EXPERIMENTAL TOXICITY OF CHROMIUM TO TWO FRESHWATER CRUSTACEANS; *DAPHNIA MAGNA* AND *MACROBRACHIUM ROSENBERGII*

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ABSTRACT

The analysis of the potential toxicity of chromium was conducted on two aquatic model systems, namely the cladoceran *Daphnia magna* and the decapod *Macrobrachium rosenbergii*. Chromium was chosen since human activity has led to a conspicuous increase of this metal in the environment. The toxicity of chromium to *Daphnia magna* was evaluated on the basis of a 48-hr 50% lethal concentration (LC50), a 3-week LC50 and a 16% decrease in the number of born young (reproductive impairment). The daphnids were exposed for concentrations ranging from 2 to 30 µg Cr/L from which 48 hr- LC50 was calculated both in absence and presence of EDTA, a chelating agent to be 8 and 27 µg Cr /L, respectively. In the static- renewal systems, daphnids were exposed to sublethal doses (13, 16, 18, 22 and 23 µg Cr/L) equivalent to LC10, LC20, LC25, LC35 and LC40, respectively, as deduced from the regression line for chromium toxicity for 48 hr. in the static system in presence of EDTA. The 3-week 16% reproductive impairment concentration for the metal ions was 17µg. Cr/L. At metal concentrations permitting survival but impairing reproduction (sublethal concentration), daphnids body length and weight were less than those of control animals. The concentration causing 50 % reproductive impairment was more or less the same as the 48-hr LC50 (in presence of EDTA), being 26 and 27 µg Cr/L respectively. The 21-day LC50 was 16.5 µg Cr/L. The time of onset of reproduction ranged from 6 days in the control to 14 days in the highest concentration. The acute toxicity of chromium to the freshwater prawn *Macrobrachium rosenbergii* revealed that the

48-hr LC50 was 425 $\mu\text{gCr/L}$. Prawn juveniles were exposed in static-renewal systems for 45 days, to sublethal doses (210, 300, 325 and 375 $\mu\text{gCr/L}$) equivalent to LC10, LC25, LC30 and LC40 respectively, as deduced from the regression line for chromium toxicity for 48 hr in the static system. The results showed that juvenile prawn of initial weight 0.255 ± 0.097 g, in higher concentration (375 $\mu\text{g Cr / L}$), had given a significant decrease ($P < 0.05$) from the control in final mean weight, weight gain, % weight gain, specific growth rate and survival, represented by 0.270 g, 0.015 g, 1.928, 0.127 and 33.3 % , respectively. Also prawn in different concentrations of chromium recorded the highest food conversion ratio represented by 3.76, 6.39, 8.1 and 8.7, respectively. Amounts of total protein and lipids of both daphnids and prawns varied with the different concentrations. This study indicated that *Daphnia magna* was more sensitive to chromium than *Macrobrachium rosenbergii* and that bioassays with these two organisms are useful to assess the toxic potential of aquatic pollutants.

INTRODUCTION

Contamination of aquatic environments, such as streams and estuaries by the emission of considerable industrial wastes, poses great threat to aquatic organisms in particular and to the whole ecosystem in general.

The effects of heavy metals on aquatic organisms depend on the supply of bio-available metal species in the water and sediments, not simply on the total concentrations of these metals (Tessier *et al.*, 1984; Jackson, 1991a). The bioavailability of a metal is in turn, a function of many environmental and biological factors and may vary widely.

Chromium is considered non essential for plants, but an essential trace element for animals (APHA, 1998). Beyersmann (1995) stated that chromium is essential for basal metabolism. Hexavalent compounds have been shown to be carcinogenic by inhalation and are corrosive to tissue. The chromium guidelines for natural water are linked to hardness or alkalinity of the water (i.e., the softer the water, the lower the permitted level for chromium). The United Nations Food and Agriculture Organization recommended maximum level for irrigation waters as 100 $\mu\text{g/L}$. The U. S. EPA primary drinking water standard MCL (maximum contamination

level) is 0.1mg/L for total chromium (APHA, 1998). Chromium is used in alloys, in electroplating, and in pigments. Chromate compounds frequently are added to cooling water for corrosion control.

