Effect of Glyphosate Herbicide on Some Hematological and Biochemical Parameters in *Carassius auratus L.*

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In recent years, commercial glyphosate herbicide formulations have been widely used in agriculture to control aquatic and terrestrial weeds. These pesticides may result in disruption of ecological balance, causing damage to nontarget organisms, including fish. Carassius auratus L. specimenes were exposed to glyphosate herbicide in concentration of 2.5mg/L and, respectively, 5 mg/L for 120 h, in laboratory condition. Thus, the effects of this chemical agent on both hematological parameters (hemoglobin, hematocrit, number of red blood cells) and on oxidative status indices (SOD, CAT, GPX, MDA) at Prussian carp experimental lots were analyzed. Our results showed, in comparison with the reference batch, the influence of glyphosate on the analyzed hematological and biochemical parameters, in close correlation with the administered pesticide dose.

Keywords: glyphosphate, oxidative stress, hematological indices, Carassius auratus L.

Organophosphorus herbicides are among the most damaging chemicals for aquatic ecosystems, as it is known that glyphosate-based herbicides are widely used in agriculture, posing a threat to many aquatic organisms, including fish [1-3]. Scientific data in recent years highlight the particular influence of this xenobiotic on aquatic organisms, due, inter alia, to high water solubility and intensive use in agriculture [4-6].

Besides, glyphosate [N-(phosphonomethyl) glycine] is a broad spectrum herbicide, being commonly used in agriculture, but also in other non-agricultural areas to stop the growth and development of undesirable terrestrial and hydrophilic plants [7-9]. It seems that, it's possible mechanism of action is to inhibit 5-enolpyruvylshikimate-3-phosphate synthase involved in the shikimate pathway, the metabolic pathway present in plants and microorganisms, but absent in animals, which is why, for years, this herbicide was considered to be non-toxic or relatively low toxicity for animal organisms, relatively recent data pointing to an increased rate of bioaccumulation, resulting in intoxication phenomena and the risk of affecting the physiological-normal state [10-12].

Literature on the field [4, 13] highlights the risk to human and animal health due to chronic and sub-chronic exposure to glyphosate, the use of this pesticide has received increased attention in recent years [14]. At the same time, there is controversy in the literature about the ability of glyphosate to induce carcinogenesis, many studies showing the carcinogenic potential of this herbicide in epidermal, renal, intestinal, hepatic, thyroid tumors [15]. It should be borne in mind that literature data indicate a moderate risk from exposure to low herbicide concentrations, suggesting, however, the need for further in-depth studies about how to use it properly, for to decrease at minimum the unwanted effects, as well as the impact on which would have glyphosate on the risks of human and animal disease [16, 17].

The goal of this study was to evaluate the toxic potential of glyphosate at concentrations of 2.5 mg/L and 5 mg/L, respectively, after 120 h exposure to *Carassius auratus* L. specimens, in term of hematological and biochemical indices.

Experimental part

Fish, food and treatment

We take into study *Carassius auratus* L. specimens, weighing of 50±2.5 g, which were divided into three experimental batches and were acclimatized under laboratory conditions for one week in 70 liter open tanks. During the experimental period, the specimens were subjected to natural day/night alternation (photoperiod 16 hours light/8h dark), permanently monitoring the water temperature (set at 22°C), the tanks being cleaned, and the water was refreshed every two days.

The fish were fed once a day with a special mixture for Prussian carp feed containing 43% crude protein, crude oils and fats 9.5%, 2% fiber, yeast, lecithin, mollusks, crustaceans, minerals, vitamins and provitamins, algae, cereals, egg and egg by-products. After acclimatization, treatment with glyphosate at 2.5 and 5 mg/L, respectively, was applied for 120 hours and, at the end of the experiment, the fish were sacrificed, blood samples and muscle tissue were taken and subsequently processed in laboratory.

