Acute Toxicity Testing of Crude Oil Using *Piaractus brachypomus*, and *Pimephales promelas*

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Abstract*- Oil industry activities such as exploration, transportation, storage, use disposal, and oil spills are sources of major contamination problems in Peru with deleterious effects on aquatic organisms. The objectives of this study were to: 1) examine reference contaminant acute toxicity on red pacu *Piaractus brachypomus*, and 2) assess the acute median lethal toxicity of crude oil on red pacu *Piaractus brachypomus* and fathead minnow *Pimephales promelas*. Results showed that median lethal concentration (LC_{50}) values for two reference toxicants of *Piaractus brachypomus* were: zinc sulfate = 5.74 mg/l, and sodium dodecyl sulfate = 11.29 mg/l. Peruvian crude oil was tested on *Piaractus brachypomus*; the LC_{50} was found to be > 4.00 mg TPH/l and the median lethal loading (LL_{50}) was found to be > 50000 mg/l; in comparison, the LC_{50} of Peruvian crude oil of *Pimephales promelas* was 1.83 mg TPH/l, and the LL_{50} was 22875 mg/l. *Piaractus brachypomus* was also exposed to Louisiana sweet crude oil and the LL_{50} was 17678 mg/l. Results suggested that the acute toxicity of the three reference toxicants on *Piaractus brachypomus* was within the range of other published studies on fish, and that this species was more tolerant to Peruvian crude oil than *Pimephales promelas*. Based on the acute toxicity tests on *Piaractus brachypomus*, the Louisiana sweet crude oil was more toxic than Peruvian crude oil. This study is one of the few toxicity studies using Peruvian crude oil and the first using *Piaractus brachypomus* as the test species. Further research on additional species and other toxicants related to oil contamination is necessary to assess the effects of this growing industry on the Amazonian aquatic environment.

***Keywords*- Acute Toxicity; Piaractus brachypomus; Crude Oil; Reference Toxicant**

I. INTRODUCTION

Oil industry activities such as exploration, transportation, storage, use disposal, and oil spills, are sources of major contamination. For instance, Occidental Petroleum (Oxy) started its oil activities more than 30 years ago in northeast Peru, in Achuar (an indigenous group) territory [1]. In 2007, a report suggested that the company had spilled 9 billion barrels of toxic oil byproduct known as “formation waters” into the Corrientes River crossing the Achuar territory during the company’s operation period. Violating international norms, the company used earthen pits that were open and leached into groundwater and soil. In addition, poor maintenance and infrastructure led to numerous oil spills. High concentrations of cadmium and lead (related to oil) were found in the adjacent human population’s blood; and oil contamination led to reduction in agricultural, fishing and hunting productivity [1]. As oil activities and incidents increase, there is an urgent need for proper management of a wide range of environmental problems.

The Amazon basin includes eight countries, and Peru represents 12% of the total area [2]. The western Amazon is a rich and still largely intact ecosystem, whose biodiversity provides services and goods of great value to the people adjacent to the river including a variety of indigenous groups. In Peru, oil exploration started in the 1920s and production peaked in the 1970s. This economic growth has posed significant opportunities to local communities and risks to the environment. Peru is entering a second oil exploration boom, and extensive areas are covered by proposed or active oil concessions [3]. Associated oil waste effluents from Pluspetrol Peru Corporation S.A. have been discharged to small tributaries of three rivers: the Pastaza, Tigre, and Corrientes [4]. Spills and incomplete clean-ups are typical in this vulnerable area; where, as recently as January, 2012, there was an oil incident with an unknown quantity of chemicals and crude oil spilled from a corroded pipeline [4]. The overall adverse effects in these rivers have yet to be determined. Thus, oil-related industrial activity has clearly become a threat to natural resources and the health of rural and indigenous communities.

