Anuário do Instituto de Geociências
Universidade Federal do Rio de Janeiro
https://revistas.ufrj.br/index.php/aigeo/

ISSN 0101-9759
e-ISSN 1982-3908

Determination of the Toxic Potential of the Formation of Oil-Suspended Particulate Matter Aggregates (OSA) in a Simulated Microscale Experiment

Determinação do Potencial Tóxico da Formação de Agregado Óleo-Material Particulado em Suspensão (OSA) em um Experimento Simulado em Microescala

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Abstract

The aggregation of suspended particulate matter (SPM) and oil droplets in environments with specific hydrodynamic energy can lead to the formation of Oil-SPM Aggregates (OSA). A laboratory simulation was conducted in order to examine the possible toxic potential of OSA formation using three different concentrations of particulate material (50, 200, 300 mg/L) in a microscale experiment. The procedure was performed through toxicological testing of acute exposure to determine the LC_{50} (lethal concentration 50%) using the microcrustacean *Artemia salina* as a test organism. Serial dilutions were made from surface and bottom samples in order to characterize different toxicity. The concentration that showed the highest potential toxicity was 200 mg/L, having the same values for surface and bottom (LC_{50} 7.91%), whereas the concentration with the least toxic potential was 300 mg/L (LC_{50} 31.5%) for surface samples. Negative correlation was found between redox potential and the hydrogenionic potential (only for samples with 200 mg/L of sediment), and positive correlation was found between toxicity factors (percent dilution and mortality) and the other monitored parameters.

Keywords: Oil-SPM Aggregates; *Artemia salina*; Geochemistry

Resumo

A agregação de material particulado em suspensão (MPS) e gotículas de óleo em ambientes com energia hidrodinâmica específica pode levar à formação de agregados de óleo-MPS (em inglês, OSA). Uma simulação de laboratório foi conduzida para examinar o possível potencial tóxico da formação de OSA usando três concentrações diferentes de material particulado (50, 200, 300 mg/L) em um experimento em microescala. O procedimento foi realizado através de testes toxicológicos de exposição aguda para determinar a CL_{50} (Concentração Letal de 50%) usando o microcrustáceo *Artemia salina* como organismo de teste. Diluições seriadas foram feitas a partir de amostras de superfície e de fundo, a fim de caracterizar diferentes toxicidades. A concentração que apresentou a maior potencial tóxico foi de 200 mg/L, com os mesmos valores para superfície e fundo (CL_{50} 7,91%), enquanto a concentração com menor potencial tóxico foi de 300 mg/L (CL_{50} 31,5%) para amostras de superfície. Foi encontrada correlação negativa entre o potencial redox e o potencial hidrogeniônico (apenas para amostras com 200 mg/L de sedimento) e a correlação positiva entre os fatores de toxicidade (porcentagem de diluição e mortalidade) e os outros parâmetros monitorados.

Palavras-chave: Agregados óleo-MPS; *Artemia salina*; Geoquímica

DOI: https://doi.org/10.11137/1982-3908_2021_44_35441

1 Introduction

The interaction between oil droplets and suspended particulate matter (SPM) has been the focus of investigation in several studies, not only in concerns to its properties, but also related to the variables that characterize its formation (Owens 1999; Lee et al. 2001, 2003; Khelifa et al. 2003, 2005; Muschenheim & Lee, 2002; Owens & Lee 2003; Loh 2020). Oil-SPM Aggregates (OSA) are effective measures to cleanse oil spills. The oil droplets are easily fragmented into smaller particles of few micrometers, increasing its surface contact and leading to a quick transfer from the water surface to the water column, (Le Floch et al. 2002; Stoffyn-Egli & Lee 2002; Leoncio et al. 2020). The formation of OSA in the environment by the oil-SPM interaction increases the oil dispersion rate, biodegradation and prevents that it continues to adhere to the sediment (Owens 1999; Lee & Stoffyn-Egli 2001).

The presence of oil droplets can also increase the concentration of soluble hydrocarbons, increasing its bioavailability and promoting an intensification in the natural degradation as well as in the oil toxicity due to the exposure to aromatics such as benzene, toluene and xylene. These compounds are highly soluble in water compared to others, which makes the marine organisms more vulnerable to absorption through the tissues, gills or by direct ingestion (through contaminated water or food) (ITOPF 2011).

Acute toxicity tests are widely used to measure possible effects of toxic compounds to some species during a short period of time compared to the life cycle of the species in test (Libralato 2016). The dose or concentration of a toxic compound that could cause a specific and measurable reaction in the test organism is evaluated in a short period of time, usually between 24-96 hours. The main measured effect in acute toxicity tests is the lethality or other behavior that precedes it such as immobility. The acute toxicity tests are expressed in EC_{50} (Effective Concentration) and LC_{50} (Lethal Concentration). These values are always related to 50% or the tested organisms because the results are then more reproducible, reliable and easily extrapolated to the whole population (Costa et al. 2008).

