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THE EFFECT OF PESTICIDES ON THE PROTEOLITIC ENZYME ACTIVITY OF FISHES

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Summary

Studies were carryed on regarding the effect of paraquat, (1,1' – -dimethyl — 4,4'-bipiridilium) $CuSO_4$ and $ZnCl_2$ on the proteolitic enzyme activity of fishes with different nutritional habits. Paraquat, $CuSO_4$ have strongly decreased the proteolitic enzyme activity in all investigated fish species (carp, silver carp and wells).

$ZnCl_2$ did not change significantly the enzyme activity. According to our results paraquat and $CuSO_4$ damaged seriously the normal activity of proteolitic enzymes even at low concentration, so they might have adverse effect on the developing and growth of fishes. Therefore the using up of these chemicals should be restricted to that fields of agriculture which can be found far from the lakes and rivers.

Introduction

During the last two decades the widespread use of pesticides in agriculture has increased. These chemicals through the rain are getting into the natural waters, where they are rapidly accumulated in different organisms living in water, especially in fishes. The effect of several antropogenic agents on the physiological and biochemical processes of fishes are for a long time investigated. These research works were mainly focused on the demonstration of damage of nervous system (SCHRECK et al., 1978, NAKONO and TOMLINSON, 1967) liver, kidney and gills (BELL, 1968; REICHENBACH-KLINKE, 1972). Several authors has already published papers about the digestive enzymes of fishes (KAWAI and IKEDA 1971, 1972, 1973; KITAMIKADO and TACHINO, 1960; NAGOSE, 1964, ONISHI and MURAYAMA, 1970). However these works are dealing with the seasonal changes, and the distribution of proteolitic enzyme activity in different organs of fishes (SAUERBIER and MEYER, 1978) and their kinetic properties (JÓNÁS et al., 1980).

So far there was not focused research work investigating the effect of environmental pollution on the digestive enzymes of fishes.

The aim of our work was to carry out studies regarding the effect of different environmental pollutant on the proteolitic enzymes in fish species with different nutritional habits.

Materials and Methods

Common carp (*Cyprinus carpio* L.) silver carp (*Hypophthalmichthys molitrix V.*) and wells (*Silurus glanis L.*) of 350—400 g were obtained from Fisheries Research Institute in Szarvas and held as it was published before (NEMCSÓK et al, 1980). Due to the Hungarian data, in permanent metal and pesticide polluted water, these chemicals might accumulate in different fish organs (muscle, fatty tissue) up to 10 ppm. Regarding this fact the treatment was carried out with 10 ppm paraquat (1,1'-dimethyl-4,4'-bipiridilium) $CuSO_4$ and $ZnCl_2$ respectively. The length of exposition was 2 hours.

The determination of proteolitic enzyme activity

The alimentary canals of fishes were removed and homogenized in 0,1 M pH 7,5 cold (5 °C) phosphate buffer in 5 ml/g dilution. The homogenate was centrifuged at 3000 g for 30 minutes. The supernatant was used as crude extract.

Total activity was determined by the method of ANSON and KUNITZ on denaturated haemoglobin substrate. Activity was calculated on the basis of the tyrosine content of peptides produced in 1 minute by proteolysis in the TCA supernatant. 1 μM tyrosine was taken as unit. The reaction mixture consisted of 0,9 ml substrate solution in 0,1 M phosphate buffer at pH 7,9, and 0,1 ml crude extract. Digestion was stopped with TCA at O, 5th and 15th minutes.

After standing for 10 minutes, theprecipitate was removed by centrifugation. The extinction was measured at 280 nm. For the evaluation of data tyrosine calibration curve was used. The data was given in percent of the controls.

Results and discussion

The effect of paraquat on the proteolitic enzyme activity

Paraquat has markedly decreased the proteolytic enzyme activity in all fishes (Fig. 1). The decrease of activity was at 1 ppm 10—20% and at 10 ppm 60—70%. These changes were about similar in each fish species.

