Bioaccumulation of cadmium in the fresh water prawn *Macrobrachium rosenbergii*

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Abstract: The effects of Cd on mortality, resistance and bioaccumulation in giant freshwater prawn *Macrobrachium rosenbergii* in Egypt were studied. Survival of prawns exposed to cadmium doses over 60 μgL^{-1} were significantly lower than of those exposed to lower doses. After 96 hours prawns exposed to >40 $\mu g\ L^{-1}$ of cadmium had a greater reduction in total haemocyte count and phagocytic activity than those exposed to lower concentrations. Bioaccumulation of Cd in the gills, hepatopancreas and muscleswas variable. Cadmium accumulated in gills and hepatopancreas, but muscles had a moderately significant Cd level increase. *Macrobrachium rosenbergii* manifested histopathological alterations in gills, hepatopancreas and muscles when exposed to different concentrations of cadmium. [Nature and Science 2010; 8(4):157-168]. (ISSN: 1545-0740).

Keywords: toxicity, survival, haemocyte count.

1. Introduction

Heavy metals are considered a major source of environmental pollution. Cadmium (Cd) which is one of these pollutants has taken considerable attention for its great different toxic effects on living individuals. Metal contamination sources are typically derived from different sources: mining, industrial waste discharges, sewage effluent, harbor activities and agrochemicals. Cadmium is unique among the other metals because of its toxicity at a very low dosage and long biologic half life (30 years in human) and its low rate of excretion from the body (Jones and Cherian 1990).

Heavy metals like cadmium are known to accumulate in marine organisms, and cause rapid genetic changes (Nimmo *et al.* 1978; Nevo *et al.* 1986). It is also possible that environmental toxicants may increase the susceptibility of aquatic animals to various diseases by interfering with the normal functioning of their immune, reproductive and developmental processes (Couch 1978; Brock 1997). In decapods crustaceans, 3 types of circulating hemocytes are recognized: hyaline, semi-granular and large granular cells (Tsing et al. 1989). They are involved in cellular immune responses that include phagocytosis and constitute the primary method of eliminating microorganisms or foreign particles (Bayne, 1990). In addition to phagocytosis, hemocytes are involved in coagulation and in the production of melanin via the prophenoloxidase system (Johansson and Söderhäll 1989 and Söderhäll *et al*. 1996). Enzymes for the prophenoloxidase system are contained in the granular hemocytes, released as proenzymes upon stimulation by microbial cell components such as 1 ,3-glucan or lipopolysaccharide from fungal cell walls, and activated by a serine protease (Söderhäll 1983, Smith *et al.* 1984, Söderhäll et al. 1996). The activity of phagocytosis has been reported for many crustaceans (Söderhäll *et al.* 1996) including the brown shrimp *Penaeus californiensis* (Hernández-López et al. 1996), the tiger shrimp *P. monodon* (Sritunyalucksana *et al.* 1999) and *Macrobrachium rosenbergii* (Cheng et al., 2002). Several physico-chemical parameters and environmental contaminants have been reported to affect the immune response in crustaceans and these have been reviewed by Le Moullac and Haffner (2000). Environmental toxicants have been reported to cause a reduction in hemocyte count in the common shrimp *Crangon crangon* (Smith and Johnston 1992)

Pollution of aquatic environments with heavy metals has seriously increased worldwide attention and under certain environmental conditions, fish may concentrate large amounts of some metals from the water in their tissues. Heavy metals such as cadmium, is potentially harmful to most organisms even in very low concentrations and have been reported as hazardous environmental pollutants able to accumulate along the aquatic food chain with severe risk for animal and human health. Bioconcentration is the increase in concentration of a chemical in an organism resulting from tissue absorption levels exceeding the rate of metabolism and excretion. Neurotoxicity on the CNS appears in a variety of neurochemical and behavioral changes due to cadmium exposure (Desi *et al.* 1998). Toxic heavy metal can cause dermatological diseases, skin cancer and internal cancers (liver, kidney, lung and bladder), cardiovascular disease, diabetes, and anaemia, as well as reproductive, developmental, immunological and neurological effects in the human body. Cd can enter into the brain

