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Effects of Chromium and Cadmium upon Respiration and Survival of *Callinectes similis*

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Studies using toxicity tests for determining the effects of heavy metals on aquatic organisms have traditionally been carried out under controlled laboratory conditions. In these tests, the organisms are studied in artificial environments, where the hardness, alkalinity and pH or salinity of the water are controlled. However, the results thus obtained are of limited value in establishing criteria for limiting the discharge of these contaminants.

One method which produces more realistic results is the performance of tests under similar conditions to those existing in the organism's original habitat. Different forms in which such studies have been carried out include the analysis of the accumulation of metals over a period exceeding two years (Amiard et al. 1986), the evaluation of the growth and reproductive processes of the population (Peternac and Legovié 1986), and the study of changes occurring in the structure of the community being studied (Weber 1981). However, due to the fact that studies based on the calculation of the indices of alteration may be excessively time consuming and expensive, the in situ measurement of the organisms metabolic rate has also been proposed as a possible way of determining the effects of heavy metal contamination (Weber 1981).

Although in Tamiahua laggon (in the state of Veracruz, Mexico) never have been registered mortality of organisms which can be associated with heavy metal pollution, the actual total concentrations in water are 246.6 ug Pb/L, 50.6 ug Cr/L and 35.0 ug Cd/L, concentrations which are above the established limits by Mexican Legislation (Rosas et al. 1988). In the southern zone of this coastal lagoon (the zone of marine influence), one may find large populations of crabs Callinectes similis, in which levels of certain heavy metal accumulation have been detected. These crabs are currently fished commercially for human consumption under conditions lacking adequate sanitary control.

For the above reasons, and for our conviction that field laboratory tests are more adequate for establishing safety criteria related to heavy metal contamination, we chose to orient this study on determination of the effects of lethal concentrations of Cd and Cr^{+6}

on survival, and more specifically on the effects on oxygen consumption and oxygen extraction mechanisms of crabs.

MATERIALS AND METHODS.

A total of 90 young Callinectes similis specimens in intermolt were collected near the southern mouth of Tamiahua lagoon in the state of Veracruz, Mexico, and transported immediately, in lagoon water, to the field laboratory located close to the collection zone. The crabs were then placed individually in 2-L polyethylene chambers which were previously filled with lagoon water at 30°C and 30°/$_{oo}$ salinity, and provided with continuous aeration. The organisms remained in these chambers for a period of 4 hr before the tests were initiated. This time was considered sufficient for reducing to a minimum any stress that could have been caused by handling (Rosas et al. 1987). The crabs were not fed during the 96-hr period. Only crabs weighing between 5 and 12 g were used in tests.

The tests for toxicity and oxygen consumption were carried out with Cd and Cr^{+6}. The organisms were separated in two groups of 45 crabs each, and exposed to 4 different concentrations of both Cd and Cr^{+6}. Control group were also formed. The $Cr^{+6}$and Cd were added to the chambers by dissolving the appropriate amount of stock solution of $K_2Cr_2O_7$ and $CdCl_2.H_2O$ (Merck), in order to obtain solutions of 27.53, 52.09, 95.26 and 195.13 mg/L of $Cr^{+6}$and 2.48, 3.96, 6.40 and 10.05 mg/L of Cd, respectively. The test solutions of $Cr^{+6}$and Cd were partially replaced every 24 hours with fresh solution. Five random samples of each concentration of each metal were taken after each replacement of fresh solution. The samples were analyzed with an atomic absorption spectrophotometer (Varian Model AA.1475). Temperature and salinity of each sample were also measured. Lagoon water from the capture zone was used for all replacement of solution, in order to conserve hydrochemical quality of the habitat. Nine organisms were used for each concentration of each metal. In addition to tests for toxicity, oxygen consumption was also measured. To obtain the latter measurement, the test chambers were used as closed respirometers, in accordance with the technique used by Rosas et al. (1986). With this technique, oxygen consumption was measured as the difference between the oxygen concentration before and after sealing the chambers. To avoid producing stress on the organisms under study the time allowed for detecting the oxygen consumption was limited to 15 and 20 min. The oxygen concentration was measured with an oxygenmeter (YSI-54 ARC$\pm$0.05 mg/L) corrected according to the temperature and salinity of the sample.