Because of the cladocerans' role as an important link in the food webs of freshwater ecosystems, as well as their high sensitivity to toxic substances, many species have been used as bio-indicators to assess heavy metal toxicity. These include *Daphnia magna*, *D. pulex* (Biesinger and Christensen, 1972; Hall *et al.*, 1986; Ghazy, 2002), *Daphnia carinata*, *Echinisca triserialis* (Chandini, 1988), *Ceriodaphnia dubia* (Belanger and Cherry, 1990), and *C. reticulata* (Hall *et al.*, 1986). Anderson (1950) concluded, "in general, the *Daphnia* and related forms are more susceptible to cations than are fish".

Trace metals may enter local food chains and so reach decapod crustaceans among others, such as the freshwater prawns considered here, which are widely consumed by local populations (Abdenmour *et al.*, 2000). *Macrobrachium rosenbergii* has been one of the main species used in freshwater prawn farming. The success of this activity is directly related to the water quality in the culture ponds. The use of various pesticides in agriculture has undoubtedly contributed to increase the crop-yield, but has also produced adverse effects in the natural environment (Lombardi *et al.*, 2001). There are other studies on heavy metal toxicity focusing on freshwater prawns and other crustaceans (Liao and Guo, 1990; Vagenase *et al.*, 1997).

Through the last decades, the detrimental effects of pollution caused by heavy metal accumulation have been repeatedly documented. Since then, heavy metal pollution and its acute effects on aquatic life have become a focus of discussion and research. At sublethal level, heavy metals may affect growth, reproduction and other physiological functions of aquatic animals (Liao and Hsieh, 1988; Ghazy, 2002). Furthermore, heavy metal pollution generates public health problems.

Crustaceans differ in their trace metal accumulation patterns among various taxa (Rainbow and white, 1989; Rainbow, 1998) between taxa. Decapods usually regulate their body concentrations of the essential elements Zn, Cu, and Fe to approximately constant levels (Rainbow and white 1989; Rainbow, 1998;), while they are net accumulators of the non-essential elements Cd and Pb (Rainbow, 1998).

At present, bioassays are increasingly used as sensitive indicators of pollutant toxicity, since they are rapid, inexpensive, applicable to a variety of toxicants and allow several acute and chronic endpoints to be assessed simultaneously. The analysis of the potential toxicity of chromium was conducted to evaluate lethal and sublethal effects on two aquatic model systems, a cladoceran, *Daphnia magna* and a decapod, *Macrobrachium rosenbergii*. Chromium was chosen since human activity had lead to a sharp increase of this metal in the environment.

MATERIALS AND METHODS

Experiments with *Daphnia magna*

Experiments with *D. magna* were carried out at a constant temperature of $24\pm 2^{\circ}\text{C}$ and with a 12-hr photoperiod. The animals were fed with the microalga, *Scenedesmus obliquus* three times a week. They were isolated from the Nile River water and maintained in the lab for more than 10 years. The food suspension was prepared by adding 14×10^8 coenobia per liter of the freshwater synthetic medium (Fayed and Ghazy, 2000) for rearing and chronic testing. The synthetic medium is characterized by pH 7.9, conductivity 260 μmhos , alkalinity 34mg/L as CaCO_3 and total hardness 90mg/L as CaCO_3 . Algae were grown in the culturing medium recommended by APHA (1989). For acute tests (batch or static system), 10<24-old daphnids were placed in triplicate 250-ml beakers, each containing 100ml medium and subjected to test conditions for 48 hr. Tests were run without food addition as recommended by standard toxicity testing (APHA, 1989) and complete immobilization or death was the end point. For chronic tests (static-renewal system), ten <24hr-old daphnids were placed in each of 250-ml beaker containing 100ml of medium. Three replicates were used for each Cr concentration as well as the control. In this static-renewal test, the surviving daphnids were counted and transferred 3 times a week with a wide flared Pasteur pipette to new medium and the number of neonates produced were recorded. The neonates were discarded after counting. After 3 weeks, the experiments were terminated, while the dry weight at 60°C for 24 hr (Lovergrove, 1966) of the adult cladocerans was determined and the carapace length of the surviving adults was measured, using a microscope with an eyepiece micrometer.

Experiments with *Macrobrachium rosenbergii*

Juvenile prawns of *M. rosenbergii* (De Man) used in the present study were obtained from the hatchery of Maryot Fish Farming Company (M.F.F.C.) near Alexandria, in August 2002. The prawn juveniles were acclimatized to laboratory conditions for about two weeks in glass aquaria. During this period, the prawns were fed on 35% protein commercial pellets. At the end of the acclimatization period, mortality was less than 2%.

