Determination of Residual Fuel Oil Contamination of Aquatic Animals

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Chromatography and spectroscopy have been used to identify and measure crude oil and petroleum products in water (1,2,3,4). Crude oil and petroleum products cause unpleasant tainting of fish and shellfish (5,6,7,15), but only a few papers describe the determination of these materials in aquatic animals. Pflaum et al. (8) used TLC for qualitative detection of petroleum products in carp exposed to a mixture of gasoline and lubricating oil. GLC was used to detect the contamination of oysters *(Crassostrea virginica)* and scallops *(Aequipecten irradians)* by light fuel oil (9). Hydrocarbons including several carcinogens were found in mussels *(Mytilus edulis)* from Toulon Harbour (10). Several polynuclear aromatic hydrocarbons have been isolated from barnacles *(Tetraclita squamosa rubescens, Mitella polymerus)* and oysters (11,12,13). This paper describes a simple spectrofluorometric method for the quantitative determination of heavy residual fuel oil (Bunker C) in aquatic animals.

EXPERIMENTAL

Bunker C oil from Venezuela was extracted with spectrograde hexane, the extract was filtered through a medium sintered-glass filter and hexane was evaporated in vacuum on a rotatory evaporator at 36°C. The resulting hexane-soluble fraction was used as the standard for the spectrofluorometric determination of Bunker C oil in aquatic animals.

A Beckman DK-2A spectrophotometer and a Perkin-Elmer MPF-2A spectrofluorometer were used to scan absorption and fluorescence spectra, respectively. Unless indicated otherwise, whole animals (shellfish without shell) were analysed. Usually 2 to 4 specimens of the same species were combined for analysis. Individual flounders and lobsters were dissected and various tissues were analysed separately. The sample was ground with anhydrous sodium sulfate and the mixture shaken with spectrograde hexane for 20 minutes on a wrist-action laboratory shaker. The ratio of hexane to wet weight of sample was approximately 4:1 V/W. The hexane extract was drawn off by a Pasteur pipet and its volume was measured. An aliquot of the

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extract was diluted with a known volume of hexane so that the absorbance at 300 nm (1 cm cell) did not exceed 0.100 and the fluorescence emission at 360 nm was used to calculate the concentration of Bunker C oil in the sample, using the hexane-soluble fraction of Bunker C oil as the standard.

RESULTS AND DISCUSSION

Fluorescence of several fuel oils, dissolved in hexane, was determined. Fluorescence emission maxima of light fuel oils occur at 310-330 nm, while residual fuel oils have maxima at 350-400 nm. Bunker C oil used in this work has a fluorescence emission maximum at 360 nm. With this material, fluorescence is a linear function of concentration at least up to 10 µg/ml. Hexane extracts of control animals have no maximum at 360 nm but they have some background fluorescence. This background fluorescence was expressed as Bunker C oil to obtain an estimate of its influence on the determination of Bunker C oil. Most animals exposed to Bunker C oil in the lab or collected in the area of a major Bunker C oil spill have a fluorescence emission maximum at 360 nm. Hexane extracts of flounders and Atlantic salmon parr, exposed to Bunker C oil in the laboratory, and hexane extracts of crabs and lobster from the contaminated area had no maximum.

Typical fluorescence emission spectra are presented in Figure 1 and the quantitative results are summarized in Table 1. As can be seen from Table 1, the background fluorescence of the hexane extracts of control animals is low and animals exposed to Bunker C oil take up the oil from their environment. Specimens were collected at different stations in the contaminated area which accounts for the differences in their Bunker C oil content.

Duplicate analyses (including extraction) of a flounder exposed to Bunker C oil in the laboratory gave the values of 90 and 108 for skin, 57 and 59 for flesh, and 108 and 144 µg/g for gut. The recovery of Bunker C oil from spiked control samples of periwinkles, mussels, lobster muscle, and crabs was 117, 45, 95, and 60%, respectively (average from two determinations).

Bunker C oil is an extremely complex mixture of compounds. The oil used in this work contains about 6% of a hexane-insoluble material which is not included in the fraction used for calibration and which is not extracted from the analysed samples under the

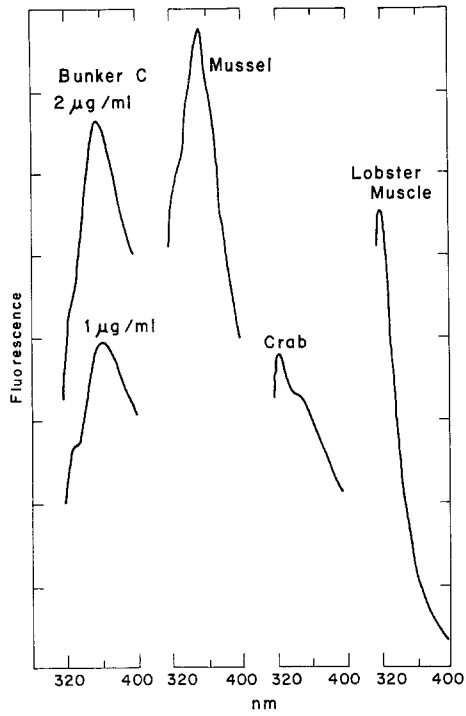

Figure 1. Typical fluorescence emission spectra.

