Accumulation and loss of tin by the mussel

Tin
Accumulation
Loss
Mussels

Etain
Accumulation
Perte
Moules

M. Ünsal
Institute of Marine Sciences, Middle East Technical University, P.K. 28, Erdemli, Içel, Turkey.

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ABSTRACT

A bivalve mollusc of Indo-Pacific origin, *Brachidontes variabilis* (Krauss), was kept in different tin concentrations (100, 250 and 500 $\mu g\ l^{-1}$) for 30 days to study the accumulation of tin, and for a further 30 days in clean sea water to determine how much tin was eliminated. All of the groups showed a significant accumulation, higher in the 100 $\mu g\ l^{-1}$ group than in the 250 and 500 $\mu g\ l^{-1}$ groups. The rate of uptake decreased with increase in external tin concentration. The subsequent rate of loss of tin was constant and independent of the internal tin concentration in all contaminated mussels. After the 30 day elimination period, the mussels contained 20% of the tin taken up during the accumulation period.

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RÉSUMÉ

Accumulation et perte d'étain par la moule *Brachidontes variabilis*

Un mollusque bivalve, *Brachidontes variabilis* (Krauss, d'origine indo-pacifique, a été maintenu pendant 30 jours dans différentes concentrations d'étain (100, 250 et 500 $\mu g\ l^{-1}$), pour étudier l'accumulation de ce métal, puis pendant 30 jours supplémentaires dans un milieu non pollué (sans étain), afin de déterminer l'élimination de l'étain par cette espèce. Toutes les moules ont montré une accumulation importante, plus élevée dans le groupe de 100 $\mu g\ l^{-1}$ que dans les groupes de 250 et 500 $\mu g\ l^{-1}$. L'accumulation diminue lorsque la concentration en étain augmente dans le milieu. Après une période d'élimination de 30 jours, le taux de perte reste constant et indépendant de la teneur en étain dans toutes les moules. Après l'élimination, les organismes exposés contenaient 20% de l'étain qu'ils ont pris pendant la période d'accumulation.

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INTRODUCTION

Brachidontes variabilis (Krauss) is a species of Indo-Pacific origin (Barash, Danin, 1972) which can live in waters having a wide range of salinity (Stern, Achituv, 1978).

This bivalve mollusc was chosen for the present study firstly because it is one of the most abundant mollusc species on the north-east Mediterranean coast of Turkey, and secondly because bivalves are known to be effective concentrators of trace elements (Majori, Petronio, 1973; Phillips, 1976*a; b*). For this reason it has been used in several studies of accumulation and transfer of heavy metals (Ünsal, 1978*a; b;* 1982; Aubert *et al.*, 1974).

Tin was one of the first metals used by man. Inorganic tin is used as a protective coating for steel and in solders, bearing metals and other alloys (Hallas *et al.*, 1982).

Organotin compounds have been increasingly incorporated into such preparations as insecticides, herbicides and fungicides (Zuckerman *et al.*, 1978) during recent years, since they have a major advantage over organomercurial and organolead compounds as biocides: the final degradation product, *e.g.* SnO_2, is an inorganic tin compound of relatively low toxicity, whereas all mercury and lead compounds present significant toxicity problems to living organisms (Thayer, 1974).

Although the chemistry, methylation and distribution of tin in the aquatic environment have been investigated by a number of workers (Smith, Burton, 1972; Macchi, Pettine, 1980; Guard *et al.*, 1981; Hallas *et al.*, 1982; Tugrul, 1982), studies of its accumulation by aquatic organisms are rare (Nicholls *et al.*, 1959; Young *et al.*, 1979).

The present study describes the accumulation and loss of tin in the mussel *Brachidontes variabilis* (Krauss), as a function of time and concentration.

MATERIALS AND METHODS

Sampling and acclimatization of organisms

In January 1982, three hundred mussels of 23 ± 1 mm were collected from their natural beds in front of our laboratory. Shells were scraped with a knife to remove encrusting organisms and then washed with tap water. The organisms were placed in three continuously aerated glass aquaria, each containing 40 l of sea water, and were acclimatized to laboratory conditions for two months prior to initiation of the experiments. During acclimatization, the sea water was changed twice daily and the mussels were fed on algae which exist naturally in sea water.

