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Tests of chronic and acute toxicity of crude oil on larvae of *Chironomus kiiensis* Tokunaga (Diptera: Chironomidae)

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Abstract

The Amazon region, known for its mega-biodiversity, also holds large reserves of petroleum and natural gas. The increasing exploitation of natural gas and crude oil in the Amazon has not been accompanied by studies evaluating the impact of these pollutants on local biological communities, particularly aquatic organisms. The aim of the present study was to determine the values of acute and chronic toxicity of crude oil from Urucu to larvae of *Chironomus kiiensis* Tokunaga, 1936. The $LD50_{48h}$ of crude oil for second-instar larvae of *C. kiiensis* was 26.5 mg/L, and mortality for the majority of concentrations tested was greatest during the first 24 hours of the experiment. The survival of eggs of *C. kiiensis* exposed to concentrations of crude oil was also evaluated but did not differ significantly among the treatments. Despite the high tolerance observed for the species in the experiments, there is a possibility that in the natural environment the oil interacts with other factors, leading to synergistic effects, so further studies are needed to assess the effects of this pollutant on aquatic insect species.

Keywords: petroleum, LC50, Chironomidae, Urucu, Amazon.

Teste de toxicidade aguda e crônica de óleo cru em larvas de *Chironomus kiiensis* Tokunaga (Diptera: Chironomidae)

Resumo

A região Amazônica, conhecida por sua megabiodiversidade, também é detentora de grandes reservas de petróleo e gás natural. A crescente exploração de gás natural e óleo cru na Amazônia não é acompanhada de estudos avaliando o impacto destes poluentes nas comunidades biológicas locais, especialmente organismos aquáticos. Neste trabalho, o objetivo das autoras foi determinar os valores de toxicidade aguda e crônica de óleo cru proveniente de Urucu para larvas da espécie *Chironomus kiiensis* Tokunaga, 1936. A $CL50_{48h}$ de óleo cru para larvas de segundo instar de *C. kiiensis* foi de 26,5 mg/L e a mortalidade para a maioria das concentrações testadas foi maior durante as 24 primeiras horas do experimento. A sobrevivência dos ovos de *C. kiiensis* expostos a concentrações de óleo cru também foi avaliada, porém sem diferença significativa entre os tratamentos. Apesar da alta tolerância da espécie observada nos experimentos, em ambiente natural existe a possibilidade do óleo interagir com outros fatores, apresentando efeito sinérgico, e mais estudos avaliando o efeitos deste poluente sobre insetos aquáticos são necessários.

Palavras-chave: petróleo, CL50, Chironomidae, Urucu, Amazônia.

1. Introduction

The Amazon rainforest is the largest tropical forest in the world, covering approximately seven million square kilometres. It is undoubtedly one of the most important regions considering biological, economic and cultural aspects, and has a disproportionate wealth of biological resources compared to the rest of the world (Killeen, 2007). For this reason, concern about the impacts caused by human activities in Amazonia is manifested not only by the countries that comprise the region, but also by the entire international community.

Underlying this biodiverse ecosystem are large reserves of oil and gas, many still unexploited. The search for oil in the Amazon began in its western portion (which comprises parts of Bolivia, Colombia, Ecuador and Peru), beginning in the 1920s in Peru; oil production increased considerably in the 1970s (Finer et al., 2008). Four decades later, many large projects have been and are currently running in the Amazon and the intensity of this activity is sure to grow rapidly, especially in the Urucu River Oil Province located 650 km southwest of Manaus (Leyen, 2008). According to Finer et al. (2008), in 2005 there were 180 oil and gas blocks covering 688,000 km^2 of forest in the Western Amazon, many of which overlap areas of high diversity, protected areas and indigenous territories. The increase in oil and gas exploitation in the Amazon is not accompanied by a comparable increase in efforts to obtain information on the consequences of this exploitation on the environment (Finer et al., 2008).

In general, oil and gas exploitation in the Amazon rainforest have caused environmental and social impacts (Killeen, 2007; Casey et al., 2008; Orta-Martínez and Finer, 2010). Oil in its raw state, also called "crude oil," is a complex mixture of hydrocarbons. The aromatic compounds, particularly polyaromatic hydrocarbons (PAHs), are considered to be the most toxic components of oil. Low-molecular-weight hydrocarbons are more soluble and therefore more toxic to aquatic organisms than are high molecular weight hydrocarbons (HMWHs). In contrast, HMWHs have very limited solubility in water and are more likely to be adsorbed into the sediments (Peterson, 1994) and are therefore potentially more toxic to benthic organisms. When oil is poured onto water, volatile substances escape quickly and the soluble fraction slowly enters through the water column (Harrison et al., 1975).

From the biological point of view, the chemical components of oil can affect organisms at different levels of organisation, from individual enzyme systems to cells, organs, individuals, populations, and entire ecosystems. As a rule, ecosystems do not respond to a single substance or parameter, but rather exhibit species-specific and situation-specific sensitivity to a variety of factors and parameters (Market et al., 2003). Information on the sensitivity and specificity of such reactions provides the basis for evaluating the biological risks and planning the safe use of oil. Studies of this nature in the Amazon region are few (Veintemilla, 2006; Duarte et al., 2010; Lopes and Piedade, 2011), and, in view of the expansion of oil exploitation in the region, it is necessary to increase our knowledge of the reactions of different organisms to the effects of oil, especially effects on native species.

