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Research Article

EFFECT OF GLYPHOSATE ON BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS IN FRESH WATER FISH, *CYPRINUS CARPIO.*

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ABSTRACT

The common carp, *Cyprinus carpio* was exposed to 1/10th of 96 hours LC50 concentrations (0.02ppm) of glyphosate for 10, 20 and 30 days. The biochemical parameters like protein, carbohydrate and lipid were measured in gill, liver, kidney and muscle both in control and experimental fishes. During various exposure periods the levels of protein, carbohydrate and lipid decreased significantly over the control. The muscle tissue showed maximum decline in protein, carbohydrate, where as the liver showed maximum decline in lipid content during the exposure periods. Monitoring of blood parameters, both cellular and non cellular may have considerable diagnostic value in assessing early warning signs of herbicide poisoning. Due to glyphosate exposure the blood parameters such as RBC, Hb, MCV, MCH, MCHC and PCV decreased whereas WBC increased with respect to control.

INTRODUCTION

Extensive use of pesticides has facilitated increase in agricultural productivity despite a decrease in the total average of land cultivated. Mass mortality of aquatic organism has often been caused by pesticide exposure especially from accidental or direct spraying of water bodies. More commonly aquatic organisms are subjected to long term stresses from exposure to sublethal concentrations. However, in the long run these sublethal concentrations also proved to have deleterious effects as do lethal concentration because sublethal and small effects on aquatic organisms may alter behaviors, feeding habits, reproduction rates etc. In addition, pesticides that do reach the water body can accumulate in fish and other aquatic fauna which are then harmful to humans when ingested.

Herbicides are a group of chemicals with high environmental risk due to their growing use, presence in aquatic environments and their capacity to affect non target organisms such as fish. Besides the active ingredient, adjuvant compounds are added to herbicide formulations in order to improve the efficacy of the commercial product. Among the large and heterogeneous group of adjuvant substances, non ionic surfactants stand out due to their wide spread use. However, there are few data on the risks and environmental toxicity of these compounds. The assessment of the toxicity of the herbicide formulations is made almost exclusively based on the active ingredient while the possible effects of adjuvant compounds are over looked by environmental toxicologists and environmental protection agencies[9]. Glyphosate based herbicides are widely used around the world and ranked first in the market of herbicides in Brazil. Government regulatory agencies and international organizations indicate low toxicity and environmental risk from direct exposure to glyphosate. However, the commercial glyphosate formulations pose higher risk than glyphosate alone[11]. Roundup® is a broad spectrum herbicide that represents one of the most commonly applied formulations in the world[6], and is used in agriculture, ornamental gardens and aquatic habitats[8].

In addition to glyphosate as the active ingredient, Roundup® has the non ionic surfactant polyoxy ethyleneamine (POEA) in its formulation, which seems to be directly related to the toxicity of the product[11]. Several studies have already demonstrated that POEA is more toxic than the active ingredient and the formulated product itself. Besides its high toxicity, the surfactant persistence in aquatic environments ranging from 21 to 42 days is longer than the half-life of the active ingredient which ranges from 7 to 14 days[8]. This surfactant adversely affect the growth and energetic reserves in the fresh water fish species[7].

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Toxicity tests with aquatic organisms represent an effective tool for assessing the effects of pollutants on organisms. Pesticides like other types of xenobiotics can induce the generation of reactive oxygen species (ROS). Due to the high reactivity, these ROS can interact with lipids, proteins, carbohydrates and nucleic acids and cause oxidative damage[10]. The herbicides are able to induce carbonylation and thiol oxidation as post translational modifications of proteins[4] and these processes are suggested to play a key role in herbicide induced toxicity[3]. Several studies suggested that among biological changes, haematological parameters are considered as a potential biomarkers of exposure to chemical agents[12,23]. It serves as an important tool for assessing how the contaminants harmfully affect the health status of an organism like fish.

In the present study, an approach examining biochemical and haematological parameters were employed in order to evaluate acute effects of the herbicide glyphosate on the fish, *Cyprinus carpio*.

