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Evaluation of Modifications of the Simple Spectrofluorometry Method for Estimating Petroleum Hydrocarbon Levels in Sea Water

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Various analytical techniques are used to measure the concentrations of petroleum hydrocarbons in environment samples. Gas chromatography, mass spectrometry, high performance liquid chromatography and thin-layer chromatography are the most common. These procedures are tedious and, with the exception of thin-layer chromatography, require expensive and complicated equipments (Albaiges et al., 1983). In some cases, a simpler though less precise and specific, technique using inexpensive equipment to monitor a large number of sea water samples, might be more desirable. This idea was realized in the assessment of the state of pollution of the Mediterranean Sea by petroleum hydrocarbons through the Med Pol I Project where the pollution of sea water with oil (dissolved and/or dispersed in sea water) was measured by spectrofluorometry (UNEP/IOC, 1988).

The use of spectrofluorometry for the estimation of pollution sea water by petroleum hydrocarbons has been published in numerous papers from analytic standpoint and applied for monitoring purposes (Lewy and Walton, 1973; Law et al., 1987; Ehrhardt and Petrick, 1989; Gonzalez-Davila et al., 1989). Application of the standard additions method for improving the estimation of petroleum hydrocarbons in sea water, sediments and biological samples has also been described (Picer and Hocenski, 1983; Picer, 1985a; Picer, 1985b). The aim of this paper is to provide an analytical evaluation of three modifications of simple the spectrofluorometry method applied on numerous sea water samples collected from various areas of the open and coastal waters of the Adriatic Sea.

MATERIALS AND METHODS

Water samples (2.8 dm^3) were collected from a boat, at a depth of 1 m, with a sampler, as recommended by IOC (IOC, 1984). The extraction of water sample was performed using 3 dm^3 separating funnel after the addition of 100 cm^3 fluorescent grade n-hexane or petroleum ether, with boiling points between 323

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and 333 K, by shaking 2 minutes. After separating the solvent phase from the water, water samples were extracted again in the same funnel with 70 cm^3 solvent. Extracts were put together and dried by passing them through a column of Na_2SO_4 anh. and evaporated to 5 cm^3 using a rotatory evaporator. The fluorescency of the extracts was measured in a 1 cm quartz cell using the Turner spectrofluorometer, Model 430 at 290 nm excitation. The emission was recorded at 350 and 390 nm. The fluorescency and concentrations of petroleum hydrocarbons measured and calculated after extraction of water samples and drying, and concentrated to appropriate volume are called "before cleaning". After fluorescency measuring, the extract is concentrated in a water bath at 313 K to 1 cm^3, and applied to a 4 m I.D. column holding 2 g alumina. The alumina is prepared by heating activated alumina (Brockman activity I) at 775 K for 12 hours and partly deactivated by adding 5 per cent distilled water by weight. Eluation was performed with 15 cm^3 30% (v/v) benzene (fluorescence grade) in distilled n-pentane. The eluate was then concentrated in a water bath to 5 cm^3 and fluorescency was measured again. Fluorescency and concentrations of petroleum hydrocarbons calculated after alumina cleaning of extract are called "after alumina cleaning" modifications.

Known volume (most frequently 50% of the total volume of alumina cleaned extract) is dilluted by the addition n-hexane until the fluorescency of the sample extract is similar to the fluorescency of Kuwait crude oil concentration (between 1 and 4 $\mu g\ cm^{-3}$ in n-hexane). Fluorescency and concentrations of petroleum hydrocarbons in sea water samples calculated after the alumina cleaning of the extract and appropriate dilution are called "after dilution" modifications.

To evaluate and accordingly correct the quenching is possible by using the standard additions method. It means that after the measurement of fluorescency of the cleaned extract a known amount of Kuwait crude oil solution is added to it. The obtained values of quenching are presented as the correction factor according to the relationship:

$$\text{Correction factor} = \frac{\text{Fs(before addition)}}{\text{Fs(after addition)}} = \frac{\text{Fs}}{\text{(Fa + S)measured - Fa}}$$

Fs - is the fluorescency of the standard solution of Kuwait crude oil,
Fa - is the fluorescency of the sample after alumina cleaning
(Fa + S)measured - is the fluorescency of the sample after the addition of a standard solution of Kuwait crude oil.

Concentrations of petroleum hydrocarbons calculated after the alumina cleaning of extract and standard additions are called "standard addition" modifications.

