TOXIC NORMAL HYDROCARBONS (NHs) IN THE FISH SAMPLES FROM DIFFERENT PARTS OF BANGLADESH

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ABSTRACT

Toxic normal hydrocarbon (NH), nonadecane in the dichloromethane-hexane crude extract of the flesh of fish samples collected from the different districts of Bangladesh was analyzed by GC-MS/MS. It was observed that NH deposition on the samples takes place in different morphological parts of the biological materials. The NH, nonadecane, was found in the fish samples collected from the roadside by the extraction of dichloromethane-hexane mixture solvents.

Keywords: *Toxic normal hydrocarbon, nonadecane, quantification, Bangladeshi fish GC-MS/MS*

INTRODUCTION

As a class of well known toxic compounds originating from incomplete combustion [1-3], polycyclic aromatic hydrocarbons (PAHs), normal hydrocarbons (NHs) are among the most important environmental contaminants in China [4] as well as all over the world. Located at the fastest growing coastal area of China, Bangladesh suffers particularly from severe contamination of PAHs and normal hydrocarbons (NHs) from various sources [5-6]. PAHs and NHs occur as contaminants in various food categories including vegetables, fish which have been documented to be one of the important contributors to human intake of PAHs and NHs [7].

This is particularly true in China given the fact that vegetables and fish are basic food in China as well as in Bangladeshi diet. It has been reported that plant, fish and other essential food uptake of PAHs and NHs is primarily from atmosphere through gas and particle-bound depositions and relative importance of these two mechanisms is driven by the gas/particle partitioning of the compound [8]. A framework for identifying the major uptake process of semi-volatile organic compounds based on octanol-air partition coefficient (K_{OA}) was developed and two separate tools for interpretation of plant uptake behavior for either gas or particle-bound chemicals were established [8]. However, knowledge gap still remains for quantitative relationship between the plant accumulation and the level in the air. The aim of the present study is to examine and determine the toxic normal hydrocarbon, nonadecane, in the crude extract isolated from the flesh of fish samples by GC-MS/MS.

EXPERIMENTAL

Chemicals

Dichloromethane and hexane (Merck, Germany), solvents used in this experiment were of HPLC grade. Anhydrous sodium sulphate (Merck, Germany) was purified by heating at 200 ^{o}C before use. Silica gel (60-120 mesh, Merck, Germany), activated at 400 ^{o}C for 12 h prior to use. Glass fiber filter paper (Merck, Germany) was used for removal of fats and lipids. Nonadecane of (Sigma-Aldrich) was used as standard in the present study.

Fish samples

Fish samples were collected from the different districts of Bangladesh in October 2006 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.

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 grinded. The paste samples (5 g) were extracted three times with dichloromethane-hexane (1:1) mixture solvents (40 mL x 3) at 80 ^{o}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.

Clean-up procedure

The cleanup column (Φ = 1 cm) was filled with cotton in the bottom. An activated silica gel (17 g) soaked with dichloromethane was loaded into the cleanup 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

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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 milliliters of dichloromethane was added to the column and allowed to flow through the column at a rate of 3–5 mL/min, and the eluent was collected. The collected eluent from the cleanup procedure was reconcentrated to 0.5 mL with K-D concentrator.