MATERIALS AND METHODS

The fish, *Cyprinus carpio* measuring 6 to 8 cm in length and 6.5 to 7.5 gm in weight irrespective of the sex were used in the experiment. Fishes were washed with 0.1 % KMnO4 solution to avoid dermal infection. The precautions for maintaining the fishes were as per APHA, AWWA and WEF standards in the study.[1] The fishes were exposed to herbicide, glyphosate to sublethal $1/10^{th}$ 96 hrs LC50 value, i.e. 0.02 ppm concentrations for various periods. When mortality occurred during the experimental period dead fishes were removed immediately to avoid depletion of dissolved oxygen (DO) level which may adversely affects other fishes. The vital tissues like muscle, liver, gill and kidney of the fishes were taken for the estimation of proteins, carbohydrates and lipids. The blood samples were also collected from these fishes.

Prior to collection of blood, fishes were hit by a blow on head to dislocate cervical. Blood was collected by cardiac puncture using disposable syringes and kept in separate vials (with EDTA and without anticoagulant). All the blood samples were handled at room temperature. EDTA containing blood was used for whole blood count parameters. Total erythrocyte count (TEC) (106 mm−3) and leucocyte count (TLC) (103mm−3) were quantified by Neubauer haemocytometer (Rohem, India). Haemoglobin concentration (Hb) (gdL−1) was determined with haemoglobin test kit (DIAGNOVA, Ranbaxy, India). Packed cell volume (PCV) (%) was estimated by Wintrobes tube method. Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular volume (MCV) were calculated using the following formulae:

$$\text{MCH (pg)} = \frac{\text{Hb (g/dL)} \times 10}{\text{TEC } (10^6\ \text{mm}^{-3})}$$

$$\text{MCHC (g/dL}^{-1}) = \frac{\text{Hb(g/dL)} \times 100}{\text{PCV (\%)}}$$

$$\text{MCV } (\mu\text{m}^3) = \frac{\text{PCV(\%)} \times 10}{\text{TEC } (10^6\ \text{mm}^{-3})}$$

RESULTS AND DISCUSSION

Environmental and chemical stress can interfere with physiological and biochemical functions such as growth, development, reproduction and circulatory systems in fish. Numerous biochemical indices of stress have been proposed to assess the health of non target organisms exposed to toxic chemicals in aquatic ecosystem[17]. However, it has been reported that apart from nervous tissue, liver and gills also contribute information in the detection of toxic symptoms caused by certain groups of pesticides.

The changes in biochemical parameters such as carbohydrates, proteins and lipids are important to indicate the susceptibility of organ systems to pollutants by altering their function. Proteins are important organic substances required by organisms in tissue building and play an important role in energy metabolism and can be expected to be involved in the compensatory mechanism of stressed organisms[19]. The result of the present study showed that when the fishes were exposed to glyphosate (0.02ppm) the protein content found decreased. The decrease was great in muscle tissue in all the exposure periods (Table1 and Figure1).

Table 1 Changes in the Protein content in the tissues of *Cyprinus carpio* on long term exposure periods to Glyphosate

Samples (mg/gwet tissue)	Exposureperiods			
	CONTROL	10DAYS	20DAYS	30DAYS
GILL	3.12±0.41	1.49±0.25	1.19±0.51	1.13±0.18
't'value		4.95**	6.16**	7.29**
%Change		+52.24	+61.86	+63.78
LIVER	4.10±0.13	1.98±0.10	1.51±0.09	1.33±0.07
't'value		18.28	23.61**	26.48**
%Change		+51.70	+63.17	+67.56
KIDNEY	2.75±0.11	1.65±0.12	1.62±0.09	0.99±0.07
't'value		9.11**	16.28**	36.03**
%Change		+40.00	+41.09	+75.85
MUSCLE	1.21±0.22	0.78±0.14	0.73±0.07	0.55±0.16
't'value		2.48*	6.18**	8.80**
%Change		+35.54	+64.04	+72.91

Values are mean SD, n = 5, Figures in parenthesis are percentage decrease over control.
*-Significant at 5%(t<0.05) ** -Significant at1%(t<0.01)
NS– Non significant

