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Toxicity of Copper on Four Chilean Marine Mussels

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Diverse bivalve molluscs attached to rocks at the intertidal-high subtidal zone live in a highly stressing environment. These species could be differentially affected by diverse environmental factors, which at the same time, could negatively affect their adaptive capacity to tolerate other less common stressing agents like heavy metal exposure. However, it has not been established with certainty whether natural environmental conditions increase the pollutant sensitivity of marine organisms. It is known that mussels are capable to support extreme environmental fluctuations (e.g., severe salinity changes, desiccation or increases in pollutant levels). This capacity is in part due to the hermetic closing of their valves for different periods of time (Davenport 1979).

In Chile there is an increased interest in copper pollution especially due to copper mine tailings running from the Andes to the sea. Copper is an essential element to living organisms but extremely low concentrations produce toxic effects on several species (Viarengo *et al.* 1994). Chilean information about environmental levels of copper and their biological effects on marine organisms are limited. Two mussels species, *Aulacomya ater* and *Perumytilus purpuratus,* have been used as test organisms in pollution assessment at specific sites in the North of the country (Zúñiga 1998). The sensitivity of a third species, *Choromytilus chorus*, to cadmium and trybutyltin was determined by Roman *et al.* (1992, 1994).

Acute toxicity tests with mortality as endpoint have largely been used to assess heavy metal effects. However, copper concentrations producing acute responses are frequently higher than those detected in natural and even polluted areas (Hall *et al.* 1998). Lately, it has been given more attention to sublethal responses using lower and almost natural copper concentrations. The goal of this work was to establish the sensitivity to copper exposure using lethal and sublethal toxicity tests in four mussel species: *A. ater, C. chorus, P. purpuratus* and *Semimytilus algosus*. Simultaneous toxicity tests were conducted, using mortality and byssal attachment as the endpoints, respectively.

MATERIALS AND METHODS

Juveniles of *A. ater* and *S. algosus* were collected from the rocky shore intertidal-

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subtidal zone of Dichato beach (36° 33' 00'' S; 72° 56' 00'' W) and juveniles of *C. chorus* and adults of *P. purpuratus* from the rocky shore intertidal zone of Purema beach (36° 26' 00'' S; 72° 53' 00'' W). Individuals were transported to the Bioassay Laboratory, University of Concepción where they were acclimated in aquaria with oxygenated seawater at 12 °C, changed every 48 hr for one week. Seawater used during acclimation and as dilution water for the toxicity tests was collected from Coliumo Bay and filtrated at 5 µm. The copper stock solution was prepared the day before running the experiments by dissolving 96 mg of copper chloride in 100 mL diluted HCl-acidified seawater. From an intermediate solution at 8.0 mg/L the final exposure concentrations were made using a dilution factor of 0.5. The experimental groups were selected by weight from a stock of cleaned specimens. Mean wet weight was 3.41± 0.69 g for *C. chorus*, 1.60 ± 0.47 g for *S. algosus*, 2.20 ± 0.62 g for *P. purpuratus* and 3.29 ± 1.34 g for *A. ater*.

Lethality tests were made in parallel with byssal attachment tests. A random design with eight experimental treatments (i.e. copper concentrations) plus a control with four replicates each was used. Each experimental unit consisted of a 0.5 L hermetic plastic chamber with five individuals of each species. Toxicity tests were run in a cool room at a constant temperature of 12 °C. Experimental seawater solutions were changed every 48 hr. Throughout the experiment, dissolved oxygen concentration was higher than 5 mg L^{-1}, mean salinity was 28 ‰ and pH was between 7.6-8.0. Mortality was estimated from the proportion of individuals with open valves and without visible activity. Byssal attachment was estimated through the percent of individuals with byssal threads fixed to the inner surface of the container. Both parameters were measured each 24 hr for a total period of 144 hr. Data processing (Wilkinson 1990) included a factorial analysis of variance with mortality (ln % mortality + 1) and byssal attachment (ln % attachment + 1) as dependent variables and copper exposure concentration, exposure time and species as independent factors. Regression coefficients with their respective slope and intercept parameters were estimated through simple lineal regressions (mortality versus copper concentration and byssal attachment versus copper concentration in different exposure times) which were used to determine lethal and sublethal toxicity trends in relation to exposure time. Estimation of LC_{50} and EC_{50} values were obtained through Spearman-Karber and lineal regressions respectively.