The microcrustacean *Artemia salina* (Leach 1819) is extensively used as a test organism for ecotoxicological studies in estuarine, marine and hyper-saline environments. Its wide salinity (5-250) and temperature (6-25°C) tolerance, short life cycle, high adaptability to adverse environmental and laboratorial conditions, high fecundity (sexual reproduction and parthenogenesis), small size, non-selective diet and high adaptability to diverse nutrients make them reliable, viable and with great cost effectiveness for laboratorial essays (Nunes et al. 2006).

The present study aims to evaluate the sensibility of *Artemia salina* in toxicological essays with static non-renewal tests to determine LC_{50} of the samples collected for both sites (surface and bottom fractions) in simulated microscale experiments of OSA formation using three different concentrations of particulate material (50, 200, 300 mg/L).

2 Material and Methods

Acute toxicological tests were carried out on serial percentage dilutions of surface and bottom from samples of simulations in microscale of the formation of OSA.

To elaborate the control chart, tests were done with a reference substance SDS (Sodium Dodecyl Sulfate, $NaC_{12}H_{25}SO_4$), in parallel to each essay. These tests were made with the purpose of illustrating the regularization of the culture sensitivity of the organisms.

To control the basic conditions of exposure and to subsidize the interpretation of the results, in the initial and final period of the tests each assay was followed by analyses of the associated physical and chemical parameters (pH, dissolved oxygen, temperature and conductivity).

The mathematical method Probit or Trimmed Spearman-Karber Method was used to analyze the data produced by the acute tests with microscale simulations and reference substances who were expressed as mortality and survival data of adults (LC_{50}), as recommended by USEPA (2002). Confidence intervals of 95% were used to produce mortality (or survival) responses that would be given for each experiment compared to the control group.

2.1 Sampling and Sediment Storage

Sediment samples were collected at six stations during low tide along the Pardo river, near the municipality of Canavieiras, southern region of Bahia State. Samples were collected in the first five centimeters respecting the limits of a transect with approximately four meters in length, where 20 portions of the mangrove substrate were randomly collected, homogenized in a glass tray and preserved in up to 4°C. The physical-chemical parameters of the sediment (pH, redox potential, temperature, salinity, conductivity, dissolved oxygen, total solids and turbidity) were measured in situ using a multiparameter sensor (Horiba 104 U-54 Multiparameter Water Quality Checker).

For the experimental simulations, each sediment sample was calcined in a muffle at 450°C for 6h, mixed homogenized, sieved with a mesh of 100 µm and kept in a refrigerator at 4°C in glass containers for a period of 24h in order to obtain a composite unit.

2.2 Oil and Sediment Characterization

The characterization of the oil matrices and sediment was carried out at the Laboratory of Petroleum Studies (IGEO/UFBA), and comprised sediment grain size analyses, API grade (density) and viscosity/fluidity, HTP and HPA in the oil. The sediment analyses did not exceed the 48h post-collection period.

In the laboratory, after lyophilization, sieving (2 mm) and homogenization of the samples, grain size analysis were performed with the particle analyzer Laser Model Cilas 1064, following the procedures recommended by Centro Nacional de Pesquisa de Solos (2009).

For the simulations, crude oil from Campos Basin was used, whose density was measured six times with DMA 5000 Density Meter at 15°C. The viscosity was also determined three times under the same temperature conditions using the rotational viscometer Haake Viscotester VT500.

The distribution of hydrocarbons was also used to characterize the oil by Gas Chromatography coupled to a Varian CP 3800 flame ionization detector (GC-FID) fitted with a DB5 model 60 mm long, 0.25 mm of internal diameter, stationary phase of 0.25 µm thick and drag gas flow (helium).

2.3 Cultivation and Acquisition of Organisms

Cysts of *Artemia salina* were kept under the light in desiccators with silica gel to avoid contaminants and retain their hatch rate. Afterwards, they were hatched in glass aquariums with 1L capacity, with a salinity 15 and average temperature of 25°C and constant aeration followed by a methodology suggested by Veiga & Vital (2002), not exceeding the concentration of 0.5 g/L of cysts.

After 48h of hatching, the organisms were collected for reference tests and toxicity assays, since at this stage they are already suitable for testing once they start filtration activities, being more sensitive and decreasing the variability of the test (Sorgeloos, Van Der Wilen & Persoone 1978).

2.4 Tests with the Reference Substance Sodium Dodecyl Sulfate (SDS)

The solution was prepared in 100 mL flasks where 10 mg of SDS was added to each 100 mL of salt water at salinity 15 (obtained by diluting 30 g of artificial sea salt in 500 mL of water). Three reference tests were performed with SDS in order to evaluate the sensitivity range of the organisms used in the test and preparation of the toxicity control chart. Samples were then diluted in volumetric flasks at concentrations of 0, 9, 12, 16, 21, 27 and 35 mg/L.