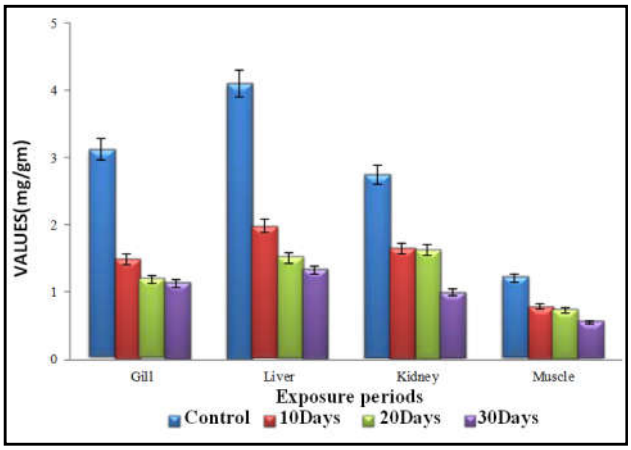


Figure1 Changes in the Protein content in the tissues of *Cyprinus carpio* on long term exposure periods to Glyphosate

The reduction of protein may be due to proteolysis and increased metabolism under toxicant stress and also could be due to its utilization to mitigate the energy demand when the fishes are under stress[19]. The results of the present findings showed a significant decrease in carbohydrate content in all the tissues studied and found maximum decline in muscle. (Table 2 and Figure 2).

Table 2 Changes in the Carbohydrate content in the tissues of Cyprinus carpio on long term exposure periods to Glyphosate.

Samples (mg/gwet tissue)	Exposureperiods			
	CONTROL	10DAYS	20DAYS	30DAYS
GILL	28.26±0.08	11.40±0.10	10.82±0.14	7.69±0.13
't'value		73.33**	66.57**	94.4**
%Change		+59.66	+61.71	+72.78
LIVER	28.40±0.05	18.20±0.13	16.80±0.11	10.60±0.14
't'value		8.232**	18.94**	58.54**
%Change		+35.91	+40.84	+62.67
KIDNEY	25.46±0.13	16.40±0.05	15.80±0.10	4.71±0.11
't'value		126.1**	78.57**	209.5**
%Change		+35.58	+37.94	+81.50
MUSCLE	28.40±0.10	8.56±0.06	7.41±0.08	5.29±0.09
't'value		63.26**	69.26**	93.22**
%Change		+69.85	+73.90	+81.37

Values are mean SD, n = 5,Figures in parenthesis are percentage decrease over control.
*-Significant at 5% (t<0.05) ** -Significant at1% (t<0.01) NS– Non significant

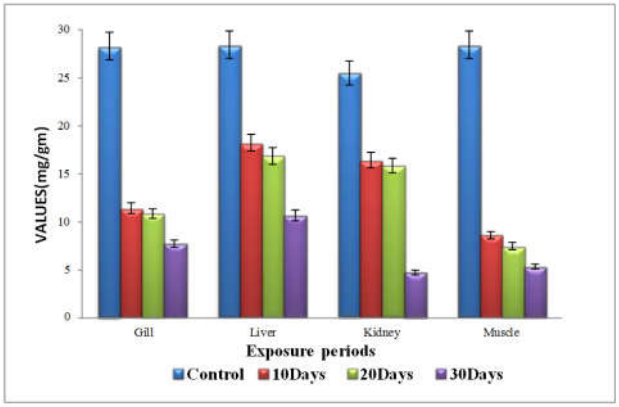


Figure 2 Changes in the Carbohydrate content in the tissues of *Cyprinus carpio* on long term exposure periods to Glyphosate.

The decrease in carbohydrate content may result in impairment of carbohydrate metabolism due to toxic effect. The carbohydrate reduction suggests the possibilty of active glycogenolysis and glycolytic path way to provide excess energy in stress condition[22]. The sublethal concentration of certain organophosphate pesticides caused glycogenolysis which produced hyperglycemia in the African food fish, *Tilapia mossambica* and the Indian cat fish, *Heteropneustes fossilis*[15]. Lipid is an important normal body constituent used in the structure of cell membranes, synthesis of bile and steroid hormones. The results presented in Table 3 and Figure 3 shows a significant decrease in lipid content in the tissues of fish, *Cyprinus carpio.* The decrease was found to be high in liver and less in kidney. This reduced lipid level may be due to the inhibition of lipid biosynthesis in the liver or due to reduced absorption of dietary cholesterol[14]. Several studies suggested similar reduction of lipids in various tissues[16,20, 21]. In the present study, the reduction in lipid content indicates the toxic nature of the glyphosate. Haematological parameters are potential biomarkers of exposure to agrochemicals due to their sensitivity to certain toxic agents.

