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Schema of Lethal Action of Copper on Mussels

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The study of the response of a marine organism to a toxic element in solution in sea water, generally takes two factors into account: the concentration of the toxic element in sea water (C) and the lethal time (LT) of the whole or a part, genrally 50%, of the contaminated population. The toxicity of the element established by these two factors is expressed by LC_{100} or LC_{50} (LC = Lethal concentration) for a given time, generally 24, 48 or 96 hours (CONNOR 1972 , OKAZAKI 1976). This notion does not take into account two other factors which seem to be essential in the study of the response of an organism to a toxic element: the level of accumulation of the element in the dead organism (A = Accumulation) and the speed of accumulation of this element by the organism (SA). In order to demonstrate what are the relationships between these different factors, C, LT, A and SA, experiments were carried out taking the mussel *Mytilus edulis* as test organism and copper as test toxic element. The results of this study show that the toxicity of copper towards mussels is regulated by a two steps reaction where these different parameters are taken into account. The difference is made between the total accumulation and the lethal accumulation.

MATERIAL AND METHODS

Eight tanks, each containing 80 mussels, from 2.5 to 6 cm length, were filled with 50 liters of sea water. Each of the tanks was contaminated by adding copper under $Cu\ Cl_2$ form. The Cu concentration in each tank was respectively 0.02, 0.05, 0.1, 0.3, 0.5, 1, 2 and 3 $\mu g\ ml^{-1}$. A ninth tank with 80 mussels and 50 liters of sea water was used as control. The average copper concentration in control mussels was 16.1 $\mu g\ g^{-1}$/dry weight. No control mussel died during the whole experiment. Contaminated and control mussels were fed with the lyophylised cyanophycea algue *Spirulina maxima*. Every 24 hours the sea water was changed and recontaminated. It has already been reported (ZIRINO and YAMAMOTO 1972) that at the normal pH of sea water, copper is mainly present as $Cu(OH)_2$. This insoluble hydroxyde precipitates. As a matter of fact, the copper concentration in

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contaminated sea water decreases with time. The water in control tank was also changed every 24 hours. In control tank, copper concentration determined by stripping anodic polarography technic, was 0.002 µg ml^{-1} (CHARLOU, personal comm.). Copper concentration in dead mussels was determined by atomic absorption spectrophotometry after drying samples at 100° C up to constant weight and ultra-pure nitric acid digestion. The conditions of analyses have been previously described (BOYDEN 1974). Regression lines were fitted by the method of least squares. Methods for statistical analysis were issued from SNEDECOR and COCHRAN (1967). Copper concentrations in dead mussels were calculated against dry weight.

RESULTS AND DISCUSSION

Figure 1 shows the relationships between the average copper concentrations in dead mussels and the mean time necessary for the 80 mussels in each tank to die. This curve shows two distinct parts, according to $C \geqslant 0.3$ µg ml^{-1} or $C \leqslant 0.1$ µg ml^{-1}.

When $C < 0.1$ µg ml^{-1}, that is to say for 0.02, 0.05 and 0.1 µg ml^{-1} Cu in sea water, the comparison of the mean A (student's test) shows no significant difference between them. Moreover, the regression analyses A over LT for each of the following concentrations of copper in sea water 0.02, 0.05 and 0.1 µg ml^{-1} show that there does not exist any significant correlation between A and LT. The three regression lines fitted for each of these three concentrations are not significantly different from each other, and for each of them the slope is not significantly different from 0 (table 1). The regression line A over LT fitted by pooling the whole values obtained for $C \leqslant 0.1$ µg ml^{-1} presents a slope which is not significantly different from 0 ($P > 0.2$). So, for $C \leqslant 0.1$ g ml^{-1}, A = Constant.

TABLE 1

Comparison between regression of total accumulation of copper over lethal time for copper concentration in sea water $\leqslant 0.1$ µg. ml^{-1}

	S^2R	by	ay
χ^2	1.95	-	-
F test	-	2.54	.70
DF	2	2/232	2/234
P	.3 < P < .5	.05 < P < .1	.25 < P < .5
	NS	NS	NS

S^2R: Residual variance ; b: slope ; a: elevation ; DF: Degre of freedom ; P: level of significance ; NS: not significant.

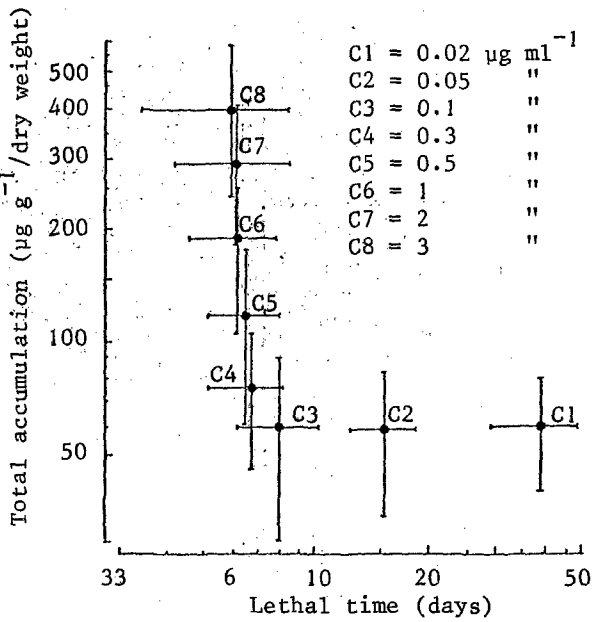

Figure 1. Relationships between the accumulation (A) in dead mussels and the lethal time (LT), at different concentrations of copper in sea water (C) expressed as µg ml^{-1}. A and LT: mean of 80 analyses ± 1 standard deviation.

