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Sublethal Effects of Copper and Mercury on Some Biochemical Constituents of the Estuarine Clam *Villorita cyprinoides* var. *cochinensis* (Hanley)

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The usefulness of sentinal organisms in environmental pollution monitoring or surveillance programs has been well established (Goldberg et al 1978; Topping 1983). A particularly significant attribute of sublethal physiological response is that it is amenable to both laboratory and field measurements unlike traditional toxicant testing. Such methods could be employed to develop environmental quality models to predict the biological effects of potential pollutants as well as to directly monitor the effects in the environment (Widdows 1985). This paper describes the effect of sublethal amounts of two well known aquatic pollutants namely Cu and Hg on an estuarine clam Villorita cyprinoides var. cochinensis.

The changes in carotenoid content (total and unsaponifiable), metabolic rate, lactic acid as well as glycogen contents of the tissues of the clam exposed to sublethal amounts of Cu(II) and Hg(II) were investigated over a range of time.

MATERIALS AND METHODS

The clams (size 25-30 mm) collected from Cochin backwaters (9° 55′ N 76° 17′ E), were acclimatized in the laboratory for 3 to 4 days (salinity=13 x 10^{-3}, temperature 28 ± 1° C, pH = 7.6 ± 0.2, dissolved oxygen = 3.6 mL/L. The animals (25 per 5-L test medium) were exposed to 300 and 600 ug/L each of Cu and Hg for 96 h. The metals were added as aqueous solutions of $CuSO_4.5H_2O$ and $HgCl_2$ (British Drug House AnalaR; India). Controls were maintained throughout. The test medium was renewed once every 24 h. Animals were not fed during the experimental period. Duplicates were run in all cases. Mortality observed was less than 10 % during the whole period. The 96 - h LC 50 was 1214 ug/L for Cu and 1570 ug/L for Hg

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(Nambisan and Lakshmanan 1982 unpublished data).

Total and unsaponifiable carotenoids were extracted and estimated by the procedure of Karnaukhov et al (1977). The total carotenoids were extracted with acetone. Saponification was carried out by means of 60% potassium hydroxide at a temperature of about 28° C for 14 h, and unsaponifiable carotenoids were extracted using light petroleum ether. Optical density was measured in 1 - cm cuvette using a Hitachi Spectrophotometer(model 200-20) at wavelength of carotenoid absorbtion maxima 450-460 nm. Oxygen uptake of the animals was measured in Erlenmeyer flasks using Winkler techniques(Strickland and Parsons 1972). Liquid paraffin was used to seal the water-air interface. Lactic acid and glycogen were determined by standard procedures (Colowick 1957). The results were analyzed statistically according to Snedecor and Cochran (1967); the significant difference between experimental groups and control groups were determined using student's t-test. The precision of all determinations was within the range ± 5%.

RESULTS AND DISCUSSION

The results are presented in Tables 1 and 2 and Figure 1. In animals exposed to 300 ug/L and 600 ug/L Cu, the carotenoid concentration increased sharply with time and reached a maximum value in about 48 h. The total carotenoid content increased 2 times in the former and 4 times in the latter, the increase being proportional to the concentration of metal ions in solutions. In 300 and 600 ug/L Hg exposed animals the total carotenoid content increased to 0.318 and 0.673 mg/100 gm (wet wt.) respectively within 48 h (control = 0.18). After 48 h the carotenoid content (total and unsaponifiable) decreased. The control values remained remarkably steady(variations ±5%) throughout the entire experimental period.

A corresponding change in metabolic rate (oxygen uptake/h/g dry body wt) of animals was noticed at all stages, with progress of time. Cu-exposed animals generally suffered a greater reduction in the oxygen uptake than Hg-exposed animals. In 600 ug/L Cu and Hg the metabolic rate was depressed by 32% and 28%, respectively, during 48 h. After 48 h, the metabolic rate in both Cu and Hg exposed animals was found to increase, being more pronounced in 300 ug/L exposed animals.

The lactic acid content in the tissue of the clam increased with progress of time and was proportional to the metal concentration. The tissue lactic acid content increased to 94.4 and 136.1 ug/g in 300 and