parenchyma and neurons causing neurological alterations in humans (Rose *et al*. 1992) and animal models (Lukawski et al. 2005). Cd is listed by the U.S. Environmental Protection Agency as one of 126 priority pollutants. Acute-Cd exposure results in pulmonary edema and respiratory tract irritation, whereas chronic exposure to Cd often leads to renal dysfunction, anemia, osteoporosis, and bone fractures (Friberg *et al.* 1986 and Goering et al. 1995). Cadmium is carcinogenic for a number of tissues (Waalkes 2000) and is classified by IARC (1993) as a human carcinogen. In laboratory animals, acute Cd poisoning produces primarily hepatic and testicular injury, whereas chronic exposure results in renal damage, anemia, and immuno- and osteotoxicity (Goering *et al.* 1995, Klaassen *et al.* 1999). It has been suggested that the mechanism of Cd toxicity involves the production of reactive oxygen species and free radicals (Manca *et al*. 1994, Stohs *etal.* 2001).

The aim of this study was to investigate the effect of Cd toxicity on mortalities and resistance in giant freshwater prawn *(Macrobrachium rosenbergii)* and also to investigate the bioaccumulation of cadmium residues in their tissues.

2- Materials and Methods

Experimental design

Freshwater was adjusted with the desired parameters according to New (1995) as followed (temperature of 20-28 °C, pH 7-7.8, dissolved oxygen 5-8 mg/L, salinity 2 ppt, hardness 100-150 ppm Ca(CO)3, total ammonia less than10 ppm, nitrate 20 ppm and nitrite 1ppm).

Stock cadmium solution: 100 mg $CdCl_2$ metal dissolve in a solution composed of 20 mL water plus 5mL concentrated HCL and make up to 1000 mL with water (1.00 mL = 100 µg Cd). Ten different concentrations of Cd were then prepared from the stock solutions (10, 20, 30, 40, 50, 60, 70, 80, 90,100 µg L^{-1}).

Macrobrachium rosenbergii were obtained from commercial farms in Alexandria and Al-Kalubia, Egypt, and acclimated in the laboratory for two days before experimentation.

The toxicity tests were conducted according to the standard procedures of FAO (1985). Ten concentrations of Cd ranged between 10 until 100 µg and a control were set up. Ten shrimps of the same size (ranged from 13.2 to 16.5 g with mean of 15.32 ±0.15g) were separately transferred from the holding tanks into the control and experimental tanks. The whole set was aerated continuously, while the test solution in each tank was changed with requisite fresh solution every 24 hrs to maintain the definite concentration of Cd for 96 hrs. Observations for mortality were made twice (10.0 am and 6.0 pm) daily.

Analysis: The 96 hrs $_{LC50}$ values were calculated using probit analysis according to Finney (1971).

Cell counts

Hemolymph (100 µL) was sampled individually at the beginning of each test and at 96 hrs post exposure to Cd. 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 using an inverted-phase contrast microscope.

Culture of *Lactococcus garvieae*

The bacterial strain *L. garvieae* isolated from diseased *Macrobrachium rosenbergii* after artificial infection 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 24 h at 28 °C as a stock culture. The stock cultures were then centrifuged at 7155 x g for 15 min at l4 °C. The supernatant fluid was removed and the sediment was resuspended in saline solution (0.85 NaCL) and adjusted at 10^{10} cfu mL^{-1} as stock bacterial suspensions for testing.

Phagocytic activity of *M. rosenbergii* to *L. garvieae*

After 72 hrs of Cd exposure in each treatment, prawns were injected in the cephalothorax with 20 µl of the bacteria suspension (10^{10} cfu ml^{-1} in 0.85% NaCI) resulting in 2 x 10_8 cfu prawn l^{-1}. After injection, the prawns were held in their respective solutions for 3 h (s). 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) and distilled water (100 ml with pH of 7.45).

