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EFFECT OF SELENIUM PRETREATMENT ON THE ACUTE TOXICITY OF MERCURY IN THE MUSSEL *BRACHIDONTES VARIABILIS*

UČINAK PREDTRETMANA SELENOM NA AKUTNU TOKSIČNOST ŽIVE KOD ŠKOLJAKA *BRANCHIDONTES VARIABILIS*

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The interaction of selenium with mercury has been studied in the mussel *Brachidontes variabilis*. The 48 hr lethal concentrations (LC_{50}) were determined for mercury in groups subjected to different selenium pretreatments (250, 500, and 1000 μg. l^{1-} Se) 24 hours before exposure to various concentrations of mercuric chloride. The LC_{50} value was also determined in the group exposed solely to mercury. Pretreatment with selenium was found to significantly decrease mercury toxicity. Thus the protective effect of selenium against the toxicity of mercury was observed in mussels. The degree of protection increased with increasing pretreatment concentrations. The LC_{50} values were calculated by using the Bliss method as well as a graphical method.

INTRODUCTION

Environmental mercury concentration is of worldwide concern because of the high toxicity of this element and its ubiquitous nature. This concern has been focused mostly on the accumulation and toxicity of this element in some seafoods, because its ability to biomagnify within the food chains.

The interaction of seienium with mercury also attracted the attention of numerous workers (Ganther *et al.*, 1972; Koeman *et al.*, 1973; Sumino *et al.*, 1977; Glickstein, 1978; Lucu and Škreblin, 1981) and it has been demonstrated that selenium, especially as selenite, is a protective agent against the toxic effect of both inorganic and organic mercury. Chen *et al.*, (1974) studied the possible mechanisms involved in the protection afforded by selenite against the toxicity of mercuric chloride and the effect of selenium pretreatment on the distribution of mercury in the tissue

of rats. The effect of selenium pretreatment on the acute toxicity of mercury has also been studied in phytoplankton (Gotsis, 1982), in invertebrates (Lucu and Škreblin, 1981) and in fish (Bower *et al.*, 1980). These authors observed the antagonistic effect of selenium on the acute toxicity of mercury.

Apart from these studies, only one paper was found in the literature (Micallef and Tyler, 1987) about the protective effect of selenium against the toxicity of mercury in mussels.

The aim of this study is to evaluate the effect of selenium pretreatment on the acute toxicity of mercury in the mussel *Brachidontes variabilis*.

MATERIALS AND METHODS

Mussels of 23 ± 0.5 mm were collected from their natural beds. Their shells were scraped to remove encrusting organisms and then washed with clean sea water. The organisms were placed in continuously aerated fiberglass aquaria, each containing 70 l sea water and were acclimatized to laboratory conditions for one month prior to the experiments. During acclimatization, the mussels were fed on mixed phytoplankton which are maintained in our laboratory. After the acclimatization period the mussels were distributed among four series of glass aquaria. Each series consisted of seven aerated aquaria with 2.5 l unfiltered test solution and 20 test organisms in each aquarium. The organisms of the first three series were pretreated for 24 hr with 250, 500 and 1000 $\mu g\ l^{-1}$ Se nominal concentration (as Na_2SeO_3), respectively These concentrations of selenium had no effect on mortality during this period of time. The organisms of the last series, not subjected to selenium pretreatment, served as a control. After a 24 hr period of pretreatment, the organisms of the first three series were transfered to selenium-free aquaria and exposed to different mercuric chloride nominal concentrations for 48 hours. The tests were repeated three times for each mercury concentration. Test solutions involving mercury and selenium were not changed and mussels were not fed during the exposure time.

Experiments were conducted in a constant-temperature room at the following conditions: pH: 8.05, salinity: 34.7‰ and temperature: 19 ± 1°C. The sea water was not analysed for the background levels of mercury and selenium. However, it is assumed that these levels were extremely low (at nanogram level) compared to the concentrations used during these tests.

The mussels were considered dead when they slowed down or failed to close their valves after mechanical stimulation (Eisler, 1973; Ahsanullah, 1976).

The 48 h LC_{50} values were calculated using the Bliss (1938) method and the statistical significance of the effect of selenium pretreatment on the acute toxicity of mercury was tested by Students's *t* test.

