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Physiological background for using freshwater mussels in monitoring copper and lead pollution

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Key words: *Anodonta cygnea* L., filtration activity, heavy metal accumulation, depuration, copper, lead

Abstract

In studying the effect of copper (10 ± 0.57 μg Cu l^{-1} and 100 ± 3.01 μg Cu l^{-1}) and lead (50 ± 1.12 μg Pb l^{-1} and 500 ± 12.5 μg Pb l^{-1}) on the filtration activity of *Anodonta cygnea* L. it was found that both heavy metals resulted in significant shortening of the active periods, but little change occurred in the length of the rest periods. The concentrations of copper and lead were measured in the gill, foot, mantle, adductor muscle and kidney for 840 hours of exposure to 10.9 ± 5 μg Cu l^{-1} and 57.0 ± 19 μg Pb l^{-1} as well as during subsequent depuration. Uptake was observed after 72 hours of exposure. The highest copper concentration (59.1 ± 16.2 μg Cu g^{-1}) was measured at 672 h in the mantle, and the highest lead value (143 ± 26.1 μg Pb^{-1}) was obtained in the kidney. Depuration of copper was fastest from the foot, and from the adductor muscle for lead. The gill had the longest half-depuration time (> 840 h for copper and 672 h for lead).

Introduction

It is well known that bivalve molluscs are able to concentrate heavy metals such as Hg, Cd, Zn, Cu, Pb and others in their tissues (Brooks & Rumsby, 1965; Pringle *et al.*, 1968), therefore they can be used in monitoring heavy-metal pollution of the environment. Among marine species *Mytilus* meets most of the requirements necessary for a monitoring system, and it is widely used as a biological indicator of metal pollution (Coleman *et al.*, 1986; Ritz *et al.*, 1982). *Mytilus* has been used in the 'mussel watch' program for indicating additional types of pollution (Farrington *et al.*, 1983; Goldberg *et al.*, 1978).

Anodonta and *Unio* species are freshwater bivalves which are very suitable for monitoring heavy-metal contamination. However, not much information is available concerning their physiological responses to pollutants, and the kinetics of uptake and depuration of heavy metals. Nevertheless, there have been papers published presenting data concerning the effects of Hg and Cd on *Anodonta cygnea* L. and kinetics of their uptake (Salánki & V.-Balogh, 1985; V.-Balogh & Salánki, 1984).

In the present study the freshwater bivalve *Anodonta cygnea* L. has been subjected to Cu and Pb treatments to measure the effects of these metals upon filtration activity and to determine the characteristics of the metal uptake and depuration.

Materials and methods

In the investigations specimens of adult (11.8 ± 1.3 cm) *Anodonta cygnea* L. collected from fish ponds located at the eastern part of Hungary were used. Before the experiments the animals were kept for four weeks in an aquarium supplied with Balaton-water rich in phytoplankton. No additional food was provided.

Two separate series of experiments were carried out (both with Cu and Pb): (a) we investigated the effect of the metals on valve activity of the animals; (b) we studied the uptake and release of the metals in different tissues. In the experiments Cu^{2+} was added as $CuCl_2 \cdot 2H_2O$ and Pb^{2+} as $PbCl_2$ via water.

Series a. The animals were placed separately in plexiglass tanks (3 l each) with running lake-water. The position and movement of the shells were recorded on a mussel actograph (Salánki & Balla, 1964). Filtering takes place when the shells are open during which period fast pumping movements occur (active period), while a persistent (longer than 60 min) closed position marks a rest period (Salánki & Lukacsovics, 1967). The shell movements were continuously recorded for 168 hours before the experiments began, during exposure to heavy metals (240 hours) and during the purging period (168 hours). Metal concentrations during exposure were 10 ± 0.57 and 100 ± 3.01 $\mu g \cdot l^{-1}$ for copper, and 50 ± 1.12 and 500 ± 12.5 $\mu g \cdot l^{-1}$ for lead. The duration of the active and that of the rest periods were measured and an average was calculated from ten parallel experiments. The duration of consecutive active and rest periods was compared before, during and after exposure.