Bioaccumulation of tin

Following the two-month acclimatization period, the mussels were distributed among four glass aquaria, each containing 8 l of sea water of 36.5 $^{0}/_{00}$ S obtained from the area of collection. Experiments started with 60 organisms in each aquarium. In the first aquarium, organisms were maintained in a medium containing 100 $\mu g\ l^{-1}$ Sn (as $SnCl_4$), while the second and third, they were subjected to 250 and 500 $\mu g\ l^{-1}$ Sn respectively. The fourth aquarium, without added tin, served as a control. Experiments were conducted at 22 ± 2*C for 30 days. During this period, the sea water was changed daily end, after each change, tin was added from a stock solution containing 1 000 mg l^{-1} $SnCl_4$ in distilled water. Six contaminated and six control mussels were sampled at time intervals of 3, 7, 14, 21 and 30 days and deep-frozen pending analyses.

Loss of tin

At the end of the 30-day bioaccumulation period, a further 30-day loss period was begun to investigate whether mussels could eliminate the accumulated tin. All aquaria were cleaned of precipitated and adsorbed material; they were scraped with a brush and washed in following order: with tap water and then with diluted (15%) HCl, again with tap water and finally with sea water. Contaminated mussels (30 mussels in each aquarium) were then placed in the tin-free aquaria. Experimental conditions were similar to those described in the previous paragraph, except that tin was not added to any aquarium. Six test organisms and six controls were removed at the same time intervals (3-7-14-21 and 30 day) as in the bioaccumulation experiment. The mussels were fed neither during the bioaccumulation nor the loss period.

Dry weights are based on the means of ten mussels taken on days 0, 30 and 60 from each aquarium. Determination of dry weight was carried out according to the procedure described by Bernhard (1976).

Analyses of samples

Frozen mussels were first thawed and removed from their shells. The soft tissues shells of each mussel were then weighed and digested in a concentrated nitric acid (Aristar) and perchloric acid (Aristar) mixture with a HNO_3:$HC10_4$ ratio of 4:1 (v/v). Digestion was carried out at 100*C. The samples were first digested in hot nitric acid for 12 h and then perchloric acid was added. After twelve hours, the liquid medium was allowed to evaporate. Following evaporation, dry samples were collected in 25 ml of distilled water and stored in a refrigerator until analysis. Samples were analysed by a Varian Techtron AA6 atomic absorption spectrophotometer, using the analytical procedure described by Braman and Topkins (1979) and Hodge *et al.* (1979), and improved by Tugrul (1982).

RESULTS

Table 1 shows the resulting metal concentrations in the mussels. The results are the mean of four individuals analysed separately.

After the 30-day accumulation period, the highest tin concentration was observed in organisms exposed to the lowest (100 $\mu g\ l^{-}$) external concentration. In this group, a slight increase was observed with time (Fig. 1 A). The accumulation pattern was similar in both the 250 and 500 $\mu g\ l^{-1}$ groups: accumulation reached its maximum value at day 3 of the experiment, after which a conspicuous equilibrium occurred in these two groups and was maintained until the end of the experiments (Fig. 1 C and E).

Table 1

Metal uptake and loss in soft tissues of Brachidontes variabilis *(ng/g wet wt) at the outset, after 30-day accumulation and after a further 30-day elimination period, and percentage of loss (×).*

Tin content in redium	% of dry wt	Tin concentration in soft tissues (ng/g wet wt) Day 0	Day 30	Day 60	% of loss
0 (control)	12.4±1.8	14	19	22	–
100 $\mu g\ l^{-1}$	–	15	641	132	79.4
250 $\mu g\ l^{-1}$	–	13	298	57	80.9
500 $\mu g\ l^{-1}$	–	14	382	69	81.9