Phagocytic activity was measured using the method described by Weeks-Perkins et al., (1995) where 200 µL *of* diluted hemolymph sample was mixed with 0.2 ml of 0.1% paraformaldehyde 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. About 200 hemocytes were counted using light microscope and the phagocytic rate was estimated as follows:

PR = [(phagocytic hemocytes) / (total hemocytes)] x 100.

Preparation and analysis of tissue samples

Procedure A: Each sample was represented by one gram of tissues dissected from the gills, hepatopancreas, and muscles, then placed in a clean screw-capped tube and digested according to the method described by Finerty *et al*. (1990).

Procedure B: The obtained solutions were then

analyzed by using Air/Acetylene Flame Atomic Absorption Spectrophotometer (UNICAM 696 AA Spectrometer) for determination of cadmium levels in examined samples.

Histopathological examination:

Tissue specimens from *Macrobrachium rosenbergii* were taken (gills, hepatopancreas and muscles) and fixed in 15 % buffered neutral formalin. They were processed to obtain five micron thick paraffin sections then stained with Hematoxylin and Eosin, (H&E) according to Bancroft *et al.*, (1996) and examined under light microscope.

Statistical analysis:

Data were analyzed using Analysis of Variance (ANOVA) and means were separated by the Duncan posthoc test at a probability level of < 0.05 (SAS, 2000).

3. Results:

After 96 h(s), mean (±SD) survival of prawns in control tanks (0 Cd) was 94±2.20% and significantly higher *(P* < 0.05) than that of prawns in all other treatments (Table 1). At 96 h(s), survival of prawns exposed to 10-50 µg/L^{-1} concentrations of cadmium were significantly greater *(P* < 0.05) than for prawns exposed to higher concentrations (60 µg/L^{-1} or greater) (P < 0.05). Survival of prawns exposed to 60, 70, 80, 90 and 100 µg/L^{-1} of cadmium was significantly lower (P < 0.05), with means *of(±SD)* 57 ± 0.70%, 50 ± 0.70%, 50 ±.70%, 40 ± 0.20% and 40 ± 0.21%, respectively as shown in Table 2. The regression analysis of prawn survival (%)was highly significant (P < 0.001; r2 = 0.964).

Table 1 and Figures B and C show that at 96 hours, prawns exposed to 40, 50, 60, 70, 80 and 90 µg/L^{-1} concentrations of cadmium were significantly had greater reduction in THC and Phagocytic activity than for prawns exposed to lower concentrations (10 – 30 µg/L^{-1}), *(P* < 0.05).

The LC50 of Cd on *M. rosenbergii*

The 96-h(s) $_{LC50}$ for cadmium-exposure in *M. rosenbergii* was calculated to be 74 µg/L^{-1}.

Bioaccumulation of Cd in different tissues of *M. rosenbergii*

The highest bioaccumulation of cadmium was observed in the organs mainly implicated in metal intoxication. Cadmium (Cd) in tissues was high in the gills > hepatopancreas> muscles. Cadmium accumulations were increased in gills, hepatopancreas and muscles, *with the increasing exposure of concentrations* respectively.

Gills: The rate of accumulation of cadmium was maximum in gills of exposed prawn .The rate of accumulation increased along with the increasing of cadmium concentration reaching up to 1.1± 0.025 µg gm^{-1} after 96 h(s) exposure for Cd at 100 µg/L^{-1} as shown in Table 2.

Hepatopancreas: Cadmium could not be traced in the hepatopancreas of control test as well as at very low concentration 10 µg/L^{-1}, even though the quantity of accumulated cadmium was less in the case of hepatopancreas when compared to gills.

Muscles: The rate of accumulation of cadmium in muscles increased along with exposure concentrations. The mean rate of accumulation at 100 µg/L^{-1} was 0.065± 0.008 µg gm^{-1}. The rate of accumulation was less as compared with other tissues, Table 2.

Histopathological alterations in different tissues of *M. rosenbergii*

Results of the present study revealed that *Macrobrachium rosenbergii* manifested histopathological changes in gills, hepatopancreas and muscles.

Gills showed mild congestion, swelling and edema at low doses of Cd intoxication. Severe edema, hyperplasia, at highest doses of intoxication was observed as shown in Figure 3.