RESULTS AND DISCUSSION

Table 1 presents basic statistical data the concentrations of petroleum hydrocarbons (expressed in equivalents of Kuwait crude oil) in seawater samples collected from coastal and open waters of the Adriatic Sea. The extract treatment and concentration calculation procedures are defined on the first row. At the beginning of Table 1, the results of the statistical calculations for all the data are presented. Separate statistical calculations for samples divided in three concentration ranges (obtained by using standard additions modification) are also presented in Table 1.

The average, median, and geometric mean measure the central tendency of the data, while the standard deviation, minimum and maximum, lower quartile, upper quartile and interquartile range show the data spread. The skewness coefficient measures the asymmetricality of the data distribution. As seen, all concentrations show positive values of skewness. This means that the upper tail of the distribution curve is longer than the lower tail. Kurtosis coefficients reveal the flatness or steepness of the data distribution with respect to a Gausian or normal distribution. For normal distribution, the kurtosis coefficient is 3.0. For the data presented, the kurtosis coefficients are all higher than 3.0 which means that the distribution curves are steep at the center or have relatively long tails. The standardized coefficients of the skewness and kurtosis test for significant deviations from normal distribution. When using relatively large samples, the standardized coefficient is approximately unit normal. When the values for the standardized coefficient are outside the range of -2.0 to +2.0, it means that the data may depart significantly from normal distribution. As seen, the data presented do depart significantly from normal distribution for investigated pollutant concentrations obtained with all modifications of the procedure used. However after the division of all the data into three concentration ranges, distribution of data in ranges lower than 2.0 μg dm^{-3} and between 2.0 and 5.0 μg dm^{-3} relatively fit normal distribution. This signifies that the median, and geometric means better indicate the central tendency of the investigated petroleum hydrocarbon concentrations in the all samples and samples with concentrations which are higher than 5.0 μg dm^{-3}. Because the data depart significantly from normal distribution, the arithmetic means of the data in mentioned groups of samples differ significantly in comparison with the median and geometric means.

Linear regression analyses of the values of fluorescency and petroleum hydrocarbon concentrations in seawater samples obtained using different modification of analytical procedure are presented on Table 2. Significant positive correlation coefficients are obtained for all comparisons, mostly at $p<0.01$, but the height of the correlation coefficient, standard error of estimation and R-squared differ significantly when comparing different modifications of the method and concentrations ranges.

Table 1. Basic statistical data of polyaromatic hydrocarbon concentrations (Kuwait oil equivalents) in sea water samples collected from the Adriatic sea open and coastal waters, obtained by using several analytical procedures

Concentration range(μg dm^{-3})	Analytical procedure	b.c.	a.c.	dil.	s.a.
All data	Number of samples	65	64	63	63
	Average	6.0	8.3	15.1	16.6
	Median	3.0	4.0	4.1	4.0
	Geometric mean	2.8	3.1	3.6	3.6
	Standard deviation	7.2	15.5	53.3	63.2
	Minimum	0.5	0.5	0.5	0.5
	Maximum	30.0	116.0	430.2	510.0
	Lower quartile	1.1	0.8	0.8	1.0
	Upper quartile	8.0	12.0	13.0	13.0
	Interquartile range	6.9	5.5	12.2	12.0
	Skewness	1.842	5.499	7.583	7.644
	Standardized skewness	6.064	18.099	4.960	25.227
	Kurtosis	2.692	36.845	59.674	60.570
	Standardized kurtosis	4.431	60.635	98.206	99.680
Lower than 2.0	Number of samples	24	24	23	23
	Average	0.9	0.8	0.8	0.8
	Median	0.7	0.7	0.6	0.7
	Geometric mean	0.7	0.6	0.6	0.6
	Skewness	0.570	0.779	1.840	0.680
	Standardized skewness	1.140	1.558	3.680	1.377
	Kurtosis	-1.028	-0.617	4.248	-0.764
	Standardized kurtosis	-1.075	-0.735	4.231	-0.670
From 2.0 to 5.0	Number of samples	10	9	9	9
	Average	2.7	2.9	2.8	2.7
	Median	2.0	3.0	2.9	2.1
	Geometric mean	2.6	2.8	2.7	2.6
	Skewness	0.340	0.269	0.279	0.902
	Standardized skewness	0.516	0.347	0.360	1.164
	Kurtosis	-0.433	-1.497	-1.486	-0.971
	Standardized kurtosis	-0.280	-0.966	-0.959	-0.627
Higher than 5.0	Number of samples	31	31	31	31
	Average	11.0	15.9	30.2	33.4
	Median	8.0	12.0	13.0	14.0
	Geometric mean	8.8	11.9	15.9	16.2
	Skewness	1.154	4.493	5.393	5.414
	Standardized skewness	2.625	10.213	12.258	12.306
	Kurtosis	0.023	22.484	29.635	29.799
	Standardized kurtosis	0.027	25.553	33.681	33.868

b.c. = Before alumina cleaning
a.c. = After alumina cleaning
dil. = Diluted alumina eluant
s.a. = Standard addition

