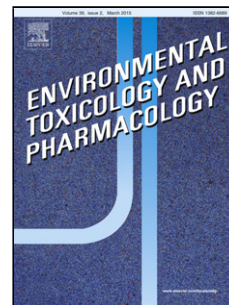


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**Chemical characterization and toxicity of water-accommodated fraction of oil on the South American native species *Hyaella curvispina***

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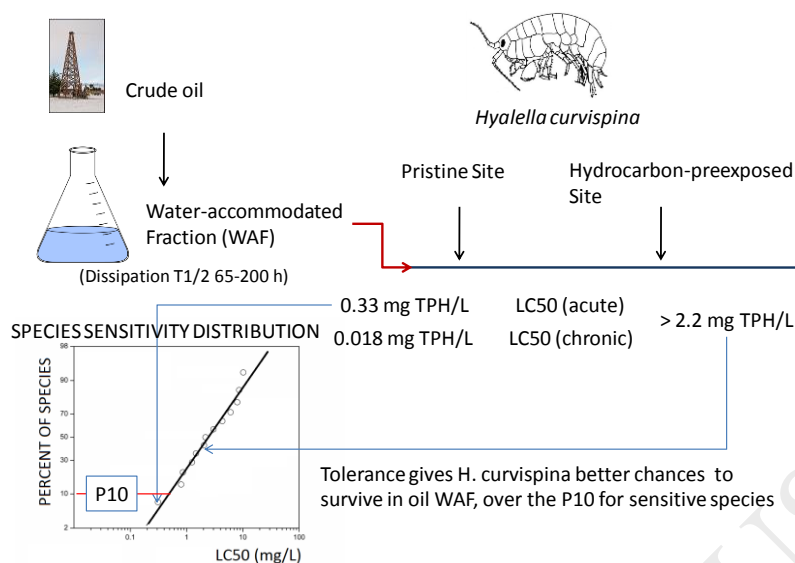
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## Graphical Abstract



## Highlights

- n-alkanes were the main fraction in WAF, followed by toluene in the aromatic group
- Main hydrocarbon components in the WAF show dissipation half-lives between 65-200 h
- TPHs are significantly higher in the oil-impacted stream than in the reference site
- Different sensitivity to WAF is determined on amphipods from both sites
- Freshwater species protection limit for WAF acute toxicity is below 0.5 mg L<sup>-1</sup>.

## Abstract

Chemical and toxicological crude oil analysis was performed on water-accommodated fraction of oil (WAF). This study characterized the chemical composition of WAF and its dissipation over a period of 192 h. Acute (96 h) and

chronic (14 d) toxicity of WAF were evaluated on *Hyaella curvispina* from both reference (Los Barreales lake, LB) and hydrocarbon-contaminated (Durán stream, DS) sites. The total hydrocarbon (TPHs) concentration in WAF was 2.18 mg L<sup>-1</sup>. The dissipation rates of hydrocarbons in WAF showed a first-order kinetics, with half-lives ranging between 65 h-200 h. Amphipods from LB showed acute and chronic LC50 values of 0.33 and 0.018 mg L<sup>-1</sup>, respectively. Amphipods from DS exposed to pure WAF showed no mortality in either acute or chronic assays. Further biochemical and molecular research is required to determine the mechanisms underlying the resistance to WAF exposure on DS amphipods.

### **Keywords**

Hydrocarbons; Water contamination; Aquatic toxicity; Amphipod; Species Sensitivity Distribution

### **1. Introduction**

Physical and chemical characterization has often been adopted to monitor the contamination levels of pollutants in various environments. They represent the water or sediment condition at the time of sampling (Springer et al., 2010). These analyses alone may not provide adequate information concerning the biological effects of most pollutants, which are crucial for assessing environmental quality (Long et al., 1995). Complementary to physical and chemical analyses, bioassays are carried out to assess the relative potency of a chemical or biological agent on living organisms. As a result, both bioassays and physical and chemical analyses have been widely used in the assessment

of environmental quality (Lee et al., 2005). Since the beginning of the 20<sup>th</sup> century, due to rapid industrial development, the hydrophobic organic pollution of the environment is one of the main ecological issues. In fact, the demand for crude oil hydrocarbons represents a growing environmental concern throughout the world due to the pollution problems derived from drilling, production and transport of crude oil (Martínez-Jerónimo et al., 2005). Crude oil is a complex mixture of aliphatic and aromatic hydrocarbons along with varying amounts of sulfur, nitrogen, oxygen and trace of metals such as nickel, vanadium and chromium (Law, 1993). The mixture composition depends on the source, age and conditions of geologic formation (Finga, 2015). Petroleum hydrocarbons can enter the aquatic systems either as products derived from petroleum industry such as fuels, lubricating oils, or products of incomplete combustion in exhaust emission (Müller, 1987). The dispersion of hydrocarbons in the environment is very complex and includes continuous transfer among compartments. Generally, low and medium molecular weight compounds of crude oil solubilize on the water column and they eventually volatilize or degrade along time. High molecular weight compounds have extremely low solubility in water and they are deposited and accumulated in sediments (Brandt et al., 2002; Sammarco et al., 2013). At the same time, petroleum hydrocarbons in sediments can be transferred to the water and the air by geochemical circulation, increasing secondary pollution (Ho et al., 1999; McLachlan et al., 2002). There are little available data regarding the persistence of hydrocarbons in the environment. Among petroleum hydrocarbon components, polyaromatic hydrocarbons (PAHs) have environmental half-lives ( $t_{1/2}$ ) ranging from weeks for

the low molecular weight fractions to years for the high molecular weight compounds (Brandt et al., 2002).

Oil contamination may cause serious problems to aquatic life. Most toxicological research of crude oil contamination has revealed that its toxicity is mainly caused by the water-accommodated fraction (WAF), rather than the dispersed droplets of the fraction (Zhou et al., 1994). Although high molecular weight compounds are the most toxic, the responsible agents for the toxic effects on aquatic organisms are those with low molecular weight because of their higher solubility in water and, as result, higher biodisponibility. The WAF is quite different from the parent oil and the concentration of components depends mainly on variables such as oil type, oil-water ratio, mixing time and exposure temperature (Faksness et al., 2008; Maher, 1986). In consequence, this could result in significant differences in their toxicity (Wells et al., 1993). Benthic organisms may accumulate hydrocarbons directly from the water column or the interstitial water from sediment as a consequence of chemical distribution between them (Bhattacharyya et al., 2003). The intake of petroleum hydrocarbons by organisms could be directly from water solution through gills and integument, from contaminated food adsorbed onto particulate matter, or directly from contaminated bottom sediments.

Pollutants such as petroleum hydrocarbons, among others, are able to cause toxic effects on growth, survival, reproduction, trophic disorder and abundance of aquatic organisms (Fleeger et al., 2003). As an environmental stress response, susceptible species are replaced by the most tolerant ones, which are able to adjust to the new conditions by physiological acclimation during the sublethal exposures at some period of their lives (Klerks and Weis, 1987).

Among freshwater benthic organisms, amphipods have relatively high sensitivity to environmental contaminants and are commonly used in toxicological studies (Borgmann et al., 2005; Jergentz et al., 2004; Wheelock et al., 2005). The freshwater amphipod *Hyaella curvispina* (Amphipoda: Hyaellidae) has a wide distribution and is often the dominant invertebrate in benthic communities of aquatic environments in South America, including Argentina (Grosso and Peralta, 1999). In addition, they are important bioindicators of aquatic ecosystem quality due to their favorable characteristics such as high-density population, their role in the ecosystem and the breeding simplicity at the laboratory (Lavarías et al., 2011). In recent years, *H. curvispina* has been used as a test organism to evaluate the toxicity of various contaminants in both laboratory and field conditions (Anguiano et al., 2008; Giusto et al., 2012; Peluso et al., 2011).