In addition, oxygen consumption was corrected with data derived from the control chamber containing no crabs. Measurements of crabs survival and oxygen consumption were taken at 1,6,12,24,48 and 72 hours after the initiation of the test for Cr^{+6}, and 96 hours for Cd. The difference in tests for Cr^{+6} was due to an electricity shut off which produced organisms mortality.

On completion of the test, surviving animals were sacrificed and dried at 60°C to a constant weight (drive weight = dw). Those organisms that died during the course of the test were dried immediately after

their death. Oxygen consumption measurements are expressed in terms of mg $O_2/hr.g^{-1}dw$.

Once the oxygen consumption was determined, the rate of oxygen extraction was calculated and used as an index of alteration in the organisms mechanism for capturing and fixing oxygen. This rate was calculated as follows:

$$\%E = \frac{VO_{2i}}{OC_i} \cdot 100$$

were %E is the percentage of oxygen extrated during the period i; VO_{2i} is oxygen consumption expressed in mg $O_2/hr.g^{-1}dw$, and OC_i is the concentration present before the chamber was sealed in time i, and expressed in mg/L. The probit method analysis was used in calculating the 72 hr and 96 hr LC_{50} for Cr^{+6} and Cd, respectively (APHA 1985). The median was used as an indicator of the central resistance trends to extreme cases of the oxygen consumption and extracting rates. The dispersion of the values obtained was expressed in terms of the confidence interval of the median with 95% degree of confidence (Tukey 1977). The Kruskal-Wallis non-parametric test was used for determining the statistical differences between the levels of oxygen consumption and extraction for the different concentrations of the two metals tested (Zar 1974).

RESULTS AND DISCUSSION.

In the tests described above the effects of different concentrations of two heavy metals were determined, using lagoon water from the capture zone. The LC_{50} for Cd was found to be one order of magnitude greater than that observed for Cr^{+6}, Cd had a 96 hr LC_{50} equal to 6.35 ± 1.05 mg/L, a level 92% lower than the value for Cr^{+6} (73.69 ± 1.96 mg/L). Although the LC_{50} obtained for both metals are too high to be found in aquatic environments, this kind of studies are useful to calculate sublethal concentrations for 21 or more days. The levels of toxicity observed in this study differ from those reported for Callinectes sapidus, resulting from tests carried out under standard laboratory conditions and 35°/₀₀ salinity (11.6 mg/LC_{50-96} for Cd and 114 mg/LC_{50-72} for Cr^{+6}) (Frank and Robertson 1979). These differences may be derive from the conditions under which both metals were tested, as well as the sensitivity of the different species studied. Callinectes similis would appear then to be more sensitive than Callinectes sapidus to both metals. Moreover Rosas et al. (1987) found that C. similis were also less tolerant than C. sapidus and C. rathbunae to salinity changes. This lack of tolerance of C. similis to changes in its environment and to the presence of contaminants may be explained by the fact that this species utilizes much of its energy in maintaining its basic metabolism, which may be twice as high as the metabolic level of C. sapidus and C. rathbunae (Rosas et al. 1987). Thus,the energy that other species use for maintaining internal equilibrium when subjected to environmental alterations must be used by C. similis for maintaining its basic metabolic functions, leaving little energy for resisting adverse environmental conditions. This physiological characteristic is in keeping with observations made

with other species of crustaceans, which like C. similis, inhabitat marine environments (Kinne 1971, Charmantier et al. 1988).

The nature of the experiments may be another key to explaining the different results obtained for the previously mentioned studies. As is common knowledge, the degree of toxicity of heavy metals depends to a significant degree on the interactions between organisms and their environment. Given the fact that in estuarine and marine environments these interactions are determined by the complexity of the physical and chemical parameters involved, any test of toxicity must take this complexity into account (Amiard et al. 1986).