Table 2. Results of the linear regression analyses of the values of polyaromatic hydrocarbons fluorescency and Kuwait oil equivalent concentrations in sea water samples obtained by using several analytical procedures (Fluorescency at 290/350 and 290/390 exc./em. lines)

Investigation conc. range	The first variable	The second variable	Number of pairs	Correlat. coeffic.	Signific. level	Stnd.error of est.	R-squared (%)
All data	290/350 b.c.	290/350 a.c	63	0.974	$p<0.01$	33.898	94.95
All data	290/390 b.c.	290/390 a.c	63	0.943	$p<0.01$	23.149	89.02
All data	Conc. b.c.	Conc. a.c.	63	0.733	$p<0.01$	10.646	53.69
All data	Conc. b.c.	Conc. dil.	62	0.557	$p<0.01$	44.659	30.99
All data	Conc. s.a.	Conc. b.c.	62	0.540	$p<0.01$	53.609	29.18
All data	Conc. s.a.	Conc. dil.	62	0.987	$p<0.01$	2.454	98.23
Lower than 2.0 $\mu g\ dm^{-3}$	Conc. b.c.	Conc. a.c.	23	0.836	$p<0.01$	0.302	69.83
	Conc. b.c.	Conc. dil.	23	0.779	$p<0.01$	0.422	60.67
	Conc. s.a.	Conc. b.c.	23	0.762	$p<0.01$	0.321	58.10
From 2.0 to 5.0 $\mu g\ dm^{-3}$	Conc. b.c.	Conc. a.c.	9	0.869	$p<0.01$	0.457	75.52
	Conc. b.c.	Conc. dil.	9	0.880	$p<0.01$	0.436	77.53
	Conc. s.a.	Conc. b.c.	9	0.689	$0.05>p>0.01$	0.696	47.54
Higher than 5.0 $\mu g\ dm^{-3}$	Conc. b.c.	Conc. a.c.	30	0.636	$p<0.01$	15.667	40.40
	Conc. b.c.	Conc. dil.	29	0.525	$p<0.01$	64.863	27.61
	Conc. s.a.	Conc. b.c.	29	0.515	$p<0.01$	77.767	26.59
	Conc. s.a.	Conc. dil.	29	0.989	$p<0.01$	2.342	97.45

b.c. = Before alumina cleaning
a.c. = After alumina cleaning
dil. = Diluted alumina eluant
s.a. = Standard addition

Figure 1 presents scatter plots of the concentrations of petroleum hydrocarbons in the sea water samples obtained before alumina cleaning of the solvent extract (independent variable) and after alumina cleaning or after dilution of the alumina eluant (dependent variable) with estimated linear regression line and two pairs of dotted lines representing 95% confidence and prediction limits. Scatter plots are given for total range of concentrations and for concentrations lower than 2.0 $\mu g\ dm^{-3}$ and for the range of concentrations higher than 5.0 $\mu g\ dm^{-3}$. Figure 2 presents scatter plots of the concentrations of petroleum hydrocarbons in the sea water samples obtained using the standard addition method (independent variable) and after the dilution of the alumina eluant modification (dependent variable). Scatter plots are given for the total range of concentrations and separately for three concentration ranges.