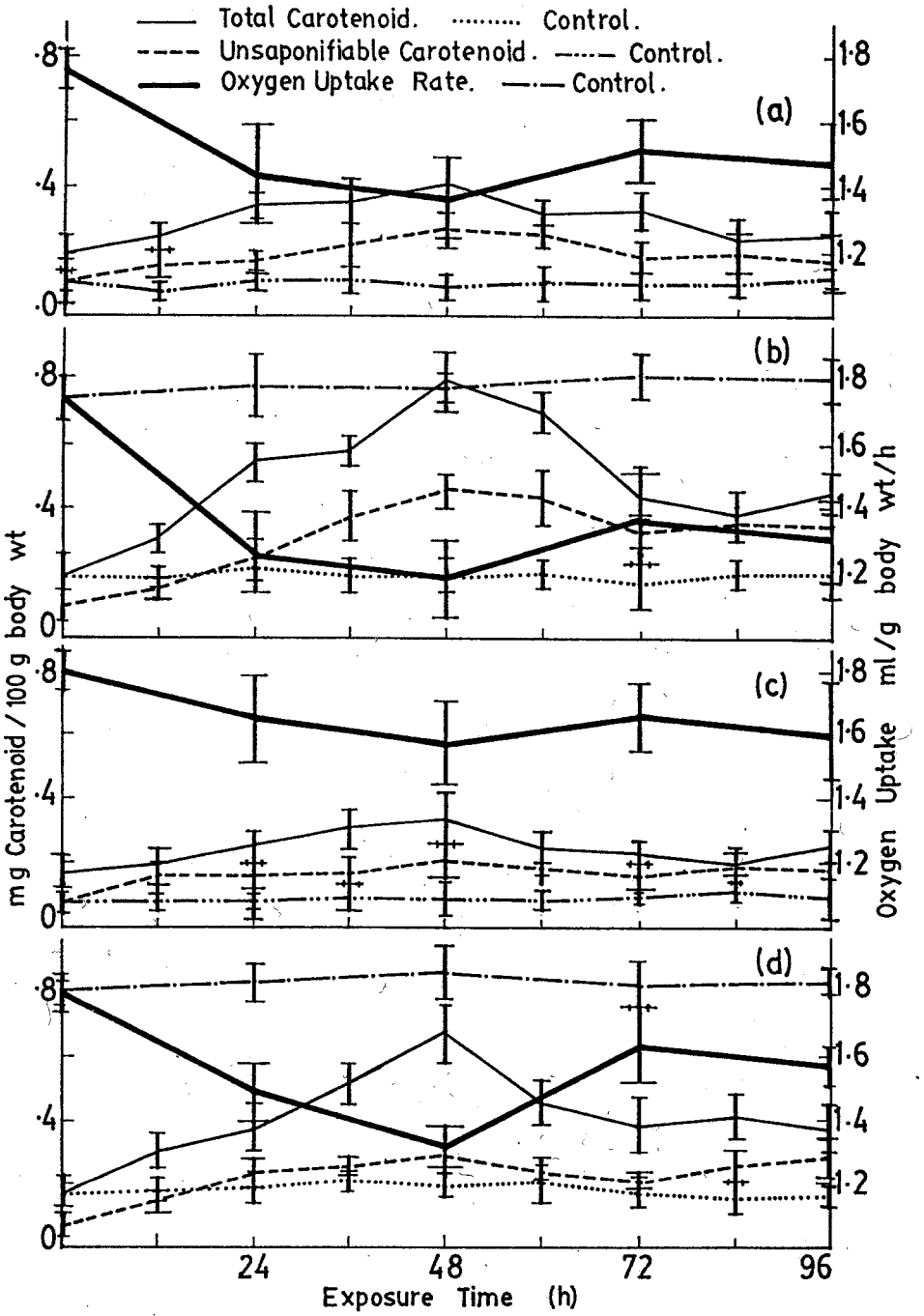

Figure.1.Carotenoid concentration and oxygen uptake rate in V.cyprinoides exposed to (a) 300 µg L^{-1} Cu (b) 600 µg L^{-1} Cu (c) 300 ug L^{-1} Hg (d) 600 ug L^{-1} Hg, Vertical bars indicate standard deviations (n = 5). Controls are same for (a) and (b), (c) and (d).

Table 1. Changes in the tissue lactic acid content of the clam V. cyprinoides exposed to sublethal concentrations of Cu and Hg for varying lengths of time.*

Conc. of metal ions ug / L	Lactic acid, ug / g wet wt; $\bar{x}\pm$ S.D. (n=5) Exposure time 3 h	6 h	9 h	12 h	24 h
Cu 300	30.3±2.2	45.3±4.4	44.6±3.5	56.5±5.2	94.4± 9.6
600	43.3±3.2	72.5±7.9	78.5±8.3	95.3±8.7	136.1±11.4
Hg 300	26.3±3.2[a]	39.7±2.7	64.9±4.3	60.5±8.3	82.1± 9.7
600	38.4±5.0	56.4±7.1	81.9±7.4	78.6±6.4	124.1±11.3
Control	22.7±2.4	21.0±1.1	18.3±2.7	22.3±1.8	19.5±2.2

Table 2. Changes in the muscle glycogen content of the clam , V. cyprinoides exposed to sublethal concentrations of Cu and Hg for varying lengths of time.*