Table 1. 48 hour LC_{50} values calculated using the Bliss method for the mussel *Brachidontes variabilis* exposed to mercuric chloride alone

Concentrations ($\mu g.\ 1^{-1}$)	Nb. of dead	% of dead	x	Y	Y'	y	$\frac{z^2}{PQ}$	w	Products				
			Log. Conc. ($\mu g.\ 1^{-1}$)	Empirical Probit	Expected Probit	Working (corrected) Probit	Weighting Coeff.	Weights	wx	wy	wxy	wx^2	wy^2
250	3	15	2.39794	3.69	3.9600	3.96421	0.43863	8.7726	21.03617	34.77639	83.39168	50.44347	137.86072
500	9	45	2.69897	4.87	4.68828	4.87810	0.61609	12.3218	33.25617	60.10697	162.22692	89.75740	293.20782
2500	15	75	3.39794	5.67	5.79149	5.66833	0.50260	10.0520	34.15609	56.97800	193.60784	116.06035	322.96984
5000	18	90	3.69897	6.28	6.29325	6.28136	0.33589	6.7178	24.84894	42.19692	156.08515	51.91549	265.05405
7500	19	95	3.87506	6.64	6.59244	6.64323	0.23753	4.7506	18.40887	31.55932	122.29433	71.33548	209.65588
							Sums	42.6148	131.76624	225.61761	717.60591	419.51220	1228.7485

$\bar{x} = 3.09062$

$\bar{y} = 5.29434$

$b = 1.63005$

$X^2 = 1.148$

$\log LC_{50} = 2.91$

$V(\log LC_{50}) = 0.0098167$

RESULTS

Table 1 shows the parameters required for the calculation of the lethal concentration according to the method of Bliss (1938).

The probit analysis method of Bliss is based on the hipothesis that in the »dose-effect« relationship, effects which are proportional to the logarithms of concentrations, are normally distributed.

The value of X^2 was computed to assess the agreement between the expected linearity and the observed data (Table 1). As the calculated value of X^2 did not exceed the critical value at the 95% confidence level, it was concluded that the curve is linear. Thus, the LC_{50} and its respective variance could be calculated.

The Bliss analysis consists in establishing a regression line expressed as $Y = y + b\ (X—x)$. The 48 hr LC_{50} values of mercury for *Brachidontes variabilis*, pretreated and not pretreated with selenium were determined from this linear regression. In this equation, the value of Y, corresponding to 50% mortality, is 5 and X, which is log LC_{50}, was calculated as folows:

$$X = x + 1/b\ (Y — y).\ \text{For } Y = 5,\ X = x + 1/b\ (5 — y)$$

Another way in which the LC_{50} value can be roughly determined, is by plotting the probits (empirical probit) against the logarithms of the concentration used and by fitting a straight dosage-mortality line (Fig. 1). The LC_{50} value is estimated by dopping a perpendicular from the intersection of the point 5 (50% mortality) on the ordinate to the abscissa. The 48 hr LC_{50} values obtained by linear regression and by a graphic method are shown in Table 2.

Table 2. The 48 hr LC_{50} values obtained for *Brachidontes variabilis* by two different methods. Values are ± standard deviation.

Toxicant	Selenium used for pretreatment ($\mu g.\ l^{-1}$)	LC_{50} obtained by linear regression ($\mu g.\ l^{-1}$)	LC_{50} obtained by graphic method ($\mu g.\ l^{-1}$)
$HgCl_2$	0	812.9 ± 1.3	891.3
$HgCl_2$	250	1560.4 ± 1.4	1584.9
$HgCl_2$	500	10647.5 ± 1.1	10715.2
$HgCl_2$	1000	16336.6 ± 1.3	16218.1

The results calculated by the two methods are very close to one another. As can be seen from Table 2, the LC_{50} values of the selenium pretreated groups were significantly different ($P < 0.001$) from those obtained for the control (Hg alone) group. The difference was very significant especially between the control and the groups pretreated with 500 and 1000 $\mu g.\ l^{-1}$ selenium, respectively (Fig. 2).

