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IDENTIFICATION AND QUANTIFICATION OF SOIL PESTICIDES IN COASTAL LAKSHMIPUR DISTRICT OF BANGLADESH

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Abstract

This study was carried out to determine the presence and quantity of some selected pesticides from soil sediments collected from some ponds and canals located in the Lakshmipur district of Bangladesh. The high performance liquid chromatography (HPLC) technique was used to determine the concentration of pesticide residues. Some soil samples were found to be contaminated with carbamate (carbofuran and carbaryl) and organophosphorus (diazinon) pesticides. The concentration of carbofuran pesticide ranged from 0.303 µg/kg to 1.851 µg/kg. The highest concentration of carbofuran pesticide was found in SSP_6 (1.851 µg/kg) and the lowest concentration was found in SSP_9 (0.303 µg/kg). Carbaryl pesticide was found to be present in the sediment of only one pond, the concentration being 1.047 µg/kg. Organophosphorus (diazinon) pesticide was found in soil samples and the concentrations ranged from 0.147 µg/kg to 0.759 µg/kg, which were higher than the EEC-recommended limit of 0.1 µg/kg.

Key words: Carbaryl, Carbofuran, Diazinon, HPLC, Soil

Introduction

Bangladesh is predominantly an agricultural country with an area of 1, 47,570 sq. km. It has only 0.31 percent of the world's total agricultural land, but 2.0 percent of the globe's total population (Hossain *et al.* 2015, Rasul and Thapa 2004). To feed 160 million people, different agrochemicals in the form of pesticides and fertilizers have been used in this limited agricultural land over the last few decades. This practice has led to the build-up of pesticide residues in the products, the destruction of beneficial insects, and pest resurgence. Pesticides have also been associated with environmental pollution (Rashid *et al.* 2015). Exposure of farm workers to pesticides has been causing various types of

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cancer. These pesticides are also causing health hazards for humans such as headache, body aches, skin rashes, poor concentration, feelings of weakness, circulatory problems, dizziness, nausea, vomiting, excessive sweating, impaired vision, tremors, cramps, psychomotor dysfunction, depression, Parkinson's disease and, in severe cases, coma (Fardous *et al.* 2015, Hancock *et al.* 2008). Barring a few, humans have no remedies for the diseases caused by pesticides. Therefore, it is necessary to determine if pesticide residues are present in environmental matrices like sediments.

The World Health Organization (WHO) estimates that there are 3 million cases of pesticide poisoning each year and up to 220,000 deaths, primarily in developing countries (WHO 2001). The application of pesticides is often not very precise, and unintended exposures occur to other organisms in an area where pesticides are applied. Children and many young and developing organisms are particularly vulnerable to the harmful effects of pesticides. Even low levels of exposure during development may have adverse health effects (Sarwar 2015).

In the past, pesticides have contributed significantly to improve the yield of crops, which ensured food security for the global population. The widely cultivated high yielding variety is highly vulnerable to pests and diseases. Thus, the use of pesticides are now an inherent part of agriculture for pest control (Bagchi *et al.* 2008). Pests pose a serious problem because of their high reproduction potential and rapid turnover of generations. Farmers use large quantities of chemical insecticides for effective control of the pest larvae. Therefore, the aim of this study was to investigate the occurrence and amount of pesticides in the soil of a coastal region of Bangladesh.

Materials and Methods

*Collection and pretreatment of samples***:** Twenty-five soil samples (15 soil samples from different ponds and 10 soil samples from different canals) were collected from Sadar Upazila of Lakshmipur district near the coastal area. Bags, permanent markers, spoons, boxes, etc., were used to collect soil samples. Before the collection of soil samples, bags were cleaned with detergent. Bags were marked by a permanent markers.After collection, the samples were brought to the lab at the Agrochemical and Environmental Research Division, Institute of Food and Radiation Biology, Bangladesh Atomic Energy Commission, Ganakbari, Savar, Dhaka. The samples were immediately preserved in a deep fridge (-20ºC) to prevent the loss of the pesticide residues. The samples were collected in August, 2016, which belongs to the rainy season in the country. Heavy rainfall washes out the topsoil and brings pesticide residues into water bodies.