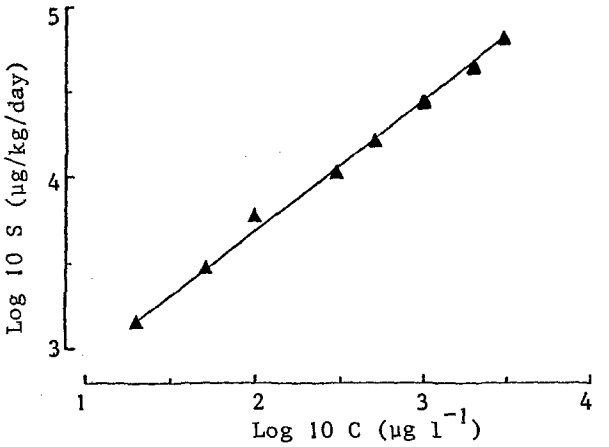

Figure 2. Relationships between the speed of accumulation of copper (S) by the mussel and the concentration of copper in sea water (C). S = (A-16100)/LT days. A = copper content in dead mussels, expressed as µg kg^{-1} ; 16100 = copper content in control mussels (µg kg^{-1}). For this regression line, r = 0.995 ; P < 0.001.

Do these two parts of the curve correspond to two different kinetics of accumulation? Figure 2 shows the relationship between the speed of accumulation of copper and the concentration of this metal in sea water. The regression line, SA over C for the whole concentration of Cu in sea water, from 0.02 µg ml^{-1} to 3 µg ml^{-1} yields a linear function:

$$\text{Log } 10\ SA = 0.75 \text{ Log } 10\ C + 2.18 \text{ (with } r = 0.995).$$

This high correlation coefficient (r) shows that there exists only one kinetic of accumulation for the concentrations ranging from 0.02 to 3 µg ml^{-1}.

From our results one can see that for concentration higher than 0.1 µg ml^{-1}, the lethal time was the same, about 6 days, whatever the concentration of copper in sae water may be. On the other hand, for concentration of Cu in sea water $\leqslant$ 0.1 µg ml^{-1}, A is constant. It seems, as a matter of fact, that the accumulation of copper by the mussel and the action of this metal on the mortality is regulated by a two steps reaction as follow:

$$\text{(a) } Cu + \text{mussel} \xrightarrow{S1} Cu - \text{mussel}$$

$$\text{(b) } Cu - \text{mussel} + R \xrightarrow{S2} R - Cu - \text{mussel}$$

Where S1 is the speed of the reaction (a) and S2 the speed of the reaction (b). The reaction (a) would correspond to the intake of Cu in the organism, the reaction (b) would correspond to the association, in the mussel, of Cu with the target organ or compound, R. Let us suppose that it is the complex R - Cu - mussel which is toxic for the organism, and that the mussel dies when R - Cu - mussel reach a certain level of accumulation LA (LA = Lethal accumulation). The speed of formation of R - Cu - mussel, that is to say S2, determines the lethal time (LT). S1 may be called speed of accumulation, S2 may be called speed of action.

If we consider that S2 > S1 in the two steps reaction described, the speed of accumulation S1 is the predominant speed to govern the whole reaction (a) + (b). The formation of the toxic complex R - Cu - mussel is equal to the accumulation Cu - mussel. That is to say that, LA corresponds to A. In that case:

$$LT = A/S1 = LA/S1 = \text{Constant}/S1$$

We have seen (fig. 2) that S = k C so, LT = Constant/kC. In that case, the time to reach the lethal level of R - Cu - mussel, that is to say LA, is inversely proportional to the concentration of Cu in sea water. That case occurs for C $\leqslant$ 0.1 µg ml^{-1}.

If we consider that S2 < S1, S2 is the predominant speed to govern the whole reaction (a) + (b), the accumulation Cu - mussel is higher than the formation of the toxic complex Cu - R - mussel. In that case, the lethal time, that is to say the time to reach LA, and consequently S2 is constant, whatever the concentration of copper in sea water, higher than 0.1 µg ml^{-1}, the speed of accumulation and the total accumulation of this metal by the mussel may be.

The response of mussels to copper at low concentration, $C \leqslant 0.1$ µg ml^{-1}, seems to be the most interesting one because these concentrations are similar to those observed in sea water in some polluted area (THORNTON et al. 1974). If C is assumed to be constant during the experiment, it can be noticed that for $C < 0.1$ µg ml^{-1} the product C x LT is constant. In effect, for

$C = 0.002$ µg ml^{-1}, $C \times LT = 0.781 \pm 0.18$

$C = 0.05$ µg ml^{-1}, $C \times LT = 0.772 \pm 0.18$

$C = 0.1$ µg ml^{-1}, $C \times LT = 0.794 \pm 0.21$

student's test shows that none of these means is significantly different from each other ($P > 0.5$).