At the highest paraquat concentration — which effected for 10—15 minutes only, because fishes were died within this time — there was not any changes in fishes regarding the proteolitic enzyme activity.

The effect of $CuSO_4$ and $ZnCl_2$ on the proteolytic enzyme activity

$CuSO_4$ has decreased the proteolytic enzyme activity in all investigated fishes already at 1 ppm.

The most significant decrease can be seen in wells (25%) and in carp (15%). There was only a slight change in silver carp (5%).

At 10 ppm $CuSO_4$ there was a remarkable decrease also in wells. However at this concentration the proteolitic enzyme activity decreased markedly (50%) in carp and silver carp as well (Fig. 2). 100 ppm $ZnCl_2$ — which is 10 times higher comparing to the highest $CuSO_4$ concentration used during our treatment — caused only 20—30% decrease of the activity. Comparatively this degree of decrease was about the same at 100 times lesser $CuSO_4$ concentration.

Due to our results the proteolitic anzyme activity after the paraquat, $CuSO_4$ and $ZnCl_2$ treatment has changed differently.

Paraquat caused the most significant decrease, and after $ZnCl_2$ treatment there was only a shight change regarding the proteolitic anzyme activity in all investigated fish species.

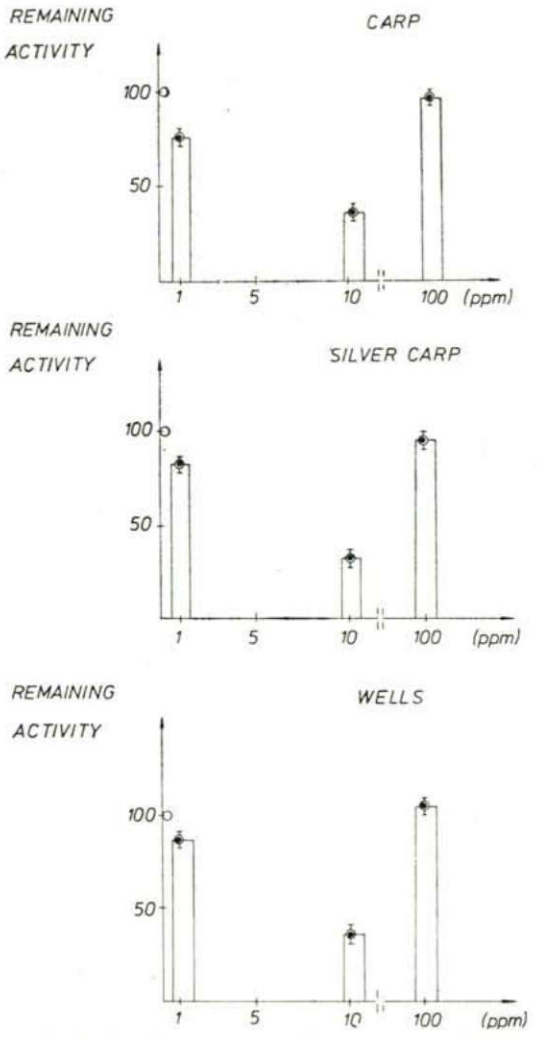

Fig. 1. The effect of 1, 10 and 100 ppm paraquat on the proteolitic enzyme activity of carp, silver carp and wells. Water temperature 20±1 °C. The values are the average of 3—9 fishes and expressed in the percent of the control ones. Exposition time 2 hours, except at 100 ppm paraquat (10—15 min.).

REICHENBACH—KLINKE (1972) reported 20—35% decrease of proteolytic enzyme activity in trout after 0,1—0,5 ppm $CuSO_4$. In our experiments such changes could be registered at higher (10 ppm) $CuSO_4$ concentration only.

The reason of this two different data might be due to the sensitivity of different fish species and the different exposure to $CuSO_4$ pollution.

