

## Models for BTEX evaluation in cases of oil spill on the sea, using Experimental Design

Modelos para avaliação de BTEX em casos de derramamento de petróleo no mar, utilizando Experimental Design

Modelos para la evaluación de BTEX en casos de derrame de hidrocarburos en el mar, utilizando Diseño Experimental

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### **Abstract**

The objective of this study was to evaluate and obtain mathematical models, using the experimental design methodology, capable of predicting the contamination of benzene, toluene, ethylbenzene and xylenes (BTEX) in cases of oil spillage at sea, and to evaluate the acute toxicity of the soluble petroleum fraction (FSA). The factorial experimental planning was developed to describe the concentration of each compound according to the variables: oil °API, contact time oil/seawater after spillage and ambient temperature. The models presented can be used to quantitatively predict BTEX contamination in seawater with accuracy greater than 99%, within the studied ranges. The contact time oil seawater was the most determining factor in the concentration/contamination of BTEX in the Petroleum FSA. According to CL50% the soluble fraction of oil of °API 39.8 showed higher toxicity, with a LC50% of 46.07 with 1 h, and 11.38 with 96 hours of contact time oil/seawater, while the oil °API 32.8 showed lower toxicity, however presented higher concentrations of BTEX in FSA, this is possibly due to the synergistic effect of other hydrocarbons that became bioavailable to *Mysidopsis juniae* during the tests. The samples demonstrated potential environmental risk in cases of oil spillage, and these results can help in the development of a risk assessment of oil spills and serve as a useful analytical tool for emergencies.

**Keywords:** Experimental design; Mathematical model; Oil spill; Acute toxicity; BTEX.

### **Resumo**

O objetivo deste estudo foi obter modelos matemáticos, utilizando a metodologia de delineamento experimental, capazes de prever a contaminação de benzeno, tolueno, etilbenzeno e xilenos (BTEX) em casos de derramamento de petróleo no mar, e avaliar a toxicidade aguda da fração solúvel do petróleo (FSA). O planejamento experimental

fatorial foi desenvolvido para descrever a concentração de cada composto em função das variáveis: °API do petróleo, tempo de contato petróleo água do mar após o derramamento e temperatura do ambiente. Os modelos apresentados podem ser utilizados para prever quantitativamente a contaminação dos BTEX em água do mar com precisão superior a 99%, dentro das faixas estudadas. O tempo de contato petróleo água do mar foi o fator mais determinante na concentração/contaminação dos BTEX na FSA do petróleo. De acordo com a CL50% a fração solúvel do petróleo de °API 39.8 apresentou maior toxicidade, com uma CL50% de 46,07 com 1 h, e 11.38 com 96 horas de contato petróleo água do mar, já o petróleo de °API 32.8 apresentou menor toxicidade, porém apresentou maiores concentrações dos BTEX na FSA, isso se deve possivelmente devido ao efeito sinérgico de outros hidrocarbonetos que se tornaram biodisponíveis aos *Mysidopsis juniae* durante os ensaios. As amostras demonstraram potencial risco ambiental em casos de derramamento desses petróleos, e esses resultados podem ajudar no desenvolvimento de uma avaliação de risco de derramamentos de óleo e servir como uma ferramenta analítica útil para emergências.

**Palavras-chave:** Delineamento experimental; Modelo matemático; Derramamento de petróleo; Toxicidade aguda; BTEX.

### Resumen

El objetivo de este estudio fue obtener modelos matemáticos, utilizando la metodología de diseño experimental, capaces de predecir la contaminación de benceno, tolueno, etilbenceno y xilenos (BTEX) en casos de derrame de petróleo en el mar, y evaluar la toxicidad aguda de los fracción del petróleo (FSA). El diseño experimental factorial se desarrolló para describir la concentración de cada compuesto en función de las variables: ° API de petróleo, tiempo de contacto petróleo agua de mar después del derrame y temperatura del ambiente. Los modelos presentados pueden utilizarse para predecir cuantitativamente la contaminación de BTEX en agua de mar con una precisión superior al 99%, dentro de los rangos estudiados. El tiempo de contacto con el petróleo y el agua de mar fue el factor más determinante en la concentración / contaminación de BTEX en el petróleo FSA. Según el CL50%, la fracción soluble del petróleo API 39.8 ° presentó mayor toxicidad, con un CL50% de 46.07 con 1 h, y 11.38 con 96 horas de contacto con petróleo en agua de mar, mientras que el API API 32.8 presentó menor toxicidad, sin embargo, presentó concentraciones más altas de BTEX en la FSA, esto posiblemente se deba al efecto sinérgico de otros hidrocarburos que se volvieron biodisponibles para *Mysidopsis juniae* durante las pruebas. Las muestras demostraron un riesgo ambiental potencial en casos de derrames de estos hidrocarburos, y estos resultados pueden ayudar en el desarrollo de una evaluación del riesgo de derrames de hidrocarburos y servir como una herramienta analítica útil para emergencias.

