

Bioaccumulation and Elimination of Copper in the Rock Oyster *Crassostrea cucullata*

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Abstract

The kinetics of copper bioaccumulation in the rock oyster *Crassostrea cucullata* Born showed that the initial rate of uptake was directly related to metal concentration in the medium. As the accumulation in the tissues increased, the oysters remained closed and the uptake rate fell. At the end of 7 weeks, the average copper concentrations in the tissue were $60.42 \mu\text{g g}^{-1}$ and $63.97 \mu\text{g g}^{-1}$ wet weight in the 0.01 and 0.05 ppm experimental groups, respectively. The net rate of uptake ranged from 1.76 to $1.97 \mu\text{g g}^{-1} \text{ week}^{-1}$ and the rate of copper loss, measured after transferring the oysters into natural sea water, was dependent on the original copper concentration in the soft parts. The concentration of copper in the tissues declined by 37.38 and 36.56% in the 0.01 and 0.05 ppm experimental groups, respectively. Even after a 7 week period of depuration (self-purification) there was some residual copper left in the tissue. This indicates that accumulation occurs in the tissue more rapidly than cleansing can eliminate it.

Introduction

Copper sulphate is widely used in the industry and for controlling unwanted vegetation. Acute toxic levels of copper rarely occur in nature, but ample evidence is available to demonstrate the increasing occurrence of copper in many organisms.

Copper toxicity in the marine environment and its severe effects on marine organisms are well documented in several reviews (Ikuta, 1967; Pringle *et al.*, 1968; Shuster and Pringle, 1969; Bryan, 1971). However, to interpret the significance of metal burden in marine animals and to predict the transport of copper through the marine ecosystem, a knowledge of accumulation dynamics is essential. Brooks and Rumsby (1965) and others have demonstrated that copper is actively transported to the tissues of marine invertebrates, and copper levels of 8 to 10 ppm have been reported in oysters from unpolluted areas and of 120 to almost 400 ppm from polluted waters.

Abnormal accumulation of copper in oysters was reported as early as 1897, and colloquially termed "green-sick" because of the presence of green pigmentation in the flesh of such oysters (Boyce and Herdman, 1897).

Much of the previous work on copper toxicity, summarized by McKee and Wolf (1963) is based on short-term exposure to determine lethal and other end-points. Information on the accumulation and depuration of copper by oysters from sea water containing appreciable levels of copper is rather limited, and hence the present report provides some data on the uptake of copper in the tissues of the rock oyster *Crassostrea cucullata*, and its subsequent clearance.

Materials and Methods

Rock oysters, *Crassostrea cucullata* Born were collected in late April from the Chapora estuary, Goa (India). Oysters of similar size (3.6 to 4.0 cm long; 2.3 to 2.4 cm wide; 1.6 to 1.8 cm high) were detached from rocks, cleaned, and acclimated in experimental tanks for 1 week. Concentrations of 0.01 and 0.05 ppm copper ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in sea water and a control to which no copper was added were used. During the 7-week accumulation period, weekly samples of 10 oysters from each tank were taken for analysis. Their soft parts were removed from the shells with a steel spatula and rinsed with distilled water before being homog-

enised. They were then subjected to wet digestion using a mixture of nitric acid and perchloric acid, and the solutions were analysed using a Hilger and Watts Atomspek HI 550. Details of the method are given in the "Manual of Methods in Aquatic Environmental Research" (see Bernhard, 1976).

After the 7-week accumulation period, a 7-week depuration period (self-purification) followed in order to determine the extent of elimination of the accumulated copper. The oysters were returned to filtered sea water in the same tanks as used during the accumulation study. The water was changed every 24 h to avoid re-absorption of lost copper from the medium. The data on copper accumulation and depuration were statistically analysed according to the procedure suggested by Schulz-Baldes (1974).

Results

Relatively high concentrations of copper occurred in *Crassostrea cucullata* after the first 5 weeks of contamination, which caused a marked "greening" of the soft tissues in both concentrations. Copper levels in the experimental oysters were consistently higher than in the controls (Table 1), clearly demonstrating that oysters exposed to copper accumulate measurable amounts thereof. Pre-experimental background concentrations of copper were not significantly different from those of the control samples determined at weekly intervals (Fig. 1c). Tissues of control oysters had a mean copper content of $22.5 \mu\text{g g}^{-1}$ wet weight (Fig. 1c).