The fish diversity in the Amazon basin is impressive, and as a whole it contains more than 3000 species [5]. Red pacu *Piaractus brachypomus*, belonging to the family Serrasalmidae, is native to the Orinoco and Amazon Rivers [6], and is commercially important in the Amazon basin. Along with black pacu *Colossoma macropomum*, a family-related species, red pacu constituted over 50% of the native Amazonian fish harvested by Peruvian farmers in 2011 [7]. While studies on aquaculture production [8], reproduction [9], and genetic variability [10] have been performed in Peru, Colombia, and Bolivia; no research has been performed evaluating the potential toxicity of contaminants on this native species.

The purpose of this study was to assess the acute median lethal toxicity of crude oil on a native Peruvian fish species red pacu *Piaractus brachypomus* with comparison to a standard aquatic toxicity test species, fathead minnow *Pimephales promelas*. Specific objectives were 1) to perform acute toxicity tests to determine the median lethal concentration (LC_{50}) of three reference toxicants (zinc sulfate, sodium dodecyl sulfate, and Louisiana sweet crude oil) and Peruvian crude oil on red pacu *Piaractus brachypomus*; and 2) to determine the LC_{50} of Peruvian crude oil on fathead minnow *Pimephales promelas*.

II. METHODS

A. Study Area

Toxicity tests on *Piaractus brachypomus* were performed at the Laboratory of Bioactive Substances, located on Iquitos-Nauta Road, 4.5 km from Iquitos, Peru. The laboratory is part of Quistococha Biological Station operated by the Peruvian Amazon Research Institute (IIAP). The toxicity tests on *Pimephales promelas* were performed at Troy University, Troy, Alabama, U.S.A.

B. Water Quality

Water quality parameters were measured before each test, using an oximeter YSI model 55® for temperature and dissolved oxygen (DO), a WTW® pH meter 330i kit for pH, and a LaMotte® freshwater test kit (model AQ-2) for total alkalinity and total hardness. Locally available (IIAP) well water was used as dilution water and for the control in acute toxicity tests in Iquitos, Peru. This local well water had 32 mg/l as $CaCO_3$ of alkalinity, 24 mg/l as $CaCO_3$ of hardness, 7.1 pH, and 4.3 mg/l DO. The dilution water used in Troy, AL was aerated tap water. It had 188 mg/l as $CaCO_3$ of alkalinity, 16 mg/l as $CaCO_3$ of hardness, 8.5 pH, and 7.5 mg/l DO.

C. Organisms

Hatchery-produced *Piaractus brachypomus* larvae individuals (one to 16 days old) were provided by IIAP for the acute toxicity tests. *Pimephales promelas* (six days old) were purchased from a commercial supplier (Marinco Bioassay Laboratory, Inc., Sarasota, Florida).

D. Preparation and Analysis of Water Accommodated Fraction (WAF)

The American Petroleum Institute (API) gravity is an inverse measure of petroleum and water. Heavy crude oil has an API gravity < 22.3 °(density 920 to 1000 kg/m^3), while the API of light oil is > 34 °[11]. Louisiana sweet crude oil (lot #WP 681), a light oil (35.6 ° API) was purchased from RT Corporation, WY. The term sweet comes from the low sulfur (< 0.42%) contained in this type of petroleum [12]. Peruvian crude oil (obtained from PetroPeru S.A. Company) is a heavy (20 ° API), sour variety with 1.2% sulfur content [13]. In order to perform the acute toxicity test on the oil, the water accommodated fraction (WAF) had to be prepared. The water accommodated fraction is a solution free of particles of bulk material (i.e., droplets > 1 µm diameter) derived from mixing (no vortex) test material and water [14]. A 2-L borosilicate glass aspirator bottle (Thomas Scientific) was used, and the sidearm was closed off with silicone tubing and a clamp. It was filled with 1 L of dilution water adding 25 g of Louisiana sweet crude oil and a second series was done for the Peruvian crude oil fraction with 1 L of dilution water adding 50 g (for *Piaractus brachypomus*), and 200 g (for *Pimephales promelas*). The mix was stirred on a magnetic stirring plate for 22 hours in darkness without vortex. The mix was used immediately after preparation [15, 16, 17].