**Palabras clave:** Diseño experimental; Modelo matemático; Derrame de petróleo; Toxicidad aguda; BTEX.

## 1. Introduction

The growing global demand for oil and gas has driven the advance of exploration and production operations (Harfoot *et al.*, 2018). In the last 20 years Brazil has experienced a great increase in maritime traffic, which has contributed to the increase in the number of accidents with oil tankers (Tournadre, 2014; Marques *et al.*, 2017). In 2019-2020 the north and southeast coast of Brazil suffered great environmental and socioeconomic impact with a spill of more than 200 tons of crude oil, reaching about 3,000 km long. Being considered the largest extension of contaminated coast in the world recorded in 30 years (IBAMA, 2019; Cardoso *et al.*, 2020). The impacts on the marine environment nature are of incalculable magnitude, this without assessing the long-term damage due to the recalcitrance of some of the heavier components of the oil (Kanjilal, 2015; Rekadwad & Khobragade, 2015; Turner *et al.*, 2014; Fingas, 2011; Stout & Wang, 2007; Peters *et al.*, 2005).

In cases of spillage or leakage of crude oil in a marine environment, the oil is immediately subject to a variety of physical-chemical and biological processes, which includes: scattering, dispersion, evaporation, solubilization, emulsification, photo-oxidation, biodegradation, chemical transformation and sedimentation (Reddy *et al.*, 2012; Liu *et al.*, 2012; McNutt *et al.*, 2012; Gutierrez *et al.*, 2013; Hall *et al.*, 2013; Liu & Liu, 2013; Kiruri *et al.*, 2013; Aeppli *et al.*, 2014; Ruddy *et al.*, 2014; Chanton *et al.*, 2014; Lewan *et al.*, 2014; Cravo-Laureau & Duran, 2014; Gros *et al.*, 2014; Bacosa *et al.*, 2015; King *et al.*, 2015; Stout *et al.*, 2016). Oil is a mixture of complex hydrocarbon compounds that vary in volatility and solubility, which are potentially toxic to marine organisms, particularly monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylenes (BTEX) that pose threats to humans and marine species (Meador and Nahrgang, 2019). In regions of intense oil-related activities, these compounds are present in appreciable concentrations (sometimes even in the milligram per liter range) (Saeed *et al.*, 2013). Brazil, being an oil producing country, is heavily involved in production, refining and transportation activities, this can inevitably result in oil spills.

During a crude oil spill accident in the ocean, an essential part of the planning and decision-making processes is driven by predictions of integrated oil and gas spill models (Nissanka & Yapa, 2018). Therefore, improvements in models for ocean oil spill cases become invaluable in the contingency planning and mitigation of environmental damage. Numerical/computational models provide a critical view of the destination and transportation of oil and subsequent weathering processes, which are important in emergency responses and oil mitigation attempts in order to minimize environmental impacts. However, there not much information regarding models of prediction of contaminants in the soluble phase of petroleum (FSA) at sea in the literature.

Therefore, the objective of this work is to develop predictive models that allow quantifying BTEX concentrations in cases of oil spillage or leakage in seawater, in order to assist in the evaluation of environmental impacts and contingency planning of accidental oil spills. In addition to the procedures for assessing the potential risk in the event of oil spill accidents, the acute toxicity assessment of the FSA was also carried out using the *Mysidopsis juniae*.

## 2. Methodology

### 2.1 Materials

The oils of °API 39.8 and 32.8 were supplied by PETROBRAS, Guamaré/RN pole, Brazil. Heptahydrate zinc sulfate ( $ZnSO_4 \cdot 7H_2O$ ) was acquired from Chemical Dynamics (São Paulo, Brazil). Seawater was acquired at the Laboratory of Aquatic Ecotoxicology of the Department of Oceanography and Limnology of the Federal University of Rio Grande do Norte, Brazil. For all experiments, the salinity of seawater was adjusted to  $34 \pm 1\%$ .

### 2.2 Experimental Planning

In order to obtain mathematical models capable of predicting the concentration of BTEX compounds in seawater after contact with crude oil, a statistical modeling was performed using a factorial experimental planning matrix  $2^3$  establishing three factors: the API degree of oil, the ambient temperature and the contact time of oil and seawater, as independent variables and as dependent variables the concentrations of BTEX. For each factor, two levels were selected: low (-1) and high (+1), according to Table 1. Independent factors were selected based on Saeed *et al.* (2013) and Saeed *et al.* (1998).

**Table 1.** Factors and levels used in the experimental matrix.

Independent variables	Symbol	Level	
		(-1)	(1)
°API	$X_1$	32.8	39.8
Time (hours)	$X_2$	1	96
Temperature (°C)	$X_3$	20	30

Source: Authors (2021).