Each dilution unit was done in triplicate with glass test vials containing 10 mL of the solution and ten organisms. The containers were placed on a tray and covered to minimize losses by evaporation. The conditions for the test were maintained respecting the photoperiod of 12h of day light and 12h of darkness at a temperature of 25°C during 48h. The observed endpoint is the LC_{50} for 48h, meaning that after a two-day exposure, living and dead organisms were counted in each test with the aid of a stereoscope or light microscope.

2.5 Microscale Simulation for the Formation of OSA

The protocol consisted of simulating the environmental conditions determining the formation of OSA, from erlenmeyers with 250 mL of artificial brackish water in salinity 15, and heavy sediment fractions for each, in order to respect the concentration value of 0.05, 0.2 and 0.3 g/L of sediment. The sediment was transferred to the erlenmeyers with artificial brackish water and each was covered with aluminum foil, placed for one minute on a shaker and left overnight at a temperature of 15 °C in the dark.

In the following day, each erlenmeyer spent an additional minute on the shaker at 2.1 Hz (126 RPM) and then it was added 0.05 g (50 mg or 0.05 cm^3) of oil, considering the expected value of 50 mg. A further three hours of stirring occurred on the shaker at 126 RPM and an additional rest of one night. Samples of OSA were used shortly after the end of the preparation and surface and bottom fractions were randomly collected.

2.6 Acute Toxicological Tests

For the acute toxicity tests of the OSA simulation experiments, samples collected from the surface and bottom were diluted with artificial brackish water with salinity 15, each dilution unit was done in triplicate. Concentrations of 0.05, 0.2 and 0.3 g/L of sediment to about 0.05 g of oil, in five serially diluted percentages (100%, 50%, 25%, 12.5% and 1%) were used for each experiment. Ten adult males or immature females were added to each sample containing 15 mL of the diluted solution.

The physicochemical parameters of all the tests were measured in the initial and final period of the assays. The temperature was measured with a thermometer coupled to the oximeter with accuracy of ± 0,05 °C; the pH were measured using a portable pH meter with an accuracy of ± 0.01 pH units; the salinity of the water was monitored using a portable refractometer and dissolved oxygen was measured with a portable micro-processed digital oximeter with ± 0.05% accuracy and conductivity with a conductivity meter, digital portable with ± 0.05% accuracy.

3 Results and Discussion

3.1 Oil and Sediment Characterization

The results concerning the sediment and oil characterization used in the present study are described in Table 1, and related to the collection schedules described therein, which followed restrictions due to the low tide (around 0.3 m). These results show the need to characterize the sediments used in the tests to control the experimental conditions.

The pH values presented a neutral to slightly basic character (with exception of station 3 that showed relative acidity), with a small variation among the six sampling stations, exhibiting the highest value at station P1 (9.91) and the lowest at P3 (5.93), which may be indicative of uniformity of characteristics between these stations, as a consequence of similar contributions from marine and fresh water.

In regards to the Eh values, a large variation was observed between the highest and the lowest observed at stations 3 and 1 (325.0 mV) and 5 (45.0 mV), respectively, all of which showing significative oxidizing characteristics. This may represent an instability during the measurement of this parameter.

The temperature variation did not exceed 4.2°C in between stations, with the highest recorded value at P4 (30.8°C) and the lowest at P1 (26.5°C). This variation in temperature may be related to the influence of climatic conditions as a function of sampling times and locations, integrated to the intrinsic characteristics of the sampled stations.

The salinity, as expected for an estuarine environment, remained relatively high in the first three stations (30, 20, 12) and significantly reduced in the last three (6, 6 and 5 respectively). It is important to observe that there is a gradual reduction in the salinity values, where the highest were found at sampling stations that are influenced by the upstream tide salinity and the lowest ones by the river influence. This behavior is explained by the tide penetration, which causes the samples collected in the mangrove regions to have greater salinities due to the proximity to the ocean.

The values of pH, Eh, and salinity are temperature-dependent, increasing and decreasing according to its variation, in a non-linear way. All these environmental factors contribute to the cations that are sorbed to the particles that constitute the mangrove substrates can become bioavailable (Queiroz & Celino 2008). The results of the physicochemical parameters in the different sampling stations are determinant in order to correlate their influential effects to the toxicity found in the sediments.

The values of dissolved oxygen ranged from a maximum of 21.04 mL/L to a minimum of 15.20 mL/L. It is influenced by sediment composition, temperature, water salinity and hydrodynamic factors and provides indications about the natural condition of the environment and makes it possible to detect possible environmental impacts such as eutrophication and organic pollution. The carbonic gas, molecular oxygen, nitrite and nitrate ions and water itself are the main sources for dissolved oxygen (Baumgarten et al. 1996).

Dissolved oxygen had a behavioral pattern of values very similar to the temperature. The temperature values at the different stations can be considered a determinant factor for the distribution of the dissolved oxygen, since the temperature determines the solubility of the gases (Fiorucci 2005).

