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Petroleum hydrocarbons, fluorescent aromatic compounds in fish bile and organochlorine pesticides from areas surrounding the spill of the Kab121 well, in the Southern Gulf of Mexico : A case study

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Abstract

In October 2007, a light crude oil spill took place in the off shore Kab121 oil well, 32 km north of the mouth of the Grijalva River, Tabasco, Mexico. In order to estimate the possible effects of oil spill on the biota in the area surrounding the spilled well, the level of different fractions of petroleum hydrocarbons were measured in fish, as well as the concentration of some chlorinated hydrocarbons and PCBs. The organisms examined were cat fish (*Ariopsis felis*), in addition fluorescent aromatic compounds in bile, the contaminants above mentioned and their relationship with cyotochrome P-450 and Ethoxyresorufin-O-deethylase, Glutathion-S-Transferase and catalase activities in liver were determined. The concentration of most pollutants were low, except PAHs. Spatial distribution of these compounds, as well as most biomarkers, reflected the highest exposure of fish to pollutants in the area adjacent to well, as well as in the proximity of rivers. The profile of exposure to this environment was chronic in nature and not temporary.

Key words

Ariopsis felis, Biomarkers, Oil pollution, PCB

Introduction

The Gulf of Mexico is an area with many natural resources, oil being one of the most important. Mexico ranks sixth world wide in oil production, and the marine area of the Gulf of Mexico contributes more than 76% of domestic production. The process of drilling and oil extraction are some of the activities that release significant amount of oil into the sea, and spills are other sources of petroleum to the environment that cause problems to the environment and organisms of the affected area. On October 2007, due to an accident there was a gas and light crude oil leak in the valve shaft of Kab-121 well, located in the Kab-101 platform 22 km north of Tabasco, Mexico. The leak was controlled 52 days later, but during this period it was estimated that approximately 16, 500 barrels were spilled into the sea.

The area of the spill is in front of the estuary of the Grijalva river, which represents about 30 to 35% of all freshwater runoff in Mexico. The plume of the river covered the spill area during the rainy season, complicating the interpretation of results. Chlorinated compounds, including polychlorinated biphenyls (PCBs), were determined to assess the influence of land-based sources of pollutants.

The aim of this study was to determine the possible effects of spill on fish of the surrounding area of the well Kab121, through identification of petroleum hydrocarbons as well as metabolites of polycyclic hydrocarbons and other biomarkers in cat fish (*Ariopsis felis*). Due to the agricultural activities conducted in coastal area, the concentrations of some organochlorine pesticides and PCBs were determined to assess the influence of

land-based sources of pollutants, and also considering that these compounds have negative effects on the organisms.

Materials and Methods

Sampling was done twice, once in March and other in June 2008. The sampling network consisted of 21 stations located around the Kab-101 platform, in which the Kab-121 well is located (Fig. 1). Five fish per station were collected by trawling and dissected to extract the bile and liver. Liver and bile samples were preserved in liquid nitrogen and later transported to the laboratory for further analysis.

Hydrocarbons and organochlorine compounds in liver were determined according to Wade *et al.* (1993). Prior to extraction, liver samples were homogenized and 100 µl of the following surrogate standards were added: biphenyl d10, phenanthrene d10, chrysene d12, benzo [a] pyrene d12 (10 mg ml^{-1}), and o-terphenil (200 mg ml^{-1}). Samples were extracted with hexane in a tissue homogenizer, and extracts were purified and separated into fractions using silica gel-alumina columns. Identification and quantification of the compounds was carried out with standards from Ultra Scientific in the case of the PAHs and organochlorine pesticides; Accu Standard for PCBs and from Chiron for deuterated PAHs. Total hydrocarbons were analyzed with an Agilent 5890 gas chromatograph equipped with a FID detector. PAHs were analyzed with a Perkin-Elmer gas chromatograph equipped with a Clarus 500 mass selective detector using a 30 m × 0.25 mm (i.d.) x 0.25 DB-5 MS fused silica capillary column (J & W Scientific) and operating in the selected ion monitoring (SIM) mode. For quality control/quality assurance in each set of samples a procedural blank and a duplicate sample were added; surrogate standards were used to evaluate recovery. Equipment calibrations were verified daily, and calibration curves were done for each set of samples analyzed. PAHs were reported as total PAHs, low molecular weight PAHs (two and three benzene ring compounds) and high molecular weight PAHs (four or more benzene rings), respectively.

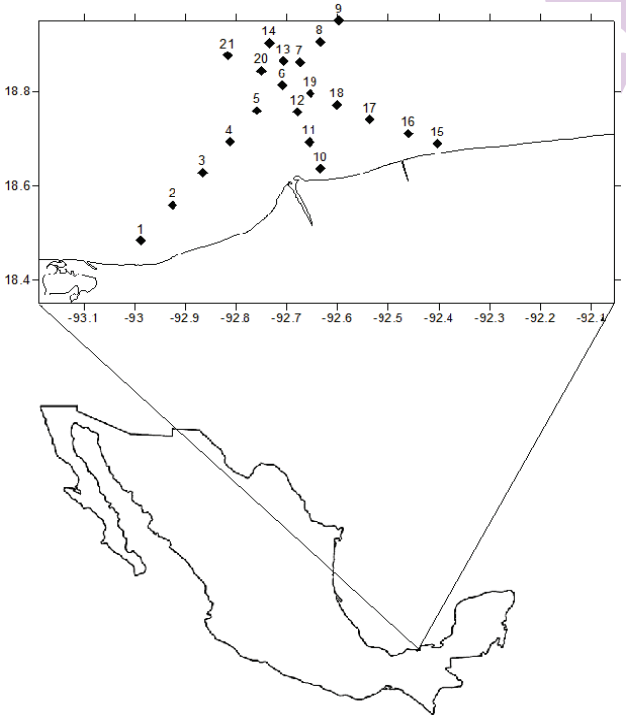

Fig. 1: Location map of sampling sites in the Tabasco coast, Mexico (station 6 location of the Kab 121 well)