Exploitation of energy resources is the main economic activity at Neuquén province, northern region of Patagonia Argentina. Despite regulations and monitoring programs, implemented at different stages of well exploration and oil refinery processes, crude oil spills have frequently caused soil and water pollution in the region (Secretaría de Estado de Ambiente y Desarrollo Sostenible de la Provincia de Neuquén, 2013). Petroleum hydrocarbons, such as naphthalene and pyrene, have been detected in water and sediment samples from different Neuquén sites (Monza et al., 2013). Thus, the objectives of this work were to: (i) characterize the hydrocarbons composition of freshly prepared WAF and its dissipation over time; (ii) evaluate the acute and chronic toxicity of WAF on *H. curvispina* collected from both an uncontaminated site and a highly impacted stream.

## 2. Materials and Methods

### 2.1. Preparation of the WAF from crude oil and dissipation studies

The WAF was obtained from crude oil extracted from Chachahuen field, located in the Neuquén basin at North Patagonia. The fraction was prepared following the methods outlined by Heras et al. (1992), with some modifications. Crude oil was added to filtered freshwater (1:100, v/v) in a 3 L glass flask and homogenized at room temperature and in the dark, using a magnetic stirrer for 24 h at 60 rpm. The mixture was then transferred to a separation funnel, where the layers were allowed to separate for 48 h. Finally, the WAF layer was collected and stored at 4°C.

The dissipation study of WAF components over time was carried out in glass flasks (1.5 L) at a constant temperature of  $21 \pm 1$  °C and 8:16 h (L:D) photoperiod. Pure WAF samples (1.2 L) were placed in glass flasks and covered with polypropylene lid to avoid the loss of highly volatile compounds. The samples (one sample = 1 glass flask) were analyzed after 0, 6, 24, 48, 96 and 192 h for hydrocarbons content. The samples were managed and treated for hydrocarbon analyses as described above. The hydrocarbons dissipation ( $t_{1/2}$ ) were calculated by a non-linear regression program (Venturino et al., 1992), using a simple exponential decay equation:

$$Y(t) = Y(0) e^{(-\ln 2 t/t_{1/2})}$$

### 2.2. Study area

The study area included two sites in the Neuquén province, Los Barreales lake (LB) and Durán stream (DS). The first one is an artificial lake where petroleum hydrocarbons have not been detected (Monza et al., 2013) and located about 100 km (S 38° 45' 34.4", W 68° 72' 91.8") from the fruit-production area with



null probability of pesticide exposure. Therefore, LB was considered as a reference area in the province. On the other hand, DS originates from an underground interconnection of an ancient course of the Limay River and flows into it after travelling 10 km through the southern area of the Neuquén city (S 38° 58' 20.60'', W 68° 06' 03.10''). The flow of the stream stems from both irrigation and alluvial drainages. Contamination with hydrocarbons occurred sometimes into DS by clandestine industrial discharges settled on the margins of the watercourse. Moreover, the presence of naphthalene and pyrene in water and sediment from DS has been previously reported (Monza et al., 2013). Upstream, DC site is surrounded by a few family orchards where farmers might use agrochemicals to enhance the crop production. Therefore, pesticide analyses were included in this site.

### 2.3. Field sampling and analytical methods

At each sample site, physicochemical parameters such as pH, conductivity, dissolved oxygen and temperature were measured with a multiparameter probe (Lutron YK 2001PH). Sediment and water samples were collected from each sampling site according to standardized methodologies (EPA, 2007). Briefly, water samples were directly collected in clean amber glass bottles (1 L), and sediment samples were gathered with a stainless-steel scoop and placed in glass jars (1 kg). Both samples were kept on ice while transported to the laboratory. Water samples were processed immediately, and sediments were cleaned from large debris such as gravel and organisms, homogenized by hand mixing and stored in freezer at -20 °C until analyses.

### 2.3.1. Hydrocarbon determinations

Hydrocarbon analyses were performed according to the Method for the Determination of Extractable Petroleum Hydrocarbons of the Massachusetts Department of Environmental Protection (MADEP), EPH-04 Revision 1 (MADEP - Massachusetts Department of Environmental Protection-State Agency, 2004). Water samples (1 L) were extracted according to the SW 846-3510C method using 3 volumes of 60 mL of methylene chloride. Sediment samples (10 g) were extracted according to the method SW 846-3550C, by Soxhlet equipment with 150 mL of methylene chloride.

The extracts were concentrated on a rotary evaporator and the solvent was exchanged with hexane. The fractionation of aliphatic hydrocarbons (AH) and PAHs was carried out using a glass chromatographic column packed with pre-activated silica gel. The AH fraction (F1) was eluted with 20 mL of n-hexane and a second fraction (F2), for the separation of PAHs, was eluted with 20 mL of dichloromethane. Each fraction was concentrated to 1 mL under a nitrogen stream, transferred to a glass vial and quantified by gas chromatography (EPA 8015D).

The quantification of AH and PAHs was performed on an Agilent 6890 Gas Chromatograph (GC), equipped with HP-05 capillary column and flame ionization detector (FID). 1-Chlorooctadecane was used as surrogate standard. The GC operating conditions were as follows: ramp 1, temperature at 95 °C, rate of 15 °C min<sup>-1</sup>; ramp 2, temperature at 300 °C, rate of 6 °C min<sup>-1</sup>; ramp 3, temperature at 35 °C, rate of 25 °C min<sup>-1</sup>. Nitrogen was used as carrier gas. Compounds were quantified by external standard method.

Water and sediment samples (10 mL and 10 g) for benzene, toluene, ethylbenzene and xylene (BTEX) determinations were collected in septum-seal vials (EPA 3810) and maintained at 70 °C until reaching equilibrium. Two mL of the headspace gas was withdrawn with a gas-tight syringe and analyzed by direct injection into a gas chromatograph. The detection limits (LOD) for water and sediment analyses were: 13  $\mu\text{g L}^{-1}$  and 1000  $\mu\text{g kg}^{-1}$  dry weight (dw) for TPHs; 5  $\mu\text{g L}^{-1}$  and 25  $\mu\text{g kg}^{-1}$  dw for BTEX; 1  $\mu\text{g L}^{-1}$  and 23  $\mu\text{g kg}^{-1}$  dw for PAHs.

### 2.3.2. Pesticide determinations

#### 2.3.2.1. Organochlorines and Pyrethroids

Pesticide determinations from water samples were performed by liquid-liquid extraction (EPA 3510C), with hexane as solvent. Compounds identification and quantification were performed by gas chromatography on the Agilent 6890 Gas Chromatograph equipped with HP-05 capillary column and electron capture detector ( $\mu\text{ECD}$ ) by external standard (calibration curves).

Organochlorine and pyrethroid extraction from sediments was achieved in conjunction with the hydrocarbons ones. Fractions F1 and F2 were pooled and the solvent was exchanged to hexane. Finally, the chromatographic scheme explained for water samples was followed. The GC run conditions were: 70 °C initial temperature, ramp 1, 20 °C  $\text{min}^{-1}$  to 160 °C; ramp 2, 4 °C  $\text{min}^{-1}$  to 270 °C, with Nitrogen as carrier gas. The detected compounds were confirmed by  $\mu\text{ECD}$  by changing the HP-05 column by a HP-17.

