DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS IN WATER SAMPLE OF THE LAGOS LAGOON

BY

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ABSTRACT

Lagos Lagoon is among the most polluted water bodies in sub-Saharan Africa. It is extensively polluted at the harbour by seepages from oil discharge terminals, spent lubricating oil from the adjoining drainage system, domestic and industrial effluents in the highly polluted Lagos metropolis, and occasional spillage from the Niger Delta where crude oil exploration is widely done. Analysis for the presence of 16 priority polynuclear aromatic hydrocarbons (PAH) were carried in water samples of the Lagos lagoon. The determination and quantification of the PAH's were done by means of soxtec extractor followed by GC-FID. From the studies carried out on the water samples, chrysene 20.33 ug/ml, anthracene 19.59 ug/ml, benzo (a) anthracene 17.15 ug/ml and pyrene 14.92 ug/ml, were detected in high concentration, while benzo (b) flouranthene showed a low concentration of 0.88 ug/ml.

INTRODUCTION

Polynuclear aromatic hydrocarbons (PAH) are a class of diverse organic compounds containing two or more fused aromatic rings of carbon and hydrogen atoms [1]. They are well known ubiquitous environemntal contaminants at low concentration found in air, water and soil, and are always found as a mixture of individual compounds [2], included in the EC and EPA priority pollutant list because of their mutagenic and carcinogenic properties [3]. PAH originate from diverse sources such as tobacco smoke, engine exhaust, petroleum distillate and coal derived products, with combusion sources predominating [4]. Beinb toxic and ubiquitous PAH pose a great danger to the environment. They are the largest class of chemical compounds known to be cancer causing agents [5]. Several works have been done on the carcinogenicity of these compounds [6-10], many of these have been found to be positive, some while not cancer causing may act as synergists [11]. PAH are usually found in the environment as a mixture of many compounds.

The concentration of individual PAH's in surface and coastal waters are generally around 50ng/liter [12]; therefore any concentration above this level indicates contamination. Due to the fact that PAH's absorb strongly to the soil organic matter, leaching from soil to groundwater is negligible [13]. Water bodies are usually contaminated by direct introduction of PAH's from various sources such as drilling operations and petroleum exploitation and production, transportation activities, coastal and riverine imputs, and combustion of all forms of fossil fuels [14-19]. PAH's in the environment are usually derived from anthropogenic sources.

Lagos lagoon is a good example of a highly polluted water body. It receives enormous amount of hydrocarbon arising from a wide variety of sources but mainly by the extensive crude oil exploration, import and export that take place in the area. The highly polluted industrial waste product and bad sewage system which contribute a lot to the level of pollution in the lagoon. This work has been necessitated by the fact that the level of PAH present in the lagoon is needed to help determine the level of treatment required before it is supplied for domestic use.

The Lagos lagoon is a wide expanse of estuarine water extending from the Lagos harbor to the Niger Delta in south west Nigeria and in fact the largest lagoon in the Gulf of Guinea [20]. It is located between longitude 3° 23′ and 3° 40′ E and between latitude 6° 22′ and 6° 48′ N as shown in figure 1. It borders the forest belts and receives a number of rivers (Yelwa, Ogun, Ona and Oshun) draining more than 103,626 square kilometers of the country Nigeria [21]. It is a highly urbanized brackish ecosystem impacted mainly by municipal and industrial activities that have significantly increased in the past decades. The Lagos lagoon is also very much impacted by crude oil due to great activities of import and export of petroleum related products [22]. The choice of the location was influenced by the fact that it is one of the most impacted environments in Nigeria

Figure 1: Map of the Lagos lagoon showing the sampling site

MATERIAL AND METHOD

Collection of samples

Water sample was collected from the Victoria Island end of the Lagos lagoon. (10x500ml) purified glass bottle was filled with water from this location and stored at low temperature until ready for use.