In the two contamination experiments (0.01 and 0.05 ppm copper), there was a significant uptake of copper during the 7-week exposure period (Fig. 1a, b). Copper residues in the 0.01 ppm group attained a maximum tissue concentration of $60.42 \mu\text{g g}^{-1}$, and copper residues in the 0.05 ppm group a maximum tissue concentration of $63.97 \mu\text{g g}^{-1}$, after 7 weeks exposure (Fig. 1a, b). Taking the

natural copper content as $20.63 \mu\text{g g}^{-1}$ (Table 1), the copper content had therefore increased by $39.79 \mu\text{g g}^{-1}$ and $43.34 \mu\text{g g}^{-1}$ in the 0.01 and 0.05 ppm groups, respectively. The "green-sick" oysters remained closed in the 0.05 ppm concentration, and a slight decrease in uptake occurred. This indicated that either the intake capacity of copper becomes significantly limited or a steady state condition is reached.

The linear relation of uptake presented in Fig. 1 (1 to 7 weeks) can be described by:

$$(A)_t = k_1 \cdot t \times (A)_0, \quad (1)$$

where $(A)_t$ = copper concentration in the oyster at time t , k_1 = factor of proportionality or rate of uptake, and $(A)_0$ = natural copper concentration in the oyster.

The linear relation of depuration from 7 to 14 weeks is given by:

$$(A)_t = (A)_i - k_2 \cdot t, \quad (2)$$

where $(A)_i$ = initial copper concentration in the oyster, and k_2 = rate of copper loss.

The time-course of copper uptake and copper loss from the oyster tissues (experimental and control) is given in Fig. 1. After the first day, a linear time-course for uptake was observed. At the end of 4 weeks, the copper concentration in the tissues has been more than doubled compared to the initial concentration. The values for the slopes of the various regression lines are given in Fig. 1.

Following the period of exposure, the oysters were kept for a further period of 7 weeks in natural sea water. During this time, a decrease in copper concentration in the soft parts was observed (Fig. 1a, b, 8 to 14 weeks). The slope of the fitted straight lines in Fig. 1a, b, indicates a net accumulation of copper, since the rate of loss of copper was lower than the rate of uptake in both the experimental tanks. Copper concentrations in the oysters could be bound to the tissues, so that their ex-

Table 1. *Crassostrea cucullata*. Copper concentration in soft parts at beginning of experiment, after 7 weeks of copper uptake from the medium, and after 7 weeks of copper loss

Cu in medium (ppm)	Cu concentration ($\mu\text{g g}^{-1}$ wet weight):			Rate ($\mu\text{g g}^{-1} \text{ week}^{-1}$) of:		% Loss	Net rate of uptake ($\mu\text{g g}^{-1} \text{ week}^{-1}$)
	Initial	After 7 weeks uptake	After 7 weeks loss	Uptake	Loss		
0.01	20.63	60.24	37.83	5.30	3.54	37.38	1.76
0.05	20.63	63.97	40.58	5.31	3.34	36.56	1.97

change with the environment is limited. The tissue concentration declined by 37.38 and 36.56% in the 0.01 and 0.05 ppm experimental groups, respectively, after 7 weeks depuration (Table 1). The rate of loss per week was 3.54 and 3.34 $\mu\text{g g}^{-1}$ of wet weight tissue, respectively (Table 1). Complete depuration was not achieved during the 7-week period. The experiments were terminated because 15%

of the oysters died in the 0.05 ppm experimental group, and the remaining were sacrificed for residue analysis.

Discussion

The most revealing aspect of this study on *Crassostrea cucullata* is the magnitude of metal accumulation resulting from a small source of contamination. Bivalve molluscs are notorious for their ability to concentrate metals, and this has been particularly apparent in our study. It is obvious that even if the concentrations in the oysters do not constitute a public-health hazard, there is always the risk of a direct toxic effect upon these particularly susceptible species. Our studies further indicate that accumulation is a more rapid process than depuration, since total elimination of all the accumulated copper from the tissues had still not been achieved after 7 weeks. The role of copper in the metabolism of the oyster is not yet clearly understood. Oysters placed in sea water of high copper concentration presumably absorb more of the element than they can possibly excrete, and thus they are forced to bind the potentially toxic copper within an organic complex. The principal copper-binding protein in oysters has been found to occur in granular haemolymph amoebocytes (George *et al.*, 1978).