The LOD were 0.001  $\mu\text{g L}^{-1}$  and 0.01  $\mu\text{g kg}^{-1}$  dw for organochlorines, and 0.01  $\mu\text{g L}^{-1}$  and 70  $\mu\text{g kg}^{-1}$  dw for pyrethroids.

### 2.3.2.2. Organophosphates and Carbamates

The extraction of organophosphates pesticides and carbamates in water was achieved by solid phase (SPE) (EPA 3535A with modifications), with reverse phase polymer cartridge (Strata™ Phenomenex). After drying in the air and under a nitrogen stream, the cartridges were eluted with hexane and methylene chloride. The extracts were concentrated and 250  $\mu$ l of hexane was added. The extracts were analyzed by gas chromatography with nitrogen-phosphorus detector (GC-NPD). Identification and quantification of the compounds were performed by calibration curves. Confirmation was performed by gas chromatography coupled to mass spectrometer (GC-MS).

Sediment samples (10 g) were placed inside polypropylene columns with paper filters. Eight mL of ethyl acetate were added to each column and sonicated in an ultrasonic bath for 15 min. The columns were then brought to the filtration system and the liquid extracts were collected in glass-graduated tubes at atmospheric pressure, allowing a slow and steady trickling. After all the liquid was collected, 8 mL of ethyl acetate were added and the procedure was repeated. Finally, 2 mL of solvent were added to each final rinse column and allowed to filter completely. The extracts were concentrated under nitrogen stream to a volume of 10 mL and then transferred to a vial. Finally, they were injected into a gas chromatograph equipped with a nitrogen and phosphorus detector (NPD). The GC operating conditions were as follows: ramp 1, temperature at 160 °C, rate of 20 °C min<sup>-1</sup>; ramp 2, temperature at 240 °C, rate of 4 °C min<sup>-1</sup> for 9 min. Nitrogen was used as carrier gas.

The LOD were 0.02  $\mu$ g L<sup>-1</sup> and 7  $\mu$ g kg<sup>-1</sup> dw for organophosphates, and 0.03  $\mu$ g L<sup>-1</sup> and 10  $\mu$ g kg<sup>-1</sup> dw for carbamates.

## 2.4. Toxicity Tests

Amphipods *H. curvispina* were collected during summer from both LB and DS sites and maintained under laboratory conditions for about 2 weeks before performing bioassays.

Groups of ten adult amphipods were exposed to a range of WAF concentrations in glass flasks (1.5 L). WAF was diluted in dechlorinated water to a final volume of 1 L. The concentrations tested were 0.011; 0.022; 0.044; 0.087; 0.17; and 0.35 mg TPH L<sup>-1</sup> for acute tests (96 h), and 0.0007; 0.0014; 0.0027; 0.0054; 0.011; 0.022; 0.044; 0.087; 0.17; 0.35; and 0.70 mg TPH L<sup>-1</sup> for chronic tests (14 d). Control groups were held in clean water without WAF. Assays were carried out in semi-static conditions, with WAF replacement in the middle of the exposure duration (48 h and 7 d for acute and chronic toxicity assays, respectively). The tests were replicated five times on different days at a constant temperature of 21 ± 1 °C and 8:16 h (L:D) photoperiod. During the experiments, the flasks were covered with polypropylene lid to minimize dissipation. Survival was monitored daily, and dead animals were recorded and removed at each time. Animals were observed under stereoscopic microscope and they were considered dead if no movement of pleopods was visible during a 20 s observation period.

Mortality data were analyzed by a non-linear regression logistic model based on least squares difference iterative approach (Venturino et al., 1992). The general logistic model included the survival percentage (%S) of the control groups, together with the LC50 and the logistic slope (m) according to the equation:

$$\% \text{ Mortality} = 100 - [\%S / (1 + (\text{TPHs Concentration}/\text{LC50})^m)]$$

The mortality curve ranges from 100 - %S to 100%. If control survival was 100%, the model was reduced to two parameters. The best model fitting to data was determined by means of the Akaike's criterion. From this approach, the LC1 and LC10 were calculated as representative of No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC), respectively (Crane and Newman, 2000; Murado and Prieto, 2013).

## 2.5. Species Sensitivity Distribution

The effects of WAF on freshwater organisms were characterized by Species Sensitivity Distribution (SSD) curve. Acute toxicity data (96 h-LC50) of WAF from species of algae, crustacean and fish (EPA, 2017) were obtained from both scientific literature and the present results. Only those bibliographic data that fulfilled the following criteria were included: acute toxicity values (LC50) reported as TPHs or equivalent, 48-96 h continuous exposure to WAF, developed in freshwater, using as life stages: larval, juvenile, or adult (not embryos). When more than one toxicological data was reported for one species, the geometric mean value was used as the estimate for this species. Physicochemical parameters should be also carefully controlled during the experiment and control groups should be included (Wheeler et al., 2002). To develop SSD curve, the species were classified in order of decreasing sensitivity and the rank of each species was transformed to a percentile value (ECOFRAM, 1999). A Probit model was fitted by linear regression on percentiles versus the log-transformed LC50 values. The WAF concentration that would affect the 10<sup>th</sup> percentile of species was estimated from the regression.

### 3. Results

#### 3.1. Physicochemical characterization of field samples

Physicochemical parameters were measured in both LB and DS sites. Water from LB showed an average temperature of 19.9 °C, pH 8.15, conductivity 182.72  $\mu\text{S cm}^{-1}$ , dissolved oxygen 7.4  $\text{mg L}^{-1}$ . Sediment from LB consisted of 1.6% fine, 10.8% sand and 87.6% gravel, with an average organic matter content of 1.66%. At DS, water exhibited an average temperature of 20 °C, pH 7.47 and a mean high conductivity of 1250  $\mu\text{S cm}^{-1}$ . DS sediment showed in average 25.0% fine, 19.6% sand and 55.4% gravel, with a high organic matter up to 10.2%.

At the reference site LB, hydrocarbons were only found in sediments (32.74  $\text{mg kg}^{-1}$ ) and chromatographic analysis showed a predominance of high molecular weight alkanes with odd-C chains. Instead, hydrocarbon concentrations at DS were 0.028  $\text{mg L}^{-1}$  in water and 900.07  $\text{mg kg}^{-1}$  dw in sediments. The sediment concentration of TPHs at DS was 27 times higher than the one at LB, showing that DS area was heavily contaminated with n-alkanes. In both DS samples, the majority of the n-alkanes were odd high molecular weight (n-C<sub>25</sub> to n-C<sub>35</sub>).

Pesticides such as organochlorides, pyrethroids, organophosphates and carbamates were below the detection limits in both sites.

#### 3.2. Analysis of WAF composition and dissipation over time

The WAF was mainly composed of C<sub>9</sub>–C<sub>36</sub> n-alkanes, which comprised about 90% of hydrocarbon mass (Table 1). Other compounds found were single-ring aromatic hydrocarbons (BTEX group: toluene, benzene, xylene, o-xylene and ethylbenzene) and PAHs, particularly naphthalene. Within the BTEX group, toluene concentration was 10-fold higher than the other components.

