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INFLUENCE OF FERTILIZERS ON PHYSIOLOGICAL FUNCTIONS OF FISHES

U.F. Hashimova [1], M.M. Akhundov [2], S.I. Mammadova [1,3]

[1] ***Academician Abdulla Garayev Institute of Physiology, Ministry of Science and Education of the Republic of Azerbaijan****, 78 Sharifzadeh Street, AZ 1100, Baku, Azerbaijan*
[2] ***Biological Resources Research Centre of the Ministry of Ecology and Natural Resources,*** *100 K.Kazimzade Street, AZ 1073, Baku, Azerbaijan*
[3] ***Baku State University, Ministry of Science and Education of the Republic of Azerbaijan,*** *23 Zahid Khalilov Street, AZ 1148, Baku, Azerbaijan*

E-mails: ulduz.hashimova@physiology.science.az; m.akhundov@eco.gov.az; sevaxalilovna@mail.ru

Fertilizers are strong pollutants in the surrounding water environment. These substances get into the water environment through the groundwater during the spraying of crops under agricultural cultivation. The toxicity of fertilizers influences growth, physiology, and a hemato-biochemical profile and causes serious changes in some tissues of aquatic organisms, including fish, which has also proved to be true in our research. According to the mentioned effects, hematological parameters serve as essential biomarkers for water pollution by fertilizers. They cause serious changes in the fish's blood as they bioaccumulate in various tissues and cause serious changes in the blood and tissues of fish (*Cyprinus Carpio*) and also in their behavioral reactions. The impact of the phosphoric fertilizers Ammophos and Kristalon in lethal concentrations of 97.2 mg/l and 265.2 mg/l on the behavioral reactions of *Cyprinus Carpio* and blood indicators was studied. It is necessary to notice that these fertilizers are widely used in the cultivation of crops, but their impact on fish was not investigated, and it is noted in their passport data. It is necessary to notice that these fertilizers influenced the behavioral reactions and the blood indicators of the fish *Cyprinus Carpio*.

Keywords: phosphoric fertilizers, lethal concentration, behavioral reactions, blood indicators, *Cyprinus Carpio*.

INTRODUCTION

One of the initial steps of the system approach to studying reservoirs is the primary diagnosis of the condition of water ecosystems. The system approach to knowledge of the laws of functioning of the hydrosphere demands a definition of the ecological capacity of the water objects that make it. Thus, the maximum anthropogenous loading falls on internal freshwater reservoirs. That is why toxicological research focused on the well-being of organisms of all trophic levels in a reservoir has paramount value in applied ecology [2, 4].

The urgency of work on a toxicological estimation of the influence of fertilizers on reservoirs does not raise doubts. Such estimation is possible because of two major problems: the autecological features of species indicators and data about pollutants cumulation in internal reservoirs [2, 7].

MATERIALS AND METHODS

The object of research was *Cyprinus Carpio*, which we got from the Neftchala Sturgeon Breeding Farm of the Azerbaijan Fish Farm, and we want to express gratitude to them for rendering assistance in carrying out this research. In each series of experiments, we used 10 fish at the age of six months. At first, we defined the lethal concentration during the first 96 hours of an exposition for these fertilizers [5]. Behavioral reactions (cough, yawn, superficial swimming, uneasy chaotic movement, balance loss, throws, movement of a tail fin, impellent activity) were defined using a method described by Al-Haem [1].

In the laboratory, the technique of blood sampling, which has considerable advantages as it allows receiving a considerable quantity of blood from fish without their killing or subsequent bleeding, has been applied. For this purpose, the fish was fixed with a paunch upwards in the machine tool consisting of two parallel thin plates, strengthened on a strong basis. A needle was used to pierce the skin behind an anal fin on the average line of a body. Then, through the stretched aperture, a glass pipette with a thin needle was inserted. The pipette end was inserted into a depth of tissue until it met (that is easily learned on resistance) the vertebra body, on the ventral part of which passes a large trunk of a tail artery. In an easy rotary motion of a pipette, an artery wall was cut, and blood started to arrive quickly in the pipette [3]. The analysis of the blood of a *Cyprinus Carpio* was carried out using the standard technique. The content of hemoglobin, the number of red blood cells, hematocrit, erythrocyte sedimentation rate, quantities of leukocytes, and red blood cell indicators were determined [3]. The obtained samples were studied under the microscope NU-2 Carl Zeiss Jena. Data were statistically processed using the Student t-test in MS Excel software.

