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Research Article

Organophosphate, carbamate and synthetic pyrethroid pesticide residues in muscle tissues of fish from Loktak Lake, a Ramsar Site in Manipur, India

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ABSTRACT

The muscle tissues of *Channa punctatus* and *Anabas testudineus* collected from the Loktak Lake (a Ramsar site) and its three major feeder rivers in Manipur, Northeastern India, were analyzed using high-performance liquid chromatography for the presence of residues of organophosphorus, carbamate, and synthetic pyrethroid pesticides. Pesticide residues of all the three typeswere detected in the fish tissues. Pesticide residues in *Channa punctatus* ranged from 0.002–0.043 µg g^{-1}, and from 0.008–0.027 µg g^{-1} in *Anabas testudineus* from Loktak Lake in pre-monsoon and post-monsoon seasons. Pesticide residues were detected only in *Anabas testudineus* (0.002–0.078 µg g^{-1}) in Nambul River, while these were detected only in *Channa puctatus* (0.001–0.032 µg g^{-1}) in Moirang River. In Nambol River, pesticide concentrations ranged from 0.002–0.026 µg g^{-1} in *Channa punctatus*, and from 0.004–0.005 µg g^{-1} in *Anabas testudineus*. Among the five pesticides detected, concentrations of dichlorvos residues detected in the present study (0.027 and 0.032 µg g^{-1} wet weight) exceeded the *Codex Alimentarius* maximum residue limit (MRL) of 0.01 mg kg^{-1} for animal tissues. The rest of the compounds were within the MRL. None of the pesticide residues was detected in the two fish species collected from the control or reference site. The present study indicates that pesticide contamination is emerging as a threat to the water quality and aquatic biodiversity of Loktak Lake, which calls for more detailed studies on the extent and magnitude of these threats.

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INTRODUCTION

Pesticides, besides killing the target pests, also kill or adversely affect the non-target organisms, contaminate the environment, and pose threats to aquatic ecosystems and human health [1]. Organophosphates, carbamates and synthetic pyrethroids are among the commonly used pesticides known to affect human health and the environment [2]. It is well established that organophosphate pesticides are the long lasting inhibitors of cholinesterases and a number of organophosphates are prohibited in many countries from being sold in the market due to their toxicity at low doses [3]. The carbamates are known to impair acetylcholinesterase (AChE) activity in a way similar to the mode of action of organophosphate pesticides. They are also known to cause reproductive failure through endocrine disruption and infertility [4]. Synthetic pyrethroids which are fast replacing organophosphates are also extremely toxic to fish because

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of their neurotoxic effects [5, 6]. Therefore, the detection of the residues of these pesticides in different environmental compartments becomes an issue of global concern.

Pesticides applied in agricultural fields can reach aquatic ecosystems via surface runoff and leave harmful residues in the ecosystems that can adversely affect aquatic biota, particularly fish. The pesticide residues in aquatic ecosystems are often transferred to humans through the food chain via phytoplankton, zooplankton and fish [7]. Several studies have shown that continuous intake of pesticide residues through food, even in lowdoses, can cause adverse effects on the environment as well as on plants, fish, wildlife, and other non-target organisms [8]. These effects could comprise endocrine disruption, neurological damages, and birth defects [9–11].