A simple exponential model was fitted using the dissipation data for each WAF component (Fig. 1). Mostly estimated initial concentrations of WAF components were similar to the measured values. However, TPHs estimated value was lower than measured one ( $1.79 \pm 0.24 \text{ mg L}^{-1}$  vs.  $2.18 \text{ mg L}^{-1}$  in the WAF). The estimated  $t_{1/2}$  of TPHs was  $71 \pm 32 \text{ h}$ . The estimated  $t_{1/2}$  of toluene ( $65 \pm 32 \text{ h}$ ), benzene ( $76 \pm 16 \text{ h}$ ) and ethylbenzene ( $88 \pm 19 \text{ h}$ ) were similar. On the other hand, m- and p-xylene showed a  $t_{1/2}$  ( $128 \pm 25 \text{ h}$ ) significantly higher than those of toluene and benzene. Finally, o-xylene showed the highest  $t_{1/2}$  ( $217 \pm 45 \text{ h}$ ), and it was significantly different from the other components. Particularly, naphthalene  $t_{1/2}$  could not be estimated because in the first 6 h the concentration was below the detection limits.

### 3.3. Toxicity tests

WAF concentration-mortality data from LB population and the fitting to the logistic model are shown in Fig. 2. Estimated 96 h-LC50 value for acute toxicity was  $0.33 \text{ mg L}^{-1}$  (0.24-0.41). From the fitted equation, NOEC and LOEC values were estimated in  $0.43 \times 10^{-3} \text{ mg L}^{-1}$  ( $0.071 \times 10^{-3}$ - $1.52 \times 10^{-3}$ ) and  $0.014 \text{ mg L}^{-1}$  (0.0049-0.028), respectively. Analyses of chronic toxicity data revealed a 14 d-LC50 of  $0.018 \text{ mg L}^{-1}$  (0.015-0.021). The NOEC and LOEC values were  $0.00081 \times 10^{-3} \text{ mg L}^{-1}$  ( $0.000092 \times 10^{-3}$ - $0.0040 \times 10^{-3}$ ) and  $0.00015 \text{ mg L}^{-1}$  (0.000048-0.00035), respectively. In both acute and chronic assays, the low slopes were indicative of a high heterogeneity in the response to WAF on LB population ( $0.69 \pm 0.13$ ;  $0.46 \pm 0.08$ ). Amphipods collected at DS showed no mortality at all, even at the pure WAF exposure, either at acute or chronic assays. Based on these results, amphipods from the LB site showed greater susceptibility to WAF than



those from DS. The control survival was 100% and 84.4% in acute and chronic assays, respectively.

### 3.4. Species Sensitive Distribution

Probability of potential WAF effects on aquatic species were obtained from SSD curve (Fig. 3). A total of 13 toxicity data points, including LB native species, were used in the development of SSDs. A Probit model was fitted to data, with a  $R^2$  value of 0.97. The highest susceptibility to WAF was detected on *H. curvispina* from LB. Furthermore, crustaceans were the most sensitivity among the aquatic invertebrates, except for *Mysidopsis almyra*. The concentration that would affect the 10<sup>th</sup> percentile of aquatic species was estimated in 0.47 mg L<sup>-1</sup> from the fitted distribution equation, showing that 96 h-LC50 of LB *H. curvispina* was below this endpoint.

## 4. Discussion

The reported composition and concentration of hydrocarbons present in WAF from different crude oils are highly variable. The concentration of WAF depends mainly on variables such as the oil type, oil-water ratio, mixing time and exposure temperature (Faksness et al., 2008; Maher, 1986). The TPHs in WAF obtained in the present study was 2.18 mg L<sup>-1</sup>. Lavarías et al. (2004) reported a concentration of 3.41 mg L<sup>-1</sup>, using the same preparation methodology for WAF from Punta Loyola crude oil. Neff et al. (2000) indicated concentrations varying from 0.008 to 38.31 mg L<sup>-1</sup> for different Australian crude oils. The analysis of WAF from the present study also showed high concentrations of BTEX and low concentrations of PAHs. A similar composition has been reported elsewhere (Anderson et al., 1974; Lavarías et al., 2004; Simonato et al., 2008). Conversely, other authors have found high concentrations of PAHs in WAF, where

naphthalene represents the highest percentage of them (Neff et al., 2000; Rodrigues et al., 2010).

The dissipation analysis of WAF hydrocarbons from the present study showed a small initial amount of naphthalene, with a fast decline below the detection limits after the first 6 h. Other authors have also showed an important decrease in naphthalene concentrations measured in water after 24 h (Laughlin et al., 1978). The vapor pressure is an important property involved in the behavior of PAHs in the environment. The PAHs with high vapor pressure such as naphthalene (11 Pa at 25 °C) will tend to be associated with the vapor phase, while those with lower vapor pressure will tend to be absorbed to particles (Abdel-Shafy and Mansour, 2016). Despite their low persistence due to the high volatility, it is also bioavailable and may cause toxicity effects in many organisms (Smreczak et al., 2008).

The dissipation rate of WAF hydrocarbons from the present study showed a first-order kinetic. O-xylene was the individual component in the WAF that exhibited the highest  $t_{1/2}$ , which correlates with its low vapor pressure. Toluene showed the lowest  $t_{1/2}$  value (60.64 h). However, its higher concentration within the BTEX group could be an important component of the toxic effects until its complete dissipation at 192 h. Previous studies have indicated that toluene rapidly volatilizes from water to air, with a short  $t_{1/2}$  at room temperature (25 °C). However, the volatilization rate depends on conditions of the water body and the atmosphere (U.S. Department of Health and Human Services. Agency for Toxic Substances and Disease Registry, 2015). Both xylene and o-xylene showed higher  $t_{1/2}$  than the other components.

The concentration of TPHs found in LB sediments was  $32.74 \text{ mg kg}^{-1} \text{ dw}$  and it was mainly composed by high molecular weight alkanes with odd-C chains. These results were in accordance with previous studies from the area (Monza et al., 2013). In general, odd alkanes come from biogenic sources while even alkanes are derived from the anthropogenic sources. Within the odd alkanes, n-C<sub>15</sub>, n-C<sub>17</sub> and n-C<sub>19</sub> are produced by marine biogenic sources whereas n-C<sub>25</sub>-n-C<sub>33</sub> hydrocarbons are from vascular plants (Rielley et al., 1991; Sakari et al., 2008). Sediment samples collected from DS showed a high concentration of TPHs ( $900.07 \text{ mg kg}^{-1} \text{ dw}$ ). Both PAHs and BTEX were below the detection limits, although previous studies showed concentrations of naphthalene and pyrene at  $40 \text{ } \mu\text{g kg}^{-1} \text{ dw}$  and  $50 \text{ } \mu\text{g kg}^{-1} \text{ dw}$ , respectively (Monza et al., 2013). These authors attributed the high levels of hydrocarbons in sediment and water to anthropogenic activity.

According to the great variability in the petroleum composition and the preparation methods of the WAF, toxicological results with WAF are difficult to compare. To obtain reproducible and comparable WAF from crude oils for acute and chronic toxicity studies, variables such as oil-water ratio, mixing and decantation time need to be controlled (Maher, 1986). Also, fresh preparations are required for assays to avoid WAF concentration changes that may occur during storage (Heras et al., 1995).

In the current study, both acute and chronic effects of WAF on a native amphipod are reported. *H. curvispina* population collected from a reference area (LB) proved to be highly sensitive compared with the one inhabiting an urban zone (DS). In fact, amphipods from DS survived to pure WAF in both acute and chronic assays. The difference in the toxicity may result in the acquisition of

increased tolerance by physiological acclimation, genetic adaptation or maternal effects. Non-genetic changes (physiological acclimation), such as detoxification by induced enzyme activity have been detected in invertebrates (Poupardin et al., 2008). In the other hand, resistance development has been shown in amphipods (Anguiano et al., 2008; Shahid et al., 2018; Weston et al., 2013). Wolf and Wade (2009) defined maternal effects as the causal influence of the maternal genotype or phenotype on the offspring phenotype. Rahman et al. (2010) observed tolerance transmitted by maternal effects in field populations of diamondback moth.