RESULTS AND DISCUSSION

Mortality in *C. chorus* was relatively low, even after 144 hr under copper exposure at 8000 µg/L (Fig. 1). Mortality in *P. purpuratus*, *S. algosus* and *A. ater* were similar but higher than in *C. chorus* (Fig. 1). After 48 hr exposure, copper concentrations higher than 62.5 µg/L produced strong lethal effects on *P. purpuratus* and copper concentrations of 125 µg/L produced strong lethal effects on *S. algosus* and *A. ater*. It was observed that *A. ater*, *P. purpuratus* and *S. algosus* exposed to copper concentrations higher than 1000 µg/L showed higher survival percentages than in lower copper concentrations (Fig. 1). In those cases, surviving individuals kept their valves closed during all exposure time.

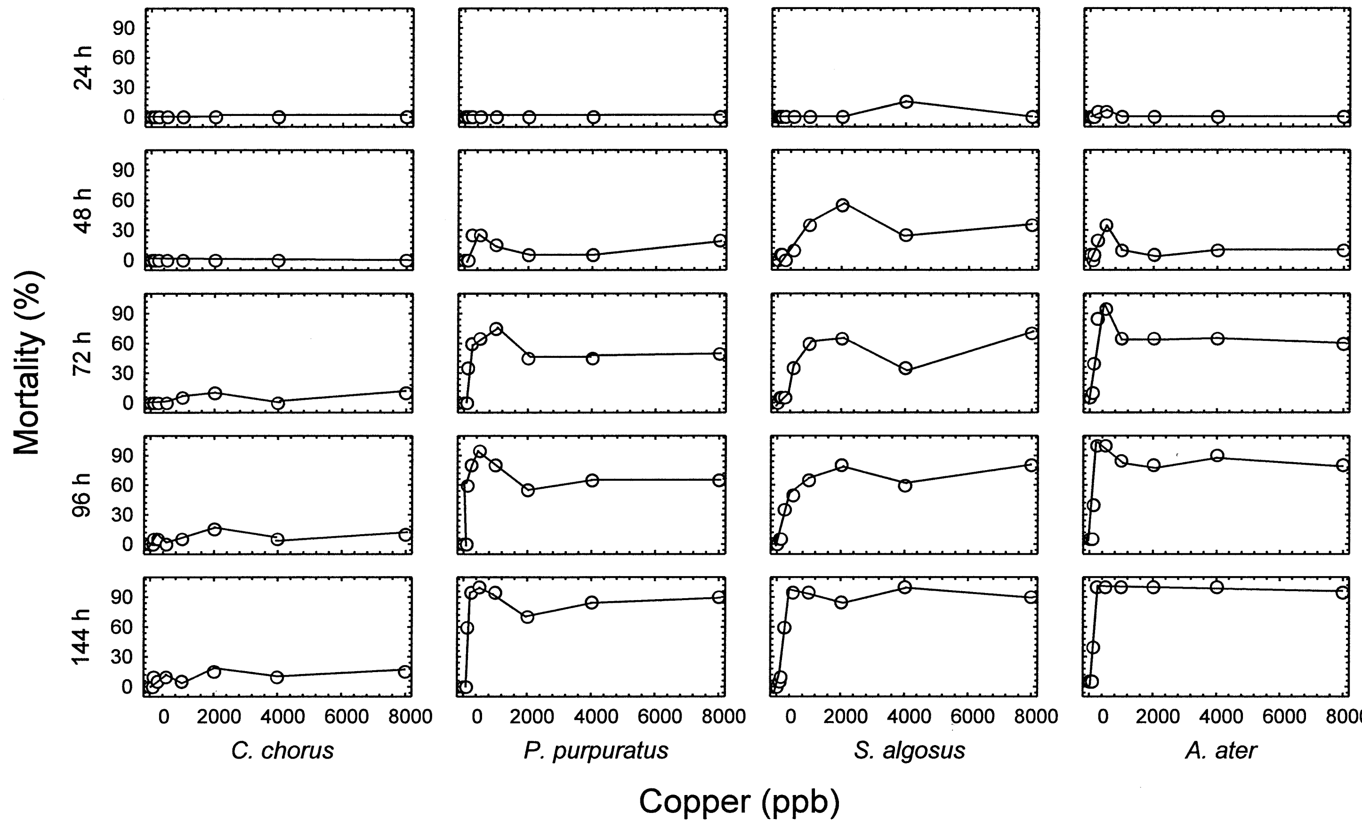

Figure 1. Mean percentage of mortality in Chilean marine mussels exposed to copper concentrations during different time periods under controlled laboratory conditions.

