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Effect of herbicides and surfactants on enzymes of energy metabolism in European carp

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The effect of herbicides (2,4-D, Roundup, Senkor) and surfactant sodium lauryl sulfate (pure and as part of detergents) on enzymes of energy pathways in the scaly carp's body was studied. Gills, brain, liver and white muscle were selected for the analysis. To determine the levels of activity of enzymes in energy metabolism, lactate dehydrogenase (EC 1.1.1.27) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) in the cytoplasmic fraction and isocitrate dehydrogenase (EC 1.1.1.42) in the mitochondrial fraction were studied. The research found that sodium lauryl sulfate, both pure and in a synthetic detergent, increase the activity of enzymes in all experimental groups. Effect of herbicides was ambiguous. Under the influence of Sencor the activity of enzymes increases in all tissues. Roundup causes inhibition of enzymes in the brain, but there is an increase in the activity of enzymes in other tissues under the influence of this toxicant. The influence of 2,4-D causes increased activity of isocitrate dehydrogenase in all experimental groups, as well as the activity of lactate dehydrogenase in the brain and the liver increases, whereas in gills and white muscle it decreases. The activity of glucose-6-phosphate dehydrogenase by the impact of 2,4-D was decreased only in the liver, in other groups - was increased. The investigations may be evidence of adaptive alterations in energy metabolism aimed at the survival of fish under conditions of herbicides and surfactants toxic effects. Also increase the activity of enzymes can be related to detoxification processes occurring in the body and require additional energy consumption. The article presents the results of the study of the influence of herbicides (2,4-D, Zenkor, Roundup) and surfactant the sodium laurylsulfate (pure and as a part of the synthetic detergent) on the activity of lactate dehydrogenase, isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase in the liver, gills, brain and white muscle of scaly carp (*Cyprinus carpio* L.). Human impact on water forcing fish to use various mechanisms of adaptation to the changed environmental conditions. Knowledge about the features of the receipt, distribution, accumulation of xenobiotics in organs and tissues, biochemical changes as a result of poisoning can be used to find out the mechanisms of fish's adaptation to toxicants, for identify the causes of death of hydrobionts in natural waters and to justify methods of controlling environmental pollution. The results of the research show that the influence of herbicides and surfactants causes significant changes in the processes of energy metabolism of scaly carp.
Key words: herbicides; surfactant; synthetic detergents; enzymes of energy metabolism

Introduction

In reservoirs of Ukraine among the many toxins first places is occupied by herbicides. They can spread beyond the cultivated land and circulate in the biosphere for a long time, which leads to high enough concentrations of toxic substances in surface waters, and cause for concern because of the harmful effect on aquatic organisms and as a possible danger to human health. Studying the effect of xenobiotics on metabolism in living organisms is a prerequisite justification of harmless levels in the environmental objects, including water fishery ponds (Ozernyuk & Isaeva, 2016; Shankar Murthy, Kiran & Venkateshwarlu, 2013). Pesticides can contaminate soil, water, turf, and other vegetation. In addition to killing insects or weeds, pesticides can be toxic to a host of other organisms including birds, fish, beneficial insects, and non-target plants. Insecticides are generally the most acutely toxic class of pesticides, but herbicides can also pose risks to non-target organisms (Aktar, Sengupta & Chowdhury, 2009). It is also known that synthetic detergents has an extremely wide range of negative effects on environmental, 'cause practically not prone to natural decomposition. The study of the influence of xenobiotics on metabolic processes in

living organisms is a prerequisite for the substantiation of harmless levels of detergents contents in environmental objects, including water of fishery ponds (Ostroumov, 2006).

As a result of human impacts on water, fish, as one of the most highly organized groups of aquatic organisms, forced to use various mechanisms of adaptation to the changed environmental conditions (Moon, Mommsen, 2005). Knowledge about characters of receipts, distribution, accumulation of xenobiotics in organs and tissues, biochemical changes resulting from poisoning can be used to explain the mechanisms of adaptation fish to toxicants, identify the causes of death aquatic organisms in natural waters and justification the pollution control methods. The study of specific and general changes in fish's body is the main objective in the way of developing criteria for biomonitoring of environmental water (Amiard-Triquet, Amiard, Mouneyrac, 2015). The impact of chemicals on the metabolism in the carp's body is diverse and depends on many factors: environmental parameters, age of fish, season etc. (Ozernyuk, 1992; Stoliar, Lushchak, 2012). According to modern concepts, the basic mechanism of metabolic regulation is changes in activity of certain enzymes or enzyme systems that ensure the normal course of metabolism (Ozernyuk and Isaeva, 2016).

The aim of this study was to investigate the effect of herbicides (2,4-D, Roundup and Senkor) and the surfactant sodium lauryl sulfate (pure and as a part of detergents) on enzymes of energy metabolism of scaly carp.