Conc. of metal ions ug / L	Glycogen, ug / g wet wt; $\bar{x}$ ± S.D. (n=5) Exposure time 12 h	24 h	36 h	48 h	60 h	72 h
Cu 300	1793±29.2	1585±32.2	1480±26.5	1409±43.2	1396±49.0	1285±32.7
600	1545±36.7	1211±44.7	1061±42.5	980±30.8	968±20.8	910_42.9
Hg 300	1866±35.8	1744±24.4	1630±42.5	1567±26.1	1560±31.6	1570±34.4
600	1674±42.2	1438±38.4	1371±32.0	1225±34.7	1193±57.0	1188±59.9
Control	1944±23.7	1950±30.5	1959±24.5	1940±31.9	1963±46.9	1954±29.2

* All values significantly different from control ($P < 0.05$) except the one superscribed[a] (student's t-test)

600 ug/L Cu-exposed animals and to 82.1 and 124.1 respectively in the Hg-exposed animals during 24 h (control = 20.2) (Table 1).

Glycogen levels, however, were generally found to decrease. The glycogen content of the 300 and 600 ug/L Cu-exposed animals decreased to 1285 and 910 ug/g respectively in 72 h (control = 1952 ug/g). The corresponding values for the Hg-exposed animals were 1570 and 1188 ug/g respectively(Table 2).

These results indicate, that Cu and Hg pollution lowers the normal metabolic rate and glycogen levels of the organism and increases the carotenoid as well as the lactic acid content in the tissues. A distinct inverse relationship is observed between carotenoid content of the clam and its metabolic rate.

The steady increase observed in the carotenoid content may be due to the physiological adaptive responses of the animal to compensate the external stress of metal concentrations and the consequent inhibiton of the respiratory processes. An increase in the carotenoid concentration with increase in environmental pollution has been reproted earlier (Karnaukhov et al. 1977; Karnaukhov 1979). Krishnakumar(personal communication) also has reported that the carotenoid concentration in the mussel Perna viridis increases directly with increase in metal concentrarion. Karnaukhov (1971) has reported that carotenoids participate in the oxidative metabolism of the mollusc Lymnaea stagnalis by providing a large number of unsaturated double bonds as an intracellular oxygen reserve. Therefore, the increase in the carotenoid content can be taken as an indication of the animal's adaptation to the hypoxic conditions.

Heavy metal - induced decrease in metabolic rate in Uca pugilator has been reported (Vernberg et al 1974). Cupric ions were shown to cause respiratory and cardiovascular depression in M. edulis and the effect was attributed to the passive binding of cupric ions with organic ligands (Scott and Major 1972).

The increase in tissue lactic acid content and decrease in glycogen levels may be due to the heavy metal intoxication, which induces appreciable mucus secretion-- even in animals exposed to 300 ug/L Cu and Hg considerable mucus secretion was observed. Lakshmanan and Nambisan (1985) have shown that Cu and Hg intoxication had caused lactic acid accumulation and glycogen depletion in tissues of V. cyprinoides and P. viridis. A similar reduction in glycogen content and increase in lactate concentration in tissues due to copper intoxication in the fresh water

teleosts(fish) was reported(Shaffi 1978).

The animals are able to adjust the initial stress or may have released partly that stress and shown symptoms of normal behaviour.The increase in oxygen consumption accompanied by a decrease in carotenoid content after 48 h may be due to this. Moreover, accumulation studies of heavy metals on many organisms have shown that the initial rate of uptake was very high at lower concentrations and the rate decreases with time. (Vernberg et al. 1974). Donaldson and Dye (1975) have observed that Sockeye salmon has adjusted the stress imposed by the low concentration of Cu within 4 h.

It is not yet clearly understood whether physiological changes during a sublethal exposure represent deleterious effects of the toxicant or merely the normal adaptive mechanism of the organisms (Dixon and Sprague 1981). As they have pointed out there is a physiological mechanism functioning within the animal to mitigate the toxic effects of heavy metals. Considerable energy was required to establish the adaptive mechanism, less energy was required to maintain it. A long period of increased oxygen consumption was necessary to restore normal metabolic process damaged by metals.

From the point of view of the organism and polluters the ability to adapt to metals seems to be a favorable aspect to the animal. However, it should be remembered that many of these tolerant organisms contain two to three orders of magnitude higher concentrations of metals than normal and, so far as we know at present, these may be transmitted to non-adapted predators, including man (Bryan 1976).

In conclusion, the animals went through two classical stages of stress response, ie; the alarm reaction and the stage of resistance (Donaldson and Dye 1975). Because metals such as copper are more toxic at low salinities, the pressure to adapt is probably greatest under these conditions. Only a detailed study with a longer time of exposure can reveal whether the animals can successfully accomodate and adapt to the stressor.

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