Series b. One hundred animals were placed in a glass aquarium containing 100 l water equipped with a perfusion system which assured a total change of the water within 8 h. Water temperature varied between 12 and 20 °C in accordance with changes in the lake's temperature. During exposure, the copper and lead concentrations were 10.9 ± 5 $\mu g \cdot l^{-1}$ and 57.0 ± 19 $\mu g \cdot l^{-1}$, respectively.

Exposure to metals took place for 840 h during which the concentrations of Cu and Pb were measured after 1, 4, 9, 24, 72, 168, 336, 504, 672, and 840 h. During the depuration period which followed, organ concentrations were measured after 48, 168, 336, 504, 672, 840 (only copper) h.

Both copper and lead concentrations were analyzed in the gills, foot (including viscera), mantle, adductor muscle and kidney. Samples were prepared for analysis by wet digestion according to the method of Krishnamurty *et al.* (1976) as described earlier (Salánki *et al.*, 1982).

Copper and lead concentrations were measured using a Zeiss AAS1 type AA spectrophotometer, by direct flame atomization in an air-acetylene flame.

The concentrations given in the figures are mean values (± standard error of mean) of three replicate samples.

Half-depuration time ($T_{1/2}$) was defined as the time necessary to reach the half-depuration concentration (HDC) using the formula $HDC = (C_e - C_c) \cdot 2^{-1}$, where C_e = the metal concentration in the organs at the end of exposure, C_c = the metal concentration in the organs of the control animals.

Results

Change in filtering activity.

During exposure to copper and lead the activity of the mussels changed depending on the metal concentration. Before Cu-treatment the length of the active periods varied between 10-20 h, but occasionally there were also shorter periods (5-6 h). After the animals were exposed to 10 ± 0.57 μg Cu l^{-1}, the duration of the active period gradually decreased from 20 h to 8 h. Exposure of the mussels to 100 ± 3.01 μg Cu l caused a sudden decrease in the duration of the active period to about 1 h, and this remained so during subsequent exposure. When copper exposure ceased, duration of the active periods suddenly increased to 30-40 h, then decreased again to control values (Fig. 1).

The duration of the rest period before copper treatment, was 10-18 h long and this did not change markedly with either concentration. Nevertheless, with 10 ± 0.57 μg Cu l^{-1} there was a cyclic shortening and lengthening of the rest period. When the copper treatment was terminated, an increase in the duration of the rest period to 30 h was observed, followed by a decrease to the level of the control values (Fig. 1).

Before the application of 50 ± 1.12 μg Pb l^{-1} the length of the active periods varied between 30-60 h. During exposure the duration of the active period decreased significantly to about 7 h. During purging there was a slight increase in the active period, but this did not reach the level of the control values even after one week.

During the same experiment, the rest period varied in the control between 6-18 h (average 10 h). It was stable at about 6 h at the beginning of the exposure, before increasing to produce the average control value. A shortening of the rest periods was observable during purging (Fig. 2).

Before exposure to 500 ± 12.5 μg Pb l^{-1}, the length of the active periods varied in the control between 6-23 h, but during exposure it decreased gradually to 4-6 h. The duration of the rest periods was also shortened at this higher lead-concentration. When the lead treatment was stopped, there was an immediate increase in the duration of both the active and the rest periods. The length of these periods then returned to low levels. Control values did not return to previous levels even after one week of purging (Fig. 2).

Uptake and depuration of copper

Exposing the mussels to 10.9 ± 5 μg Cu l^{-1}, the concentration of this metal increased in almost all organs within 1 h, but dropped below the control value after 4 h. A significant uptake could be measured only after 72 h, but different accumulation patterns were found in different organs.