Biliary metabolites (FACs) were analyzed according to Aas *et al.* (1998) using fixed-wavelength spectrofluorometry. Ethoxyresorufin-O-dethylase (EROD) activity was measured fluorometrically in the microsomal suspension in the presence of NADPH using ethoxyresorufin (ethoxyphenoxazone) as substrate. Enzyme activity was estimated from the resorufin formed per unit of time, which was monitored in a spectrofluorometer using excitation and emission wavelengths of 530 nm and 572 nm, respectively. A resorufin molar extinction coefficient of 73.2 nM^{-1} cm^{-1} was used. Results were expressed per mg of microsomal protein using bovine serum albumin as standard, according to the method described by Burkeetal. (1974). Glutathione-S-transferase (GST) activity was determined according to Habig *et al.* (1974). Briefly, 1 mM CDNB was added to buffer containing 1 mM GSH and an aliquot of sample to be tested. Upon addition of CDNB, the change in absorbance at 340 nm was measured as a function of time. The extinction coefficient for this reaction was 9.6 $mM^{-1}cm^{-1}$. GST activities with 1-chloro-2, 4-dinitrobenzen (CDNB) and 3, 4-dichloronitrobenzene (DCNB) were assayed spectrophotometrically at room temperature by monitoring the change in absorbance at 344, 270, and 344 nm, respectively by the method of Habig *et al.* (1974). Activity with CDNB was carried out in the presence of 0.1 M HEPES (pH 7.6), 1 mM GSH, 1 mM CDNB, 1 mg purified protein or 50 mg cytosolic protein. GST–DCNB activity was measured using 0.1 M HEPES (pH 7.6), 5 mM GSH, 1 mM DCNB, 0.1–0.2 mg purified protein or 1.0–1.5 mg cytosolic protein. GST activity toward EA was measured in 0.1 M sodium phosphate (pH 6.5), 0.15–0.3 mg purified or 0.5 mg cytosolic protein, 0.25 mM GSH, and 0.2 mMNaEA (freshly prepared from equimolar amounts of EA and sodium bicarbonate in deionized water). Activities were calculated using absorptivities of 9.6 mM^{-1} cm^{-1}, 8.5 mM^{-1} cm^{-1} and 5.0 mM^{-1} cm^{-1}, respectively.

Catalase (CAT) activity was evaluated according to the method proposed by Aebi (1984) in which the disappearance of H_2O_2 [initial concentration: 0.04% (v/v) H_2O_2] in a phosphate buffer (0.0068 g l^{-1} KH_2PO_4, 0.0175 g l^{-1} Na_2HPO_4, pH 7.0) was monitored at 240 nm. The reaction mixture contained 30 mM H_2O_2 in 50 mM phosphate buffer pH 7.0 and 0.1 ml enzyme in a total volume of 3 ml. CAT activity was estimated by decrease in absorbance of H_2O_2 at 240 nm. The enzyme activity [I.U.] was defined as 1 m mol of H_2O_2 decomposed per minute. The final activity was expressed in

units per milligram of total soluble proteins. Total protein was quantified by the method of Lowry *et al.* (1954). Fish livers were homogenized in a boron buffer (pH 8.7), in a refrigerated centrifuge at 5000´g for 15 min. The absorbence of the solution was determined in the presence of Folin reagent at 750 nm wavelength. The protein content was determined following a standard curve obtained using bovine serum albumin as standard.

Statistical analyses : Data that did not meet the assumptions of normality and homogeneity of variance, non-parametric analyses were carried out using Mann-Whitney U test and Spearman correlation. All statistical analyses were done with R version 3.0.0 (http://www.r-project.org/).

Results and Discussion

Tables 1, 2 shows the average concentration of hydrocarbon fractions in the liver and metabolites of PAHs in bile of catfish from the study area. Low molecular weight PAHs showed a mean of 39.7 ± 24.6 µg g^{-1} and 11.3 ± 7.9 µg g^{-1} for first and second samplings. High molecular weight PAHs showed a mean of 1.47±1.67 µg g^{-1} for first sampling and 1.19±1.21 µg g^{-1} for second sampling. Total hydrocarbons showed a mean of 133.5 ± 84.9 µg g^{-1} and 75.9 ± 50.2 µg g^{-1} in the first and second sampling, respectively.

Low molecular weight PAHs concentrations were significantly higher (Mann-Whitney U = 22.2; P = 2.4 X 10^{-6}) during the first sampling as compared to second (Fig. 2), probably due to metabolism and excretion, and also because sediment concentrations also decreased (data not shown); enough time passed between the two samplings to detect the decrease in the level of hydrocarbons. High molecular weight PAHs showed no significant differences (Mann-Whitney U = 0.11; P = 0.74) between samplings, probably due to lower contribution of these compounds in the oil spilled in the study area, because the oil spilled is light and high molecular weight PAHs are less abundant compounds in petroleum than low molecular weight PAHs (De Luca *et al.,* 2005).