GC-MS/MS Analysis

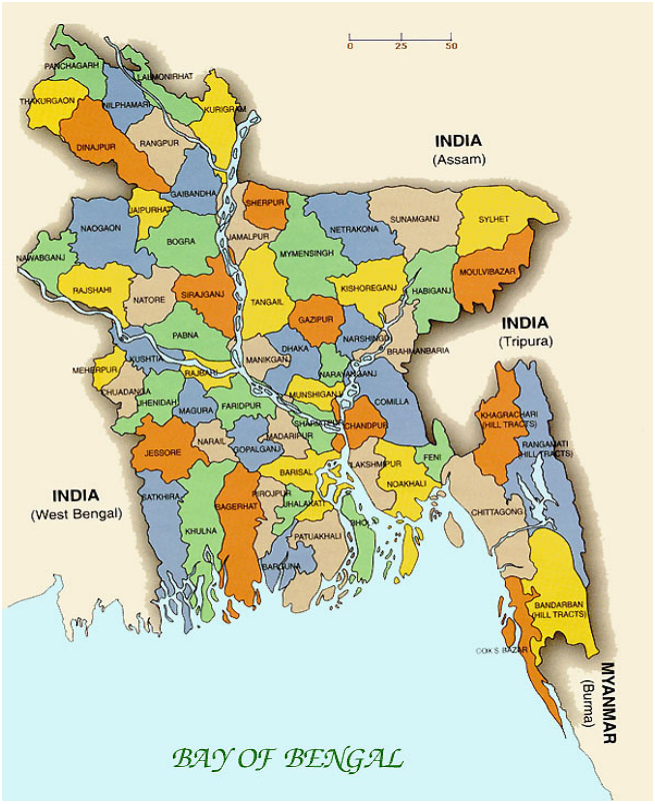
The GC-MS/MS analysis of the crude extract of fish samples was performed using a Varian GC-MS/MS (Model Varian CP 3800) equipped with a VF-5 fused silica capillary column (30m x0.25 i. d., film thickness 0.25 μm). For GC-MS/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Injector and mass transfer line temperature were set at 250 and 300 ^{o}C, respectively. The oven temperature was programmed from 50 to 200 at 8 ^{o}C/min, and then held isothermal for 20 min. and finally raised to 300 ^{o}C at 10 ^{o}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 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/MS systems) and, whenever possible, by co-injection with authentic compounds [9].

Preparation of standard

Calibration graphs for the samples treated according to the described analytical procedure were made using the SIM mode. Different concentrations of nonadecane (0.5, 1, 5 and 100 ng/mL) were used for calibration curve.

RESULT AND DISCUSSION

Bangladesh is an agricultural country, which comprises of sixty four districts. On the basis of land it has been divided into two regions. One is hilly area and the other is plane land. Vegetables, 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 as 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. So considering the fact, the need for checking any toxic compounds



Sampling site

Fig 1. Six Fish samples collected from some district of hilly areas of Bangladesh.

contaminating it or not cannot be overlooked. For this reason, the objective of this work is to check the toxic hydrocarbons in the crude extract isolated from the fish sample by GC-MS/MS.

On the basis of its importance as food we have collected six different types of fish samples (small, moderate and large) from some districts of hilly areas of Bangladesh in the month of January 2006 Fig 1. The chemical composition of all kind of fruits, vegetables, fish and plants depends on the geographical distribution such as temperature, weather, soil condition etc [10].

The flesh of the fish sample was extracted with dichloromethane-hexane and filtered. The filtrate was cleaned up to remove the animal fats and oily or gummy compounds. 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/MS.

The quantitative determination of nonadecane was done by external calibration curve method. The calibration curve already prepared with known concentration of nonadecane is detailed in Fig 2.

The nonadecane is identified by comparing its retention time (RT) on the total ion chromatogram (TIC) of the substance in the samples Fig. 3-8 with that of the

respective compound in a standard solution analyzed under the same conditions. The existing GC-MS/MS library database (NIST) shows the RT of nonadecane from the fish samples in Fig. 3-8. as -22.256 (base peak, 57.1).

The crude extract contains a complex mixture consisting of mainly flavonoids, alkaloids, caffice acid, oxygenated mono, di and triterpenes, and mono and sesqueterpene hydrocarbons [11].

Firstly for our experiment, we took six different types of small and large fish samples, collected from the different districts of Bangladesh. The collected samples were processed during the winter season. The concentration of nonadecane, a normal hydrocarbon, in the five different types of fish samples were measured by GC-MS/MS and the results were calculated from the external curve method Table 1.