Table 3 Changes in the Lipid content in the tissues of Cyprinus carpio on long term exposure periods to Glyphosate

Samples(mg/gwet tissue)	Exposureperiods			
	Control	10days	20days	30days
GILL	28.47±0.09	14.60±0.08	11.67±0.10	7.81±0.12
't'value		29.36**	57.08**	87.59**
%Change		+48.71	+59.00	+72.56
LIVER	30.5±1.01	11.20±0.61	7.3±0.49	4.7±0.35
't'value		9.42**	23.35**	37.62**
%Change		+63.28	+76.07	+84.59
KIDNEY	26.54±0.10	20.60±0.14	18.80±0.15	13.50±0.11
't'value		18.08	31.38**	88.47**
%Change		+22.38	+29.16	+49.13
MUSCLE	22.6±0.75	12.8±0.57	10.3±0.39	10.1±0.53
't'value		10.47**	9.54**	9.53**
%Change		+43.36	+54.42	+55.22

Values are mean SD, n= 5,Figures in parenthesis are percentage decrease over control.
*-Significant at 5% (t<0.05)** -Significant at1% (t<0.01)
NS– Non significant

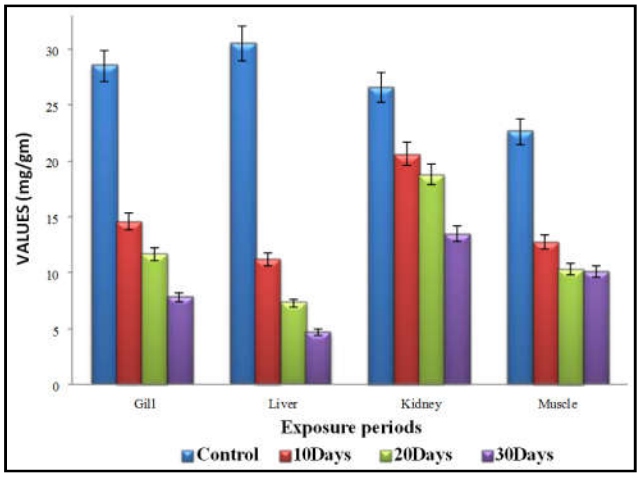


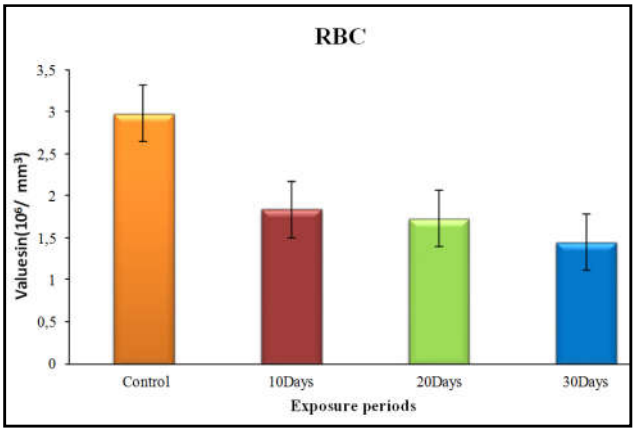
Figure 3 Changes in the Lipid in content in the tissues of *Cyprinus carpio* on long term exposure periods to Glyphosate

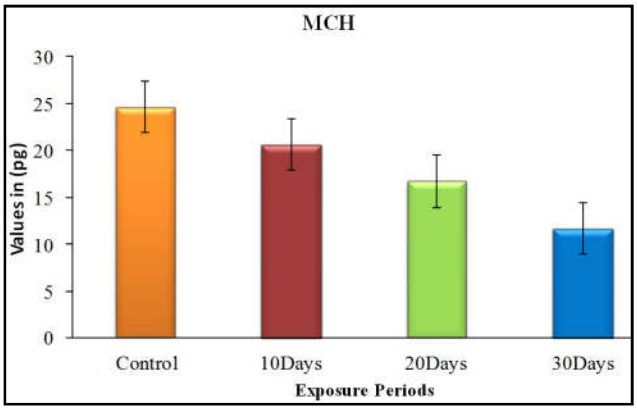
The fishes exposed to glyphosate presented a significant decrease in PCV, haemoglobin (Hb), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) (Table 4 and Figure 4).