Reagents

All chemicals and reagents were of analytical grade and of highest purity possible. Dichloromethane and n-Hexane used for the extraction were obtained from Koch Light Laboratories Ltd England. Silica gel used in the cleaning up of the extract was supplied by BDH Laboratories England. PAH standard mixture PM-525a (EA EPA method 525 PAH mixture) was obtained from Ultra Scientific, North Kingstown. The mixture contains 12 PAHs which are acenephthylene, anthracene, benz(a)anthracene, benzo(k)flouranthene, benzo(b)flouranthene, benzo(g,h,i)perylene, benzo(a)pyrene, chrysene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene, phenanthrane, and pyrene, each at 100ug/ml in acetone.

Apparatus

The HP 6890 GC used was equipped with flame ionization detector, a pre-fractionator, a HP 6890 series autosampler, a restrictor column (5 A mole sieve, 80/80 mesh 1/8" x 1' SS), an analytical column (25m x 320um x 0.52um methyl siloxane capillary column) and a split injector. A tudor Vap II concentration workstation used for concentrating the extract was supplied by Zymark USA. The oven was supplied by Linderg. A silli-Therm heating module supplied by Pierce Chemical Company was also used. High precision weighing balance obtained from Ohaus Corporation was used in all the necessary weighing during this work. The carrier gas for the GC was helium and it was supplied by Air Liquide Nigeria. The same company also supplied the hydrogen gas used. Kimax separating columns were used for sample clean up.

Sample preparation

A centrifuge assisted liquid-liquid extraction was performed. 100ml of the water sample and 100ml of n-hexane were added in a centrifuge bottle and centrifuged at 3000rpm for 15 minutes. The organic phase was removed and 100ml of n-hexane was again added and the process repeated. All the organic layers were combined and the extract concentrated in Tudor Vap Concentration Workstation until a volume of 0.5ml was obtained.

Analysis

The extract was analyzed in a HP 6890 series GC system. The injection volume was 3 microlitre. The GC oven temperature was 50°C for 2 minutes and ramped at 8°C/min till it attained 290°C and this temperature was maintained for 10 minutes. Carrier gas (Helium) was at 1ml/min, make up gas (Helium) was at 16ml/min, Hydrogen gas was at 40ml/min and the air flow was at 450ml/min. The split inlet vent was at 100psi while the split column head pressure was at 27 psi. The run time was programmed to 42 minutes and the retention time was the basis for identification while peak area is the basis for quantification. The same GC conditions were employed in analyzing the standard mixture of PAHs used in this study.

RESULTS

Chromatographic study

Taking into account previous studies, the GC oven temperature was programmed at 50°C for 2 minutes and ramped at 8°C/min till it attained 290°C and this temperature was maintained for 10 minutes. This programme was to achieve an optimum separation. Figure 2 and 3 show the chromatogram of a standard mixture of PAH containing 12 compounds and the water samples respectively.

Recovery studies of PAH from the sample

The extraction procedure employed in this study was applied to the recovery of PAH from water sample. 5ml of water was spiked with PAH standard mixture solution containing 100ug/ml of individual PAHs. The recovery 72.3% was obtained. The recovery was done by spiking the water sample with a mixture of PAHs of known concentration and employing the same extraction procedure as the unspiked sample.

Analytical characteristic of the method

Calibration graphs were obtained by preparing PAH solutions at the different concentrations. Six concentrations ranging from 100ug/ml to 5ug/ml were prepared by diluting the stock solution with dichloromethane. Linearity was found in all cases. Relative standard deviations (RSD %) were around 11%. The detection limit is particularly low for acenaphthylene and highest for Benzo(b)Flouranthene.

Determination of PAH in the water sample

The above method was used to determine some PAHs in the water sample of the Lagos lagoon. The PAHs detected include anthracene 19.57ug/ml; pyrene 14.92ug/ml. Benzo(a)anthracen 17.15ug/ml; chrysene 20.33ug/ml and benzo(b)flouranthene 0.88ug/ml. The cumulative PAH concentration is 72.87ug/ml which is considered as very high. Figure 3 shows the chromatogram of the water sample extract. Figure 4 shows a graphical distribution of the PAHs in the studied water sample.