Despite the differences in the composition of different WAF of oils, LB population has shown to be more sensitive than other amphipods, such as adults of *Gammarus fasciatus*, which have a LC50 of 0.80 mg L<sup>-1</sup> (Mayer and Ellersieck, 1986). Higher 96 h-LC50 values were reported for other crustaceans such as *Homarus americanus* (0.86 mg L<sup>-1</sup>) (Wells and Sprague, 1976) and *Mysidopsis almyra* (7.65 mg L<sup>-1</sup>) (Anderson et al., 1974). Within BTEX group, toluene was the major component of WAF with a concentration of 0.24 mg L<sup>-1</sup>. Acute toxicity data of toluene from ECOTOX vary from 447 mg L<sup>-1</sup> (*Gammarus minus*) to 58 mg L<sup>-1</sup> (*Diaptomus forbesi*) for crustaceans, whose values are several orders of magnitude higher than WAF acute toxicity (0.31 mg L<sup>-1</sup> to 7.65 mg L<sup>-1</sup>). This could indicate that the mixture of compounds present in the WAF is more toxic than the individual compounds. The exposure to chemical mixtures of differing solubility, likely produce reactions that may not be associated with the individual chemicals. Generally, exposures to chemical mixtures produces an effect greater than the sum of their individual effects (Zeliger, 2003). Synergistic

effects were found in amphipods (Boese et al., 1999) and in fish early life-stage for polycyclic aromatic hydrocarbon mixtures (Barron et al., 2004).

The aim of SSD is to determine the concentration of a toxicant that is protective of most species in the environment. The concentrations corresponding to 10 of the affected species in the SSD curve should be paid special attention because the former ascertain the safety for most species below the corresponding concentration (Xin et al., 2015). The 10<sup>th</sup> percentile of the distribution of acute toxicities to species is generally used as the assessment endpoint according with the Aquatic Risk Assessment and Mitigation Dialog Group (SETAC, 1994). Although the SSDs are frequently used in ecologic risk assessments for the formulation of water quality guidelines (Wheeler et al., 2002), their use in oil regulation and impact assessment has been limited. Previous studies have shown that the development of oil SSDs is feasible and can be useful to evaluate species sensitivity to oil (de Hoop et al., 2011; Smit et al., 2009). In addition, Barron et al. (2013) developed SSDs for 5 different oils and combined oil, and their findings showed similar 5<sup>th</sup> percentile hazard concentrations values for crude oils. These results suggest that oil SSDs is feasible combining toxicity values from different oils. In the present study, the resulting 10<sup>th</sup> percentile value from SSDs could then serve as the basis for establishing toxicity benchmarks. In this sense, the present results suggest that TPHs concentrations in freshwater must not exceed 0.5 mg L<sup>-1</sup> to avoid acute mortality in more than a 10% of total aquatic species. A maximum allowed concentration of 50 µg TPHs L<sup>-1</sup> is proposed as an environmental quality standard for seawater (Țigănuș et al., 2016). Considering that relatively long term exposures may arise from oil spills or drill operations, chronic effects have to be considered. Endpoints from

*H. curvispina* bioassays suggest that TPHs concentrations in the order of  $10 \mu\text{g L}^{-1}$  are enough to cause adverse effects (14 d-LC50), while  $0.2 \mu\text{g L}^{-1}$  should not be exceeded to avoid chronic toxicity for this species.

## 5. Conclusion

The 96 h-LC50 value of *H. curvispina* from LB, comprised within the 10<sup>th</sup> percentile estimated from the SSD, indicates its high susceptibility to WAF compounds from oil contamination. Nevertheless, the occurrence of a decrease susceptibility to WAF of amphipods from DS may result in the acquisition of increased tolerance. Further biochemical and molecular research is required to determine the mechanisms underlying the toxicity of this species to WAF exposure. The SSD may be a reasonable approach to establish oil toxicity benchmarks, but additional toxicity data for aquatic species are needed performed in standardized test conditions.

## Conflicts of Interest

The authors have no competing interests to declare.

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## References

- Abdel-Shafy, H.I., Mansour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egypt. J. Pet.* 25, 107–123. <https://doi.org/10.1016/j.ejpe.2015.03.011>
- Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E., Hightower, G.M., 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Mar. Biol.* 27, 75–88. <https://doi.org/10.1007/BF00394763>
- Anguiano, O.L., Ferrari, A., Soleño, I.J., Martínez, M.C., Venturino, A., Pechen de D'Angelo, A.M., Montagna, C.M., 2008. Enhanced esterase activity and resistance to azinphosmethyl in target and nontarget organisms. *Environ. Toxicol. Chem.* 27, 2117–23. <https://doi.org/10.1897/07-655.1>
- Barron, M.G., Carls, M.G., Heintz, R., Rice, S.D., 2004. Evaluation of fish early life-stage toxicity models of chronic embryonic exposures to complex polycyclic aromatic hydrocarbon mixtures. *Toxicol. Sci.* 78, 60–67. <https://doi.org/10.1093/toxsci/kfh051>
- Barron, M.G., Hemmer, M.J., Jackson, C.R., 2013. Development of aquatic toxicity benchmarks for oil products using species sensitivity distributions. *Integr. Environ. Assess. Manag.* 9, 610–615. <https://doi.org/10.1002/ieam.1420>
- Bhattacharyya, S., Klerks, P.L., Nyman, J. A., 2003. Toxicity to freshwater organisms from oils and oil spill chemical treatments in laboratory microcosms. *Environ. Pollut.* 122, 205–215. [https://doi.org/10.1016/S0269-7491\(02\)00294-4](https://doi.org/10.1016/S0269-7491(02)00294-4)

- Boese, B. L., Ozretich R. J., Lamberson J.O., Swartz R.C., Cole F.A., Pelletier J., Jones J., 1999. Toxicity and phototoxicity of mixtures of highly lipophilic PAH compounds in marine sediment: can the  $\Sigma$ model be extrapolated?. Arch. Environ. Contam. Toxicol. 36, 270–280.
- Borgmann, U., Grapentine, L., Norwood, W.P., Bird, G., Dixon, D.G., Lindeman, D., Chapman, P.M., Goulet, R.R., Wang, F., 2005. Sediment toxicity testing with the freshwater amphipod *Hyalella azteca*: Relevance and application. Chemosphere 61, 1740–1745.  
<https://doi.org/10.1016/j.chemosphere.2005.04.011>
- Brandt, C. A., Becker, J.M., Porta, A., 2002. Distribution of polycyclic aromatic hydrocarbons in soils and terrestrial biota after a spill of crude oil in Trecate, Italy. Environ. Toxicol. Chem. 21, 1638–1643. [https://doi.org/10.1897/1551-5028\(2002\)021<1638:dopahi>2.0.co;2](https://doi.org/10.1897/1551-5028(2002)021<1638:dopahi>2.0.co;2)
- Crane, M., Newman, M.C., 2000. What level of effect is a no observed effect? Environ. Toxicol. Chem. 19, 516–519.  
[https://doi.org/10.1897/15515028\(2000\)019<0516:wloeia>2.3.co;2](https://doi.org/10.1897/15515028(2000)019<0516:wloeia>2.3.co;2)
- de Hoop L., Schipper A. M., Leuven R. S. E. W., Huijbregts M. A. J., Olsen G. H., Smit M. G. D., Hendriks A. J., 2011. Sensitivity of polar and temperate marine organisms to oil components. Environ Sci Technol. 45, 9017–9023.
- ECOFRAM, 1999. Ecological Committee on FIFRA risk assessment methods: Report of the Aquatic Workgroup, Washington, DC.
- EPA, 2007. SW-846. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, Chapter 12<sup>th</sup> LAND TREATMENT MONITORING.  
<http://www.epa.gov/epaoswer/hazwaste/test/pdfs/chap12.pdf>