Muscular tissues

Figure 5 shows the normal structures of the muscles. Several histopathological alterations were seen in the muscles *Macrobrachium rosenbergii.* The pathological findings 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 were seen.

Table 1: Effect of cadmium on survival, total hemocyte count (THC) and phagocytic % of freshwater prawns, *Macrobrachium rosenbergii,* exposed to cadmium at different concentrations for 96 hours post-treatment. Values are means± SD (n =4 prawns in each case).

Cd[1] Con.	Survival %	Immune response	
		THC[2]	Phagocytic%
0	94±2.20	196±70	90±7.70
10	86±1.70*	195±16	90±.8.70
20	86±1.60*	199±12*	84±7.00
30	70±1.67*	170±9.0*	70±7.00*
40	63±0.87*	170±8.0 *	62±7.00*
50	60±0.30*	145±11*	50±2.70*
60	57±0.70*	138±9.0*	40±0.70*
70	50±0.70 *	136±8.0*	40±0.70*
80	50±.70*	130±12*	40±3.00*
90	40±0.20*	130±8.0*	40±0.00*
100	40±0.21*	120±0.0*	30 ±3.00*

[1]: [1]: Cd^{2+} µgL^{-1} , [2]:x $10^5 ml^{-1}$, *Significant($P < 0.05$).

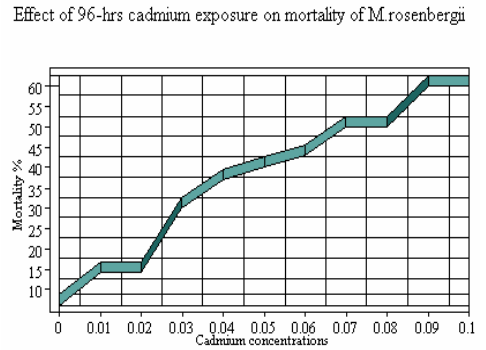
Table 2. The residual analysis of cadmium in freshwater prawns, *Macrobrachium rosenbergii,* exposed to cadmium at different concentrations for 96 hours post-treatment. Values are means± SD.

Conc. of Cd[1]	Bioaccumulation in tissues[2]		
	Gills	Hepatopancreas	Muscles
0	-	-	-
10	-	-	-
20	0.05 ±0.008	0.02±0.006	0.005±0.001
30	0.05 ±0.009	0.025±0.008	0.01±0.001
40	0.06 ±0.018	0.03±0.012	0.02±0.003
50	0.065 ±0.021	0.04±0.009	0.02±0.005
60	0.08± 0.022	0.06±0.011	0.03±0.01
70	0.90±0.011	0.065±0.011	0.05±0.009
80	1 ±0.011	0.08 ±0.012	0.055±0.011
90	1.1±0.02	2± 0.01	0.06 ±0.022
100	1.1±0.025	2.2±0.02	0.065±0.008

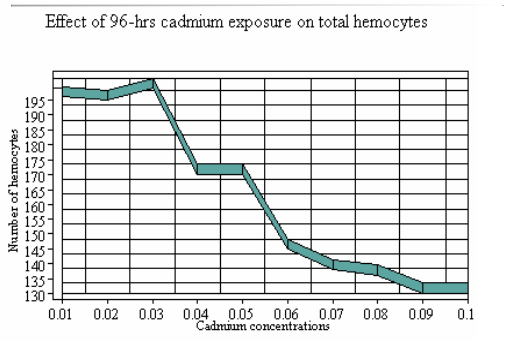
Cd^{2+} µg gm^{-1}. = mg kg^{-1} = ppm. :[2] [1]: [1]: Cd^{2+} µg L^{-1},