Selection of the study area: Lakshmipur is a small southeastern district of Bangladesh. Chandpur District borders it to the north, Bay of Bengal and Noakhali district to the south, Noakhali District to the east, and Meghna river to the west. About 47.51% people are engaged in agriculture for their occupation in Lakshmipur district. The main crops in this district are paddy, wheat, potato, pulse, sugarcane, etc. But paddy is the most cultivated crop. Our study area is Lakshmipur Sadar Upazila, which is located at 22.95°N and 90.82°E (Fig. 1). In the Sadar Upazila, paddy and vegetables, especially cucumber are cultivated the most. 'Basudin', an organophosphorus pesticide, is used by the farmers which contains 'Diazinon'. Another brand of pesticide, which contains mostly 'Carbaryl' and 'Carbofuran' of 'Carbamate' is also used by the farmers. Most of the paddy fields are located near ponds, and most of the time, the drainage water of those paddy fields flows to the nearest ponds. Some ponds are lying, and their banks are too low to prevent the incoming water from the nearby paddy fields in rainy seasons. The vegetable fields are located near the canals and the drainage water flows to the canals.

Sample processing in laboratory: Fifty (50) gm soil sample was taken in a conical flask (250 ml) and 100 mL of solvent (a mixture of hexane and acetone at 1:1) was added to the conical flask. The conical flask was then shaken for 6-7 hrs using a mechanical shaker and the contents were allowed to settle down. Finally, the extract (hexane: acetone mixture) was collected from the conical flask. Similarly, the extract mixture was collected two more times with 25 ml solvent by hand shaking for 5 min. Ten gm of anhydrous sodium sulphate Na_2SO_4 was added to the combined extract and the contents were allowed to settle. The solvent was then decanted and subsequently evaporated by a rotary vacuum evaporator to dryness. Two (2) mL of HPLC-grade acetonitrile was added in three portions and the sample extracts were collected into a vial for clean-up. The extract was subjected to clean-up using florisil column chromatography, where necessary. The top 1.5 cm of the florisil column was packed with anhydrous Na_2SO_4. Elution was done with 2% diethyl ether in hexane (5 ml/min). The eluate was concentrated in a rotary vacuum evaporator and transferred to glass-stoppered test tubes. The solvent was completely removed under mild nitrogen flow. The evaporated sample was dissolved in acetonitrile and the volume was made up to 1 ml in a volumetric flask for analysis in high performance liquid chromatography (HPLC) (Uddin *et al.* 2016, 2018).

Identification and quantification procedures: The analysis was conducted by an HPLC (Shimadzu, Japan) LC-10ADvp, equipped with an SPD-M 10 Avp attached to a photodiode array detector (Shimadzu SPD-M 10 Avp, 200-800 nm). A C18 Reverse Phase Alltech (250 × 4.6 mm, 5 µm) was used as the analytical column, and the column temperature was maintained at 30^0C. Acetonitrile in distilled water (70:30) was used as

the mobile phase at a flow rate of 1.0 ml/min. Prior to HPLC analysis, the samples were filtered through 0.45 µm nylon (Alltech Associates, IL, USA) syringe filters. The chromatograms were obtained following manual injection (20 µl) of both standard and sample solution. The suspected pesticides were identified based on the retention times of the respective standard pesticide preparation.

For the preparation of the calibration curve, equal volumes of several different concentrations of standard solutions were injected into the HPLC machine. Tentative identification of the suspected pesticides was carried out in relation to the retention time (RT) of the pure analytical standards. Quantification was performed according to the calibration method described by Bhattacharjee *et al.*, (2012). For this purpose, the injection of equal volumes of differently concentrated standard solutions into HPLC prepared calibration curve for each pesticide. To determine the residual levels of pesticides, the following equation was used:

$$R' = \frac{H_A V_{END} W_{ST}}{H_{ST} V_i G}$$

Where,

R' = mg/l for water and mg/Kg for soil

G = Sample weight (l or Kg)

V_{END} = Terminal volume of the sample solution (mL)