- EPA, 2017. ECOTOX User Guide: ECOTOXicology Knowledgebase System. Version 4.0. <http://www.epa.gov/ecotox/> (accessed 2017).
- Faksness, L.G., Brandvik, P.J., Sydnes, L.K., 2008. Composition of the water accommodated fractions as a function of exposure times and temperatures. *Mar. Pollut. Bull.* 56, 1746–1754. <https://doi.org/10.1016/j.marpolbul.2008.07.001>
- Finga, M., 2015. Handbook of Oil Spill Science and Technology. Wiley & Sons Inc.
- Fleeger, J.W., Carman, K.R., Nisbet, R.M., 2003. Indirect effects of contaminants in aquatic ecosystems. *Sci. Total Environ.* 317, 207–233. [https://doi.org/10.1016/S0048-9697\(03\)00141-4](https://doi.org/10.1016/S0048-9697(03)00141-4).
- Giusto, A., Somma, L.A., Ferrari, L., 2012. Cadmium toxicity assessment in juveniles of the Austral South America amphipod *Hyaella curvispina*. *Ecotoxicol. Environ. Saf.* 79, 163–169. <https://doi.org/10.1016/j.ecoenv.2011.12.020>.
- Grosso, L.E., Peralta, M., 1999. Anfípodos de agua dulce Sudamericanos. Revisión del género *Hyaella* Schmitt. *Acta Zool. Lilloana* 45, 79–98.
- Heras, H., Ackman, R.G., Macpherson, E.J., 1992. Tainting of Atlantic salmon (*Salmo salar*) by petroleum hydrocarbons during a short term exposure. *Mar. Pollut. Bull.* 24, 310–315.
- Heras, H., Zhou, S., Ackman, R.G., 1995. Plastic bags for stable storage of the water-soluble fraction of crude petroleum used in aquatic environment toxicity and tainting studies. *Bull. Environ. Contam. Toxicol.* 55, 597–602.
- Ho, K., Patton, L., Latimer, J.S., Pruell, R.J., Pelletier, M., McKinney, R., Jayaraman, S., 1999. The chemistry and toxicity of sediment affected by oil

- from the North Cape spilled into Rhode Island Sound. *Mar. Pollut. Bull.* 38, 314–323. [https://doi.org/10.1016/S0025-326X\(98\)00151-9](https://doi.org/10.1016/S0025-326X(98)00151-9)
- Jergentz, S., Pessacq, P., Mugni, H., Bonetto, C., Schulz, R., 2004. Linking in situ bioassays and population dynamics of macroinvertebrates to assess agricultural contamination in streams of the Argentine pampa. *Ecotoxicol. Environ. Saf.* 59, 133–141. <https://doi.org/10.1016/j.ecoenv.2004.06.007>
- Klerks, P.L., Weis, J.S., 1987. Genetic adaptation to heavy metals in aquatic organisms: a review. *Environ. Pollut.* 45, 173–205. [https://doi.org/10.1016/0269-7491\(87\)90057-1](https://doi.org/10.1016/0269-7491(87)90057-1)
- Laughlin, R.B., Young, L.G.L., Neff, J.M., 1978. A long-term study of the effects of water-soluble fractions of No. 2 fuel oil on the survival, development rate, and growth of the mud crab *Rhithropanopeus harrisi*. *Mar. Biol.* 47, 87–95. <https://doi.org/10.1007/BF00397022>
- Lavariás, S., Heras, H., Pedrini, N., Tournier, H., Ansaldo, M., 2011. Antioxidant response and oxidative stress levels in *Macrobrachium borellii* (Crustacea: Palaemonidae) exposed to the water-soluble fraction of petroleum. *Comp. Biochem. Physiol. - C Toxicol. Pharmacol.* 153, 415–421. <https://doi.org/10.1016/j.cbpc.2011.02.002>.
- Lavariás, S., Heras, H., Pollero, R.J., 2004. Toxicity, uptake, and release of the water-soluble fraction of crude oil in different developing stages of the prawn *Macrobrachium borellii*. *Arch. Environ. Contam. Toxicol.* 47, 215–222. <https://doi.org/10.1007/s00244-004-3025-1>
- Law, E.A., 1993. *Aquatic pollution: An introductory text*, second ed. Wiley, New York.

- Lee, J., Lee, K., Park, G.S., 2005. Acute toxicity of heavy metals, tributyltin, ammonia and polycyclic aromatic hydrocarbons to benthic amphipod *Grandidierella japonica*. *Ocean Sci.* 40, 61–66. <https://doi.org/10.1007/BF03028586>
- Long, E.R., Macdonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environ. Manage.* 19, 81–97. <https://doi.org/10.1007/BF02472006>
- MADEP - Massachusetts Department of Environmental Protection-State Agency, 2004. Method for the Determination of Extractable Petroleum Hydrocarbons (EPH). Boston, Massachusetts.
- Maher, W.A., 1986. Preparation of water soluble fractions of crude oils for toxicity studies. *Bull. Environ. Contam. Toxicol.* 36, 226-229.
- Martínez-Jerónimo, F., Villaseñor, R., Ríos, G., Espinosa-Chavez, F., 2005. Toxicity of the crude oil water-soluble fraction and kaolin-adsorbed crude oil on *Daphnia magna* (Crustacea: Anomopoda). *Arch. Environ. Contam. Toxicol.* 48, 444–449. <https://doi.org/10.1007/s00244-003-0220-4>
- Mayer, F.L.L., Ellersieck, M.R.M.R., 1986. Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. Resource Publication 160 Fish and Wildlife Service US Department of Interior Washington DC.
- McLachlan, M.S., Czub, G., Wania, F., 2002. The influence of vertical sorbed phase transport on the fate of organic chemicals in surface soils. *Environ. Sci. Technol.* 36, 4860–4867. <https://doi.org/10.1021/es025662y>