The WAF prepared with 200 g/l of Peruvian crude oil was sent to Sitelab Corporation in West Newbury, MA to be analyzed for total petroleum hydrocarbons (TPH) and total polycyclic aromatic hydrocarbons (PAH) concentrations on a UVF-3100 analyzer according to the protocol available online at http://www.site-lab.com/water_TPH_procedure.htm. The sample was weighed and methanol was added as solvent.

E. Acute Toxicity Testing (Static)

A preliminary toxicity range-finding test was done for zinc sulfate and sodium dodecyl sulfate (SDS). Groups of three organisms were exposed to several concentrations (zinc sulfate ranged from 0.5 mg/l to 30 mg/l; and SDS ranged from 0.625 mg/l to 90 mg/l) for 24 hours. Once the approximate range to be used was determined, acute toxicity bioassays were performed for 96 hours [18]. The concentrations used for zinc sulfate were: 1.875 mg/l, 3.75 mg/l, 7.5 mg/l, 15 mg/l, and 30 mg/l; for SDS: 5 mg/l, 10 mg/l, 15 mg/l, 20 mg/l, and 25 mg/l; and for both oils the percentages of WAF were 6.25%, 12.5%, 25%, 50%, and 100%. Dilution water in Iquitos, Peru for *Piaractus brachypomus* was locally available (IIAP) well water, and for *Pimephales promelas* it was aerated tap water from Troy, Alabama [19]. New plasticware was rinsed with dilution water, while new glassware was washed with 10% hydrochloric acid and rinsed with deionized water, and dilution water. All containers and equipment were flushed with dilution water before using. Borosilicate glass beakers of 250 ml were used as exposure chambers with 200 ml of respective test solutions. The temperature was kept at 28 ℃ ± 1 ℃ for *Piaractus brachypomus* and 25 ℃ ± 1 ℃ for *Pimephales promelas*. Three replicates of each concentration with 10 organisms each were run concurrently [18].

Three reference toxicants were used: zinc sulfate, SDS purchased from Sigma-Aldrich Co., LLC, and Louisiana sweet crude oil. Peruvian crude oil available from the vicinity of Iquitos and Louisiana sweet crude oil were used to prepare the WAF. Mortality was assessed every 24 hours, dead fish were removed and discarded. Control survival was equal to or better than

90%. Results were reported as LC_{50}, defined as the concentration of a substance that causes mortality in 50% of test organisms in a specific time period [18]. For Peruvian crude oil, the LC_{50} was calculated using the TPH concentration found by Sitelab Corporation in West Newbury, MA. For Louisiana sweet crude oil, the TPH concentration could not be determined due to lack of availability. For both crude oils, which are complex and poorly water-soluble mixtures, the median lethal loading rate (LL_{50}), defined as the amount of the substance resulting in 50% mortality of population [20], was also reported.

F. *Statistical Analysis*

The LC_{50} and 95% confidence intervals for each toxicant were calculated using the Trimmed Spearman Karber (TSK) version 1.5 software [21], available online at http://www.downloadplex.com/Scripts/Matlab/Development-Tools/Download-trimmed-spearman-karber-method-scripts_427751.html. Values were reported as mg/l (ppm) for zinc sulfate and sodium dodecyl sulfate, and as WAF percentage and LL_{50} in mg/l for Louisiana sweet crude oil and Peruvian crude oil.