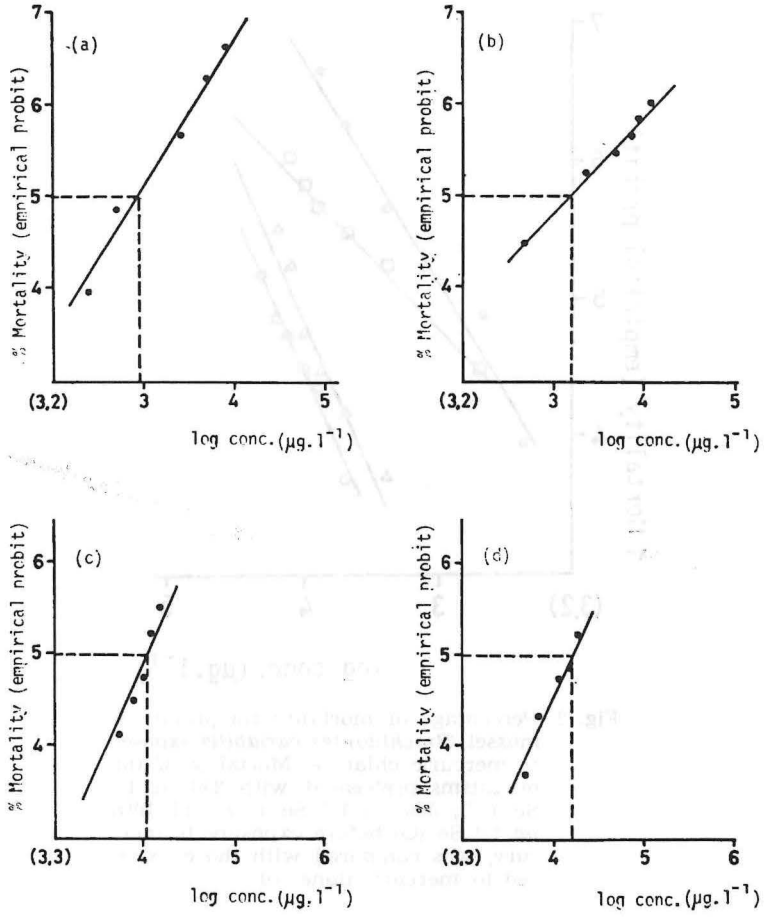

Fig. 1. Linear regression between the percentage of mortality (empirical probit) and the logarithms of the concentrations of (a) mercury alone, (b) mercury pretreated with 250 $\mu g.\ l^{-1}$ Se, (c) 500 $\mu g.\ l^{-1}$ Se and (d) 1000 $\mu g.\ l^{-1}$ Se.

DISCUSSION

The interaction of mercury and selenium has been investigated in the mussel *Brachidontes variabilis*. A bivalve mollusc was chosen for this study because it is one of the most abundant bivalves on the Eastern Mediterranean coast of Turkey, and secondly, bivalves are known to have a capacity for accumulating trace elements in significant amounts (Phillips, 1976a, b; Ünsal, 1978, 1984) and for transfering them within the food chain (Aubert *et al.*, 1974; Ünsal, 1982). The only information about a seleniummercury interaction is that given by Fowler and Benayoun (1976).

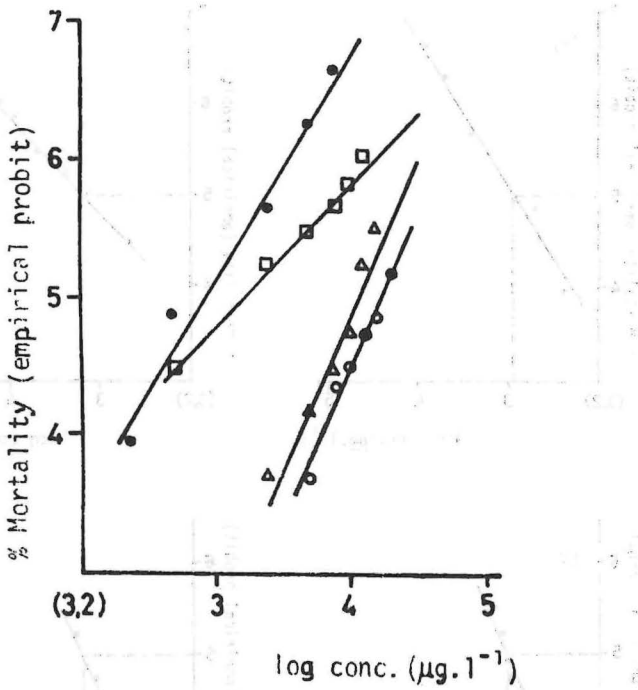

Fig. 2. Percentage of mortality (in probit) of mussel, *Brachidontes variabilis* exposed to mercuric chlaride. Mortality of the organisms pretreated with 250 $\mu g.\,l^{-1}$ Se (□), 500 $\mu g.\,l^{-1}$ Se (△) and 1000 $\mu g.\,l^{-1}$ Se (O) before exposure to mercury, was compared with those exposed to mercury alone (o).