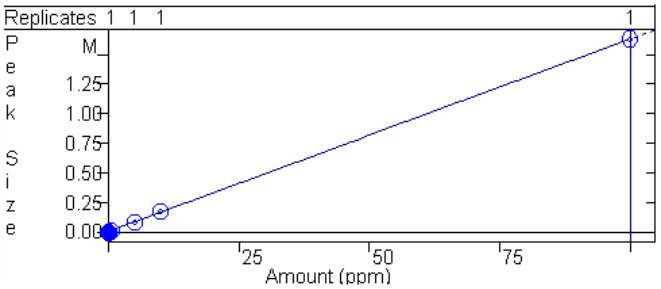
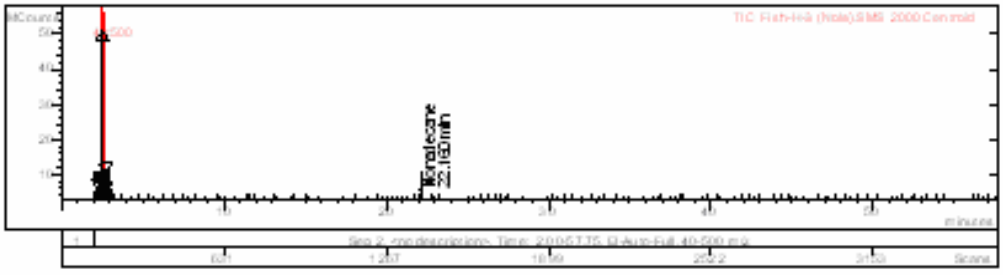
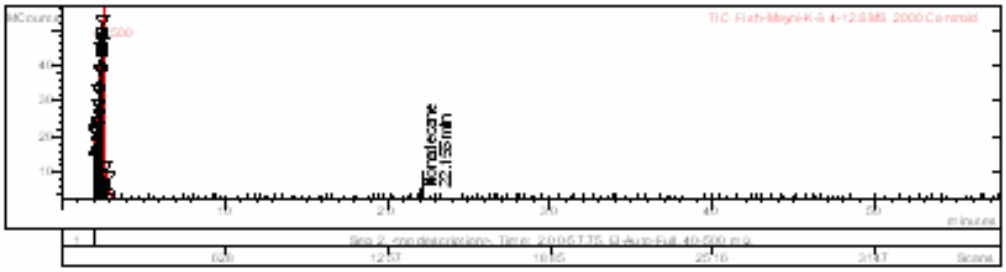


Fig 2. Calibration curve of nonadecane standard. Column: VF-5 (l. 30 m, i.d. 0.25, film thickness 0.25 µm); delay: 3min; Temperature Program: 50 °C(1)—› 200 °C (8 °C/min) —›300 °C (10 °C/min); Injector Temperature: 250 °C; Split: 20%; Injection volume: 0.2 µL; Carrier gas: He; Flow rate: 1 mL/min.



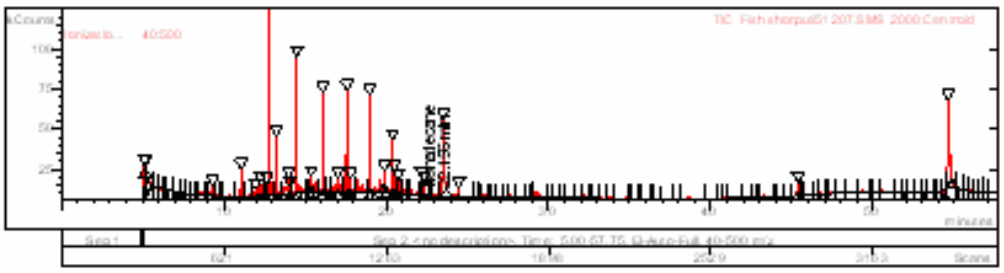
Column: VF-5 (l. 30m, i.d. 0.25, film thickness 0.25 µm); delay: 3min; Temperature Program: 50 °C(1)—› 200 °C (8 °C/min) —›300 °C (10 °C/min); Injector Temperature: 250 °C; Split: 20%; Injection volume: 0.2 µL; Carrier gas: He; Flow rate: 1mL/min.