Table 1 Geographic coordinates and descriptive physicochemical parameters at the sampling stations on the river margin of Pardo river, municipality of Canavieiras, southern Bahia. Legend: pH = hydrogenionic potential; Eh = redox potential; D.O. = Dissolved Oxygen; T.D.S. = Total dissolved solids; NTU = Nephelometric Turbidity Unit.

	Geographic coordinates	**Time**	**physical-chemical parameters (*in situ*)**							
			pH	**Eh (mV)**	**Temperature (°C)**	**Salinity**	**Condtuctivity. (S/m)**	**D.O. (mL/L)**	**T.D.S. (mg/L)**	**Turbidity (NTU)**
P1	S 15° 41' 58" W 38° 55' 54"	07:35	9.91	45	26.56	30	30.8	15.2	17.4	121.0
P2	S 15° 41' 36" W 38° 56' 21"	08:57	8.55	163	29.62	20	35.7	18.3	21.8	26.6
P3	S 15° 40' 52" W 38° 56' 24"	09:22	5.93	325	30.02	12	23.4	17.66	14.5	30.6
P4	S 15° 41' 26" W 38° 57' 05"	10:03	7.89	245	30.82	6	15.5	21.04	9.61	49.1
P5	S 15° 41' 25" W 38° 57' 35"	10:56	7.61	240	30.37	6	14.8	19.63	9.16	99.2
P6	S 15° 40' 58" W 38° 58' 25"	11:47	7.91	222	30.08	5	12.3	18.95	7.62	179

The conductivity showed a large variation and the highest observed value was at station P2 (35.7 mS). Also was found an inverse relation between the behavior of Eh and compared to the conductivity, since the presence of dissolved ions can exert an enormous influence on the recorded values of conductivity (Queiroz & Celino 2008).

The highest value found in total dissolved solids was seen at P2 (21.8 mg/L) and the lowest value at P6 (7.6 mg/L) so the total solids values were directly proportional to the conductivity. Sampaio et al. (2007) had already developed studies demonstrating that there is a strong linear correlation between these two parameters, which can be explained by the fact that they are in solution and in a colloidal state (Braile & Cavalcanti 1993).

According to Pina et al. (2003), the high turbidity found in estuaries is a consequence of the high tidal flow, the fluvial load, the great extension of intertidal areas and the great susceptibility to the generation of waves that intensify the resuspension. The highest values of turbity was found at station P6 (179.0 NTU) followed by P1 (121.0 NTU) and the lowest value was recorded at P2 (20.6 NTU). These values show that a set of environmental conditions in the study area could enable and sometimes attenuate the formation of OSA in these sites, considering the factors that influence the formation of these aggregates in the natural environment as temperature, turbidity, grain size and salinity.

Regarding the grain size at the sampling stations, the mean values show that there is a predominance of the fractions of very fine sand (62.5 - 125 µm) and silt (3.9 - 62.5 µm) according to the grain size classification proposed by the Krumbein scale (Krumbein & Sloss 1951) (Figure 1). According to Sun & Zheng (2009) and Li et al. (2020), granulometry is a determining factor for the formation and destination of OSA and that it is attenuated in sediments with particle size of less than 5 µm, due to its larger available surface area. The predominance of the silt fraction in the sampled sediment confirms the high viability for the formation of OSA in the study area.

The crude oil from the Campos Basin used to perform the tests had a density of 0.8823 g/mL, the average of viscosity found was 33.44 mPa.s. The Campos Basin oil contain a higher composition of low molecular weight n-alkanes (nC10-nC17), suggesting typically marine oils. The value of the relationship Pristane/Fitane (Pr/Ph)>1 is an indicative of a depositional environment with oxidizing conditions (oxides). The ratio nC17/nC29 >1 (nC29 is a terrestrial indicator and n-C17 is an indicator of marine algal material), indicates predominantly origin of marine material input. The Pr/nC17 and Ph/nC18 ratios being less than 1, and the value of IPC=1.07, can indicate the maturity for this oil (Barragan 2012). There was no evidence of biodegradation for the oil, justifying its use for the tests performed.