Fig. 3 shows the spatial distribution of low molecular weight PAHs in liver, which shows influence of spill and contribution of Grijalva river. It should be noted that the point of intersection of the transects (station 6) is the area where the spill originated. For total PAHs, significantly higher concentrations in the first sampling were found, where more than 90% of these compounds consisted of low molecular weight PAHs. This indicated that source of these hydrocarbons was petrogenic (originating from petroleum), but it is possible that high molecular weight PAHs were metabolized to low molecular weight PAHs (Gagnon and Holdway, 2002). Al-Hassan *et al.* (2000) found that two, three and four benzene ring compounds were more common in sharks from Arabian Gulf, compared to higher-membered ring compounds. This may be due to relatively higher solubility of

Table 1 : Average concentrations of hydrocarbons (µg g^{-1} wet weight) in *Ariopsis felis* liver and metabolites in bile of PAHs (µg ml^{-1}) collected during first sampling

Station	Low molecular weight PAHs	High molecular weight PAHs	PAHs	Total HC	OH-P	BaP	OH-N	PHE
1	78.552	1.789	79.267	343.216	0.262	0.270	104.184	85.282
2	55.522	1.882	55.898	185.578	0.320	0.317	128.759	76.925
3	42.174	ND	42.174	96.741	0.297	0.346	108.259	63.473
4	36.772	ND	36.772	228.198	0.305	0.259	101.942	95.113
5	40.080	1.723	40.769	42.950	0.270	0.211	92.732	91.627
6	26.700	1.453	27.281	27.281	0.207	0.460	323.359	89.661
7	48.064	ND	48.064	170.620	0.338	0.440	159.283	72.992
8	38.136	ND	38.136	242.088	0.293	0.207	169.513	53.150
9	56.388	3.616	57.111	100.562	0.235	0.217	250.287	81.796
10	42.316	ND	42.316	78.715	0.328	0.256	278.571	87.069
11	53.931	3.047	54.541	138.997	0.222	0.175	185.366	79.472
12	28.153	5.103	29.174	80.166	0.292	0.200	223.502	87.996
13	21.859	ND	21.859	37.708	0.209	0.204	210.797	86.265
14	28.999	1.811	29.361	39.146	0.229	0.460	296.299	99.224
15	24.453	2.819	25.017	190.447	0.307	0.231	194.495	65.038
16	117.971	4.383	118.848	235.388	0.127	0.191	231.601	75.093
17	26.012	ND	26.012	186.340	0.185	0.188	227.302	72.232
18	6.017	3.306	7.339	76.095	0.266	0.236	236.186	74.154
19	17.732	ND	17.732	151.940	0.225	0.246	371.408	90.063
20	27.791	ND	27.791	39.673	0.262	0.203	90.450	100.520
21	15.209	ND	15.209	111.559	0.229	0.317	316.727	77.104

Total HC = Total hydrocarbons, OH-P = Hydroxipyrene, BaP = Benzo a pyrene, OH-N = Hydroxi naphthalene, PHE = Phenantrene ND = Not detected

Table 2 : Average concentration of hydrocarbons (µg g^{-1} wet weight) in *Ariopsis felis* liver and metabolites in bile of PAHs (µg ml^{-1}) collected during second sampling

Station	LPAHs	HPAHs	PAHs	THC	OH-P	BaP	OH-N	PHE
1	10.857	2.303	11.778	99.266	1.417	0.068	107.932	24.306
2	6.996	ND	6.996	81.431	0.115	0.043	103.730	16.044
3	15.862	2.267	17.676	33.191	0.194	0.076	122.182	16.112
4	27.367	3.502	29.468	135.340	0.144	0.024	102.488	10.905
5	21.148	3.096	23.006	70.367	0.190	0.081	122.912	14.674
6	21.864	2.857	23.578	89.036	0.143	0.030	121.889	20.046
7	6.031	ND	6.031	34.947	0.123	0.028	110.270	13.532
8	25.706	2.381	27.135	35.340	0.129	0.065	122.401	22.407
9	11.136	1.571	11.765	126.401	0.193	0.074	99.346	39.688
10	18.550	1.330	19.082	205.632	0.197	0.072	108.590	18.789
11	7.474	ND	7.474	48.303	0.072	0.019	88.147	9.272
12	13.861	2.050	15.091	37.150	0.123	0.106	113.376	26.256
13	8.720	ND	8.720	16.176	0.198	0.146	143.848	17.058
14	13.210	1.586	13.845	13.845	0.410	0.093	58.899	9.838
15	8.782	1.194	9.260	24.070	0.167	0.132	60.762	3.890
16	4.688	ND	4.688	112.482	0.170	0.107	54.953	10.103
17	1.572	ND	1.572	57.887	0.229	0.152	75.487	9.626
18	3.341	ND	3.341	77.771	0.100	0.117	65.512	7.123
19	4.580	1.042	4.788	132.834	0.168	0.115	75.341	15.711
20	3.883	ND	3.883	126.974	0.112	0.087	76.108	20.881
21	1.387	ND	1.387	35.878	0.125	0.150	102.999	21.718

Total HC = Total hydrocarbons, OH-P = Hydroxi pyrene, BaP = Benzo a pyrene, OH-N = Hydroxi naphthalene, PHE = Phenantrene ND = No detected

these compounds in water, which facilitates their uptake by marine animals (Baumard *et al.,* 1999). It is difficult to determine the origin of hydrocarbons in catfish, because these compounds are rapidly metabolized by fish (Malins and Hodgins, 1981) and therefore the level found in organisms reflect only part of the concentration present in sediments.