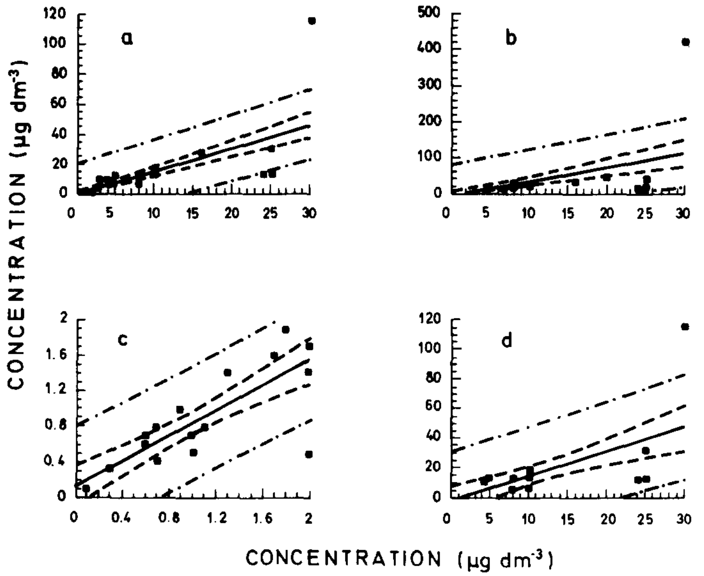

Figure 1. Scatter plots of the concentrations of petroleum hydrocarbons in the sea water samples obtained before alumina cleaning of the solvent extract (independent variable) and after alumina cleaning (depended variable - figures a and c) or after the dilution of the alumina eluant (dependent variable - figures b and d). Figures a and c all data; Fig c - conc. range lower than 2.0 μg dm^{-3}; Fig d - conc. range higher than 5 μg dm^{-3}.

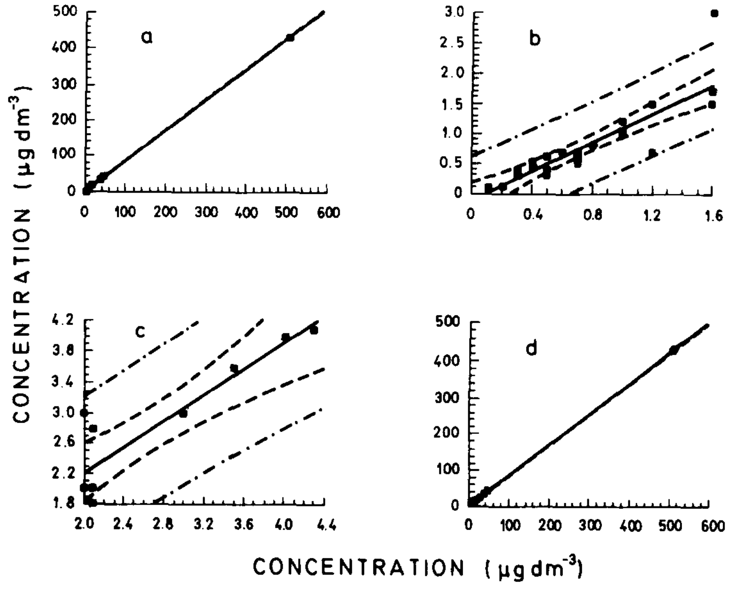

Figure 2. Scatter plots of the concentrations of petroleum hydrocarbons in the sea water samples obtained using the standard addition (independent variable) and after the dilution of the alumina eluant (dependent variable). Fig a - all data; Fig b - conc. range lower than 2.0 μgdm^{-3}; Fig c - conc. range between 2.0 and 5.0 μg dm^{-3}; Fig d - conc. range higher than 5 μg dm^{-3}.

Table 3 presents the results of a one-way analysis of the variance of petroleum hydrocarbon concentrations in sea water samples obtained with modifications of simple fluorescency methodology, depending upon the applied methodology modifications. Calculations are made using 95% confidence intervals. Figure 3 presents the means of the concentrations of petroleum hydrocarbons in sea water samples, the intervals for the means using 95% confidence intervals for all the data and for three concentration ranges separately. There are no statistically significant differences between the concentrations of the petroleum hydrocarbons in the investigated sea water samples obtained with all three modifications in samples concentrations lower than 5 ppb but for higher concentration ranges dilution and standard additions modification show higher results in comparison with the simple extraction and alumina cleaning modification.

Table 3. One way analysis of variance of polyaromatic hydrocarbons concentrations in sea water samples depending upon applied methodology

Response variable	Variance homogenity		Homogenous groups (applied methodology)	Significans level for mean difference
	Hi2	p		
All data	2.8961	$0.30<p<0.50$	All groups are homogenous	0.4056
Concentration Lower than 2.0 $\mu g\ dm^{-3}$	1.0301	$0.70<p<0.80$	All groups are homogenous	0.7644
Concentration From 2.0 to 5.0 $\mu g\ dm^{-3}$	1.0745	$0.50<p<0.70$	All groups are homogenous	0.9205
Concentration Higher than 5.0 $\mu g\ dm^{-3}$	3.4316	$0.20<p<0.30$	All groups are homogenous	0.3756

Analysis of petroleum hydrocarbons in sea water samples from the eastern Adriatic coastal and open waters using the simple solvent extraction fluorescency method and three modifications of there of led the following conclusions:

Linear regression analyses of the values of fluorescency and petroleum hydrocarbon concentrations in sea water samples obtained using different modification of the simple extraction method show statistically significant positive correlation coefficients for all comparisons, mostly at $p<0.01$. The

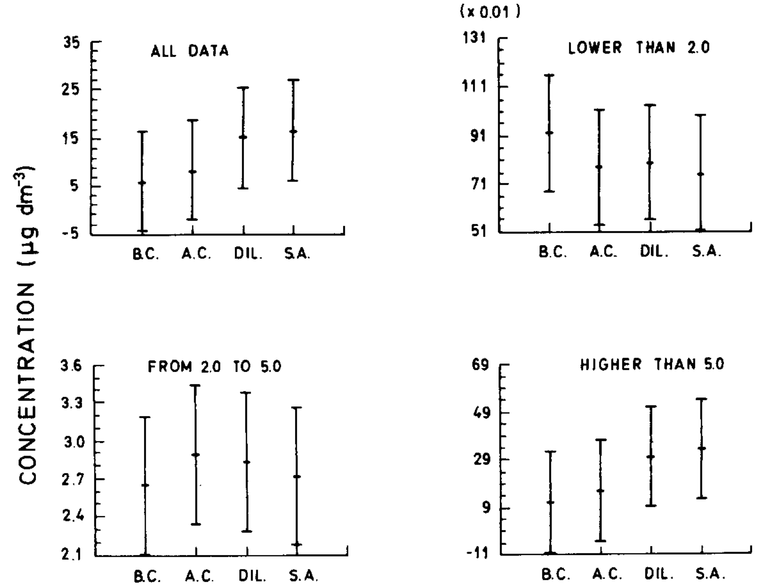

Figure 3. The means with the intervals for the means using 95% confidence intervals of petroleum hydrocarbons of concentrations in sea water samples obtained using the simple extraction method and its three modifiactions, for all the data and for three concentration ranges separately.

height of the correlation coefficients, standard error of estimation and R-squared differ significantly when comparing different modifications of the method and concentrations ranges.

There are no statistically significant differences between the concentrations of petroleum hydrocarbons in the investigated sea water samples obtained with all three modifications in samples with concentrations lower than 5 ppb. But for higher concentration ranges, dilution and standard additions modifications yield higher results than simple extraction and alumina cleaning modifications.

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REFERENCES

Albaiges J., Frei R.W. and Merian E. (1983) Chemistry and analysis of hydrocarbons in the environment, Gordon and Breach Sci. Pub., New York, London, Paris, Montreaux, Tokyo, p. 314.

Ehrhardt M. and Petrick G. (1989) Relative concentrations of dissolved/dispersed fossil fuel residues in Mediterranean surface waters as measured by UV fluorescence, Mar Pollut Bull 20:560-565.

Gonzalez-Davila M., Perez-Pena J., Santana-Casiano M. and Hernandez-Brito J. (1989) Dissolved/dispersed hydrocarbons in sea water determined by infrared and fluorescence spectroscopy, Int J Environ Anal Chem 37:277-285.

IOC (1984) Manual for monitoring oil and dissolved/dispersed petroleum hydrocarbons in marine waters and on beaches, Manuals and Guides 13, UNESCO, Paris, 1984 p. 35.

Law R.J., Marchand M., Dahlmann G. and Fileman T.W. (1987) Results of two bilateral comparisons of the determination of hydrocarbon concentrations in coastal seawater by fluorescence spectroscopy. Mar Pollut Bull 18:486-489.

Levy E.M. and Walton A. (1973) Dispersed and particulate petroleum residues in the Gulf of St. Lawrence J Fish Res Bd Can 30:261-267.

Picer M. (1985a), Dissolved, dispersed petroleum hydrocarbons in the waters of the Krka river estuary and Kornati archipelago, J Etud Pollut CIESM 7:567-571.

Picer M. (1985b), Improvement in the estimation of polyaromatic hydrocarbons in seawater, marine sediments and organisms by spectrofluorometry by using standard additions method, Proc. of the Fourth European Symp. "Organic micropollutants in aquatic environment", Vienna, 1985, Dordrecht, Boston, Lancester: D. Reidel Pub. Co., 1985, 114-117.

Picer M. and Hocenski V. (1982), Improvement in the estimation of petroleum hydrocarbons in marine sediments and organisms by spectrofluorometry by using the standard additions method. J Etud Pollut CIESM 6:177-182.

UNEP/IOC (1988) Assesment of the state of pollution of the Mediterranean Sea by petroleum hydrocarbons. MAP Technical Reports Series No. 19. UNEP, Athens, 1988, p.130.

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