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Toxicity evaluation of produced formation waters after filtration treatment

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ABSTRACT

During the last years many authors have characterized the produced formation waters (PFW_s) with respect to chemical compounds and toxicity. Most of data are related to PFW_s collected on offshore platform after treatment process. The available results showed that the particulate phase had an influence on PFW toxicity. Assuming the toxicity of PFW_s treated on platform, the aim of this paper is to study the toxicity of these PFW_s after a further filtration treatment carried out in laboratory. For this purpose PFW_s were sampled from three natural gas platforms located in the Adriatic Sea (Italy) below treatment system. The eco-toxicological bioassays have been conducted on test-organisms belonging to different trophic levels such as bacteria, algae, crustaceans and fishes. The PFW_s resulted toxic according to an overall assessment obtained through the bioassays. Furthermore, it has been possible to identify the species that were more sensitive to the tested PFW_s, namely Tigriopus fulvus, Dicentrarchus labrax and Vibrio fischeri. Besides, a chemical characterization was reported related to the contaminants present in the PFW_s to go with eco-toxicological assessment. Barium, zinc and manganese showed the most concentrations among the metals and the lower molecular weight components were common among the organic compounds. Some differences among PFW_s were observed both for toxicity and chemical composition. The highest toxicity was recorded in PFW_s (PFW1 and PFW2) containing the highest concentrations of some metals (Ba, Mn and Zn) and/or BTEX.

Keywords: Adriatic Sea (Italy); Offshore Platforms; Natural Gas Production Fields; Produced Formation Waters; Toxicity Assessment; Bacterium (Vibrio Fischeri); Algae (Dunaliella Tertiolecta and Phaeodactylum Tricornutum); Crustaceans (Artemia Franciscana and Tigriopus Fulvus); Fish (Dicentrarchus Labrax); Chemical Characterization

1. INTRODUCTION

Produced formation water (PFW) is water naturally present in sedimentary formations from which oil and gas are mined. It is piped to the surface during the production process and may be discharged into the sea when the rejection is not possible. Before discharge, PFW_s are treated directly on platform to reduce oil and solid suspended content [1]. In spite of this treatment, PFW_s still include oil and particles.

In Italy, like in other Countries, the legislation binds to control the oil content in the PFW when they are discharged into the sea [2]; for this reason, the PFW characterization has been limited for several years to measurement of "oil in water", which means analysis of non polar aliphatic hydrocarbons. However, PFW contains a variety of compounds such as metals (i.e. barium, copper, zinc, and iron), volatile aromatic compounds (benzene, toluene, ethylbenzene, xylenes, called BTEX), semi-volatile compounds (naphthalene, phenanthrene, dibenzothiophene and their C1-C3 alkyl homologues), phenols alkylated up to C7, organic acids (C1-C6 compounds) and some additives of possible employment (i.e. diethylene glycol, called DEG) [3].

For this reason, during the last years many authors have characterized PFW_s with respect to these compounds (metals, aromatic and aliphatic hydrocarbons, phenols and additives) and to the toxicity of PFW_s.

The most data-gathering is related to PFW_s collected on platform after treatment process and untreated in laboratory later on [4-8]. Few data are referred to PFW_s collected on oil platforms below treatment system and filtered in laboratory subsequently [3,9].

There are scattered data about PFW_s originated from Italian offshore gas installations (Adriatic Sea): some

chemical analyses were made on seawaters and mussels near Adriatic platforms [10]; a methodological approach was proposed to study the environmental impact of oil and gas offshore platforms [11]; preliminary results were published on metal content and toxicity of PFW_s coming from gas Adriatic platforms [12-15].

In these studies, the PFW_s generally showed to be toxic and their toxicity was higher for samples unfiltered in laboratory. This effect was observed probably because of particulate phase influence. The effects of particulate could be to mechanic level (oral ingestion and digestion) and/or a chemical process (adsorption) [7,12].