The tests were carried out in duplicates in a total of 16 trials. Moreover, to evaluate the statistical significance of the proposed models, a study of variance analysis (ANOVA) was carried out at 95% confidence. All analyses, statistical calculations and graphs were obtained with the software STATISTICA 7.0.

### 2.3 Soluble fraction of oil

The soluble fraction of oil in seawater was based on Saeed *et al.* (2013) and Saeed *et al.* (1998). First, the oil was added into a glass jar with about 2 Liters of seawater (adjusted to  $34 \pm 1\%$ ), being transferred slowly with the aid of a glass rod, until it formed a 5 mm film (oil:water ratio 1:25 v/v). The system was maintained in low agitation without vortex formation, using a magnetic stirrer.

In order to evaluate the concentration of monoaromatic hydrocarbons (BTEX) in the soluble fraction of petroleum (FSP) in seawater, the contact time of oil and seawater of 1h and 96h and at room temperature of 20 °C and 30 °C ( $\pm 0.5$  °C) was varied, according to the predetermined experimental matrix previously determined. After the defined times, water samples were collected with the aid of a BT 100S peristaltic pump (Lead Fluid, Hebei, China) with low flow (50 mL/min) and transferred to 44 mL glass vials, with PTFE/silicone thread and septum. A sample was also collected to analyze the toxicity of the FSA of the petroleum in seawater in amber glass vials of 1 L.

### 2.4 Analysis of BTEX by chromatography

The chemical analysis of BTEX in the soluble fraction of the oils was based on the USEPA 8260B (Volatile Organic Compounds by Gas Chromatography-Mass Spectrometry (GC/MS) method, using the gas chromatograph coupled to a Clarus 600 MS mass spectrometer (PerkinElmer, Waltham, USA), and in the USEPA 5021A. Chromatographic separation performed with a stationary phase TG-624 SILMS (Thermo Fisher Scientific, Massachusetts, USA) column of 6% cyanopropylphenyl and 94% dimethylpolysiloxane measuring 30 m in length, 0.25 mm in internal diameter and 1.40  $\mu$ m film thickness. The drag gas used was helium (99.999% purity, Linde Gases Ltda, Brazil) constant flow of 1 mL/min.

The chromatographic conditions used were: initial temperature of 40 °C maintained for 2 min, following a heating ramp of 100 °C, rate of 10 °C/min. The temperature was then raised to 200 °C, rate of 30 °C/min, remaining at this temperature for 4 min. The running time was 15.33 minutes, the temperature of the injector and the transfer line were 220 °C and 200 °C, respectively. The detection of the BTEX was performed in a quadrupole mass spectrometer, with electrical ionization at a temperature of 200°C, in the total scanning mode of the spectra (full scan), with mass-over-load ratio (m/z) ranging from 35~270. Quantification was performed with external standardization and the identification of analyses was performed comparing the retention and confirmation times of the mass spectra with the compounds of the standard mixture.

### 2.5 Acute toxicity test

*Mysidopsis juniae* were cultivated in the ECOTOXLAB (Aquatic Ecotoxicology Laboratory) of the Primary Processing and Reuse Center of Produced Water and Waste at UFRN. The crop was maintained under controlled conditions in aquariums (8 L) with filtered natural seawater (filter of 0.5  $\mu$ m) and gender ratio of 20 males to 60 females. The abiotic conditions were as follows: salinity of sea water at  $35 \pm 1\%$ ; photoperiod of 12h light / 12h dark; temperature of  $25 \pm 2$  °C; constant aerate. *Mysidopsis* were fed with the nauplios of the microcrustacean *Artemia sp.* enriched with cod liver oil and fish oil (age: 48h) daily.

The acute toxicity tests were based on NBR 15308 of the Brazilian Association of Technical Standards (ABNT, 2017). Ten juveniles of *M. juniae* (1–8 days of age) were exposed in triplicate for 96 hours to five concentrations of the soluble fraction of oil in seawater (6.25, 12.5, 25, 50 and 100%), in addition to a negative control of filtered seawater. The test organisms were fed daily with *Artemia sp.* (age: 48h). Organisms were considered dead when immobilized after mild mechanical stimulation. The pH, salinity and dissolved oxygen concentration (DO) were measured at the beginning and end of each experiment using a Hanna Multiparameter - HI9829 (Hanna Instruments, Limena, Italy). The validation criteria of the test

were based on the survival of the control, which should be higher than 90%, and on the sensitivity to zinc as a reference substance, at concentrations of 0.10; 0.18; 0.32 and 0.56 mg/L (ABNT, 2017).