For acute tests, ten juvenile prawns of *M. rosenbergii* with a mean length 3.1 ± 0.552 cm and a mean weight 0.255 ± 0.097 g, were randomly distributed in triplicate (for each treatment and a control) of rectangular-glass aquaria, Each containing 5L of holding water with a pH of 7.4 ± 0.25 , dissolved oxygen 7.3 ± 0.2 mg/L, alkalinity 106 ± 5.29 mg/L as CaCO_3 , hardness 110.7 ± 1.15 mg/L as CaCO_3 , and water temperature was maintained at 24 ± 2 °C. The aquaria were covered with a plastic film to prevent volatilization. Aeration was provided by air pumps and individual stone diffuser. The bioassays were carried out according to the static tests (batch-system); where no solution replacements and no food was offered during 48 hours of exposure. Mortalities were recorded after 48hr of exposure and the criterion for death was judged as total immobility in response to gentle touching with a glass rod.

For chronic tests, fifteen aquaria each containing 5L holding water were used and a total of 10 juvenile prawns of the same size and weight as those used in acute tests, were placed in each aquarium. Three containers were maintained as the unexposed control groups and all Cr concentrations were conducted in triplicates.

The juvenile prawns were fed daily with commercial feed pellets (35% protein) at a rate of 5% biomass. The remaining feed, fecal materials and dead organisms were removed regularly every morning from the test solution and a quarter of the water volume was changed. The water was replaced completely once every 10 days. At 15 days intervals, survival and prawn measurements { weight, length, weight gain, specific growth rate (SGR) and food conversion ratio (FCR) } were estimated. The experiments were ended after 45 days.

Biochemical Analyses

At the end of the experiments samples of prawns and daphnids were pooled from each treatment for biochemical analysis, total protein was determined according to the method of Daughady *et al.* (1952) and total lipids according to Knight *et al.* (1972).

Test Chemical

The chromium metal used in toxicity experiments as potassium dichromate ($K_2Cr_2O_7$) had a chemical purity >99% (Adwic, Prolabo). Fresh stock solutions were prepared in distilled water for each experiment. The concentrations of Cr tested were: 0, 10, 15, 20, 30 $\mu g/L$, and 0, 200, 400, 800, 1000 $\mu g/L$ for acute tests for 48hr, in case of *Daphnia magna* and *Macrobrachium rosenbergii*, respectively. Another short-term (48hr) experiment was conducted to study the toxic effect of Cr on daphnid neonates in absence of EDTA of the holding water and the applied concentrations were 0, 2, 5, 10, 15 $\mu g/L$.

The series 13, 16, 18, 22, 23 $\mu g/L$ and 210, 300, 325, 375 $\mu g/L$ were used for chronic tests for the two test animals, respectively. The above series of sub-lethal chromium concentrations were equivalent to LC10, LC20, LC25, LC35, LC40 and LC10, LC25, LC30, LC40 which were deduced from the regression lines for chromium toxicity for 48 hr in static systems to *Daphnia magna* and *Macrobrachium rosenbergii*, respectively.

Data Processing

LC50 values for daphnids and prawns were determined by a statistical method according to Finney (1977). The Terminology recommended by Sprague (1969), lethal concentration (LC) was used for survival and, as given here, represents an interpolation from three or more partial-effect concentrations.

Reproductive effects for daphnids were analyzed in a similar manner, but are given in terms of reproductive impairment by percentage. Both 50 and 16% reproductive impairment concentrations are given. These values refer to reduction in the total number of produced young as compared with the controls or the maximum production at a low metal concentration. Reproduction was usually stimulated by small metal additions. The 16% reproductive impairment concentration was given since it represented a minimal reproducible value below which the variability in reproduction could not be detected from controls.

All data were put to statistical verification by analysis of variance "ANOVA" (Gomez and Gomez, 1984). Least significant difference (LSD) was used to test for the differences among treatment means when F-values from ANOVA were significant. Significance of differences was evaluated at 5% level of probability.