DISCUSSION

By all standards the PAHs concentration obtained is very high even though the result is to be expected considering the level of activity in the said lagoon. According to the World Health Organization study in 1997, the concentration of individual PAHs in surface and coastal waters are generally in the neighbourhood of 50ng/L and concentration above this point indicate some contamination. This level of contamination observed in this sample gives a clue on how much the aquatic life is affected. PAHs are generally insoluble in water therefore oil rich sediment and marine organism will contain much more.

Of the five compounds detected benzo(a)anthracene and benzo(b)flouranthene are the most carcinogenic [23,24] due to their relatively higher ability to bind covalently to DNA thereby initiating tumors. Even though the most carcinogenic PAH namely Benzo(a)pyrene [25,26] was not detected or rather is present at a concentration lower than the detection limit, the cumulative concentration of PAHs detected is of great concern. Several works done on rat have shown that BaA is genotoxic producing tumors in mice treated dermally, intraperitioneally and subcutaneously [27,28]. Nevertheless there is ample evidence for enhancement or inhibition of carcinogenicity by other PAHS [31]. It is worth noting that all the studies done on the

carcinogenicity of PAHs have been done with mice and rodent because it is not possible to assess the risk to human for obvious reasons. Therefore, we rely on the animal data to estimate the risk of exposure to humans. The extrapolation of risk to human from animal data may not be absolutely accurate due to the possibility of interspecies differences in the enzyme that activate PAHs [32]. Most available human data are from inhalation and percutaneous absorption of PAHs from a large range of occupational exposures [33-35] but since there is also exposure to other chemicals correlation is a bit less absolute.

CONCLUSION

The amount of PAHs found in the Lagos lagoon is so much that swimming or water activity in the lagoon may have a long term negative effect on human health. Water for domestic purposes from the lagoon could also be found to be hazardous to man. It is therefore recommended that measures be taken to minimize all forms of industrial waste and sewage discharge that could lead te increased concentration of PAHs in the area.

Figure 2: The chromatogram of a standard PAH mixture containing acenephthylene, anthracene, benz(a)anthracene, benzo(k)flouranthene, benzo(b)flouranthene, benzo(g,h,i)perylene, benzo(a)pyrene, chrysene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene, phenanthrane, and pyrene. Conditions:Methyl siloxane capillary column (25m x 320um x 0.52um), the GC oven temperature was programmed at 50°C for 2 minutes and ramped at 8°C/min till it attained 290°C and this temperature was maintained for 10 minutes