After 96h of exposure the tests with the *M. Juniae* were terminated and the final count of the individuals was performed, followed by the calculation of the median lethal concentration at 50% of the tested organisms (LC50), using the Trimmed Spearman-Kärber program (Hamilton *et al.*, 1977). The data of the LC50 were calculated for 96h, with a 95% confidence interval. The percentage of survival of the organisms at the end of each test was also evaluated and for the validation of the results, only trials in which the mortality of the organisms in the control was less than or equal to 10% was adopted as a criterion of acceptability.

### 3. Results and Discussion

#### 3.1 BTEX concentration in soluble petroleum fraction in seawater

Table 2 presents the configuration of the experiments, as well as the experimental concentrations obtained for benzene (B), toluene (T), ethylbenzene (E), xylenos (X) and the sum of BTEX in the soluble fraction oil in seawater, related to factorial planning.

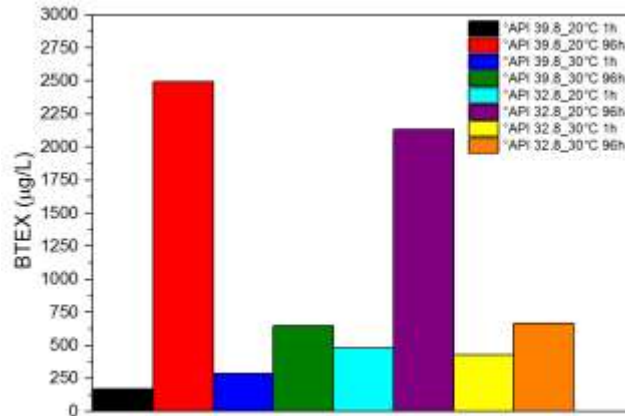
**Table 2.** Experimental planning matrix 2<sup>3</sup>.

Run	Trails			Experimental results (µg/L)				
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	B	T	E	X	BTEX
1	1	1	-1	331,5	1204,6	99,8	867,7	2503,6
2	1	1	-1	328,4	1209,5	88,1	868,4	2494,4
3	-1	1	-1	525,3	999	83,9	523,8	2132,0
4	-1	1	-1	516,9	1003,1	94,9	529	2143,9
5	1	1	1	32,4	241,4	11,4	305,9	591,1
6	1	1	1	36,9	286,4	15,7	375,6	714,6
4	-1	1	1	27,4	327	28,1	345,6	728,1
8	-1	1	1	39,2	249,1	23,5	307,6	619,4
9	1	-1	-1	57,33	77,96	4,82	34,8	174,91
10	1	-1	-1	57,96	77,92	4,7	35,1	175,68
11	-1	-1	-1	215,3	209,5	12,4	59,5	496,7
12	-1	-1	-1	214,6	192,3	11,6	54,6	473,1
13	1	-1	1	79,9	135,3	8,7	65,3	289,2
14	1	-1	1	85	138,6	8,5	68,5	300,6
15	-1	-1	1	206,6	179,8	10,5	49,5	446,4
16	-1	-1	1	193,4	167,6	10,2	47	418,2

Source: Authors (2021).

The levels of significant factors and effects of interactions between the factors that influence the responses were analyzed by factorial planning 2<sup>3</sup>. Three important factors were selected, as described in the literature: the oil °API, contact time of oil/seawater and temperature of the environment, both at two levels, planned according to the matrix described in Table 2. Figure 1 shows the average concentration of BTEX in FSA's, one can observe that concentrations increased with the increase of the contact time of oil and seawater from 1 hour to 96 hours, for the oils of °API 39.8 and °API 32.8. On the other hand, the increase in temperature from 20 °C to 30°C in the contact time of oil and seawater of 96h, there was a decrease in dissolved BTEX, possibly due to the high volatility of these hydrocarbons.

**Figure 1.** Average concentration of BTEX in FSA of 32.8 °API and 39.8 °API, in seawater oil contact times from 1 h to 96h, and ambient temperatures of 20 °C and 30°C.



Source: Authors (2021).

### 3.2 Regression Analysis

Regression analysis was applied in order to obtain mathematical models that resulted in second-order polynomials equations and that express the relationship between each response and significant factors and interactions. The following models were obtained by experimental design.

$$\text{Benzene}(\mu\text{g/L}) = 184.26 - 58.08X_1 + 45.49X_2 - 96.65X_3 + 10.63X_1X_2 + 9.03X_1X_3 - 99.12X_2X_3 - 19.09X_1X_2X_3 \quad \text{Eq.1}$$

$$\text{Toluene}(\mu\text{g/L}) = 418.692 + 2.76X_1 + 271.32X_2 - 203.04X_3 + 42.69X_1X_2 - 7.99X_1X_3 - 210.99X_2X_3 - 39.54X_1X_2X_3 \quad \text{Eq.2}$$

$$\text{Ethylbenzene}(\mu\text{g/L}) = 32.30 - 2.09X_1 + 23.38X_2 - 17.73X_3 + 0.16X_1X_2 - 1.41X_1X_3 - 18.27X_2X_3 - 2.79X_1X_2X_3 \quad \text{Eq.3}$$

$$\text{Xylenes}(\mu\text{g/L}) = 283.62 + 44.04X_1 + 231.83X_2 - 87.99X_3 + 44.91X_1X_2 - 35.84X_1X_3 - 93.78X_2X_3 - 46.03X_1X_2X_3 \quad \text{Eq. 4}$$

$$\text{BTEX}(\mu\text{g/L}) = 918.87 - 13.36X_1 + 572.02X_2 - 405.42X_3 + 98.39X_1X_2 - 26.22X_1X_3 - 422.17X_2X_3 - 69.27X_1X_2X_3 \quad \text{Eq. 5}$$

