

17 ABSTRACT

18 Artificial weathering of Angolan crude and a Heavy Fuel Oil (HFO) was performed by evaporation and
19 photooxidation. The aliphatic, aromatic, polar and asphaltene fractions of the fresh and weathered oils
20 were isolated. The toxicity of the water accommodated fraction or an oil/fraction dissolved in DMSO
21 was assessed using the sea urchin embryo test. Photooxidation was observed to decrease the aromatics
22 content and increase polar compounds. A slight reduction in the toxicity of Angolan crude was observed
23 following weathering for the water-accommodated fraction and the extract in DMSO, but no effect was
24 seen for the Heavy Fuel Oil. For aliphatic compounds, the toxicity decreased in the order
25 Fresh>Evaporated>Photooxidated for both Angolan crude and HFO. Weathering slightly increased the
26 toxicity of the aromatic and polar fractions of the oil. The aromatic fractions were responsible for most
27 of the toxicity and the polar compounds were the second most important toxic components, despite
28 having less or similar abundance than the aliphatic fraction. The toxic contribution of the aromatic
29 compounds was higher for the HFO than for the Angolan crude. A decrease in the toxicity of Angolan
30 crude following weathering correlated with a reduction in the toxicity of the aliphatic fraction.

31 KEYWORDS oil spill, weathering, fraction, toxicity, bioassay.

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34 **1. Introduction**

35 Petroleum consists of a highly complex mixture of organic compounds, predominantly hydrocarbons
36 and can be characterized by the relative content of the fractions of saturated hydrocarbons, aromatics,
37 resins and asphaltenes (SARA analysis) because its physico-chemical properties are determined by the
38 proportions of these major components. Saturated hydrocarbons (paraffins, iso-paraffins and
39 naphthenes) have low aqueous solubility and toxicity. The aromatic fraction has been identified as
40 containing the compounds mainly responsible for the acute toxicity of petroleum, and polycyclic
41 aromatic hydrocarbons (PAHs) as the family of compounds of greater environmental concern [1].
42

43 The European Chemicals Agency (ECHA) recommends the hydrocarbon block method to assess the
44 ecological risk of oil [2]. The method is based on the grouping of known compounds of similar
45 properties into blocks to estimate the predicted environmental concentration (PEC), the Predicted No
46 Effect Concentration (PNEC), and subsequently the PEC/PNEC risk ratio of each individual block and
47 the whole oil. This requires that the properties of individual compounds related to their fate in the
48 environment (solubility, volatility) and toxicity have to be known or estimated. For this reason, it is
49 solely applicable to those compounds that can be resolved by chromatographic techniques (mainly
50 compounds from the saturated and aromatic fractions) but not so for compounds unresolved by gas
51 chromatography such as the unresolved complex mixture (UCM). The proportion of the UCM in a spilt
52 oil is determined by its origin, degree of weathering and/or biodegradation [3]. The UCM of saturated,
53 aromatic and polar fractions have all been shown to be toxic [4-6], so it is important not to ignore
54 unresolved components just because the exact composition cannot be determined by the available
55 analytical methods [7]. The difficulty in establishing causality between composition and effects has
56 already been reported. For example, PAHs may not be the main cause of toxicity for certain oils [8], and
57 the toxic contribution of the polar organic fraction may also be important [7, 9, 10]. Recently Bellas et

al. [11] have shown that the increased toxicity of certain weathered oils cannot be explained on the basis of the changes in total PAH content.

The term "weathering" involves a series of processes (evaporation, dissolution, dispersion, emulsification, photooxidation, biodegradation, spreading, and adsorption) that alter the physical and chemical characteristics of spilled oil. Evaporation is a crucial process in terms of material balance that results in losses of lighter saturate and aromatic compounds (monoaromatic and light PAHs), which is more marked for light crude oils. For this reason, the proportion of PAHs and their toxic contribution increases with evaporation [12]. The photooxidation of aliphatics and aromatics fractions generates more polar and water soluble compounds such as ketones, aldehydes, carboxylic acids and esters [12].

The present study was aimed at better understanding the effects of weathering on oil composition and toxicity. Artificial weathering of oil was performed by evaporation and photooxidation, and chemical fractionation to characterize compositional changes. The toxicity of the selected oils and their fractions were quantified by the sea urchin embryo test.

2. Materials and Methods

The following oils were used: Angolan crude oil (Dalia, API 23.14), Heavy Fuel Oil (HFO, API 11.47) [13].

