

## **Acute toxicity of cassava residues in *Hyphossobrycon eques* Steindachner (1982)**

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### **Abstract**

Detoxification of Cassava is a fundamental process for processing the roots into by-products, but this process occurs stocking roots in water. However, its toxicity released into aquatic environment still is unknown. For this reason, this study evaluated the acute toxicity of effluent with cassava root residue on the fish *Hyphossobrycon eques*. Therefore, sensitivity tests were performed with five concentrations of KCl (0.4; 0.9; 1.4; 1.9; and 2.4 g.L<sup>-1</sup>) and acute toxicity test (LC<sub>50-96h</sub>) with eight effluent concentrations with cassava root residue (0; 3.8; 4.7; 5.7; 6.6; 7.6; 8.5 and 9.5 mg.L<sup>-1</sup>), and four replicates. During the toxicity test, behavioral changes and mortality rate were observed. The estimated sensitivity for *H. eques* was 1.12 g.L<sup>-1</sup>, and the lethal concentration at 50% (LC<sub>50-96h</sub>) of the effluent with cassava root residue was 6.2 mg.L<sup>-1</sup>, classified as moderately toxic xenobiotic. During the experimental period, 100% mortality was observed at the highest concentration (9.5 mg.L<sup>-1</sup>) and behavioral changes such as agitation, erratic swimming and increased opercular beating of fish exposed to the xenobiotic. Therefore, the effluent with cassava root residue is a risk to the aquatic environment, causing changes in the behavior and mortality of *H. eques*.

**Keywords:** toxicity, hydrocyanic acid, cassava

### **Introduction**

Cassava (*Manihot esculenta*) stand out as one of the most culture vegetables among the farmers due to the easy collect, low cost of production, resistant to adverse condition on the ground, dry season and resistance against insects or diseases <sup>[1]</sup>. Meal is one of the several by-products of cassava, but for its production, farmers put the cassava

into water (rivers) during 2 or 8 days to release all toxic compounds and make it softer (named pubagem) [2].

The cyanuric acid (HCN) released into water during the “pubagem” is generated by the cyanogenic glycoside compounds linamarin and lotaustralin, responsible for root toxicity [3]. Therefore, submerged roots in water can release 50% of cyanide acid in less than 24 hours, and when it is put directly in rivers or streams of running water, the presence of unpleasant odors is observed as well as yellow coloration of the water [4,5].

The residue of cassava roots released into the water cause damage to the aquatic environment, which can cause biochemical and tissue changes and even fish mortality due to cyanide intoxication [6,7]. However, there is no information on the toxicity of this cassava root residue during the cyanide detoxification process to fish in the Amazon region, which may be a risk to these organisms, which may lead to fish mortality in rivers and streams.

The toxicity test can be used as an efficient tool in monitoring the risks caused by xenobiotics to aquatic organisms [8,9]. The Amazonian species *Hyphossobrycon eques* is used as a biological model in toxicity tests due to its rapid response in the presence of contaminants [10,11]. Therefore, this study evaluated the acute toxicity of the effluent with cassava root residue in the fish *Hyphossobrycon eques*.

## Material and method

Specimens of *H. eques* were captured (Sisbio 19515) at Santa Ana station (01° 08' 40.12" S and 46° 34' 04.8" W) of the Chumucuí River microbasin, Bragança, Pará, Brazil. All fish were acclimatized into 300L water tanks remaining in feeding deprivation 24 hours before the toxicity test [9].

The effluent containing cassava root residues (CRR) was obtained by adding 2kg of cassava root in 12L of water during 4 days [2]. Preliminary tests (Screening) were carried out to determine the effluent concentration with residue from cassava roots (RRM) that would cause zero and 100% mortality (IBAMA, 1987). A sensitivity test (LC<sub>50-96h</sub>) was performed with potassium chloride (KCl), a reference substance, with five concentrations of KCl (0.4; 0.9; 1.4; 1.9; and 2.4 g.L<sup>-1</sup>), with one control and four replicates, containing 16 fish (1.12 ± 0.11 g) per replicate.

To perform the definitive acute toxicity test (LC<sub>50-96h</sub>) of cassava root residue (CRR), a completely randomized design occurred with eight concentrations (0; 3.8; 4.7;

5.7; 6, 6, 7.6, 8.5 and 9.5 mg.L<sup>-1</sup>), and four replicates containing 20 fish (1.14±0.10 g) per treatment.

The test was conducted in static system lasting 96 hours evaluating behavioral changes according to the methodology of Couto et al. [8] and Sousa et al. [9]. The physicochemical variables of water such as dissolved oxygen, temperature (Oxygenmeter LT lutron DO-5519), pH (QUIMIS® Q-400BC/BD), electrical conductivity (HANNA, HI-8733) and total ammonia (HANNA HI 93715) were measured daily.

Mortality was observed every hour, dead fish were removed with the aid of nets and counted. At the end of the tests, the mean lethal concentration (LC<sub>50-96h</sub>) was determined by the Trimmed Spearman Karber method [12]. After obtaining the LC50 value, the xenobiotic was classified according to the description adapted from Zucker [13], which classifies xenobiotic compounds as: highly toxic (when the LC50 is less than 0.1 mg/L), in very toxic (0.1 mg/L > LC50 > 1 mg/L), moderately toxic (1mg/L > Cl50 > 10mg/L), slightly toxic (10 mg/L > Cl50 > 100 mg/L) and practically non-toxic (Cl50 > 100 mg/L). Pearson's correlation (5%) was performed between mortality rate and xenobiotic concentrations.