### 3.3 Statistical validation of the models obtained

A very important step is the validation of the constructed models, that is, their adequacy to the experimentally obtained responses, which will dictate their predictability (Breitkreitz *et al.*, 2014). Moreover, the validation of the models was determined by the Variance Analysis (ANOVA), presented in Table 3, and the correlation measure used to estimate the model was the coefficient of determination ( $R^2$ ).

**Table 3.** Experimental planning matrix 2<sup>3</sup>.

Source of Variation	Quadratic Sum	*GL	Quadratic Mean	F <sub>calc</sub>	F <sub>calc</sub> /F <sub>tab</sub>	Model adjustment
Benzene (R <sup>2</sup> - 0.999)						
Regression	414890	7	59270.03	2151.38	614.68	Significative and predictive
Residue	220,4	8	27.55			
Total	415110,6	15				
Toluene (R <sup>2</sup> - 0,998)						
Regression	2609244	7	372749.14	694.31	198.37	Significative and predictive
Residue	4295	8	536.86			
Total	2613539	15				
Ethylbenzene (R <sup>2</sup> 0,992)						
Regression	19338	7	2762.56	148.16	42.33	Significative and predictive
Residue	149,16	8	18.64			
Total	19487,12	15				
Xylenes (R <sup>2</sup> - 0.998 )						
Regression	1242298	7	177471.14	445.75	127.36	Significative and predictive
Residue	3185	8	398.14			
Total	1245483	15				
BTEX (R <sup>2</sup> - 0.998)						
Regression	10962280	7	1566040	870.72	248.78	Significative and predictive
Residue	14388	8	1798.56			
Total	10976668	15				

\*GL – Graus de Liberdade; F Test (F 7.8 = 3.5). Source: Authors (2021).

The F test was applied to verify the significance of the regression, since a F calculated (F<sub>calc</sub>) greater than F table (F<sub>tab</sub>) indicates a good regression and, consequently, a mathematical model that satisfactorily represents its experiment (Barros Neto *et al.*, 2010). The models showed significant regression at a 95% confidence level (F<sub>calc</sub> > F<sub>tab</sub>) with R<sup>2</sup> > 0.99 for all models, showing that they explained above 99% of the variation of the experimental data (Table 3). The F<sub>calc</sub> value of the regressions ranged from 42.33 to 614.68 times higher than F<sub>tab</sub>, to a confidence level of 95% for all cases analyzed, implying useful models to perform predictions within the studied range (predictive models).

### 3.4 Quantitative evaluation of proposed models

In order to quantitatively evaluate the prediction of the proposed models, it was chose to use the mathematical equations to calculate the concentrations of BTEX compounds in the experimental conditions previously performed in this study. Thus, the values of the variables °API, time and temperature in the mathematical equations and the concentration of BTEX compounds then calculated were replaced. Table 4 shows a relationship between the actual values, obtained by the mean between the experimental tests in duplicate, and the values calculated by the models.

**Table 4.** Experimental mean values and model calculated values for BTEX.

Variable			Experimental results (µg/L)					Calculated results (µg/L)				
X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	B	T	E	X	BTEX	B	T	E	X	BTEX
1	1	-1	329.9	1207	94.0	868.1	2499.0	330.0	1207.1	94.0	868.1	2499.0
-1	1	-1	521.1	1001	89.4	526.4	2137.9	521.1	1001.1	89.4	526.4	2138.0
1	1	1	34.7	263.9	13.6	340.8	653.0	34.7	263.9	13.5	340.8	652.9
-1	1	1	33.3	288.1	25.8	326.6	673.7	33.3	288.1	25.8	326.6	673.8
1	-1	-1	57.6	77.9	4.8	34.9	175.2	57.6	77.9	4.8	35.0	175.3
-1	-1	-1	215.0	200.9	12.0	57.1	485.0	215.0	200.9	12.0	57.1	484.9
1	-1	1	82.5	137.0	8.6	66.9	295.0	82.5	137.0	8.6	66.9	294.9
-1	-1	1	200.0	173.7	10.3	48.2	432.2	200.0	173.7	10.4	48.3	432.3

Source: Authors (2021).