- Monza, L.B., Loewy, R.M., Savini, M.C., Pechen de d'Angelo, A.M., 2013. Sources and distribution of aliphatic and polyaromatic hydrocarbons in sediments from the Neuquen River, Argentine Patagonia. *J. Environ. Sci. Heal. Part A Toxic/Hazardous Subst. Environ. Eng.* 48, 370–9. <https://doi.org/10.1080/10934529.2013.728097>
- Müller, H., 1987. Hydrocarbons in the freshwater environment: a literature review. *Arch. Hydrobiol., Adv. Limnol.* 24, 1–69.
- Murado, M.A., Prieto, M.A., 2013. NOEC and LOEC as merely concessive expedients: two unambiguous alternatives and some criteria to maximize the efficiency of dose-response experimental designs. *Sci. Total Environ.* 461–462, 576–586. <https://doi.org/10.1016/j.scitotenv.2013.04.098>
- Neff, J.N.M., Ostazeski, S., Gardiner, W., Stejskal, I., 2000. Effects of weathering on the toxicity of three offshore Australian crude oils and a diesel fuel to marine animals. *Environ. Toxicol. Chem.* 19, 1809–1821. [https://doi.org/10.1897/1551-5028\(2000\)019<1809:EOWOTT>2.3.CO;2](https://doi.org/10.1897/1551-5028(2000)019<1809:EOWOTT>2.3.CO;2)
- Peluso, L., Giusto, A., Rossini, G.D.B., Ferrari, L., Salibián, A., Ronco, A.E., 2011. *Hyaella curvispina* (amphipoda) as a test organism in laboratory toxicity testing of environmental samples. *Fresen. Environ. Bull.* 20, 372-376.
- Poupardin, R., Reynaud, S., Strode, C., Ranson, H., Vontas, J., David, J.-P., 2008. Cross-induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito *Aedes aegypti*: impact on larval tolerance to chemical insecticides. *Insect Biochem. Mol. Biol.* 38, 540–551.
- Rahman, A.M.M., Baker, G., Powis, K.J., Roush, R.T., Schmidt, O., 2010. Induction and transmission of tolerance to the synthetic pesticide emamectin

- benzoate in field and laboratory populations of diamondback moth. *J. Econ. Entomol.* 103, 1347-1354. <https://doi.org/10.1603/EC09171>
- Rielley, G., Collier, R.J., Jones, D.M., Eglinton, G., 1991. The biogeochemistry of Ellesmere Lake, U.K.-I: source correlation of leaf wax inputs to the sedimentary lipid record. *Org. Geochem.* 17, 901–912. [https://doi.org/10.1016/0146-6380\(91\)90031-E](https://doi.org/10.1016/0146-6380(91)90031-E)
- Rodrigues, R.V., Miranda-Filho, K.C., Gusmão, E.P., Moreira, C.B., Romano, L.A., Sampaio, L.A., 2010. Deleterious effects of water-soluble fraction of petroleum, diesel and gasoline on marine pejerrey *Odontesthes argentinensis* larvae. *Sci. Total Environ.* 408, 2054–2059. <https://doi.org/10.1016/j.scitotenv.2010.01.063>
- Sakari, M., Zakaria, M.P., Annuar, N.A., Yun, H.Y., Heng, Y.S., Zainuddin, S.M.H.S., Chai, K.L., 2008. Spatial distribution of petroleum hydrocarbon in sediments of major rivers from east coast of peninsular Malaysia. *Coast. Mar. Sci.* 32, 9–18.
- Sammarco, P.W., Kolian, S.R., Warby, R.A.F., Bouldin, J.L., Subra, W.A., Porter, S.A., 2013. Distribution and concentrations of petroleum hydrocarbons associated with the BP/Deepwater horizon oil spill, Gulf of Mexico. *Mar. Pollut. Bull.* 73, 129–143. <https://doi.org/10.1016/j.marpolbul.2013.05.029>
- Secretaría de Estado de Ambiente y Desarrollo Sostenible de la Provincia de Neuquén, 2013. <http://w2.neuquen.gov.ar/>, Artic (accessed November 2010).
- SETAC, 1994. Aquatic dialogue group: pesticide risk assessment and mitigation. Society for Environmental Toxicology and Chemistry and SETAC Foundation for Environmental Education. Pensacola, FL.

- Shahid, N., Becker, J.M., Krauss, M., Brack, W., Liess, M., 2018. Adaptation of *Gammarus pulex* to agricultural insecticide contamination in streams. *Sci. Total Environ.* 621, 479–485.  
<https://doi.10.1016/J.SCITOTENV.2017.11.220>
- Simonato, J.D., Guedes, C.L.B., Martinez, C.B.R., 2008. Biochemical, physiological, and histological changes in the neotropical fish *Prochilodus lineatus* exposed to diesel oil. *Ecotoxicol. Environ. Saf.* 69, 112–120.  
<https://doi.org/10.1016/j.ecoenv.2007.01.012>
- Smit M.G.D., Bechmann R.K., Hendriks A.J., Skadsheim A., Larsen B.K., Baussant T., Bamber S., Sanni S., 2009. Relating biomarkers to whole-organism effects using species sensitivity distributions: a pilot study for marine species exposed to oil. *Environ. Toxicol. Chem.* 28, 1104–1109.
- Smreczak, B., Maliszewska-Kordybach, B., Klimkiewicz-Pawlas, A., 2008. Assessing the bioavailability of phenanthrene to soil microorganisms using the Tenax extraction method. *Environ. Geochem. Health* 30, 183–186.  
<https://doi.org/10.1007/s10653-008-9141-5>
- Springer, M. 2010. Biomonitoring acuático. *Rev. Biol. Trop. San José de Costa Rica* 58, 59-59.
- Țigănuș, D., Coatu, V., Lazăr, L., Oros, A., 2016. Present level of petroleum hydrocarbons in seawater associated with offshore exploration activities from the Romanian black sea sector. *Cercetări marine: Recherches marines* 46, 98-108.
- U.S. Department of Health and Human Services. Agency for Toxic Substances and Disease Registry, 2015. Toxicological Profile for Toluene - Draft.

- Venturino, A., Gauna, L.E., Bergoc, R.M., Pechen de D'Angelo, A.M., 1992. Effect of exogenously applied polyamines on malathion toxicity in the toad *Bufo arenarum* Hensel. *Arch. Environ. Contam. Toxicol.* 22, 135–9.
- Wells, P.G., Engelhardt, F.R., Blackman, R.A.A., Howgate, P., Butler, J.N., 1993. Impact of oil and related chemicals on the marine environment. *Rep. Stud. GESAMP.*
- Wells, P.G., Sprague, J.B., 1976. Effects of crude oil on American lobster (*Homarus americanus*) larvae in the laboratory. *J. Fish. Board Canada* 33, 1604–1614.
- Weston, D.P., Poynton, H.C., Wellborn, G.A., Lydy, M.J., Blalock, B.J., Sepulveda, M.S., Colbourne, J.K., 2013. Multiple origins of pyrethroid insecticide resistance across the species complex of a nontarget aquatic crustacean, *Hyalella azteca*. *Proc. Natl. Acad. Sci. U. S. A.* 110, 16532–7. <https://doi.org/10.1073/pnas.1302023110>
- Wheeler, J.R., Grist, E.P.M., Leung, K.M.Y., Morritt, D., Crane, M., 2002. Species sensitivity distributions: data and model choice. *Mar. Pollut. Bull.* 45, 192–202. [https://doi.org/10.1016/S0025-326X\(01\)00327-7](https://doi.org/10.1016/S0025-326X(01)00327-7)
- Wheelock, C.E., Miller, J.L., Miller, M.J., Phillips, B.M., Gee, S.J., Tjeerdema, R.S., Hammock, B.D., 2005. Influence of container adsorption upon observed pyrethroid toxicity to *Ceriodaphnia dubia* and *Hyalella azteca*. *Aquat. Toxicol.* 74, 47–52. <https://doi.org/10.1016/j.aquatox.2005.04.007>.
- Wolf, J.B., Wade, M.J., 2009. What are maternal effects (and what are they not)? *Philos. Trans. R. Soc. B Biol. Sci.* 364, 1107–1115. <https://doi.org/10.1098/rstb.2008.0238>

- Xin, Z., Wenchao, Z., Zhenguang, Y., Yiguo, H., Zhengtao, L., Xianliang, Y., Xiaonan, W., Tingting, L., Liming, Z., 2015. Species sensitivity analysis of heavy metals to freshwater organisms. *Ecotoxicology* 24, 1621–1631. <https://doi.org/10.1007/s10646-015-1500-2>
- Zeliger, H.I., 2003. Toxic effects of chemical mixtures. *Arch. Environ. Health* 58, 23–9. <https://doi.org/10.3200/AEOH.58.1.23-29>
- Zhou, S., Heras, H., Ackman, R.G., 1994. Preparation and characterization of water-soluble fraction of crude oil by a karr reciprocating plate countercurrent extraction column. *Arch. Environ. Contam. Toxicol.* 26, 527–533.