Several organophosphate, carbamate and synthetic pyrethroid pesticides are being increasingly usedfor controlling agricultural pests in Manipur - a state in the northeastern region of India - during the past several decades. Many studies have suggested that indiscriminate application of pesticides in agricultural activities such as in paddy fields and other cultivated areas near aquatic ecosystems have increased the probability of the entry of pesticide residues into the lakes where the rivers finally drain [12–14]. In this regard, Loktak Lake in Manipur, which is a "wetland of international importance" (Ramsar Site) and the largest freshwater Lake in northeast India, is highly vulnerable to contamination by pesticide residues through its three major feeder rivers: the Nambul, the Moirang and the Nambol [15]. These rivers receive agricultural and domestic wastes from inhabited areas to ultimately drain into the Loktak Lake, a Ramsar site in Manipur, India. The heavy influx of such wastes threatens the biodiversity of the lake, especially aquatic macrophytes and indigenous freshwater fishes. The situation has been made more precarious by the construction of the Ithai barrage, which has obstructed the outlet of the lake. This has affected the normal movement of water in the lake, thereby raising the possibility of accumulation of pesticide residues in water, sediment and biota, particularly fish, in the Lake. It is also pertinent to mention here that different species of fish which were once abundantly available in the Lake such as (state fish of Manipur) etc. are now becoming threatened [16]. It is possible that reproductive failure due to continuous exposure to low concentrations of pesticide residues accumulated in the Lake over a period of time could be one of the reasons for the decimation of fish fauna in the Lake.

Among aquatic organisms, fish is considered a useful component for monitoring environmental contaminants and an important source of animal protein to humans. Contamination of fish by pesticide residues, therefore, has proven to be a serious risk to its consumers including humans [17]. The two fish species, *Channa punctatus* and *Anabas testudineus* selected in this study are abundantly available in Loktak Lake, and also comprise favorite food items of the local people. While *Channa punctatus* is a carnivore, *Anabas testudineus* is an omnivore [18, 19].

Several analytical methods have been used for determination of pesticide residues in foodstuffs including fisheries products [17, 20]. Among these methods, high-performance liquid chromatography (HPLC) is regarded as a useful tool in the laboratory because of its highly precise quantitative results, very low detection limits and an exceptional degree of selectivity for qualitative identifications of target analytes [21]. Moreover, HPLC with a diode-array detector (DAD) is considered as an advanced method which has the capability of collecting chromatographic data and UV spectra simultaneously [22].

In the last few years, several studies have been conducted worldwide to analyze the presence of pesticide residues in freshwater ecosystems [20, 23–33]. On the other hand, investigations on detection of pesticide residues in freshwater ecosystems in India are relatively rare and more so in the freshwater lakes and wetlands of North-east India [34]. In this context, this study investigates the presence of pesticide residues in two fish species *Channa punctatus* and *Anabas testudineus* representing two different trophic levels, and collected from the Loktak Lake and its three major feeder rivers. Six pesticides including three organophosphates (dichlorvos, malathion and monocrotophos), one carbamate (carbofuran), and two synthetic pyrethroids (deltamethrinand cypermethrin), which are commonly used in the study area, were selected for analysis and possible detection in fish muscle. This is probably the first study that aims to ascertain the status of pesticide residues in fish of Loktak Lake. The findings are expected to throw light on the status of pesticide contamination of Loktak Lake and serve as an 'early warning report' of the threats posed by pesticides to this important ecosystem along with potential health risks to the people of Manipur who are consumers of its fish.

MATERIALS AND METHODS

Study Sites

Loktak Lake (24°33′N 93°47′E) of Manipur, North East India and its three major feeder rivers, viz., the Nambul River (northern feeder), the Nambol River (western feeder), and the Moirang River (south western feeder), were selected for the present study. Loktak Lake, about 45 km from Imphal, the capital of Manipur, is a "wetland of international importance" (Ramsar Site) since 1990 and is well-known for being the only natural habitat of the endangered brow-antlered deer (Ru*cervus eldii eldii*) (Fig. 1). It is also the largest freshwater lake in the northeastern region of India. The central part of the lake was selected for the study because most of the central areas of the lake are covered by a large number of 'phumdis' (heterogeneous mass of floating vegetation) due to the construction of the Ithai Barrage across the natural outlet of the lake. Hence, the possibility of the concentration of pesticide residues was high in this part of the lake. Study sites selected in the three tributaries were near their point of entry into the lake after traversing extensively cultivated and inhabited areas. A fish pond, which was about 5.7 km away from paddy fields or other cultivated areas and 35 km away from the study sites of Loktak Lake, was selected as the control or reference site.