III. RESULTS

The LC_{50} values and 95% confidence intervals for three reference toxicants (zinc sulfate, SDS, and Louisiana sweet crude oil), and Peruvian crude oil on *Piaractus brachypomus* are reported (Table 1). The LC_{50} values for zinc sulfate and SDS are based on nominal concentrations as chemical analyses were not conducted to characterize concentration in exposure media. In addition, the LL_{50} for both crude oils are reported. In general, it is indicated that the percent mortality of *Piaractus brachypomus* increased as the concentration of the toxicant increased. The LC_{50} for zinc sulfate was 5.74 mg/l, and for SDS it was 11.29 mg/l. For zinc sulfate and SDS, the mortality in the control was 10%, and within the first 24 hours of exposure, all individuals died in the highest concentration (25 mg/l) of SDS.

TABLE 1 MEDIAN LETHAL CONCENTRATIONS (LC_{50}) AND 95% CONFIDENCE INTERVALS FOR 96-HOUR TOXICITY TESTS ON *PIARACTUS BRACHYPOMUS.* MEDIAN LETHAL LOADINGS (LL_{50}) ONLY FOR CRUDE OILS. NOTE: WAF = WATER ACCOMMODATED FRACTION, TPH = TOTAL PETROLEUM HYDROCARBONS, N/A = NOT AVAILABLE

Toxicant	**96 h - LC_{50}**	**96 h – LL_{50} (mg/l)**
Zinc sulfate (mg/l)	5.74 (3.62 - 9.08)	N/A
Sodium dodecyl sulfate (SDS) (mg/l)	11.29 (8.36 - 15.26)	N/A
Louisiana sweet crude oil (WAF)	N/A	17678
Peruvian crude oil (WAF) (mg TPH/l)	> 4.00 (N/A)	> 50000

Within the first 24 hours of exposure, almost 50% of the test fish (*Piaractus brachypomus*) died in the highest concentration (100%) of Louisiana sweet crude oil. The LC_{50} found for Louisiana sweet crude oil was 70.71% using 25 g/l and the LL_{50} was 17678 mg/l. Regarding the Peruvian crude oil, the TPH concentration of the WAF using 200 g/l of oil was 16 mg/l, and it was used to calculate the LC_{50} values, while the total PAH concentration for the aquatic fraction of this mixture was 0.47 mg/l. The concentration of Peruvian crude oil (50 g/l) used to prepare the WAF was not sufficient to cause 50% mortality; therefore, the actual LC_{50} value could not be calculated. However, based on these data, the LC_{50} was estimated to be > 4 mg TPH/l or > 50000 mg/l of crude oil. The LC_{50} for Peruvian crude oil on *Pimephales promelas* was 11.46% (1.83 mg TPH/l) with 95% confidence intervals of 6.32% – 20.79% (1.01 – 3.33 mg TPH/l). Low organisms mortality (6.5%) was observed in the highest concentration (100%) within the first 24 hours of exposure.

IV. DISCUSSION

A. *Zinc Sulfate*

Zinc is an essential trace constituent of natural water and is required in the metabolism of most organisms. Nevertheless, high concentration (400 µg/l) has toxic effects on fish causing gill damage [22], reduced sexual dimorphism, liver degeneration, and muscle underdevelopment [23]. In addition, Ololade and Ogini (2009) [24] found a decrease in leucocytes, erythrocytes and hemoglobin with increasing concentrations of zinc in an African catfish, *Clarias gariepinus*. In toxicity tests, zinc sulfate, one of the inorganic forms of zinc, is used as a reference toxicant to demonstrate acceptable laboratory performance, and to assess the sensitivity and health of organisms [18].