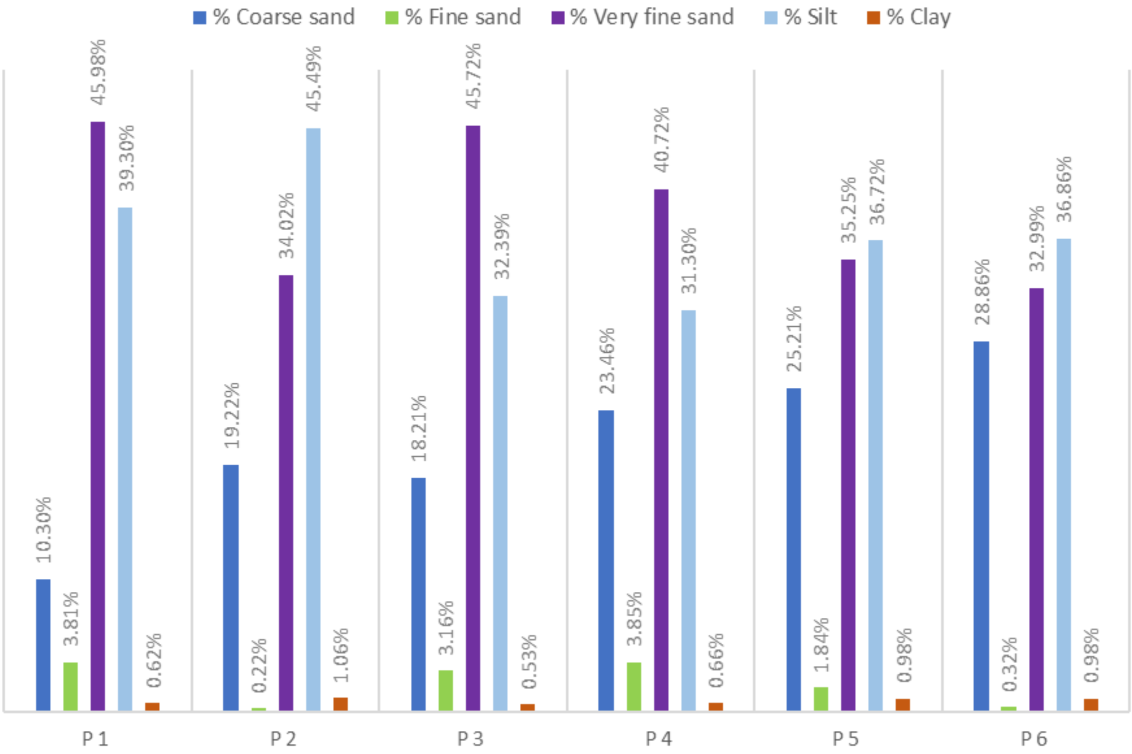

Figure 1 Graphical representation of the grain size values along the Pardo river, at different sampling stations.

3.2 Tests with the Reference Substance Sodium Dodecyl Sulfate (SDS)

The acute toxicity tests with the reference substance SDS were performed concurrently with the sensitivity tests and showed results within the described acceptability limits (13 - 30.9 mg/L) (Veiga & Vital 2002). The LC50-48h values were 27.06; 14.19 and 14.51 mg/L, being therefore within the established sensitivity range. Table 2 specifies the LC50 values found for each test, as well as the confidence intervals (95%), and the physicochemical parameters measured.

3.3 Acute Toxicology Tests

Acute toxicity tests were performed with the aqueous phase of the OSA formation simulation protocol (Moreira 2014). Each of these assays was characterized according to the number of partial mortalities adjusted to produce LC50 values associated with a 95% confidence interval. The toxicity test was performed between February 22-24, 2014 (48h), and the results of the analyses and their adjustments were performed using the Probit and Trimmed Spearman-Karber statistical methods shown in Table 3. Table 4 shows the physicochemical parameters recorded at the beginning and at the end of the analyses.

According to Table 3, it can be concluded that the concentration with the highest toxic potential was 200 mg/L with equal values for surface and bottom (LC_{50} 7.91%). The concentration with the lowest toxic potential was 300 mg/L (LC_{50} 31.5) for surface samples.

The intensity of the linear associations of the test results with the physicochemical parameters was verified through the Pearson's Linear Correlation Coefficient. The correlation coefficient is positive if the variables are directly related, and negative if they are inversely related. Their maximum modular value being 1.

For the toxicological analysis, the values marked in red were significant considering p>0.05. This analysis was used to verify the parameters that contribute the most to toxicity, and strong positive correlations were found in all tested sediment concentrations (50, 200 and 300 mg/L) between the percent dilution and mortality.

The Pearson correlation analysis (ACP) shows an expressive correlation between pH and Eh (oxy-reduction potential) in all tested concentrations, expressing positive and negative influences depending on the concentration of sediment used. At the concentration of 200 mg/L, an anomalous behavior of these variables was observed, with pH having a strong negative influence and temperature as a factor of positive influence for the increase of toxicity. It is important to note that the redox potential is directly related to the bioavailability of toxic substances in the environment, especially metals associated with estuarine sediments (Nizoli & Luiz-Silva 2009).

A Principal Component Analysis (PCA) was used to estimate the similarity of the data, representing them into two orthogonal dimensions. The two factors represented in Figure 2 (A, B and C) explain the largest proportion of total variance among all linear combinations of the original data, having the second lowest proportion of the total variance in comparison to the first.

For the toxicological analysis, considering the sediment concentration of 50 mg/L, the two main PCA axes - Factors 1 and 2, explain 87.33% of the total variance (18.22% and 69.11%). For the sediment concentration of 200 mg/L, 96.53% of the total variance of the data is explained by the first two factors (83.25% and 13.28%) and at 300 mg/L, the variation of 86.51 % of the data is explained by factor 1 and 2 in 70.68% and 15.83%, respectively.