In this study the performance of toxicity tests and measurements made to determine oxygen consumption levels were carried out simultaneously. The respiratory rate was calculated for crabs exposed to Cd (Tables 1 and 3) as well as for those exposed to Cr^{+6} (Tables 2 and 4). In the control group was observed a significant increase of oxygen consumption between the 6 and 12 hr of exposition ($P<0.05$) follow by decrease and finally by its stabilization (Table 1). In contrast, this increase of respiratory rate was only observed on experimental crabs exposed to Cd at 6 hr interval. The only case which a significant increase in oxygen consumption was not observed during the mentioned period was for 6.40 mg/L concentration ($P>0.05$) (Table 1). In terms of metabolic levels, no significant differences were observed between those crabs exposed to Cd and the control, except for the 3.96 mg/L concentration. The variations in oxygen extraction observed throughout the test showed similar patterns to those obtained for oxygen consumption (Table 3). The percentage of oxygen extraction registered higher levels for the control case during the first 12 hours of the test, as compared to the test cases ($P<0.05$).

For crabs exposed to Cr^{+6} oxygen consumption (Table 2) showed significantly different patterns of behavior than those for crabs exposed to Cd. The test crabs exposed to Cr^{+6} had considerably lower metabolic rates when compared to the control ($P<0.05$), registering a downward tendency in this area throughout the test period. On the other hand, the control was observed to reach its oxygen consumption maximum levels during the first part of the test period, as well as toward the end of the period. The behavior of oxygen extraction percentage, however, showed more similar patterns to those observed for the crabs exposed to Cd: a significant increase in this variable was observed during the first hours of the test, followed by the stabilization of these levels thereafter (Table 4).

According to some authors, the effects of both heavy metals on metabolic rates of crabs depends to a considerable degree on the organism's reaction mechanisms, the entrance speed of substances to the organism and the competition which may occur for an active place in several enzymes (Hughes 1976).

Any alteration in the oxygen consumption and extraction in animals exposed to heavy metals is necessarily accompanied by modifications in all respiratory mechanisms. Due to the fact that the primary effects of these metals are first observed in the external areas of aquatic organisms, as in the case of crabs, the first areas to suffer

Table 1. Oxygen consumption (mg O_2/h•g.d.w.) of *Callinectes similis* exposed to Cd^{+2} for 96 hours (median ± confidence interval to 95%). *Medians with N ≤ 4

	Time, hours						
mg/l	1	6	12	24	48	72	96
Cd^{+2} Control	0.35 ± 0.21	0.45 ± 0.17	0.57 ± 0.16	0.33 ± 0.16	0.38 —	0.28 ± 0.08	0.32 ± 0.08
2.48	0.38 ± 0.04	0.58 ± 0.16	0.47 ± 0.08	0.42 ± 0.14	0.38 ± 0.14	0.36 ± 0.02	0.38 ± 0.02
3.96	0.52 ± 0.08	0.67 ± 0.16	0.61 ± 0.10	0.57 ± 0.12	0.47 ± 0.08	0.37 ± 0.14	0.46* —
6.40	0.31 ± 0.04	0.39 ± 0.02	0.37 ± 0.04	0.33 ± 0.06	0.36 ± 0.08	0.36*	0.35*
10.05	0.39 ± 0.02	0.53 ± 0.12	0.51 ± 0.04	0.33 —	0.45* —	0.22* —	—

Table 2. Oxygen consumption (mg O_2/h•g.d.w.) of *Callinectes similis* exposed to Cr^{+6} for 72 hours (Median ± confidence interval to 95%).

	Time, hours					
mg/l	1	6	12	24	48	72
Cr^{+6} Control	1.15 ± 0.32	0.85 ± 0.41	0.66 ± 0.48	0.62 ± 0.31	0.71 ± 0.39	1.18 ± 0.82
27.53	0.47 ± 0.10	0.43 ± 0.16	0.43 ± 0.10	0.43 ± 0.10	0.42 ± 0.12	0.52 ± 0.14
52.09	0.71 ± 0.14	0.60 ± 0.12	0.62 ± 0.22	0.53 ± 0.08	0.39 ± 0.02	0.45 ± 0.04
95.26	0.43 ± 0.18	0.49 ± 0.05	0.46 ± 0.02	0.47 ± 0.12	0.39 ± 0.10	0.35* —
195.13	0.52 ± 0.16	0.43 ± 0.16	0.38 ± 0.10	0.29 ± 0.04	0.28* —	

Table 3. Percent extraction of oxygen by *Callinectes similis* exposed to Cd^{+2} for 96 hours (Median ± confidence interval to 95%).