RESULTS AND DISCUSSION

As can be seen from Table 1, in our research studying behavioral reactions, we have found out that in controls, a *Cyprinus Carpio* superficial swimming made 4.8±0.533 moves/min, coughed 3.6±0.670 moves/min, yawned 2.6±0.306 moves/min, had uneasy chaotic movement 2.9±0.379 moves/min, lost balance 1.9±0.233 moves/min, moved by throw 7.2±0.306 moves/min, had a tail fin movement of 54.0±3.400 moves/min, and had impellent activity 5.2±0.611 moves/min.

Table 1. Influence of Ammophos and Kristalon fertilizers on changes in fish behavioral responses.

Behavioral responses (moves/min)	Control	Ammophos (97.21 mg/l)	Kristalon (265.18 mg/l)
cough	3.6 ±0.670	8.8 ±0.323	4.9 ±0.674
P		<0.001	>0.2
yawn	2.6 ±0.306	5.4 ±0.164	4.2 ±0.200
P		<0.001	<0.001
superficial swimming	4.8 ±0.533	6.9 ±0.396	5.5 ±0.373
P		<0.01	>0.2
uneasy chaotic movement	2.9 ±0.379	3.6 ±0.164	4.2 ±0.618
P		<0.02	≤0.05
balance loss	1.9 ±0.233	4.9 ±0.314	5.1 ±0.277
P		<0.001	<0.001
throws	7.2 ±0.573	4.2 ±0.730	4.5 ±0.601
P		<0.001	<0.01
tail fin movement	54.0 ±3.400	33.0 ±1.719	56.0 ±4.269
P		<0.001	>0.5
impellent activity	5.2 ±0.611	8.8 ±0.323	4.9 ±0.458
P		>0.5	>0.5

The next stage of our research aimed at studying the influence of phosphoric fertilizers at lethal concentrations during the 96-hourly expositions of Ammophos 97.21 mg/l and Kristalon 265.18 mg/l. As a result of the influence of these fertilizers, we have revealed sharp changes in the behavioral reactions of

Cyprinus Carpio. So under the influence of Ammophos and Kristalon, cough indices rose twice to 8.8±0.323 moves/min and 4.9±0.674 moves/min; balance loss was up to 4.9±0.314 moves/min and 5.1±0.277 moves/min; yawn was up to 5.4±0.164 moves/min and 4.2±0.200 moves/min; superficial swimming was up to 6.9±0.396 moves/min and 5.5±0.233 moves/min; uneasy chaotic movement was up to 3.6±0.164 moves/min and 4.2±0.618 moves/min; throws decreased to 4.2±0.730 moves/min and 4.5±0.601 moves/min. Impellent activity under the influence of Ammophos rose to 6.3±0.111 moves/min, but under the influence of Kristalon, it decreased to 4.9±0.458 moves/min. Tail fin movement under the influence of the first preparation decreased to 33.0±1.729 moves/min, but after the second preparation, it rose to 56.0±4.269 moves/min in comparison with the control.

Analyzing the above-stated data, it is possible to conclude that the influence of these preparations had a different impact on the behavioral reactions of *Cyprinus Carpio*. Oppression of impellent activity can be related to the toxic influence of the dominant operating substance in these preparations. A decrease or increase can be a consequence of an infringement on gas exchange because of gill damage. It led to the oppression of the process of metabolism and, hence, to a decrease in the power supply of an organism, which was reflected in the behavior of fish [7]. Infringement of impellent activity could be related to the influence of phosphoric fertilizers on the nervous system, which led to infringement of signaling from the central nervous system to muscles, and could also be a consequence of enzymatic dysfunctions that led to infringements of the respiratory center [2]. Besides that, *Cyprinus Carpio* is a group of fish capable of regulating oxygen levels, but only to a certain level. A decrease in the maintenance of oxygen in the blood (based on hemoglobin indicators in our research) led to hypoxia in the organism, which led to power exhaustion, which was shown in the change in impellent activity [8].

The study of the physiology of blood cells of fish in control and after the influence of investigated phosphoric fertilizers of lethal concentration (Table 2) was the next stage of our research.

Table 2. Blood parameters in control and experimental conditions (M±m, n=10, P).