Table 3 .The permissible limits of Cd

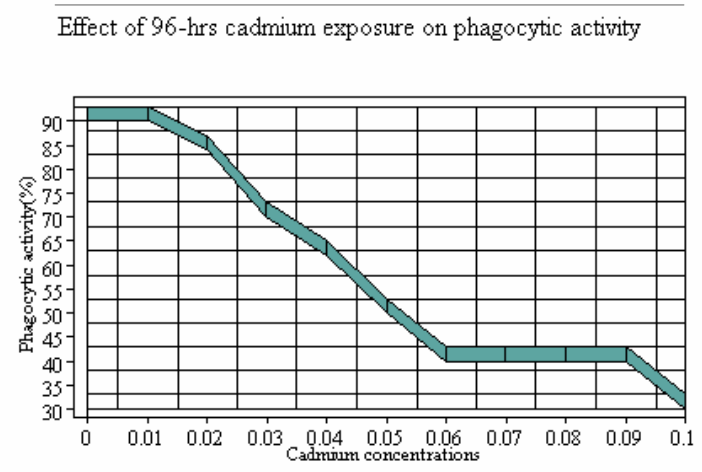
Metal	Permissible	Country and references
Cadmium	0.005 ppm	WHO (1984)
	0.05 ppm	FAO/WHO (1992)
	0.1 ppm	Egypt, E.O.S.Q.C. (1993)
	1.0 pg/g^{-1}	Spain: Boletin Official del Estado (1991)



A-Regression Equation(y) = a + bx=33.09+58.30x
Slope (b) = (NΣXY - (ΣX) (ΣY)) / (NΣX^2 - $(\Sigma X)^2$)
Intercept (a) = (ΣY - b (ΣX)) / N



B-Regression Equation(y) = a + bx=157.18+1999.48x
Slope (b) = (NΣXY - (ΣX) (ΣY)) / (NΣX^2 -



C-Regression Equation(y) = a + bx=57.82+640.52x Slope (b) = (N ΣXY - (ΣX) (ΣY)) / (NΣX2 - (ΣX)2)
Intercept (a) = (ΣY - b (ΣX)) / N.Correlation coefficient =-0.96

Figure1 (A, B, C) The relationship of mortality and immune response (total hemocyte count and phagocytic activity) to different concentrations of Cd^{2+}

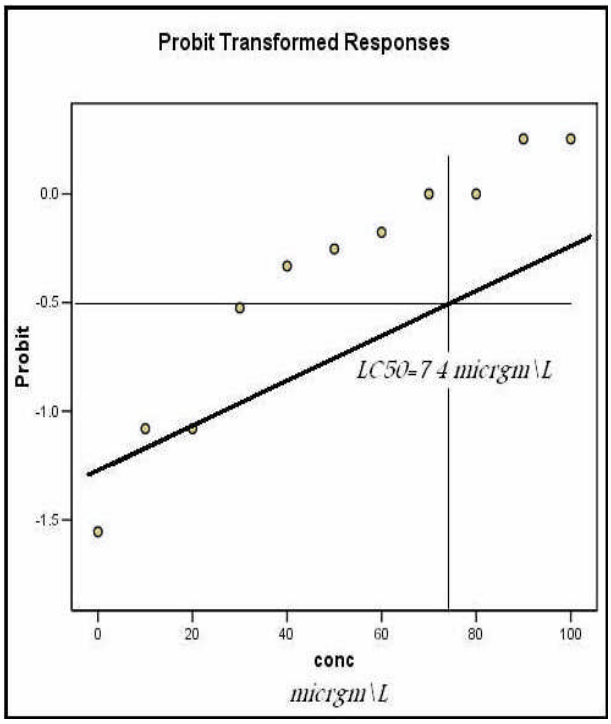


Figure2: LC50 of cadmium on *M. rosenbergii* for 96-h exposure using the resulting regression equation, in *M. rosenbergii*, the 96-hours $_{LC50}$ for cadmium was calculated to be 7 4 µg L^{-1}, cadmium.

