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EFFECT OF COPPER AND LEAD COMPOUNDS ON THE ACTIVITY OF THE FRESH-WATER MUSSEL

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Waste materials of industrial or agricultural origin polluting natural waters very often contain various metallic compounds, damaging living organisms by affecting various physiological processes. In most cases the harmful effect becomes obvious only when mass death of some organisms occurs, while chronic disturbances remain masked for a long time. Biological tests are restricted mainly to the determination of the lethal concentrations.

It is well known from earlier investigations that marine and fresh-water mussels are very sensitive to changes in temperature (LOOSANOFF, 1958), to some organic compounds (KORRINGA, 1952), to SH-blocking agents like Hg and Cd (SALÁNKI, 1961) and to the lack of oxygen (SALÁNKI, 1965; 1966). The harmful effect manifests itself in the decrease of life functions, especially of the filtering activity and finally in the death of the animal. Any change in filtering activity can be monitored by registering the valve movement or measuring the cleaning of the water. There are various methods for such purpose (COLE and HEPPER, 1953; SALÁNKI and BALLA, 1964; HOGGARTH and TRUEMAN, 1967; VÉRÓ and SALÁNKI, 1969). The change in the activity to the damaging effect of various external influences appears much earlier than the death of the animals, thus by investigating this process the effect of sublethal doses can be discovered.

In the present experiments the effect of copper sulphate, lead chloride and lead nitrate was investigated on the rhythmic and periodic activity of the fresh-water mussel. Copper and lead compounds contaminate surface waters as plant protecting agents or as industrial waste materials and can cause damage in living organisms. The aim of the present work was to investigate the effect of different concentrations of the above substances, whether they can evoke a noticeable change in the mussel's life phenomena. We wanted also to clear up whether these typical filtering organisms may be suitable for signalling the effect of sublethal concentrations of copper and lead compounds.

Material and methods

The experiments were carried out on 10–14 cm long *Anodonta cygnea* specimens, during summer and autumn. The animals were collected from fish

ponds, but before treatment they were kept in Balaton water either in natural conditions or in aquaria with running water.

For the investigation the mussels were placed in separate vessels containing 3 litre of Balaton water. The temperature of the water was not controlled, thus varied between 15 and 24 °C according to laboratory conditions in summer and autumn. The water was changed in the vessels every day in the morning hours. Activity of the animals was registered continuously by a mussel actograph (SALÁNKI and BALLA, 1964) for several weeks. From the record the duration of the open and closed position of the valves can be measured with great accuracy; the former corresponds to the active, filtering period while the latter to the rest period (SALÁNKI and LUKACSOVICS, 1967). The alteration of the active and rest periods lasting both for several hours and being the result of the functioning of the adductor muscles is called as periodic activity. The duration of the active and rest periods can show significant variability even in control conditions, therefore at counting the mean values we took in consideration at least one week duration. Further on, we kept one animal without treatment in each series of experiments to monitor any change in the activity caused by uncontrolled circumstances. If more than 10 per cent difference occurred in the activity of this control animal, the results of the given series were not evaluated.

The chemicals used in the experiments were dissolved in Balaton water and diluted further in the vessels in which the animals were placed. Before adding the substances the activity of mussels was recorded for one–two weeks. The daily change of water was performed after the addition of the chemicals as before, but also the tested substance was solved in it. The concentration value was each day the same, regardless of the possible accumulation process.

Evaluation of the data was carried out on the basis of actograms. In each animal the mean duration of the active periods was determined for the control period and for the period following the treatment. The mean values were compared taking the control value as 100 per cent. This way the fall of activity below 100 per cent means the shortening of the active periods and *vice versa*. The effect of various concentrations was evaluated from 5 – 8 experiments carried out on different mussels.

The changes of the frequency of the rhythmic, fast contractions observable during active periods were not analyzed, since this frequency depends also in the length of the active period (SALÁNKI et al., 1970), nor were the mean of the rest times and its changes investigated.

The death of the animals is accompanied by the loss of the contractility of the adductor muscles resulting in the complete opening of the valves. This was the criterion we used in our experiments for determining mortality.

Results

The effect of $CuSO_4$ was investigated in $10^{-3} - 10^{-9}$ g/l concentrations, 10^{-3} g/l proved to be lethal. In this concentration the death of animals occurred usually within 10 hours. Following the addition of $CuSO_4$ the immediate closure of the shells was observed. In some cases this was followed by their opening and the animal performed rhythmic activity for a few hours *(Fig. Ia)*, while in other cases the relaxation of the adductor muscles after long closure

was the sign of the death of the animal *(Fig. 1b)*. In the former case the amplitude of the rhythmic contractions became less and less before the death of the animal, while in the latter case after opening no rhythmic valve movement was observed.

