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Acute toxicity of the water-soluble fraction of diesel in *Prochilodus vimboides* Kner (Characiformes: Prochilodontidae)

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Diesel oil can be a source of contamination in aquatic environments, mainly as a result of spills. The effects of the water-soluble fraction of diesel (WSF) on *Prochilodus vimboides* were assessed. Fish were exposed to three different WSF dilutions for up to 96 h and were compared to a control group. Damages in the fragments of DNA were analyzed using the Comet assay. The presence of erytrocyts abnormalities was assessed by micronucleus test. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity and the accumulation of copper in gills were also analyzed. Fish exposed for 96 h had higher rates of damage than those exposed for 24 h. There was no significant difference regarding the presence of micronuclei between exposed and control fish and between 24-h and 96-h exposures. For AST, no significant difference was observed between samples collected at the two exposure times. Fish exposed to a 1:100 dilution of WSF showed higher activity of the enzyme ALT than the control fish after a 24-h exposure period. There was no bioaccumulation of copper in the gills. We conclude that the genotoxic effects of WSF in the cells are more evident in *P. vimboides* during an acute exposure.

O óleo diesel pode ser uma fonte de contaminação em ambientes aquáticos, principalmente como resultado de derrames acidentais. Foram avaliados os efeitos da fração solúvel do óleo diesel (FSO) em *Prochilodus vimboides*. Os peixes foram expostos a três diferentes diluições da FSO por até 96 horas e comparados com um grupo controle. Os danos nos fragmentos de DNA foram analisados utilizando o ensaio Cometa. A presença de anormalidades nos eritrócitos foi avaliada pelo teste do micronúcleo. A atividade da Aspartato aminotransferase (AST) e alanina aminotransferase (ALT) e da acumulação de cobre nas brânquias também foram analisados. Os peixes expostos por 96 h tiveram maiores taxas de danos do que aqueles expostos por 24 h. Não houve diferença significativa quanto à presença de micronúcleos entre os peixes expostos e controle e entre 24 e 96 h exposições. Para AST, não foi observada diferença significativa entre as amostras coletadas em dois tempos de exposição. Os peixes expostos a uma diluição de 1:100 do FSO apresentaram maior atividade da enzima ALT do que os peixes do controle após um período de exposição de 24 horas. Não houve bioacumulação de cobre nas brânquias. Os efeitos genotóxicos nas células foram os mais evidentes em *P. vimboides* durante a exposição aguda a FSO.

Key words: Comet assay, Curimba, Diesel, Micronucleus.

Introduction

In recent years, the rivers of South America have been contaminated by diesel oil. This oil derivate exhibits low solubility in water, and the water-soluble fraction contains several toxic constituents like hydrocarbons and metals. The water-soluble fraction (WSF) of crude oil and their derivatives products contains a mixture of polycyclic aromatic hydrocarbons (PAHs), monoaromatic hydrocarbons often referred to as BTEX (benzene, toluene, ethylbenzene and xylenes), phenols and heterocyclic compounds, containing nitrogen and sulfur (Saeed & Al-Mutairi, 1999), and also heavy metals (Rodrigues *et al*., 2010). The most frequently metals found in the diesel oil and lubrificant oils were copper and nickel (Silveira *et al*., 2006). Due their toxicity and potential to accumulate, copper deserves more attention among then.

The polyaromatic hydrocarbons (PAHs), such as naphthalene, fluorine and phenanthrene (Simonato *et al*., 2008) are among the chemical components of diesel oil that pose the greatest environmental hazard (Lee & Anderson, 2005). The acute toxicity of hydrocarbons is known to cause damage in several organisms, including in their DNA (Zhang *et al*., 2004; Kennedy & Farrel, 2005). Comet assay it is a rapid, sensitive and relatively inexpensive method providing the opportunity to study DNA damage (including oxidative damage), repair and cell death (apoptosis) in different cell types without prior knowledge of

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karyotype and cell turnover rate (Jha, 2008). For that this analyze has been shown to be a relatively sensitive and broad specificity indicator of genotoxic pollutant exposure (Mitchelmore & Chipman, 1998). The micronucleus test is one of the most popular tests of environmental genotoxicity, has been used as an indicator of cytogenetic damage for more than 30 years and is considered to be a well-established indicator of genotoxicity (Fenech *et al*., 2003; Çavas *et al*., 2005). The PAHs also cause damage to fish tissues thereby leading to leakage of enzyme to the extracellular fluid increasing the serum activity of several enzymes like those from amino group (Adeyemi *et al*., 2009).

