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Extraction, Fractionation, and Toxicity Determination of Organic Contaminants in Sediment from Coastal Area Receiving Industrial Effluents in Kuwait

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The Shuaiba industrial area in Kuwait houses refineries and other petroleum-based industries therefore, contamination of effluents with oil-related pollutants is inevitable. Discharged wastewater contains diverse organic and non-organic compounds that have been examined in earlier studies (Shunbo et al. 1983; Shuaiba Area Authority 1990; Al-Muzaini et al. 1995; 1997). Among these contaminants designated polycyclic aromatic hydrocarbons (PAHs) are considered to be priority pollutants (Baumard et al. 1998). PAHs are hydrophobic in nature and tend to rapidly adsorb on suspended material and settle to constitute a reservoir in sediment (Karcher 1988). These chemicals can produce a variety of toxicological effects (Neff 1979; Marvin et al. 1999). Several studies suggested that extraction, fractionation, and bioassays are useful tools in detection of anthropogenic toxic and mutagenic compounds in sediment (Grifoll et al. 1990; Fernandez et al. 1992; Ho and Quinn 1993; Burgess et al. 1997). In this study, sediment samples were collected from heavily polluted area in Shuaiba coast (Beg et al. 2000) and their toxicity was ascertained by direct Microtox solid phase assay (Kwan and Dutka 1995). The samples were extracted with acetonitrile and fractionated by silica gel column chromatography (SGCC) and acid-base fractionation (Ho and Quinn 1993). In order to characterize the nature of the toxicant, each fraction was tested for toxicity by Microtox assay and also for the presence of PAHs by GCMS. The objective was to identify the causative organic fraction(s) in sediment from Shuaiba coast.

MATERIALS AND METHODS

Sediment samples were collected from the Shuaiba coast between Mina Al-Ahmadi Refinery and Harbor area. The area of sampling has been identified as a heavily polluted area in Shuaiba coast (Beg et al. 2000). The samples were collected by using a van Veen grab from three points, each five meters apart from the other two, forming a triangle, and pooled to constitute one sample for each site at three distances from the shore; ST-A,

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500 m (N29° 03.174; E 048° 09.271, depth 5 m), ST-B, 1 km (N29° 03.086; E 048° 09.638; depth 12 m), and ST-C, 1.5 km (N29° 02.811; E 048° 10.093; depth 19 m) on the transect line. Samples were transported to the laboratory in an ice box.

The procedure for organic solvent extraction and fractionation was as described by Ho and Quinn (1993), that was adopted from the method of extraction and fraction of Gardner et al. (1987). Fresh sediment samples (90 g) were extracted 3 times with 150 mL acetonitrile, and combined extracts were treated with equal volume of pentane in a separate funnel. A portion of the pentane extract (150 mL) was reduced and dried over anhydrous sodium sulfate, and exchanged with DMSO by evaporating the pentane to dryness under nitrogen over a warm water bath. A small volume of DMSO was added, and the mixture was left for 12 h to dissolve. The whole extract thus obtained in DMSO was tested by bioassay.

For silica gel column chromatography (SGCC) fractionation, 150 mL pentane extract was reduced under nitrogen and subjected to SGCC. Four fractions, one each in 45 mL pentane (F1); 36 mL 30:70 methylene chloride:pentane (F2), 36 mL 30:70 methanol:methylene chloride (F3), and 36 mL methanol (F4), were collected reduced under nitrogen and exchanged in DMSO for bioassay.

For acid-base fractionation, 150 mL pentane extract was partitioned with 30 x 3 mL of 0.2 N NaOH in organic (neutral and basic) and aqueous (acidic) fractions. The aqueous acidic fraction was treated with 3 mL 10N HCl, pH set to 2 and back extracted with pentane (30 mL x 3), reduced and exchanged into DMSO providing acidic fraction. The organic fraction was further partitioned with 0.1 N HCl (30 mL x 3) in aqueous basic and organic neutral fraction. The organic fraction was reduced and exchanged in DMSO to give neutral fraction. The aqueous basic fraction was treated with Na OH, pH adjusted to 13.5 and back extracted with pentane (30 mL x 3), the volume was reduced and exchanged into DMSO providing basic fraction. Thus, three fractions, acidic, basic and neutral were separated. The PAHs in different fractions were determined by monitoring selected ions for the target compounds in GCMS (Saeed et al. 1999) using external standards.

The toxicity was determined after transferring the fractions in dimethyl sulfoxide (DMSO) by Microtox assay (Microtox Manual 1992). The system records the light output of luminescent bacteria, *V. fischeri* before and after exposure to test samples and process the raw data statistically to produce reports on the toxicity (EC_{50} values) of the samples. Fractions toxicity was quantified by calculating the concentration of extract required for Microtox EC_{50} and converting it to equivalent quantity of sediment.