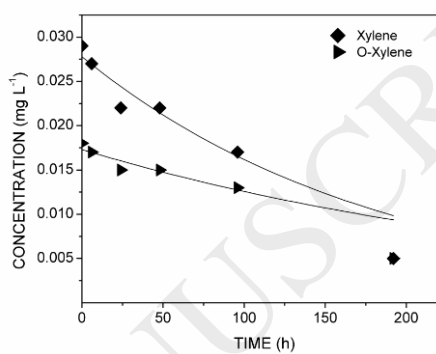
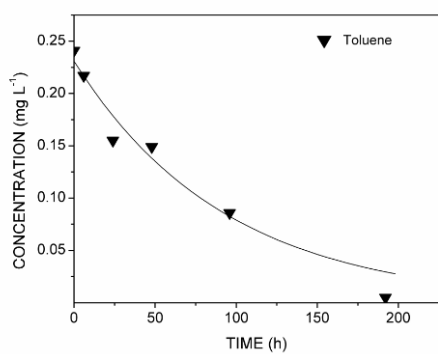
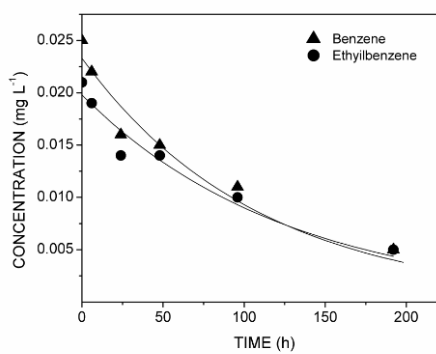
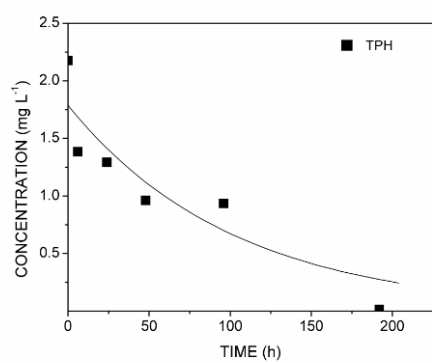
## Figure Captions

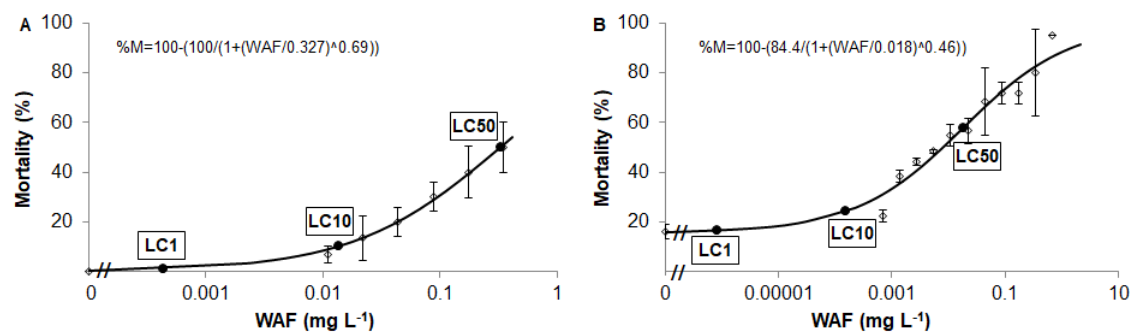
**Fig. 1.** Dissipation rates of WAF components over time.

Total petroleum hydrocarbons (TPHs) and BTEX were analyzed by gas chromatography and simple exponential decay models were fitted to data (solid lines).

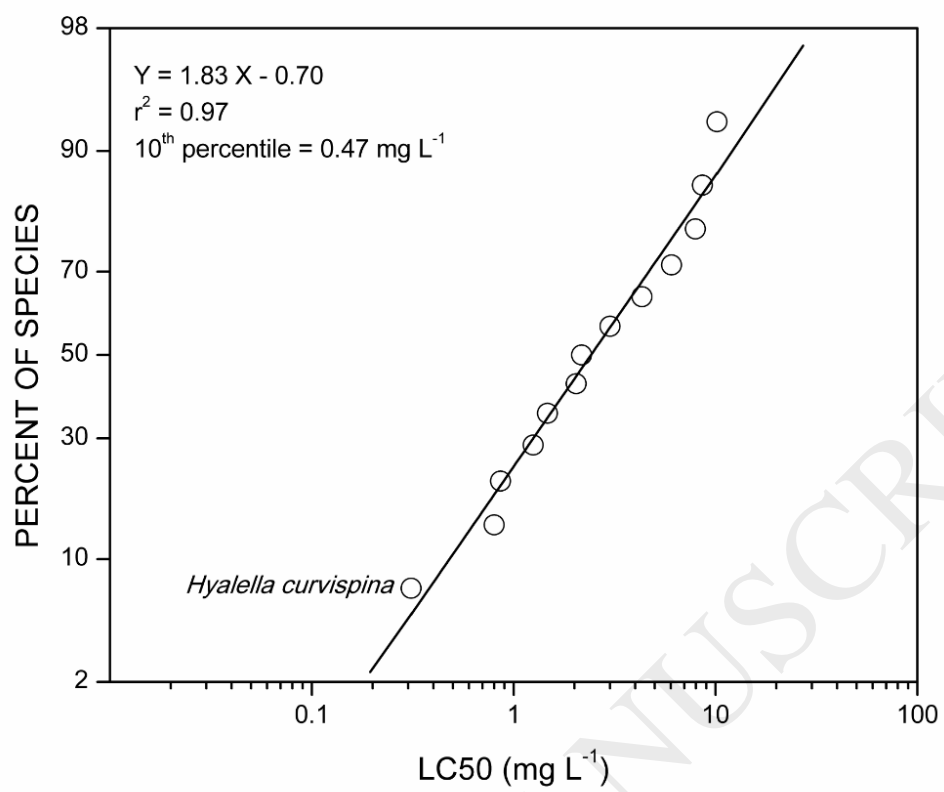
**Fig. 2.** Acute (A) and chronic (B) toxicity of WAF on *Hyaella curvispina* from LB. Mean toxicity data (N = 5 or higher) with SD are shown for WAF concentrations, expressed as TPHs. Logistic mortality models were fitted to data using a non linear regression approach (solid lines).

**Fig. 3.** Species sensitivity distributions for TPHs. Toxicity value for *H. curvispina* (native species) is identified.





ACCEPTED MANUSCRIPT



**Table 1**

Composition of WAF extracted from Chachahuen crude oil.

<b>Hydrocarbon</b>		<b>Concentration (mg L<sup>-1</sup>)</b>
TPHs	C <sub>9</sub> -C <sub>36</sub>	2.18
BTEX	Benzene	0.025
	Toluene	0.24
	Ethylbenzene	0.021
	m- p-Xylene	0.029
	o-Xylene	0.018
PAHs	Naphthalene	0.022