Prochilodus vimboides is a freshwater migratory fish species from Southern Brazil used as human food that present high sensitivity to metal contamination (Gomes *et al*., 2009). This fish species were subjected to fast exposures to contaminant dissolved in the water due their ability to escape and the lotic characteristics of their habitat. This study tested the hypothesis that *Prochilodus vimboides* show several genetic and tissue damage and has potential to accumulate copper even under acute exposure to WSF.

Material and Methods

Animals and acclimation. *Prochilodus vimboides* juveniles (n = 72) were obtained from the Instituto Federal de Colatina, Espírito Santo, Brazil. The fish (9.83 ± 5.84 g; 9.48 ± 1.60 cm; mean ± SD) were transported to the laboratory and kept in a 500-L tank for 15 days, with water supplied by local water company and constant aeration.

In the tanks, fish were fed twice a day on a 40% protein pellet fish food (Nutriave, ES, Brazil). During acclimation, the physicochemical parameters of the water were monitored every 3 days using a multiparameter YSI 85 instrument and a digital pH meter. The parameters analyzed were (mean ± SD) temperature (25.6 ± 0.4ºC), conductivity (148.6 ± 33.3 µS/cm), dissolved oxygen (6.2 ± 0.4 mg/L), pH (7.3 ± 0.2) and total hardness (27.75 ± 0.01 mg/L $CaCO_3$).

Preparation of the diesel water-soluble fraction. Commercial diesel oil (B2; density 0.832 g/cm³) was purchased at a gas station. The container used to transport the diesel oil was lined with a black plastic bag to prevent any sort of alteration in its physiochemical parameters.

The water-soluble fraction of diesel (WSF) was prepared strictly according to the methodology described by Singer *et al*. (2000). Initially, a 5-g sample of diesel oil was weighed and transferred to a 1-L volumetric flask, which was filled with water from the acclimation tank to the 1-L mark. The water and oil solution was then transferred to an amber flask and shaken for 24-h, with care to prevent the formation of a vortex. The resulting WSF was removed using a pipette. The composition of the WSF was analyzed by gas chromatography (Perkin Elmer, Clarus 500) to detect total PHA, benzene, toluene, xylene, naphthalene, phenanthrene and fluorene. A sample of WSF was digested and analyzed in a graphite furnace atomic absorption spectrometer (EAA-FG; GBC Avanta 932, IL, USA) to establish copper and nickel contents.

Toxicity tests. After the acclimation period, the fish (n = 72) were transferred to 72 aquaria (2-L) with constant aeration. One fish was placed in each aquarium. Four treatments were defined according to WSF dilutions: 1:1,000; 1:500; 1:100 and one control group. WSF dilutions were chosen based on previous studies on fish exposure to WSF (Santos *et al*., 2010). In total, the exposure period lasted 96-h and fish were sampled at 24 and 96-h (n = 9 fish per treatment on each time). Half the contents of aquaria containing fish exposed to the three different WSF dilutions for 96-h was replaced at 48-h during the exposure period by an equal volume of the respective WSF dilution (Santos *et al*., 2010).

At the end of the experiment, fish were killed in a solution containing a lethal concentration of benzocaine (300 mg/L). Blood samples were collected from each specimen by caudal vein puncture using heparinized syringes. This methodology is in accordance with the procedures described in the guide for the use of fish in research published by the American Fisheries Society (Nickum *et al*., 2004). The gills of the fish were excised and placed in a refrigerator at -20ºC for copper bioaccumulation analysis.

During the experiment, water physicochemical parameters were measured using a multiparameter YSI 85 instrument. The measurements obtained included (mean ± SD) temperature (25.5 ± 0.3ºC), dissolved oxygen (6.9 ± 0.5 mg/L), conductivity (87.5 ± 2.9 µS/cm), pH (7.05 ± 0.3) and total hardness (28 ± 0.3 mg/L $CaCO_3$).