V_i = Portion of volume V_{END} injected into HPLC (µL) column

W_{ST} = Amount of standard pesticides injected with standard solvent (µg)

H_A = Peak area obtained from V_i (mm^2)

H_{ST} = Peak area obtained from W_{ST} (mm^2)

Extraction efficiency/recovery: The validation of the analytical method was performed according to the European Commission (EC) guidelines in terms of the accuracy, precision, and limit of quantification (LOQ) (DG SANCO, 2010). Accuracy was calculated by analyzing the samples of known concentration (n =3) and comparing the estimated values with the actual values. Within our experimental limit, the mean recovery for accuracy should be within 70-120%. For accuracy experiments, soil (50 g) was utilized as a matrix after homogenization and the addition of an appropriate amount of pesticide standards at two different fortification levels (0.02 and 0.20 ppm). Control samples were processed along with spiked ones. Both sample and standard preparation

were allowed to stand for one hour to permit equilibration. Equilibration was followed by the extraction and clean up process, as described above. Percentage recovery was calculated by the following equation:

Percentage recovery = [CE/CM × 100]

Where CE is the experimental concentration determined from the calibration curve and CM is the spiked concentration. The precision was estimated by monitoring the repeated (n=6) peak response and expressed by the relative standard deviation (RSD). The acceptance criterion for precision is RSD ≤ 20%. Analytical procedures employed are found to be satisfactory and average recoveries between 72% and 95% were obtained for Carbofuran, Carbaryl and Diazinon pesticides from the soil samples (the fortifications were made in the range 0.02-0.2 ppm level), indicating the suitability of the methodology. The LOQ and LOD were evaluated as signal-to-noise ratios (S/N) of 10:1 and 3:1, respectively. In the present study, the LOD and LOQ were 0.01 mg/kg and 0.05 mg/kg, respectively.

Results and Discussion

Toxic pesticides are widely used in agricultural lands for prevention of valuable crop losses by pests. Pesticides improve yield as well as the quality of the product. However, pesticides pose serious health risks to farmers exposed to pesticides when they mix and apply for pesticides or work in the pesticides-treated fields. People in general also get exposed to the pesticide residues in food and drinking water. In this investigation, Sadar Upazila of Lakshmipur district was taken as the study area. The outer part of Sadar Upazila belongs to “lotic” ecosystem because of the presence of the Meghna River, while the inner part of it belongs to “lentic” ecosystem because of the presence of many man-made ponds and some natural water sources such as pools, canals, and lakes. Most of the man-made ponds and canals are in close proximity to these agricultural fields. Therefore, when soils of agricultural fields are contaminated by pesticides, the nearby water bodies are also contaminated. Pesticide residues find their way to these water bodies through surface run-off during precipitation and after irrigation.

Tables 1 and 2 show the average concentration of pesticide residues in soil samples collected from different ponds and canals of Sadar Upazilla, Lakshmipur. The retention time and area for carbofuran, carbaryl, and diazinon are presented in Tables 3-5. Fifteen soil samples were collected from ponds and ten soil samples were collected from canals of the area. Six soil samples representing ponds and canals were found to be

contaminated with residues of carbofuran. Carbofuran was present in SSP_6, SSP_7, SSP_9 and SSC_6, SSC_7, SSC_{10}. The concentration of carbofuran ranged from 0.303 to 1.851 µg/kg. The highest concentration (1.851 µg/kg) was found in SSP_6 and the lowest concentration (0.303 µg/kg) was in SSP_9. Carbaryl residue was observed only in the SSP_8 sample with a concentration of 1.047 µg/kg. Table 1 and Table 2 also show the soil samples that were found to be contaminated with diazinon. Four soil samples, namely SSC_9, SSP_1, SSP_{13}, and SSP_{15}, were contaminated with diazinon. The highest concentration (0.759 µg/kg) was found in SSP_{15}, and the lowest concentration (0.147 µg/kg) was found in SSP_1.Data of the present study indicate the contamination of soil with an organophosphate. Organophosphorus pesticides have been widely used in Bangladesh since 1990 because organochlorine insecticides were banned due to their persistence as well as acute toxicity in the environment (Chowdhury *et al.* 2012). The use of organophosphorus and carbamate pesticides such as chlorpyrifos, diazinon, malathion, carbofuran, and carbaryl has greatly increased because of their less detrimental effects on the environment (Chowdhury *et al.* 2012). Many studies reported very high concentration of organophosphate and carbamate residues in the soil and water of Bangladesh (Bhattacharjee *et al.* 2012, Chowdhury *et al.* 2012, Chowdhury *et al.* 2013, Shammi *et al.* 2014). In our previous study, carbofuran was detected at a higher concentration of 3.21 µg/kg, carbaryl at 2.52 µg/kg and diazinon at 0.235 µg/kg in samples collected from vegetable and paddy field in the coastal district Feni of Bangladesh (Uddin *et al.* 2018). Carbofuran and carbaryl concentrations in the present study were lower than those of the previous study. On the other hand, diazinon concentration was found to be at a higher level than our previous study. However, the contamination level of organophosphorus and carbamate was relatively low compared to the IAEA/FAO/Codex Alimentarius Guideline values.