The aim of this paper was to investigate the toxicity of PFW_s originated from three natural gas platforms in the Adriatic Sea (Italy). These PFW_s were collected below treatment system occurred on platforms and then filtered in laboratory. Their toxicity was evaluated using the integrated response of many species. The eco-toxicological battery included six species belonging to different trophic levels: a bacterium (*Vibrio fischeri)* representative of the debris chain, two algae species (*Dunaliella tertiolecta* and *Phaeodactylum tricornutum*) as primary producers, two crustaceans (*Artemia franciscana* and *Tigriopus fulvus*) as primary consumers and a fish (*Dicentrarchus labrax*) as representative of the highest trophic level (secondary consumer).

Besides, a chemical characterization of PFW_s was reported to go with eco-toxicological assessment. We investigated the metals, BTEX, PAHs and DEG. Metals and PAHs were analyzed both in filtered and particulate PFW_s, because these compounds are present above all as particles [7,16]. BTEX and DEG were recorded directly in the whole sample of PFW_s. This choice was necessary because BTEX are partitioned between gas and liquid phases, therefore the particulate matter does not influence their concentration; moreover they are volatile compounds and the filtration procedure causes loss of analytes. DEG was also analyzed in unfiltered sample because it is highly soluble in water, not much volatile and it does not tend toward absorption on particles [17].

Analytical methods by themselves were not able to give information on what happens when organisms are exposed to PFW_s, which concentrations are toxic and which is ecological impact of a PFW discharge. The use of bioassays, together with the classic chemical analyses, can contribute to the understanding of these aspects.

2. MATERIALS AND METHODS

2.1. Sampling and Sample Treatment of Produced Formation Water (PFW)

PFW samples were collected from three different gas platforms situated at about 20 km off the Adriatic coast (Pescara and Rimini, Italy): one of these (PFW1) was collected in October 2005 and the other two (PFW3 and PFW2) in June 2006.

On offshore platforms PFW is stored in a tank which empties when it is full load. All PFW_s were sampled from a tap located on the platform, which receives the PFW after this has had a physical-chemical treatment (depressurization, gravity separation techniques, activated carbon filtration). The physical-chemical parameters of PFW_s (salinity, pH, conductibility, ORP and oxygen dissolved) were measured in laboratory by multi-parameter probe (YSI, mod. 556MPS) (**Table 1**).

For the bioassays, about ten litres of PFW were immediately filtered (Millipore®, 0.45 µm) and refrigerated in polystyrene vessels at 4°C until their execution. The bioassays were carried out in 72 hours.

The PFW_s were stored in different containers according to type of chemical analyses. For the metals two litres were filtered (Millipore®, 0.45 µm), acidified with high purity nitric acid and refrigerated at 4°C until analysis; the filters were stored at -20°C. For BTEX 10 mL of PFW were stored in SPME dark vials (Varian S. p. A); a magnetic stirrer bar was inserted in each vial prior to sealing the vial by magnetic steel closures equipped with Teflon septa. The vials were refrigerated at 4°C and the samples were acidified at pH=2 with HCl and saturated with NaCl. For the PAHs analysis, one litre of PFW was immediately filtered (Millipore®, 0.45 µm) and, together with the filters, stored at 4°C. For the DEG analysis one litre of sample was collected in dark glass bottles, saturated with mercury chloride and refrigerated at 4°C to avoid photochemical and bacterial activity.

2.2. Bioassays

The bioassays were carried out on filtered samples, according to the methods reported in **Table 2** and summarized for each taxon as follows:

Bacteria: Controls and different concentrations for each PFW sampled (dilution ratio 1:2) were tested according to the Basic Protocol [18] and the method ISO [19] with

Table 1. Physical-chemical parameters of production formation water and information on platforms.

