

Histo Hepatic Alterations in Commercially Important Fish (*Pseudoplatystoma corruscans* Spix & Agassiz, 1829) Exposed to a Glyphosate-Based Herbicide

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Abstract

The presence of herbicides in the aquatic environment can cause different effects at all levels of biological organization. This study aimed to evaluate the hepatic alterations of the herbicide Roundup WG® on juvenile *Pseudoplatystoma corruscans* exposed to three different concentrations of this chemical compound: 0.25g/1000L, 0.50g/1000L, and 0.75g/1000L, plus control treatment (0.00g/1000L). The experiment lasted sixty days and, in the end, liver fragments were collected for further histological processing, using the hematoxylin-eosin (HE) technique. Only the control group showed a statistically significant increase in body mass and total length during the experiment. Blood glucose also showed no difference among the sample groups. The lesions found in the liver considered severe were hemorrhage, vacuolization, and hypertrophy of hepatocytes and the presence of free melanomacrophages, recorded in the groups exposed to 0.50 and 0.75g/1000L. The results obtained in the present study indicate that the herbicide Roundup WG® can promote liver alterations in *Pseudoplatystoma corruscans*.

Keywords: Hepatic histopathology, Spotted sorubim, Water pollution, Chronic toxicity

1. Introduction

1.1 Herbicide Consumption

In Brazil, the consumption of chemical compounds for pest control in plantations has grown in proportion to the growth of agribusiness, that is, due to the significant increase in monoculture cultivation, making it the largest consumer of pesticides in the world (Sousa et al., 2020).

The increase in agricultural activity in the vicinity of watershed areas has caused great concern regarding its potential for contamination, due to the intense application of herbicides and other chemicals that help control pests in plantations (Silva et al., 2020).

When applied directly to the soil, herbicides are subject to physical, chemical, and biological processes that will influence their activity and determine their permanence or not. Molecules with a low degradation rate can remain in the environment without undergoing any change and, if not absorbed by plants, can become strongly adsorbed to organic matter, be carried by rainwater, or even suffer leaching and reach the water table (Silva et al., 2017).

Once in the aquatic environment and depending on their physicochemical characteristics, the herbicide molecules can bind to suspended particulate matter, deposit at the bottom of the sediment, or even be absorbed by aquatic organisms (Silva et al., 2020) and in fish to accumulate in the liver.

1.2 Herbicides and Aquatic Organisms

Herbicides present in water bodies can penetrate organisms through several entry ports and their degree of accumulation depends on the availability and persistence of the contaminant in the water. Fish and invertebrates can accumulate at concentrations far above those found in the waters in which they live, as they can be ingested along with suspended particulate matter. In addition, herbicides or other water-soluble chemical agents can penetrate an organism through the entire surface of the body, gills, and mouth and can be bioaccumulated and biomagnified along the food chain and/or web (Rand Netter, 1985).

Histological changes in fish tissue are a valuable tool to detect the direct toxic effects of chemical compounds on target organs and are therefore considered excellent indicators of exposure to environmental stressors (Hinton et al., 1992; Schwaiger et al., 1997). Fish are relatively sensitive to changes in the environment, so the harmful effects of pollutants may be evident in cells and tissues, before significant changes in behavior or outward appearance can be identified (Silva et al., 2020).

In fish, the liver is one of the first organs to undergo the action of xenobiotics and metabolize them for later excretion. For this reason, it has been widely used as a model to study the effects of contaminants on liver functions and structures (Santos et al., 2018).

1.3 Glyphosate

Glyphosate is an organic and non-selective post-emergence herbicide that is used in both agricultural and non-agricultural areas around the world, and in agriculture, it is widely used

in genetically modified crops, and with the release of commercial production of transgenic soy, its use became potentially larger (Merotto Jr et al., 2015).

In this work, we chose to use Roundup WG®, a commercial formulation based on glyphosate (Grisolia, 2005), since in agriculture commercial formulations are used and not the active ingredients.

Thus, considering that Roundup WG® is a herbicide that, since the 70s, has been widely used in Brazil and also the scarcity of studies on possible impacts on the aquatic environment caused by this herbicide, the present work aimed to evaluate the histological alterations of this chemical compound in the liver of spotted sorubim (*Pseudoplatystoma corruscans*), one of the most cultivated native species of freshwater fish in the country, due to the excellent quality of its fish meat.