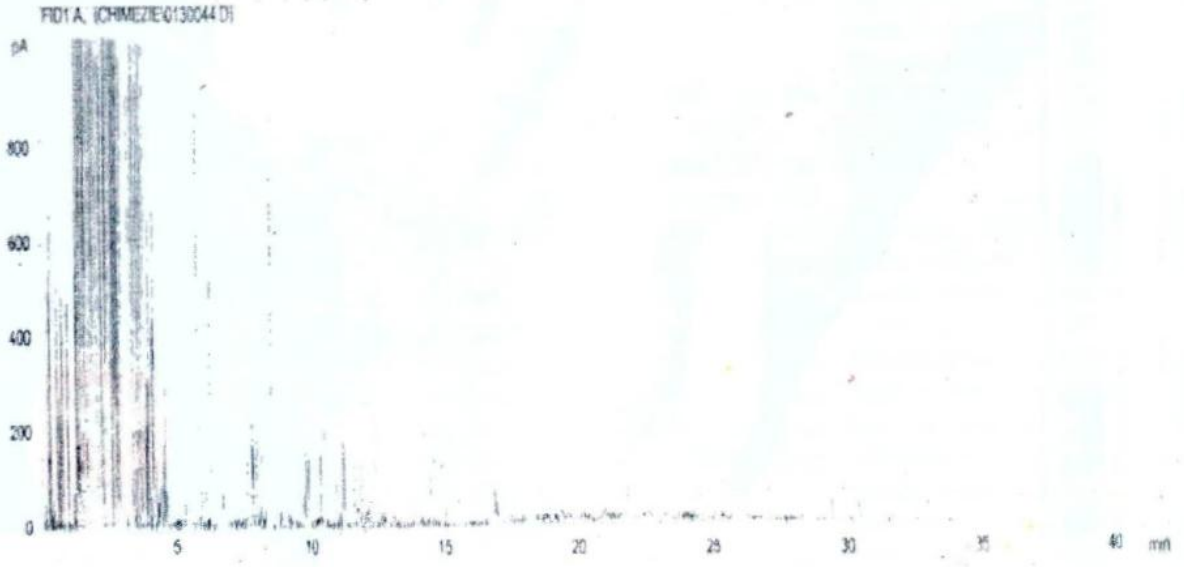

Figure 3: The chromatogram of extract from the Lagos lagoon. Conditions: Methyl siloxane capillary column (25m x 320um x 0.52um), the GC oven temperature was programmed at 50°C for 2 minutes and ramped at 8°C/min till it attained 290°C and this temperature was maintained for 10 minutes

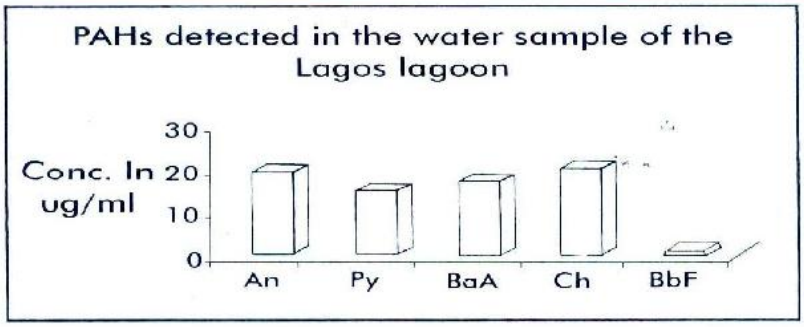

Figure 4: PAHs detected in the water sample of the Lagos lagoon. An = Anthracene; Py=Pyrene; BaA= Benz(a)Anthracene; Ch=Chrysene; BbF=Benzo(b)Flouranthene

	Compound	Retention time (mins)	Peak Area	Peak Height	Peak Width
1	Acenaphthylene	4.85	7.27	3.80	0.03
2	Phenanthrene	17.34	69.04	28.00	0.04
3	Anthracene	19.60	74.68	30.11	0.04
4	Pyrene	22.58	85.07	32.35	0.04
5	Benz(a)Anthracen	22.73	83.60	31.07	0.04
6	Chrysene	27.07	96.75	34.46	0.04
7	Benzo(b)Flouranthene	30.98	100.44	32.43	0.05
8	Benzo(k)Flouranthene	31.08	101.69	34.83	0.05
9	Benzo(a)Pyrene	34.64	97.98	23.96	0.06
10	Indeno(1,2,3-cd)Pyrene	34.72	99.72	24.70	0.06
11	Dibenzo(a,h)Anthracene	35.85	95.07	20.30	0.07
12	Benzo(g,h,I)Perylene	41.84	171.93	13.15	0.16

Table 1: The chromatographic characteristics of the PAH standard mixture employed in this study.

	Compound	Retention time (mins)	Peak Area	Peak Height	Peak Width
1	Anthracene	19.60	145.02	28.28	0.07
2	Pyrene	22.59	127.29	25.65	0.06
3	Benz(a)Anthracen	22.74	143.39	21.79	0.09
4	Chrysene	27.08	197.87	80.45	0.04
5	Benzo(b)Flouranthene	30.94	8.76	2.8	0.04

Table 2: The chromatographic characteristics of the detected PAHs in the water sample.