Fig 3. Chromatogram of the Large fish samples



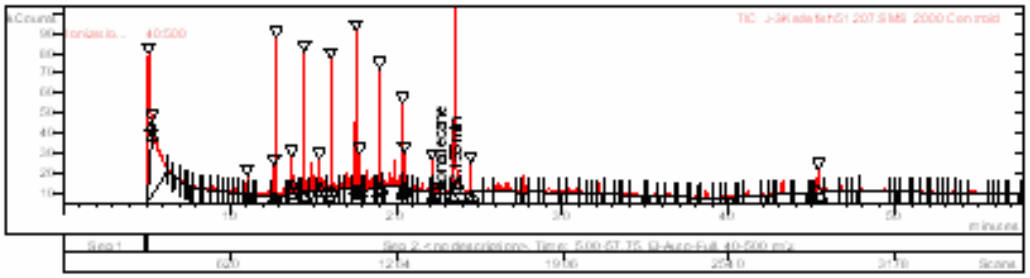
Column: VF-5 (l. 30m, i.d. 0.25, film thickness 0.25 µm); delay: 3min; Temperature Program: 50 °C(1)—› 200 °C (8 °C/min) —›300 °C (10 °C/min); Injector Temperature: 250 °C; Split: 20%; Injection volume: 0.2 µL; Carrier gas: He; Flow rate: 1mL/min.

Fig 4 . Chromatogram of the small fish samples



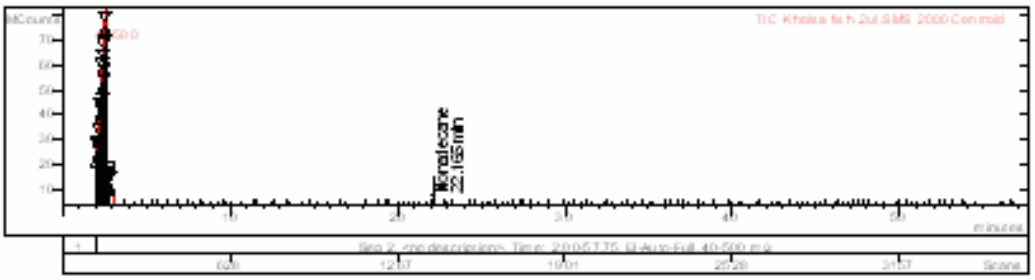
Column: VF-5 (l. 30m, i.d. 0.25, film thickness 0.25 µm); delay: 3min; Temperature Program: 50 °C(1)—› 200 °C (8 °C/min) —›300 °C (10 °C/min); Injector Temperature: 250 °C; Split: 20%; Injection volume: 0.2 µL; Carrier gas: He; Flow rate: 1mL/min.

Fig 5. Chromatogram of the moderately large fish samples



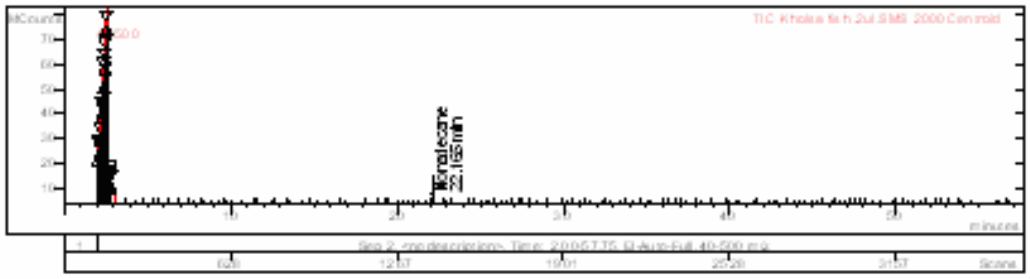
Column: VF-5 (l. 30m, i.d. 0.25, film thickness 0.25 µm); delay: 3min; Temperature Program: 50 °C(1)—› 200 °C (8 °C/min) —›300 °C (10 °C/min); Injector Temperature: 250 °C; Split: 20%; Injection volume: 0.2 µL; Carrier gas: He; Flow rate: 1mL/min.