2. Method

All procedures exposed in this work are in accordance with the ethical principles adopted by the Brazilian College of Animal Experimentation (COBEA) and were analyzed and approved by the Committee on Ethical Conduct in the Use of Animals in Experiments of the State University of Maringá (Protocol No. 014 /2014).

The experiment lasted 60 days and was carried out with juvenile spotted sorubim (*Pseudoplatystoma corruscans*) acquired from a fingerling production and trade farm in the state of Mato Grosso do Sul, transported together with the Animal Transit Guide (GTA) valid throughout the national territory, issued by the Ministry of Agriculture, Livestock and Supply (MAPA) and the State Agency for Animal and Plant Sanitary Defense of Mato Grosso do Sul.

32 specimens of the species were used, with an average weight of 32.48 ± 1.77 grams and 18.60 ± 0.68 centimeters in total length. Before the beginning of the experiment, the animals were distributed in four asbestos tanks (n=8) with waterproofing paint and artificial aeration, for acclimatization, for a period of two weeks, being fed twice a day with extruded commercial feed with 40% from crude protein to apparent satiety. Before the beginning of the experiment, feeding was suspended for 24 hours, in accordance with the standards for toxicity testing with fish established by the Brazilian Association of Technical Standards (ABNT, 1993). The tanks were monitored throughout the experimental period, the temperature was kept between 20 and 24 Celsius degrees, and the oxygenation of the water was carried out by an aeration pump.

After this period, the fish were subjected to three different concentrations of Roundup WG®: 0.25g/1000 L, 0.50g/1000 L, and 0.75g/1000 L, according to the group to which they belonged, plus the control group (0.00g/1000 L). The concentrations used were chosen taking into account the maximum allowed value of glyphosate in water regulated by Ordinance MS 2914/2011 (approximately 500 µg/L of water), using a concentration between this range, a 50% above and a 50% below. Two contaminations were made, one at the beginning and the other after 30 days of the experimental period. At the end of the experiment, the animals of all groups were anesthetized with benzocaine (2g/150ml

alcohol/20 L water) and proceeded to euthanasia through the medullary section.

The liver fragments used for histological analysis were fixed in common formalin for a period of 48 hours, and the solution was subsequently replaced by 70% alcohol. Routine histological processing followed, using hematoxylin-eosin (H.E.) as dyes (Luna, 1968). The sections were analyzed under a light microscope and the alterations found were classified individually according to the degree of frequency of the tissue lesion, and for this purpose, the following score was established: 0 - no alteration observed; 1 - discrete alteration (up to two occurrences of alteration per blade); 2 - moderate alteration (three to five occurrences of alteration per lamina) and 3 - intense alteration (above five occurrences of alteration per lamina). From these values, the mean of the degree of alteration for each group was calculated, being classified as mild (0.1 to 1.0), moderate (1.1 to 2.0), and intense (above 2.1). The mean values obtained were compared to each other employing analysis of variance ANOVA, after a normality test. When indicated, differences were identified by Tukey's t-test. Values of $P \leq 0.05$ were considered significant.

3. Results and Discussion

Regarding biometric parameters, only the control group (0.00 g/1000 L) had a statistically significant increase in body mass during the experiment. In the other groups, at the end of the experimental period, despite the weight gain, the difference was not significant in relation to the beginning of the experiment (Table 1). The same occurred in relation to the length of the animals, where the difference was only considered statistically significant in the group that was not exposed to the herbicide (Table 1).

Table 1. Means and standard deviations of weight and length of fish from groups exposed to different concentrations of the herbicide at the beginning of the experiment (Day 0) and at the end of the experiment (Day 60)

	Period	Weight (g)	Length (cm)
Control	Day 0	32,85±1,61 ^a	18,36±0,51 ^a
	Day 60	43,99±5,68 ^b	22,08±1,46 ^b
0,25g/1000L	Day 0	32,72±1,60 ^a	18,88±0,82 ^a
	Day 60	38,56±13,06 ^a	21,18±2,31 ^a
0,50g/1000L	Day 0	32,14±1,78 ^a	18,48±0,84 ^a
	Day 60	37,25±13,30 ^a	20,89±1,99 ^a
0,75g/1000L	Day 0	32,24±2,11 ^a	18,68±0,58 ^a
	Day 60	36,20±12,94 ^a	20,65±2,06 ^a

Means followed by different letters, of the same degree of contamination and in the same column, differ from each other by Tukey's t-test ($P \leq 0.05$).