Pringle *et al.* (1968) proposed that the rate of loss in oysters is determined by the rate of uptake. Our results indicate that the rate of loss is far more closely correlated with the total internal copper concentration of the oyster than with the rate of uptake. In the case of mercury loss from the American oyster *Crassostrea virginica*, Cunningham and Tripp (1973) observed that the concentration in the tissues declined only during the first 18 to 30 days. For 160 days thereafter, the concentration remained constant, indicating that a permanent fixation of mercury had occurred in the tissues. Schulz-Baldes (1974) has suggested that the rate of uptake of lead in *Mytilus edulis* may always exceed the rate of loss, resulting in a permanent fixation of the metal. A similar situation exists for copper fixation in the rock oyster (see Table 1 and equations in Fig. 1).

Pringle *et al.* (1968) established that *Crassostrea virginica* is able to accumulate and purge itself of lead under constant experimental conditions. In the natural environment, shell-fishes would not be exposed to controlled conditions such as those in the laboratory, and hence the rate of accumulation or elimination of

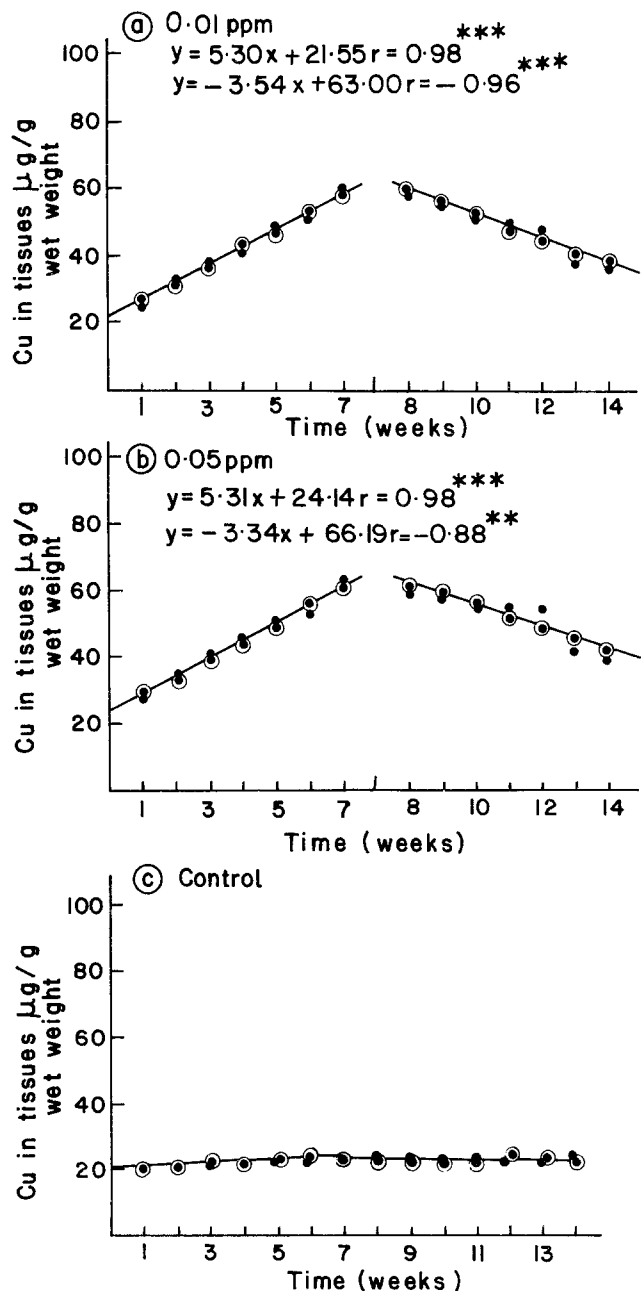


Fig. 1. *Crassostrea cucullata*. Copper uptake at two different concentrations in the medium, and copper loss after transfer to natural sea water. Significance of correlation (valid for a, b and c) = *** $P < 0.1\%$; ** $P < 1\%$

heavy metals occurring naturally in the molluscs will not be easy to assess. However, after about a year of constant exposure, a net accumulation of residual copper will occur in oyster tissues, since total elimination of copper is not possible, even after a prolonged cleansing period. This will lead to what has often been described in the literature as the "green-sick" condition in oysters.

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