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ORIGINAL ARTICLE

Naphthalene, a polycyclic aromatic hydrocarbon, in the fish samples from the Bangsai river of Bangladesh by gas chromatograph–mass spectrometry

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Abstract Naphthalene, a polycyclic aromatic hydrocarbon (PAH), was detected and quantified in the selected varieties of fishes collected from the Bangsai river, one of the contaminated rivers located at Savar near the Dhaka Export Processing Zone (DEPZ), Bangladesh, during the period October 2009. Naphthalene, a carcinogenic compound, was analyzed by GC–MS as it was in the mixture of dichloromethane–hexane (1:1) crude extract of the flesh of fish samples collected from the aforesaid river. A suitable and reliable procedure for the extraction of naphthalene from the fish sample has been developed. A multi-layer clean-up (silica gel) column was used, followed by glass fiber filter (GFF) paper to eliminate the interfering organic compounds as well as the lipids and fat. It was observed that PAHs deposition on the samples takes place in different morphological parts of the biological materials. The PAH, naphthalene, was found in almost all of the fish samples and the concentration of which was in the range 0.030–1.004 μg/g. Recovery studies with fortified samples indicated that the recovery efficiency for naphthalene was about 79.14%. This concentration is within the range of values reported for other comparable regions of the world.

1. Introduction

The contamination of the environment by polynuclear aromatic hydrocarbons (PAHs) is becoming a rising environmental concern. They have a widespread distribution in the environment and the carcinogenicity and mutagenicity of several of these compounds have been proven (Alonge, 1988; Simko, 2002; Koyano et al., 2001; Bouloubassi and Saliot, 1991, 1993; Literathy et al., 1991; Malins et al., 1984; Liu and Kor-

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enga, 2001). In 2001 PAHs were ranked the ninth most threatening compounds to human health (King et al., 2002). Several epidemiological studies on PAHs especially among workers exposed to these compounds in a number of countries have been carried out (Grimmer et al., 1988). PAHs comprise the largest class of chemical compounds known to be cancer-causing agents and are included in the European Union and United States Environmental Protection Agency (EPA) priority pollutant list due to their mutagenic and carcinogenic properties.

PAHs consist of several hundred compounds containing two or more condensed rings. Among the several hundred different PAHs already identified, 16 are considered as priority because they are supposed to be more harmful than the others; there is more information available on them and there is a greater possibility of people being exposed to them. Both natural and anthropogenic sources contribute PAHs to the environment. But crude oil and other petroleum based products have been found to contribute significant amount of PAHs to the environment. Other sources of PAHs in the environment include natural fires, volcanic eruptions, thermal geological reactions, industrial processes (aluminum production, iron and steel production, foundries), transportation, burning (e.g. forest, straw, agriculture, cooking), waste incineration, combustion of fossil fuel, exhausts from vehicles, tobacco smoke, domestic heating using wood, coal and mineral oil, etc. (Anyakora et al., 2004, 2009; Nieva-Cano et al., 2008; Grova et al., 2002; Guillen et al., 2000; Govers, 1990; Gibbs et al., 1986; Al Yakoob et al., 1993).

In Bangladesh the marine and coastal areas are of a major economic significance. Marine resources are exploited for local consumption as well as for export. The report of Haskoning suggested (Haskoning, 1999) that the main impacts presently affecting Bangladesh marine environment are pollution and overexploitation of certain natural resources. Qualitative and quantitative data are still lacking on the expected source/s of pollution, and their impacts on the coastal environment.

Thus, we present here, for the first time to the best of our knowledge, a detailed analytical study using gas chromatography and mass spectrometry (GC–MS) of anthropogenic PAHs, naphthalene, in fish samples from the Bangsai river of Bangladesh. This investigation involves screening of PAHs in several fish species from the Bangsai river to determine if these animals show evidence of oil contamination.

Polycyclic aromatic hydrocarbons (PAHs) are of special concern because they are widely distributed in the environment and many of them have toxic and carcinogenic properties (Anyakora et al., 2009). They can be generated and introduced into the environment by various processes. For this reason, the objective of this work is to check the toxic polycyclic aromatic hydrocarbons in the crude extract isolated from the fish sample by GC–MS.

2. Materials and methods

2.1. Chemicals

Dichloromethane, hexane and other solvents used in this experiment were of HPLC grade. Anhydrous sodium sulfate (Merck, Germany) was cleaned by heating at 200 °C before use. Silica gel (60–120 mesh, Merck, Germany) was activated at 400 °C for 12 h prior to use. Glass fiber filter paper (Merck, Germany) was used for removal of fats and lipids. Naphthalene (Sigma–Aldrich) was used as the standard in the present study.

2.2. Fish samples

Seven different varieties of fish samples were collected from the Bangsai river, Savar, Dhaka, Bangladesh, in October 2009 and initially identified by morphological features and database present in the library at the herbarium of the Department of Biology, University of Dhaka, Dhaka, Bangladesh.

2.3. Isolation and preparation of crude extracts

Fish samples were at first washed with tap water and then with de-ionized water. The washed samples were flayed to collect flesh, which again was washed by de-ionized water to remove blood, dusts and any other foreign particles. The collected flesh samples were ground by mortar pestle. The paste samples (5 g) were extracted three times with dichloromethane–hexane (1:1) (40 ml × 3) at 80 °C for 30 min. It was then filtered by glass fiber filter paper and the filtrate was evaporated near to dryness by Kuderna-Danish evaporator.

2.4. Clean-up procedure

The clean-up column (i.d. = 1 cm) was filled with cotton in the bottom. An activated silica gel (17 g) soaked with dichloromethane was loaded into the clean-up column (5 cm), which was then topped with 1.5 cm of anhydrous sodium sulfate. Five milliliters of dichloromethane was added to wash the sodium sulfate and the silica gel. The dried 1 ml sample was then transferred into the column; the vessel was rinsed twice with 2 ml dichloromethane, which was also added to the column. Sixty millimeters of acetone was added to the column and allowed to flow through the column at a rate of 3–5 ml/min, and the effluent was collected. The collected effluent from the clean-up procedure was reconcentrated to 0.5 ml with K-D concentrator.

2.5. GC–MS analysis and program

The GC–MS analysis of the crude extract of fish samples was performed using a Varian GC–MS (Model Varian CP 3800) equipped with a VF-5 fused silica capillary column (30 m × 0.25 i.d., film thickness 0.25 μm). For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. Injector and mass transfer line temperatures were set at 250 and 280 °C, respectively. The oven temperature was programmed from 50 to 200 at 8 °C/min, and then held isothermal for 20 min and finally raised to 300 °C at 10 °C/min. Diluted samples (1/100, v/v, in methanol) of 0.2 μl were manually injected in the split less mode. Identification of compounds of the crude fish extract was based on GC retention time on VF-5 capillary column, computer matching of mass spectra with standards (Mainlab, Replib and Tutorial data of GC–MS systems) and, whenever possible, by co-injection with authentic compounds (Lawless, 1995).

The system suitability of the method was evaluated by the intra- and inter-day precision and accuracy of replicates. The accuracy was evaluated through recovery studies by adding