Hematological parameters

The hematocrit was determined by the Guest method through centrifugation of the capillaries for 2 minutes at a rate of 12,000 rpm, and the hemoglobin with the Gowers-Sahli hemoglobinometer, by comparing a solution of hydrochloride hemoglobin with a brown glass standard. Red blood cells were counted directly at the microscope, preceded by pre-dilution of blood with Hayem reagent - isotonic solution with the erythrocytes [18].

Biochemical parameters

The activity of superoxide-dismutase (SOD) was determined on the basis of the ability of the enzyme to inhibit the reduction of Nitro-Blue-Tetrazolium (NBT) by superoxide radicals, catalase (CAT) by the Sinha spectrophotometric method, glutathione peroxidase (GPX) by the 5'-dithiobis-2-nitrobenzoic acid which reacts with

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the remaining reduced glutathione forming a complex colored in yellow, the malondialdehyde concentration (MDA) by the 2-thiobarbituric acid method and proteins concentration by the Bradford method [19-21].

Results and discussions

Literature on the filed suggests that glyphosate, considered to be non-toxic to humans and animals so far [22], can bioaccumulate in animal organisms leading to intoxication, various degrees of disease and types of multiple diseases [23-25]. Moreover, the excessive use of glyphosate-based pesticides in agriculture has led to the disruption of the health of the aquatic environment, the increase in the bioaccumulation rate being due to the high solubility of this compound in water [5, 6].

Kreutz *et al.* [26] highlights that hematological parameters can be affected by stress factors and pollutants from water, the authors point out that massive use of glyphosate in agriculture determines its presence in soil and water and is, therefore, indirectly an impediment to fish production, aquatic organisms being more susceptible to glyphosate than other species.

Considering that the evaluation of fish blood parameters could be a useful tool to understand the impact of herbicide and pesticide chemical compounds on population health, a first objective of our study was the determination of hematocrit, hemoglobin and red blood cells in the experimental groups of *Carassius auratus*.

Hematocrit values in the blood of the specimens from the reference group reached the median threshold of 25.15±1.02% (table 1), while in the variants treated with glyphosate herbicide in the concentration of 2.5 and 5.0 mg/L, respectively, values mean of 24.95±0.5% and 24.2±0.4%, respectively, was estimated. The results obtained in what concerns the hematocrit values in Prussian carp individuals' concord with the literature data [27] which show slightly lower values for glyphosate application compared to the control group, the influence of the pesticide on hematological parameters reflecting a moderate hemorrhagic anemia.

The hemoglobin, a respiratory pigment that helps to maintain the acid-base balance of the body, is a hemoprotein that is part of, along with catalase, peroxidase and chloroplastins, from class of porphyrin chromoproteins and is consisting of a protein component - globin and a prosthetic component - hem [28]. The mean hemoglobin concentration was highlighted in the Prussian carp from the reference batch (10.26±0.58 g/dL), and the lowest of 8.98±0.64 g/dL was found in the experimental variant containing 2.5 mg glyphosate/L, while in the treated group with a herbicide concentration of 5 mg/L, hemoglobinemia reached the level of 9.32±0.75 g/dL.

The average number of erythrocytes slightly decreased from the control variant to the highest glyphosate concentration used, the values being of 1.93±0.04 mL/mm^3 in the control group, 1.89±0.05 mL/mm^3 in the sample containing 2.5 mg glyphosate/L water and 1.72±0.08 million/mm^3 for the variant treated with 5 mg of glyphosate/L.

Our results are in line with literature data that noticed severe hematological changes in treated fish, anemia can be occur due to inhibition of erythrocytes production, but also of hemodilution, a condition state that is reflected by the reduction of hematological parameters values [29-31].

The oxidative stress is an indispensable component of aquatic life, the reactive oxygen species (ROS) being the result of various physiological-normal processes, and appearing even more so as a direct effect of xenobiotisc action by altering certain biochemical mechanisms such as electron transport at the membrane level, facilitation of Fenton reaction, inactivation of antioxidant enzymes and depletion of free scavengers [32].