Table 4 shows that the values calculated by the models are close to the actual values, corroborating the results of significance and prediction presented and ensuring that all models satisfactorily represent the experimental data. Furthermore, the model error was also calculated in percentage to evaluate how true the estimated values are. Table 5 presents the errors of the models.

**Table 5.** Model errors for BTEX, in percentage.

Variables			Errors (%)				
X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	B	T	E	X	BTEX
1	1	-1	0.0003	0.0001	0.0000	0.0000	0.0000
-1	1	-1	0.0000	0.0001	0.0000	0.0000	0.0000
1	1	1	0.0000	0.0000	0.0074	0.0000	0.0002
-1	1	1	0.0000	0.0003	0.0000	0.0000	0.0001
1	-1	-1	0.0000	0.0000	0.0000	0.0029	0.0006
-1	-1	-1	0.0000	0.0000	0.0000	0.0000	0.0002
1	-1	1	0.0000	0.0000	0.0000	0.0000	0.0003
-1	-1	1	0.0000	0.0000	0.0097	0.0021	0.0002

Source: Authors (2021).

Table 5 shows a maximum difference of 0.0097% between the experimental results and the results calculated by the model for ethylbenzene. The models were able to predict the experimental results with very low errors for all BTEX.

### 3.5 Effect of variables on BTEX solubilization

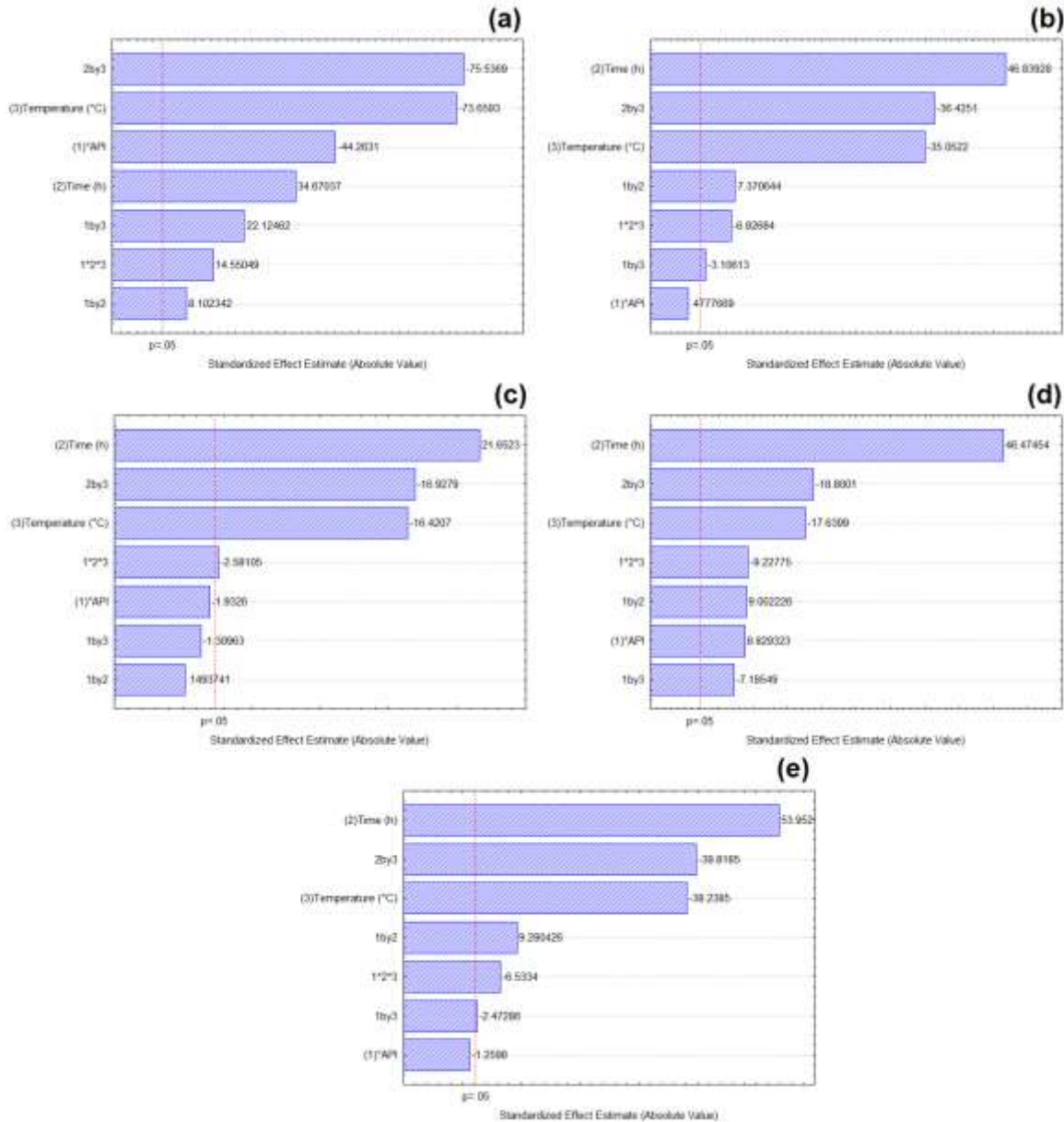
The effects of the oil °API, contact time oil/seawater after spillage and ambient temperature, were evaluated using Pareto diagrams. Variables with statistical significance in the process were identified for a confidence level of 95%. Figure 2 shows the Pareto diagrams for BTEX.

