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Research Article

Carcinogenic polycyclic aromatic hydrocarbon (PAH), anthracene in cabbage samples from Bangladesh

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Abstract

The carcinogenic polycyclic aromatic hydrocarbon (PAH), anthracene, in the methanolic crude extract of the leaves of cabbage samples collected from different districts of Bangladesh was analysed by GC-MS. It was observed that PAH deposition on the samples takes place in different morphological parts of the biological materials. The PAH, anthracene, was only found in the cabbage samples collected from the roadside by the extraction of methanolic solvents.

Keywords: pollution, contamination, *Brassica oleracea* var. *capitata* f. *alba*, GC-MS

Introduction

As a class of well known carcinogenic compounds originating from incomplete combustion [1-3], polycyclic aromatic hydrocarbons (PAHs) are among the most important environmental contaminants in China [4], particularly around the fast growing coastal areas, where there is evidence of severe contamination from various sources [5-6] (TEPB, 1996, 2001). PAHs occur as contaminants in various food categories including vegetables, which have been documented to be one of the important contributors to human intake of PAHs [7].

This is particularly true in China given the fact that vegetables are both a basic food and a significant export. Vegetables also play a prominent part in the Bangladeshi diet. It has been reported that plant uptake of PAHs is primarily from the atmosphere through gas and particle-bound depositions and the 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 behaviour for either gas or particle-bound chemicals were established [8]. However, a knowledge gap still remains for establishing a quantitative relationship between the plant accumulation and the level in the air. The aim of the present study is to examine and determine the carcinogenic polycyclic aromatic hydrocarbon, anthracene, in the methanolic crude extract isolated from the leaves of cabbage samples by GC-MS.

Materials and Methods

Chemicals

Methanol and dichloromethane (Merck, Germany), solvents used in this experiment were of HPLC grade. Anhydrous sodium sulphate (Merck, Germany) was cleaned by heating at 200^{o}C before use. Silica gel (60-120 mesh, Merck, Germany) activated at 400^{o}C for 12 hr. prior to use. Phenanthrane of (Sigma-Aldrich) was used as standard in the present study.

Plant material

The cabbage samples were collected from the different districts of Bangladesh in the winter season of 2008 and initially identified by morphological features using a database present in the library at the herbarium of the Department of Pharmacy, University of Dhaka, Dhaka, Bangladesh.

Isolation and preparation of crude extracts

The leaves of the collected cabbage samples were washed by tap and deionized water to removed dust and any other foreign particles. After washing, the leaves were cut into small pieces and dried by sunlight or an oven at below 40^{o}C. The dried cabbage samples were pulverized into powder form. The dried powder (2 g) was extracted three times with methanol (50 ml x3) at 120^{o}C for 1 hr. It was then filtered and the filtrate was evaporated near to dryness by Kuderna-Danish evaporator.

Clean-up procedure

The cleanup column (i. d. = 1 cm) was filled with cotton in the bottom. An activated silica gel (17 gm) soaked with dichloromethane was loaded into the cleanup column (5 cm), which was then topped with 1.5 cm of anhydrous sodium sulphate. Five millilitres of dichloromethane was added to wash the sodium sulphate 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. Fifty millilitres 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 then collected. The collected eluent from the cleanup procedure was further reconcentrated to 0.5 ml with K-D concentrator.

GC-MS analysis

The GC-MS analysis of the methanolic crude extract of cabbage samples was performed using a Varian GC-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 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 280^{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 280^{o}C at 10^{o}C/min. Diluted samples (1/100, v/v, in methanol) of 0.2μl were manually injected in the splitless mode. Identification of compounds of the methanolic 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 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 anthracene (0.5 ng/ml, 1ng/ml, 5 ng/ml, 10 ng/ml and 20 ng/ml) were used for calibration curve.

Results and Discussion

Bangladesh is mainly an agricultural country. Vegetables, crops and fruit are grown here in abundance, mainly in the winter season. Cabbage is one of the most commonly used vegetables because it is cheap and available all over Bangladesh throughout the winter season. Again, to use it for other seasons, sometimes villagers harvest the cabbage samples, cut them into small pieces and dry under sunlight for storage. This harvested and dried product is also used as animal feed. Thus the opportunity also exists for passing contamination through the food chain. For this reason, the objective of this work is to check the highly carcinogenic polycyclic aromatic hydrocarbon, anthracene, in the methanolic crude extract isolated from whole cabbage samples by GC-MS.

The dried cabbage powder sample was extracted with methanol and filtered. The filtrate was cleaned up to remove the vegetable fats and oily or gammy compounds. The methanol solvent was evaporated to a dry state by Kuderna-Danish evaporator. The cabbage samples mainly contained flavonoids, alkaloids, caffice acid and mono, di and triterpene and their respective hydrocarbons [9]. From the concentrated extract only 0.2 μl was injected to the GC-MS. Cabbage samples were collected from ten districts; Dhaka, Barishal, Pabna, Khulna, Kushtia, Rajshahi, Chittagong, Commilla, Jessore and Manikgonj in the winter season of 2008. Ten districts were chosen as it is known that the chemical composition of all kinds of fruit, vegetables and plants depends on the geographical distribution such as temperature, weather, soil condition etc. [10].

The quantitative determination of anthracene was done by external calibration curve method. The calibration curve already prepared with known concentration of anthracene is detailed below in Figure 1.