RESULTS AND DISCUSSION

The median lethal concentrations (48-hr LC50) of chromium to *Daphnia magna* and *Macrobrachium rosenbergii* were 27 and 425 µg/L, respectively (Tables 1&2). As expected, daphnid neonates were considerably more sensitive to chromium than were prawn juveniles; chromium toxicity to daphnids was 16 times greater than that for prawns. Magliette *et al.* (1995) stated that white shrimp; *Penaeus setiferus* was susceptible to the toxic effects of zinc than *Daphnia magna* and *Ceriodaphnia dubia*, whose 48-hr LC50 values were 1.22 and 0.32 mg/L, respectively. There were no significant difference in LC50 values ($P > 0.05$) as exposure time of prawns increased from 48hr to 21 day, but as time increased from 48hr to 45 day, the LC50 was 440 µg Cr/L. That the cladocerans (*Daphnia* and *Bosmina*) are more sensitive to toxic materials than are the cyclopoid copepods. This may be ascribed to differences in mechanisms of food collection as well as in the intensity of contact with water having heavy metal in ionic form. The periodic molting of *Daphnia* and *M. rosenbergii* accompanied by loss of exuvia may lead to a greater absorption through body surface of the toxic materials in solution which may increase the vulnerability of these animals to heavy metal (Gulati *et al.*, 1988).

Metal toxicity even to the same organism, can vary widely in different waters because of the influence of water chemistry and metal speciation on metal bioavailability.

Acute LC50 values for chromium in the absence and presence of EDTA in the holding medium are shown in Table 1. Na₂EDTA. 2H₂O is one of the ingredients of the holding medium, the presence of which at 100 µg/L on the lethality of *Daphnia magna* neonates in the static system for 48hr was investigated. The results show that the 48hr-LC50 values of Cr were 27 and 8 µg/L with and without EDTA, respectively. The regression lines for chromium toxicity to *Daphnia magna* showed nearly the same slope with and without EDTA and indicates that the relative toxicity remained the same for the two cases while the absolute concentrations decreased in absence of EDTA.. It seems quite evident that chelating agents, of whom EDTA is a model compound, competing with other ligands in water on metal and can control the concentrations of free metal ion (Stumm and Morgan, 1981). This must interfere with metal availability and toxicity to exposed organisms as well (Elendt and Bias, 1990).

In foregoing studies on toxicity of various metals to *D. magna* where the dilution water was the same medium as that used in the present study, the following order of toxicity of heavy metals was reported: copper > cadmium > zinc. The LC50s were 9 and 26 µgCu/L, 102 and 144 µgCd/L, and 350 and 740 µgZn/L in absence and presence of Na₂EDTA (Ghazy, 1997 and 2002). Biesinger and Christensen (1972) investigated the chronic toxicity of various metals to *Daphnia magna*. and found the following toxicity succession of heavy metals: cadmium > copper > zinc.

The effect of chromium on survival, growth and reproduction of *Daphnia magna* at 21st day of exposure are shown in Figures 1 & 2 and Table 3. Percent survivals at 21st day were reduced from 88% for control to 80, 45, 38, and 13% at 13, 16, 18, 22, and 23 µgCr/L, respectively. Mean cumulative progeny per female were slightly decreased at those toxicant concentrations from 38 in the control to 35, 37, 30, 28 and 25. It was noticed that the mean cumulative progeny per female at 16 µgCr/L was more or less the same as control. The mean length and average weight of adult daphnid female at 21st day in control and different concentrations are shown in Table (3). The mean length decreased gradually from 3.69 mm in the control until it reached 3.18 mm at the highest concentration (23 µg Cr/L). The average weight of 21-day-old female decreased from 306 µg in the control to 207 µg in the highest concentration. The time to the first brood of daphnids ranged from 6 days for those of control to 14 days for daphnids used in the highest concentration.

The impairment of biochemical and physiological functions of an organism may be taken as the first indication showing the effect of the environmental perturbation. This impairment could result in measurable changes in the behavior of the organism (Sprague, 1976). These changes may be used as rapid and sensitive indicators of toxic stress and may help to explain other observed changes in the survival, growth and reproduction of non-target organisms.

Survival and reproduction are considered as sensitive endpoints on the traditional twenty-one day's chronic toxicity bioassays with daphnids. Reproductive impairment was found to be a sensitive measure of toxicity like survival. Chromium concentration for 50% reproductive impairment (Fig. 3) was higher than 3-week LC50. Reproductive impairment level; 16% (Fig. 3 and Table 4), when compared with 48-hr LC50 (Table 1) revealed that the chronic safe level for chromium of 17 µg/L was considerably lower than 48-hr LC50 which was 27 µg Cr/L.