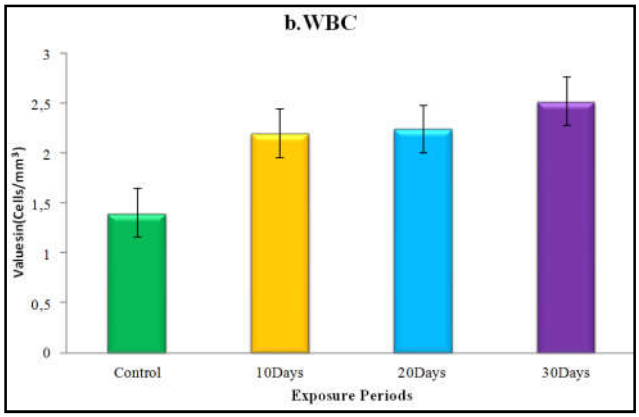
Table 4 Haematological changes in the fresh water fish, Cyprinus carpio on long term exposure periods to Glyphosate.

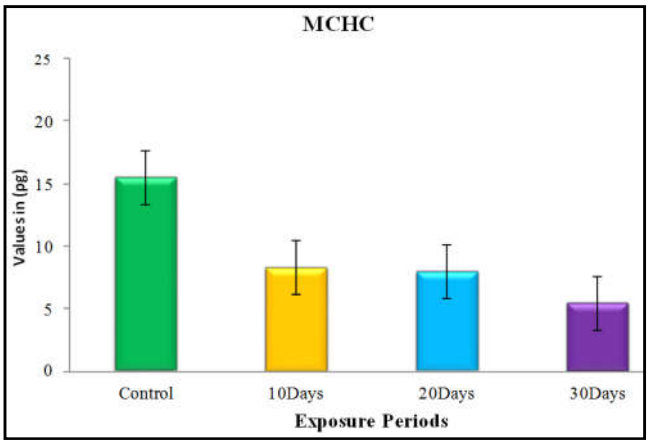
Blood Parameters	Exposure periods			
	Control	10DAYS	20DAYS	30DAYS
RBC	2.98±0.12	1.84±0.10	1.73±0.11	1.45±0.13
't'value		10.32**	10.86**	10.65**
%Change		+38.25	+41.94	+51.34
WBC	1.40±0.22	2.20±0.45	2.24±0.31	2.52±0.53
't'value		2.43*	2.30*	2.86*
%Change		-57.14	-*60.00	-80.00
Hb	3.88±0.07	3.77±0.12	2.83±0.12	2.66+ 0.14
't'value		1.08**	10.61**	10.60**
%Change		+2.83	+27.06	+31.44
MCV	26.47 ±0.03	20.68±0.01	18.62±0.04	14.30±0.03
't'value		39.28**	52.58**	73.84**
%Change		+21.87	+29.65	+45.97
MCH	24.60±0.16	20.60±0.16	16.70±0.16	11.70±0.16
't'value		35.78**	73.34**	115.00**
%Change		+16.26	+33.30	+76.25
MCHC	15.50±0.71	8.31±0.39	7.98±0.05	5.43±0.07
't'value		17.67**	21.0**	28.08
%Change		+46.38	+48.51	+64.96
PCV	12.40±0.09	8.60±0.10	7.89±0.11	6.71±0.11
't'value		39.978**	44.88**	28.88**
%Change		+30.64	+36.37	+45.88

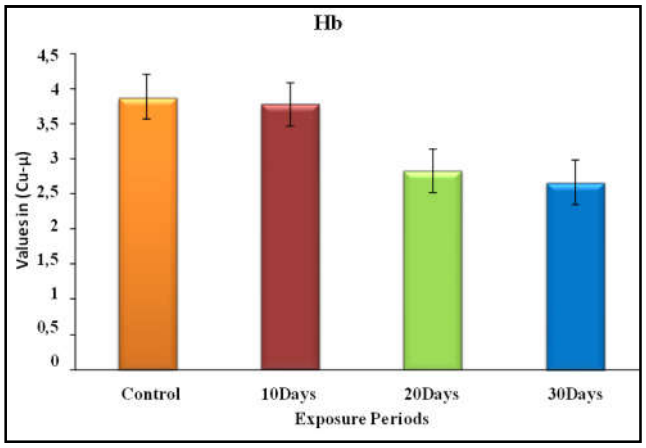
Values are mean SD, n= 5, Figures in parenthesis are percentage decrease/increase over control.*-Significant at 5% (t<0.05) ** -Significant at1% (t<0.01) NS– Non significant