Procurement of Fish, Selection of Sampling Season and Experimental Design

Specimens of *Channa punctatus* and *Anabas testudineus* were collected from the five sampling sites in three seasons, viz., pre-monsoon (April–June), monsoon (July–September) and post-monsoon (October–December). Different types of nets, especially conventional gill nets were used to capture the fish with the help of local fishermen. The net was hauled at short intervals and trapped fish, if any, were immediately transferred to polythene bags containing water of the given site and saturated with extra oxygen. Since both the fish species are air-breathing, this practice of sampling exerted minimum stress. Fish samples comprising of six individuals of similar size (length and weight 24±1 g and 15±1 cm, respectively, for *Channa punctatus*; 22±1 g and 11±1 cm, respectively, for *Anabas testudineus*) belonging to both species were collected from each study site in the three seasons. The fish were brought to the laboratory and immediately sacrificed by injecting a high dose of Tricaine Methanesulfonate (MS-222) after taking their morphometric measurements. Each fish was dissected and muscles from the mid dorsal side were collected. The analysis of pesticide residues in the sampled fish muscles tissues was conducted by using high-performance liquid chromatography (HPLC) with diode-array detector (DAD).

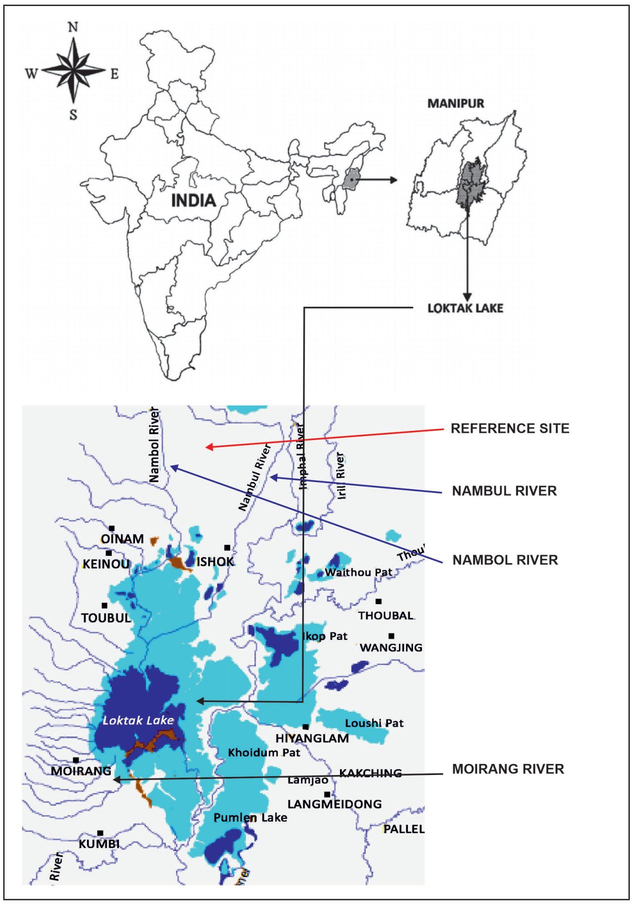

Figure 1. Map of Loktak Lake showing its feeder rivers of Nambul, Nambol and Moirang along with reference or control site.

High-Performance Liquid Chromatography Analysis of Pesticide Residues

Procurement of Pesticide Standards and Reagents

Altogether six (6) pesticide standards belonging to three major pesticide groups were purchased from the Sigma-Aldrich Laborchemikalien GmbH D – 30918 Seelze, Quality Management SA-LC. These comprised three organophosphates (malathion, monocrotophos, dichlorvos), one carbamate (carbofuran), and two synthetic pyrethroid (deltamethrin and cypermethrin) pesticides. The purity of all these pesticide standards was over 99% except for Cypermethrin (94.3%). Besides, HPLC grade water, acetone and acetonitrile were also purchased from Merck.