Table 1. Copper LC_{50} (µg/L), EC_{50} (µg/L) and lethal/sublethal ratio for Chilean marine mussels during different exposure times under controlled laboratory conditions.

Time (hr)	24	48	72	96	144
C. chorus					
LC_{50}	<8000	<8000	<8000	<8000	<8000
EC_{50} (**)	390 (331-458)	415 (363-474)	369 (304-447)	321 (278-371)	226 (199-256)
LC_{50}/EC_{50} *	-	-	>20	>20	>20
S. algosus					
LC_{50} (**)	-	-	1708 (619-1213)	479 (336-800)	247 (219-279)
EC_{50} (**)	291 (259-328)	298 (264-336)	268 (240-300)	234 (209-263)	220 (194-249)
LC_{50}/EC_{50} *	-	-	6.4	2.0	1.1
P.purpuratus					
LC_{50} (**)	-	-	228 (197-263)	122 (101-146)	126 (107-147)
EC_{50} (**)	181	76 (66-86)	99 (94-105)	93 (88-98)	89 (85-93)
LC_{50}/EC_{50} *	-	-	2.3	1.3	1.4
A. ater					
LC_{50} (**)	-	-	165 (131-207)	147 (133-162)	139 (129-149)
EC_{50} (**)	192 (179-206)	132 (110-158)	108(99-118)	97(92-102)	119(111-28)
LC_{50}/EC_{50} *	-	-	1.5	1.5	1.2

* Lethal/Sublethal ratio, (**) (95 % confidence interval)

From the comparative analysis of mortalities (LC_{50} values in Table 1) it is clear that *C. chorus* showed higher copper tolerance than the other mussel species. It was also found that *S. algosus* showed an intermediate tolerance to copper. Finally, *P. purpuratus* and *A. ater* showed the highest copper sensitivity.

Regression analysis between mortality and copper concentrations in different exposure times, generally showed low regression coefficients, explaining less than 50 % of mortality variance (Table 2). From the comparative analysis of mortalities it is clear that *C. chorus* showed higher copper tolerance than the other mussel species. It was also found that *S. algosus* showed an intermediate tolerance to copper. Finally, *P. purpuratus* and *A. ater* showed the highest copper sensitivity. It is necessary to point out that mortality, measured as valve aperture, was difficult to measure in *P. purpuratus*, situation that could induce a statistical error in the estimation of LC_{50}s. On the contrary, this acute response was more easily detected in *S. algosus* and *A. ater*, where dead individuals relaxed completely their adductor muscles, providing a constant and evident opening of the valves after death.