The level of PAHs in the liver of cat fish in the present study were higher than those reported by Al-Hassan *et al.* (2001) in the liver of other species of catfish (*Arius bilineatus* Val.) in the Arabian Gulf (0.0078 – 0.126 µg g^{-1}), a system characterized by intense oil activity; likewise the concentrations found in this study were higher than the values reported by Swapan *et al.* (2000) for fish obtained from Hiroshima Bay (0.25 – 13.6 µg g^{-1}), which is reported to be of toxicological concern to other trophic components of the coastal ecosystem.

Total hydrocarbon were significantly (Mann-Whitney U = 6.02; P = 0.014) higher during first sampling than second (Fig. 2); UCM contributed approximately 90% of hydrocarbons, which indicates petrogenic sources since UCM is a common feature of oil, certain refined products, and extracts of samples

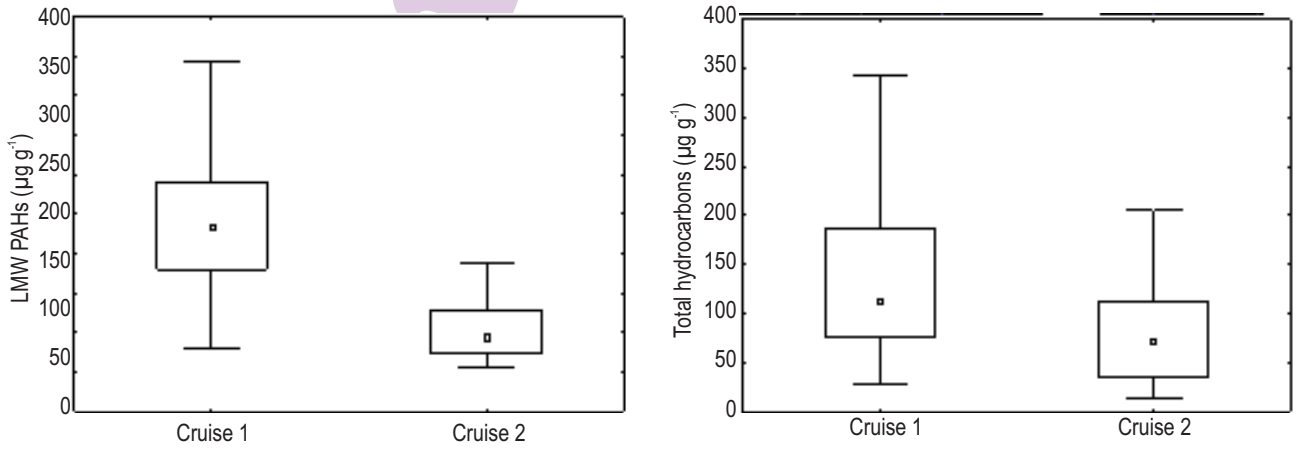

Fig. 2 : Medium concentrations of low molecular weight PAHs and total hydrocarbons in liver of *A. felis per cruise*

contaminated by oil, and also an indication of weathering of oil.

Table 3 and 4 shows the concentration of organochlorine pesticides in the liver of catfish. Total organochlorine pesticides showed a mean of 19.9 ± 14.9 ng g^{-1} and 40.1 ± 27.1 ng g^{-1} in the first and second samplings, respectively. PCBs showed a mean of 18.1± 15.8 ng g^{-1} for the first and 24.7 ± 14.5 ng g^{-1} for the second sampling.

All groups of organochlorine pesticides showed higher concentration in the second sampling, and with exception to chlorobenzenes and PCBs, all showed significant differences (Fig. 4). The second sampling was conducted during the rainy season, and increased discharge from the rivers could have provided these pollutants. Cyclopentadienic pesticides ("Drins") were the most abundant group.

Fig. 5 shows the spatial distribution of organochlorine pesticides in liver, showing the influence provided by the contribution of river Grijalva. Predominant direction of sea currents was East-West during the sampling period.

Comparing these results with those reported by Colombo *et al.* (2011) for fish from Rio de la Plata, Argentina, which varied from 10 to 100 ng g^{-1}, it was determined that concentration of these compounds in fish in the study area were low. Also, organochlorine pesticide level in fish of this study were lower than those reported by Adu-Kumi *et al.* (2010) in fish from a lagoon in Ghana, whose concentrations were considered low (average 442 ng g^{-1}).