2.1. Oil weathering

2.1.1. Photooxidation

Photooxidation was performed on the oil samples, to simulate the weathering of oil at sea, using SUNTEST® CPS flatbed xenon exposure system from Atlas (Chicago, USA). It is equipped with 1500B NrB4 Xenon lamp that was operated at a potential of 507.5 W/m². An appropriate mass of each

83 oil sample (ca. 0.4 g) was transferred to pre-weighed petri dishes and spread to obtain uniform layer.

84 Each sample was irradiated for 6 hours.

85

86 2.1.2. Evaporation

87 Evaporation was performed to simulate the weathering of oil over the short-term (ca. 2h) following a

88 spill [14]. Crude oil (1L) was placed in a pre-weighed crystallising dish (2L) in a fume hood (air flow

89 0.5 m/s) at room temperature for 24h. Evaporative loss was calculated for each oil.

90

91 2.2. Coarse fractionation

92 Open column liquid solid chromatography was used to fractionate the oils. A glass column packed

93 with silica gel (60-100 mesh; 5% H₂O; 40 g) under alumina (grade 1 neutral; 1.5% H₂O; 20 g) was

94 loaded with oil (1 g), and the column eluted with hexane (2 column volumes), dichloromethane (2

95 column volumes) and methanol (2 column volumes) to provide aliphatic, aromatic and polar fractions

96 respectively. The obtained fractions were used for toxicity tests.

97

98 The oil samples were also analysed using thin layer chromatography with flame ionisation detection

99 (TLC-FID) which is well established as an efficient, fast and cost effective method to obtain quantitative

100 data on the composition of oils, more specifically the relative contents of saturates, aromatics, resins and

101 asphaltenes (SARA analysis) [15, 16].

102

103 Using a sample spotter SES 3202/IS-02 (Ses GmbH, Nieder-Olm, Germany), 0.8 µL of the DCM oil

104 solution was spotted onto silica-coated quartz rods (ChromaRod®-SIII). A three-step separation was

105 performed using 100% n-hexane to 10 cm, 20:80% n-hexane:toluene to 5 cm and 5:95%

106 methanol:dichloromethane to 2 cm, respectively. All eluents used were analytical grade (Suprasolv

107 grade). After elution, the Chromarods® were dried at 40°C for 5 min to remove solvents and transferred

108 into a MK-5 TLC-FID Iatroscan® apparatus (Iatron Labs, Tokyo, Japan) where each Chromarod® was

109 scanned with the FID to detect the oil compound classes separated on the silica. The hydrogen flow rate
110 was 160-180 mL min⁻¹, the airflow rate was 2000 mL min⁻¹ and the scanning speed was 30 s per
111 Chromarod® burned.

112 113 *2.3. Preparation of water accommodated fractions and oil extracts*

114 Water accommodated fractions (WAFs) of weathered and unweathered oils (Angolan crude oil and
115 HFO) were prepared by adding 3.5 g of to 87.5 mL of 0.22-µm-filtered seawater (FSW) in a 250 mL
116 bottle with a teflon cap (loading rate, 40 g/L). The mixture was kept in the dark and shaken (150 rpm)
117 for 24 h at 20 °C. The aqueous phase was separated and used to obtain the FSW dilutions to be tested.
118 The experimental concentrations tested (from 1.25 to 1000 mL/L in geometric increments) were
119 obtained by dilution of the WAF in FSW.

120
121 DMSO extraction for each fraction or unweathered/weathered oil (Angolan crude oil and HFO) was
122 performed at a ratio 1:9 (m:m) by orbital shaking (150 rpm) for 16 h at 50°C and dilutions of the extract
123 in DMSO. For the sea urchin embryo test, 1 mL/L of the extract was added to each vial with FSW. The
124 experimental concentrations tested were 0, 10, 20, 50, 100, 250, 500 and 1000 µL/L.

125 126 *2.4. Sea urchin embryo test*

127 The sea urchin embryo test was performed in accordance with the method of Saco-Álvarez et al. [17].
128 Gametes of *Paracentrotus lividus* were obtained by dissection of two of the adults and their maturity
129 (ovum sphericity and sperm mobility) checked with a microscope. The ova were transferred to a 100-
130 mL graduated cylinder containing seawater (5-10 ova/µL), a few drops of sperm (30-100 µL) taken
131 from the male gonad were added through a Pasteur pipette, and the mixture shaken gently to facilitate
132 fertilisation. The fertilisation rate was determined in a Sedgewick-Rafter counting chamber in
133 quadruplicate (n=100), as the proportion of eggs with a fertilisation membrane (> 97%). Within 30
134 minutes, the fertilised eggs were transferred to vials with 10 mL (WAF) or 4 mL (extracts of oil/fraction

in DMSO) of FSW dosed with the oil or fraction to be tested. Each vial received 40 eggs per mL and each dose was performed in quadruplicate.