Materials and methods

The research object was a two-year carps (*Cyprinus carpio* L.). According to ichtyopatological observations fish's skin parasites weren't found. Tapeworm parasites also were absent.

Experiments were performed in 200-liter aquarium with tap water, the fish were placed by principle of one exemplar in 40 dm^3 water. A constant hydrochemical regime of water were monitored and maintained in all cases. The pH was 7.30 ± 0.27; oxygen content – 5.6 ± 0.4 mg/dm^3, the temperature was kept close to natural. Concentration of the xenobiotic (that equaled 2 maximum permissible concentration, MPC) was created by making the calculated amounts of amine salt 2,4-dyhlorfenoksyacetic acid solution (0.2 mg/dm^3). Senkor concentration was 0,2 mg/dm^3 (by adding 70% Senkor powder). Roundup concentration was 0.004 mg/dm^3 and achieved by adding 36% solution in calculated quantities. Sodium lauryl sulfate, recalculated for lauryl sulfate containing substance, added to the aquarium water in the amount that corresponded to 2 MAC.

To determine the enzyme activity tissue homogenates were prepared in 0.25 M sucrose at a ratio of 1:10. The nuclei, mitochondria and microsomes extracted by generally accepted methods (Schachman, 1959).

For the purpose of establish the activity level of energy metabolism enzymes were studied lactate dehydrogenase (EC 1.1.1.27) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity in the cytoplasmic fraction and isocitrate dehydrogenase (EC 1.1.1.42) activity – in the mitochondrial fraction. Extracting of mitochondria was performed by the standard method (Zinich, 1986).

Lactate dehydrogenase activity was determined spectrophotometrically by the change of absorption at oxidation of NADH at 340 nm. For LDH incubation mixture (3 cm^3) containing 100 mM K^+ – phosphate buffer, pH 7,0; 23 mM pyruvate; 12 mM NADH; 0,02 cm^3 suspension of studied enzyme (Biochemica information, 1975). The enzyme activity is expressed in mmol NADP/mg protein per minute.

Determination of glucose-6-phosphate dehydrogenase activity was performed spectrophotometrically at a wavelength 340 nm. The incubation solution contained: 0.1 M K^+ – phosphate buffer, pH 7.6, 35 mM solution of glucose-6-phosphate, 11 mM $NADP^+$ (Biochemica information, 1975). Activity was shown in mmol NADP/mg protein per minute.

In studying the activity of NADP-dependent isocitrate dehydrogenase (NADP mmol/mg protein per minute) incubation solution containing in 3 cm^3: HCI-Tris buffer – 50 mmol, pH = 7.4, magnesium chloride – 2.0 mmol, isocitrate – 1.0 mmol, and NADP – 0.025 mmol (Biochemica information, 1975) The protein content of enzyme preparations was determined by the method of Lowry et al (1951).

All interventions and slaughter of animals were carried out according to the national General Ethical Principles of Experiments on Animals (Ukraine, 2001) and the requirements of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). All results were statistically processed due to Oyvin I. A. (1960). Differences between compared groups were considered to be significant at $P < 0.05$. Correlation analysis and single-factor analysis of variance was performed according to Lakin (1990).

Results and discussion

During the study the data were obtained regarding the activity of enzymes basic pathways of energy metabolism in the gills of Common carp (Table 1).

The analysis of the data showed a tendency to increase the activity of the studied enzymes in the gills of fish that were under the influence of the three pesticides (except LDH influenced 2,4-D (2,4-dichlorophenoxy). Activation of enzymes of glycolysis, Krebs cycle and pentose - phosphate cycle can be explained by intense energy intensive processes excretion of toxins and their metabolites which formed by biological transformation through the gills and increased energy metabolism processes of result in toxicity. ICDH of gills was the most sensitive to 2,4-D that caused to the increase of enzyme activity by 3.7 times compared with the control.

Table 1. The activity of enzymes in the gills of two-year carp in the conditions of influence by xenobiotics (M ± m, n = 5)

Conditions of living	The enzyme activity		
	LDH. NADH mmol/mg protein per minute.	ICDH. NADP mmol/mg protein per minute.	G6PD. NADP mmol/mg protein per minute.
Control	0.059±0.010	0.038±0.002	0.060±0.006
2,4-D	0.052±0.012 (P>0.5)	0.141±0.020 (P<0.001)	0.064±0.012 (P>0.5)
Senkor	0.065±0.011 (P>0.5)	0.059±0.010 (P<0.1)	0.090±0.010 (P<0.05)
Roundup	0.478±0.083 (P<0.01)	0.040±0.004 (P>0.5)	0.383±0.046 (P<0.01)
Sodium lauryl sulfate	0.243±0.055 (P<0.01)	0.095±0.015 (P>0.05)	0.084±0.034 (P>0.5)
SDA	0.145±0.045 (P<0.05)	0.080±0.010 (P>0.05)	0.094±0.024 (P>0.5)

The maximum increase in the activity of LDH and G6PD (by 8.1 and 6.4 times, respectively) were observed at Roundup toxicosis. In conditions of Senkor toxicity the activity of all three enzymes increased: LDH - by 1.1 times (nonsignificant), ICDH - by 1.6 times, G6PD – by 1.5 times compared with the control. Thus, the herbicide stress causes an increase in the activity of enzymes in carbohydrate metabolism in gills of experimental fish. Listed changes directed to protect from toxins.