It was observed, for the sediment concentration of 50 mg/L, a strong positive correlation between the toxicity factors (percentage dilution and mortality) and the dissolved oxygen being the least influent among the monitored physicochemical parameters. It was also possible to observe the strong negative correlation of Eh with the toxicity factors and the other parameters, in agreement with the values shown in the Pearson correlation.

The PCA related to the concentration of 200 mg/L once again shows a strong and positive correlation between the toxicity factors, with pH being the parameter that shows an inverse influence to the results, as shown in the

Table 2 LC_{50} values with the confidence limits (95%) and physicochemical parameters representative of the reference test with SDS followed by average, standard deviation and coefficient of variance.

Reference Test (SDS)	LC_{50} (mg/L)	Confidence limits	pH	D.O. (mg/L)	Temperature (°C)
TR1	27.06	23.18 – 31.58	7.12	6.57	25.1
TR2	14.19	12.5 – 16.1	7.33	6.65	24.8
TR3	14.51	11.75 – 18.17	7.06	6.93	25.3
Average	18.58	**	7.17	6.71	25.06
Stand. Deviation	7.33	**	0.14	0.18	0.25
Coef. Vari.	0.39	**	0.01	0.02	0.01

Pearson correlation matrix (Table 5). The toxicity factors (concentration and mortality) strongly influenced the results just as temperature and dissolved oxygen.

In the graphic representation corresponding to the sediment concentration of 300 mg/L, it is possible to confirm the negative correlation between the oxy-reduction potential and a positive correlation between the toxicity factors (percentage dilution and mortality) and the other monitored parameters, verified through Pearson's correlation. Once again, the dissolved oxygen was considered a factor of low influence to the analysis.

These results corroborate the relationships found between the physicochemical parameters and the toxicity factors verified in previous studies (Amaral 2012) where the toxicity showed strong relationships with the sensitivity to the physicochemical parameters, observing a negative correlation between pH and the biochemical demand of oxygen. Considering the several processes that combined can conduct the observed results, it is possible to associate the observed toxicity in surface samples with results demonstrated in previous reports.

Table 3 Percentage of mortality and survival of the acute toxicity tests from the aqueous simulation phase of the OSA formation used to determine LC_{50} including adjusted ratios for calculation and confidence limit (95%) at different sediment concentrations (50, 200, 300 mg/L).

50 mg/L		**Surface**			**Botton**	
Percent Dilution	**Mortality**	**Survival**	**Adjusted ratios**	**Mortality**	**Survival**	**Adjusted ratios**
1%	3.4	6.6	0.334	4.4	5.6	0.44
12.50%	3	7	0.3	4.4	5.6	0.44
25%	5.4	4.6	0.54	4.7	5.3	0.47
50%	7	3	0.7	5	5	0.5
100%	8.67	1.33	0.867	5.7	4.3	0.57
CL_{50} (%)		25			70.71	
Confidence limit		13.01 - 48.03			**	
200 mg/L		**Surface**			**Botton**	
Percent Dilution	**Mortality**	**Survival**	**Adjusted ratios**	**Mortality**	**Survival**	**Adjusted ratios**
1%	4.7	5.3	0.47	4.4	5.6	0.44
12.50%	5.7	4.3	0.57	5.4	4.6	0.54
25%	6	4	0.6	6	4	0.6
50%	6.7	3.3	0.67	6.67	3.33	0.667
100%	7	3	0.7	6.67	3.33	0.667
CL_{50} (%)		7.91			7.91	
Confidence limit		0.31 - 204.80			0.31 - 204.80	
300 mg/L		**Surface**			**Botton**	
Percent Dilution	**Mortality**	**Survival**	**Adjusted ratios**	**Mortality**	**Survival**	**Adjusted ratios**
1%	4	6	0.4	3.34	6.66	0.334
12.50%	4.4	5.6	0.44	5	5	0.5
25%	5	5	0.5	5	5	0.5
50%	5.4	4.6	0.54	6.67	3.33	0.667
100%	8	2	0.8	7.7	2.3	0.77
CL_{50} (%)		31.5			13.5	
Confidence limit		7.10 - 139.68			2.28 - 77.51	

Table 4 Initial and final physical-chemical parameters (representative of the acute toxicity test with the microcrustacean *Artemia salina* from the aqueous simulation phase of the OSA formation, followed by Average, Standard Deviation and Coefficient of Variance at different sediment concentrations (50, 200, 300 mg/L).