Equipments

The equipments used in various analytical procedures were: Shimadzu AUW 220D analytical balance, micropipette 200 μL and 1000 μL (Oxford, Ireland), rotary evaporator (Buchi, model 011, Switzerland), centrifuge (Remi Instrument Ltd.), and HPLC Agilent 1260 Infinity - DAD detector (Germany).

Procedure for Extraction of Pesticide Residues from Fish Muscle Tissues

The pesticide residue extraction procedure was performed by following a slight modification of the standard protocol of Sun et al. [17]. Six replicates of the two fish species *Channa punctatus* and *Anabas testudineus* collected from each study site were dissected and 2 g of muscle tissues for each fish species (either *Channa punctatus* or *Anabas testudineus*) in a pool were homogenized in a mortar and pestle with 5 ml of HPLC grade acetone. The resultant extracts were centrifuged at 4000 rpm for 10 minutesto remove all the cell debris and unwanted materials [35]. Two ml of the extracts were then evaporated to dryness under reduced pressure in a rotary evaporator at 40 °C. Finally, the dry extract was dissolved in 2 ml of HPLC grade acetonitrile and the resulting extracts were then centrifuged at 4000 rpm for 10 minutes and the supernatant was used for HPLC analysis. The sample preparation or sample extraction was repeated thrice to have three replicates of each sample from a study site of a particular season.

HPLC Conditions and Analysis

The standards and samples were analyzed for pesticide residues by using HPLC Agilent 1260 Infinity having DAD detector. Standard calibration curves of each pesticide were drawn with three concentrations i.e. 1 ppm, 50 ppm and 100 ppm for each standard pesticide (dichlorvos, malathion, monocrotophos, carbofuran, deltamethrin and cypermethrin) respectively. Identification of pesticides was executed by comparing sample retention times with those obtained for the standards, while quantification of different pesticides was done by comparing the areas of sample peaks with those of their respective standards. All the glassware was washed with detergent,

Table 1. Concentrations of organophosphorus, carbamate and synthetic pyrethroid pesticides (μg g^{-1} wet weight) in muscles of two fish species from Loktak Lake and its three major feeder rivers along with a reference site in three seasons

Concentration in muscle tissue (μg g-1 wet weight)						
Fish species	**Season**	**Loktak lake**	**Moirang river**	**Nambol river**	**Nambul river**	**Ref. site**
Channa punctatus	Pre-monsoon	Mt: 0.002±1E-04**	Mt: 0.001±5.77E-05**	ND	ND	ND
	Monsoon	Dv: 0.027±0.0006	Dv: 0.032±0.0006	ND	ND	ND
		Cf: 0.043±0.0006		Mt: 0.002±5.77E-05**		
	Post-monsoon	Mt: 0.027±0.0006**	ND	Cf: 0.026±0.0006	ND	ND
Anabas testudineus	Pre-monsoon	Dm: 0.009±5.77E-05*	ND	Cm: 0.004±0.0001*	Mt: 0.019±0.0004**	ND
					Cm: 0.002±5.77E-05*	
	Monsoon	ND	ND	ND	ND	ND
	Post-monsoon	Mt: 0.008±5.77E-05**	ND	ND	Mt: 0.078±0.0004**	ND
				Mt: 0.004±5.77E-05**		
				Cf: 0.005±5.77E-05		

The values represent mean±SD {significance level: p≤0.05(*), p≤0.01(**)}. Ref: Reference; Cf: Carbofuran; Cm: Cypermethrin; Dv: Dichlorvos; Dm: Deltamethrin; Mt: Malathion; ND: Not detected.