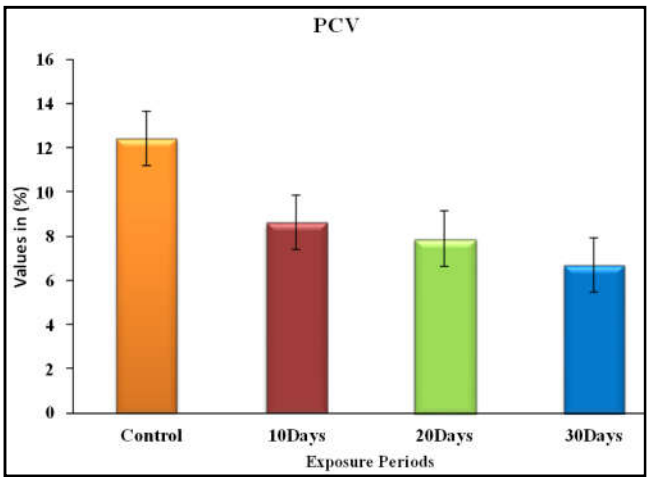












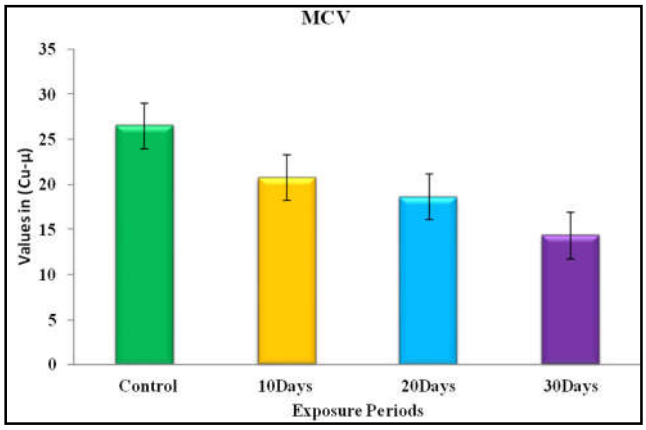


Figure 4 Haematological changes in blood due to Glyphosate in the fresh water fish, *Cyprinus carpio* on long term exposure periods.

Herbicides has been reported to cause PCV to decrease in the Neotropical fish species *Rhamdia quelen* exposed to concentrations of 0.5 and 1mgl−1 for 96 hours[5]. These results express a probable condition of anemia since it is known that many chemical pollutants including agro chemicals can induce anemia in fish and this anemia may be due to ROS induced oxidative injury via oxidation of haemoglobin or other cellular components[2]. The increase in WBCs count in the present study indicates the stress condition of the fish caused by glyphosate. This might produce damages to gill and other organs which denote a cellular response of tissue alteration generated by the herbicide or a high sensitivity of neutrophils to environmental changes[13].In this study, the RBC count decreased significantly in the glyphosate treated fish. This decrease in RBC count

levels may be due to the inhibition of erythropoiesis, haemosynthesis or osmoregulatory dysfunction or due to an increased rate of erythrocyte destruction in the haematopoietic organ[24] as in the fishes exposed to chlorpyrifos[18, 25] . The decrease in MCV values may be considered as an index of RBC destruction in the present study.

CONCLUSION

The present work indicates that glyphosate caused alterations in the biochemical metabolism in the gill, liver, kidney and muscle of the fresh water fish, *Cyprinus carpio*. The treated fish tissues showed more decrement in biochemical levels and this may be due to more herbicidal stress. The low content of proteins, carbohydrate and lipid reflects a change in the rate of synthesis and degradation of these, lowered working capacity under the impact of accumulation of pollutants leading to an alteration in function which indicates the vulnerability of the organs. The variations of haematological parameters also denote the stressful nature of glyphosate.

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