In Brazil, the invertebrate organisms most tested in toxicological studies are mollusks, crustaceans and echinoderms, while fish is the only group of vertebrates tested. The worse situation is observed in Northern Brazil, where toxicological studies are restricted to the state of Amazonas, and only fish species have been evaluated. With the aim of obtaining more realistic results for our environment, researchers began using Brazilian native species in toxicology essays in the early 1980s. In the case of fish and amphipods, native species are considered to be more sensitive to pollutants when compared with exotic species (Martins and Bianchini, 2011). Most studies of the harmfulness of hydrocarbons for the aquatic biota have been conducted *in situ* comparing impacted and not impacted areas (Martins and Bianchini, 2011).

Among the organisms used in biological monitoring, insects deserve special attention. The aquatic insect community responds quickly to disturbances found in natural environments by the decrease in species richness and by the dominance of a few tolerant generalist species, such as species of the genus *Chironomus* (e.g. Couceiro et al., 2007). The advantages of using insects in biological monitoring of aquatic environments include: the functional importance of these organisms (which range from secondary producers to top predators), the ability to identify most aquatic insects at a satisfactory level and the predictability and facility of detecting the responses of many aquatic insects to specific disorders, and finally, the facility of reproducing populations of some taxa in the laboratory (Gullan and Cranston, 2005).

In fact, insects in the order Diptera are considered to be very tolerant of pollutants and some taxa show a positive correlation with the presence of oil (Rych and Duchrow, 1973; Rosenberg and Wiens, 1976; Woodward and Riley, 1983). Rosenberg and Wiens (1976) investigated the abundance of two species of *Cricotopus* on artificial substrates in the presence and absence of oil, and both species were more abundant on substrates with oil. In one case an intermediate concentration of crude oil was responsible for the greatest increase in the abundance of Chironomidae (Cushman and Goyert, 1984). These studies in natural environments or in mesocosms attribute this positive relationship between the larvae and the concentration of oil to the loss of macrophytes coupled with the increase of benthic algae and, thus, more detritus would favour the Chironomidae species. Another explanation for the situation in the natural environment would be the depletion of predators in the presence of oil and the consequent increase in the population of benthic organisms (Crunkilton and Duchrow, 1990). Only two studies evaluating the effect of oil derived from petroleum on aquatic macroinvetebrates have been performed in the Amazon (Couceiro et al., 2006, 2007). Couceiro et al. (2006) found that both the richness and the abundance of aquatic insects were generally higher in the non-impacted stream.

Larvae of species in the family Chironomidae (Diptera) represent one of the most abundant aquatic insects in most ecosystems, and they have demonstrated their importance as test organisms for studies on risk assessment of substances such as pesticides (Taenzler et al., 2007), heavy metals (Watts and Pascoe, 1996; Harrahy and Clements, 1997; Bechard et al., 2008) and oil (Rosenberg and Wiens, 1976; Cushman, 1984; Crunkilton and Duchrow, 1990; Hatch and Burton Junior, 1999). However, toxicity studies using petroleum-derived products on animals have only been conducted for Amazonian fish (Veintemilla, 2006; Duarte et al., 2010), there being no studies assessing effects on aquatic insects. The use of a single species in toxicological bioassays allows us to observe the survival, behaviour and physiological parameters. Thus, these tests provide information on the direct impact of substances on organisms, including information on species sensitivity and mode of action (Altenburger and Schmitt-Jansen, 2003).

This study aimed to determine the sensitivity of an aquatic insect, *Chironomus kiiensis* Tokunaga, 1936, to the pollutant crude oil from the Urucu Oil Province, using the median lethal concentration (LC50) of second-instar larvae and to determine chronic toxicity of this pollutant on eggs. Few freshwater species in the Amazon region have had their sensitivity studied (Veintemilla, 2006; Duarte et al., 2010; Lopes and Piedade, 2011) and these data are of considerable interest for evaluation of the potential environmental impacts of oil on the aquatic biota and the implications of these impacts for the preservation of the Amazon ecosystem.

2. Material and Methods

The larvae of *C. kiiensis* used in the experiments came from a colony maintained in cages in the Aquatic Insect and Cytotaxonomy Laboratory, Department of Biodiversity, National Institute for Research in Amazonia (INPA). The experiments were performed in October and November 2011 at 30°C temperature. The positions of the trays (replicates of the treatments) on the shelves were randomised. INPA artesian water was used for dilution of the concentrations of crude oil. The water used has low concentrations (and very low availability) of humic substances (Veintemilla, 2006). The composition of Urucu crude oil used in the experiments is shown in Table 1.