Comet assay. The blood samples were diluted 1:120 (v/v) with RPMI 1640 medium and used immediately. The alkaline Comet assay was performed as described by Tice *et al*. (2000). Briefly, 5 µL of each diluted blood sample was added to 95 µL of 0.75% (w/v) molten low melting point agarose, and a portion of the mixture was spread on a microscope slide pre-coated with 1.5% (w/v) normal melting point agarose and covered with a coverslip. After the agarose solidified, the coverslips were removed, and the slides were immersed in a lysis solution (2.5 M NaCl, 100 mM EDTA and 10 mM Tris, pH 10.0–10.5) containing 1% Triton X-100 and 10% DMSO. The slides were kept frozen in lysis solution (4°C), and protected from light for at least 2 h and at most 3 h. Subsequently, slides were incubated in freshly prepared alkaline buffer (300 mM NaOH and 1 mM EDTA, pH ≥ 13, which was experimentally determined) for 20 min for DNA unwinding. Electrophoresis (15 min at 300 mA and 25 V [0.8 V/cm]) was performed in the same buffer. Every step was carried out under indirect yellow light. After electrophoresis, slides were neutralized in 400 mM Tris (pH 7.5), rinsed three times in distilled water, and left to dry overnight at room temperature. Slides were then fixed for 10 min in 15% trichloroacetic acid (w/v), 5% zinc sulfate (w/v), and 5% glycerol (v/v), rinsed three times in distilled water, and dried for 2 h at 37°C. The dry slides were rehydrated for 5 min in distilled water and then stained (5% sodium carbonate (w/v), 0.1% ammonium nitrate (w/v), 0.1% silver nitrate (w/v), 0.25%

tungstosilicic acid, 0.15% formaldehyde (w/v), freshly prepared in the dark) with constant shaking for 35 min. The stained slides were rinsed twice with distilled water and then submerged in the stop solution (1% acetic acid), rinsed again, and immediately coded for analysis. To calculate image length, 200 cells from each fish were randomly chosen (100 from each duplicate slide) and analyzed under an optical microscope (100× magnification). We analyzed 200 cells per animal and utilized the following visual classification groups based on the migration of DNA fragments from the nucleus: class 0 (no damage), class 1 (little damage - less or equal to the diameter of one nucleus), class 2 (medium damage - greater than the diameter of two nuclei), class 3 (extensive damage - greater than two times the diameter of one nucleus) and class 4 (apoptosis - cell death) (Collins *et al.*, 1995; Kobayashi *et al.*, 1995). The data are presented as the frequency of cells per class of damage. The comet score (CS) for each fish was calculated as the sum of the number of nucleoids observed in each damage class multiplied by the value of its respective damage class (0, 1, 2, 3, or 4). The results are expressed as comet score for each treatment group, where 0 represents the absence of damage and 400 indicates the highest damage score.

Micronucleus test. Peripheral blood samples obtained from the caudal vein of the specimens and smeared on clean slides. Slides were left to dry in the laboratory environment overnight, and then the smears were fixed in methanol for 15 min, stained for 20 min with 5% (w/v) Giemsa, washed with distilled water and left to dry in the laboratory. After drying, 2,000 erythrocytes per fish (1,000 from each duplicate slide) were analyzed for the presence of micronuclei at 100× magnification under a light microscope (Al-Sabati and Metcalfe, 1995).

Aspartate aminotransferase and alanine aminotransferase (AST and ALT). The blood samples were centrifuged at 151.2 x g for 10 min. The plasma was used to quantify the enzyme activity of aspartate aminotransferase (AST) by kinetic method. The AST catalyzes the transfer of amino groups of aspartate for α-ketoglutarate, leading to formation of glutamate and oxalacetate. Oxalacetate in the presence of MDH reacts with NADH, reducing itself into Malate NADH oxidates to NAD+. The rate of oxidation is proportional to AST activity in the sample. The alanine aminotransferase (ALT) was also determined according to kinetic method. The ALT catalyzes the transfer of amino groups of alanine for α-ketoglutarate, leading to formation of pyruvate and glutamate. Pyruvate in the presence of LDH reacts with NADH, reducing the Lactate and oxidizes NADH to NAD+. The rate of oxidation is proportional to ALT activity in the sample. Both enzymes were analyzed using the enzyme kit Bioclin (Bioclin, MG, Brazil) and a UV/Visible spectrophotometer.

Copper bioaccumulation. The digested samples were analyzed in a graphite furnace atomic absorption spectrometer (GF AAS - ZEEnit 700, Analytik Jena, Germany) equipped with a transversely heated graphite atomizer (THGA) and a transverse background corrector system based on the Zeeman effect (2-field mode with maxima strength of 1.0 Tesla). An autosampler MPE 60, platform graphite tubes and a hollow cathode lamp (Cu -Analytik Jena - operated at a current of 2.0 mA) were used. The measurements were performed in peak area (N = 3) at 328.8 nm, with the spectral band pass at 0.8 nm. The results were expressed as μg Cu/g fresh tissue. Several metals were assessed in WSF, such as nickel and copper. It was choose to study the bioaccumulation of copper in the gill of the fish because of its toxicity and their abundance detected in the analysis of the WSF.