The toxicity of zinc, as well as other heavy metals, is influenced by chemical factors including magnesium, calcium, pH, hardness, and ionic strength [25]. In general, heavy metals are more toxic in soft water because they are more soluble. Zinc is less toxic in harder water because zinc ions' activity decreases since the ions contributing to hardness (calcium and magnesium) compete with zinc for binding sites and uptake in biological tissues [26]. In previous studies with different fish species using about the same hardness (24 mg/l as $CaCO_3$) as the one used in this study, the LC_{50} values for zinc sulfate ranged from 0.6 mg/l to 6.4 mg/l (Table 2). Ebrahimpour et al. (2010) [27] tested waters of different hardness, finding that zinc toxicity generally increased with softer water. However, toxicity varies among individuals, species and larger phylogenetic groups [26]. For instance, a toxicity study on mottled sculpin *Cottus bairdi* suggested that this species had the lowest acute toxicity to zinc (0.156 mg/L) than any other fish tested to date [28]. Similar hardness (20 mg/l as $CaCO_3$) to the one in the present study was used by Pickering and Henderson (1966b) [29], who reported similar LC_{50} values for bluegill *Lepomis macrochirus* (5.82 mg/l), and goldfish *Carassius auratus* (6.4 mg/l) compared to *Piaractus brachypomus*. Pickering and Henderson (1966b) [29] also found that the LC_{50} at similar hardness values to ours for guppy *Poecilia reticulata* was 1.27 mg/l, and for *Pimephales promelas* it was 0.78 mg/l, suggesting that these species were more sensitive to zinc toxicity.

TABLE 2 MEDIAN LETHAL CONCENTRATIONS (LC_{50}) AND 95% CONFIDENCE INTERVALS (IF AVAILABLE) FOR ZINC SULFATE TOXICITY TESTS ON DIFFERENT FISH SPECIES

Fish species name	**Hardness (as** $CaCO_3$)	**96- h LC_{50} (mg/l)**	**Reference**
African catfish *Clarias gariepinus*	193.3	36.7	[24]
Siah mahi *Capoeta fusca*	40	13.7 (7.0 - 22.3)	[27]
Mottled sculpin *Cottus bairdi*	48.6	0.156 (0.125 - 0.193)	[28]
Fathead minnow *Pimephales promelas*	20	0.78	[29]
Fathead minnow *Pimephales promelas*	360	33.4	[29]
Guppy *Poecilia reticulata*	20	1.27	[29]
Bluegill *Lepomis macrochirus*	20	5.82	[29]
Goldfish *Carassius auratus*	20	6.4	[29]
Red pacu *Piaractus brachypomus*	24	5.74 (3.62 - 9.08)	Present study

B. Sodium Dodecyl Sulfate (SDS)

Sodium dodecyl (lauryl) sulfate is an anionic surfactant used in household products, pesticides, herbicides, emulsion polymerization, and as a reference toxicant in toxicological studies [18]. Barbieri et al. (1998) [30] remarked that SDS negatively affected the swimming capacity and metabolism of common carp *Cyprinus carpio*. In gilthead bream *Sparus aurata*, morphological changes such as loss of normal structure in the kidney and an increase of red blood cells and leucocytes infiltration were observed in toxicity studies [31]. The SDS 96-h LC_{50} for *Piaractus brachypomus* reported herein is 11.29 mg/l, which is slightly higher than the value reported for other fish species such as the inland silverside *Menidia beryllina* (9.5 mg/l) [32], and *Pimephales promelas* (8.6 mg/l) [18], but less than the killifish *Cynopoecilus melanotaenia* (14.9 mg/l) [33] (Table 3).

TABLE 3 MEDIAN LETHAL CONCENTRATIONS (LC_{50}) AND 95% CONFIDENCE INTERVALS (IF AVAILABLE) FOR SODIUM DODECYL SULFATE (SDS) TOXICITY TESTS ON DIFFERENT FISH SPECIES

Species	**96- h LC_{50} (mg/l)**	**Reference**
Fathead minnow *Pimephales promelas*	8.6	[17]
Inland silverside *Menidia beryllina*	9.5 (8.7 - 10)	[32]
Killifish *Cynopoecilus melanotaenia*	14.9	[32]
Red pacu *Piaractus brachypomus*	11.29 (8.36 - 15.26)	Present study

