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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

known amounts of the standard solution to the extract. The recovery experiment was performed at three different standard concentrations.

2.6. Preparation of standard

Calibration graphs for the samples treated according to the described analytical procedure were made using the SIM mode. Different concentrations of naphthalene (0.625, 1.25, 2.5 and 5 μg/ml) were used for calibration curve.

3. Results and discussion

Bangladesh is an agricultural country which comprises 64 districts. On the basis of land it has been divided into two regions. One is hilly area and the other is plane land. Vegetables, fish, crops and fruits are grown in both areas in plenty, mainly in the winter season. Whereas fish is locally cultivated across the country all the year round and it is the second main food in Bangladesh. It is one of the most commonly used food because it is cheap and available all over Bangladesh throughout the rainy season. Again, to use it for other seasons, sometimes villagers and fishermen catch fish and dry it under sunlight for storage. This dried product is also used as animal feed. It is also exported to the foreign countries. So considering the fact, the need for checking any toxic compounds contaminating it or not cannot be overlooked.

On the basis of its importance as food we have collected seven different types of fish samples (small and moderate) from the nearest Bangsai river of Dhaka industrial areas of Bangladesh in the month of October 2009 (Fig. 1). The chemical composition of all kinds of fruits, vegetables, fish and plants depends on the geographical distribution such as temperature, weather, and soil condition (Haward and Fazio, 1980). The crude extract contains a complex mixture consisting of mainly flavonoids, alkaloids, fats, caffice acid, oxygenated mono, di and triterpenes, and mono and sesqueterpene hydrocarbons (Kipopoulou et al., 2001).

The flesh of the fish sample (small and moderate) was extracted with mixture of dichloromethane and hexane (1:1) and then filtered. The filtrate was cleaned up to remove the animal fats and oily or gummy compounds by glass wool filter paper. The water was removed from the filtrate by anhydrous sodium sulfate. The filtrate solvent was evaporated to dryness by Kuderna-Danish evaporator. From the concentrated extract only 0.2 μl was injected to the GC–MS. The reference marker was present in the chromatographic profiles of the various fish samples from the Bangsai river when the sample solutions were analyzed by GC–MS (Fig. 2). The peak of naphthalene was confirmed by comparison of their retention times with reference standard.

The quantitative determination of our aim target polycyclic aromatic hydrocarbon compound, naphthalene, was done by external calibration curve method. The calibration curve already prepared with known concentration of naphthalene is detailed below (Fig. 3). Standard curves for naphthalene generated by plotting the area of four spots vs. the concentration gave high correlation coefficients. Linear responses were achieved for naphthalene in the concentration range for fish samples. Over this concentration range, the linear regression analysis of peak areas (y) in function of concentration (x), calculated by least square method, leads to the following equations: $y = +1.6683e^{+4x} + 5116.0039$ ($r^2 = 0.97820$) for naphthalene.

Naphthalene was identified by comparing its retention time (RT) on the total ion chromatogram (TIC) of the substance in the fish samples with that of the respective compound in a standard solution analyzed under the same conditions. The existing GC–MS library database (NIST) shows the RT of naphthalene from the fish samples.

For our experiment, we took seven different types of small and large fish samples, collected from the Bangsai river at Savar of Bangladesh. The collected samples were processed during the winter season. Normally fish is contaminated by various aspects such as industrial effluents, air, highway vehicle exhaust, and highway tar samples. The concentration of naphthalene in the seven different types of fish samples was measured by GC–MS and the results were calculated from the external curve method (Table 1). Naphthalene is a two member ring compound and as such is found in the middle of the water. Among the naphthalene accumulations by seven different fishes, it is observed that bata fish receives the highest

Figure 1 Seven types of fish samples collected from the Bangsai river of Bangladesh.