Significant increase in enzyme activity was observed under the influence of sodium lauryl sulfate, as pure, and as part of SDA. Probable changes in activity were observed for LDH ($P < 0.01$ for pure substances and $P < 0.05$ for SDA). Interestingly, the use of pure sodium lauryl sulfate had greater changes enzyme activity compared to SDA.

The enzyme activity of basic ways of energy supply were determined in the brain tissues studied fish. The data presented in Table 2.

Table 2. The enzyme activity in the two-year carp's brain under conditions of influence of xenobiotics (M ± m, n = 5)

Conditions of living	The enzyme activity		
	LDH, NADH mmol/mg protein per minute.	ICDH, NADP mmol/mg protein per minute.	G6PD, NADP mmol/mg protein per minute.
Control	0.077±0.010	0.042±0.008	0.069±0.007
2,4-D	0.083±0.019 (P < 0.2)	0.093±0.009 (P < 0.05)	0.093±0.009 (P < 0.2)
Senkor	0.113±0.023 (P < 0.2)	0.072±0.006 (P < 0.05)	0.073±0.009 (P > 0.2)
Roundup	0.053±0.009 (P < 0.2)	0.038±0.004 (P > 0.5)	0.030±0.008 (P < 0.01)
Sodium lauryl sulfate	0.104±0.010 (P < 0.2)	0.164±0.018 (P < 0.01)	0.163±0.009 (P < 0.5)
SDA	0.094±0.029 (P < 0.2)	0.086±0.008 (P < 0.05)	0.096±0.012 (P < 0.2)

We observed in the brain of fish that were under the influence of Roundup the inhibition of LDH (by 1.45 times), but the differences are insignificant. Also we registered inhibition of ICDH and G6PD (by 1.1 and 2.3 times, respectively), but the difference probable only for G6PD. According to the data presented in Table 2, among three studied enzymes the most favorable to the action of xenobiotics is ICDH. The maximum change in enzyme activity was observed under the influence of sodium lauryl sulfate.

The activity of enzymes in the liver of two-year carp in conditions of influence of xenobiotics shown in Table 3. The study found that rates of enzyme activity in the liver increased under the influence of Roundup, but changes in activity of LDH and D6PD fluctuated insignificant, and the figures for LDH were nonsignificant. ICDH activity was higher by 2.1 times compared with the control.

Effects of surfactant causes a slight increase in activity of all studied enzymes, but the differences are nonsignificant.

In white muscle of carp that was in aquarium with a solution of Roundup, the activity of enzymes of carbohydrate metabolism changed ambiguous. LDH activity increased in small limits, while the activity ICDH quite significantly decreased, and the activity of G6PD quite significantly increased (respectively by 5 and 2.9 times).

The other two studied herbicides did not cause any significant changes of enzyme activity, but there was a tendency to increase the action of enzymes. Pure Sodium lauryl, and as part of SDA causing activation ICDH and G6PD in fish.

Table 3. The activity of enzymes in the liver of two-year carp in conditions of influence of xenobiotics, (M ± m, n = 5)

Conditions of living	The enzyme activity		
	LDH, NADH mmol/mg protein per minute.	ICDH, NADP mmol/mg protein per minute.	G6PD, NADP mmol/mg protein per minute.
Control	0.111± 0.023	0.020± 0.004	0.063± 0.008
2,4-D	0.118±0.024 (P > 0.5)	0.022±0.002 (P > 0.5)	0.044±0.008 (P < 0.5)
Senkor	0.124±0.022 (P > 0.5)	0.036±0.006 (P < 0.5)	0.064±0.012 (P > 0.5)
Roundup	0.116±0.040 (P > 0.5)	0.042±0.002 (P < 0.001)	0.083±0.012 (P < 0.1)
Sodium lauryl sulfate	0.180±0.014 (P > 0.5)	0.022±0.004 (P > 0.5)	0.120±0.014 (P < 0.1)
SDA	0.168±0.064 (P > 0.5)	0.021±0.005 (P > 0.5)	0.144±0.034 (P < 0.1)

Table 4 shows the results of the study of enzyme activity in basic energy pathways of carp's white muscle under conditions of influence of herbicides and surfactants.