Statistical analysis. All obtained data were submitted to a Kolmogorov-Smirnov test to verify their distribution. The micronucleus and damage frequencies results did not presented a normal distribution even after log transformation. Therefore, the micronucleus and damage frequencies in the erythrocytes of the fish exposed to dilutions of WSF were compared to the respective control fish using a Kruskal-Wallis ANOVA and a Dunn's test ($p<0.05$). Difference between the two exposure times (24 and 96 h) for a particular dilution were compared using a Mann-Whitney test. The CS, copper concentration in the gills as well as AST and ALT of the fish exposed to the WSF solutions were compared to the respective controls using ANOVA and a Dunn's test ($p<0.05$) and difference between the two exposure times (24 and 96 h) for a particular dilution were compared using a T-test.

Results

During the experiment there was no mortality and no change in fish behavior during the exposure.

Diesel water-soluble fraction content. The composition of the WSF was: total PHA - 4.3 mg/L, benzene - 0.10% v/v, toluene - 0.56% v/v, xylene - 2.0% v/v, naphthalene - 4.6 mg/L, phenanthrene - 0.31 mg/L and fluorene - 0.24 mg/L. The copper and nickel contents were 3.904 ng/mL and 4.00 ng/mL, respectively.

Comet assay. With respect to class 1 damage (which defines little damage to the DNA of erythrocytes), except for *P. vimboides* exposed to a 1:1,000 dilution of WSF for 24-h, no significant differences were observed in fish treated with the other WSF concentrations (1:500 and 1:100) in comparison to the respective controls. For class 2 damage (which denote moderate damage), *P. vimboides* exposed to 1:500 and 1:100 WSF presented the highest number of damaged cells in comparison to the respective controls. For class 3 (extensive damage), only the fish exposed to 1:100 WSF presented significantly more damaged cells in comparison to the control. For class 4 (apoptosis feature), the median was zero for all WSF dilutions, with no significant differences in comparison to the control (Table 1).

With regard to the 96-h exposure period, *P. vimboides* exposed to all WSF dilutions (1:1,000, 1:500 and 1:100) presented a lower occurrence of class 0 cells in comparison to the respective controls; however, a significant difference in comparison to controls was observed for the fish exposed

to the 1:100 dilution of WSF. Fish exposed to this WSF concentration presented lower frequencies of class 1 cells compared to the respective controls, and these differences were statistically significant. On the other hand, the fish exposed to 1:500 and 1:100 dilutions of WSF presented higher frequencies of class 2 and 3 cells compared to the respective controls. The fish exposed to these WSF dilutions also presented significantly higher numbers of class 4 cells than their respective controls (Table 1).

The fish exposed to all WSF dilutions (1:1,000, 1:500 and 1:100) for 24- and 96-h treatments presented higher CS than the control fish (Fig. 1). Fish exposed to WSF for 96-h presented significantly higher CS than those exposed for 24-h.

Micronucleus test. The micronucleus median was 0 for all dilutions and times of exposure. There were no significant differences in the micronuclei in erythrocytes of *P. vimboides* exposed to WSF dilutions (1:1,000, 1:500 and 1:100) for 24-h or 96-h compared to the respective control. Similarly, there was no difference in fish exposed to a particular WSF dilution for either exposure times.

Aspartate aminotransferase and alanine aminotransferase (AST and ALT). The AST activity varied between 290 and 390 URF/mL. No significant differences were observed in AST activity in *P. vimboides* exposed to the WSF dilutions 1:1,000, 1:500 or 1:100 for 24-h ($p = 0.121$) or 96 h ($p = 0.572$). Similarly, no significant differences were observed in fish exposed to a particular WSF dilution between the two exposure times (control: $p = 0.428$ and $F = 0.622$; 1:1,000: $p = 0.312$ and $F = 1.088$; 1:500: $p = 0.789$ and $F = 0.740$; and 1:100: $p = 0.365$ and $F = 0.870$).