	Time, hours						
mg/l	1	6	12	24	48	72	96
Cd^{+2} Control	53.06 ± 11.94	69.45 ± 6.04	70.67 ± 9.48	35.56 ± 2.65	40.00 ± 7.22	30.10 ± 6.92	35.31 ± 9.61
2.48	29.81 ± 6.38	53.48 ± 7.02	39.88 ± 9.97	34.44 ± 6.79	34.10 ± 7.60	29.13 ± 11.71	31.73 ± 3.89
3.96	35.11 ± 1.48	38.27 ± 9.69	51.25 ± 17.74	33.04 ± 9.29	30.53 ± 7.66	35.35 ± 8.61	20.38*
6.40	25.19 ± 9.52	28.97 ± 5.86	31.86 ± 3.46	23.14 ± 2.85	25.75 ± 5.91	26.16*	19.85*
10.05	33.41 ± 5.22	40.88 ± 3.10	44.46 ± 2.83	23.73 ± 2.99	43.46 ± 1.80	46.40*	

Table 4. Percent extraction of oxygen by *Callinectes similis* exposed to Cr^{+6} for 72 hours (Median ± confidence interval to 95%).

	Time, hours					
mg/l	1	6	12	24	48	72
Cr^{+6} Control	11.11 ± 1.80	38.91 ± 4.33	30.30 ± 5.11	29.08 ± 3.84	26.68 ± 2.11	30.83 ± 7.21
30.0	39.44 ± 5.13	52.46 ± 4.81	30.38 ± 4.14	24.71 ± 6.35	32.31 ± 3.56	28.17 ± 7.12
52.0	46.57 ± 4.10	57.53 ± 3.16	48.15 ± 5.40	34.89 ± 9.83	34.81 ± 7.11	32.78 ± 4.11
95.26	17.50 ± 6.31	26.25 ± 2.33	24.09 ± 4.08	23.81 ± 7.60	23.08 ± 4.04	20.80* —
195.13	23.18 ± 2.08	28.57 ± 4.17	18.57 ± 7.18	15.00 ± 4.69	18.39* —	

functional alteration are the gills (Hernandez et al. 1986).

For all the concentrations tested, Cd produced an increase in oxygen consumption at least for two of the measurements taken during the course of the 96 hr test (Table 1). This increase could have been the result of an increase in locomotor activity and in the ventilating system; an attempt on the part of the crab to escape an adverse environment in which it had found itself.

After the first 24 hr of the test, these high rates of oxygen consumption were observed more frequently in those organisms exposed to the two highest metal concentrations. This finding agrees with those obtained by Moraitou-Apostolopoulou et al. (1982) for Cr^{+6} and Cd, tested on Palaemon elegans. However, for sublethal concentrations an increase in the metabolic rate of an organism may have graver consequences because the range of metabolic activity of the population may be reduced,thus limiting the functioning of the species within its community. An example of an extreme sublethal concentration of this kind is that of 2.48 mg/L of Cd (Fig.1). The rate of oxygen extraction is an indicator of the way in which those mechanisms governing the capture and diffusion of oxygen into the blood are operating. In this study, we observed that, the rate of oxygen extraction was lower than normal for those organisms exposed to Cd. These results suggest that an alteration occurs in the mechanisms governing oxygen transfer, which may be a result of a reduction in the efficiency of oxygen transfer over the gill, or of vascular constriction in the gill capillaries, resulting, in turn, in an inhibited ability for gas interchange. Thus, one of the causes of stress, which is one of the causes of the organisms death, together with intoxication per se, may be anorexia produced by Cd (Frank and Robertson 1979).