2.1. Acute toxicity test - determination of LC5048H

For the experiment to determine the $LC50_{48H}$ each ovigerous mass, after being removed from the colony, was placed in a plastic tray containing 1 L of well water and 200 g of sand that had been burned in a muffle furnace with constant aeration. After hatching, larvae were fed every 48 hours with a small amount of commercial flacked feed for fish (TetraMin Plus Tropical Flakes) until the sixth day post-hatching (second-instar) when they were transferred to glass trays 2.5 L in size. In the experiments we used 12 trays, each with 1.4 L of well water, a thin layer of sand (100 g) in the bottom and 10 *C. kiiensis* larvae. All trays were maintained under constant homogeneous aeration by aquarium pumps. Although the acute toxicity tests are usually carried out for 96 hours, the time of larval development forced us to reduce the time to 48 hours. The experiment was conducted with second instar larvae so that they could be easily visualised in the sampling units, even in the presence of crude oil. In a pilot experiment above 48 hours, some larvae have reached the pupal stage. Thus, we chose to perform bioassays in 48 hours to ensure that the larvae were evaluated before the pupal stage.

Before working with the concentrations of crude oil diluted in water to determine the LC_{48h}, a preliminary experiment was performed with eight larvae per tray, with the following concentrations (mg/L) and respective mortalities: 0, 0; 0.1, 0; 0.2, 1; 0.4, 2; 0.8, 2; 1.6, 1; 3.2, 3; 6.4, 3; 12.8, 5; 25.6, 6; 51.2, 8, to determine the concentration that resulted in the mortality of all larvae. The concentration of crude oil that caused the death of all larvae within 48 hours was 51.2 mg/L. Graphical estimates indicated a concentration of 38.4 mg/L for 10% survival of the larvae. With these data, we determined 10 concentrations of crude oil in the final LC_{48h} experiment, with mortalities between 0 and 100%, observing the criteria of geometric progression with ratio of 0.9 (Sprague, 1971) and no replications for the concentrations (Hamilton et al., 1977). The concentrations tested were 16.5, 18.4, 20.4, 22.7, 25.2, 28.0, 31.1, 34.6, 38.4 and 42.7 mg/L. Larval mortality was quantified every 12 hours.

The water variables (temperature, pH, conductivity and dissolved oxygen) were estimated daily at 8:00 am, using a conductivity meter (Oakton® Model 35630-62) and a portable oxymeter (Oakton® Model DO110). The measurements began three days before the beginning of each experiment. After addition of the oil, only temperature and pH were estimated every 24 hours during the experimental period using pH tapes and analogue thermometers. According to Crunkilton and Duchrow (1990), the presence of oil in the water column does not significantly alter levels of dissolved oxygen and conductivity in streams.

2.2. Chronic toxicity test – 6 days

The chronic toxicity test was conducted by exposing eggs of *C. kiiensis* (previously quantified) to the concentrations 0, 6.6 and 13.0 mg/L (0, 25% and 50% of the $LC50_{48h}$, respectively) of crude oil for six days. The aim was to investigate whether different concentrations of crude oil are responsible for differences in the percentage of individuals reaching the sixth day (second-instar).

Table 1. Composition of Urucu crude oil used in the experiments (Petrobras, 1997).

Compounds	Values %
Motor octane number (distillation cut point 16–180°C)	38.0
Hydrocarbon types (% of total; distillation cut point 16–180°C)	
Paraffinic	64.8
Naphthalenic	20.2
Aromatic	15.0
Hydrocarbon types (% of total; distillation cut point 144–244°C)	
Saturates	85.7
Aromatics	13.2
Oleffins	1.1

At the end of the tests the contents of the trays were placed in 50 L plastic Jerry cans and sent to a private company for treatment.

2.3. Statistical analysis

In the acute toxicity test, the mean lethal concentration ($LC50_{48h}$) was determined by the Trimmed Spearman Karber method (Hamilton et al., 1977) using the Spearman program available at http://www.epa.gov/eerd/stat2.htm (accessed in 10-30-2011). To analyse the results of the chronic toxicity test we used an analysis of variance, with prior processing of the data expressed in percentage to arc sin $\sqrt{P\%/100}$. Since the distribution of survival data did not meet the assumptions of the binomial distribution (Shapiro-Wilk W=0.87, p=0.068), the Kruskal-Wallis test was used (α=0.05).

3. Results

The parameters estimated in the trays with crude oil in the two experiments were not significantly different from the values in the control trays, thus reducing the possibility of death of the larvae due to other factors (Table 2).

3.1. Acute toxicity test - determination of LC5048h

The concentrations and mortality data obtained in the acute toxicity test after 48 hours are shown in Table 3. Dead larvae showed absence of mobility and faded yellow colour. The median lethal concentration of crude oil for the larvae of *C. kiiensis* was 26.5 mg/L (±5.00). The dose-response curve had a tendency to form a typical sigmoid curve, except for concentration 6 (28.0 mg/L), which did not follow the monotonic increase in mortality (Figure 1). Mortality for the majority of concentrations was highest within the first 24 hours of the experiment (Table 4).

3.2. Chronic toxicity test – 6 days

Number of eggs and larvae before and after the experiment and survival rates are shown in Table 5. The analysis of variance with prior processing of the data showed a slight drop in the percentage survival of larvae subjected to the highest concentration of crude oil. However, this difference was not significant (H=0.12, p=0.94) (Figure 2).