The eggs were incubated in the dark at 20 °C for 48 hours, and the larvae fixed by adding a few drops of 40% formalin. In each vial the maximum length of 35 individuals was measured using an inverted microscope and Leica QWIN image analysis software, version 3.4.0 (Leica Microsystems, Germany). The inhibition of growth in length was quantified as [18]:

$$R_i = 1 - \frac{\Delta L_i}{\Delta L_0} \quad (1)$$

where ΔL_0 and ΔL_i are the mean length increases in the control and the i^{th} dose, respectively.

2.5. Statistical analyses

The dose-response relationship for each heavy metal was described using the modified Weibull model [19]:

$$R = K \left\{ 1 - \exp \left[- \ln 2 \left(\frac{D}{m} \right)^\alpha \right] \right\} \quad (2)$$

where R is the response (with K as the maximum value), m is the dose corresponding to the semi-maximum response and α is a shape parameter related to the maximum slope of the response.

Fitting procedures and initial parametric estimations were performed by minimisation of the sum of quadratic differences between experimental and predicted values using the non-linear least-squares (quasi-Newton) method provided by the ‘Solver’ macro of the Microsoft Excel spreadsheet. Parametric

159 estimates were confirmed in the non-linear section of the Statistica 6.0 pack, which was also used to
160 calculate the parametric confidence intervals and model consistency (Student's t and Fisher's F tests,
161 respectively, in both cases with $\alpha=0.05$).

162

163 The EC_{50} of each oil was estimated from the fractions by Concentration Addition (CA) and
164 Independent Action (IA) models. CA model can be formulated as Junghans et al. [20]:

165

$$166 \quad ECx_{mix} = \left(\sum \frac{p_i}{ECx_i} \right)^{-1} \quad (3)$$

167

168 where ECx_{mix} is the total concentration of a mixture corresponding to the effect x , p_i are the relative
169 proportions of the compounds in a mixture and ECx_i are the equivalent effect concentration of the single
170 substances.

171

172 Independent Action (IA) model [21] is usually written as:

173

$$174 \quad E(c_{mix}) = 1 - \prod_{i=1}^n (1 - E(c_i)) \quad (4)$$

175

176 where $E(c_{mix})$ is the total effect corresponding to the mixture and $E(c_i)$ are the effects of the individual
177 constituents.

178

179 Toxic units (TU) for each fraction were calculated according to the CA model and the following
180 expression:

181

$$182 \quad TU = \frac{p_i ECx_{mix}}{ECx_i} \quad (5)$$

3. Results

3.1. Coarse fractionation

TLC-FID analysis of the weathered samples and their comparison to the original samples shows that the effects of weathering are somewhat different depending on the type of oil (Table 1). Simulated weathering resulted in losses of monoaromatics in the two oils. Aromatics content is decreasing due to photooxidation, while after evaporation in HFO its content is increased (Table 1). The percentage of resins increased following irradiation in all samples due to the photooxidative transformation of aromatics ($p < 0.05$, Table 1), while evaporation caused a slight decrease of the percentage of resins for HFO ($p < 0.05$). Asphaltenes are the most resistant to weathering with both oils showing only slight changes in their content.

3.2. Sea urchin embryo test

The WAF of the fresh Angolan crude showed similar toxicity to the evaporated oil suggesting very little change as would be expected 2h post spill. The photooxidised oil was less toxic ($p < 0.05$) (Table 2 and Figure 1). The toxicity of the HFO WAFs was remarkably low, and it was not possible to discern a clear trend (Table 2 and Figure 1).

For the Angolan crude, no significant differences were found between the EC_{50} for the extract of fresh oil and evaporation-weathered oil, although oil weathered by Xenon lamp showed less toxicity than fresh oil ($p < 0.05$) (Table 3 and Figure 2). No significant differences were detected in the EC_{50} for the extracts of fresh and weathered HFO (Table 3 and Figure 2).