Table 1. Toxicity of sediment extracts, SGCC-fractions and acid-base fractions

Fractions	Microtox, EC_{50}, ≡ mg Sediment		
	ST-A	ST-B	ST-C
Whole Extract	2.22	0.63	0.51
Pentane	(1.34-3.69)	(0.11-3.50)	(0.17-1.49)
SGCC Fractionation			
Fraction 1	6.39	1.22	0.91
Pentane	(3.06-13.32)	(0.49-3.04)	(0.19-4.43)
Fraction II	218.10	82.30	162.70
Methylene chloride:	(196.0-242.7)	(38.9-174.4)	(22.0-1203.7)
Pentane			
Fraction III	153.00	165.00	64.60
Methylene chloride:	(101-229)	(93.3-291)	(41.2-101)
Methanol			
Fraction IV	3354.80	892.40	648.87
Methanol	(exceeds limit)	(exceeds limit)	(exceeds limit)
Acid-Base Fractionation			
Neutral Fraction	6.50	2.50	2.07
	(3.30-12.82)	(1.79-3.50)	(0.94-4.58)
Basic Fraction	915.00	825.00	715.00
	(exceeds limit)	(exceeds limit)	(exceeds limit)
Acidic Fraction	100.50	140.80	101.00
	(16.03-630)	(39.08-507)	(62.0-165)

( ) Values in parentheses represent 95% confidence range

RESULTS AND DISCUSSION

The data for Microtox EC_{50} of whole extracts, SGCC fractions and acid-base fractions are given in Table 1. The whole extracts showed extreme toxicity to Microtox. The four SGCC fractions showed toxicity of variable degree. Fraction 1 that contained mostly non-polar hydrocarbons showed consistently highest toxicity in all the sediment samples irrespective of distance from the shore line. Similarly, most polar fraction 4 was least toxic. The other two fractions, F2 and F3 were moderately toxic and their toxicity was comparable to each others. Among the three fractions, obtained on acid-base fractionation, neutral fraction contained most of the toxicity. From the toxicity point of view, the neutral fraction obtained in acid-base fractionation and the non-polar fraction obtained in SGCC fractionation showed similarity in response. Likewise, the basic fraction and most polar fraction IV were least toxic. It was difficult to determine

Table 2. PAHs in SGCC-fractions and acid-base neutral fraction of sediment extracts *

	PAHs, μg/kg Fresh Sediment					
	SGGC Fractions				Sum of	Neutral
	F-1	F-2	F-3	F-4	F1-F4	Fraction
Naphthalene	0.94	0.00	0.00	0.13	1.07	0.58
Methyl Naphthalenes	3.73	0.00	0.45	0.24	4.42	1.20
Dimethyl Naphthalenes	8.80	0.72	1.50	0.22	11.24	13.36
Trimethyl Naphthalenes	8.77	1.93	0.30	0.56	11.56	6.56
Total Naphtalenes	**22.24**	**2.65**	**2.25**	**1.15**	**28.29**	**21.67**
Fluorene	0.00	2.04	0.02	0.28	2.34	0.77
Methyl Fluorenes	0.00	0.00	0.00	0.00	0.00	0.00
Total Fluorenes	**0.00**	**2.04**	**0.02**	**0.28**	**2.34**	**0.77**
Dibenzothiaphene	0.53	0.93	0.00	0.00	1.46	0.61
Methyl Dibenzothiaphenes	1.30	1.91	0.18	0.50	3.89	4.63
Total Dibenzothiaphenes	**1.83**	**2.84**	**0.18**	**0.5**	**5.35**	**5.24**
Phenanthrene	0.00	36.76	0.07	0.37	37.2	5.43
Methyl Phenanthrenes	0.00	13.83	1.60	1.45	16.88	0.00
Dimethyl Phenanthrenes	0.00	11.87	0.30	0.21	12.38	11.50
Total Phenanthrenes	**0.00**	**62.46**	**1.97**	**2.03**	**66.46**	**16.93**
Fluoranthene	0.54	30.10	0.10	0.12	30.86	28.44
Pyrene	0.18	22.32	0.11	0.14	22.75	21.99
Benzo(a)anthracene+ Chrysene	0.00	30.28	0.39	0.15	30.82	35.83
Benzo(b&k)fluoranthene	0.00	24.53	0.33	0.00	24.86	29.15
Benzo(a)pryene	0.00	107	1.08	0.00	107.08	11.81
Total other PAHs	**0.72**	**214.23**	**2.01**	**0.41**	**216.37**	**127.22**
Total PAHs	24.79	284.22	6.43	4.37	318.81	171.83

*from ST-B; Neutral fraction on acid-base fractionation

EC_{50} in these two fractions. The acidic fraction, however, contained the compounds of moderate toxicity. The pattern of toxicity in SGCC fractions and acid-base fractions was the same in sediment from near shore line stations A or at increasing depths i.e., stations B and C. However, the sample from ST-A was less toxic than from ST-B and ST-C. This comparison is, however, relative between the three samples. Otherwise the solvent extracts of sediment exerted extreme toxicity to Microtox.