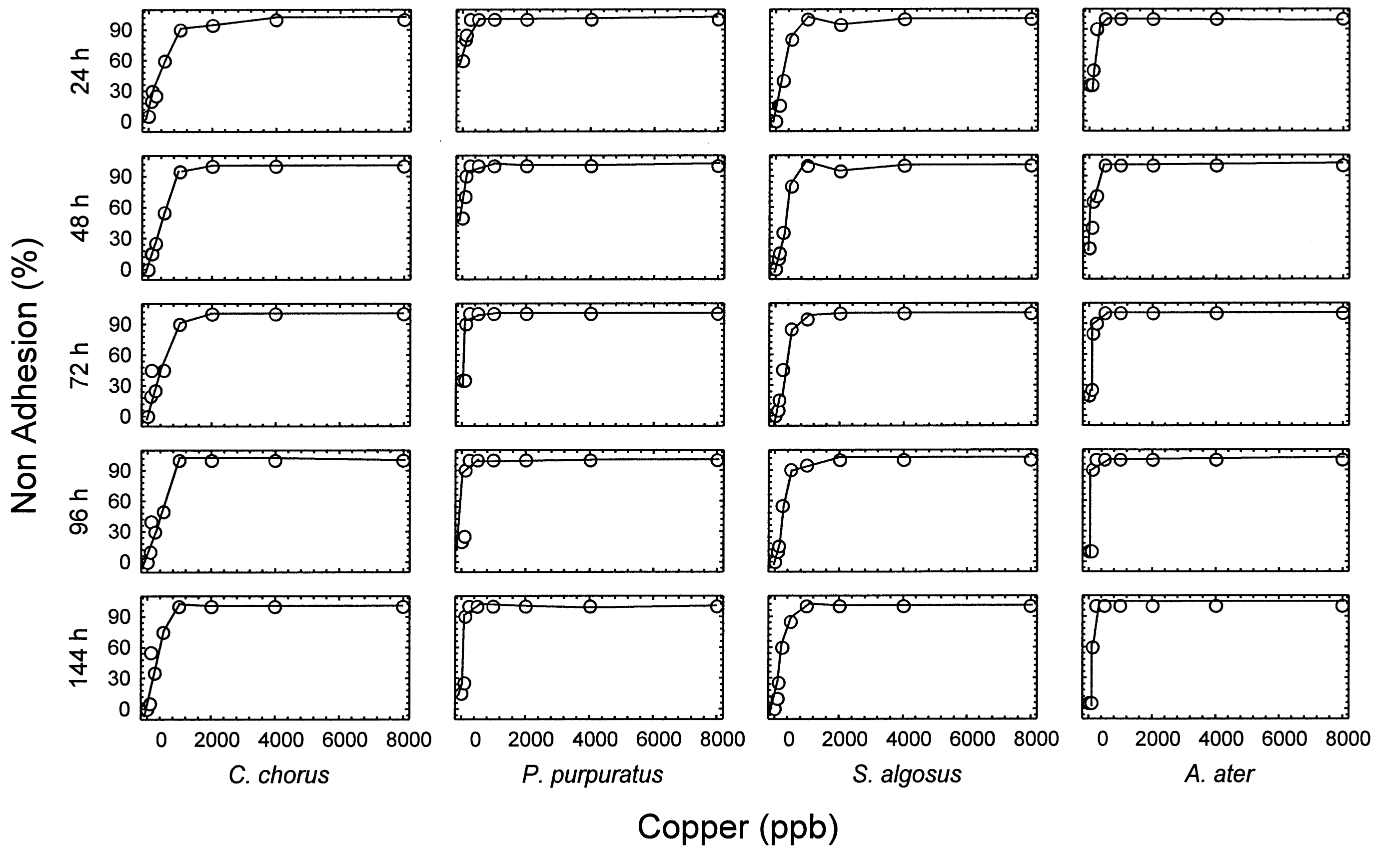

Figure 2. Mean percentage of non-adherence in Chilean marine mussels exposed to copper concentrations during different time periods under controlled laboratory conditions.

Table 2. Simple linear regression analysis for survival (ln survival+1) versus copper concentration in four marine mussels during different exposure times.

Time (hr)	24	48	72	96	144	24	48	72	96	144
Species	*C. chorus*					*S. algosus*				
a	1,79	1,79	1,81	1,81	1,8	1,81	1,9	2,03	2,08	2,14
b	0.00	0.00	-0.01	-0.01	-0.02	-0.01	-0.05	-0.10	-0.14	-0.23
r^2	1.00	1.00	0.12	0.12	0.12	0.08	0.33	0.42	0.51	0.59
p	0.990	0.990	0.040	0.037	0.038	0.085	<0.001	<0.001	<0.001	<0.001
Sig diff	no	no	no	no	no	no	yes	yes	yes	yes
Species	*P. purpuratus*					*A. ater*				
a	1,79	1,78	1,76	1,64	1,78	1,78	1,73	1,65	1,78	1,95
b	0.00	-0,02	-0,08	-0,11	-0,18	0.00	-0.01	-0.11	-0.17	-0.24
r^2	1.00	0.07	0.24	0.2	0.41	0.00	0.02	0.20	0.39	0.62
p	0.990	0.130	0.002	0.005	<0.001	0.980	0.460	0.006	<0.001	<0.001
Sig diff	no	no	no	no	yes	no	no	no	yes	yes

a: intercept; b: slope; r^2: regression coefficient; p: probability; sig diff: significant differences between treatments

Byssal adherence in the four species showed significant differences ($p<0.01$) in relation to controls, independent of exposure time (Fig. 2). Percentage of attachment in *C. chorus* controls was maximal at 48 hr, being constant until 144 hr. A notorious decrease in adherence was observed after 24 hr exposed to 125 µg/L, being null over 1000 µg/L. Adherence behaviour of *S. algosus* was also sensitive to copper. This species showed low attachment percentages at copper concentrations higher than 250 µg/L. Adherence behaviour of *P. purpuratus* and *A. ater* was similar between them (Fig. 2), but in opposition to the other species they both showed a lower percentage of attachment in controls, increasing gradually with exposure time. Maximal attachment values of 80 % and 90 %, respectively, were obtained only after 144 hr in control seawater. Copper effects on attachment capacity were stronger for these species, decreasing to zero at copper concentrations higher than 250 µg/L in *A. ater* and higher than 125 µg/L in *P. purpuratus*.