The most evident effect of weathering on oil toxicity was obtained for the aliphatic fraction (Table 3 and Figure 2), namely an increase in toxicity parameter values (EC_{10} , EC_{50} , NOEC, LOEC). It can be concluded from the EC_{50} values obtained for this fraction that weathering decreases toxicity ($p < 0.05$) in

209 the order fresh>evaporated>photooxidated for both Angolan oil and HFO. The decrease in toxicity was
210 more marked for the medium oil (Angolan crude) than for the heavy oil. Weathering caused an increase
211 in the toxicity (EC₅₀ values) of the aromatic fraction, although the differences between fresh and
212 weathered oils were not statistically significant ($p < 0.05$) (Table 3). For this fraction, no significant
213 differences were found between Angolan oil and HFO. The toxicity of the polar fraction was
214 significantly higher for weathered oil than for fresh oil ($p < 0.05$) (Table 3).

215

216 The toxicity of the aromatic and polar fractions were both significantly greater than that of the
217 aliphatic fraction in all cases tested ($p < 0.05$) except for fresh Angolan crude (Table 3 and Figure 2).

218

219 Toxic units were calculated for each fraction according to the concentration addition model, the
220 relative proportions of each fraction in oil and assuming that the toxicity of the asphaltenes was
221 negligible (Table 4). Aromatics explain most of the toxicity and polars are the second most important
222 component, despite having less or similar abundance than aliphatics. The value of toxic units for the
223 aliphatic fraction decreases in the order Fresh<Evaporated<Xenon and increases in reverse order for the
224 polar fraction. The predicted EC₅₀ values were calculated based on the toxicity of their fractions and CA
225 and IA models (Table 5).

226

227 **4. Discussion**

228 Aromatic hydrocarbons, as defined by the aromatic fraction, were the main oil components
229 responsible for the toxicity observed for the sea urchin embryo test. A reduction in the toxicity of the
230 aliphatic fraction was observed following weathering and it is probably due to a loss of compounds of
231 low molecular weight. The toxic contribution of this fraction was medium in Angolan crude (0.37-0.03
232 TU) and low (0.07-0.02 TU) for a heavy oil. The toxicity of the aromatic components increased
233 following artificial weathering, in particular for the HFO. The toxic contribution of the polar fraction

234 increases with weathering (Figure 2), and a greater contribution of the polar fraction was observed in the
235 weathered oils compared to the aliphatic fraction (Table 4).

236

237 A slight reduction in the toxicity of the medium Angolan crude (Dalia) was observed following
238 weathering in the sea urchin tests performed with the water-accommodated fraction and the extract in
239 DMSO, but no clear effect of weathering was found for the HFO. Photooxidative treatment with the
240 Xenon lamp produces a slight reduction in toxicity for the Angolan crude in both WAF and extract in
241 DMSO tests, and this correlates with a decrease of the toxic contribution of the aliphatic fraction (Table
242 4). Artificial weathering methods do not substantially change the toxicity of the HFO since the toxic
243 contribution of aromatics is decisive (~0.7-0.8 TU).

244

245 Embryo-larval toxicity obtained for the WAF of a medium oil (Angolan crude) was higher than that
246 of a heavy oil (HFO) (Table 2). However, the toxicity for DMSO extracts of these oils were similar
247 (Table 3), possibly due to the higher capacity and rate of extraction of DMSO compared to water. The
248 low toxicity of the WAF of a HFO is possibly caused by a low transferring rate of hydrocarbons from
249 oil to water and a low concentration of total petroleum hydrocarbons as suggested by Tsvetnenko and
250 Evans [22]. These authors found that: a) the WAF of a heavy oil showed a maximum concentration of
251 total hydrocarbons (TPH) at 600 hours while the maximum TPH for medium and light crude oils were
252 achieved within 24 hours; b) maximum TPH for light and medium crude oils were higher than that of
253 the heavy oil.

254

255 The water accommodated fraction has been considered more suitable for oil toxicity testing than to
256 use a carrier because of a greater resemblance to natural conditions and the impossibility to find a low
257 toxicity solvent capable of dissolving all compounds of oil [23]. The chemical analysis of water-
258 accommodated fraction has raised questions about the class of compounds causing toxicity [8, 24], and
259 these findings underline the need of using other approaches for identification purposes. In this paper,

fractionation and biological testing were used to establish cause-effect relationships between the toxicity of coarse fractions and oil. The use of an extract in DMSO of an oil fraction or oil has made possible to: a) test the potentially active compounds in water [7]; b) consider all the compounds of a fraction irrespective of the proportion that can be solved by chromatographic methods; c) obtain reproducible results with low sample volumes; and d) evaluate the changes of toxicity for a fraction/oil caused by the artificial weathering methods. However, it was not possible to establish causality between the compounds belonging to each fraction and the toxic response; thus, finer fractionation procedures may be recommended in future research.