With decreasing concentrations of the $CuSO_4$ the mortality of the mussels decreased, nevertheless the death of animals occurred sometimes after long

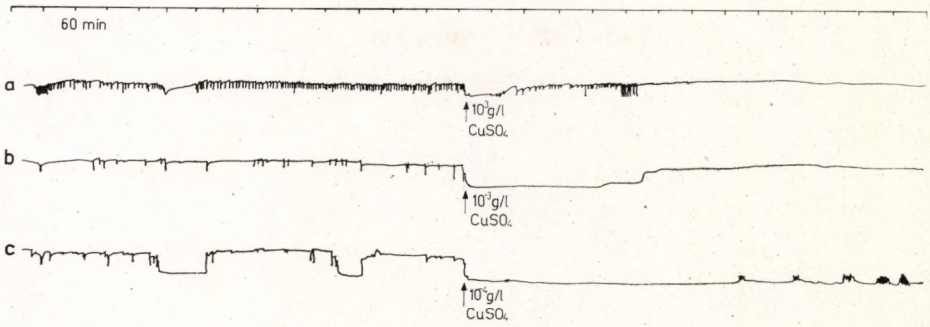

Fig. 1. Effect of $CuSO_4$ on periodic activity. *a* and *b* — 10^{-3} g/l; *c* — 10^{-4} g/l

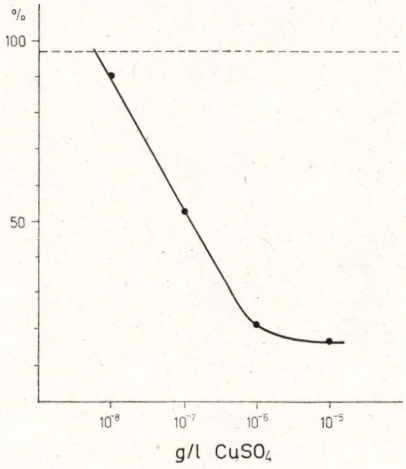

Fig. 2. Decrease of the duration of active periods plotted against $CuSO_4$ concentration

lasting (7 – 17 days) treatment in 10^{-4} g/l concentrations, too. In $10^{-4} - 10^{-5}$ g/l concentrations the duration of the active periods became shorter, the duration of activity reached only 5 – 20 per cent of the control. The duration of active periods varies between 10 and 60 minutes, and in this range it depends on the concentration only to a slight degree.

From 10^{-6} to 10^{-8} g/l the effect depends linearly on the concentration of the $CuSO_4$ *(Fig. 2)*. The threshold was 10^{-8} g/l, at which concentration the shortening of the active periods was about 10 per cent. The effect of $CuSO_4$ was permanent, adaptation was not observed within two weeks long exposure

(Fig. 3). On the record also the lengthening of the rest periods and the increase of the fast rhythmic contractions during active periods are well observable.

Washing (returning to fresh-water) restores control activity. Total restoration requires about 24—48 hours, when the original values of active periods are again observable.

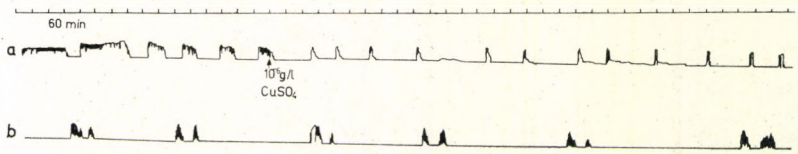

Fig. 3. Effect of 10^{-6} g/l $CuSO_4$ on the periodic activity of *Anodonta* when adding the solution (*a*) and on the 12th day (*b*)

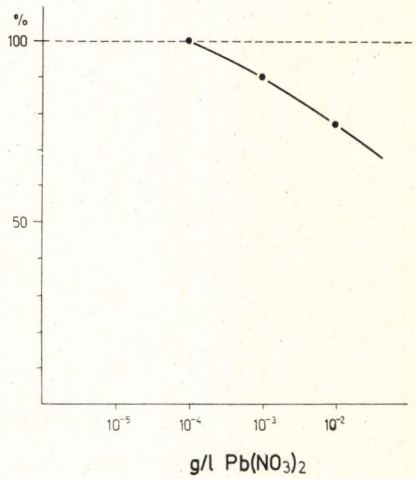

Fig. 4. Decrease of the duration of active periods plotted against $Pb(NO_3)_2$ concentration

Among lead compounds the effect of $PbCl_2$ and $Pb(NO_3)_2$ were investigated in the range of $10^{-2}-10^{-6}$ g/l concentration. Lead chloride has no effect on the activity of the mussels. Lead nitrate caused a similar but much weaker effect than copper sulphate. In 10^{-3} g/l concentration of lead nitrate the decrease in the duration of active periods was about 10 per cent, and even in 10^{-2} g/l only about 20 per cent decrease was observed *(Fig. 4)*. At the same time, in contrast to the effect of copper sulphate, the restoration was not complete after lead nitrate within 24 hours.