PFW	Salinity (PSU)	pH	Conductibility (mS/cm^2)	ORP	Oxygen dissolved (%)	Volume flux of PFW (mc/year)	Platform Installation (year)	Platform distance from coast (Km)	Water depth (m)
PFW1	34	7	51	-100	93	6000	1991	36	116
PFW2	37	7	56	-105	96	3000	1972	15	18
PFW3	37	8	55	-70	86	3000	1991	21	23

bacteria coming from freeze-dried SDI. PFW salinity was not adjusted prior to testing. The Software Microtox Omni™ v. 1.16 was utilized to calculate the EC_{50} and EC_{20} values (effect concentration of 50% and 20% respectively) and the Dunnett test was used to calculate the NOEC value (no observed effect concentration).

Algae: One control and some concentrations for each PFW sampled were tested according to the ISO method [20] with *Phaeodactylum tricornutum* strain 1090-1° and *Dunaliella tertiolecta* strain 13.86, obtained from the Plant Physiology Institute of Gottingen University (Germany). Algal growth medium was prepared with artificial seawater [20]; for *D. tertiolecta* nutrients, according to the IRSA-CNR method [21] and vitamins according to the ISO method [20] were added. The algal inoculum had an initial density of 10000 cells mL^{-1} ± 10% for *P. tricornutum* and 2000 cells mL^{-1}± 10% for *D. tertiolecta*. Regression analysis technique was performed for the determination of EC_{50} and EC_{20}; the Dunnett test was used to calculate the NOEC value.

Crustaceans: A control and some concentrations for each PFW sampled were tested with nauplii of *Artemia franciscana,* according to the APAT IRSA-CNR method [22], and with nauplii of *Tigriopus fulvus,* according to the ISO/FDIS method [23] as modified by Faraponova *et al.* [24,25]. Reference cysts of *A. franciscana* were obtained from the Quality Assurance Research Division U. S. Environmental Protection Agency (Cincinnati OH 45268, USA) or from the Laboratory for Biological Research in Aquatic Pollution, University of Ghent (Belgium). The eggs of *A. franciscana* were hatched in synthetic seawater and the nauplii were used within 48 hours of hatching [22]. Synchronized nauplii (24-48h) of *T. fulvus* were collected from a culture of two hundred females taken from a mass laboratory culture originated from the Italian coast (Calafuria, Livorno) and supplied with an algal mixture (*Tetroselmis suecica* and *Isochrysis galbana,* ratio 1:2). Probit analysis was performed for the determination of EC_{50} and EC_{15}, the Dunnett test was used to calculate the NOEC value.

Fish: One control and some concentrations for each PFW sampled were tested with juveniles of *Dicentrarchus labrax* (80 days old, length of 3.74±0.28 cm and weight of 0.48±0.08 g), according to the EPA [26] and OECD [27] methods. Organisms were supplied by the hatchery production plant ASA (Rome), stabled in synthetic seawater with salinity of 20±1 PSU for 15 days and fed with granulated food until 24 hours before the test. Probit analysis was performed for the determination of EC_{50} and EC_{15}, the Dunnett test was used to calculate the NOEC value.

The results were compared to a toxicity scale reported in **Table 3**. On the basis of this toxicity scale, the samples were classified as follows: 1) toxic $10 \leq EC_{50} < 100$, $20 \leq EC_{20} < 50$, effect percentage ≥50; 2) weakly toxic $EC_{50} > 100$, $EC_{20} > 50$, 20≤effect percentage<50; 3) no toxic EC_{50} no calculable, $EC_{20} > 100$, effect percentage 20.

2.3. Analysis of Metals

Determination of iron (Fe), copper (Cu), zinc (Zn), lead (Pb), chromium (Cr), manganese (Mn), nickel (Ni), barium (Ba), arsenic (As), cadmium (Cd) and mercury (Hg) was carried on both filtered and particulate samples. The filtered sample was directly analyzed. The metal dissolution of particulate fraction collected on the filters was conducted using microwave-assisted digestion (Milestone MLS Ethos TC) with 3 mL of HNO_3 and 9 mL of HCl. The metal concentrations were determined by a graphite furnace atomic absorption with Zeeman background correction technique (SpectrAA-220Z, Varian) and by coupled emission plasma ICP-OES (Liberty AX, Varian). For Hg analysis a Direct Mercury Analyzer (DMA-80, FKV) instrument was used (EPA Method [28]). All samples were run in triplicate. The quantification limits (LOQ) were: 0.0005 mg/l for Hg and Cd, 0.01 mg/L for the other metals.