Blood glucose showed no statistical difference between groups, although it did show an increase in glycemic levels in animals exposed to different concentrations of the herbicide (Table 2).

Table 2. Means and standard deviations of blood glucose in fish from groups exposed to different concentrations of the herbicide at the end of the experiment

	Glucose (mg/dL)
Control	57,88±13,18 ^a
0,25g/1000L	71,75±10,28 ^a
0,50g/1000L	74,88±11,75 ^a
0,75g/1000L	77,88±19,74 ^a

Means followed by equal letters in the same column do not differ by Tukey's t-test ($P \leq 0.05$).

The little weight gain and the low total growth rate of the fish suggest that the search for the maintenance of the organism, under the action of the herbicide, requires a great demand for energy and, one of the central aspects of adaptation to stress is the reallocation of this energy away from high energy demand activities, including growth. Such dynamics can considerably reduce fish performance capacity during both acute and chronic stress (Schreck, 1990; Kebus et al., 1992; Pankhurst and Kraak 1997; Mommsen et al., 2009; Santana and Cavalcante, 2016).

In addition to this aspect, Blier (2002) suggests that growth can be determined by the availability of oxygen for tissue metabolism and protein synthesis. It is already known that pesticides, in general, can interfere with tissue oxygen availability and protein synthesis, as shown by other studies (Gimeno 1995; Oruç and Uner, 1999; Begum, 2004; Zeni et al., 2016). In this study, the nutrients from digestion probably had their use compromised by the action of the herbicide since in the control group the weight of the animals was significantly higher at the end of the experiment, as well as the total length. Comparable results to this work were also found in studies conducted by Salbego et al. (2010) and Albinati et al. (2009) with fish exposed to glyphosate and Roundup, respectively.

Although blood glucose was not significantly different between groups, the results indicate an increase in glycemic levels in animals exposed to the herbicide compared to the control group. Langiano and Martinez (2008) also found hyperglycemia in *curimatás* (*Prochilodus lineatus*) exposed to 10 ppm of Roundup. The concentration of 100 mg.L⁻¹ of Difluzuron, in acute exposure, also caused an increase in glycemia in *Prochilodus lineatus* (Maduenho and Martinez, 2008).

A quite common response in fish under some stress conditions is the increase in plasma glucose as an energy source for the maintenance of body homeostasis. As fish require a large demand for energy in these situations, the mobilization of energy reserves through endocrine pathways can be considered an adaptive mechanism that allows the organism to meet its needs during exposure to stressful factors (Tavares-Dias and Moraes 2004; Kavitha, 2012).

In the histopathological evaluation of the liver, the three groups that were exposed to the herbicide showed some degree of tissue alteration, and the lesions considered severe were hemorrhage, vacuolization, and hypertrophy of hepatocytes and the presence of free

melanomacrophages (Figure 1), recorded in the exposed groups at 0.50 and 0.75 g/1000 L. Tissue necrosis was recorded discretely in the group exposed to 0.75 g/1000 L (Figure 1). In the groups exposed to 0.50 and 0.75 g/1000 L, sinusoid congestion was also recorded slightly (Figure 1). Bleeding, vacuolization of hepatocytes, and the presence of free melanomacrophages were moderately recorded in the tissue of fish exposed to 0.25 g/1000 L (Table 3).

Figure 1 Photomicrographs of the liver tissue of *Pseudoplatystoma corruscans* exposed to Roundup WG® herbicide for 60 days. (A) liver tissue from a control group animal, showing the arrangement of hepatocytes (B) bile duct of the liver parenchyma from a control group animal (asterisk) (C) Hypertrophy and vacuolization (arrow) of the hepatocytes of the liver tissue from an animal exposed to 0.75 g/1000 L of water (D) Liver hemorrhage in an animal exposed to 0.75 g/1000 L of water (black arrow) (E) Centers of melanomacrophages (circle) and free melanomacrophages (thick arrow) in the liver of animal exposed to 0.75 g/1000 L of water and (F) focal necrosis in the liver tissue of an animal in the group exposed to 0.75 g/100 L of water. HE, 400X.