Table 2. Mean values and standard deviation of physical and chemical variables of water estimated at every 12 hours for the tests of acute and chronic toxicity. Significant differences between the control and the different concentrations of crude oil were not observed.

Experiment	Concentration (mg/L)	pH	T (C°)	DO (mg/L)	Conductivity
Acute Toxicity	Control	7.09±0.45	24.90±0.87	6.57±0.15	16.57±0.40
	16.5	6.94±0.35	25.20±0.96	6.90±0.10	16.40±0.75
	18.4	6.94±0.35	25.30±0.96	7.00±0.17	16.43±1.00
	20.4	6.94±0.44	25.30±1.06	6.93±0.15	15.80±0.46
	22.7	7.00±0.47	25.23±1.10	7.00±0.30	16.20±1.15
	25.2	6.96±0.41	25.07±1.02	7.00±0.17	17.27±1.76
	28.0	6.94±0.29	24.97±0.93	7.00±0.10	19.40±0.70
	31.1	6.96±0.31	25.27±1.40	6.97±0.06	16.83±1.58
	34.6	6.97±0.37	25.20±0.96	6.93±0.12	16.17±0.86
	38.4	6.96±0.26	25.03±1.03	7.00±0.10	17.07±1.46
	42.7	6.99±0.56	25.17±1.12	6.97±0.32	17.67±1.35
Chronic Toxicity	Control – A	7.11±0.23	24.18±1.55	6.70±0.36	22.93±1.83
	Control – B	7.13±0.27	25.22±1.78	7.10±0.36	24.93±4.91
	Control – C	7.14±0.26	24.85±1.49	7.17±0.25	22.03±3.82
	Control – D	7.04±0.21	25.20±1.62	6.93±0.42	22.83±4.38
	6.6 – A	7.13±0.27	24.85±1.58	6.40±0.10	24.27±3.75
	6.6 – B	7.08±0.17	25.07±1.75	6.00±1.47	22.60±1.08
	6.6 – C	7.09±0.19	25.30±1.66	6.87±0.42	23.00±1.15
	6.6 – D	7.04±0.21	25.55±1.88	6.80±0.50	22.00±2.86
	13.3 – A	7.09±0.19	25.42±1.95	6.60±0.44	22.20±3.47
	13.3 – B	7.12±0.22	25.53±1.74	7.10±0.30	21.47±1.16
	13.3 – C	7.12±0.24	25.01±1.69	7.00±0.30	22.30±2.07
	13.3 – D	7.09±0.21	25.39±1.82	6.97±0.21	22.27±2.51

Table 3. Concentrations (Mg/L), number of exposed larvae and mortality in the 48h acute toxicity test with *Chironomus kiiensis* larvae (Diptera: Chironomidae) on the sixth day after hatching.

Concentration	Control	16.5	18.4	20.4	22.7	25.2	28.0	31.1	34.6	38.4	42.7
Exposed larvae	10	10	10	10	10	10	10	10	10	10	10
Dead larvae	0	0	2	6	6	6	5	7	8	10	10

4. Discussion

Among the aquatic insects, species in the family Chironomidae are considered to be good organisms for toxicological testing, mainly because this is the most abundant group in the benthic community (Pinder, 1986). In nature, these organisms are exposed to different toxic compounds, either directly through physical contact or indirectly through ingestion of contaminated food. Another feature of Chironomidae that makes them very suitable for toxicological studies is the ease of maintaining and manipulating them in the laboratory (Fonseca and Rocha, 2004).

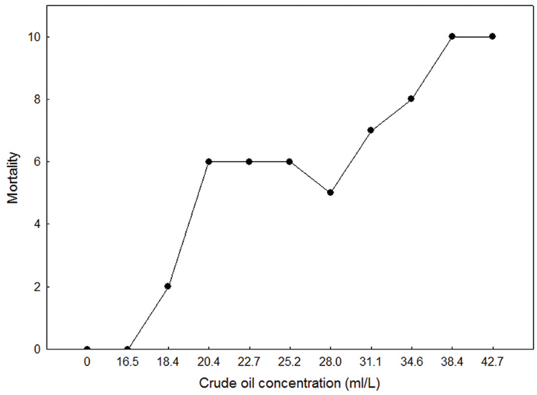

Figure 1. Dose-response curve of the acute toxicity test with *Chironomus kiiensis* (Diptera: Chironomidae) larvae and crude oil.

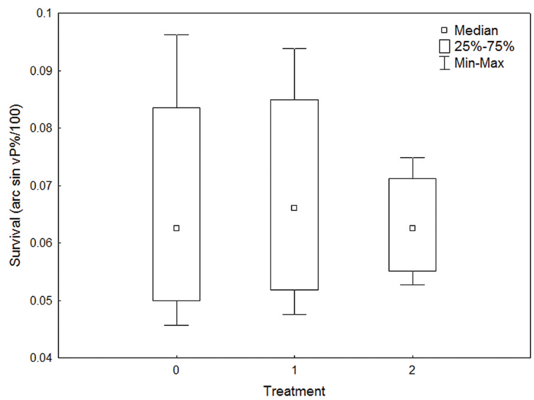

Figure 2. Chronic toxicity test using eggs and larvae of *Chironomus kiiensis* (Diptera: Chironomidae) subjected to different concentrations of Urucu crude oil.