Table 3. Microtox EC_{50} with fresh sediment and solvent extract assay

Assay	Microtox EC_{50}, mg fresh sediment		
	ST-A	ST-B	ST-C
SPT-Fresh Sediment *	12.30	22.20	25.70
95% CL	6.9-21.8	14.1-34.8	17.9-37.0
Solvent Extract**	2.22	0.63	0.51
95% CL	1.34-3.69	0.11-3.50	0.17-1.49

Solid Phase Toxicity Assay with fresh sediment, ** Whole extract

If PAHs distribution in various fractions is considered (Table 2), the maximum content of total PAHs was found in F-2 of SGCC and neutral fraction of acid-base fractionations. Other fractions contained low PAHs content. The F-1, that contained mainly naphthalenes, was the most toxic fraction in Microtox acute toxicity assay because they are soluble above the toxic threshold. Acute toxicity of PAHs is based on the neutral narcosis mechanism of action and related to water solubility. The solubility limits the availability of the phenanthrenes and more so the higher molecular weight PAHs in F-2. The neutral fraction contained naphthalenes beside other higher molecular weight PAHs and its toxicity was comparable with F-1 suggesting that toxicity was the function of naphthalenes. Earlier, Schiewe et al. (1985) reported a significant correlation of aromatic hydrocarbons, naphthalenes and chlorinated hydrocarbon concentrations with Microtox toxicity data but subsequent studies (True and Heyward 1990) conducted in similar fashion revealed no such correlation between chemical concentrations and toxicity. Such discrepancies were possible because of sediment from different locations with different types and degrees of contamination. Ho and Quinn (1993) in their studies with four different sediment samples on SGCC fractionation found that the most toxic fraction was F-1 with two samples and F-2 with the other two samples. Our results showing highest toxicity in non polar fraction (F-1) are close to the findings of Schiewe et al. (1985) and matches with two sediment samples analyzed by Ho and Quinn (1993).

A comparison of the toxicity obtained with solvent extracts and the toxicity obtained in solid phase direct sediment assay is given in Table 3. The data shows that in terms of sediment weight, the toxicity was relatively more in solvent extracts than in whole sediment. In the direct sediment assay, the sediment is suspended in aqueous Microtox diluent and the hydrophobic nature of organic compounds possibly prevented the bioavailability of all the compounds to exert their effects or other compounds present in the whole sediment exerted antagonistic effects. The process of aging also reduces the rate of desorption that correlates with a net reduction in

bioavailability of the contaminants (Tang and Alexander 1999). In aqueous elutriation studies reported elswhere (Beg et al. 2000), the toxicity was not found in sea water elutriates even after 24h of vigorous shaking of sediment in sea water. It was also found that aqueous elutriates contained only traces of TPH and trace elements. Whereas, solvent extraction brought all the organic compounds in the extraction medium and collectively assayed in whole extracts toxicity assay. At this point, it is important to mention that by solvent extraction assay only organic compounds are accounted for toxicity.

The toxicity data with whole sediment and solvent extracts also revealed that whole sediment Microtox assay was efficient in identifying the toxic nature of a sediment sample with precision. Fractionation and separation of the group of compounds in different fractions also indicated that all the solvent extractable contaminants of sediment were not toxic in nature. The SGCC fractions corresponds to different chemical classes, therefore, their toxicity was also different (Gardner 1987). The stability of some of these compounds was found to be affected by the changes in sediment environment, but most of the chemical compounds found in sediment were fairly stable (Ho and Quinn 1993).

It is concluded that exhaustive organic solvent extraction, SGCC-fractionation revealed that all the organic contaminants in sediment were not of toxic nature. Fraction 1 that contained mostly non-polar hydrocarbons showed consistently highest toxicity in all the sediment samples irrespective of distance from the shoreline. Similarly, most polar fraction 4 was least toxic. The other two fractions, F-2 containing most of the PAHs and F3 containing low levels of PAHs were moderately toxic and their toxicity was comparable to each other. From the toxicity point of view, the neutral fraction obtained in acid-base fractionation and the non polar fraction obtained in SGCC fractionation showed similarity in response. Likewise, basic fraction and most polar fraction IV were least toxic. The toxicity data revealed partitioning of toxicants in various fraction contrary to any single fraction and some of the toxic components were different in nature that were not detected. Therefore, more exhaustive analysis is needed to define the exact nature of causative chemical contaminants in sediment and their toxic effects, thereby, better regulation of their discharges is possible.

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