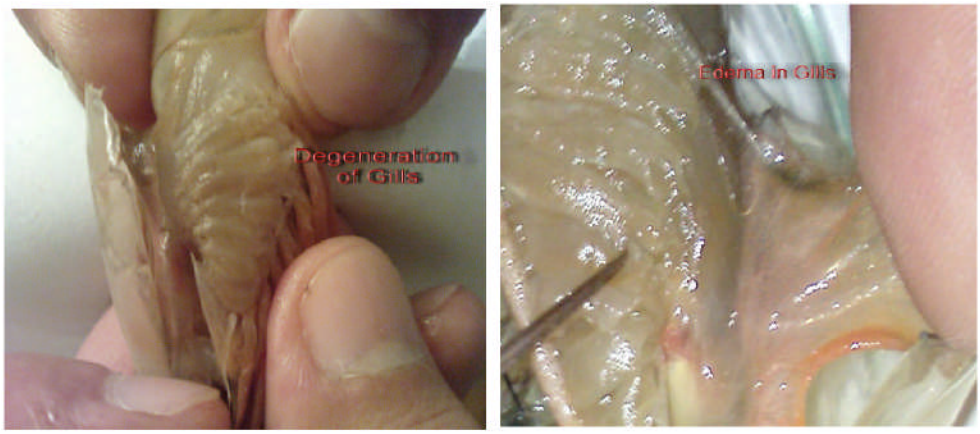


Figure 3: Gills showed congestion, swelling, edema and hyperplasia, at highest doses of intoxication

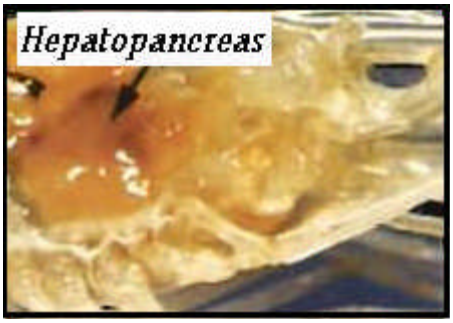
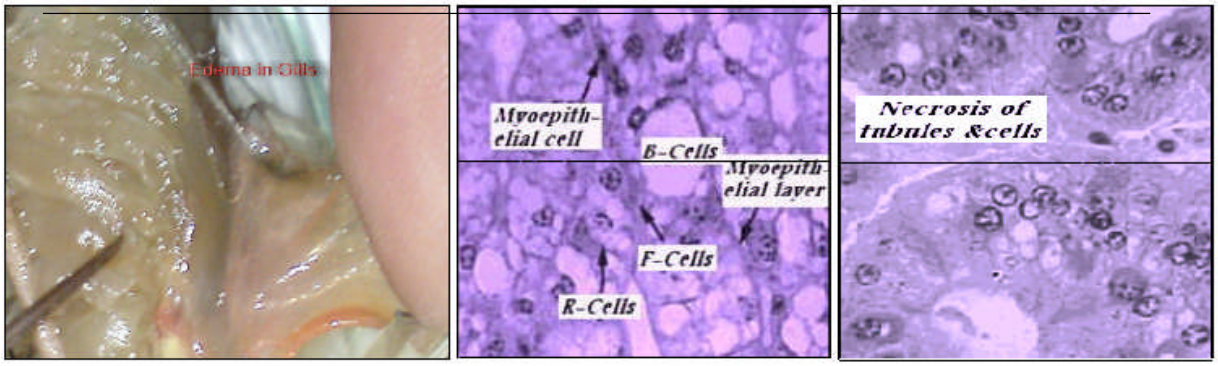


Figure 4: Hepatopancreas showed degeneration of the hepatocytes and haemolysis (the findings were apparent as the hepatospleen consider the organ of detoxification, excretion and binding proteins such as metallothionein).