Certainly, the fraction of the oil that is deposited at the bottom of the tray in our experiment came into contact with food of the larvae and with the larvae themselves when they were not occupying their sand tubes. The negative effects of oil extracted in the Amazon region have been experimentally verified in aquatic plants (Silva and Camargo, 2007; Lopes and Piedade, 2011) and fish (Brauner et al., 1999; Brix et al., 2011). However, the effect of pollutants on aquatic invertebrates have not been evaluated experimentally.

Chironomus species have high tolerance to a variety of compounds. Brix et al. (2011), in their review of the toxicity of metals, indicated aquatic insects, especially *Chironomus*, as a group that is relatively insensitive when compared to other organisms. Santos et al. (2007) evaluated the effect of NaCl concentrations as a reference toxic on *Chironomus xanthus* Rempel, 1939, *Daphnia magna* Straus, 1820, *Hydra attenuata* Pallas, 1766, and *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák, 1990, and *C. xanthus* was considered to be the most resistant to the contaminant. Woodward et al. (1987) examined the composition of the benthic community of substrates exposed to crude oil for 96 hours, where the most common genera were *Baetis*, *Isoperla*, *Brachycentrus*, and members of Chironomidae (more than 50% of the individuals collected); the most sensitive of these groups were *Baetis* and *Isoperla*. Bhattacharyya et al. (2003) assessed the toxicity of oil and of oil-spill chemical treatments in microcosms, using three freshwater benthic species: the amphipod *Hyallela Azteca* (Saussure, 1858), the oligochaete *Tubifex tubifex* (Müller, 1774) and *Chironomus tentans* Fabricius, 1805; the latter species exhibited an intermediate level of sensitivity to the

Table 4. Mortality of larvae of *Chironomus kiiensis* (Diptera: Chironomidae) every 12 hours during acute toxicity test using one control and 10 concentrations of Urucu crude oil.

Time (hours)	Concentration of crude oil (mg/L)										
	0	16.5	18.4	20.4	22.7	25.2	28.0	31.1	34.6	38.4	42.7
12	0	0	1	1	1	2	2	2	3	3	4
24	0	0	1	3	3	3	3	4	4	4	4
32	0	0	0	0	1	0	0	1	1	2	1
48	0	0	0	2	1	1	0	0	0	1	1

Table 5. Number of eggs in each tray, number of larvae after six days of experiment and survival rates of *Chironomus kiiensis* during the chronic toxicity test.

	Crude oil (mg/L)											
	0				6.6				13.3			
Number of eggs	77	88	78	78	92	71	90	85	94	93	72	68
Number of larvae	16	26	72	39	29	16	79	49	31	52	20	31
Survival (%)	20.8	29.5	92.3	50.0	31.5	22.5	87.8	57.6	33.0	55.9	27.8	45.6

pollutants. Unfortunately, there are no studies on the lethal or acute doses and chronic effects of crude oil on aquatic insects for use as a comparison. There is, however, the study by Merchant and Walton (1985) on the toxicity of crude oil to a terrestrial insect, the large milkweed bug *Oncopeltus fasciatus* (Dallas, 1852) (Hemiptera, Lygaeidae), where the LD was estimated by extrapolation from low-toxicity data to be 100 milligrams per gram of individual, applied to the abdominal sternum of each insect. The same authors found that crude oil did not result in significant reduction in egg viability, as observed in the presented study.

Tolerance of *C. kiiensis* to the crude oil was considered high, as evidenced by the median lethal concentration ($LC50_{48h}$=26.5 mg/L) compared to the values responsible for the chronic toxicity of the same pollutant using young fish (*Colossoma macropomum* Cuvier, 1816) as test organisms (3.96 mg/L) (Kochhann et al., 2009). Verrhiest (2001) apud Clément (2012) determined the LC50 of phenanthrene in the sediment for *Chironomus tentans* of 0.490 mg/l, and the LC50 of fluoranthene for *Chironomus tentans* and *C. riparius*, 0.030 to 0.060 mg/l, and 0.029 to 0.041 mg/l, respectively, during experiments of ten days. The major difference in the values found by Verrhiest (2001 apud Clément, 2012) and the present study may be explained by the duration of the experiments (2 and 10 days, respectively), and because the authors used isolated hydrocarbons, which are more soluble than the crude oil and present higher bioavailability. Although the sensitivity of *C. kiiensis* to crude oil was found to be low in the present study, other authors have shown that other species in the genus experience high mortality in the presence of oils and dispersants during a 6-month period (Bhattacharyya et al., 2003) and were totally eliminated even more than two years after an oil spill (Harrel, 1985). Al-Shami et al. (2012) tested concentrations of Zn, Cu and Cd below the LC50 in *C. kiiensis* larvae and no mortality was observed during the 24-hour exposure period they used in all treatments. However, these authors observed that all heavy metals were proven to have a genotoxic effect on *C. kiiensis* larvae after short-term exposure. According to Bhattacharyya et al. (2003), chironomids are not overly sensitive and therefore probably represent a good indicator for the overall effects of oil toxicity on the benthic community. The crude oil has low solubility and consequently low bioavailability, and this could be responsible for the high lethal concentrations observed. Determination of the LC50 for *C. kiiensis* constitutes an advance in ecological knowledge of aquatic insects and can serve as a basis for future studies investigating the effect of crude oil in the Amazon region. The tested concentrations may have relevance in the environment, especially if the oil spill reaches small streams and water bodies. In 1999 there were two oil spills in the Amazon region. On August 6th, 3,000 litres of crude oil leaked from the pipeline that supplies Manaus, reaching the Cururu stream and Negro River. The second accident, less than a month later, on August 29th, was a new oil spill of least one thousand litres, which contaminated the Negro River.