The concentration of copper in the gill increased linearly up to 672 h, reaching a value of 12.8 ± 2.33 μg Cu g^{-1} before stabilizing. There was strong binding of copper by the gill since no release of copper was evident after the bivalves were placed in metal-free water. Moreover, half-depuration was not achieved after 840 h of purging (Fig. 3A).

Similarly, copper concentrations increased linearly up to 672 h in the mantle, reaching a value of 59.1 ± 16.2 μg Cu g^{-1}. A linear depuration of copper was also observed, with $T_{1/2}$ being reached in 420 h (Fig. 3B). In the foot, the concentration of copper increased linearly for the entire period of exposure (840 h), then a linear depuration pattern was observed. The $T_{1/2}$ for the foot was 195 h (Fig. 3C).

Accumulation in the adductor muscle proceeded slowly. The elevation of Cu concentrations was measured after 336 h, then saturation occurred between 15-20 μg Cu g^{-1}. There was slow depuration from this muscle, and half-depuration was not achieved during the 840 h purging period (Fig. 3D).

Only a slight concentration increase (from 15 to 27 μg Cu g^{-1}) was observed in the kidney which was reduced by half within 48 h (Fig. 3E).

Uptake and depuration of lead

No significant uptake was measured during the first 72 h of exposure to 57.0 ± 19 μg Pb l^{-1}. Considering individual organs, two accumulation patterns could be distinguished: a linear pattern for the kidney and a logarithmical type for all the other organs.

The concentration of lead increased during the 672 h exposure period to 62.7 ± 6.67 μg g^{-1} in the gill; and this organ did not release Pb during 672 h of depuration (Fig. 4A). The foot (Fig. 4B), the mantle (Fig. 4C) and the adductor muscle (Fig. 4D) showed the same type of saturation uptake for lead, and reached 24.9 ± 3.0 μg Pb g^{-1}, 49.3 ± 19.4 μg Pb g^{-1}, and 38.3 ± 2.64 μg Pb g^{-1}, respectively.

The depuration of lead was fastest from the adductor muscle ($T_{1/2}$ = 100 h), followed by the mantle ($T_{1/2}$ = 145 h), and the foot ($T_{1/2}$ = 672 h). However, control levels were not achieved by any of them during the experimental period.

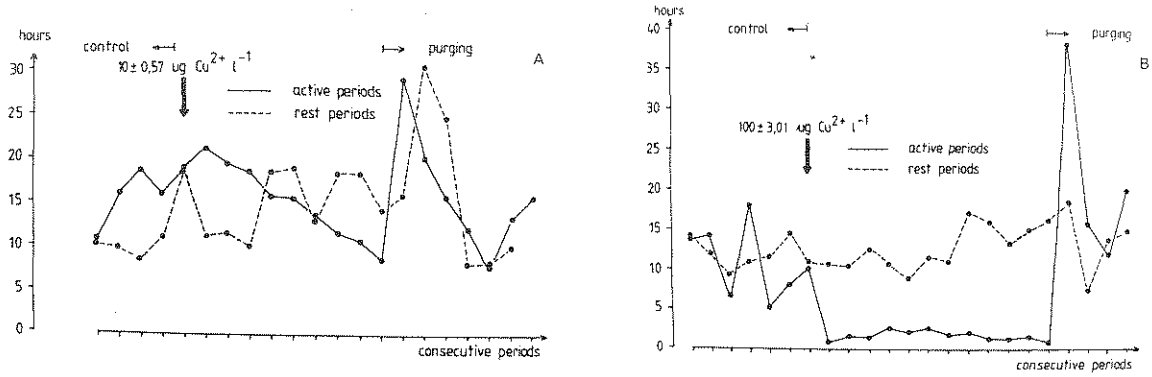

Fig. 1. Change of the duration of consecutive active and rest periods of the mussel *Anodonta cygnea* L. in control, under 240 h exposure to 10 ± 0.57 µg Cu l^{-1} (A), 100 ± 3.01 µg Cu l^{-1} (B) and during purging