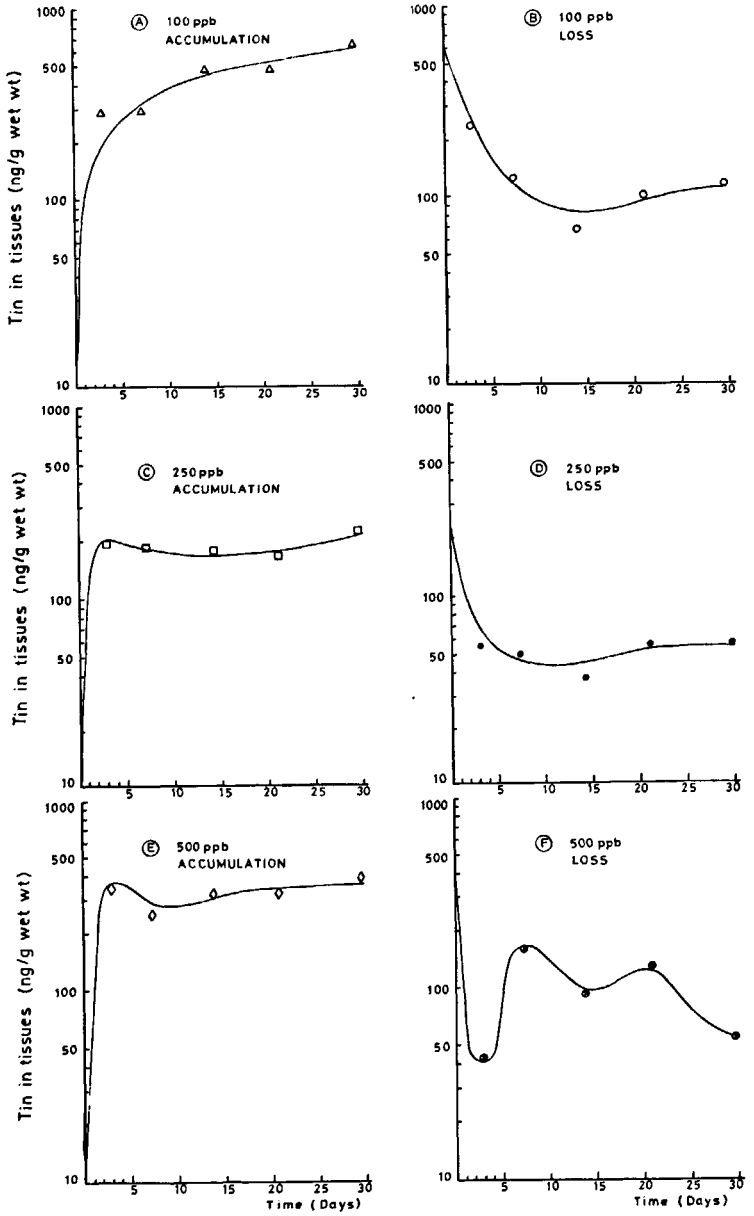
(*) The results are means of four mussels.

In control mussels the tin concentration was slightly increased by the end of a 60-day experimental period. The concentration factor was higher in the 100 $\mu g\ l^{-1}$ group than in either of the other two groups (Fig. 2).

The uptake of tin decreased with increasing concentration of $SnCl_4$ in the sea water. Though the tin concentration in the 500 $\mu g\ l^{-1}$ groups (Fig. 1 E) was higher than that in the 250 $\mu g\ l^{-1}$ group (Fig. 1 C), it may be seen from Figure 2 that the latter group established a greater accumulation factor relative to the sea water than the former. In all test groups, shells contained a significant amount of tin (Tab. 2).

By the end of the 30-day elimination period, a significant loss of tin had occurred in all contaminated groups. After 3 days of elimination a sharp decrease was observed in the tin concentration in all exposed mussels; this decrease continued for 14 days in the 100

Figure 1
Accumulation and loss of tin in soft tissues of Brachidontes variabilis *at different test concentrations and as a function of time.*



and 250 µg l^{-1} group, after which the concentration remained fairly constant until the end of the experiment. Thus, the pattern of the elimination curves of these two groups was more or less similar, while the elimination curve of the 500 µg group was quite different. The organisms of this group were probably unable to equilibrate their metal content throughout the elimination experiment. Although the 100 µg l^{-1} group always contained the highest tin content, after the 30-day loss period the percentage of loss was almost the same in all group (Tab. 1).