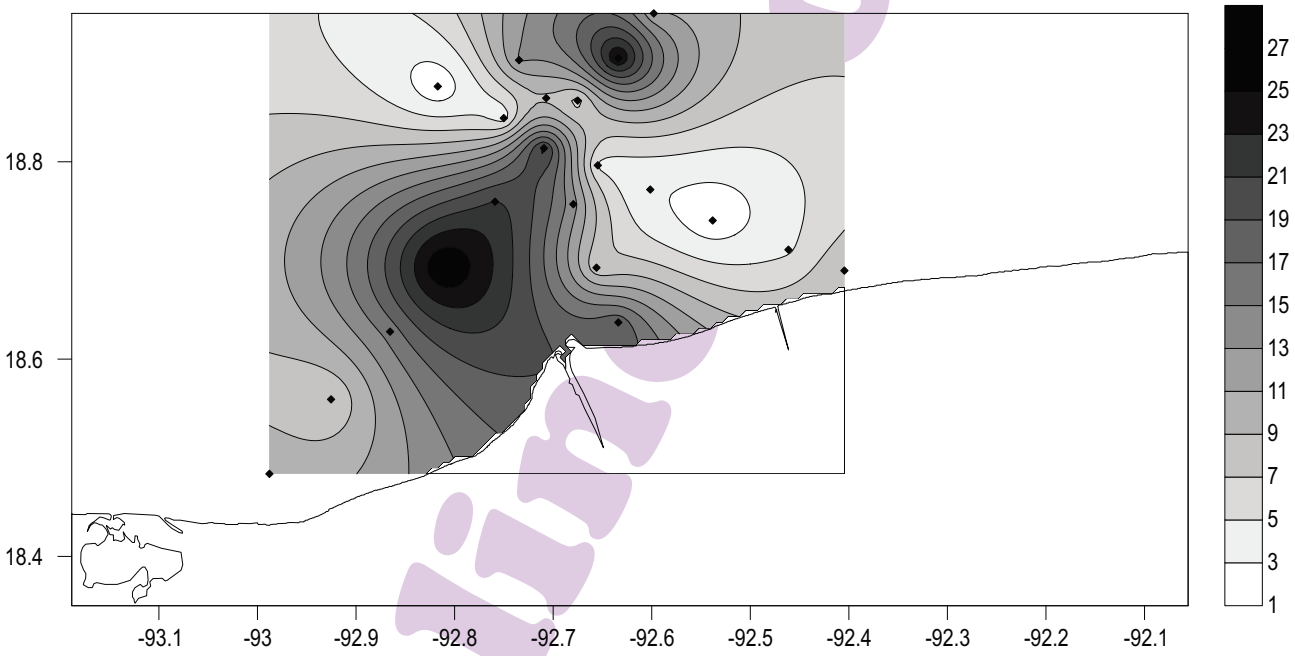

Fig. 3 : Spatial distribution of low molecular weight PAHs in the liver *A. felis*

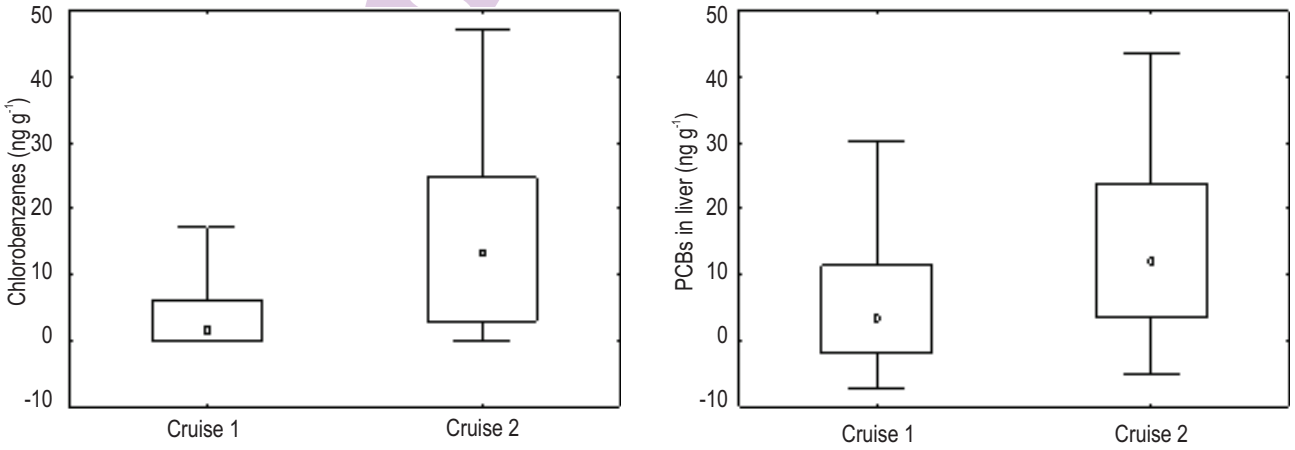

Fig. 4 : Concentration of chlorobenzenes and PCBs in liver of *A. felis per cruise*