There was no significant reduction in the viability of the eggs in the chronic toxicity experiment and difference in the development of larvae was not observed. Future studies may investigate the possibility of reduction on the number of eggs from the adults of the bioassays, or even a reduction of fertility in future generations.

In conclusion, *C. kiiensis* proved to be tolerant to high concentrations of crude oil. In addition to being resistant to high doses of the oil over a short period, doses equivalent to 25% and 50% of the average lethal concentration did not affect development from eggs to the second larval stage. However, it is important to remember that the oil can interact with other factors in the natural environment and cause synergistic effects, but this type of information is absent for the Amazon region. Studies using aquatic insect species and crude oil are important for evaluating the sensitivity of species to different pollutants. The composition of the oil also varies depending on the region of origin. Considering the growing oil-extraction activity in the Amazon, risk assessment of this practice using aquatic insects is a necessary step in order to understand the behaviour of the aquatic community in the face of this challenge.

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References

AL-SHAMI, SA., RAWI, CSM., AHMAD, AH. and NOR, SAM., 2012. Genotoxicity of heavy metals to the larvae of Chironomus kiiensis Tokunaga after short-term exposure. *Toxicology and Industrial Health*, vol. 28, no. 8, p. 734-739. http://dx.doi.org/10.1177/0748233711422729. PMid:22025505

ALTENBURGER, R. and SCHMITT-JANSEN, M., 2003. Predicting toxic effects of contaminants in ecosystems using single species investigations. In MARKET, BA., BREURE, AM. and ZECHMEISTER, HZ. (Eds.). *Bioindicators & biomonitors: principles, concepts and applications*. Oxford: Elsevier Science. p. 153-198. http://dx.doi.org/10.1016/S0927-5215(03)80135-2.

BÉCHARD, KM., GILLIS, PL. and WOOD, CM., 2008. Acute toxicity of waterborne Cd, Cu, Pb, Ni, and Zn to first-instar Chironomus riparius larvae. *Archives of Environmental Contamination and Toxicology*, vol. 54, no. 3, p. 454-459. http://dx.doi.org/10.1007/s00244-007-9048-7. PMid:17917758

BHATTACHARYYA, S., KLERKS, PL. and NYMAN, JA., 2003. Toxicity to freshwater organisms from oils and oil spill chemical treatments in laboratory microcosms. *Environmental Pollution*, vol. 122, no. 2, p. 205-215. http://dx.doi.org/10.1016/S0269-7491(02)00294-4. PMid:12531308

BRAUNER, CJ., BALLANTYNE, CL., VIJAYAN, MM. and VAL, AL., 1999. Crude oil exposure affects air-breathing frequency, blood phosphate levels and ion regulation in an air-breathing teleost fish, Hoplosternum littorale. *Comparative Biochemistry and Physiology*, vol. 123, no. 2, p. 127-134. PMid:10442821.

BRIX, KV., DEFOREST, DK. and ADAMS, WJ., 2011. The sensitivity of aquatic insects to divalent metals: a comparative analysis of laboratory and field data. *The Science of the Total Environment*, vol. 409, no. 20, p. 4187-4197. http://dx.doi.org/10.1016/j.scitotenv.2011.06.061. PMid:21820156

CASEY, JF., KAHN, JR. and RIVAS, AAF., 2008. Willingness to accept compensation for the environmental risks of oil transport on the Amazon: A choice modeling experiment. *Ecological Economics*, vol. 67, no. 4, p. 552-559. http://dx.doi.org/10.1016/j.ecolecon.2008.01.006.

CLÉMENT, B., 2012. Bioavailability of Polycyclic Aromatic Hydrocarbons Studied Through Single-Species Ecotoxicity Tests and Laboratory Microcosm Assays. In PUZYN, T. and MOSTRAG-SZLICHTYNG, A. (Eds.). *Organic pollutants ten years after the stockholm convention: environmental and analytical update*. Rijeka: InTech, p. 89-112.. http://dx.doi.org/10.5772/33304.

COUCEIRO, SRM., FORSBERG, BR., HAMADA, N. and FERREIRA, RLM., 2006. Effects of an oil spill and discharge of domestic sewage on the insect fauna of Cururu stream, Manaus, AM, Brazil. *Brazilian Journal of Biology*, vol. 66, no. 1A, p. 35-44. http://dx.doi.org/10.1590/S1519-69842006000100006. PMid:16680304

COUCEIRO, SRM., HAMADA, N., FERREIRA, RLM. and SILVA, JO., 2007. Domestic Sewage and Oil Spills in Streams: Effects on Edaphic Invertebrates in Flooded Forest, Manaus, Amazonas, Brazil. *Water, Air, and Soil Pollution*, vol. 180, no. 1-4, p. 249-259. http://dx.doi.org/10.1007/s11270-006-9267-y.