The present study has shown that selenium pretreatment has a significant effect against the toxicity of inorganic mercury in *Brachidontes variabilis*. Similar findings were obtained by Lucu and Škreblin (1981) in a study of the shrimp, *Palaemon elegans*. They reported that a dose of 7.9 ug Se per gramme fresh weight, injected 12 hrs before exposure of the shrimp to the various mercuric chloride solutions produced a slight increase in the tolerance of this shrimp when compared with those treated with mercury alone. They also observed that shrimps pretreated for 4 days with a sublethal level of selenium and then exposed to mercury showed a substential delay in median lethal time when compared with those of a non-pretreated group.

The degree of significance of the selenium pretreatment differs depending on the taxonomic group of the organism. In a previous study (Ünsal, 1987), it was observed that the effect of selenium pretreatment was not as significant in fish larvae *(Mugil auratus)* as was observed in mussels. This may be due to various effect of selenium on the retention of mercury in dif-

ferent organisms or to the distribution of this metal among the tissues. Lucu and Škreblin (1981) showed that release of $HgCl_2$ from shrimps pretreated with selenium was significantly decreased compared with those of a non — pretreated group, whereas Sheline and Schmidt — Nielsen (1977) found that pretreatment with selenite caused no changes in overall body retention of mercury in killifish, but caused a redistribution of mercury among the various organs of this species.

In our experiment, the mortality rate in the selenium pretreated groups were markedly lower than in those which did not receive selenium prior to the mercuric chloride treatment: For example, treatment with mercury resulted in a 75% mortality in the group which did not receive selenium as a pretreatment and was subjected to 250 ug. l^{-1} mercury, whereas for the same concentration the mortality was 60, 10 and 0% in the groups pretreated with 250, 500 and 1000 ug. l^{-1} selenium, respectively.

Micallef and Tyler (1987) studied the interactions of mercury and selenium in *Mytilus edulis* and observed a decrease in mercury accumulation at moderate selenium pretreatment concentrations (0.32, 0.56 and 1.0 mg. l^{-1} which are very similar to our pretreatment concentrations.

Several explanations have been given for the mechanism by which selenium protects against mercury toxicity. Burk *et al.*, (1974), who studied the mercury-selenium interaction in rat plasma protein, suggested that selenium is attached to a sulphydryl group of the protein and that mercury is attached to selenium. This protein may play a role in preventing acute inorganic toxicity by preventing a large part of the mercury from reaching target tissues. Likewise, Micallef and Tyler (1987) suggested that mercury and selenium compete for the same binding site and if selenium only present, as in the case of pretreatment, the binding site is occupied by the selenium. In the presence of both ions, the binding site is occupied preferentially by mercury with the possible displacement of selenium. The observation of Chen *et al.*, (1974) showed that selenium counteracts mercury toxicity by altering the tissue concentration of this element and by diverting tissue mercury to presumably less critical components. Wrench and Campbell (1981) have determined the total selenium levels associated with protein in several marine organisms and have found in the clam, *Mya arenia*, which is also a mollusc species, 98.7% of the selenium was bound to protein in the hepatopancreas. The role of the liver in the protection of mercury toxicity has been previously studied by Bowers *et al.*, (1974). They pointed out that selenium pretreatment, prior to liver formation, in Japanese ricefish failed to protect them against mercuric chloride; but following liver formation, pretreatment was protective against the toxicity of this element. From the above results it is highly probable that in our experiment the selenium was accumulated in the hepatopancreas of the mussels, as in clams, and bound to proteins. These proteins, to which mercury was bound, played a substantial role in the mercury detoxification.

CONCLUSIONS

The results obtained in this study, showed that selenium pretreatment significantly decreases mercury toxicity in the mussel *Brachidontes variabilis*. The degree of protection increased with increased Se concentrations during pretreatment.

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UČINAK PREDTRETMANA SELENOM NA AKUTNU TOKSIČNOST ŽIVE KOD ŠKOLJAKA *BRANCHIDONTES VARIABILIS*

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KRATKI SADRŽAJ

Međudjelovanje selena i žive proučavano je na školjkama *Brachidontes variabilis* određivanjem 48-satnih letalnih koncentracija žive (LC_{50}) na grupama školjaka tretiranih 24 sata različitim koncentracijama selena (250, 500 i 1000 $\mu g.\ l^{-1}$ Se) pred izlaganje živinom kloridu. Također je LC_{50} određen u grupi školjaka izloženih samo živi. Ustanovljeno je da je predtretman selenom značajno smanjio toksičnost žive iz čega je očito njegovo zaštitno djelovanje kod ovih vrsta školjaka.

Stupanj navedene zaštite proporcionalan je koncentraciji selena. LC_{50} vrijednosti izračunate su Blissovom metodom i grafički.