2.4. Analysis of Organic Compounds

BTEX: The analyses were extracted and pre-concentrated by means Solid Phase Micro Extraction (SPME) using a stable flex fiber of divinilbenzene-carboxen-poly-dimethylsiloxane (film thickness: 55/30 µm) (Supelco®) by head space sampling. The analytical determinations of BTEX were carried out in unfiltered samples using a modified EPA method [29]. A gas chromatography coupled with mass spectrometry (GC HP 5790 Agilent Technologies® and MS 5973 Network Agilent Technologies®) were used. The method detection limits were 1 µg/L for benzene and 0.1 µg/L for toluene, ethyl benzene and xylenes.

PAHs: The analyses were investigated in filtered samples and on the particulate matter retained by the filter (Millipore®, 0.45 µm). The analyses were extracted by the filtered samples by means Solid Phase Extraction technique. The treatment of filters was carried out by ultrasonic extraction for 20 minutes with 10 mL of dichloromethane. Then, both the extraction phases were evaporated at 1-2 mL with a gentle nitrogen flow. Afterward, 1 mL of toluene was added and the residual dichloromethane was completely removed. All solvents were capillary GC grade supplied by Sigma-Aldrich. The analyses of PAHs were carried out in gas chromatography coupled with mass spectrometry (GC HP 5790® and MS 5973 Network Agilent Technologies). The LOQ was 1 µg/L for each analysis.

DEG: An extraction procedure of DEG was carried out with 2 mL SPE cartridges packed with 200 mg of EN V+stationary phase (International Sorbent Technology,

Table 2. Experimental conditions of bioassays (*for PFW1 were tested the concentrations: 10-20-40-80%).

	Vibrio fischeri	Phaeodactylum tricornutum	Dunaliella tertiolecta	Artemia franciscana	Tigriopus fulvus	Dicentrarchus labrax
Organisms/ Life stage	cells	unialgal culture	unialgal culture	nauplii 48h	nauplii 48h	juveniles
Strain/origin	commercially available	culture	culture	commercially available	culture	hatchery
Type of test	static	static	static	static	static	static
Time exposure	5-10-15min.	72h	72h	96h	96h	96h
Intensity of lux	Not required	7000	7000	3000-4000	500-1200	500-800
Photoperiod (L:D)	Not required	24:0	24:0	14:10	16:8	16:8
Dilution water/ control	synthetic seawater	synthetic seawater	synthetic seawater	synthetic seawater	synthetic sea-water	synthetic seawater
Salinity (PSU)	35	32	32	35	38	20
Temperature (°C)	15±1	20 ± 2	20 ± 2	25 ± 2	18 ± 2	20 ± 1
pH	8.0 – 8.2	8 ± 0.5	8 ± 0.5	6.5 – 8.5	8.0 ± 0.3	7.5 ± 0.5
Vessel	5mL	100mL	100mL	50 mL	culture plates 12 wells	2000 mL
Volume/well	1mL	25mL	25mL	40mL	3mL	1800mL
N°organisms/well	-	10000/mL	2000/mL	10	10	5
N°of concentrations (Range)	5 (6-11-22-45-90%)	5 (6-12-25-50-100%)*	5 (6-12-25-50-100%)	5 (6-12-25-50-100%)	5 (5-10-20-40-80%)	5 (6-12-25-50-100%)
N°of replicates	3	3	3	3	3-4	3
Feeding during the test	absent	absent	absent	*D. tertiolecta*	absent	absent
Endpoint/Effect	bioluminescence inhibition rate	growth inhibition rate	growth inhibition rate	immobilization rate	mortality rate; moult release rate	mortality rate
Expression of endpoint	EC_{50};EC_{20} NOEC	EC_{50};EC_{20} NOEC	EC_{50};EC_{20} NOEC	EC_{50};EC_{15} NOEC	EC_{50};EC_{15} NOEC	EC_{50};EC_{15} NOEC
Acceptability (effect control)	≤10%	>0.04/h	>0.04/h	≤10%	≤10% (mortality) ≤20% (moult release)	≤10%