Fig 6. Chromatogram of the small fish samples



Column: VF-5 (l. 30m, i.d. 0.25, film thickness 0.25 µm); delay: 3min; Temperature Program: 50 °C(1)—› 200 °C (8 °C/min) —›300 °C (10 °C/min); Injector Temperature: 250 °C; Split: 20%; Injection volume: 0.2 µL; Carrier gas: He; Flow rate: 1mL/min.

Fig 7. Chromatogram of the small fish samples



Column: VF-5 (l. 30m, i.d. 0.25, film thickness 0.25 µm); delay: 3min; Temperature Program: 50 °C(1)—› 200 °C (8 °C/min) —›300 °C (10 °C/min); Injector Temperature: 250 °C; Split: 20%; Injection volume: 0.2 µL; Carrier gas: He; Flow rate: 1mL/min.

Fig 8. Chromatogram of the small fish samples

Table 1. Concentration of nonadecane in the six different types of small and large fish samples

Sl. No	District	Name of fishes	Location	Concentration (mg/g)
1	Dhaka	Nola	Away from road	0.78 µg/g
2	Comilla	Moiya	Away from road	ND*
3	Chittagong	Sharputi	Away from road	0.63 µg/g
4	Rangamati	Katla	Road side	0.253 µg/g
5	Kushtia	Talapia	Highway road side	0.278 µg/g
6	Feni	Kholsha	Away from road	0.86 µg/g
7	-	Blank	-	N/A

*ND=Not detectable

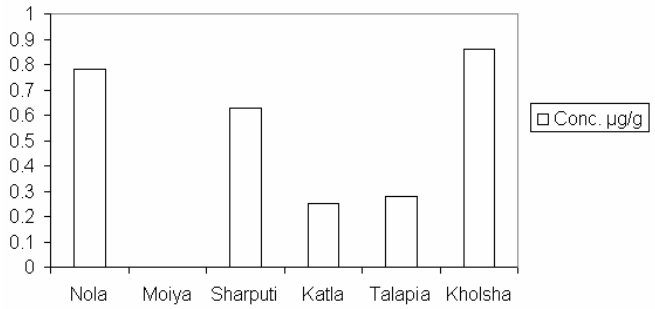


Fig 9. Concentration of nonadecane in the six different types of fish samples.

From experiment we have found that two large fish samples out of six contain toxic nonadecane, but the concentration is too low to reach the permissible limit [11]. We have also seen that the NHs contamination in fish is mainly depended on the sample collection site. At the roadside, the fish samples are normally contaminated by the NHs, but away from the roadside, no NHs were detected by the GC-MS/MS in our

experiment Table 1 and the results are shown in the bar graph Fig 9. On the other hand, large or moderately large fish easily absorbs hydrocarbons with taken oxygen into their flesh, liver etc. So from our experiment we conclude that the fish sample was contaminated mainly by the highway vehicle exhaust, highway tar samples etc. For small fish, it also absorbs hydrocarbons but it is not detectable by our GC-MS/MS instruments.

CONCLUSION

The developed method, combination of liquid-phase extraction with gas chromatographic–mass spectrometric towards analysis of trace normal hydrocarbon, nonadecane in fish samples, was reported for the first time. The method has favorable extraction effect and higher enrichment factor, especially to normal hydrocarbons. This reliable, rapid and convenient method was applied successfully to determine other normal hydrocarbons and polycyclic aromatic hydrocarbons in the fish sample. From the data of nonadecane, it is confirmed that the vehicular emission is the major source of NHs and it has polluted our environment and foodstuff. Therefore, future assessment of the health risk associated with exposure to the NHs content of environmental air and foodstuff sample is recommended.

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