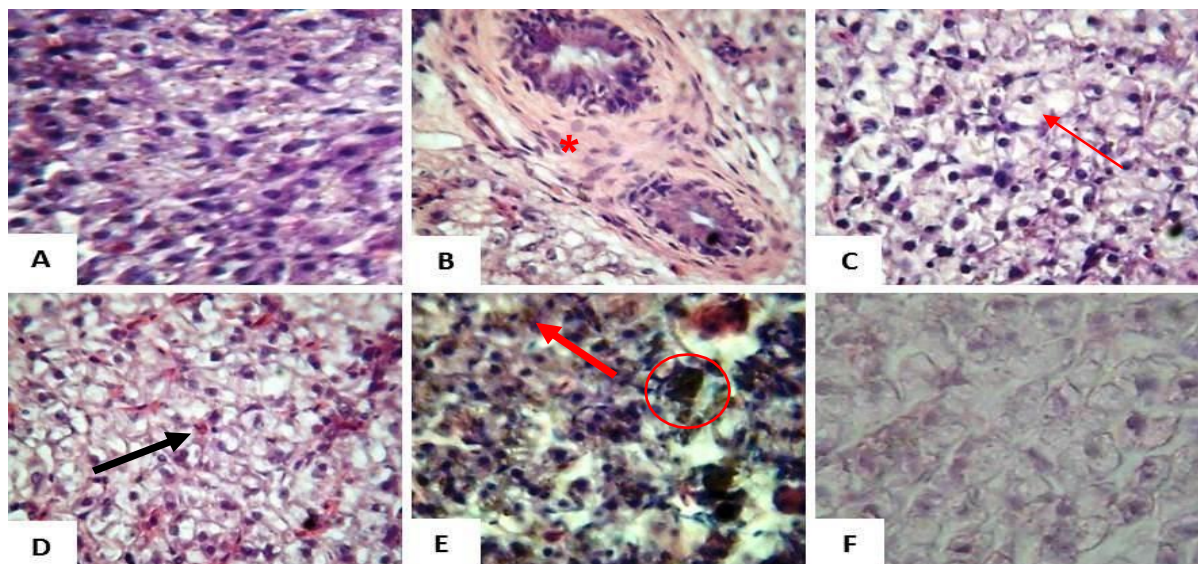


Figure 1. Photomicrographs of the liver tissue of *Pseudoplatystoma corruscans* exposed to Roundup WG® herbicide for 60 days

Table 3. Means and standard deviations of the degree of histological lesions observed in liver sections from fish exposed to different concentrations of the herbicide after 60 days of exposure

	Control	0,25g/1000L	0,50g/1000L	0,75g/1000L
Necrosis	0,00±0,00 ^a	0,00±0,00 ^a	0,00±0,00 ^a	0,75±0,55 ^b
Congestion	0,00±0,00 ^a	0,00±0,00 ^a	0,25±0,29 ^a	0,87±0,58 ^b
Bleeding	0,00±0,00 ^a	1,25±0,59 ^b	2,12±0,66 ^c	3,60±0,67 ^d
Vacuolization	0,00±0,00 ^a	1,87±0,35 ^b	3,37±0,74 ^c	5,12±1,88 ^d
Hypertrophy of hepatocytes	0,37±0,35 ^a	0,87±0,23 ^a	2,00±0,53 ^b	3,37±0,51 ^c
Melanomacrophage center	0,00±0,00 ^a	0,12±0,13 ^a	0,37±0,29 ^a	0,87±0,50 ^b
Free melanomacrophages	0,50±0,35 ^a	1,75±0,66 ^b	2,62±0,83 ^c	4,00±0,55 ^d

Means followed by different letters on the same line differ by Tukey's t-test ($P \leq 0.05$).