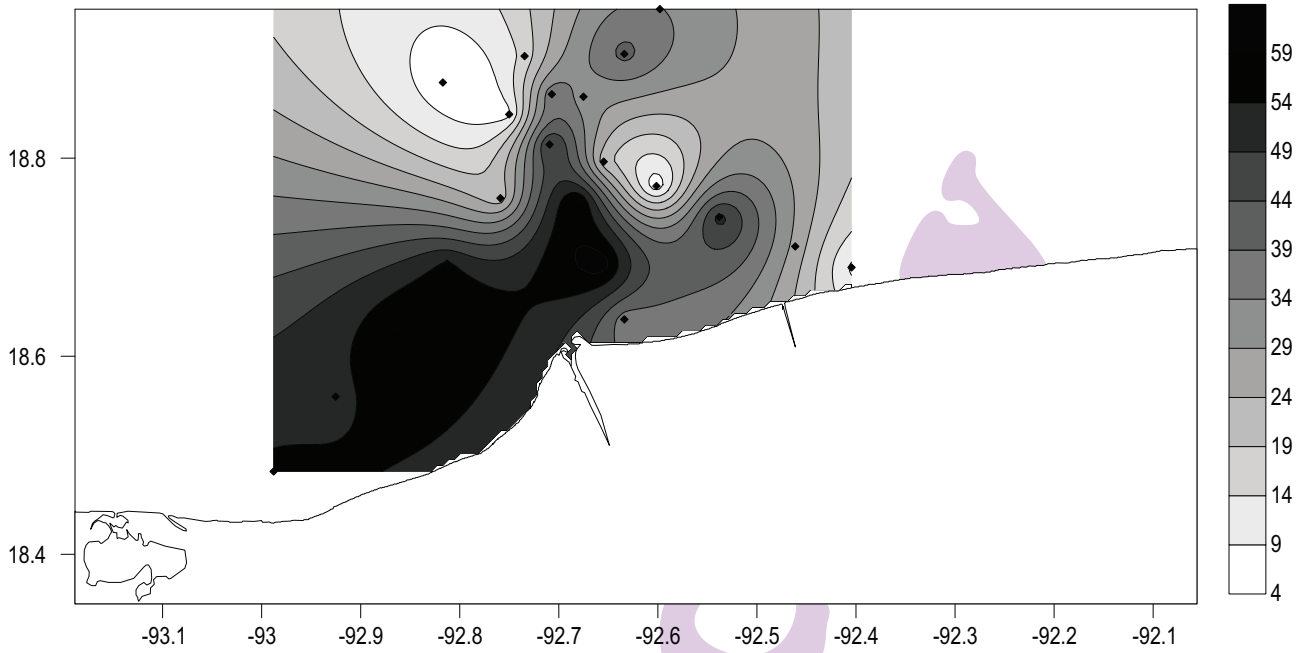

Fig. 5 : Spatial distribution of total chlorinated pesticides in the liver of *A. felis*

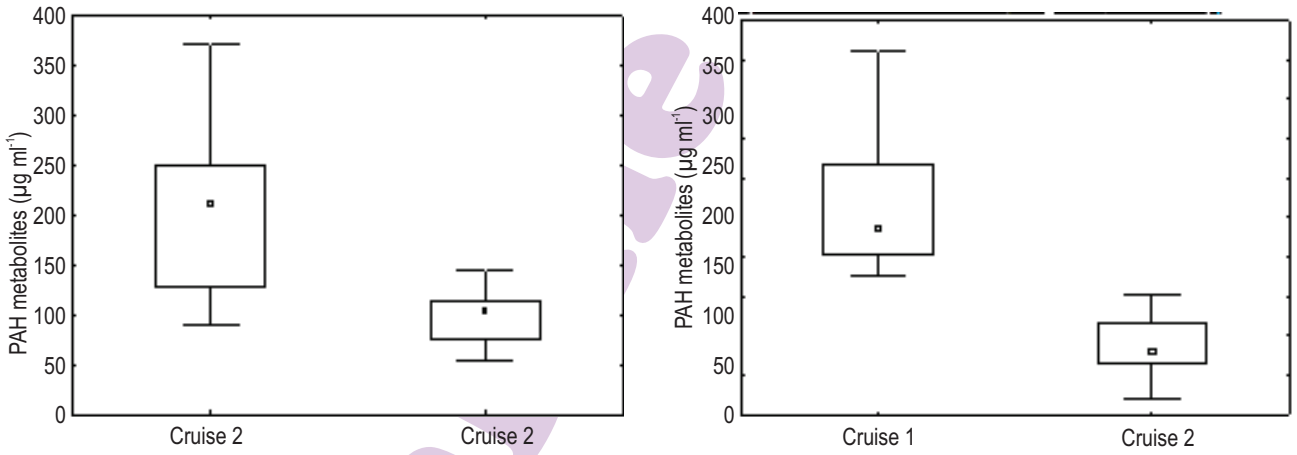

Fig. 6 : Median concentrations of PAH metabolites in bile of A. felis per cruise

The four metabolites tested had higher concentrations in fish from the first sampling, which is similar pattern than hydrocarbons in liver, and thus reflects exposure to hydrocarbons from the spill (Fig. 6). Statistical differences were highly significant for Benzo (a) Pyrenes (Mann-Whitney U = 30.8; P = 2.9 X 10^{-8}), for Pyrenes (Mann-Whitney U = 15.5; P = 8.3 X 10^{-5}), Fenanthrenes (Mann-Whitney U = 30.8; P = 2.9 X 10^{-8}) and Naphthalenes (Mann-Whitney U = 17.1; P = 3.5 X 10^{-5}). The concentration of all metabolites decreased for the second sampling, possibly because PAH metabolites were excreted from the fish.

Over all there was a predominance of low molecular weight metabolites (2 and 3 benzene rings) compared to high molecular weight (4 and more benzene rings), which is consistent and statistically correlated with the presence of more hydrocarbons of low molecular weight than high molecular weight found in the sediments. Krahn *et al.* (1992) reported the same relationship in areas where low molecular weight hydrocarbons were predominant, and hydrocarbons were supplied from petrogenic sources. High proportions of naphthalenes indicate exposure to petroleum products or crude oil, while low proportions of naphthalenes indicate contribution of pyrogenic hydrocarbons, such as the combustion of petroleum, wood, etc. (Krahn *et al.,* 1992; A as *et al.,* 2000; Gagnon and Holdway, 2002). McDonald *et al.* (1995) presented a very similar value of low and high molecular weight FACs in the fish *Nototheniagibberifrons* exposed to diesel oil from the Arctic to those reported in this study, indicating that the source of FACs is petrogenic.