C. Crude Oil

In the current study, the LC_{50} for Peruvian crude oil on *Piaractus brachypomus* was higher than the LC_{50} value found for *Pimephales promelas*, suggesting that the Peruvian species might be less sensitive to this crude oil (Table 4). *Piaractus brachypomus* was tested with two crude oils, and the LL_{50} for the Louisiana sweet crude oil was lower than the Peruvian crude oil, indicating higher toxicity. This was expected since the two crude oils had different density (API), therefore, different properties. The Peruvian crude oil was heavy, which USEPA (2011) [34] describes as viscous, black, and having low toxicity. The Louisiana sweet crude oil was light, and described as highly fluid and toxic. The higher toxicity of lighter oils is related to the ease of bioavailability/uptake of lower molecular weight compounds (volatile) over higher molecular weight ones contained in heavy crude oils [35].

TABLE 4 MEDIAN LETHAL CONCENTRATIONS (LC_{50}), MEDIAN LETHAL LOADINGS (LL_{50}), AND 95% CONFIDENCE INTERVALS (IF AVAILABLE) FOR DIFFERENT CRUDE OIL TOXICITY TESTS ON DIFFERENT FISH SPECIES. NOTE: TPH = TOTAL PETROLEUM HYDROCARBONS, N/A = NOT AVAILABLE

Fish species name	**Crude oil type**	**96- h LC_{50} (mg TPH/l)**	**96- h LL_{50} (mg/l)**	**Reference**
Marine species				
Inland silverside *Menidia beryllina*	Louisiana sweet	>2.9	N/A	[17]
Inland silverside *Menidia beryllina*	Alaska North Slope	26.36 (25.54 - 27.22)	3520 (3326 - 3725)	[37]
Inland silverside *Menidia beryllina*	Prudhoe Bay	> 19.86	> 8152	[37]
Inland silverside *Menidia beryllina*	Arabian medium	> 14.5	N/A	[39]
Sheepshead minnow *Cyprinodon variegatus*	Arabian medium	> 6.1	N/A	[39]
Inland silverside *Menidia beryllina*	Kuwait	> 1.32	> 25000	[36]
Turbot *Scophthalmus maximus*	North Sea Forties	> 1.33	> 23471	[36]
Freshwater species				
Crimson-spotted rainbowfish *Melanotaenia fluviatilus*	Australian	1.28	N/A	[40]
Red pacu *Piaractus brachypomus*	Louisiana sweet	N/A	17678	Present study
Red pacu *Piaractus brachypomus*	Peruvian	> 4.00	> 50000	Present study
Fathead minnow *Pimephales promelas*	Peruvian	1.83 (1.01 - 3.33)	22875 (12625 - 41625)	Present study

Different crude oils tested on fish species are compared to the Peruvian and Louisiana crude oil in the present study (Table 4). Previous toxicity studies range from the Kuwait crude oil (> 1.32 mg TPH/l) [36] to Alaska North Slope crude oil (26.36 mg TPH/l) [37]. However, comparisons on effects of crude oil WAF are difficult since the composition of hydrocarbons in the oils varies depending on their density and origin. Other factors influencing the widely different results is the preparation method of the WAF between studies, which include room temperature, water chemistry, mixing energy, settling period, and the tolerance to crude oil of the species tested [16]. Furthermore, toxicity of crude oil seems to be lower in marine species compared to freshwater species, due to hydrocarbon solubility and lower bioaccumulation in fish in waters with increased salinity. At higher salinities, there is a reduction of PAH uptake probably due to PAH and water efflux in response to osmotic gradients [38]. Inland silverside *Menidia beryllina* is an estuarine and EPA approved marine species commonly used in toxicity testing [32]. Several crude oils have been tested on this species such as Arabian medium (LC_{50} = > 14.5 mg TPH/l) [39], Alaska North Slope (LC_{50} = 26.36 mg TPH/l) [37], and Kuwait (LC_{50} = > 1.32 mg TPH/l) [36] showing the high variability of LC_{50} values for different crude oils.