## Results

During the experimental period, pH values were 5.93 ± 0.32, dissolved oxygen of 6.22 ± 0.11 mg.L<sup>-1</sup>, temperature 28.1 ± 0.150 °C, conductivity of 137 ± 10.9 μS.cm<sup>-1</sup> and total ammonia of 0.48± 0.09 mg.L<sup>-1</sup>.

The estimated sensitivity (CL<sub>50-96h</sub>) for the species *H. eques* to potassium chloride (KCl) was 1.12 g.L<sup>-1</sup>, with a lower limit of 0.95 g.L<sup>-1</sup> and an upper limit of 1.34 g.L<sup>-1</sup>. Regarding the acute toxicity of the effluent with cassava root residue (CRR), the estimated lethal concentration at 50% (LC<sub>50-96h</sub>) was 6.2 mg.L<sup>-1</sup> with a lower limit of 5.8 mg.L<sup>-1</sup> and an upper limit of 6.6 mg.L<sup>-1</sup>, being classified as a moderately toxic xenobiotic.

During the exposure of *H. eques* specimens to cassava root residue (CRR), a positive correlation ( $r^2 = 0.9858$  and  $p=0.0032$ ) was observed between the mortality rate and the xenobiotic concentration (figure 1). Total mortality (100%) was observed in the highest concentration evaluated (9.5 mg.L<sup>-1</sup>) in less than 24 hours. Regarding behavioral changes, there was an increase in opercular beating, erratic swimming, surface positioning, agitation and lethargy, mainly in the two highest concentrations (Table 1).

Figure 1: Pearson correlation ( $r$  and  $p$  value) between mortality rate (%) and effluent concentration with cassava root residue during the acute toxicity test on *H. eques* species.

	Concentration of Cassava roots residues							
	0.0	3.8	4.7	5.7	6.6	7.6	8.5	9.5
Shaking	-	-	-	-	+	+	+	+
Erratic swimming	-	-	+	+	+	+	+	+
Remaining in water surface	-	+	+	+	+	+	+	+
Rapid opercular beating	-	-	-	-	+	+	+	+
Letargy	-	-	-	-	-	-	+	+

(+) presence and (-) absence of signs of behavioral changes in fish.

## Discussion

Aquatic ecosystem has been altered by human actions as discarding pesticides, industrial residues and other xenobiotics [8, 14, 15] making it a problem for fish causing tissue, hematological and behavioral alterations. In specific reports, there is a mortality of some more sensitive fish which have no ability to recover the normal status [8, 9, 11, 16].

Sensibility of fish during the toxicity assays can indicate the relationship between organism and concentration of xenobiotic, and this could be used as an efficient tool to determine biological and bioindicator models [9, 11]. In the present study, estimated value of sensitivity for *H. eques* ( $1.12 \text{ g.L}^{-1}$ ) exposed to potassium chloride (KCl) was similar to that reported by [10] and Fujimoto et al. [17]. Emphasizing that sensitivity is different between species and even with the stage of development of the organism [11], and animals with greater sensitivity respond more quickly to the harmful effects caused by xenobiotics, and may even result in mortality [9, 18].

There are few reports on the toxicity of effluent with cassava root residues for Amazonian species, where rivers and streams are used as stores of cassava roots for the hydrocyanic acid detoxification process [19]. According to Oghenejoboh [20], in one week, roots submerged in water can release up to  $38.2 \pm 3.4 \text{ mg.L}^{-1}$  of cyanide, which can change the quality of the water causing fish mortality [19, 21].

The toxicity of contaminated effluent with cassava root residues has already been reported in the species *Clarias gariepinus* [6, 21] and *Oreochromis niloticus* [22]. In the

present study, the highest concentration (9.5 mg.L<sup>-1</sup>) of effluent with cassava root residues in the species *H. eques* caused 100% mortality with lethal concentration (LC<sub>50</sub>) estimated at 6.2 mg. L<sup>-1</sup>, for the species *C. gariepinus*, with an estimated value of (CI<sub>50</sub>) 9.765 mg.L<sup>-1</sup>, with 100% mortality at the highest concentration tested (30 mg.L<sup>-1</sup>) [7].

Behavioral changes are quick and sudden responses of the animal to the stress caused by intoxication with the xenobiotic [7, 8, 9, 23]. In the present study, shaking, erratic swimming, increased opercular beating and remain in water surface were observed, mainly at the highest concentrations tested (6.6 to 9.5 mg.L<sup>-1</sup>). Similarities were observed for *C. gariepinus* [7], *O. niloticus* [8] and *Pterophyllum scalare* [9] exposed in toxicity tests. Such changes are possible adaptation responses, such as increased opercular beating, to increase gas exchange and thus provide oxygen to internal organs, allowing the animal to survive in the contaminated environment [8, 9].

## Conclusion

Residues of Cassava released in the water during the pubagem process is a risk factor for aquatic organism with lethal concentration (CI<sub>50</sub>) of 6.2 mg.L<sup>-1</sup> for *H. eques*, causing behavioral changes and mortality of the species.

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