50 mg/L	**Surface**								**Botton**							
	pH		**D.O. (mg/L)**		**Temperature (°C)**		**Eh (mV)**		**pH**		**D.O. (mg/L)**		**Temperature (°C)**		**Eh (mV)**	
Conc. (%)	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
12.50%	7.15	6.6	9.18	9.56	21.1	21.9	-24.6	12.8	7.62	6.68	9.23	9.52	21.5	22.8	-46.8	-2.3
25%	7.19	6.02	9.13	6.51	21.5	22.7	-27.6	19.8	7.64	6.15	9.23	9.4	21.4	22.5	-43.1	4.9
50%	6.84	6.55	9	9.41	23.7	23.1	-19.3	11.7	7.55	6.48	9.24	9.39	21.4	22.6	-37.4	-3.4
100%	7.07	8.45	9.14	9.63	22.2	22.9	-21.2	-88.7	7.27	6.26	9.26	9.44	21.5	21.3	-36.1	16.3
Average	7.0625	6.905	9.112	8.777	22.125	22.65	-23.15	-11.1	7.52	6.392	9.24	9.437	21.45	22.3	-40.8	3.875
S.D.	0.1564	1.062	0.078	1.514	1.1441	0.5259	3.675	51.857	0.171	0.235	0.0141	0.059	0.0577	0.6782	4.997	9.064
C.V.	0.0221	0.153	0.008	0.172	0.0517	0.0232	-0.158	-4.671	0.022	0.036	0.0015	0.006	0.0026	0.0304	-0.12	2.339

200 mg/L	**Surface**								**Botton**							
	pH		**D.O. (mg/L)**		**Temperature (°C)**		**Eh (mV)**		**pH**		**D.O. (mg/L)**		**Temperature (°C)**		**Eh (mV)**	
Conc. (%)	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
12.50%	7.61	6.6	9.25	9.04	21.9	20	-45.7	3.9	7.59	6.77	9.22	8.89	22	21.4	-46.4	5.9
25%	7.29	6.82	9.13	9.12	21.8	20.8	-46.3	0.8	7.52	6.58	9.21	8.92	21.8	21.2	-43.4	9.9
50%	7.6	7.07	9.25	9.16	21.9	24.9	-47.5	-22.6	7.54	6.61	9.18	8.91	21.9	22.8	-44.3	12.6
100%	7.6	7.04	9.27	9.33	21.6	24.4	-47.3	-15.7	7.57	6.36	9.22	9.09	22.1	24.1	-46.5	15.6
Average	7.525	6.882	9.225	9.162	21.8	22.525	-46.7	-8.4	7.555	6.58	9.207	8.952	21.95	22.375	-45.15	11
S.D.	0.1567	0.218	0.0640	0.122	0.1414	2.4837	0.8485	12.79	0.0310	0.168	0.018	0.092	0.1290	1.3524	1.545	4.120
C.V.	0.0208	0.031	0.0069	0.013	0.0064	0.1102	-0.018	-1.52	0.0041	0.025	0.002	0.010	0.0058	0.0604	-0.034	0.374

300 mg/L	**Surface**								**Botton**							
	pH		**D.O. (mg/L)**		**Temperature (°C)**		**Eh (mV)**		**pH**		**D.O. (mg/L)**		**Temperature (°C)**		**Eh (mV)**	
Conc. (%)	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
12.50%	7.79	6.92	9.24	8.73	21.7	25	-52.2	-4.3	7.77	7.04	9.22	8.79	21.7	22.2	-55.6	-18.9
25%	7.72	6.67	9.25	8.88	22	21.7	-52.6	8.2	7.67	7.01	9.23	8.63	21.7	21	-52.2	-19
50%	7.71	6.74	9.19	8.85	22.2	23.2	-52.8	-4.9	7.67	7.09	9.23	8.51	21.9	21.7	-51.3	-14.7
100%	7.61	6.1	9.22	8.78	22.3	25	-48.9	-4.3	7.61	6.75	9.21	8.5	22.2	21.4	-49.6	-13.1
Average	7.7075	6.6075	9.225	8.81	22.05	23.725	-51.625	-1.325	7.68	6.9725	9.2225	8.6075	21.875	21.575	-52.175	-16.425
S.D.	0.0741	0.3543	0.0264	0.0678	0.2645	1.5945	1.8337	6.3562	0.0663	0.1519	0.0095	0.1352	0.2362	0.5058	2.5250	2.9881
C.V.	0.0096	0.0536	0.0028	0.0076	0.0119	0.0672	-0.035	-4.7972	0.0086	0.0217	0.0010	0.0157	0.0108	0.0234	-0.0484	-0.18193