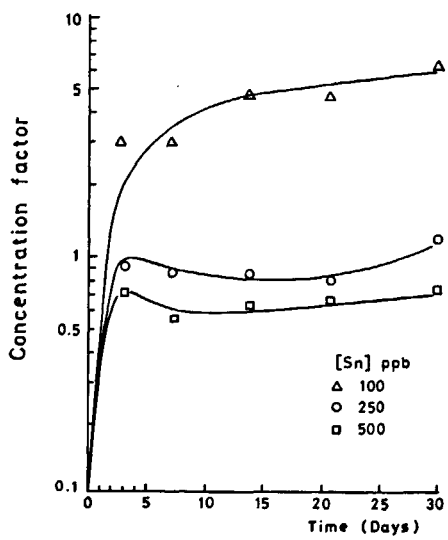


Figure 2
Concentration factor of tin in tissues of mussels during a 30-day accumulation period.

Table 2
Concentration factors in solft tissues and shell of B. variabilis. *Concentration factor is based on the ratio, tin concentration in contaminated organisms concentration in the medium* (*).

	Concentration factor		
Tissues	100 mg/l	250 mg/l	500 mg/l
Soft tissues	6.4	1.2	0.7
Shell	5.8	2.5	1.1

(*) The results are means of four mussels.

DISCUSSION

We have demonstrated the accumulation and release of tin by mussels in aquaria under controlled conditions. The results showed that in all test groups, except controls, tin was accumulated in significant amounts by mussels. As an example, the amount of tin in the 100 $\mu g\ l^{-1}$ group attained a maximum concentration of 640 $\mu g\ l^{-1}$ in the soft tissue after a 30-day exposure period (Fig. 1 A). Heavy metals are taken up by marine organisms from two main sources: the medium (water) and the food. The relative importance of these two sources has been investigated (Schulz-Baldes, 1974; Bouquegneau, Noël-Lambot, 1977; Ünsal, 1978 *b*; 1983). Furthermore, several mechanisms are involved in the accumulation of heavy metals from these sources (Romeril, 1971; Bryan, 1971) and a number of internal and external factors are known to affect the uptake of metals. Thus Delhaye and Cornet (1975), studying the effect of copper on *Mytilus edulis* during its reproductive period, observed that the spawning period was accompanied by an acceleration of copper uptake. Since, at this time, the animal's metabolism is very high and the renewal of water caused by ventilation of the gills is rapid, so is the copper accumulation. The results found by Phillips (1976 *a*) showed that seasonal weight variations are strongly related to the annual reproductive cycle of *Mytilus edulis* and that concentration of zinc and other metals reciprocated these weight variations. The present experiments were conducted from April to June, which is the spawning period for many marine animals. During dissection, we found most mussels, removed in April (start of experiment) and in May, to contain ripe eggs, thus proving that *Brachidontes variabilis* spawns during these months. We therefore suggest that this spawning was a contributory factor to the accumulation of tin in mussels.

Significant individual variations were observed in the rate of uptake of tin. These were probably due to variations in the stage of development of the gonads in different mussels. Simpson (1979) suggested that comparisons between mussels at different stages of the reproductive cycle would show different concentrations of metals. Our observations support this suggestion. The ability to accumulate tin decreased with increasing tin concentration in the medium, as was observed by Miramand *et al.* (1980). These authors observed that there was a tendency towards decreased vanadium uptake by mussels in sea water containing elevated vanadium concentrations. Similar results were observed by Bryan (1971) in lobster. Showing that the rate of zinc uptake in whole lobsters and in the isolated gills was not proportional to the sea water concentration of zinc, and that relatively more zinc was taken up from low concentrations and relatively less from high concentrations, Bryan presumed that this phenomenon probably occurred because adsorption, the rate of which is not proportional to the sea water concentration of zinc, is involved.

As the uptake of heavy metals in marine bivalve molluscs takes place by either active processes (filtration) or passive processes -adsorption on bysus and gills), it is probable that some part of the tin in soft tissues of *Brachidontes variabilis* resulted from passive accumulation, and consequantly that the uptake of tin is not proportional to external concentrations. Bryan and Hummerstone (1973) have similarly suggested from the results of radionuclide experiments that in *Nereis diversicolor*, the rate at which Zn is absorbed is proportional to the degree of adsorption at the surface of the body, rather than to the external concentration. On the other hand, Schulz-Baldes (1974) showed that the rate of uptake of Pb in the soft parts of *Mytilus edulis* was proportional to the lead concentration in the medium and that accumulation increased with increasing external concentrations. The same relationship was found in different organisms by other authors (Pringle *et al.*, 1968; Bryan, 1969; 1971; D'Silva, Kureishy, 1978).