Glamorgan, UK). The off-line solid-phase extraction/ pre-concentration technique was followed by a nano-scale flow injection/direct-electron ionization (EI) mass spectrometric analysis. A quadrupole mass spectrometer (Palo Alto, CA) was coupled with a Direct-Electron Ionization (EI) [30-32]. Using this approach, DEG was detected within a concentration of 31 µg/L [33].

Table 3. Toxicity scale used in this paper to classify the toxicity of Production Formation Water (PFW).

Effect (%)	EC50 (%)	EC20 or EC15 (%)	TOXICITY ASSESSMENT
%<20	n.c.	>100	no toxic
20≤%<50	>100	>50	weakly toxic
≥50	10≤%<100	20<%<50	toxic

3. RESULTS

3.1. Bioassays

The results of the eco-toxicological battery are reported in **Table 4**. The three filtered PFW_s resulted toxic according to the overall assessment related to bioassays. The species showed different sensitivity to PFW: the two microalgae and *Artemia franciscana* showed higher values of EC_{50} and EC_{20} (EC_{15}) than the other organisms, indicating weak toxicity. In particular, *Artemia* did not record toxicity for PFW1 (no efficient concentration was calculable). The other crustacean *T. fulvus* showed toxic effects for all PFW_s (PFW2>PFW1≥PFW3) related to both mortality and moult release. The sub-lethal effect was already observed at PFW concentrations that were not causing mortality of nauplii (20-80) %. The fish species *D. labrax* showed a toxic response similar to the one of *T. fulvus,* with a minimum value of EC_{50} equal to 15% and maximum value of 47% as follows: PFW2>PFW1≥

PFW3. The bacterium *V. fischeri* recorded lower toxicity than *T. fulvus* and *D. labrax* but yet a toxic effect was observed for all PFW_s (PFW1>PFW3≥PFW2).

3.2. Analysis of Metals

In filtered sample, Ba, Mn and Zn showed detectable concentrations. There were not significant differences between PFW2 and PFW3 exclusive of Ba and Mn. These two metals showed higher concentrations of one order in PFW2 than PFW3. In particulate sample, all metals were detectable except Pb and Hg. Ni, Cd and As registered detectable concentrations only in PFW3. Ba, Zn and Fe showed highest concentrations and some significant differences among the PFW_s analyzed. Ba concentration was higher of one order in PFW1 and PFW2 than PFW3; Zn was higher of two orders in PFW2 and PFW3 than PFW1; Fe was higher of one order in PFW3 than PFW1 and PFW2.

3.3. Analysis of Organic Compounds

As reported in introduction section, BTEX and DEG were analysed on unfiltered PFW_s. The analyses of the three platforms pointed out that the volatile organic compounds (BTEX) were detected at very high concentrations by the following ranking: PFW1 (1281.8 μg/L) >PFW3 (66.5 μg/L)>PFW2 (48.0 μg/L) (**Table 6**), showing for PFW1 values almost twenty times higher than ones of PFW2 and PFW3. The DEG showed concentrations ranging from 2400 to 13000 μg/L by the following ranking of the fields: PFW3>PFW2>PFW1 (**Table 6**). PAHs were investigated both in filtered and particulate sample but they were lower than LOQ (1 μg/L) in filtered PFW_s. In particulate sample PAHs showed the trend as follows: PFW1 (150.0 μg/L)>PFW3 (126.0 μg/L)>PFW2 (100.0 μg/L), with values about of the same magnitude order. The congeners with two and three rings recorded the following concentrations: 87 μg/L in PFW1 compared to 150 μg/L of the total PAHs content; 54 μg/L in PFW2 compared to 100 μg/L of total PAHs concentration, and 66 μg/L in PFW3 compared to 126 μg/L of total PAHs. The detected concentrations were of the same order of magnitude for all three PFW investigated.