By analyzing the Pareto diagrams (Figure 2) one can observe that the oil °API, oil contact time in seawater after the spill and temperature have significant effects, in isolation and/or combined in the solubilization of BTEX in seawater, within the studied intervals. Taking into account the effect of the isolated variables and combined with the other factors, it is observed that the time variable showed greater influence on the solubilization process of BTEX, followed by temperature and oil °API, respectively. For all contaminants, the variable time presented an effect directly proportional to the response, that is, the longer the time, the higher the concentration of BTEX in seawater after the oil spill. According to Meador and Nahrgang (2019), Marques *et al.* (2017) and Sammarco *et al.* (2013), about 15% of the oil mass is composed by volatile hydrocarbons (VOCs) that are quickly lost by evaporation in cases of oil spillage at sea. In this study, we noticed the increase in BTEX



concentrations in the soluble fraction of oil in seawater with the increase of contact time oil from seawater from 1 h to 96 h, from  $\cong 2$  to 15 times. This may have occurred possibly, because all experiments were carried out in a laboratory with controlled ambient conditions, without weathering.

**Figure 2.** Pareto chart: (a) Bezene, (b) Toluene, (c) Ethylbenzene, (d) Xylees and (e) BTEX.



Source: Authors (2021).

For the temperature variable, the inversely proportional effect was perceived for all models (Figure 2), which implies that the lower the temperature, the higher the solubilization of BTEX in seawater. As BTEX are volatile compounds, temperature directly influences the volatilization of these compounds, due to the high vapor pressures (Gebara *et al.*, 2013), thus for marine environments with temperatures higher than 20 °C, to a tendency of greater volatilization of BTEX, consequently decreasing the amount solubilized in water. In relation to the oil °API, different behaviors were verified: for benzene, the higher the oil °API, the lower the presence of these contaminants, for Xylees, the higher the oil °API, the greater

the presence of this contaminant in seawater after oil spillage. For toluene, ethylbenzene and BTEX, °API does not influence the process in isolation. In these cases, only when combined with the other variables, the °API will influence the solubilization of these compounds in seawater.

### 3.6 Acute toxicity - *Mysidopsis juniae*

Using the test method with *Mysidopsis juniae*, according to NBR 15308 (ABNT, 2017), it allowed to determine the LC50%, which is the median lethal concentration that causes mortality or immobility at 50% of *M. juniae* after the 96-hour exposure period, being it calculated by the Trimmed Spearman-Kärber method (Hamilton *et al.*, 1977). Making inference about the acceptable internal laboratory quality control, in all experiments, mortality did not exceed 10% in the negative control and sensitivity of *M. juniae* to zinc, a LC50–96 h of  $0.26 \pm 0.05$  mg/L was reached, within the values reported by Figuerêdo *et al.* (2016a), Figuerêdo *et al.* (2016b), Nilin *et al.* (2019) and Nascimento *et al.* (2020), ranging from 0.25 to 0.46 mg/L. Thus, the sensitivity of organisms is a determining factor of environmental impact, as well as the intensity and frequency of exposure (Connon *et al.*, 2012).

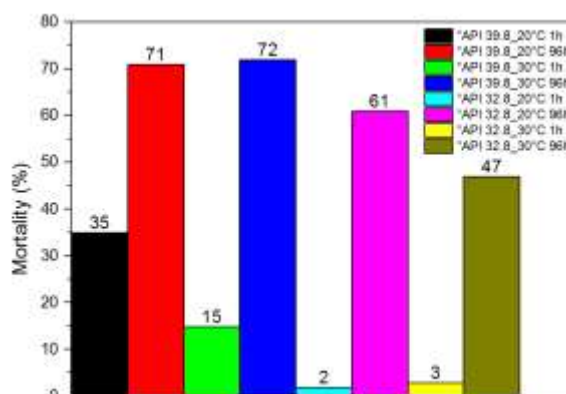
Table 6 and Figure 3 show the number of dead organisms and the mortality rate of *Mysidopsis juniae* after exposure to the soluble fraction of oil in seawater at 6.25, 12.5, 25, 50 e 100% (v/v).

**Table 6.** Number of dead organisms and the mortality rate of *Mysidopsis juniae* after exposure to the soluble fraction of oil (FSA) in seawater at 6.25% at 100% (v/v).