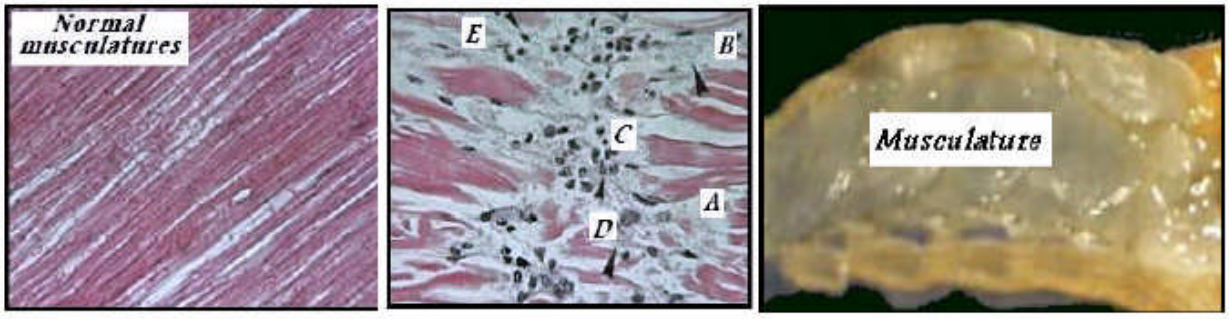


Figure 5: Muscular tissues showed pathological alterations; included degeneration in muscles with infiltration and aggregations of hemocytes between them and focal areas of necrosis. Also, atrophy of muscle bundles, edema, hyaline degeneration and splitting of muscle fibers. A; splitting of muscle fibers,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

4. Discussion:

After 96 hrs, survival of prawns exposed to 10-50 µg L^{-1} concentrations of cadmium were significantly greater ($P < 0.05$) than prawns exposed to higher concentrations (60 µg/L^{-1}or greater)

Cheng (1979) tested Hg, Cu, Cd and Zn in *Penaeus monodon* and found that Hg was the most toxic of all metals, followed by Cu, Cd and Zn and he added that Cd toxicity was the most rapid one.

Kuo *et al* (1984) suspected that Cd and Cu 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.

Prawns exposed to 40, 50, 60, 70, 80 , 90 and 100 µgL^{-1} concentrations of cadmium were significantly had greater reduction in THC and Phagocytic activity than for prawns exposed to lower concentrations (10 – 30 µg/L^{-1}), ($P < 0.05$).

Several scientists have investigated the effects of environmental parameters on crustacean defense mechanisms. Dean and Vernberg (1966) reported that temperature affects hemolymph clotting time, hemocyte counts and serum protein concentration in the hermit crab *Uca pugilator*. Truscott and White (1990) found tide-associated rhythms in the total hemocyte count for freshly captured shore crab *Carcinus maenas*, with peak count occurring at high tide. Increased hemocyte numbers provide an enhanced immune capability during periods of high activity. Hauton *et al.*, (1995) reported a significant negative correlation between phenoloxidase activity and tidal height in *C. maenas*, and this indicated cyclical changes in immunocompetence. An increased prevalence in the shell disease of marine decapods crustaceans has been reported to result from polluted environments, also suggesting a decrease in immunocompetence (Gopalan and Young ;1975, Young and Pearce; 1975).

Carolina (2009) studied the effect of Manganese on the immune system of marine invertebrates and found that Mn severely suppresses the number of circulating hemocytes in *Nephrops norvegicus* by inducing apoptosis. However, Mn increased the number of circulating hemocytes in *Asterias rubens* and at the same time affected their ability to phagocyte. The sensibility of exposed gills to bacterial infection has been previously described in other shrimps exposed to cadmium (Couch 1977, Darmono 1990). Their presence has also been observed in gills of *P. japonicus* (Souheil 1995) and of the crayfish *Astacus leptodactylus* (Maesteracci and Vey 1989) infected by fungi.

A significant reduction in phagocytosis of *Bacillus cereus* was observed in the shore crab *Carcinus maenas* following 14 day exposure to 500 µg/L^{-1} Cd (Truscott and White 1990).

The 96-hour LC_{50} for cadmium in *M. rosenbergii* was calculated to be 7 4 µg/$L^{-1.}$ However, Fafioye and Ogunsanwo (2007) found that the lethal concentration (LC_{50}) for 96 hrs exposure to cadmium for *M. rosenbergii* post larvae was 3.23 m/L .The 96 h(s) LC_{50} values of 2.88, 3.02 and 3.11 mg/ L of Cd reported to be toxic to *P. monodon* (Diaz 1995), *P. pencillatus* and *P. indicus* (Chinni and Yallapragda 2000), respectively.