Blood parameters	Control	Ammophos (97.21 mg/l)	Kristalon (265.18 mg/l)
RBC ($\times10^{12}$/l)	1.7 ±0.152	2.8 ±0.899	1.8 ±0.326
P		>0.2	>0.5
WBC ($\times10^{9}$/l)	25.1 ±3.844	39.0 ±7.952	25.1 ±4.762
P		>0.1	<0.001
Hb (g/l)	67.0 ±1.528	74.1 ±4.838	47.0 ±3.667
P		>0.1	<0.001
HCT (%)	26.0 ±2.667	23.5 ±2.926	28.0 ±2.000
P		>0.2	>0.2
ESR (mm/hour)	2.6 ±0.334	3.2 ±0.200	5.0 ±0.447
P		>0.1	<0.001
MCH (pg)	39.0 ±4.583	45.9 ±9.725	34.0 ±5.764
P		>0.5	>0.5
MCV (fl)	180.0 ±35.904	131.0 ±22.564	168.0 ±29.090
P		>0.2	>0.5
MCHC (g/l)	28.08 ±2.613	35.5 ±4.540	17.9 ±2.120
P		>0.1	<0.001

In the study of blood indicators, we have revealed that the level of red blood cells (RBC) in controls was 1.7±0.152×10^{12}/l, white blood cells (WBC) were 25.1±0.152×10^{9}/l, hemoglobin (Hb) was 67.0±1.528 g/l, hematocrit (HCT) was 26.0±2.667%, erythrocyte sedimentation rate (ESR) was 2.6±0.334 mm/hour, the mean concentration of hemoglobin (MCH) was 39.0±4.583 pg, the mean corpuscular volume (MCV) was 180.0±35.904 fl, the mean corpuscular hemoglobin concentration (MCHC) was 28.08±2.613 g/l.

Under the influence of Ammophos, with a lethal concentration of 97.21 mg/l, and

Kristalon, whose lethal concentration was 268.18 mg, the following situation was observed: the Ammophos affected the level of red blood cells and leukocytes, which were raised to 2.8±0.899×1012/l and 39.0±0.899×109/l, but the Kristalon impact kept the indicators up to standard. Hemoglobin levels under the influence of fertilizer Ammophos rose 1.2 times, and those under the influence of Kristalon decreased compared with control. In experimental conditions studying hematocrit, we observed a return pattern of hemoglobin data. The erythrocyte sedimentation rate compared with the control has been raised to 3.2±0.200 mm/hour and 5.0±0.447 mm/hour, respectively. The levels of the MSH and MCHC under the influence of Ammophos were above control, and after Kristalon's influence, they have been lowered. But, in MCV indicators, Ammophos and Kristalon caused a sharp decrease.

Changes in hemoglobin concentration, a quantitative increase of red blood cell indicators, and hematocrit can be associated with a sensitivity of erythroid cells to the toxic effect of the used preparations. It can be associated with the erythropoiesis suppression process. The indicator of the concentration of hemoglobin in the blood reflects the possibilities of an organism's oxygen supply [6]. However, the toxicant influenced hemoglobin to decrease, indicating that oxygen levels in the blood fell and there was anemia [8]. The influence of toxins led to insufficient oxygen content in the water, which caused an increase in hemoglobin levels as a physiological response of *Cyprinus Carpio*, which mobilized all forces of the body to maintain hemoglobin levels. Indicators MCH and MCHC state a saturation estimation of erythrocyte hemoglobin and are used for the definition of hyperchromic and hypochromic erythrocytes. The values of MSH and MCHC did not depend on the volume of blood or the number of red cells in the blood. Changes in the values of the given indicators explain how many new cells, which are thrown out into the circulating blood, are sated by oxygen in our research, both hyperchromic and hypochromic. An increase in leukocyte numbers was a fish immunological attempt to mobilize the protective reserves of an organism against toxins [4]. The erythrocyte sedimentation rate directly depends on the physiological condition of an organism and indicates the presence of inflammatory processes in the organism.

CONCLUSIONS

1. The influence of phosphoric fertilizers Ammophos and Kristalon on *Cyprinus Carpio* has been studied for the first time.
2. The lethal concentration of these preparations had been defined.
3. The obtained data show that these preparations have a toxic impact.
4. Toxicity is shown both in the behavioral reactions of fish and in blood indicators.
5. It is recommended to reduce the quantity of these fertilizers used in the cultivation of agricultural crops.

