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Toxicity identification and evaluation of a refinery wastewater from Brazil (Phase I)

S.D.A. Daflon[1]; I.L. Guerra[1]; M.V. Reynier[2]; C.M.R. Botta[3] & J.C. Campos[1].

[1]Escola de Química, Universidade Federal do Rio de Janeiro. Av. Athos da Silveira Ramos,
149 Sala E-206, Rio de Janeiro, Brasil.CEP:21941-909,

[2]LABTOX – Laboratório de Análises Ambientais. Av. Carlos Chagas Filho, nº 791,
Bio-Rio – Laboratório 4, - Rio de Janeiro, Brasil. CEP: 21.941-904.

[3]Universidade de São Carlos. Av. Trabalhador Sãocarlense, 400 CP 359 São Carlos,
São Paulo, Brasil. CEP: 13566-590.

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Abstract

Petroleum refineries generate a large amount of effluents, which if released without appropriate treatment can cause chronic effects to organisms. Most studies show that many contaminants can be responsible for the toxicity, among them ammonia, sulfide, cyanide, phenol and hydrocarbons. The present study evaluated the cause of the chronic toxicity of a refinery wastewater from Brazil using the organism *Ceriodaphnia dubia* in short-term test. The results suggest that metals such as barium, manganese and strontium can be contributing for the toxicity of the wastewater. Besides this, conductivity could be contributing to the toxicity too, since the level is high for the test organism evaluated.

Keywords: *C. dubia*, chronic tests, refinery wastewater, toxicity identification.

INTRODUCTION

Much attention has been given to the impact of oil refinery wastewaters. This industry discharges large volumes of wastewaters containing significant amounts of toxic substances and compounds, which need to be removed at the refinery (Avci *et al*., 2005).

Burks (1982) stated that chemicals in oil refinery wastewaters which cause acute short-term toxicity can be removed by good biological treatment systems. However, while some refinery treatment plants can eliminate the problem of acute toxicity, the contaminant levels still remain high enough to cause chronic toxicity (Dorris *et al*., 1972).

Toxicity identification and evaluation (TIE) studies aim at identifying the agents responsible for wastewater toxicity, isolating the sources of this toxicity, evaluating and implementing actions to control and confirm the effectiveness of measures taken to reduce the toxic effects (USEPA, 1991).

Tischler (2013) applied TIE to refinery effluents in the USA and pointed out that the main toxicants contained in these effluents were total dissolved solids (TDS), fluoride, nitrite, ammonia, organic chemicals and metals such as copper, lead, zinc, and nickel. The author also stated that multiple waste constituents such as hardness and TDS can also reduce the bioavailability of other toxicants, e.g., metals.

Other studies suggest that compounds such as sulphides, phenol, cyanide, ammonia and hydrocarbons are responsible for refinery wastewater toxicity (Dorris *et al.,*1974; Hall *et al*., 1978; Westlake *et al*., 1983; Hartmann, 2004; Wake, 2005). However, few works cite metals as responsible for toxicity (Dorris *et al.,* 1961; Pessah *et al.,* 1973; Badaró-Pedroso, 1999; Tischler, 2013).

The composition of refinery wastewater cannot be generalized because it depends on the refinery, the type of crude oil and the units that are in operation at any specific time (Wake, 2005). Therefore, it is difficult to predict what effects

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*Corresponding author: Sarah D. A. Daflon; e-mail: sarah@eq.ufrj.br

the wastewater may have on the environment, especially on local species.

Brazil's National Environmental Council (CONAMA) issued Resolution 430, (BRASIL, 2011) covering the conditions and standards for wastewater discharge. This resolution requires industries to perform toxicity tests of their wastewater using at least two trophic levels. Besides this, the environmental agency of each state sets the permitted levels for these toxicity tests. Some environmental agencies require the refinery wastewater to have no chronic toxicity (PETROBRAS, 2005).

This article describes Phase I TIE studies performed with wastewater from a Brazilian refinery which showed chronic toxicity in a previous study. The refinery wastewater treatment plant consists of an API (American Petroleum Institute) oil-water separator (which separates oil from water by gravity), followed by flotation, aerated ponds and RBCs (rotating biological contactors).

MATERIAL AND METHODS

Wastewater sample and chemical analysis.

Grab sample was collected from the final stage of the treatment plant on July17, 2014. In the laboratory, after measurements of physical and chemical parameters, the sample was stored at 4°C (USEPA, 1992) for two weeks for further chemical analyses and TIE tests. The toxicity tests were initiated at the day after the arrival of the sample.

The physical and chemical parameters analyzed were: pH, biological oxygen demand (BOD), chemical oxygen demand (COD), sulfate, nitrate, conductivity, hardness, chloride, total dissolved solids (TDS) and ammonia nitrogen. The metals analyzed were aluminum, barium, calcium, strontium, iron, magnesium, manganese, sodium and zinc.

All the parameters were measured according to the method described in the Standard Method for the Examination of Water and Wastewater (APHA, AWWA, WEF, 2005).