Fig. 7 shows the significant positive correlation (Spearman

Rho = 0.49; P = 0.0011) between the metabolites of Naphthalenes and low molecular weight PAHs in liver, which is to be expected since naphthalenes are low molecular weight PAHs.

Tables 5 and 6 shows the result of biomarkers for cruises 1 and 3, respectively. Catalase activity showed a mean of 36.3 ± 6.8 pmol mg^{-1} protein in the first cruise and 53.5 ± 9.4 pmol mg^{-1} protein in the second cruise. The difference was highly significant (Mann-Whitney U = 22.7; P = 1.9 X 10^{-6}). EROD had a mean activity of 43.1 ± 10 pmol min^{-1} mg^{-1} protein in the first cruise, and 61.1 ± 14 pmol min^{-1} mg^{-1} protein in the second cruise, respectively. The difference was highly significant (Mann-Whitney U = 14.9; P = 0.00011). GST showed a mean activity of 33.2 ± 3.9 pmol mg^{-1} protein in the first and 64.2 ± 7.5 pmol mg^{-1} protein in the second cruise. The differences were highly significant (Mann-Whitney U = 30.8; P = 2.9 X 10^{-8}). Since hydrocarbon concentration decreased in the second cruise, but organo chlorine compounds increased, it seems that biomarkers were responding to these latter compounds. Low molecular weight PAHs showed a significant negative correlation with CAT activity (Spearman Rho = -0.64; P = 4.5 X 10^{-6}), which may

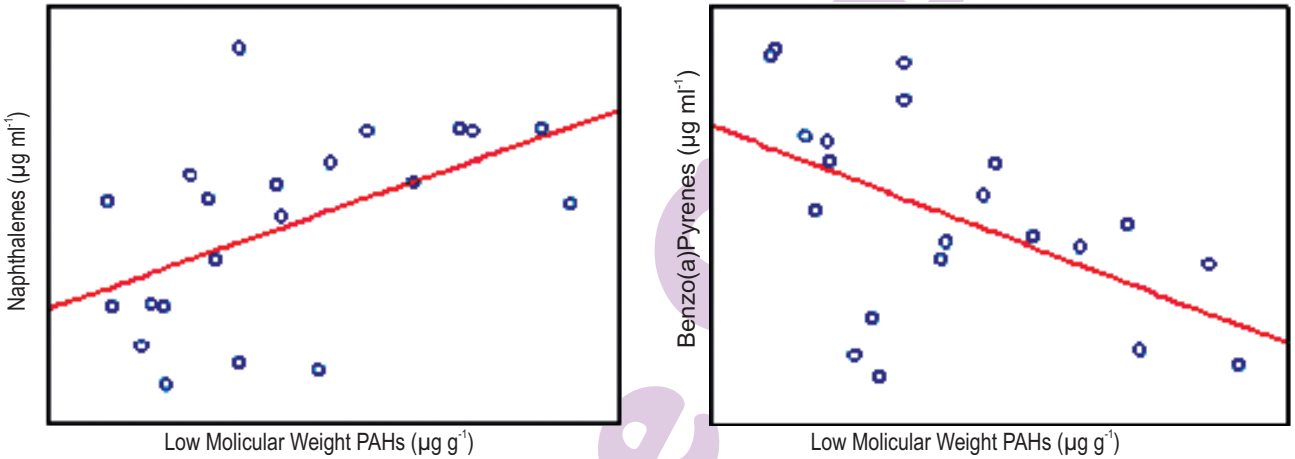

Fig. 7 : Correlation of Naphthalene metabolites with low molecular weight PAHs in liver (r=0.53) and of Benzo(a)Pyrene metabolites with low molecular weight PAHs (r=0.56) of *A. felis*.

Table 3 : Concentration of organochlorinated hydrocarbons (ng g^{-1} wet weight) in the liver of *Ariopsis felis* during first sampling

Station	Chloro benzenes	HCHs	Drines	DDTs	Chorinated pesticides	PCBs
1	8.140	7.098	65.157	14.263	59.176	73.178
2	16.832	3.516	15.726	6.443	34.184	30.914
3	0.556	1.243	30.704	4.134	35.309	18.922
4	2.994	ND	13.657	14.225	28.481	17.754
5	ND	2.889	11.047	8.771	18.068	19.797
6	ND	1.208	12.070	18.262	26.367	12.561
7	1.709	ND	10.348	5.336	15.643	9.974
8	0.958	6.839	7.716	24.347	17.462	25.599
9	2.264	ND	7.705	6.287	14.522	6.878
10	2.350	ND	7.330	3.676	10.674	4.111
11	17.244	ND	22.101	13.167	47.680	8.773
12	ND	ND	11.795	2.099	13.369	2.812
13	1.513	4.398	2.896	ND	3.973	11.109
14	0.110	6.804	ND	1.473	4.166	25.314
15	ND	ND	8.466	3.429	9.324	4.595
16	ND	0.693	34.814	ND	26.284	40.061
17	13.267	6.890	7.546	2.684	23.236	8.107
18	5.922	10.461	10.763	5.893	14.460	15.962
19	4.047	ND	ND	ND	4.047	13.465
20	ND	ND	1.278	3.187	2.871	21.415
21	12.423	1.091	6.006	3.874	9.056	8.131

HCHs = Hexachlorocyclohexanes, Drins = Aldrin + Endrin + Dieldrin, DDTs = DDT + DDD + DDE