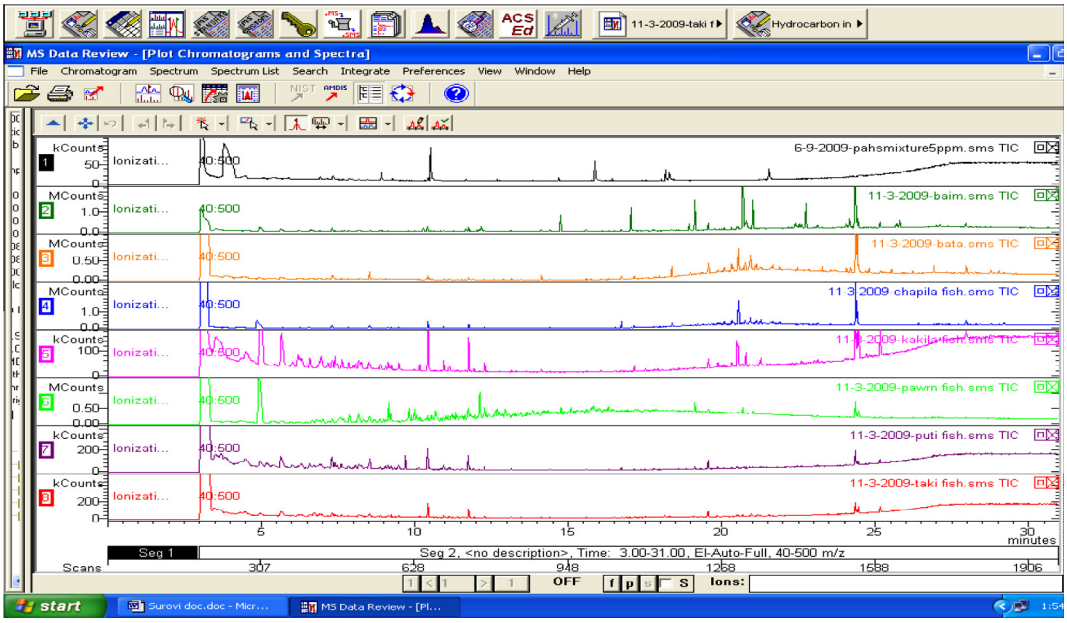

Figure 2 A typical overlaid chromatogram of fish samples and standard.

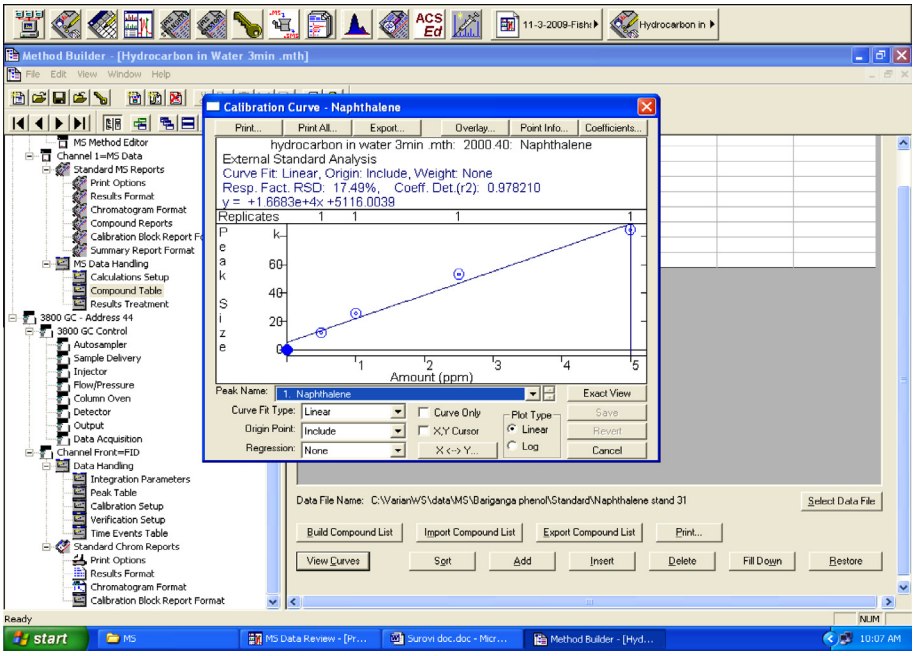

Figure 3 Calibration curve of naphthalene standard.

amount of naphthalene as it normally stays and lives in the middle position of the water and the lowest in taki. The others have naphthalene in their body well above the detection limit with the exception of kakila. The concentration of naphthalene in the fish samples is within the permissible limit reported by the European Union and United States Environmental Protection Agency (EPA).

4. Conclusion

From the experiment, it is concluded that six fish samples such as baim, bata, puti, chapilla, prawn and taki were contaminated by various aspects such as industrial effluents, air, highway vehicle exhaust, and highway tar samples. The findings thus suggest that awareness should be grown among the Bangladeshi nationals who live nearby the Bangsai river for not to eat these naphthalene biased fishes. The experiment also reveals the fact that the most probable source of PAHs is oil contamination originating from the effluents from different industries such as tannery, dye, plastic, chemical, and fertilizer or spillages and/or heavy ship traffic. However, the high concentration of carcinogenic naphthalene encountered in these fishes should be considered seriously as it is hazardous to human health.

Table 1 Concentration of naphthalene in the seven different types of small and moderate fish samples.

Sl. No.	Local name of fishes	Concentration (μg/g)
1	Baim	0.057
2	Bata	1.004
3	Puti	0.516
4	Chapila	0.903
5	Kakila	ND[a]
6	Prawn	0.405
7	Taki	0.030
8	Blank	ND[a]

[a] Not detectable.

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