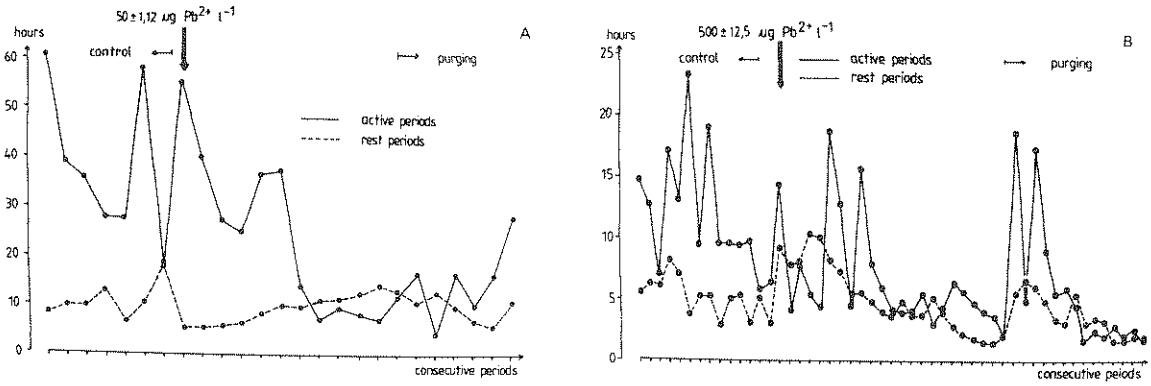

Fig. 2. Change of the duration of consecutive active and rest periods of the mussel *Anodonta cygnea* L. in control, under 240 h exposure to 50 ± 1.12 µg Pb l^{-1} (A), [illegible] and during purging