rinsed with HPLC grade water and heated at 180 °C for 2 h before use. We tested different wavelengths between 202 and 290 nm for the optimum identification of pesticides in different samples. The wavelengths of 202 nm for deltamethrin, cypermethrin and malathion; 220 nm for dichlorvos; and 272 nm for carbofuran and monocrotophos were observed to be the optimum. Based on their respective wavelengths detected, pesticides were grouped as group 1, group 2 and group 3 employing standard HPLC conditions. Group 1 compriseddeltamethrin, cypermethrin and malathion, group 2 dichlorvos, and group 3 carbofuran and monocrotophos. The column and injection volume for all the three groups were Column (SB-C18), 250 × 4.6 mm, 5.0 μm and 20 μL, respectively. The flow rate was 1 ml min^{-1} for group 1, and 1.4 ml min^{-1} for both group 2 and 3. Mobile phase ratio for group 1 was acetonitrile/water (10% acetonitrile for 5 minutes, gradually increased to 100% acetonitrile within 15 minutes and then maintainedat 100% acetonitrile for 5 minutes) with a run time of 20 minutes; for both group 2 and 3, it was acetonitrile/water (40:60) with a run time of 10 minutes each. Sample analysis of three replicates of a particular fish (*C. punctatus* or *A. testudineus*) sample from a study site in a particular season was done consecutively following above conditions of HPLC.

Data Analysis

The data were first checked for normality with the Shapiro-Wilk test. Since the data were not normally distributed, these were normalized by log transformation. Statistical significance of differences in concentration of pesticide residues among the sampling sites and fish species were determined by One-Way Analysis of Variance (ANOVA) using SPSS 20 software. The results were denoted significant at p ≤0.05, and highly significant at p ≤0.01.

RESULTS AND DISCUSSION

Standard Calibration Curves and Chromatogram

Calibration graph of the standard solution concentrations of each pesticide are shown in Appendices 1–6. The cal- culations were made following the equation y = mx + b, where m is the regression co-efficient, y is the area, x is the amount and b is the residual standard deviation. The retention times of the standard pesticides were found to be 2.13, 13.55, 0.74, 2.74, 16.28 and 16.14 minutes for dichlorvos, malathion, monocrotophos, carbofuran, deltamethrin and cypermethrin, respectively. Moreover, the standard calibration curves of peak area against concen tration as well as chromatograms of dichlorvos, malathion, monocrotophos, carbofuran, deltamethrin and cyper methrin are shown in Appendices 1–6.

Pesticide Residue Analysis in Fish Muscle Tissue

The occurrence of the residues of the six pesticides in the muscle tissues of *Channa punctatus* and *Anabas testudineus* are presented in Table 1. Of the three organophosphate pesticides analyzed in the present study, dichlorvos residues were detected only in *Channa punctatus* collected from Loktak Lake (0.027±0.0006 μg g^{-1}) and Moirang River (0.032±0.0006 μg g^{-1}) during monsoon season. Malathion residue was detected in both *Channa punctatus* and *Anabas testudineus* collected from Loktak Lake (0.002±1E-04**μg g^{-1}; 0.027±0.0006**μg g^{-1}, 0.008±5.77E-05**μg g^{-1}), and Nambol River (0.002±5.77E-05**μg g^{-1}; 0.004±5.77E-05**μg g^{-1}) during pre-monsoon and post-monsoon seasons (p≤0.01), however, in Moirang River malathion residue was found only in *Channa punctatus* (0.001±5.77E-05**μg g^{-1}) in pre-monsoon; Nambul River only in *Anabas testudineus* collected during pre-monsoon (0.019±0.0004**μg g^{-1}) and post-monsoon (0.078±0.0004**μg g^{-1}) seasons respectively (Table 1). Monocrotophos residue was not detected in fish collected from any study site in the three seasons. Residues of none of the organophosphorus pesticides were detected in the samples of the two fish species collected from the control or reference site (Table 1).