Carbamate pesticides were observed in soils of both pond and canal near the paddy and vegetable fields of Sadar Upazila. Among the carbamate and organophosphate pesticides, diazinon, carbofuran and carbaryl are frequently used in Bangladesh. When the soil samples were collected, farmers said, they had been using diazinon, carbofuran, and carbaryl. Therefore, the study was carried out to determine the presence and quantity of diazinon, carbofuran, and carbaryl pesticides. A better understanding about degradation of these pesticides in soil and the factors affecting the degradation process may help in the judicious application of these pesticides and the consequent mitigation of environmental pollution (Chowdhury *et al.*, 2002). There is no data on pesticide pollution in the studied area. Therefore, this study provides some baseline data to help future investigation on pesticide pollution in the area.

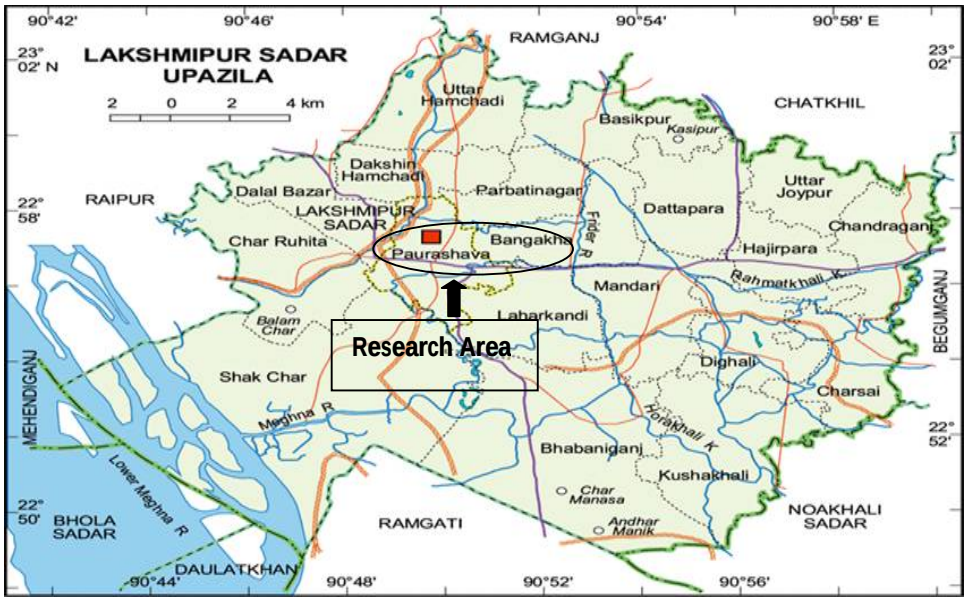

Fig. 1. Map of Sadar Upazila showing the sampling locations.

Table 1. The average concentration of pesticide residues in soil samples which were collected from different ponds of Sadar Upazila, Lakshmipur.