In the past, similar approaches have been used to assess the contribution of toxic fractions of oil. The purpose of those studies was not to evaluate the effect of weathering on the toxicity of fractions or oil, the fractionation procedures were different, and the results are not fully comparable. Some authors [7, 9] found that aromatic and polar fractions showed significant toxicity in oil and extracts of affected sediments. Other authors [6, 10] performed an extraction and fractionation of the water soluble fraction of a weathered oil and found that the greatest toxic contribution was obtained for the polar fraction. The present paper shows that the polar fraction may play an important role in the effect of weathering on oil toxicity (Figure 2 and Tables 3 and 4). An increase in polars content and a slight decrease in the proportion of total aromatics have been observed in the photooxidative treatment for both Angolan and HFO (Table 1), and this agrees with the results found by Garrett et al [25] for UV treatment of oil. The photooxidation of hydrocarbons is a mechanism that could generate compounds of higher polarity, solubility and reactivity (hydroxylated derivatives, ketones, aldehydes, carboxylic acids and esters) [12] and thus explain the increase of the polar fraction toxicity for the Xenon lamp treatments. However, the authors of the present study have not been able to suggest a process responsible for the increase of toxicity in this fraction for weathered oils by evaporation. Small amounts (1.1-5.1 mg) of the polar fraction were obtained and the relevance of the results suggests performing oil fractionation at a larger scale in future research. Some authors [26] have found that oxygenated hydrocarbons generated by

286 weathering were eluting in the polar and asphaltene fractions defined by TLC-FID chromatograms,
287 which raises doubts about the assumption of negligible toxicity for the asphaltene fraction.
288

289 It is generally accepted that weathering decreases oil toxicity due to the loss of more volatile and
290 soluble compounds [27, 28]. This is moderately in agreement with the results shown for the extracts of a
291 fresh and weathered medium oil but not for the HFO. Neff et al. [24] conclude that monocyclic aromatic
292 hydrocarbons (MAHs) are primarily responsible for the acute toxicity of light oils and toxic contribution
293 of PAHs increases with weathering. In this study, the toxicity of the aromatic fraction to sea urchin
294 embryos slightly vary with weathering for medium and heavy oil (Table 3); hence the decrease of
295 overall toxicity seems to be related to the drop of toxicity of the aliphatic fraction.
296

297 The interpretation of the toxicity observed in a sample of water containing hydrocarbons will depend
298 on the available analytical information (resolved plus unresolved) for the compounds of each fraction
299 and the whole set. Oil weathering causes a decrease of the peaks resolved by chromatography and an
300 increase of the UCM which depends on the oil or oil fraction considered [3]. Neff et al. [24] and Barron
301 et al. [8] proposed that other group of components besides MAHs and PAHs might have contributed to
302 the toxicity of the WAF of middle volatility oils; and the polar compounds and UCM were the most
303 likely candidates. Bellas et al. [11] also found that weathering of a standard fuel oil in seawater by
304 magnetic stirring and photooxidation caused an increase of the WAF toxicity with time which was not
305 related to aromatic hydrocarbon levels. The HFO mainly contains a residual fuel and a smaller
306 proportion of lower boiling components; hence the significant presence of the UCM in the
307 chromatograms normally obtained by GC-MS. Some authors [29, 30] have shown that the increase of
308 toxicity with light observed for the WAF of residual fuel oils cannot be explained exclusively by the
309 PAH concentration and suggested that unidentified chemicals, in particular the heterocyclic compounds,
310 could present potent phototoxicity. For these reasons, parental PAHs should not be the only group of
311 compounds monitored after an oil spill.

312

313 The predicted EC₅₀ for the sea urchin assay by CA and IA models is quite close to the observed values
314 (Tables 3 and 5). This would be in line with the widespread assumption of a non-specific mode of
315 action, called narcosis, for petroleum compounds [31]. Some authors [32] have questioned that the
316 toxicity of PAHs may be due to nonspecific mechanisms. The experimental design of this work is not
317 adequate to discern this, and additivity between compounds and/or petroleum fractions deserves further
318 research.

319

320 **Conclusions**

321 Weathering decreases the toxicity of the aliphatic fraction and increases the toxicity of the aromatic
322 and especially the polar fractions of the oil. Therefore, the present results stress the role of polar
323 compounds in the toxicity of weathered oils.