The relation between LT and C can be expressed in that case by the equation:

$$LT \times C = \text{Constant} \; ; \; LT = \simeq 0.78/C$$

If we apply this equation for mussels growing in unpolluted coastal waters, where C generally ranges from 0.002 to 0.005 µg ml^{-1}, the lethal time should be between 156 and 390 days. Now, mussels in unpolluted areas live longer than 390 days. That means that in natural conditions the speed of "decontamination" is higher or, at least equal to the speed of action S2 in the global equation

$$\text{Cu + mussel} \xrightarrow{S1} \text{Cu - mussel}$$

$$\text{Cu - mussel + R} \xrightarrow{S2} \text{R - Cu - mussel}$$

$$\text{R - Cu - mussel} \xrightarrow{S3} \text{R - mussel + Cu}$$

where S3 is the speed of decontamination. Figure 3 synthesizes the global reaction of the toxicity of copper towards mussels. In normal condition, that is to say when $S1 < S2 < S3$, the turnover of copper in the mussels is equilibrated, and when in abnormal conditions, $S1 \simeq S2 > S3$ or $S3 < S2 < S1$, the turnover of copper in mussel is in a desequilibrated state.

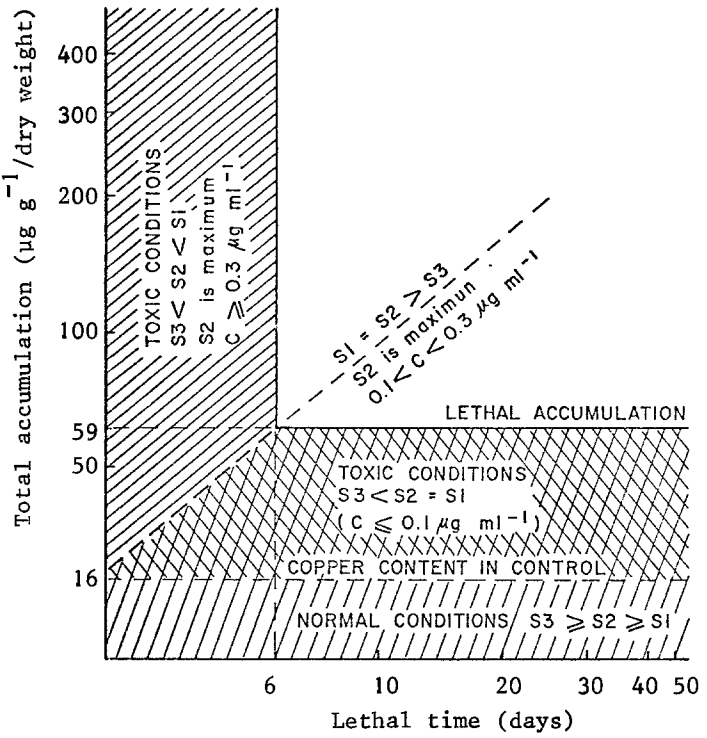

Figure 3. General schema of the relationships between the different parameters: copper total accumulation (A), lethal accumulation (LA), concentration of copper in sea water (C), lethal time (LT), speed of accumulation (S1), speed of action (S2) and speed of decontamination (S3).

Most of the values observed in the literature are in accordance with our results, that is to say that in most cases the concentration of copper in *in situ* mussels is lower than 59 µg g^{-1}/dry weight (BROOKS and RUMSBY 1965 , EUSTACE 1974 , PHILLIPS 1976 , SEGAR et al. 1971 , SHEPPARD and BELLAMY 1974 , STEELE 1973 , STENNER and NICKLESS 1975). These values range generally from 2 to 20 µg g^{-1}/dry weight. Nevertheless, it appears that values higher than 59 µg g^{-1}/dry weight have been found in a few samples (FOWLER and OREGIONI 1976). It must be noticed on one hand, that mussels with a copper content higher than 59 µg g^{-1}/dry weight are, in all cases, from polluted area. On the other hand, for mussels from the same polluted area, the copper content can vary widely according to the season of sampling, from 10 to 95 µg g^{-1}/dry weight (for example, FOWLER and OREGIONI 1976). According to these observations, it is possible that the level of lethal accumulation (LA) vary according to the season, in relation to the physiological cycle of the mussels, perhaps gametogenesis. LA could also vary and

could be higher than 59 μg g^{-1}/dry weight, in relation to the nutrition cycle of the mussel. It is also possible that this level of lethal accumulation may vary with the faculty of adaptation of the mussel to polluted waters. Mussels growing in polluted areas would have a higher level of lethal accumulation, corresponding to a physiological and perhaps a genetical adaptation. Further studies to determine if the schema of toxicity of copper towards mussels may apply to other toxic elements and to other marine animals, merit investigations.

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