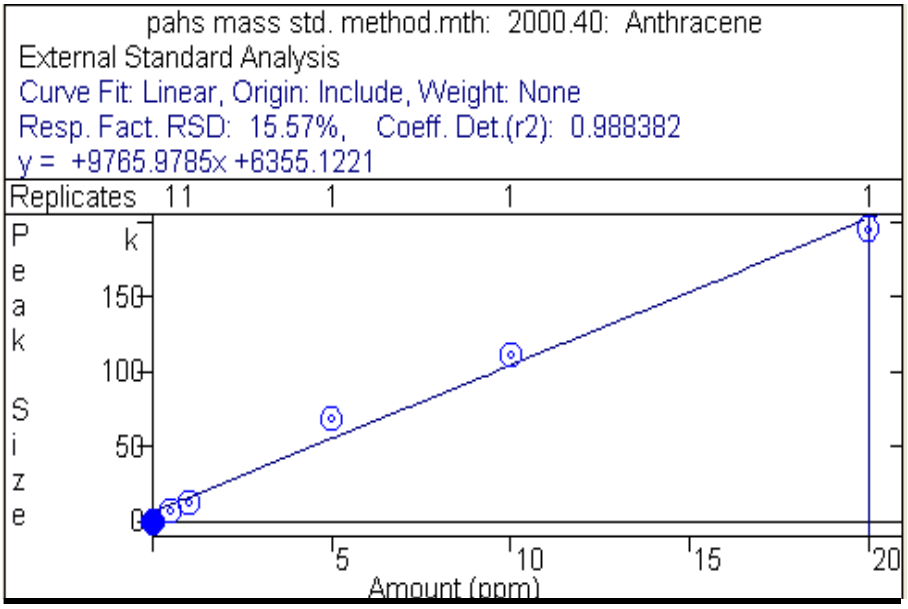

Figure 1. Standard calibration curve of anthracene.

Anthracene is identified by comparing retention time (RT) on the total ion chromatogram (TIC) of the substance in the samples (Fig. 2) 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 anthracene from the cabbage samples in Figure 2 to be 20.782 (base peak, 178.2).

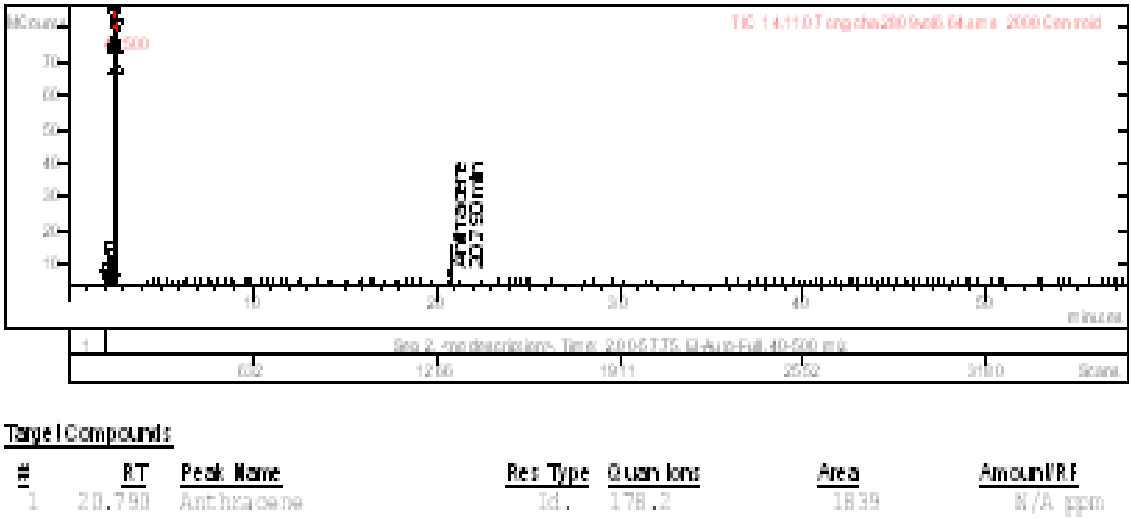

Figure 2. Total Ion Chromatogram of Cabbage Sample

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) —›280°C (10°C/min); Injector Temperature: 250°C; Split: 20%; Injection volume: 0.2 µl; Carrier gas: He; Flow rate: 1ml/min.

Table 1. GC-MS result of anthracene in the ten cabbage samples.

Sl No.	District (Cabbage sample collection area)	Location site	Concentration of anthracene µm/gm
1	Dhaka	Roadside	0.006 µg/g
2	Barishal	Away from roadside	ND*
3	Pabna	Away from roadside	ND*
4	Khulna	Roadside	0.012 µg/g
5	Kushtia	Roadside	0.003 µg/g
6	Rajshahi	Away from roadside	ND*
7	Chittagong	Roadside	0.009 µg/g
8	Comilla	Roadside	0.013 µg/g
9	Jessore	Away from roadside	ND*
10	Manikgonj	Roadside	0.010 µg/g

*ND=Not detectable

Conclusion

For this experiment, ten cabbage samples were collected from both roadside and farms in different districts of Bangladesh. The collected samples were cultivated and harvested during the winter season. The concentration of anthracene, a polycyclic aromatic hydrocarbon, in the ten cabbage samples was measured by GC-MS and the results calculated from the external curve method (Table-1). From the experiment it was found that six cabbage samples collected from the roadside of the districts of Khulna, Kushtia, Comilla, Dhaka, Chittagong and Manikgonj out of ten contain carcinogenic anthracene but the concentration is too low to reach the permissible limit [7]. It was also learnt that PAH contamination in vegetables, fruit and plants is mainly dependent on the sample collection site. From the roadside, the cabbage samples are normally contaminated by PAHs, but away from the roadside, no PAHs were detected by the GC-MS in this experiment (Table 1). It may be concluded from these results that the cabbage samples found to be contaminated was mainly due to vehicle exhaust.

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