324

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427 FIGURE CAPTIONS

428

429 Figure 1. Inhibition of sea urchin larval growth by the water accommodated fraction of Angolan crude
430 oil (up) and HFO (down). The symbols represent fresh oil ($\text{—}\diamond\text{—}$), weathering by evaporation ($\cdots\square\cdots$),
431 and weathering by Xenon lamp ($\text{--}\Delta\text{--}$). Dose in mL/L.

432 Figure 2. Inhibition of sea urchin larval growth by a extract of DMSO of Angolan crude oil (left) and
433 HFO (right). The symbols represent fresh oil or fraction ($\text{—}\diamond\text{—}$), weathering by evaporation ($\cdots\square\cdots$),
434 and weathering by Xenon lamp ($\text{--}\Delta\text{--}$). The fraction or oil is indicated by text printed on each graph.
435 Dose in $\mu\text{L/L}$.

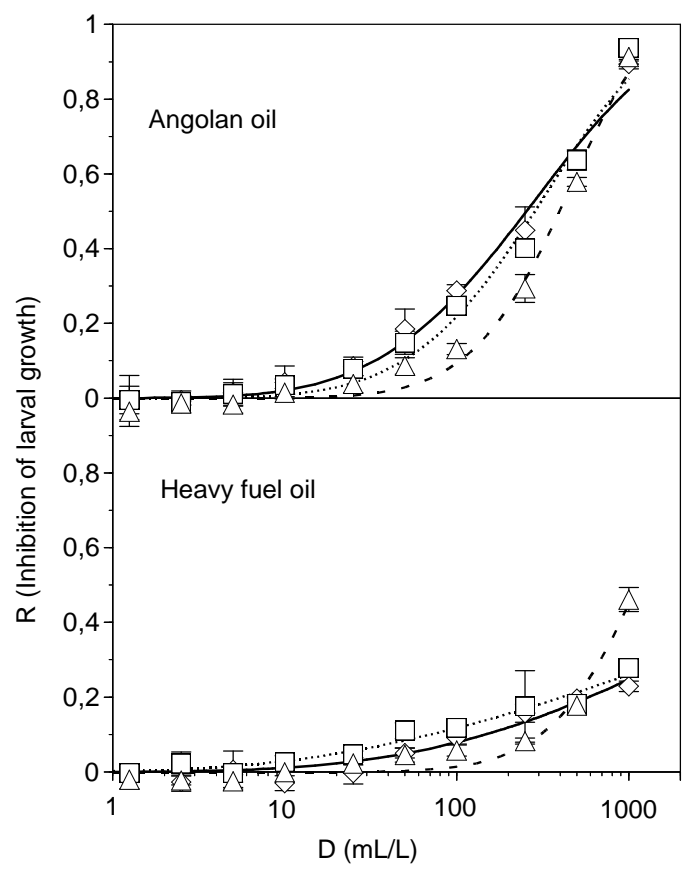
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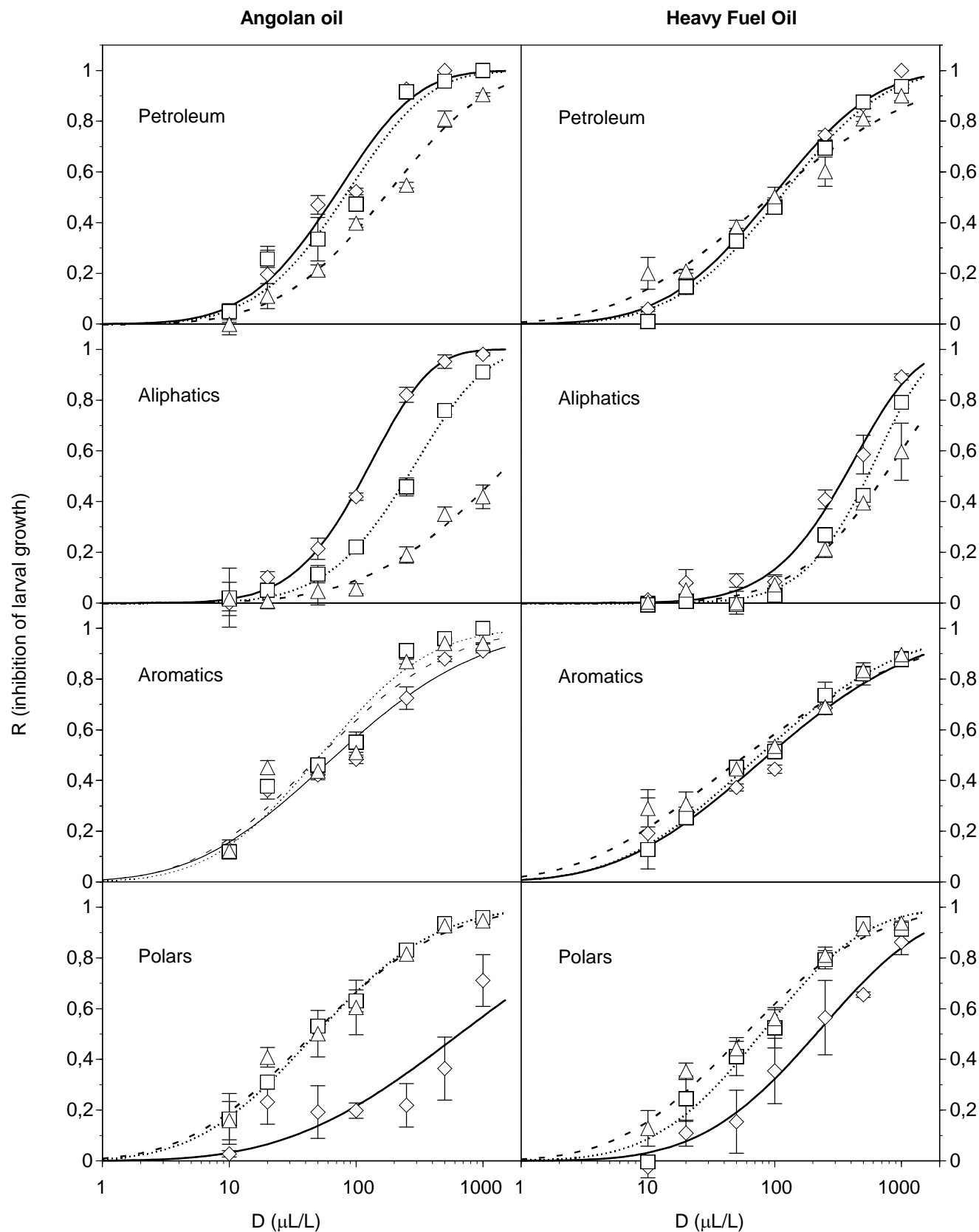
437 Figure 1