For comparison, daphnid chronic toxicity value in the present study and those reported by other investigators are presented in Table 4. The 16% reproductive impairment level for Cr to daphnids in moderately hard water in the present study was much more toxic than that to daphnids and fish, (*Salvelinus fontinalis*) in soft water in other studies.

Experiments with *Macrobrachium rosenbergii*

Table (5) and Fig (4) summarized the effect of different concentrations of chromium (0, 210, 300, 325 and 375 $\mu\text{g/L}^{-1}$) on the growth of the freshwater prawn, *Macrobrachium rosenbergii* after 45 days of exposure. It was found that prawn in zero concentration (control group) showed the best growth ($P < 0.05$) final weight, weight gain and percent weight gain represented by 0.825 g, 0.570 g and 48.270, respectively. Meanwhile, the prawn in the highest concentration of chromium (375 $\mu\text{g/L}^{-1}$) showed a significant decrease in growth parameters, final weight, weight gain and percent weight gain which are represented by 0.270 g, 0.015 g and 1.928, respectively. There was no significant difference in growth ($P > 0.05$) between the three groups of prawn living in higher concentrations (300, 325 and 375 $\mu\text{g/L}^{-1}$). This finding was in agreement with those obtained by Chen (1975); Conner (1972) and Wisely and Blick (1967) who reported that the toxicity of the heavy metals increases with their increased concentrations and this affect the growth of aquatic animals. Moreover, the heavy metals ions dissolved in water permeate through the gill surface incessantly (Bryan, 1976) and then become transported through blood to each part of the body, producing their toxic effect.

Specific growth rate (SGR) in weight was gradually decreased from 2.6 in the control group until it reached 0.127 in the highest concentration of 375 $\mu\text{g/L}$. In contrast, the Food conversion ratio (FCR) increased gradually from 1.6 for the control group until it reached 8.7 in the highest concentration. This may be due to the toxicant effect of feed utilization, so group of prawns in control (zero concentration of Cr) convert the feed more efficiently, showing the best SGR (higher value) and improvement of FCR (lower value) than those reared in other treatments (Table 5).

Furthermore, the lowest survival rate of prawns at the highest concentration of chromium (375 $\mu\text{g/L}$) was represented by 33.3 %. After about 15 days nearly half of prawns died at this concentration, while control group showed the best significant survival ($P < 0.05$)

represented by 73.3% at the end of experiment. This may be due to the higher concentration of toxicant leading to its higher accumulation in different tissues of prawn, which affected the absorption and assimilation of food, causing inhibition of growth and survival (Fig 5). Also prawns in the highest concentrations of metal showed marked toxicity symptoms such as black spots on the shell especially cephalothorax, and abnormal behavior before death as reported by Liao and Hsieh (1990). The latter authors studied the toxicity of three heavy metals (Cu, Cd and Zn) to *M. rosenbergii* and found that the growth and survival of the tested prawns were significantly less than those in the control ones ($P < 0.05$). Also Ismail *et al.* (1990) demonstrated the acute toxicity of copper on larval and post larval *M. rosenbergii*, and suggested that copper level in rearing water should not exceed 0.05 mg/L. However, Chan *et al.* (1992) showed that the eggs of *M. rosenbergii* have a relatively high tolerance for the heavy metals but recommended that the levels of cadmium, copper and zinc in larval rearing water should not exceed 0.1, .057 and 2.97 mg /L⁻¹, respectively. Over this ratio, shorter, ill and curved larvae manifested heavy metal toxicity. Also larvae showed inhibition in growth.

Other researches proved that decapods regulate the body concentration of some metals (e.g. Zinc and copper) to relatively constant levels, but when the regulation breaks down, the metals have the potential to exert a toxic effect (White and Rainbow, 1982; Nugegoda and Rainbow, 1989 a). The toxicity of cadmium and zinc in white shrimp, *P. setiferus* juveniles were studied by Vanegas *et al.* (1997) who found that the median lethal concentrations (96 hr. LC 50) for cadmium and zinc were 0.99 and 43.87 mg/L⁻¹, respectively. Cadmium toxicity was 44 time greater than for zinc. In this respect, Rainbow and White (1989) stated that the sensitivity of aquatic organisms, to heavy metals is related to the metal biological activity. In this context, the greater toxicity of cadmium might be expected since zinc is an essential metal that is regulated by decapod crustaceans, whereas cadmium has no known biological function.