4. DISCUSSIONS

The bioassays showed that the PFW_s were toxic even if filtered. EC_{50} values ranged between the minimum of 14.8 % and values higher than 100 %. Some of the samples did record no toxicity at the EC_{50} level observed

Table 4. Bioassay results related to three Italian Production Formation Waters and toxicity evaluation according to toxicity scale (n.c. not calculable; n.d. not determined).

Species (time of exposure, end point)	%	PFW1	PFW2	PFW3	PFW1	PFW2	PFW3
Vibrio fischeri (15 min. bioluminescence)	EC_{50}	67 (59-75)	> 90	> 90	toxic	toxic	toxic
	EC_{20}	20(18-23)	29(27-31)	28(25-31)			
	NOEC	-	-	-			
Phaeodactylum tricornutum (72h growth)	EC_{50}	> 80	> 100	> 100	weakly toxic	weakly toxic	weakly toxic
	EC_{20}	> 80	52 (46-58)	68 (24-111)			
	NOEC	40	25	25			
Dunaliella tertiolecta (72h growth)	EC_{50}	> 80	n.d.	n.d	weakly toxic	n. d	n. d.
	EC_{20}	> 80	n.d	n.d			
	NOEC	40	n.d	n.d			
Artemia franciscana (96h immobilization)	EC_{50}	> 100	> 100	> 100	no toxic	weakly toxic	weakly toxic
	EC_{15}	> 100	86 (55-259)	77 (42-462)			
	NOEC	n.c.	50	25			
Tigriopus fulvus (96h mortality)	EC_{50}	29	15	44	toxic	toxic	toxic
	EC_{15}	19	8	29			
	NOEC	10	5	20			
Tigriopus fulvus (96h moult release)	EC_{50}	23	25	77	toxic	toxic	toxic
	EC_{15}	n.c.	n.c.	n.c.			
	NOEC	10	5	10			
Dicentrarchus labrax (96h mortality)	EC_{50}	32 (27-39)	15 (n.c)	47 (n.c)	toxic	toxic	toxic
	EC_{15}	23 (15-28)	11 (n.c)	15 (n.c)			
	NOEC	13	6	6			

Table 5. Metal concentrations in filtered samples of two Produced Formation Waters, total suspended solids and metal concentrations in particulate samples of Produced Formation Water from three offshore gas platforms in the Adriatic Sea (Italy).

Parameters	Unit	PFW2	PFW3	PFW1	PFW2	PFW3
		FILTERED			PARTICULATE	
Total suspended solid	mg			177.42	367.05	398.10
Ba	mg/L	1.63	0.13	309.65	237.75	13.50
Cr	mg/L	<0.01	<0.01	0.12	1.34	0.45
Cu	mg/L	<0.01	<0.01	0.05	0.55	0.42
Mn	mg/L	0.34	0.04	0.84	1.05	0.77
Ni	mg/L	<0.01	<0.01	0.07	<0.01	0.35
Pb	mg/L	<0.01	<0.01	<0.01	<0.01	<0.01
Zn	mg/L	0.18	0.37	0.14	61.92	59.77
Cd	mg/L	<0.0005	<0.0005	<0.0005	<0.0005	0.62
Fe	mg/L	<0.10	<0.10	242.40	775.70	1335.00
As	mg/L	<0.01	<0.01	0.01	<0.01	9.25
Hg	mg/L	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005

Table 6. Organic compound concentrations in Produced Formation Water (PFW) samples from three offshore gas platforms in the Adriatic Sea (Italy).