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ВОЗДЕЙСТВИЕ УДОБРЕНИЙ НА ФИЗИОЛОГИЧЕСКИЕ ФУНКЦИИ РЫБ

Улдуз Фаизи гызы Гашимова [1], Мехман Магсуд оглу Ахундов [2], Севиндж Ибрагимхалил гызы Мамедова [1,3]

[1] Институт физиологии им. академика Абдуллы Гараева, Министерство науки и образования Азербайджанской Республики, Баку, Азербайджан
[2] Центр Исследования Биологических Ресурсов, Министерство Экологии и Природных Ресурсов Азербайджанской Республики, Баку, Азербайджан
[3] Бакинский государственный университет, Министерство науки и образования Азербайджанской Республики, Баку, Азербайджан

Удобрения относятся к сильнодействующим загрязнителям окружающей водной среды. Эти вещества попадают в водную среду при выращивании сельскохозяйственных культур, когда опрыскивают эти культуры, а также за счёт грунтовых вод. Токсичность удобрений оказывает влияние на рост, физиологию, гемато-биохимический профиль и вызывает серьёзные изменения некоторых тканей водных организмов, включая рыб, что подтвердилось и нашими исследованиями. Из упомянутых эффектов гематологические параметры служат важными биомаркерами загрязнения вод удобрениями. вызывают серьёзные изменения в крови рыб, так как они биоаккумулируются в различных тканях рыб и тем самым вызывают серьёзные изменения в кровяной ткани рыб, а также в поведенческих реакциях рыб (сазан). В своих исследованиях мы использовали фосфорные удобрения Ammophos и Kristalon летальной концентрации, которые соответствовали 97,21 мг/л и 265,18 мг/л. Мы изучали поведенческие реакции сазана и показатели крови. Надо отметить, что эти удобрения широко используются при выращивании сельскохозяйственных культур, но эти препараты не исследовались на рыбах и об этом написано в их паспортных данных. Надо отметить, что эти удобрения оказывают влияние на поведенческие реакции сазана, а также на показатели крови рыб**.**

Ключевые слова: фосфорные удобрения, летальная концентрация, поведенческие реакции, показатели крови, сазан

GÜBRƏLƏRİN BALIQLARIN FİZİOLOJİ FUNKSİYALARINA TƏSİRİ

Ulduz Fayızi qızı Həşimova [1], Mehman Maqsud oğlu Axundov [2], Sevinc İbrahimxəlil qızı Məmmədova [3]

[1] Akademik Abdulla Qarayev adına Fiziologiya İnstitutu, Azərbaycan Respublikası Elm və Təhsil Nazirliyi, Bakı, Azərbaycan
[2] Bioloji Resursların Tədqiqatı Mərkəzi, Azərbaycan Respublikası Ekologiya və Təbii Sərvətlər Nazirliyi, Bakı, Azərbaycan
[3] Bakı Dövlət Universiteti, Azərbaycan Respublikası Elm və Təhsil Nazirliyi, Bakı, Azərbaycan

Gübrələr su mühitinin güclü çirkləndiriciləridir. Bu maddələr su mühitinə kənd təsərrüfatı bitkilərinin becərilməsi zamanı, bu bitkilərə səpildikdə, həmçinin yeraltı sular hesabına daxil olur. Gübrələrin toksikliyi böyüməyə, fiziologiyaya, hemato-biokimyəvi göstəricilərə təsir edir və su orqanizmlərinin, o cümlədən balıqların bəzi toxumalarında ciddi dəyişikliklərə səbəb olur ki, bu da tədqiqatlarımızla təsdiq edilmişdir. Qeyd olunan təsirlərdən hematoloji parametrlər suyun gübrələrlə çirklənməsinin mühüm biomarkerləri kimi çıxış edir. balıqların qanında ciddi dəyişikliklərə səbəb olur, çünki onlar balıqların müxtəlif toxumalarında bioakkumulyasiya olunur və bununla da balıqların qan toxumasında, eləcə də balıqların (çəki balığı) davranış reaksiyalarında ciddi dəyişikliklərə səbəb olur. Tədqiqatlarımızda 97,21 mq/l və 265,18 mq/l-ə uyğun gələn Ammofos və Kristalon fosfat gübrələrinin letal konsentrasiyalarından istifadə etdik. Çəki balığının davranış reaksiyalarını və qan göstəricilərində ciddi fəsadlar yaratmışdır. Qeyd edək ki, bu gübrələrdən məhsul becərilməsi zamanı geniş istifadə olunur, lakin bu preparatlar balıqlar üzərində tədqiq olunmayıb və bu, onların pasport məlumatlarında yazılıb.

Açar sözlər: fosfat gübrələri, letal konsentrasiya, davranış reaksiyaları, qan göstəriciləri, çəki balığı

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