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REFERENCES

1. Harvey, G. R. (1997), Polynuclear Aromatic Hydrcarbons, Wiley-VCH, New York P1-27.
2. Sabrina Moret, Lanfranco Conte and Daniela Dean (1999), J. Agric. Food Chem. 47, 1367-1371.
3. M. J. Nieva-Cano, S. Rubio-Barroso and M. J. Santos-Delagado (2001). The Analyst, 126, 1326.
4. K. D. Bartle, C. Creaser and R. Purchase (1991), Food Contaminants, Sources and Surveillance, The Royal Society of Chemistry, Cambridge, 41.
5. P. Simko (2002), J. Chromaogr. 3, 770.
6. Michiko Koyano, Shigeru Mineki, Yurie Tsunoda, Osamu Endo and Sumio Goto, Journal of Health Science, 47 (5) 452 (2001).
7. Alonge, D. O., J. Sci. Food Agric 1988, 43, 167-173.
8. Dungel, N. J. Am. Med. Assoc., 1961, 178, 93-95.
9. Xiaoxing Liu and Takashi Korenaga, Journal of Health Science, 47 (5) 446-451 (2001).
10. Ohshima H., Friesen M., Malaveille C., Brovet I., Hautefeutlle A., And Bartsch H. (1989) Food Chemistry, 27, 193-203.
11. Teal, J. M., Farrington, K. A., Burns, J. J., Stegeman, B. W., Tripp. B. W., Phinnley, C., (1992), Mari. Poll. Bull., 24 (12) 607-614.
12. WHO (1997) Environmental Health Critieria 202.
13. Stuermer D. H., Ng. D., J. Morris C. J., (1982) Environmental Science and Technology 16: 582-587.
14. Nwachukwu, S. C. U., (2000), J. Environ. Biology 21 (3), 241-250.
15. Baek, S. O., Field, R. A., Goldstone, M. E., Kirk, P. W., Lester, J. N., Perry, R. A., (1991), Water, Soil and Air Pollut. 60, 279-300.
16. Yang, H. H., Lee, W. J., Chen, S. J., Lai, S. O. 91998) J. Hazard, Mater. 60, 159-174.
17. Lorber, M., Cleverly, D., Schaum, J., Phillips, L., Schweer, G., Leighton, T., (1994), Environ. Sci. Technol. 156, 39-65.
18. Grova, N., Feidt, C., Crepineau, C., Laurent, C., Lafargue, P. E., Hachimi, A., Rychen, G., (2002), J. Agric. Food Chem., 50, 4640-4642.
19. Nwachukwu, S. C. U., James P., Gurney, T. R., (2001), J. Environ Biol. 22 (1), 29-36.
20. Odiete, W. O. (1999), Hydrocarbon Pollution In: Environmental Physiology of Animals and Pollution. Diversified Resources Ltd. Lagos, Nigeria, vol. 1, 180-192.
21. Atlas R. M., (1981), Microbiol. Rev., 45, 180-209.
22. Amund, O. O. And Igiri C. U., (1990), World J. Microbiol. Biotechnol., 6, 225-262.
23. Klein M. (1963), Cancer Research, 23:1701-1707.
24. Bock, F. G., King D. W., (1959), Journal of the National Cancer Institute, 23:8233-838.
25. Neal J. Rigdon R. H., (1967), Texas Reports on Biology and Medicien, 25:553-557.
26. Rugen P. J., Stern C. D., Lamm S. H. (1989) Regulatory Toxicology and Pharmacology, 9: 273-283.
27. Wynder E. L. And Hoffmann D., (1959), Cancer, 12:1079-1086.
28. Wynder E. L. And Hoffmann D., (1959), Cancer, 12:1194-1199.
29. Habs M., Schulting F. L., (1985), Science of the Total Environment, 43: 193-219.
30. Kalberlah, F., Frijus-Plessen, N., Hassauer, M. (1995), Altlasten-Spektrum, 5: 231-237.
31. Warshawsky D., Barkley W., Bingham E. (1993), Fundamental And Applied Toxicology 20: 376-382.
32. Guengerich F., Shimada T. (1991) Chemical Research in Toxicology, 4: 391-407.
33. Armstrong B., Tremblay C., Baris D., Theriolt G., (1994), American Journal of Epidermiology 139: 250-262.
34. Partanen T., Boffetta P., (1994), American Journal of Industrial Medicine 26: 721-740.
35. Constantino J. P., Redmond C. K., Bearden A., (1995), Journal of Occupational and Environmental Medicine 37: 597-604.