metal storage and or in detoxification process by a metal binding component [50]. The bioconcentration factor (BCF) is the ratio of a substance’s concentration in tissue of an aquatic organism to its concentration in the ambient water, in situations where the organism is exposed through the water only and the ratio do not change substantially over time. BCF is a dimensionless number representing how much of a chemical is in a tissue relative to how much of that chemical exists in the environment. The BCFs were increased in gills, hepatopancreas and muscles, with increasing exposure concentrations, respectively. The mean contents of Cd, Cu, Pb and Zn of the white shrimp (*Litopenaeus vannamei*) were lower in the muscle than in the corresponding hepatopancreas samples [51], which is in agreement with most literature on the metal contents in the tissues of different aquatic organisms because the hepatopancreas is the main organ for metal accumulation [52,53] .

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V. CONCLUSION

The present study reveals an important precaution for prawn cultivation. Knowledge of the toxicity of copper will be helpful to water quality management in fish farms with specialty to prawn cultures. It affects the immune response and resistance in *Macrobrachium rosenbergii* due to reduction in hemocyte count and phagocytic activity that make it susceptible to infectious agents and death. Caution should be exercised against water source contamination and exposure to fertilizer and industrial pollution.

VI. ACKNOWLEDGMENT

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AUTHOR'S PROFILE

Dr.Hussein A. Kaoud

He is full professor of Veterinary Hygiene and Environmental pollution and Chairman of Department of Veterinary Hygiene and Environmental Pollution, Faculty of Veterinary Medicine, Cairo University, Giza-Egypt, Dr. Kaoud has written 80 books in Arabian and English language and many Scientific Articles and 10 patents. He has guided many students at Ph.D.and P.G.level and attended many conferences .Dr. Kaoud is the member of several Egyptian and international society's. He is the editor and reviewer of many international journals. He is received many awards.