CRUNKILTON, RL. and DUCHROW, RM., 1990. Impact of a massive crude oil spill on the invertebrate fauna of a Missouri Ozark stream. *Environmental Pollution*, vol. 63, no. 1, p. 13-31. http://dx.doi.org/10.1016/0269-7491(90)90100-Q. PMid:15092329

CUSHMAN, RM., 1984. Chironomid deformities as indicators of pollution from a synthetic, coal-derived oil. *Freshwater Biology*, vol. 14, no. 2, p. 179-182. http://dx.doi.org/10.1111/j.1365-2427.1984.tb00031.x.

CUSHMAN, RM. and GOYERT, JC., 1984. Effects of a synthetic crude oil on pond benthic insects. *Environmental Pollution*, vol. 33, no. 2, p. 163-186. http://dx.doi.org/10.1016/0143-1471(84)90176-4.

DUARTE, RM., HONDA, RT. and VAL, AL., 2010. Acute effects of chemically dispersed crude oil on gill ion regulation, plasma ion levels and haematological parameters in tambaqui (Colossoma macropomum). *Aquatic Toxicology*, vol. 97, no. 2, p. 134-141. http://dx.doi.org/10.1016/j.aquatox.2009.12.020. PMid:20097436

FINER, M., JENKINS, CN., PIMM, SL., KEANE, B. and ROSS, C., 2008. Oil and gas projects in the Western Amazon: threats to wilderness, biodiversity, and indigenous peoples. *PLoS ONE*, vol. 3, no. 8, p. e2932. http://dx.doi.org/10.1371/journal.pone.0002932. PMid:18716679

FONSECA, AL. and ROCHA, O., 2004. Laboratory cultures of the native species *Chironomus xantus* Rempel, 1939 (Diptera-Chironomidae). *Acta Limnologica Brasiliensia*, vol. 16, no. 2, p. 153-161.

GULLAN, PJ. and CRANSTON, PS., 2005. *The insects: an outline of entomology*. Oxford: Blackwell Publishing. 511 p.

HAMILTON, MA., RUSSO, RC. and THURSTON, RV., 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environmental Science & Technology*, vol. 11, no. 7, p. 714-719. http://dx.doi.org/10.1021/es60130a004.

HARRAHY, EA. and CLEMENTS, WH., 1997. Toxicity and bioaccumulation of a mixture of heavy metals in *Chironomus tentans* (Diptera: Chironomidae) in synthetic sediment. *Environmental Toxicology*, vol. 16, no. 2, p. 317-327. http://dx.doi.org/10.1897/1551-5028(1997)016<0317:TABOAM>2.3.CO;2.

HARREL, RC., 1985. Effects of a crude oil spill on water quality and macrobenthos of a southeast Texas stream. *Hydrobiologia*, vol. 124, no. 3, p. 223-228. http://dx.doi.org/10.1007/BF00015238.

HARRISON, W., WINNIK, MA., KWONG, TY. and MACKAY, D., 1975. Disappearance of aromatic and aliphatic components from small sea-surface slicks. *Environmental Science & Technology*, vol. 9, no. 3, p. 231-234. http://dx.doi.org/10.1021/es60101a006.

HATCH, AC. and BURTON JUNIOR, GA., 1999. Photo-induced toxicity of PAHs to Hyalella azteca and Chironomus tentans: effects of mixtures and behavior. *Environmental Pollution*, vol. 106, no. 2, p. 157-167. http://dx.doi.org/10.1016/S0269-7491(99)00079-2. PMid:15093043

KILLEEN, TJ., 2007. *A Perfect Storm in the Amazon Wilderness: Development and Conservation in the Context of the Initiative for the Integration of the Regional Infrastructure of South America (IIRSA)*. Arlington: Conservation International. 99 p. Advances in Applied Biodiversity Science, no. 7.

KOCHHANN, D., BRUST, SMA. and DOMINGOS, FXV. and VAL, AL., 2009. Exposição aguda do tambaqui ao petróleo: marcadores fisiológicos e comportamentais. In Registros da 61ª Reunião Anual da SBPC: Amazônia Ciência e Cultura, 2009. Manaus. Manaus: UFAM.

LEYEN, BC., 2008. *Eco-eficiência na Exploração e Produção de Petróleo e Gás em Regiões de Florestas Tropicais Úmidas: O Caso da Petrobras na Amazônia.* Rio de Janeiro: Universidade Federal do Rio de Janeiro. 202 p. Dissertação de Mestrado em Planejamento Energético.

LOPES, A. and PIEDADE, MTF., 2011. Time of exposure and oil dosage affectingbiomass production of the Amazonian semi-aquatic grass *Echinochloa polystachya*. *Rodriguésia*, vol. 62, no. 4, p. 901-908.