The most important enzymes involved in cell detoxification are SOD, CAT, GPX and a series of transferases, xanthine oxidase and glucose 6-phosphate dehydrogenase.

In individuals treated with 2.5 and 5.0 mg/L glyphosate, the SOD activity, an oxidoreductase involved in the first line of defense against free radicals, reached an average thresholds of 51.35±1.623 USOD/mg protein and, respectively, 40.64±1.88 USOD/mg protein, as compared to the control group, where the enzyme recorded an average activity of 45.68±1.514 USOD/mg protein (fig. 1). Decreased activity in the samples treated with the maximum pesticide concentration may be due to massive transitory hydrogen peroxide accumulation at the tissue level, our results may be correlated with those from the literature data which show variations in the enzyme activity in the treated groups as compared to the reference variant [33].

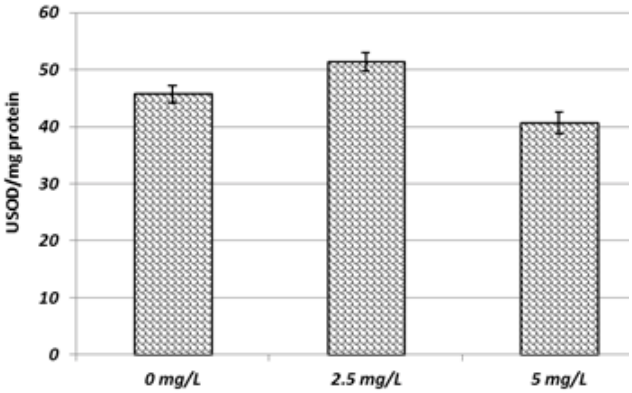

Fig. 1. SOD activity in *Carassius auratus*

If SOD is responsible for catalyzing the conversion of the superoxide anion into hydrogen peroxide, it then degrades into water and molecular oxygen following CAT action, the two enzymes constituting a family present mainly in peroxisomes.

The CAT activity in the control group (fig. 2) achieved an average activity level of 68.27±1.812 UCAT/mg protein, whereas in the experimental variant with a 2.5 mg glyphosate/L concentration, the enzyme exhibited an average activity of 59.48±2.843 UCAT/mg protein, so that at the maximum herbicide concentration, it achieves an activity threshold of 52.19±1.537 UCAT/mg protein.

A series of literature studies [34] show a significant increase in CAT activity in close dependence on the type of herbicide applied, the exposure period and its concentration in the environment, while other authors [35, 36] signal a 45% decrease in the activity of this oxidoreductase compared to the reference group.

	Herbicide concentration (mg/L)		
	0.0	2.5	5.0
Hematocrit (%)	25.15 ± 1.02	24.95 ± 0.5	24.2 ± 0.4
Hemoglobin (g/dL)	10.26 ± 0.58	8.98 ± 0.64	9.32 ± 0.75
Erythrocytes (x 10^6/mm^3)	1.93 ± 0.04	1.89 ± 0.05	1.72 ± 0.08

Table 1
HEMATOLOGICAL PARAMETERS VALUES IN *Carassius auratus*

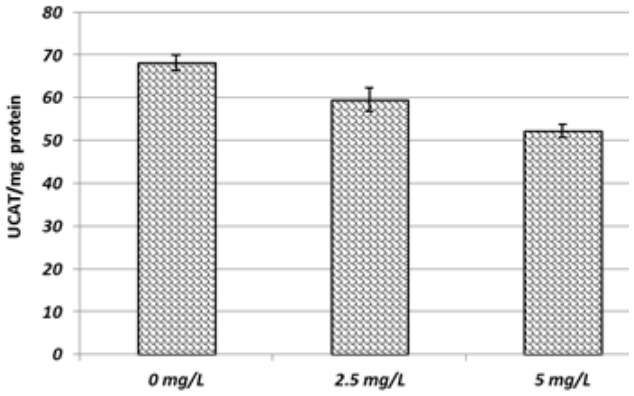

Fig.2. CAT activity in *Carassius auratus*

An important peroxidase in the metabolization of numerous organic peroxides, including hydrogen peroxide, is GPX, the activity values for this enzyme in the experimental variants treated with glyphosate being lower compared to the control batch, at a concentration of 2.5 mg/L the mean activity being equal with 1.94±0.15 UGPX/mg protein, while in the group treated with 5.0 mg glyphosate/L, the median activity threshold was of 2.45±0.18 UGPX/mg protein, a value slightly lower than that of the untreated group (2.85±0.11 UGPX/mg protein).