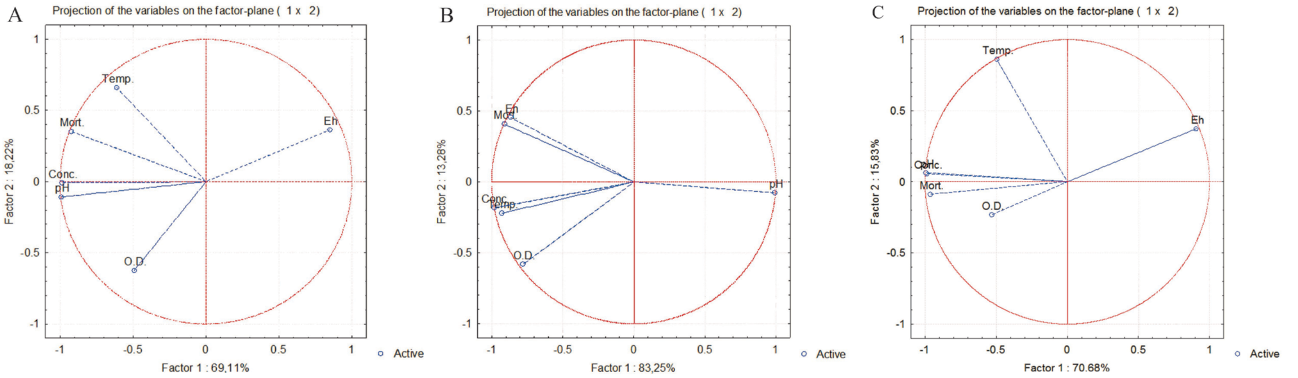
A. 50 mg/L **B.** 200 mg/L **C.** 300 mg/L

Figure 2 Graphical representation of the principal components (1 and 2) of the principal component analysis of the correlation matrix (percentage dilutions. mortality and physico-chemical parameters) for different sediment concentrations (A. 50 mg/L; B. 200 mg/L; C. 300 mg/L).

Table 5 Pearson correlation matrix for physicochemical parameters and toxicity tests for different sediment concentrations (50, 200 and 300 mg/L).

50 mg/L	**Conc.**	**Mort.**	**pH**	**D.O.**	**Temp.**	**Eh**
Conc.	1.00					
Mort.	0.93	1.00				
pH	0.97	0.88	1.00			
D.O.	0.39	0.15	0.59	1.00		
Temp.	0.52	0.74	0.56	0.16	1.00	
Eh	-0.90	-0.71	-0.86	-0.41	-0.11	1.00
200 mg/L	**Conc.**	**Mort.**	**pH**	**D.O.**	**Temp.**	**Eh**
Conc.	1.00					
Mort.	0.82	1.00				
pH	-0.96	-0.93	1.00			
D.O.	0.86	0.45	-0.74	1.00		
Temp.	0.96	0.78	-0.90	0.78	1.00	
Eh	0.76	0.95	-0.90	0.46	0.63	1.00
300 mg/L	**Conc.**	**Mort.**	**pH**	**D.O.**	**Temp.**	**Eh**
Conc.	1.00					
Mort.	0.98	1.00				
pH	0.97	0.93	1.00			
D.O.	0.39	0.33	0.59	1.00		
Temp.	0.52	0.37	0.56	0.16	1.00	
Eh	-0.90	-0.96	-0.86	-0.41	-0.11	1.00

Some studies have evaluated the oil persistence and ecosystem response 14 years after the accident with the Exxon Valdez oil tanker and concluded that surface oil-derived compounds persisted in surprising amounts in their most toxic form, being sufficiently bioavailable to induce biological exposures and that impacts are strictly associated with the existence of surface sediments (Peterson et al. 2003). Thus, residual concentrations of toxic chemical compounds on the surface added to the existence of surface sediment induce indirect effects in the trophic webs for example, transmitting impacts that go well beyond the acute mortality.

4 Conclusions

The present work aimed to evaluate the potential toxic character of the OSA formation from three microscale simulation experiments using the microcrustacean Artemia salina. A comparative study was carried out with three different sediment concentrations from the percentage dilution of its aqueous phase in surface and bottom fractions.

The concentration with the highest toxic potential was 200 mg/L, with equal values for surface and bottom (LC50 7.91%). The concentration with the lowest toxic potential was 300 mg / L (LC50 31.5) for surface samples.

It was observed a negative relationship between the oxy-reduction potential and the hydrogenionic potential (only in samples with 200 mg / L of sediment) and the other parameters studied. A positive correlation between the toxicity factors (percentage dilution and mortality) and the other monitored parameters was verified. Given the complexity and variability of hydrocarbons and water insoluble compounds, it is important to highlight the relevance of the relationship between toxicity analyses and the monitoring of the physicochemical parameters, since the confidence limits of the effects are not very small and in one of the tests it could not be calculated.

5 Acknowledgments

This study was conducted and financed during a scholarship supported by the CNPq (National Council of Scientific and Technological Development) at the Federal University of Bahia within the Brazilian Ministry of Science, Technology, Innovation and Communications.

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Received: 10 June 2020

Accepted: 05 July 2021

How to cite:

Silva, A.C.M., Azevedo, I.H.S. & Milazzo, A.D.D. 2021, 'Determination of the Toxic Potential of the Formation of Oil-Suspended Particulate Matter Aggregates (OSA) in a Simulated Microscale Experiment', *Anuário do Instituto de Geociências,* vol. *44*: 35441. https://doi.org/10.11137/1982-3908_2021_44_35441