The liver alterations observed were more accentuated according to the increase in the dose to which the animals were exposed and, although most studies are carried out with acute exposure, the results are similar to the findings of this study (Table 3).

In a study by Jiraungkoorskul et al. (2002) with tilapia exposed to Roundup® at 36 ppm, liver alterations such as hypertrophy, vacuolization of hepatocytes, degeneration, and tissue necrosis were observed. Congestion, hepatocyte hypertrophy, and vacuolization were alterations found by Henares et al. (2008) in *Oreochromis niloticus* exposed to the herbicide Diquat. In a study with *Oncorhynchus mykiss* exposed to 1.3 µg/L of Endosulfan for 21 days, hepatocyte hypertrophy, necrosis, hepatocyte cord disarray, melanomacrophage centers, and free melanomacrophages in the liver of animals were recorded (Altinok and Capkin, 2007). Albinati et al. (2009) recorded congestion, hemorrhage, and necrosis in the liver of piauçu (*Leporinus macrocephalus*) exposed to 7.5ppm of Roundup. According to Filho et al. (2014), the main alterations found in fish livers are vacuolization, hypertrophy of hepatocytes, increased frequency of melanomacrophages, and congestion, which confirms the results of this work.

Hepatocytes are uninucleate cells with a polygonal shape that have important metabolic functions. The hypertrophy of these cells may be related to an increase in the number of organelles responsible for detoxification and is a reversible or transitory phase. The increase in liver cells reflects its physiological functional state, which can present itself in a hyperfunctional state, where there is an increase in cell volume due to increased metabolism, or hypofunctional, where there is a decrease in cell volume (Lins et al., 2010).

The liver is an organ sensitive to intoxication and metabolic changes. Changes such as vacuolization of hepatocytes can be interpreted as responses to environmental stress, being considered histopathological indicators of environmental quality (Thomas, 1990; Teh et al.,

1997). Vacuolization may also be related to an increased accumulation of glycogen in the liver. In fish, although hepatocytes are usually full of glycogen or neutral fat, if the food is sufficient, during the stress response phase there may be a mobilization of liver glycogen to meet the body's energy needs, which in turn increase under conditions of stress (Heath 1995; Oliveira et al., 2010).

Changes such as congestion, hemorrhages, and necrosis are related to intoxication processes, and the extent and severity of the injury are proportional to the type, duration, severity, and physiological state of the cell involved (Robbins et al., 2005). Fish livers are especially sensitive to the action of chemicals due to the slowness of blood flow. Therefore, the toxic elements that reach the liver through the bloodstream exert their effects on hepatocytes for a longer time than they would in mammals (Campos, 2008). Congestion is an injury that, despite causing damage to tissue function, can be reversible in case of improvement in water quality or progressive in cases of persistent contaminant. Changes such as necrosis are processes in which there is a reduction or functional loss of the organ, therefore, irreversible (Nešković et al., 1996).

The presence of a melanomacrophage center in the liver parenchyma may be related to the presence of chemical agents in the body (Rabitto et al., 2006; Leknes, 2007; Mela et al., 2007; Hinton, 2008). Melanomacrophage centers are believed to accumulate pigments like melanin within them. Elevated levels of free melanin, resulting from the rupture of melanomacrophages, may have a protective effect against pollutants, as this compound can absorb or neutralize free radicals derived from the degradation of phagocytosed material and, thus, protect the body against cell damage (Agius and Roberts, 2003). Mela et al. (2007) suggest that free melanomacrophages have a tendency to aggregate forming melanomacrophage centers, therefore, it is assumed that with a longer exposure time, an even greater number of melanomacrophage centers would be formed. Thus, the results of this study suggest that the fish suffered the action of the herbicide, since it stimulated melanomacrophage responses, thus justifying its presence in the liver.

4. Conclusion

In summary, the results obtained in this work show that the herbicide Roundup WG® promotes important biometric and histological alterations in *Pseudoplatystoma corruscans*. Severe liver damage can impair tissue function. Although the changes occurred more frequently in animals exposed to the highest concentration of the herbicide, even when the level of contamination was below the maximum allowed limit of glyphosate for water, the animals showed some kind of interference in fundamental processes for the maintenance of their body homeostasis.

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