We have found that the Cu^{2+} reduced the proteolytic activity of these fish species. Though it is known that the Cu^{2+} ions accelerate the oxidation of SH-groups of pro-

teins, in our cases the inhibiting effect of this metal ion may be taken as a secondary effect followed by the changes in metabolic provesses after Cu^{2+} treatment of fishes rather than a direct action on the active centre of these enzymes, because as it was shown earlier (JÓNÁS et al., 1980) these enzymes were not SH-type proteases.

$ZnCl_2$ up to 10 ppm has not changed significantly the proteolitic enzyme activity in investigated fishes.

Presumably $ZnCl_2$ is not toxic for this enzyme in this concentration range. It was published by other outhors that Zn is lesser toxic for fishes than that of the Cu

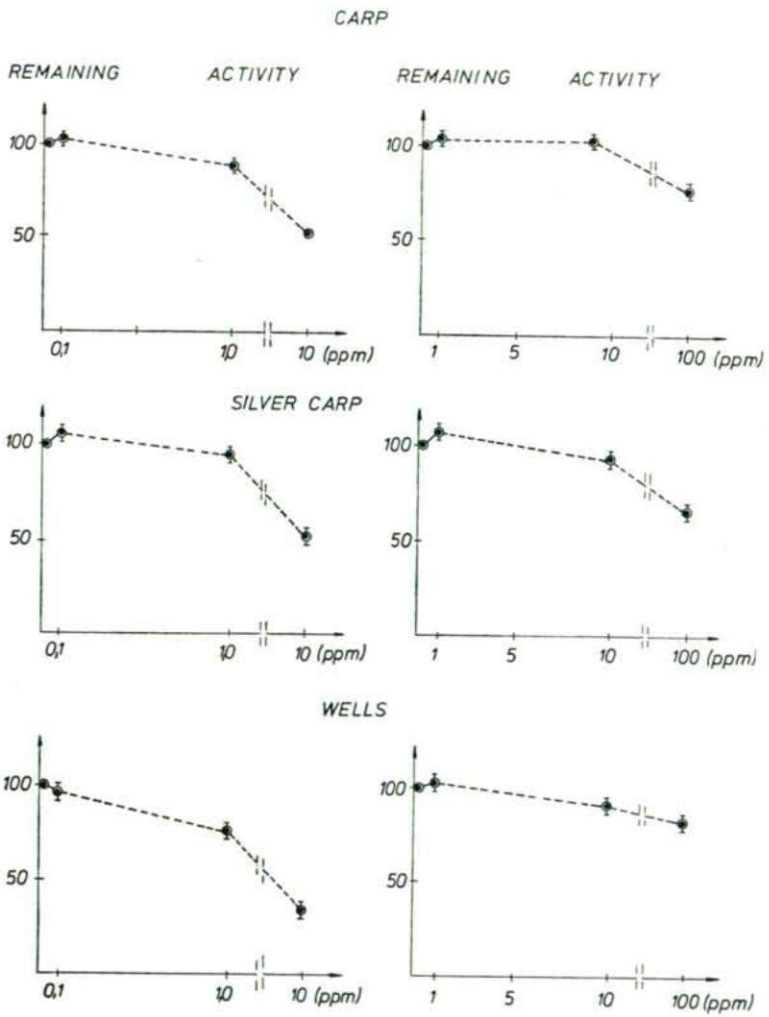

Fig. 2. The effect of 0,1, 1 and 10 ppm $CuSO_4$ (left) and 1, 10, 100 ppm $ZnCl_2$ (right) on the proteolitic enzyme activity of carp, silver carp and wells.

(MOLNÁR and SZAKOLCZAY, 1973). It was observed as well, that Zn was able inhibiing or decreasing the toxic effect of other metals (FINELLI and EL-GAZZÁR, 1977).

According to our findings, paraquat and $CuSO_4$ damaged seriously the normal functions of proteolitic enzymes even at low concentration so they might have adverse effect on the developing and gowth of fishes. Therefore the using up of these chemicals should be restrieted to that fields of agriculture which can be found far from the lakes and rivers.

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