Crude oil contains poorly soluble components that are influenced by changes in temperature or chemical changes due to weathering. Therefore, it is recommended to report the results of materials with low solubility components as the LL_{50} rate, defined as the amount of the substance used in the WAF resulting in 50% mortality of the population [20]. The loading rate used for Peruvian crude oil on *Piaractus brachypomus* (50 g/l) was not enough to kill 50% of the test organisms; therefore neither the LC_{50} nor LL_{50} could be calculated. However, the result was extrapolated to > 50000 mg/l (our highest concentration used), almost twice as high as the LL_{50} for Kuwait and North Sea Forties crude oils tested on inland silverside *Menidia beryllina* and turbot *Scophthalmus maximus*, respectively [36]. Alaska North Slope crude oil had the lowest LL_{50} (3520 mg/l) for *Menidia beryllina*, suggesting high toxicity [37]. Brand et al. (2001) [41] found that the WAF from the Alaskan crude oil caused stress and morphologic lesions in gills, hepatic and kidney tissues on pink salmon fry *Oncorhynchus gorbuscha*.

This study is the first and only attempt to compare relative sensitivity of a Peruvian species larvae using WAF to other freshwater and marine fish species. It is also the first toxicity study for *Piaractus brachypomus*, and the species was chosen due to its commercial importance and its availability in the hatchery at the time. We believe that tests should be performed to find a better and more sensitive test species that has year-round availability. On the other hand, *Pimephales promelas* is a standard test species that was used to compare the effects of Peruvian crude oil. However, there are several other standard freshwater test species, but most of them are non-native in the Amazonian area and would not be appropriate to provide a high level of protection for native fish populations.

Acute toxicity bioassays are a prescreening tool for the chemical assessment of polluted water [42], and in Peru the effects of crude oil on the environment and aquatic organisms are not well understood. Oil-contaminated wastewater contains PAHs and other harmful substances that may have chronic effects, including genotoxic impacts on DNA structure [43]. Furthermore, with the characteristic of PAHs to bioaccumulate in tissues; bivalve mollusks and other edible aquatic organisms (especially invertebrates) exposed to PAH contamination endanger the public through consumption and represent appreciable human exposure to potential carcinogens [44]. Acute and chronic tests would be extremely relevant since Peru does not have standard limits for PAHs as a class in drinking water. Therefore, it is necessary to further test the toxicity of these contaminants in the

Peruvian Amazon and use the information as part of the basis for public health and regulatory decisions concerning toxic chemicals. The present research is only one of several studies that would be needed to make a complete hazard evaluation taking into consideration invertebrate and vertebrate species, not only of PAHs but heavy metals as well.

V. CONCLUSIONS

This study reported LC_{50} values on a native fish species, *Piaractus brachypomus*, for three reference toxicants, zinc sulfate = 5.74 mg/l, SDS = 11.29 mg/l, and Louisiana sweet crude oil = 2.05 mg TPH/l. When testing crude oil, it is recommended to report the LL_{50} to better compare the results to other studies. Peruvian crude oil was tested on *Piaractus brachypomus*, and the LC_{50} was found to be > 4.00 mg TPH/l, and the LL_{50} was estimated to be > 50000 mg/l. The same Peruvian crude oil was tested on *Pimephales promelas* and the LC_{50} was 1.83 mg TPH/l, while the LL_{50} was 22875 mg/l.

Piaractus brachypomus was more tolerant to Peruvian crude oil than *Pimephales promelas*. Based on the acute toxicity tests in *Piaractus brachypomus*, the Louisiana sweet crude oil was more toxic than the Peruvian crude oil, due to the properties of the oils since the Peruvian crude oil is considered heavy and less toxic compared to light crude oils.

Bioassays are an important tool used to provide background information for risk assessment of chemicals. This study is one of the few toxicity studies using Peruvian crude oil and the first one using *Piaractus brachypomus*. However, further research on other species and other toxicants related to oil contamination such as lead, cadmium and mercury, is necessary to more fully assess the effects of this industry on the aquatic environment.

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