Table 4 : Concentration of organochlorinated hydrocarbons (ng g^{-1} wet weight) in the lives of *Ariopsisf elis* during second sampling

Station	Chloro benzenes	HCHs	Drines	DDTs	Chorinated pesticides	PCBs
1	40.565	ND	21.720	11.089	54.919	27.789
2	46.995	ND	ND	5.702	52.698	5.061
3	13.189	ND	92.286	7.179	57.873	41.424
4	9.713	ND	44.425	20.257	55.077	42.266
5	10.066	ND	0.564	12.264	20.765	33.684
6	25.866	ND	31.477	11.089	50.283	32.579
7	13.311	1.683	21.337	7.057	31.020	13.592
8	13.405	3.869	24.356	7.890	51.138	14.030
9	42.762	ND	21.697	8.137	34.230	8.430
10	18.559	ND	ND	15.833	36.152	18.445
11	ND	88.881	3.819	6.518	123.497	6.304
12	22.208	32.009	8.402	15.273	59.490	53.436
13	24.637	12.853	0.602	14.374	38.162	32.049
14	13.708	3.824	1.650	ND	15.643	37.138
15	ND	1.258	ND	3.707	9.981	46.670
16	ND	37.559	ND	1.872	45.591	21.393
17	ND	48.181	ND	5.389	63.872	6.470
18	ND	5.638	4.236	ND	8.488	10.917
19	29.539	13.907	2.913	2.722	20.779	31.125
20	5.202	5.162	0.103	5.314	7.294	21.940
21	3.015	4.741	0.661	ND	5.866	14.388

HCHs = Hexachlorocyclohexanes, Drins = Aldrin + Endrin + Dieldrin, DDTs = DDT + DDD + DDE

Table 5 : Activities of biomarkers in the liver of *Ariopsis felis* during first sampling

Station	EROD*	GST**	CAT**
1	29.8	31.7	33.6
2	24.9	34.6	29.8
3	34.5	31.3	31.4
4	30.1	26.7	30.6
5	38.5	35.1	37.3
6	41.0	38.9	44.7
7	44.1	36.6	30.5
8	33.6	30.4	29.4
9	57.5	26.2	51.2
10	48.4	29.4	36.2
11	46.8	35.2	31.5
12	46.1	27.2	35.8
13	54.2	35.3	47.7
14	50.9	38.2	44.8
15	54.1	38.5	38.6
16	62.7	38.0	44.9
17	42.5	35.2	31.5
18	48.1	31.4	39.8
19	30.6	34.2	31.0
20	44.1	30.5	32.0
21	41.4	33.2	29.3

Table 6 : Activities of biomarkers in the liver of *Ariopsis felis* during in the second sampling

Station	EROD*	GST**	CAT**
1	64.0	61.7	48.6
2	47.3	64.6	44.8
3	40.0	51.3	41.4
4	38.0	46.7	40.7
5	47.0	55.1	50.3
6	74.8	68.9	59.7
7	80.5	66.6	40.5
8	61.4	60.4	44.4
9	73.8	61.2	66.3
10	63.7	64.4	51.2
11	62.5	71.2	50.9
12	42.9	67.5	49.2
13	61.0	66.6	50.4
14	53.4	68.3	46.5
15	82.9	76.5	61.7
16	89.4	78.0	62.7
17	51.8	60.5	64.6
18	57.0	57.7	67.1
19	63.3	66.8	70.4
20	65.3	68.7	52.1
21	62.7	66.4	59.8

*Units for EROD are pmol min^{-1} mg^{-1} protein; **Units for GST and CAT are pmol mg^{-1} protein.

be due to the metabolic breakdown of high molecular weight PAHs (Oliva *et al.,* 2010). A similar profile was observed in a correlation between Benzo (a) pyrene metabolites and CAT activity. A significant negative correlation (Spearman Rho = -0.51; P = 0.00054) between Naphthalene metabolites with CAT was found. Part of the variation recorded for CAT activity could be linked to modification of food availability, spawning period, kind of pollution or environmental factors as season and temperature (Sheehan and Power, 1999).

GST activity in this study showed a significant correlation with HCHs in liver (Spearman Rho = 0.33; P = 0.034). Hepatic GST activity appeared to be induced in rainbow trout exposed to other halogenated compounds, such as PCB 153 or DDE, but was not induced after 2,3,7,8-TCDD exposure. This observation indicates that this parameter may be a suitable biomarker for exposure to nonplanar PCBs and organochlorines (Machala *et al.,* 1998).GST activities reveal that the responses to pollutant mixtures are complex, since absence of a specific effect does not always reflect the absence of contamination.

As expected, EROD was highly correlated with low molecular weight PAHs ((Spearman Rho = -0.55; P = 0.00018), since low molecular weight PAHs are classic inducers of EROD (Krahm *et al.,* 1982; Machala *et al.,* 1998; Sheehan and Power, 1999; Barra *et al.,* 2001; Jewett *et al.,* 2002; Oliva *et al.,* 2010).

Hydrocarbon concentrations and particularly PAHs concentrations were higher in the first sampling, which together with their spatial distribution show that the spill is the source of these pollutants. The fact that level of organochlorinated pesticides and PCBs were higher in the second sampling, and their spatial distribution, showed that the source of these compounds was land-based. Positive and negative correlation between enzymatic activities and pollutants were observed and its explanation is hard to elucidate due to the its complex mixture present in the environment.

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