The results obtained with regard to the effects of Cr^{+6} on oxygen consumption show an inhibition of respiratory metabolism (Fig. 2). This inhibition may be explained by the manner in which Cr^{+6} is incorporated into the organisms system for obtaining energy. It has been reported that at high concentrations Cr^{+6} may form complexes with the ATP-ADP system, which in turn may produce the inhibition of some enzymes related to the energetic metabolism (Peternac and Legovié 1986; del Ramo et al. 1987, Rosas 1984).

Given the patterns observed for the rate of oxygen extranction, it is possible to infer the existance of mechanisms of compensation for metabolic inhibition. These compensation mechanisms appear to operate by increasing the efficiency of the mechanisms in the respiratory system governing the oxygen capture (Fig. 2). As may be observe, in 27.53 mg/L and 52.09 mg/L, these mechanisms for transferring and transporting oxygen tend to stabilize at levels close to those observed in the control group, after 72 hr of exposure to the contaminant. This may be achieved if, after exposure to Cr, the organism is able, in the short run, to increase its efficiency with regard to the capture and transport of oxygen in the hemocyanin, in a similar manner to that described for the hemoglobin in fish (Larson et al. 1976) However, a limit to this compensatory process apparently exists, after which point these mechanisms can no longer counteract their inhibited respiratory capacity (95.26 and 195.13 mg/L. concentration of Cr^{+6} . Fig 2).

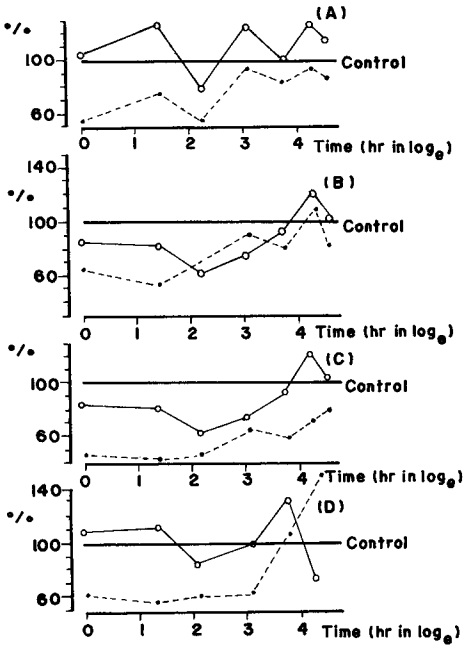

FIGURE 1. Oxigen consumption (—o—) and extraction rates (---•---) of *Callinectes similis* exposed to Cd^{+2} for 96 hours. Letters indicates concentrations of Cd^{+2}: A=2.48; B=3.96; C=6.40; D=10.05 mg $Cd^{+2} \cdot L^{-1}$.

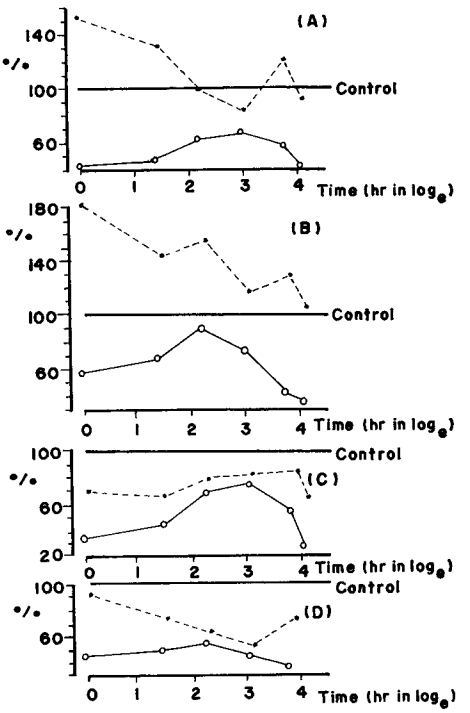

FIGURE 2. Oxigen consumption (—o—) and extraction rates (---•---) of *Callinectes similis* exposed to Cr^{+6} for 72 hours. Letters indicates concentrations of Cr^{+6}: A=27.53; B=52.09; C=95.26; D=195.13 mg $Cr^{+6} \cdot L^{-1}$.

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