Chronic TIE characterization scheme

A preliminary toxicity test was performed to determine the IC25 of the sample. Based on these results, subsequent Phase I TIE manipulations were performed according to procedures developed by USEPA (1992). All manipulations were conducted with 100%, 50% and 25% (v/v) concentrations plus control. Water used for dilution and control was natural water with hardness of 40 mg L^{-1} of $CaCO_3$ and pH of 7.0 (adjusted to these parameters when necessary).

Baseline test was performed each time additional manipulation test was started to evaluate the toxic effects over time and to obtain data to compare with the TIE results.

As recommended by USEPA (1992) for short-term chronic tests, the sample was tested using 72-hour-old *Ceriodaphnia dubia* (Cladocera) in a 96-hour static-renewal test and the endpoint was reproduction. The tests were conducted with a 16:8-h light:dark photoperiod at 25±2 °C. Test chambers were 12-mL plastic tubes filled with 10 mL of test solution, into which one female was placed and five replicates was done.

The objective of phase I TIE procedures is to characterize classes of compounds causing toxicity in an aqueous sample. In Phase I toxicity characterization, sample manipulations included EDTA chelation, sodium thiosulfate ($Na_2S_2O_3$) reduction, pH adjustment, pH adjustment/filtration, pH adjustment/aeration and pH adjustment/solid-phase extraction (SPE). Tests with pH adjustment were conducted at three pH levels: pHi (initial pH), pH 3, and pH 10. The samples were adjusted to pH 3 and 10 by the addition of reagent-grade HCl and NaOH, respectively. After manipulations (aeration, filtration, solid-phase extraction), the samples were readjusted to pHi by the addition of NaOH and HCl, respectively, prior to testing. Two types of membranes were used in the filtration test, glass fiber (1 μm) and cellulose acetate (metal binding). Only the glass fiber membrane filter was used for filtration/pH adjustment. Both types of filter were conditioned with 25 mL of pure water and 50 mL of dilution water. The samples were filtered through a glass fiber membrane before passing through the column for extraction. The columns (1000 mg; 6 mL; J.T. Baker) were conditioned with 25 mL of methanol followed by 25 mL of high pure water at a flow rate of 10 mL min^{-1}. The fractionation method involved passing a 250 mL sample through a C18 SPE column with the post column sample being used in the chronic tests.

Statistical analysis

The 25% inhibition concentration (IC25) was estimated for each test using a linear interpolation technique (USEPA, 1994). The level of inhibition of 25% (IC25%) is proposed for chronic short-terms tests used in Phase I TIE studies and is generally suggested as an equivalent for the NOEC (USEPA, 1994). The computer program used was ICPIN.EXE (Version 2.0).

RESULTS AND DISCUSSION

Chemical analyses of original wastewater sample

Table 1 shows the results of physical and chemical analyses of the original sample and Table 2 shows the results of metals analyses.

The wastewater presents relatively high levels of hardness and conductivity (Table 1), since the test organisms are cultivated in soft water with hardness about 40 mg L^{-1} and conductivity about 160 μS cm^{-1}. According to the USEPA (1992), when conductivity exceeds 1000 μS cm^{-1} in tests with *C. dubia,* it can be suspected of causing toxicity, and according to the American Petroleum Institute (1998), conductivity above 2000 μS cm^{-1} is high enough to cause adverse effects on freshwater species.

Goodfellow *et al*. (2000) highlighted that some wastewaters are toxic because of imbalance in the ion environment to which the test organisms are exposed. The authors also said that this salinity tolerance issue is more important for chronic toxicity testing because of growth and reproductive endpoints that are more sensitive to energy-taxing requirements of osmoregulation. Still according the same institute, specific ions important to freshwater species include Ca^+, K^+, Mg^+, Na^+, Cl^-, HCO_3^- and SO_4^{2-}.and .

In the present study, total ammonia nitrogen (TAN) was present at a low level of 0.7 mg (measured as N-NH3 L^{-1}). According to USEPA (1979) when the sample has pH of 8.0 and 25°C of temperature, the percentage of un-ionized ammonia is 5.38%. In this case, un-ionized ammonia of the sample was present at 0.037 mg L^{-1}.

According to Johnson (1995) chronic toxicity of un-ionized ammonia generally seemed to decrease as hardness increased. The author reported IC25% of 0.81 mg L^{-1} of un-ionized ammonia at pH 8.05 and 168 mg L^{-1} of $CaCO_3$.

Nimmo *et al*. (1989) reported a LOEC for reduction in reproduction of 0.88 mg L^{-1} for C. *dubia* at pH 8 in river water. Cowgill & Milazzo (1991) reported a NOEC of 0.73 mg L^{-1} of NH_3 in medium water at pH 8.3 for the same organism.

According to the USEPA (1992), ammonia is suspected of causing toxicity if it is present at minimum level of 5 mg L^{-1} (total ammonia) and it toxicity will be confirmed at graduated pH test.

Table 1. Physical and chemical properties of original wastewater sample.