Table 4. The enzyme activity in white muscle of two-years carp in conditions of influence of xenobiotics (M ± m, n = 5)

Conditions of living	The enzyme activity		
	LDH, NADH mmol/mg protein per minute.	ICDH, NADP mmol/mg protein per minute.	G6PD, NADP mmol/mg protein per minute.
Control	0.109 ±0.010	0.136 ±0.030	0.012±0.001
2,4-D	0.108±0.025 (P > 0.5)	0.148±0.025 (P > 0.5)	0.017±0.005 (P > 0.5)
Senkor	0.227±0.025 (P < 0.5)	0.154±0.055 (P > 0.5)	0.018±0.003 (P > 0.5)
Roundup	0.117±0.015 (P > 0.5)	0.027±0.002 (P < 0.001)	0.035±0.004 (P <0.001)
Sodium lauryl sulfate	0.150±0.045 (P > 0.5)	0.336±0.016 (P < 0.05)	0.048±0.006 (P <0.05)
SDA	0.144±0.018 (P > 0.5)	0.240±0.054 (P <0.5)	0.040±0.006 (P <0.05)

Adaptation of fish to changes in environmental conditions leads to changes in intracellular bioenergetic processes, resulting in changes of the energy generation intensity. Information on the activity of enzymes of glycolysis, TCA and G6PD as the main energy pathways of the body, along with the study of changes in the concentration of metabolites needed for a comprehensive explanation ways of ensuring adaptation of carp to toxic effect. It is known that one of the functions of the pentose-phosphate pathway is the formation of the reduced form $NADPH+H^+$ with G6PD. Recovered $NADPH+H^+$ used in the biosynthesis of fats. Last needed for the fishes body not only for energy but also for the biosynthesis of glucose, particularly during the winter starvation when no monosaccharide present in the environment during the winter.

Thus we can say about activation of catabolism in tissues and organs of carp during intoxication by xenobiotics, because of energy detoxification processes.

Conclusions

The results of the conducted research show that the impact of herbicides and surfactant causes significant changes in the processes of energy exchange of Scaly carp. There was an increase in the activity of enzymes under the influense of sodium lauryl sulfate (pure and in the synthetic detergent) and Senkor herbicide in all experimental groups. Roundap causes inhibition of enzymes in the brain, but in other tissues was obsorved an increase in the activity of enzymes under the effects of this toxicant. The influence of 2,4-D causes ambiguous changes in fish tissues. The investigations may be evidence of adaptive alterations in energy metabolism aimed at the survival of fish under conditions of herbicides and surfactants toxic effects . Also increase the activity of enzymes can be related to detoxification processes occurring in the body and require additional energy consumption.

References

Aktar, Md.W, Sengupta, D., Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicology, 2(1), 1-12. https://doi.org/10.2478/v10102-009-0001-7
Amiard-Triquet, C., Amiard, J.C., Mouneyrac, C. (2015). Aquatic Ecotoxicology. San Diego: Academic Press Inc.
Lakin, G. F. (1990) Biometry. Moscow. Vyshaya shkola (in Russian).
Lowry, O. H., Rosebrough, N. I., Farr, A. I., Rendall, R. I. (1951). Journal Biological Chemistry, 193, 265– 275.
Moon, T. W., Mommsen, T.P (2005). Environmental Toxicology (Vol. 6). Oxford: Elsevier Science Ltd.
Ostroumov, S.A. (2006). Biological effects of surfactants. Boca Raton: Press Taylor & Francis.
Oyvin, I. A. (1960). Statistical analysis of the results of experimental studies. Pathological physiology and experimental therapy, 4, 76–85.
Ozernyuk, N.D. (1992). Mechanizmy adaptacij. Moscow: Nauka (in Russian).
Ozernyuk, N.D., Isaeva, V.V. (2016). Evolution of ontogenesis. Moscow: KMK Scientific Press (in Russian).
Schachman, H. K. (1959). Ultracentrifugation in Biochemistry. New York: Acad. Press.
Shankar Murthy, K., Kiran, B.R., Venkateshwarlu, M.V. (2013). A review on toxicity of pesticides in Fish. International Journal of Open Scientific Research, 1(1), 15-36.
Stoliar, O.B., Lushchak, V.I. (2012). Environmental Pollution and Oxidative Stress in Fish. In V.I. Lushchak (Ed.). Oxidative Stress - Environmental Induction and Dietary Antioxidants, (pp. 131-166). InTech. Available from: http://www.intechopen.com/books/oxidative-stress-environmentalinduction-and-dietary-antioxidants/ environmental-pollution-and-oxidative-stress-in-fish/ Accessed on 22.01.2018
Zinich, V.N. (1986). Method of measuring 2-oxiglutarate dehydrogenase activity of intact mitochondria. The Ukrainian Biochemical Journal, 58(2), 73–77. (in Russian).

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