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446 TABLES.

447 Table 1 Aliphatics, monoaromatics, polyaromatics, total aromatics, polars and asphaltene content for
448 fresh and weathered oils. An asterisk indicates a content statistically different from the unweathered
449 fraction (* $p < 0.05$).

450 Table 2. Toxicity thresholds (NOEC, LOEC and EC_{10}) and EC_{50} obtained with sea urchin embryo test
451 for the water accommodated fraction of fresh and weathered Angolan and HFO.

452 Table 3. Toxicity thresholds (NOEC, LOEC and EC_{10}) and EC_{50} obtained with sea urchin embryo test
453 for the extracts in DMSO of fresh and weathered Angolan and HFO and their fractions.

454 Table 4. Toxic units (TU) for the dose corresponding to 50% effect (EC_{50}) calculated according to the
455 concentration addition model for the fresh and weathered oils.

456 Table 5. Predicted EC_{50} values for the DMSO extracts of oil according to concentration addition (CA)
457 and independent action (IA) models applied to the isolated fractions.

458

Table 1

		Content (%)					
Oil		Aliphatics	Monoaromatics	Polyaromatics	Total aromatics	Polars	Asphaltenes
Angolan	No weathering	39.94±8.48	16.04±8.84	23.67±9.37	39.71±5.46	14.51±2.66	5.84±2.17
	Evaporation	41.55±2.23	12.41±2.47	27.64±4.58	40.05±2.20	14.01±2.18	4.39±1.38
	Xenon lamp	37.48±3.41	10.54±2.38	16.91±0.50	27.45±2.32*	30.65±2.32*	4.43±3.55
Heavy fuel	No weathering	16.44±2.70	10.96±8.13	39.22±11.26	50.18±3.27	17.76±2.46	15.63±3.28
	Evaporation	17.16±1.47	10.65±9.27	44.64±12.70	55.28±4.87*	14.93±1.51*	12.63±4.32
	Xenon lamp	17.03±0.85	8.26±0.46	39.30±2.25	47.55±1.95	22.22±1.87*	13.20±1.25

Values ± 95% confidence intervals.

Table 2.