Discussion

The mussel-killing effect of different heavy metal compounds and of industrial waste materials is well established (KORRINGA, 1952) and the threshold of toxicity was described among others for copper salts, too. MARKS (1938) found that the highest concentration of Cu which can be tolerated by

Mytilus is 0.1 – 0.2 mg/l. This same range of concentration was described by SCOTT and MAJOR (1972) as the threshold of toxicity in *Mytilus* using Cu-chloride dihydrate. On the larvae of *Mytilus* 3.5×10^{-4} M Cu-citrate caused 50 per cent mortality (WISELY and BLICK, 1967) which corresponds to 2.2×10^{-2} g/l concentration. According to our data $10^{-4} - 10^{-3}$ g/l $CuSO_4$ kill *Anodonta*.

Our experiments were conducted primarily with the aim to establish whether Cu and Pb influence the main physiological functions of mussels in sublethal concentrations or not and that the effect to which degree can be followed by recording the motor activity of the animals. In the case of $CuSO_4$ 10^{-8} g/l proved to be the threshold of sensitivity, decreasing the activity by about 10 per cent; 50 per cent decrease was reached in 10^{-7} g/l $CuSO_4$ solution. $PbCl_2$ and $Pb(NO_3)_2$ did not cause decrease in activity at the concentration of 10^{-4} g/l. This value is much higher than the level occurring even in polluted natural waters.

The long lasting closure of the shells to $CuSO_4$ was observed in *Mytilus* by CLARKE (1947) and SCOTT and MAJOR (1972) keeping the animals in 10^{-2} and 3×10^{-4} g/l concentration of Cu solution, respectively. In these concentrations, however, in 3 – 4 days the death of the animals was observed.

Our results show that the effect of low, sublethal Cu concentrations influencing the physiological functions can be investigated by recording the periods of activity and rest, and that this latter procedure can be used as a test method. The decrease of activity means the decrease of the feeding, oxygen uptake, growth and also that of water cleaning. According to our data the presence of 10^{-8} g/l $CuSO_4$ is already harmful in this respect.

Similarly to other water organisms mussels accumulate a lot of inorganic compounds in the body, among them also Cu (SCOTT and MAJOR, 1972). A low amount of copper is present in the various tissues of mussels even in physiological conditions, its concentration reaches 0.1 – 0.5 mg/100 g wet tissue in the muscle, mantle and gill of *Mytilus* (MARKS, 1938). These values can increase as a result of accumulation, and through acute or chronic effect similarly to other metal ions, Cu becomes toxic for intracellular enzyme processes. The inhibition of tissue respiration was described in mollusc among other substances for mercury and cadmium (LUKACSOVICS and SALÁNKI, 1964; SALÁNKI and LUKACSOVICS, 1965), while a similar effect in total animal for copper was reported by SCOTT and MAJOR (1972). SH-blocking agents cause the shortening of the active periods in mussels (SALÁNKI, 1961; 1976), therefore it can be supposed that Cu ions evoke the inhibitory action on the duration of the active periods also through influencing the respiratory processes.

The toxic effect of copper on the living organisms can be modified by various properties of the water (pH, temperature, presence of other substances) (BRUNGS et al., 1976). To clear up the role of these conditions and also to throw light on the mechanism of the effect of Cu on the mussel's activity require further investigations.

Summary

The rhythmic movement of the valves and the duration of their open and closed position was recorded by an actograph. It was found that $CuSO_4$ in 10^{-8} g/l concentration decreases the duration of the active periods by 10

per cent, while 10^{-7} g/l results in the 50 per cent reduction of the active periods. Death of animals occurred at $10^{-3}-10^{-4}$ g/l.

$PbCl_2$ and $Pb(NO_3)_2$ did not cause any noticeable change in the activity at 10^{-2} and 10^{-4} g/l concentration respectively.

The applied method is suitable for testing the harmful, but sublethal effect of substances influencing already in lower doses basic physiological processes. The observed phenomenon reflecting the filtering activity too, is probably the consequence of the damage of the respiratory system.

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RÉZSZULFÁT ÉS ÓLOMVEGYÜLETEK HATÁSA A TAVIKAGYLÓ (*ANODONTA CYGNEA* L.) AKTIVITÁSÁRA

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Összefoglalás

Szerzők aktográffal regisztrálták a kagylók ritmikus héjmozgását, valamint azok nyitott és zárt állapotának időtartamát.

Rézszulfát 10^{-8} g/l koncentrációban 10%-kal csökkenti a tavikagyló aktivitását, 10^{-7} g/l pedig a több órás aktivitási szakaszok 50%-os redukciójához vezet. Az állatok pusztulása 10^{-3}—10^{-4} g/l $CuSO_4$ alkalmazásakor következik be.

Ólomklorid és ólomnitrát még 10^{-2}, ill. 10^{-4} g/l koncentrációban sem okozott észrevehető változást az aktivitásban.

Az alkalmazott eljárás alkalmas különböző károsító anyagok subletális, de életfolyamatokat befolyásoló dózisainak tesztelésére. A megfigyelt hatás, mely a filtrációs aktivitás alakulásának tükröződése is, valószínűleg a légző rendszer károsodásának következménye.