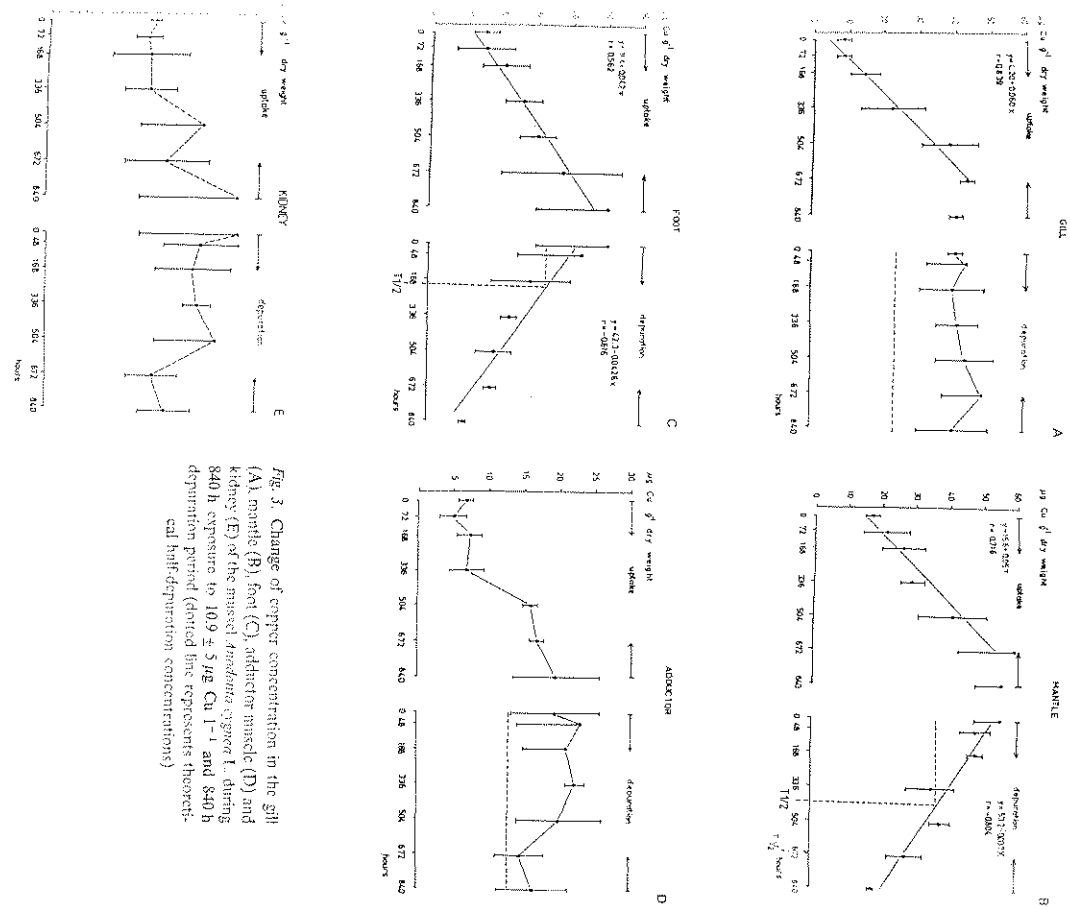

Fig. 3. Change of copper concentration in the gill (A), mantle (B), foot (C), adductor muscle (D) and kidney (E) of the mussel *Anodonta cygnea* L. during 840 h exposure to 10.9 ± 5 µg Cu l^{-1} and 840 h depuration period (dotted line represents theoretical half-depuration concentrations)

Table 1. Concentration of copper in the organs of *Anodonta cygnea* L. in control and after 840 h exposure (μg Cu g^{-1} dry weight, mean ± SEM)

Organ	Control concentration (C_o)	Concentration after exposing to copper (10.9 ± 5 μg l^{-1}) (C_e)	$C_e \cdot C_c^{-1}$	P
Gill	8.42 ± 2.32	40.0 ± 1.70	4.75	< 0.001
Foot	14.9 ± 3.45	50.1 ± 19.2	3.36	< 0.001
Adductor muscle	6.75 ± 1.02	19.3 ± 6.65	2.86	< 0.001
Mantle	16.9 ± 1.73	55.4 ± 7.45	3.28	< 0.001
Kidney	14.8 ± 0.784	26.9 ± 13.9	1.82	> 0.05

The kidney showed linear uptake and depuration characteristics. The highest lead concentration, 143 ± 26.1 μg g^{-1}, was measured after 672 h exposure. Binding of lead was weak, as shown after 672 h depuration, when the concentration reached the initial, 29.9 ± 8.06 μg Pb g^{-1} value ($T_{1/2}$ = 295 h) (Fig. 4E).

Rate of bioconcentration

At the end of the exposure period, metal concentrations in the mussels were significantly higher in all organs as compared to their pre-exposure concentrations. The rate of copper bioconcentration was organ specific (Table 1), and ranged from 1.82 (kidney) to 4.75 (gill). The value of 1.82 was not significant. The bioconcentration rate of lead (Table 2) ranged between 2.35 (gill) and 4.18 (kidney).

Discussion

Measurement of metal concentrations in living organisms is an acceptable approach for detecting the level of pollution in the environment. Although this simple method is accurate for practical purposes, nevertheless, it does not offer a possibility for the more generalized evaluation of the data obtained. The combined methods of analyzing both the behavioural effects of pollutants and checking their uptake and release kinetics may increase the usefulness of biomonitoring organisms.

Mussels are remarkable because they can change their filtration rate under the effect of heavy metals and other toxicants (Abel, 1976; Salánki & Varanka, 1976). Since the activity of the animals corresponding to filtration activity can be recorded both in laboratory conditions as well as in the natural environment (Véró &

Table 2. Concentration of lead in the organs of *Anodonta cygnea* L. in control and after 840 h exposure (μg Pb g^{-1} dry weight, mean ± SEM)