Oil	Weathering	EC ₅₀ (mL/L)	EC ₁₀ (mL/L)	NOEC (mL/L)	LOEC (mL/L)
Angolan	No weathering	254.4 (216.7-298.7)	33.4 (24.2-45.9)	10	25
	Evaporation	283.1 (235.1-340.9)	48.5 (32.9-71.5)	10	25
	Xenon Lamp	394.2 (349.4-444.7)	104.0 (78.7-137.3)	25	50
Heavy Fuel	No weathering		147.3 (90.7-238.8)	50	100
	Evaporation		70.3 (47.3-104.4)	25	50
	Xenon Lamp		305.9 (231.3-404.6)	25	50

Values reported with 95% confidence intervals in parentheses.

Table 3

Oil	Oil/Fraction	Weathering	EC ₅₀ (µL/L)	EC ₁₀ (µL/L)	NOEC (µL/L)	LOEC (µL/L)
Angolan	Bulk	No weathering	66.8 (50.6-88)	13.2 (7.7-22.2)	10	20
		Evaporation	79.9 (57.1-111.6)	15.2 (8-28.4)	10	20
		Xenon Lamp	166.6 (139.2-199.4)	23.2 (16.4-32.6)	10	20
	Hexane (aliphatics)	No weathering	115 (103.8-127.4)	28.9 (23.6-35.3)	10	20
		Evaporation	254.6 (230.1-281.7)	52.1 (42.1-64.4)	50	100
		Xenon Lamp	> 1000	112.7 (74.9-169.2)	100	250
	Dichloromethane (aromatics)	No weathering	69.6 (48.1-100.6)	6.2 (3.2-11.3)		10
		Evaporation	52.1 (35.2-76.8)	7.2 (3.5-14)		10
		Xenon Lamp	52.2 (29.8-91)	5.7 (2-14.1)	10	20
	Methanol (polars)	No weathering	654.2 (226.6-1885.1)	32.8 (5.7-170.3)	10	20
		Evaporation	47.9 (42.8-53.5)	5.9 (4.8-7.1)		10
		Xenon Lamp	45.7 (34.4-60.6)	4.8 (2.9-7.6)		10
Heavy Fuel	Bulk	No weathering	94.5 (81.3-109.8)	13.6 (10.4-17.8)		10
		Evaporation	109.8 (95.8-125.7)	15.8 (12.3-20.2)	10	20
		Xenon Lamp	93.9 (69.6-126.4)	7.3 (4.3-12)		10
	Hexane (aliphatics)	No weathering	358.1 (294.7-435)	80.9 (52.1-125.5)	20	50
		Evaporation	537.8 (476.9-606.4)	145 (108.4-193.9)	100	250
		Xenon Lamp	727.5 (645.6-819.8)	125.3 (96.2-163.1)	100	250
	Dichloromethane (aromatics)	No weathering	89.4 (67.3-118.5)	7 (4.2-11.3)		10
		Evaporation	75.8 (65.2-88.2)	6.7 (5.1-8.6)		10
		Xenon Lamp	59.3 (42.8-82.1)	3.9 (2.2-6.5)		10
	Methanol (polars)	No weathering	212.2 (170.4-264.1)	25.6 (16.6-39.1)	50	100
		Evaporation	77.9 (58.5-103.5)	11.2 (6.7-18.5)	10	20
		Xenon Lamp	57.9 (44.2-75.6)	6.4 (4-10)	10	20

Values reported with 95% confidence intervals in parentheses.

Table 4

Oil	Weathering	Fraction	TU
Angolan	Fresh	Aliphatics	0.37
		Aromatics	0.61
		Polars	0.02
		Asphaltenes	0
	Evaporation	Aliphatics	0.13
		Aromatics	0.63
		Polars	0.24
		Asphaltenes	0
	Xenon lamp	Aliphatics	0.03
		Aromatics	0.43
		Polars	0.54
		Asphaltenes	0
Heavy fuel	Fresh	Aliphatics	0.07
		Aromatics	0.81
		Polars	0.12
		Asphaltenes	0
	Evaporation	Aliphatics	0.03
		Aromatics	0.77
		Polars	0.20
		Asphaltenes	0
	Xenon lamp	Aliphatics	0.02
		Aromatics	0.66
		Polars	0.32
		Asphaltenes	0

Table 5

Oil	Weathering	Predicted EC50 _{oil} (μ L/L)	
		CA	IA
Angolan	Fresh	106.4	96.5
	Evaporation	81.6	74.8
	Xenon Lamp	80.8	61.8
Heavy fuel	Fresh	144.7	131.7
	Evaporation	104.9	93.9
	Xenon Lamp	82.7	59.1