In comparison with other toxic compounds, Natarajan *et al.* (1992) reported that endosulfan is more toxic to postlarvae of *M. rosenbergii* than malathion and copper sulphate. Accordingly, Conte and Parker (1975) found malathion affected juveniles of *Penaeus astecus* and *P. setiferus* at a concentration of 0.25 ppm. Organophosphate pesticides are known to inhibit growth and sex development in *M. lamerii* (Mary *et al.* 1986). Thinning and softening

of shells in prawns was reported by Cecilia *et al.* (1991) due to inhibition of chitin synthesis. Metcalf *et al.* (1971) found that cupric pesticides damage Mg^{2+} ATPase in animals.

Biochemical measurements

The different concentrations of chromium were found to affect the total protein and lipid contents for both *Daphnia magna* and final prawn carcasses (*M. rosenbergii*), where in control group, protein content increased while lipid showed a significant decrease ($P < 0.05$) for *D. magna* (Table 6). For the prawn, the protein content did not differ statistically between different groups in different concentrations of chromium. But the highest value of protein was shown in control group and decreased gradually as the concentrations of toxicant increase till reaching a minimum value at the highest concentration ($375 \mu\text{g Cr/L}^{-1}$). Lipid content decreased significantly ($P < 0.05$) in control group than other groups of prawn at different concentrations (Table 7). This may be explained as the control group of prawns and *D. magna* (without metal) make a best utilization and absorption of food so have a higher protein and lower lipid contents. Similar finding was observed with fish by Love (1970); Bardach *et al.* (1975) and Gad (2003).

The results of the present experiments indicate that chromium is toxic to both *D. magna* and freshwater prawn, *M. rosenbergii* and it affected growth, reproduction and survival of both crustaceans.

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Table (1): Comparison between toxicity of chromium to *Daphnia magna* in acute (48 hr) test (in presence and absence of EDTA) and chronic 21 days-test.

Toxicity (LC)	Short-term exposure (48 hr)		Long-term exposure (21 days)
	In presence of EDTA* without food, $\mu\text{g Cr / L}$	In absence of EDTA* without food, $\mu\text{g Cr / L}$	In presence of EDTA* with food, $\mu\text{g Cr / L}$
LC 10	13	3.5	11.0
LC 20	16	4.5	12.5
LC 25	18	5.0	13.0
LC 35	22	6.2	14.5
LC 40	23	6.6	15.0
LC 50	27	8.0	16.5
Slope	3.9781	3.5282	7.0827

*Na₂ EDTA. 2H₂O concentration is 100 $\mu\text{g / L}$ = 0.2686 $\mu\text{mole / L}$

Table (2): Comparison between toxicity of chromium to *Macrobrachium rosenbergii* after 48-hr acute test and at 21st and 45th days as chronic tests.

Toxicity (LC)	Short-term exposure (48 hr) without food $\mu\text{g Cr / L}$	Long-term exposure (21st days) with food.	
		(21 st days) $\mu\text{g Cr / L}$	(45 th days) $\mu\text{g Cr / L}$
LC 10	210	140	170
LC 20	270	200	230
LC 25	300	240	270
LC 30	325	275	300
LC 40	375	350	360
LC 50	425	430	440
Slope	4.2724	2.5162	3.1245

Table (3): Effect of chromium on % survival, growth, and reproduction rate of *Daphnia magna* cultured in static- renewal system for 21 days.

Cr addition to exposure medium ($\mu\text{g Cr / l}$)	% survival at 21 st day	Time to the first brood (days)	Number of progeny per female at 21 st day (mean $\pm$ SD)	Mean length of adults at 21 st day in mm (mean $\pm$ SD)	Average weight of adults females at 21 st day (μg)
Control	88	6	37.88 $\pm$ 2.79	3.69 $\pm$ 0.14	306
13	80	9	34.73 $\pm$ 1.16	3.47 $\pm$ 0.29	260
16	45	9	37.00 $\pm$ 2.27	3.51 $\pm$ 0.07	268
18	30	12	30.05 $\pm$ 5.51	3.33 $\pm$ 0.06	233
22	23	12	27.8 $\pm$ 2.40	3.250.07	219
23	13	14	24.65 $\pm$ 0.92	3.18 $\pm$ 0.22	207

Table (4): "Safe" concentration of chromium ions for aquatic animals tested through a complete generation under laboratory conditions.