MARKET, BA., BREURE, AM. and ZECHMEISTER, HZ., 2003. Definitions, strategies and principles for bioindication/biomonitoring of the environment. In MARKET, BA., BREURE, AM. and ZECHMEISTER, HZ. (Eds.). *Bioindicators & biomonitors: principles, concepts and applications*. Oxford: Elsevier Science . p. 3-39.

MARTINS, SE. and BIANCHINI, A., 2011. Toxicity tests aiming to protect Brazilian aquatic systems: current status and implications for management. *Journal of Environmental Monitoring*, vol. 13, no. 7, p. 1866-1875. http://dx.doi.org/10.1039/c0em00787k. PMid:21643562

MERCHANT, ER. and WALTON, BT., 1985. Lethal and sublethal effects of petroleum and two coal-derived oils on the large milkweed bug *Oncopeltus fasciatus* (Dallas) (Hemipt., Lygaeidae). *Zeitschrift Angewandte Entomologie*, vol. 99, no. 1-5, p. 253-260. http://dx.doi.org/10.1111/j.1439-0418.1985.tb01986.x.

ORTA-MARTÍNEZ, M. and FINER, M., 2010. Oil frontiers and indigenous resistance in the Peruvian Amazon. *Ecological Economics*, vol. 70, no. 2, p. 207-218. http://dx.doi.org/10.1016/j.ecolecon.2010.04.022.

PETERSON, DR., 1994. Calculating the aquatic toxicity of hydrocarbon mixtures. *Chemosphere*, vol. 29, no. 12, p. 2493-2506. http://dx.doi.org/10.1016/0045-6535(94)90052-3.

PETROBRAS, 1997. *Características do petróleo Urucu*. Rido de Janeiro: CNPES/SEPESQ/DIQUIM/SETAV. 10 p. Anexo V.

PINDER, LCV., 1986. Biology of freshwater Chironomidae. *Annual Review of Entomology*, vol. 31, no. 1, p. 1-23. http://dx.doi.org/10.1146/annurev.en.31.010186.000245.

ROSENBERG, DM. and WIENS, AP., 1976. Community and Species Responses of Chironomidae (Diptera) to Contamination of Fresh Waters by Crude Oil and Petroleum Products with Special Reference to the Trail River, Northwest Territories. *Journal of the Fisheries Research Board of Canada*, vol. 33, no. 9, p. 1955-1963. http://dx.doi.org/10.1139/f76-249.

RYCH, FM. and DUCHROW, RM., 1973. Oil pollution and the aquatic environment in Missouri, 1960-1972. *Transactions of the Missouri Academy of Science*, vol. 7-8, p. 164-171.

SANTOS, MAPF., VICENSOTTI, J. and MONTEIRO, RTR., 2007. Sensitivity of four test organisms (*Chironomus xanthus, Daphnia magna, Hydra attenuata* and *Pseudokirchneriella subcapitata*) to NaCl: An alternative reference toxicant. *Journal of the Brazilian Society of Ecotoxicology*, vol. 2, no. 3, p. 229-236. http://dx.doi.org/10.5132/jbse.2007.03.004.

SILVA, RS. and CAMARGO, AFM., 2007. Effect of Urucu crude oil on the aquatic macrophyte *Pistia stratiotes*. *Acta Limnologica Brasiliensia*, vol. 19, no. 2, p. 155-161.

SPRAGUE, JB., 1971. Measuremnent of pollutant toxicity to fish. III. Sublethal effects and safe concentrations. *Water Research*, vol. 3, p. 793-821.

TAENZLER, V., BRUNS, E., DORGERLOH, M., PFEIFLE, V. and WELTJE, L., 2007. Chironomids: suitable test organisms for risk assessment investigations on the potential endocrine disrupting properties of pesticides. *Ecotoxicology*, vol. 16, no. 1, p. 221-230. http://dx.doi.org/10.1007/s10646-006-0117-x. PMid:17268839

VEINTEMILLA, CAC., 2006. *Impactos do fenantreno sobre o tambaqui Colossoma macropomum Cuvier, 1818: CL50, crescimento e hematologia*. Manaus: Instituto Nacional de Pesquisas da Amazônia. 142 p. Dissertação de Mestrado em Biologia Tropical e Recursos Naturais.

WATTS, MM. and PASCOE, D., 1996. Use of the freshwater macroinvertebrate *Chironomus riparius* (Diptera: Chironomidae) in the assessment of sediment toxicity. *Water Science and Technology*, vol. 34, no. 7-8, p. 101-107. http://dx.doi.org/10.1016/S0273-1223(96)00730-5.

WOODWARD, DF., LITTLE, EE. and SMITH, LM., 1987. Toxicity of five shale oils to fish and aquatic invertebrates. *Archives of Environmental Contamination and Toxicology*, vol. 16, no. 2, p. 239-246. http://dx.doi.org/10.1007/BF01055805. PMid:3566344

WOODWARD, DF. and RILEY, RG., 1983. Petroleum hydrocarbon concentrations in a salmonid stream contaminated by oil field discharge water and effects on macrobenthos. *Archives of Environmental Contamination and Toxicology*, vol. 12, no. 3, p. 327-334. http://dx.doi.org/10.1007/BF01059410.