Only the fish exposed to the 1:100 WSF solution for 24-h presented higher activity values of ALT (410 URF/mL) compared to the control (181 URF/mL). For the 96-h exposure period, no significant differences were observed between WSF dilutions and controls ($p = 0.946$). Similarly, no significant differences were observed when the two exposure times are compared in terms of a particular WSF concentration, (control: $p = 0.442$ and $T = -0.790$; 1:1,000: $p = 0.361$ and $T = -0.941$; 1:500: $p = 0.302$ and $T = -1.071$; and 1:100: $p = 0.204$ and $T = 1.329$).

Table 1. DNA damage frequency (%) in erythrocytes of *Prochilodus vimboides* exposed to different water soluble fractions of diesel (1:1,000, 1:500 and 1:100) for 24 and 96 h. Median values are presented. *: Significant difference in one dilution in comparison to respective controls using a Kruskal-Wallis ANOVA and the Dunn's test ($p<0.05$).

	Comet class				
Dilution	0	1	2	3	4
24-h exposure					
Control	69.50	30.00	0.00	0.00	0.00
1:1000	30.00	43.00*	24.00	0.00	0.00
1:500	26.00*	32.00	41.00*	0.50	0.00
1:100	21.50*	31.50	44.00*	2.50*	0.00
96-h exposure					
Control	67.00	32.50	0.50	0.00	0.00
1:1000	14.50	57.50	26.50	0.50	0.00
1:500	10.50*	25.00	48.00*	15.50*	0.50*
1:100	6.50*	15.50*	49.00*	45.25*	0.50

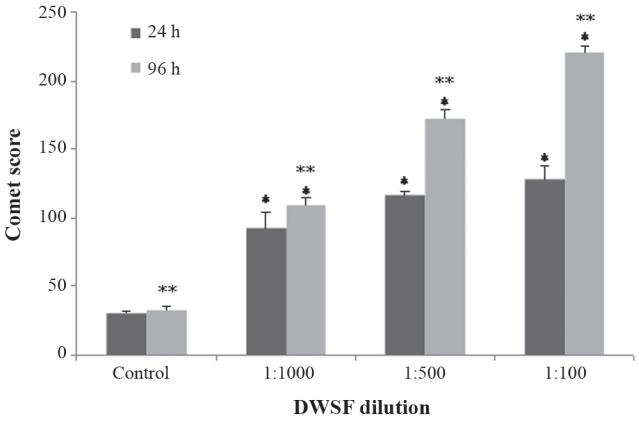

Fig. 1. Comet score (CS) in *Procholidus vimboides* exposed to different water soluble fractions of diesel (1:1,000, 1:500 and 1:100) for 24 and 96 h. The results are expressed as the mean ± standard deviation. *: Significant difference between one dilution and the respective control after 24- or 96-h exposure periods using an ANOVA and the Dunn's test ($p<0.05$). ** Significant difference between the two exposure times (24 and 96 h) for the same dilution using the T- test ($p<0.05$).

Copper bioaccumulation. Copper in the gills ranged between 23 and 33 µg/g fresh tissue. There was no significant differences in the copper concentration in the gills of the *P. vimboides* exposed to the three WSF dilutions (1:1,000, 1:500 and 1:100) for 24-h ($p = 0.160$) or 96-h ($p = 0.356$). Similarly, there was no difference in fish exposed to a particular WSF dilution for either exposure times (control: $p = 0.603$ and $F = 0.286$; 1:1,000: $p = 0.881$ and $F = 0.234$; 1:500: $p = 0.248$ and $F = 1.472$; and 1:100: $p = 0.125$ and $F = 2.668$).

Discussion

Comet assay. According to Maria *et al*. (2004), the process of biotransformation of PAHs in fish, often, converts these xenobiotics into reactive intermediate substances, which are highly toxic and cause an oxidative damage to DNA. At the cellular level, a sensitive indicator of genetic damage is the breakage of DNA that form fragments of different lengths and can be detected by Comet assay (Buschini *et al*., 2004). According to Speit and Hartmann (1995), several mechanisms have been proposed to explain DNA breaks in vertebrates, particularly in the case of exposure to PAHs. The WSF is composed mainly of PAHs, probably these compounds have influenced the damages observed in the cells of *P. vimboides*.

In the present study, the Comet assay revealed high mean frequencies of damaged cells detected in the exposed groups in comparison to control fish. When samples collected from *P. vimboides* exposed to WSF for 24-h were compared to controls,

it was observed that the lower the WSF dilution was (1:100) the higher the number of class 3 (extensive damage). In samples treated with 1:100 or 1:500 WSF for 96-h, class 4 (apoptosis feature) were detected. These data show the occurrence of damaged cells and demonstrate the high genotoxicity of WSF, which confirms previous results of genotoxic damage in *P. lineatus* exposed to WSF (Vanzella *et al*., 2007).