Compound	Safe concentration ($\mu\text{g/l}$)	Water hardness (as CaCO_3 in mg/l)	Species	References
$\text{K}_2\text{Cr}_2\text{O}_7$	17	90	<i>Daphnia magna</i>	Present study from Fig.3, 16% reproductive impairment concentration
CrCl_3	330	45	<i>Daphnia magna</i>	Biesinger and Christensen (1972)
Na_2CrO_7	200-400	45	<i>Salvelinus fontinalis</i>	McKim and Benoit (1971)

Table (5): Mean values of growth parameters and survival of *Macrobrachium rosenbergii* at different concentrations of chromium at the end of experiment.

Growth parameters	Different concentrations of chromium ($\mu\text{g/L}$)				
	Zero (control)	210	300	325	375
Initial weight (g) $\pm$ SD	0.255 $\pm$ 0.097	0.255 $\pm$ 0.097	0.255 $\pm$ 0.097	0.255 $\pm$ 0.097	0.255 $\pm$ 0.097
Final weight (g) $\pm$ SD	0.825 $\pm$ 0.191 ^A	0.540 $\pm$ 0.113 ^B	0.364 $\pm$ 0.076 ^C	0.338 $\pm$ 0.062 ^C	0.270 $\pm$ 0.061 ^C
Weight gain (g)	0.570 ^A	0.285 ^B	0.109 ^C	0.083 ^C	0.015 ^D
% weight gain	48.270 ^A	29.238 ^B	12.648 ^C	9.89 ^C	1.928 ^D
SGR in weight	2.6 ^A	1.7 ^{AB}	0.79 ^C	0.63 ^C	0.127 ^D
FCR	1.6 ^A	3.76 ^B	6.39 ^C	8.1 ^D	8.7 ^D
Initial length (cm) $\pm$ SD	3.1 $\pm$ 0.552	3.1 $\pm$ 0.552	3.1 $\pm$ 0.552	3.1 $\pm$ 0.552	3.1 $\pm$ 0.552
Final length (cm) $\pm$ SD*	4.5 $\pm$ 0.554	4.1 $\pm$ 0.495	3.8 $\pm$ 0.329	3.7 $\pm$ 0.287	3.7 $\pm$ 0.535
SGR in length*	0.828	0.621	0.452	0.393	0.393
% survival	73.3 ^A	56.7 ^B	43.3 ^C	40 ^C	33.3 ^C

Means have the same superscript in the same horizontal row are not significant difference ($P>0.05$).

Parameters have * are not statistical.

SD = Standard deviation

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Table (6): Protein and lipid content of *Daphnia magna* at different Concentrations of chromium at the end of experiment.

Parameters	Different concentrations of chromium ($\mu\text{g} / \text{L}^{-1}$)					
	Zero (Control)	13	16	18	22	23
Protein, g /100g wet weight	2.9 ^A	2.1 ^B	1.7 ^C	1.6 ^{CD}	1.5 ^{CD}	1.4 ^D
Lipid, g /100 g wet weight	0.3 ^A	0.6 ^B	0.6 ^B	0.4C ^A	0.5 ^{BC}	2.0 ^D

Means having the same superscript in the same horizontal row are not significantly different ($P>0.05$)

Table (7): Protein and lipid contents of *Macrobrachium rosenbergii* (flesh) at different concentrations of chromium at the end of experiment.

Parameters	Different concentrations of chromium $\mu\text{g} / \text{L}^{-1}$				
	Zero (control)	210	300	325	375
Protein g /100 g wet weight	10.6 ^A	9.1 ^A	9.0 ^A	7.7 ^A	7.3 ^A
Lipid g / 100 g wet weight	1.4 ^A	2.0 ^D	1.7 ^{CB}	1.9 ^{DB}	1.7 ^C

Means having the same superscript in the same horizontal row are not significantly different ($P>0.05$).

Figure (1) Survivorship curves of *Daphnia magna* at five chromium concentrations after 21 days of exposure in static-renewal system.

Figure (2) Mean Cumulative progeny per female of *Daphnia magna* after 21 days of exposure to different chromium concentrations.

Figure (3) Reproductive impairment at 21st day of exposure to different chromium concentrations in Static-renewal system.

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Figure (4) Growth in weight (g) of *M. rosenbergii* larvae exposed to different concentrations of chromium during the period of experiment

Figure (5) The survival rates of *M. rosenbergii* larvae exposed to different concentrations of chromium during the period of experiment