Organ	Control concentration (C_o)	Concentration after exposing to lead (57 ± 19 μg l^{-1}) (C_e)	$C_e \cdot C_c^{-1}$	P
Gill	26.50 ± 4.00	62.2 ± 9.14	2.35	< 0.001
Foot	8.68 ± 4.46	24.9 ± 3.00	2.87	< 0.001
Adductor muscle	10.8 ± 9.28	38.3 ± 2.64	3.55	< 0.001
Mantle	15.7 ± 1.42	49.3 ± 19.4	3.14	< 0.001
Kidney	29.9 ± 8.06	125 ± 14.5	4.18	< 0.001

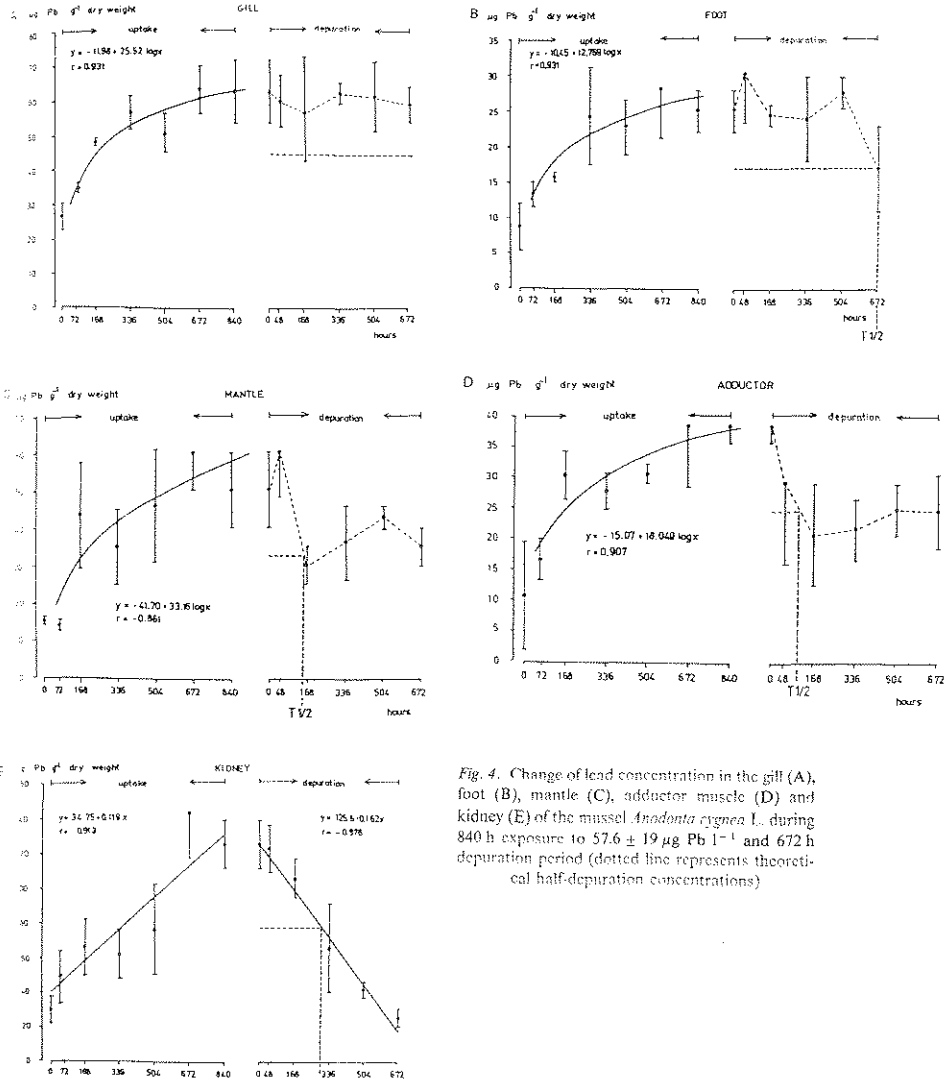

Fig. 4. Change of lead concentration in the gill (A), foot (B), mantle (C), adductor muscle (D) and kidney (E) of the mussel *Anodonta cygnea* L. during 840 h exposure to 57.6 ± 19 μg Pb l^{-1} and 672 h depuration period (dotted line represents theoretical half-depuration concentrations)

Salánki, 1969), monitoring of the activity is a suitable method of predicting the biological effect of many water-soluble chemicals and hence of pollutants.