In our experiment, accumulation was relatively important after 3 days in all exposed mussels. After this time, tin concentration reached a steady state in both the 250 and 500 $\mu g\ l^{-1}$ groups, but continued to increase in the 100 $\mu g\ l^{-1}$ group. Although a steady state occurred in the 250 $\mu g\ l-1$ and 500 $\mu g\ l^{-1}$ groups, the concentration of tin was 10 to 20 times higher than that in the controls. If we compare the concentration factors of the three contaminated groups, it can be readily seen from Figure 2 that the 100 $\mu g\ l^{-1}$ group accumulated 6 to 8 times more tin than the 250 and 500 $\mu g\ l^{-1}$ groups respectively. Cunningham and Tripp (1973), who studied the accumulation and depuration of mercury in the American oyster, *Crassostrea virginica*, found that when *C. virginica* was exposed to an experimental sea water concentration of 10 and 100 ppb mercury the biological enrichment factors were 2 800 and 1 400 respectively.

During our experiments no mortality was observed in any experimental group. However, the appearance of mussels kept in the 500 $\mu g\ l^{-1}$ tin concentration was not as healthy as that of the other groups and their body colour started to become pale towards the end of the experiments. This poor condition might also have had some bearing on the accumulation of tin in the group concerned.

Because some heavy metals are so readily accumulated by marine organisms, the main issue for most organisms is often not one of uptake but one of removal (Bryan, 1971). This author stated four different ways by which metals could be removed from the body of marine organisms: 1) loss of metal from the body surface or gills; 2) excretion into the gut; 3) excretion in the urine; and 4) storage in a particular tissue.

In the present study, an important decrease of tin in the 100 and 250 $\mu g\ l^{-1}$ groups was observed in the first 14 days of the elimination experiment (Fig. 1 B, D). The tin concentration decreased by 89% in the 100 $\mu g\ l^{-1}$ and 87% in the 250 $\mu g\ l^{-1}$ group in 14 days. After this time, the concentration remained fairly constant for a further 16 days. Our results are quite similar to those of Cunningham and Tripp (1973). They observed that during the depuration period, mercury concentrations in a 100 ppb group declined by 43% in 18 days, and remained at this concentration for a further 14 days; in the 10 ppb group, there was a significant decline for the first 18 days but no further

decline occurred for the remainder of a 160-day depuration period. Elimination of tin in the 500 $\mu g\ l^{-1}$ group was very irregular (Fig. 1 F); several fluctuations followed each other and an undulating curve was obtained. Probably the mussels of this group reaccumulated the tin they eliminated. Irrespective of the internal concentrations, the loss of tin was constant (about 80%), and thus independent of internal concentrations in all test groups (Tab. 1).

Schulz-Baldes (1974) pointed out that the rate of loss was proportional to the lead concentration in the tissues of *Mytilus edulis* and that the rate of loss increased with higher internal concentration. Although loss of tin was quite high in all contaminated mussels, the concentrations remaining after the 30-day elimination period were 3 to 6 times higher than those in the controls. Cunningham and Tripp (1973) showed that total elimination of all accumulated mercury from the tissues of *Crassostrea virginica* was not achieved, even after 6 months of depuration.

Different bivalve mollusc species have been proposed as indicator organisms by a number of authors (Majori, Petronio, 1973; Schulz-Baldes, 1974; Phillips, 1976 *a; b;* Bryan, Uysal, 1978), since they possess certain specific characteristics required of such organism. These characteristics have also been described by several investigators (Bittel, Lacurly, 1968; Butler *et al.*, 1971; Haug *et al.*, 1974). We suggest that *Brachidontes variabilis* presents most of these characteristics and, having observed that it accumulated tin in significant amounts from its environment even though the tin concentration was 10000 times greater than that found in unpolluted sea water, that it may be used as an indicator of tin in areas polluted by this metal.

To summarize, in the present experiment we observed the accumulation and loss of tin in the mussel *Brachidontes variabilis.* The degree of accumulation was found to be highest at the lowest concentration used, and to decrease with increase in the concentration of tin in the medium. The rate of loss was constant in all contaminated mussels, and may thus be considered to be independent of internal concentrations.

We consequently suggest that *Brachidontes variabilis* can be used as an indicator organism of pollution by tin.

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