Sample ID	Sampling location	Carbofuran (µg/kg)	Carbaryl (µg/kg)	Diazinon (µg/kg)
SSP_1	Kachari Bari pond	BDL	BDL	0.147
SSP_2	Lakshmipur Markaz Mosque Pond	BDL	BDL	BDL
SSP_3	Monir Uddin Patwary Bari Pond	BDL	BDL	BDL
SSP_4	Tomehar Bazar Pond	BDL	BDL	BDL
SSP_5	Tomehar Bazar Mosque Pond	BDL	BDL	BDL
SSP_6	West Shayedpur Mosque Pond	1.851	BDL	BDL
SSP_7	Pearapur Bazar Pond	0.167	BDL	BDL
SSP_8	Vobanigonj Bazar Pond	BDL	1.047	BDL
SSP_9	Mia Bari Big Pond	0.303	BDL	BDL
SSP_{10}	Chourasta Bazar Pond	BDL	BDL	BDL
SSP_{11}	Jakshin Road Point Pond	BDL	BDL	BDL
SSP_{12}	Vobachat Bazar Pond	BDL	BDL	BDL
SSP_{13}	Torabgonj Borobari Pond	BDL	BDL	0.395
SSP_{14}	Torabgonj Bazar Pond	BDL	BDL	BDL
SSP_{15}	Koroitola Bazar Pond	BDL	BDL	0.759

Note: SSP = Soil Sample of Pond, BDL = Below Detection Limit and µg/kg = microgram per kilogram.

Table 2. The average concentration of pesticides residues in soil samples which were collected from different canals of Sadar Upazila, Lakshmipur.

Sample ID	Sample Sources	Carbofuran (µg/kg)	Carbaryl (µg/kg)	Diazinon (µg/kg)
SSC_1	Meghna River	BDL	BDL	BDL
SSC_2	Wapdar Khal West Point	BDL	BDL	BDL
SSC_3	Wapdar Khal Middle Point near Voberhat	BDL	BDL	BDL
SSC_4	Wapdar Khal North Point near Torabgonj	BDL	BDL	BDL
SSC_5	Wapdar Khal near Lakshmipur Bas Terminal	BDL	BDL	BDL
SSC_6	Rahmat Khali Khal near Meghna River	1.732	BDL	BDL
SSC_7	Rahmat Khali Khal near West Point	1.657	BDL	BDL
SSC_8	Rahmat Khali Khal near Cucumber field (Koroitola)	BDL	BDL	BDL
SSC_9	Rahmat Khali Khal north Point	BDL	BDL	0.222
SSC_{10}	Lakshmipur Khal near Ramgong Highway Road	1.069	BDL	BDL

Note: SSC = Soil Sample of Canal, BDL = Below Detection Limit and µg/kg = microgram per kilogram.

Table 3. Sample Analysis Report of Carbofuran.

Sample No	Retention Time	Area	Concentration (µg/kg)
SSC_6	3.334	332133	1.732
SSC_7	3.351	317882	1.657
SSC_{10}	3.367	205149	1.069
SSP_6	3.357	355086	1.851
SSP_7	3.304	32159	0.167
SSP_9	3.344	58121	0.303

Table 4. Sample Analysis Report of Carbaryl.

Sample No	Retention Time	Area	Concentration (µg/kg)
SSP_8	3.549	604601	1.047

Table 5. Sample Analysis Report of Diazinon.

Sample No	Retention Time	Area	Concentration (µg/kg)
SSC_9	3.329	76875	0.222
SSP_1	3.296	51027	0.147
SSP_{13}	3.296	136634	0.395
SSP_{15}	3.284	262320	0.759

[SSC=Soil Sample of Canal; SSP= Soil Sample of Pond].

The wide use of pesticides in Bangladesh causes major health and environmental problems. It is necessary to learn more about the problems caused by exposure to pesticides with respect to safety, health and the environment. A guideline should be provided for farmers on pesticide usage, safety instructions, preparation, application and disposal, and washing of equipment. As a result, the health hazards of pesticides may be reduced to a great extent. The environment and ecosystems will also be protected from the scourge of pesticide pollution. The present study has implications considering the health hazard associated with these pesticides. A further exhaustive investigation needs to be carried out to assess the status of these pesticides in foodstuff and in the food chain.

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