The highest bioaccumulation of cadmium was observed in the organs mainly implicated in metal intoxication. Cadmium (Cd) in tissues was high in the gills > hepatopancreas> muscles.
The highest Cd concentration in gills might be related to the important quantity of this metal in the hemolymph and or the necrosed tissues, or these organs might constitute the entry sites of the metal and act as a transient store for accumulated Cd (Martin and Rainbow 1998). The relatively higher Cd concentration in the hepatopancreas could originate from a progressive transfer of Cd from gills to the hepatopancreas could originate from a progressive transfer of Cd from gills to the hemolymph (Bjerregaard 1990), and/or from a process of differentiation of hepatopancreatic epithelium as observed by AliKhan (1989) 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 Brown (1982) in Cray fish. However, the higher Cd concentration in hepatopancreas suggested that this organ plays a role in metal storage and or in detoxification process by a metal binding component (White and Rainbow 1986)

cadmium accumulation in muscles of *M. rosenbrgii* was ranged from 0.005-0.065 (ppm) and the maximum permissible limits recommended by WHO, (1984) is 0.005 ppm. The recorded results of cadmium concentrations in muscles of*M. rosenbrgii* were higher than the permissible limits intended by Boletin Official del Estado (1994) in Spain [l.0 pg/g^{-1}] and FAO/WHO (1992)[0.05 p.p.m] but lower than Egyptian Organization for Standardization and Quality Control (E.O.S.Q.C) (1993) [0.1 mg kg^{-1}].

Cadmium is highly toxic non essential heavy metal and it does not have a role in biological processes in living organisms. Thus even in low concentration, cadmium could be harmful to living organisms (Burden *et al.*, 1998). High accumulation of cadmium in liver may be due to its strong binding with cystine residues of metallothionein (Klaassen *et al*. 1999).

Agricultural activities are likely to add important amounts of Cd to the natural levels. Fertilizers are important sources of Cd based agrochemicals which are widely used in intensive agriculture (Alloway 1990).

The histopathological alterations in different tissues of *M. rosenbergii*

Gills showed mild congestion, swelling and edema at low doses of Cd intoxication. Severe edema, hyperplasia, at highest doses of intoxication. Similar effects such as necrosis, cell proliferation, epithelial lifting and dilated lamellae were observed in gills of

fish exposed to metals, including cadmium as observed by Malia (1985). Since high Cd concentrations result in serious damage to the gills, the metal may consequently inhibit the physiological functions of these organs. Since the gills of the shrimp are probably involved in gas exchange, we suppose that these alterations resulting in disruption of respirations (Thurberg 1973).

The effects of Cd on fish gill morphology have been studied in some species (Gardner and Yevich 1970; Karlsson-Norrgren *et al.* 1985; Pratap and Wendelaar Bonga 1993and Thophon *et al.* 2003).

Hepatopancreas showed degeneration of the hepatocytes and haemolysis (Fig.4). These findings were apparent as the hepatospleen consider the organ of detoxification, excretion and binding proteins such as metallothionein (MTs). The metal-binding proteins were present in the nuclei of hepatocytes suggested that the increase in the cell damages (De Smet, Blust 2001). Similar results were observed by Van Dyk (2003) and Mela *et al.* (2007).

Frías-Espericueta et al., (2008) studied the effect of three concentrations of Cu (3.512, 1.756 and 0.877 mg\ l- 1) on the juvenile *Litopenaeus vannamei* and he found that there were severe time- and dose-dependent structural damages, such as necrosis, loss of regular structure and infiltration of hemocytes in the gill tissues, as well as atrophy, necrosis and irregular tubular structure in the hepatopancreas.

4. Conclusions:

This study reveals an important precaution for prawn cultivation. Knowledge of the toxicity of cadmium will be helpful to water quality management in fish farms with specialty to prawn cultures; they affect the immune response and cause a reduction in hemocyte count in *Macrobrachium rosenbergii*. Caution should be exercised against water source contamination and exposure to fertilizer and industrial pollution. For this reason, the assessment of risk and the safe levels of toxic substances added to any natural environment through human or natural sources, should not neglect the effects on biological systems caused by the interaction of minute amounts of toxicants.

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