Lemos *et al*. (2005) showed the efficacy of the Comet assay in *Tilapia rendalli*. The organisms kept in a contaminated environment presented twice the amount of CS compared to the control group. In another study, high CS have also been reported in *Zoarces viviparus* collected in an estuary where the sediment was contaminated with several compounds, such as PAHs (Frenzilli *et al*., 2004). Pandrangi *et al*. (1995) showed an increase in DNA damage in erythrocytes of the fish species *Ameirurus nebulosus* and *Cyprinus carpio* captured in waters contaminated with PAHs. The results showed that CS was an efficient approach to evaluate WSF toxicity in *P. vimboides* at both exposure periods evaluated (24- and 96-h).

Micronucleus test. The importance of nuclear changes as biomarkers of genotoxic damage in fish has been demonstrated both in the laboratory and in the field (Al-Sabati & Metcalfe, 1995; Çavas & Ergene, 2003). In this scenario, the micronucleus test is now a key tool in the assessment of environmental pollution and of the effects of different compounds on organisms (Ergene *et al*., 2007). The data obtained in the micronucleus test in several fish species confirm the usefulness and efficiency of this test in biomonitoring studies (Rao *et al*., 1997). According to Buschini *et al*. (2004), micronuclei are considered the endpoint of genotoxicity because they may emerge after cell division. However the results of this study was not in agreement to the above affirmation, as *P. vimboides* exposed to WSF do not presented a significantly increase in MN values.

Prochilodus lineatus acute and subchronically exposed to WSF, revealed a sharp increase in micronucleus frequencies mainly in subchronic (15 days) exposure (Vanzella *et al*., 2007). Sea horse (*Hippocampus reidi*) showed an increase in MN frequency after 96-h of exposition to WSF (Santos *et al*., 2010). In the present study, there was no increase in micronucleus frequencies in *P. vimboides* exposed to a WSF for 96-h. According to Udroiu (2006) 96-h was sufficient to detect an increase in MN, therefore the main explanation to the obtained results was the low genotoxic damage of the tested dilutions.

Aspartate aminotransferase and alanine aminotransferase AST and ALT). AST and ALT are enzymes that catalyze the transfer of one amino group (NH_3) and act both in the degradation and in the biosynthesis of amino acids. These enzymes have been used as indicators of damage in tissues or metabolic changes induced by contaminants (Teles *et al*., 2003) mainly in liver. Adeyemi *et al*. (2009) reported that high levels of these serum enzymes are seen in cases of cellular death arising from toxicity.

Previous results showed that fish exposed either PAHs (Adeyemi *et al*., 2009) or metals showed an increase in the ALT and AST activity, therefore both WSF constituents can cause tissue damage. The absence of differences in ALT and AST in the present study in most *P. vimboides* samples was also observed in liver of *Oreochromis mossambicus* (Dangé, 1986) acutely exposed to PAHs. On the other hand *Oreochromis mossambicus* exposed to PAHs for 10 weeks presented a significantly alteration on ALT and AST activity in the liver (Dangé, 1986). These findings suggest that acute exposure do not cause a tissue damage or metabolic change in *P. vimboides* at used dilutions.

Copper bioaccumulation. The main sources of copper and nickel in WSF were the crude oil used to produce the diesel combustible and the additives added to the diesel formulation (Silveira *et al*., 2006). In the present study, copper was one of the more abundant metals in WSF, which suggests a great potential to accumulate in the gills of the organisms tested. Due to the direct exposure of the gills to the contaminants present in aquatic environments, this organ is considered the first choice in the analysis and monitoring of water pollution. However, the present results showed that the metal did not accumulate in the fish analyzed. It is possible that contact with metals activates the hypersecretion of gill mucus, which is a protection mechanism against metal absorption by chelation (*i.e.*, the formation of a copper complex to reduce the metal's toxicity) (Heath, 1995). Probably the metal concentration in the WSF does not produce a toxic effect in *P. vimboides*.

The frequencies of damaged cells and the CS obtained in the Comet assay were greater in lower dilutions and increase with exposure times (24- and 96-h). The micronucleus test, AST and ALT were not efficient at detecting contamination by WSF in *P. vimboides* under acute exposure. No copper bioaccumulation was observed in *P. vimboides*. Finally, the genotoxic effects of WSF in the cells were the most evident in *P. vimboides* during acute exposure.

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