Parameter	Results
pH	8.0
BOD	8.6 mg O_2 L^{-1}
COD	112 mg C L^{-1}
Sulfate	120 mg SO_4^{-2} L^{-1}
Nitrate	82 mg NO_3^- ou N-NO_3^- L^{-1}
Conductivity	1970 µS cm^{-1}
Hardness	180 mg de $CaCO_3$ L^{-1}
Chloride	490 mgCl$^-$ L^{-1}
Total dissolved solids (TDS)	930 mg L^{-1}
Total ammonia nitrogen (TAN)	0.7 mg N-NH_3 L^{-1}

Table 2. Metals found in the original wastewater sample.

Parameter	Results (mg L^{-1})
Aluminum	<0.05
Barium	0.51
Calcium	70
Strontium	2.4
Iron	<0.01
Magnesium	9.5
Manganese	0.06
Sodium	359
Zinc	<0.01

The levels of barium and strontium were respectively 0.51 mg L^{-1} and 2.4 mg L^{-1} (Table 2), above the levels proposed by Buchman (2008) as causing chronic effects to freshwater organisms (0.0039 mg L^{-1} and 1.5 mg L^{-1}, respectively). Also, the concentration of manganese was 0.06 mg L^{-1}, higher than the acute toxicity level reported by Stubblefield et al. (1990) for *C. dubia* (LC_{50} 48h of 0.0288 mg L^{-1}).

The other metals analyses, zinc (<0.01 mg L^{-1}), iron (<0.01 mg L^{-1}) and aluminum (<0.05 mg L^{-1}), were under the chronic levels proposed by Buchman (2008), which are <0.12 mg L^{-1}, 1.0 mg L^{-1} and 0.087 mg L^{-1}, respectively.

Chronic TIE manipulations

The inhibition concentrations of 25%t of the organisms (IC25%) for the original and for the treated samples are listed in Table 3.

The addition of 0.06 mg L^{-1} of EDTA and 0.5 mg L^{-1} of $Na_2S_2O_3$ removed the wastewater toxicity, suggesting that metals are responsible for this toxicity. Hockett & Mount (1996) achieved substantial reduction of toxicity caused by copper, cadmium and mercury (metals not analyzed in present study) by adding EDTA and $Na_2S_2O_3$.

Table 3. Toxicity of original and manipulated wastewater samples according to the chronic TIE tests.

Manipulations	pH	CI25(%)
Baseline test 1	pHi	65.82
pH adjustment	pH3	65.13
	pH10	70.12
pH adjustment/Aeration	pH3	63.28
	pHi	Non toxic
	pH10	Non toxic
pH adjustment/ C18 SPE	pH3	60.13
	pHi	67.96
	pH9	65.50
pH adjustment/Filtration glass fiber	pH3	62.10
	pHi	67.53
	pH10	93.75
Baseline test 2	pHi	58.82
EDTA addition (0.06 mg L^{-1})	pHi	Non toxic
EDTA addition (0.16 mg L^{-1})	pHi	73.68
$Na_2S_2O_3$ addition (0.2 mg L^{-1})	pHi	30.26
$Na_2S_2O_3$ addition (0.5 mg L^{-1})	pHi	Non toxic
pH graduation	pH6	61.02
	pH9	67.50
Filtration cellulose acetate	pHi	Non toxic

Note: pHi = 8.0

Manganese, barium and strontium, as stated earlier in this study, were found to be present in concentrations capable of causing chronic toxicity and they were also chelated by EDTA addition, although they are not chelated by addition of sodium thiosulfate.

Other manipulations that removed toxicity were pH adjustment/aeration at initial pH and pH adjusted to 10. These results suggest that some metals could have been precipitated after oxidation at this pH value, like manganese, for example (Di Bernardo, 1993).

Filtration with the pH adjustment to 10 also reduced toxicity, which can also be explained by the precipitation of the metal(s). According to Van Sprang & Janssen (2001), some metals form insoluble precipitates at high pH and can be easily removed by filtration.

As metals were the main suspects of the wastewater toxicity according to the previous TIE tests, filtration through a cellulose acetate filter (0.45 µm) was conducted, since according Weltje *et al*. (2003), this filter has high affinity for metals. As shown in Table 3, the toxicity was totally removed by passing the sample through this filter. In order to recover the toxicants retained by the filter, dilution water adjusted to pH 3 was used. After that, the pH was readjusted to initial pH and tested. The test toxicity was recovered, confirming that metals are the main toxicity agents in this wastewater.

Nitrogen ammonia was measured at low level and the pH graduation test did not show an increase in toxicity when the sample was adjusted to pH 9. Thus, ammonia did not contribute to the toxicity of the sample studied.

CONCLUSIONS

Although the physical and chemical levels were below those established by the Brazilian regulation (CONAMA Resolution 430/2011), they were not sufficient to prevent the chronic toxicity of the refinery's wastewater. The TIE tests indicated that metals such as barium, strontium and manganese are present at levels capable to cause toxicity to the organism tested, but their toxicity is only reduced by EDTA addition, suggesting that other metals such as copper and cadmium (not analyzed at present study) might be contributing to the toxicity of the sample studied. In addition, special attention should be given to the high levels of conductivity, since this can also be contributing to the toxicity of the sample.

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