Our results show that under exposure to copper and lead the activity of the mussels changed depending on the metal concentrations. The filtering activity was reduced in both cases by shortening the duration of active periods. The rate of decrease was two and greater than ten times for copper at 10 and 100 μg Cu l^{-1}, respectively, while lead was six and ten times at 50 and 500 μg Pb l^{-1}, respectively. The decrease in duration of the active periods was gradual at both lead concentrations and at the lower copper concentration; but immediate at 100 $\mu g \cdot l^{-1}$ copper concentration.

Davenport and Manley (1978) investigated the valve closure mechanisms of *Mytilus edulis* on exposure to copper sulphate. Our findings are in good accordance with their results. They also suggested that mussels would be reasonably efficient indicators of copper pollution up to concentrations of 160-200 $\mu g \cdot l^{-1}$.

In our earlier investigations we showed that other heavy metals such as Hg and Cd not only reduced the active periods in *A. cygnea*, but also caused the elongation of the rest periods (V.-Balogh & Salánki, 1984). In the present experiments we found that under the effects of copper and lower concentration of lead only the active periods became shorter, the rest periods did not change. This suggests that different mechanisms are involved in the regulation of activity and rest.

On the basis of our results, it is also suggested that there are differences between the effects of copper and of lead on the mechanisms which regulate activity in *A. cygnea*. In contrast to lead exposure, the duration of the active periods reached the control values after stoppage of the copper treatment, suggesting that mussels are more tolerant to copper fluctuations than to lead fluctuations.

Although filtration activity of the mussels was re-established within 168 hours after copper exposure, the concentrations of copper did not decrease to the control level. Following lead treatment, neither the activity nor the lead concentration of organs was restored even after 168 hours of purging. This residual metal content can have a marked effect on an additional bioaccumulation.

In spite of changes in the filtration activity there was no significant copper and lead uptake by the freshwater mussel in the first 72 h of exposure. This suggests that on the basis of bioaccumulation one cannot detect copper or lead pollution in less than three days using mussels.

After 72 h exposure, the concentration of Cu increased significantly in all organs, except the kidney. The highest concentration rate occurred in the gill. Tallandini *et al.* (1986) found similar results while studying the uptake and distribution of Cu in various tissues of the freshwater bivalves, *A. cygnea* and *Unio elongatulus*. They reported that although copper uptake was not very marked, the highest uptake was exhibited by the gill in both organisms.

Lead uptake also remained in the range of a 2-4 fold increase and the highest lead bioconcentration rate occurred in the kidney, and the lowest in the gill. The accumulation pattern in the kidney differed from that in other organs, since there was a linear uptake. Schulz-Baldes (1974) described a similar linear uptake pattern for lead in *M. edulis*. In his experiments, however, the lead concentration increased in a linear way in all organs.

The degree of time-integration capacity of an indicator organism is an important factor in its usefulness for monitoring heavy metal contaminants (Phillips, 1980). Beside the concentration ability, the storage ability is also important. The rate of elimination and the storage ability may be characterized by half-depuration time. If $T_{1/2}$ is long, the animal or its tissues can reflect the average concentration of metals in the environment for a longer time.

The half-depuration times for different organs of *A. cygnea* treated with copper and lead show that the gill has the most prolonged retention of both metals. In our earlier investigations we found slow depuration in the gill for mercury ($T_{1/2} > 840$ h), and for cadmium ($T_{1/2}$ between 504 and 672 h), (Salánki & V.-Balogh, 1985). Consequently, the gill represents the best biomonitoring organ in the mussel, while the time lapse between two samplings should not be less than 672 h for a reliable, permanent indication of copper and lead pollution.

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