described conditions. About one-half of the hexane-soluble fraction consists of aliphatic hydrocarbons, the other half contains unidentified fluorescent compounds, very likely polynuclear aromatic hydrocarbons. Thus a great deal of uncertainty is involved in the determination of Bunker C oil in aquatic animals. The described method determines only the fluorescent fraction of the oil and gives no data on the aliphatic fraction. However, aliphatic hydrocarbons are quite inert biologically, while aromatic hydrocarbons are toxic, some of them are carcinogenic and may have much more pronounced effects on aquatic fauna than aliphatic hydrocarbons (14). It is therefore the fluorescent fraction of the oil which is more important in the study of oil contamination of aquatic animals.

TABLE 1

Determination of Bunker C oil in aquatic organisms.

Species	Bunker C oil, µg/g: Control area or laboratory control	Bunker C oil, µg/g: Contaminated area or laboratory exposure
Crab *(Cancer irroratus)*	7	17+,61+,53+
Hermit crab *(Pagurus)*	2	
Mussel *(Mytilus)*	3	103, 77
Periwinkle *(Littorina littorea)*	11	231, 57, 66
Starfish *(Asterias vulgaris)*	11	158, 31, 14+
Sea urchin *(Strongylocentrotus)*	22	39, 21
Clam *(Mya arenaria)*	8*	87*
Atlantic salmon parr *(Salmo salar)*	10*	113*+
Winter flounder *(Pseudopleuronectes americanus)*		
Skin	0*	182*
Flesh	0*	7*+
Gut	21*	622*+
Lobster *(Homarus americanus)*		
Abdominal muscle	5, 3*	4+,137*
Claw muscle	4, 4*	3+, 33*
Gut	57, 149*	130+,1810*
Stomach	19, 116*	230+,2840*

+No maximum at 360 nm
*Laboratory control or exposure

The complexity of Bunker C oil must be borne in mind when interpreting the data in Table 1. It can be seen that aquatic animals take up large quantities of Bunker C oil and distribute it throughout the tissues. This is in agreement with the literature reports (8,9) on the uptake of other petroleum products by aquatic animals. The analysis of flounder and lobster tissues indicate very high concentration of the oil in the gut which may be the site of the initial accumulation of the oil. A thin film of oil on the stomach wall and black discoloration of the gut were observed in lobsters exposed to Bunker C oil (15). It is not clear at the moment why only an increased background fluorescence of the extracts of exposed animals was observed in some cases. It is possible that in these cases fluorescent compounds from Bunker C oil are either metabolized or irreversibly bound in the tissue and different fluorescent compounds are generated in the process.

Oils are taken up readily by aquatic animals. Further study is required to determine the fate of the oil in the animals and in the food chain. From the analytical point of view the effort should be concentrated on the isolation and identification of individual compounds in oils and on the determination of those compounds in the environment. The described method is suitable for the determination of the general patterns of uptake, excretion, and metabolism of Bunker C oil in aquatic animals and is being used in a study of the contamination of aquatic animals following a major spill of Bunker C oil.

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REFERENCES

1. MATTHEWS, P. J., J. Appl. Chem. 20, 87 (1970).
2. RAMSDALE, S. J., and R. E. WILKINSON, J. Inst. Petroleum 54, 326 (1968).
3. LIVELY, L., A. A. ROSEN and C. I. MASHNI, Conf. Purdue Univ., Enging Extn. Ser. #118, 657 (1965).
4. KAWAHARA, F. K., Environ. Sci. & Technol., 3, 150 (1969).
5. MANN, H., Fette, Seifen, Anstrichmittel 71, 1021 (1969).
6. SIMPSON, A. C., Biol. Eff. Oil Pollut. Littoral Communities, Proc. Symp. 1968, 91.

7. KRISHNASWAMI, S. K., and E. E. KUPCHANKO, J. Water Pollut. Contr. Fed. 41, R 189 (1969).
8. PFLAUM, W., T. KEMPF, and D. LUEDEMANN, Motortechnische Zeitschrift 29, 84 (1968).
9. BLUMER, M., G. SOUZA, and S. SAAS, Marine Biology 5, 195 (1970).
10. GREFFARD, S., and J. MEURY, Cah. Oceanogr. 19, 457 (1967).
11. ZECHMEISTER, L., and B. K. KOE, Arch. Biochem. Biophys. 35, 1 (1952).
12. KOE, B. K., and L. ZECHMEISTER, Arch. Biochem. Biophys. 71, 396 (1952).
13. CAHNMANN, J. H., and M. KURATSUNE, Analyt. Chem. 29, 1312 (1957).
14. REIMANN, K., Öle und Detergentien im Wasser und Abwasser, Munchner Beiträge zur Abwasser-, Fischerei- und Flussbiologie, 9, 60 (1962), R. Oldenbourg, Munich 1962.
15. WILDER, D. G., Fisheries Research Board of Canada, MS Report No. 1087. The tainting of lobster meat by Bunker C oil alone or in combination with the dispersant Corexit (1970).