Concentration % (v/v) of FSA	Number of dead organisms								
	° API				32.8				
	Temperature (°C)		39.8		20		30		
	Time (h)		1h	96h	1h	96h	1h	96h	
6.25		2	5	3	7	0	5	2	1
12.5		1	19	0	13	2	11	1	2
25		6	23	1	28	0	16	1	9
50		14	30	6	30	0	30	0	29
100		29	30	12	30	1	30	1	30
<b>Dead total</b>		52	107	22	108	3	92	3	71

Source: Authors (2021).

**Figure 3.** Pareto chart: (a) bezeno, (b) toluene, (c) ethylbenzene, (d) xylees and (e) BTEX.



Source: Authors (2021).

The parameters (salinity, pH and DO) were measured during the ecotoxicological tests to verify the minimum survival conditions of *M. juniae*. The physical-chemical parameters were measured at the beginning and end of each experiment (including controls), and were within the acceptable range (ABNT 2017). The mean pH was  $8.0 \pm 0.3$  (n = 24), the mean DO concentration was  $4.8 \pm 0.6$  mg/L (n = 24) and the mean salinity was  $34.2 \pm 0.6$  (n = 24). Therefore, the authors chose not to renew the test solutions, aiming to achieve exposure conditions that most closely resemble dweller in a case of accidental oil spillage in the environment.

Petroleum hydrocarbons are chemical compounds widely known for their toxicity and mobility in water. In order to evaluate the toxicity of the soluble fraction of the oils studied in seawater, the acute toxicity was evaluated by the mortality rate of *Mysidopsis juniae* organisms in 96 h of exposure to soluble fraction of petroleum. Figure 3 showed an increase in the mortality rate of organisms with an increase in the contact time of oil and seawater from 1 h to 96 h, corroborating the results found in the Concentrations of BTEX of FSA. However, it was observed (Figure 3) that the FSA of the oil sample of °API 39.8 with a temperature of 30 °C and contact time oil seawater of 96h, which presented average concentration of benzene 33.3 µg/L, toluene 288.0 µg/L, ethylbenzene 25.8 µg/L, xylenes 326.6 µg/L and total BTEX 673.7 µg/L much lower than the FSA sample of the same oil with room temperature of 20°C and the contact time oil sea water of 96h, but presented a similar mortality rate of 72%. This high mortality rate is possibly due to other larger chain hydrocarbons, such as polyaromatics (HPAs) that have been solubilized in seawater with increased temperature, or due to petroleum compounds called complex mixtures of unresolved aromatics (MCU), as reported by Melbye *et al.* (2009), which are persistent pollutants, biocomulant and toxic, generally neglected in petroleum contamination (Booth *et al.* 2007).

Table 7 shows the median lethal concentration (LC%) calculated with a 95% confidence limit for all oil soluble fraction samples studied. There is an increase in toxicity with the contact time of oil in seawater from 1 hour to 96 hours, this condition occurs since with the increase of time, hydrocarbons become more bioavailable to *Mysidopsis juniae*, possibly causing greater toxicity of the soluble fraction of the oil.

**Table 7.** Median lethal concentration (LC%) for all oil soluble fraction samples.

Sample	CL50%
°API 39.8_20 °C 1h	46.07
°API 39.8_30 °C 1h	*NC
°API 39.8_20 °C 96h	11.38
°API 39.8_30 °C 96h	12.68
°API 32.8_20 °C 1h	*NC
°API 32.8_30 °C 1h	*NC
°API 32.8_20 °C 96h	18.55
°API 32.8_30 °C 96h	28.29

\*NC – Not calculated; Source: Authors (2021).

#### 4. Conclusion

The proposed models can be used to quantitatively predict BTEX contamination in seawater with accuracy greater than 99%, within the range of contact time seawater oil, ambient temperature and °API addressed in this work. The approach of the statistical modeling technique using experimental planning was constituted, in this experiment, as a potential tool in the prediction and dimension of contamination of BTEX compounds in seawater after oil spill age and its possible environmental impacts. From the developed models, it is possible to estimate the concentration of BTEX during an oil spill or spill, and this information can assist in emergency plans, as well as in the taking of mitigation and remediation actions of contaminated areas. Moreover, regarding toxicity, it was verified that the contact time of oil with seawater directly influences the increase in

toxicity due to hydrocarbons becoming more bioavailable to *Mysidopsis juniae*, possibly causing greater toxicity of the soluble fraction of the oil. The experimental analyses indicate that the concentrations of BTEX in seawater and the toxicity of FSA are favored at the lowest temperatures, because at 30°C it is observed the volatilization of these compounds with 96h, decreasing their concentration in seawater, this information being a warning point for regions with temperatures between 20 °C and 30 °C, where accidents occur with these types of oil.

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