Regression analyses between byssal attachment and copper concentration gave high and significant regression coefficients (Table 3). In the case of *C. chorus* and *S. algosus* the regression model showed that attachment was constant in time, with values near to the experimental measurements, meantime intercepts for *A. ater* and *P. purpuratus* showed lower values but increasing with time, however, their maximum fall below those of the other two species. Comparing slopes it was found that *C. chorus*, *S. algosus* and *A. ater* showed a similar and constant behaviour through exposure time. On the contrary, *P. purpuratus* showed a gradual increase in the slope with respect to exposure time. Slopes indicate effect intensity, where the increases indicate a decrease in copper tolerance with time.

Table 3. Simple linear regression analysis for byssal attachment (ln byssal attachment +1) versus copper concentration in Chilean marine mussels during different exposure times.

Time (hr)	24	48	72	96	144	24	48	72	96	144
Species	*C. chorus*					*S.algosus*				
a	2.25	2.35	2.27	2.29	2.20	2.28	2.31	2.30	2.25	2.20
b	-0.24	-0.25	-0.24	-0.25	-0.25	-0.25	-0.26	-0.26	-0.26	-0.26
r^2	0.65	0.66	0.66	0.66	0.66	0.69	0.69	0.70	0.71	0.71
Species	*P. purpuratus*					*A. ater*				
a	0.99	1.13	1.40	1.58	1.63	1.68	1.81	1.73	1.71	1.90
b	0.13	0.15	0.18	0.21	0.21	0.20	0.22	0.22	0.22	0.24
r^2	0.62	0.63	0.59	0.62	0.65	0.64	0.73	0.71	0.64	0.69

a: intercept; b: slope; r^2: regression coefficient

Table 1 shows copper lethal to sublethal ratios for the four mussel species. *C. chorus* showed the higher lethal-sublethal ratio, due mainly to its low lethal sensitivity. *S. algosus* showed decreasing ratios with exposure time, due to a notorious increase in lethal sensitivity between 48-144 hr of copper exposure. *P. purpuratus* and *A. ater* showed relatively low ratios (near 1), indicating a narrow range of copper concentrations between lethal and sublethal toxicity.

Bivalve molluscs deploy many sublethal responses as consequence of toxicant exposure. Neuhoff and Theede (1984) determined that copper exposure produce deleterious effects that in general lead to a shorter life span. Among the responses, valve closure was described as a self-protection mechanism when mussels were exposed to adverse environmental conditions. This behaviour is immediate and produces a total individual isolation, preventing any contact with the external environment (Akberali and Trueman 1986; Brown and Newell 1972). Valve closure affects directly byssal thread production with a consequent decrease in substrate adherence capacity.

The use of valve aperture as mortality endpoint in mussels has never been established as a clear acute endpoint. Our results suggest that valve aperture has a low significance as mortality predictor. In the four species, all regression analysis of mortality versus copper concentration showed low regression coefficients, which support the low predictive value of valve closure in mortality evaluations. Until now, there is no information about other visible biological responses that could be used as mortality indicators in mussels. For this reason, we strongly recommend the use of sublethal responses, which allow a better prediction of copper biological effects and probably of other heavy metals as well.

From an ecological point of view, the four studied species show a differential distribution in the intertidal-subtidal rocky shore environment. *P. purpuratus* live in the upper fringe where is exposed to a more stressing environment (extreme exposure to waves and desiccation in low tide). Castilla (1981) reported that this species does not have an appropriate morphology to live in that environment, which would force it to be always associated with crevices. The fact that this species has a low capacity to produce byssal threads, as was shown in our control vessels, supports this observation. In synergistic conditions with pollutants (e.g., mining water discharges) it is possible that this species will be easily removed from the intertidal.

An application of this test could be in the evaluation of the potential failure or success in mussel transplanting experiments to restore intertidal ecosystems of areas affected by heavy metal pollution.

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