The scientific literature [37] shows a decrease in the activity of antioxidant SOD, CAT, GPX and glutathione-S-transferase enzymes in fish exposed to the action of glyphosate-based pesticides, enzyme inhibition being a mechanism by which can be put into evidence the degree of the oxidative stress produced by this herbicide.

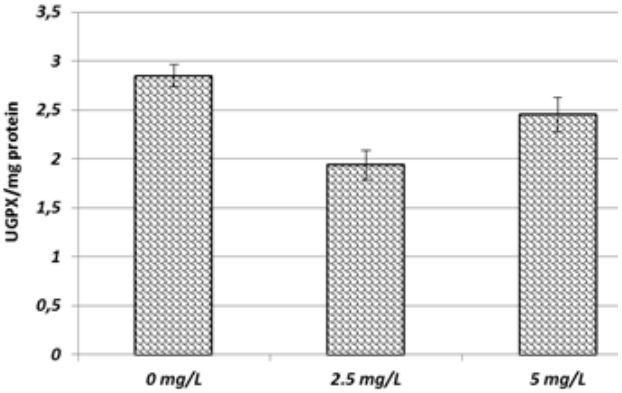

Fig.3. GPX activity in *Carassius auratus*

The antioxidant defense due to oxidative stress enzyme activity fails to neutralize enough free radicals produced during biotransformation processes, resulting in the installation of membrane lipid peroxidation, implicitly the accumulation of reactive aldehydes of the MDA type, marker of the oxidant status of the animal organism [38]. Thus, the use of glyphosate in the concentration of 2.5 and 5.0 mg/L, respectively, led to the installation of oxidative stress, confirmed by the high level of MDA detected in the muscle tissue (148.69±10.64 nM and 155.97±12.87 nM, respectively) compared to the untreated experimental variant (124.68±9.15 nM).

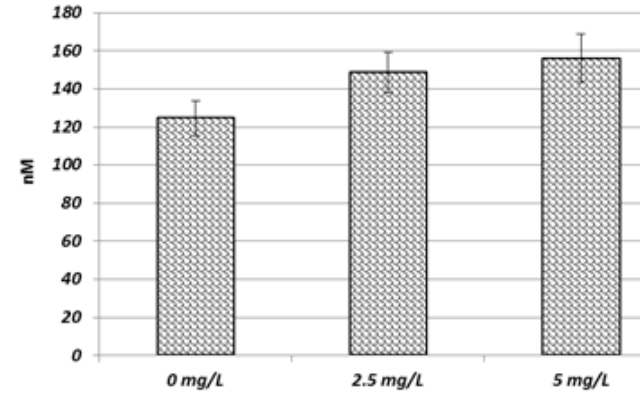

Fig.4. MDA concentration in *Carassius auratus*

Conclusions

The analysis of the experimental results on the influence of glyphosate in the concentration of 2.5 and 5.0 mg/L, respectively, on some hematological and biochemical parameters in *Carassius auratus* L., allowed us to formulate the following general conclusions:

- the hematocrit and hemoglobin values, as well as the erythrocytes number have slightly decreased by comparison with the reference batch, indicating the occurrence of a moderate anemia, those three hematological indices being considered markers of the body's anemic profile.

- the oxidative stress enzyme activity, as well as MDA concentration - as a marker of lipid peroxidation, varied more or less significantly as compared to the control sample, in close correlation with the administered glyphosate concentration.

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