Analytes		PFW1 (µg/L)	PFW2 (µg/L)	PFW3 (µg/L)
BTEX (unfiltered sample)	Benzene	256.0	10.4	20.4
	Toluene	50.6	14.1	12.1
	Ethyllbenzene	115.2	7.7	13.8
	Xilenes (o,m,p-xylene)	860.0	14.8	20.2
	BTEX	1281.8	47.0	66.5
DEG (unfiltered sample)	Diethylene glycol	2400	9600	13000
PAHs (particulate sample)	Naphtalene	14	8	11
	Acenaphtylene	21	15	17
	Acenaphthene	19	15	15
	Fluorene	16	4	6
	Phenanthrene	13	8	10
	Anthracene	4	4	7
	Fluorantrene	12	10	12
	Pyrene	10	7	10
	Benzo(a)anthracene	8	4	7
	Crysene	5	6	7
	Benzo(b)fluorantene	6	4	4
	Benzo(k)fluorantene	6	5	6
	Benzo(a)pyrene	5	3	3
	Dibenzo(a,h)anthracene	4	4	4
	Benzo(g,h,i)perylene	4	3	4
	Indenopyrene	3	< 1	3
	PAHs 2- 3 ring congeners	87	54	66
	PAHs	150	100	126

butdid show evidence of a toxic response at the EC_{20} level. The EC_{50} data lied within or were higher than the range (5.54-20.73%) previously reported for another filtered PFW originated from an Italian gas platform and assayed by bacteria and sea urchins [14]. Ours EC_{50} data also were higher than values related to unfiltered PFW_s coming from the North Sea platforms [4-8]. This shows that the filtered samples have generally lower toxicity than the untreated samples.

The difference of sensitivity among the species has been quite remarkable: *T. fulvus* and *D. labrax* showed the highest toxicity (EC_{50}<50%), followed by *V. fischeri* (EC_{20}<30%). *Artemia* and the two algae did not record significant toxic effect (EC_{20}>50%).

In addition to the toxicity assessment, we analyzed chemically PFW (metals, BTEX, PAHs and DEG). Appreciable concentrations of Ba, Mn and Zn were recorded in filtered samples while also high quantities of Fe were registered in particulate samples. Ba is probably related to drilling fluid residuals of PFW [7], Zn may be derived from corrosion or chipping of galvanized structures on the platform or in the oil/water separator system [34] and Fe could have natural origin or derive from corrosive events. The lower weight aromatic hydrocarbons (BTEX) were found by significant concentrations in liquid phase, while the PAHs were recorded only on particulate samples. DEG concentrations also were of milligram order in liquid phase but very low compared to the threshold of 3500 mg/L imposed by the PFW discharge authorization decrees issued by the Italian Ministry of the Environment.

An integrated evaluation of the eco-toxicological and chemical results showed that test-organisms were especially sensitive when exposed to PFW_s containing Ba, Mn, Zn and BTEX. *T. fulvus* and *D. labrax* showed the highest toxicity in PFW2 containing high concentrations of Ba, Mn and Zn. *V. fischeri* showed the highest toxic effect in PFW1 that recorded the highest quantities of BTEX. Nobody among the test-organisms indicated a preference for PFW3 containing the highest value of DEG. Moreover, DEG is not toxic alone but could determine co-solvent effects with other chemical compounds [35].

5. CONCLUSIONS

Our results indicate that a filtration treatment might diminish PFW toxicity. If a similar treatment was carried out on the platform before the PFW discharge, the ecological risk associated to the discharge would be probably reduced.

Besides, the results confirm the different sensitivity of test-organisms belonging to different trophic levels. Because PFW chemical composition is so variable for type and concentration of contaminants, test-organisms that are tolerant to a type of PFW could be sensitive to others. For this reason, we think that it is not correct to establish a single species to investigate the PFW_s but a battery of species should always be applied in order to have integrated responses.

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