Dichlorvos residues detected in the present study (0.027 and 0.032 μg g^{-1} wet weight) were above the MRL of 0.01 mg kg^{-1} in animal tissue and products such as edible mammalian offal, eggs, mammalian fats, meat, milk, and fat, meat and offal of poultry, as laid down in the *Codex Alimentarius* [36]. An earlier study revealed that the strongest inhibition of brain AChE was found in association with high dichlorvos residues [37]. This indicates that the bioaccumulation of dichlorvos residues in fish tissues in the study area even in small quantities may alter or inhibit brain AChE- enzyme activities in fish which in turn could similarly affect its consumers including larger carnivorous fish, fish-eating mammals like otters, and humans, through the food chain. Brodeur et al. [26] also detected dichlorvos in tissues of one-sided livebearer fish (*Jenynsia multidentata*) which further revealed an inhibition in the activity of ChE in association with reduced body condition. The *Codex* limit for malathion in animal products is not available, although its concentrations were found to be higher on two occasions in *Anabas testudineus* than the MRL prescribed for some plant products such as sweet corn and tomato juice (0.02 and 0.01 mg kg^{-1}, respectively) [36]. Among all the pesticides, malathion residues were detected more frequently and in more study sites and seasons ($p \leq 0.01$)in both the fish species (Table 1), reflecting the fact that it is one of the most commonly applied pesticide in the study area. Amaraneni and Pillala [38] reported the detection of malathion residue in tissues of fish *Channa striata* and *Catla catla* collected from Kolleru Lake in India where the value was found to be 2.5 μg g^{-1} wet weight, which was considerably higher than the highest concentration of malathion residue detected in the present study, i.e. 0.078 μg g^{-1} wet weight in *Anabas testudineus* collected from the Nambul River during post-monsoon season (Table 1). Maurano et al. [39] detected malathion residues in the muscle tissues of two fish species *Carassius carassius* and *Mugil cephalus* collected from Sele River in SouthItaly where the concentrations were found to be 480 pg g^{-1} and 582 pg g^{-1} wet weight, respectively, which were comparatively much less than the lowest concentration of malathion residue detected in the present study i.e. 0.001 μg g^{-1} wet weight in *Channa punctatus* collected from the Moirang River during pre-monsoon season (Table 1). Possible reasons for not detecting monocrotophos residues in the present study include its rapid hydrolysis and conjugation in fish tissues [40].

Carbofuran is a broad spectrum carbamate pesticide used world-wide to control insects, mites and nematodes, which has been widely detected in surface, ground and rain waters and is extremely toxic to aquatic organisms including fish [20, 41]. In our study, carbofuran was detected in *Channa punctatus* collected from Loktak Lake (0.043±0.0006 μg g^{-1}) and Nambol River (0.026±0.0006 μg g^{-1}) and in *Anabas testudineus* collected from Nambol River (0.005±5.77E-05 μg g^{-1}) during post-monsoon (Table 1). The value of 0.043 μg g^{-1} recorded in *Channa punctatus* in Loktak Lake in post-monsoon was close to its *Codex* MRL of 0.05 μg g^{-1} in animal products such as cattle, goat, sheep, pig and horse fat, and offal of cattle, goat, horse, sheep and pig [36]. Thus, carbofuran posed a health risk to the consumers of this fish in this area. Jabeen et al. [20] detected carbofuran residues in muscle tissues of fish *Labeo rohita* and *Channa marulius* collected from the Indus River around Mianwali where the concentrations ranged from 0.0425–0.066 μg g^{-1} and 0.613–0.946 μg g^{-1}, respectively. Carbofuran concentrations in *Labeo rohita* were comparable to its levels in *Chana punctatus* in our study. Vryzas et al. [42] also mentioned about the detection of carbofuran at the highest concentration on a regular basis in the three trophic levels: algae, aquatic invertebrates and fish in drainage canals of two transboundary rivers of northeastern Greece, where its extreme concentrations were observed just after the occurrence of high rainfall during pesticide application, thus posing a threat to aquatic organisms and subsequently to the fish-consumers. Mahboob et al. [43] also recorded concentrations of carbofuran residue in the muscle tissues of fish, *Catla catla* at 1.23 and 8.53 μg g^{-1} lipid-normalized weight. However, carbofuran is known to undergo rapid degradation and elimination from fish tissues [40].

In the last two decades, synthetic pyrethroids have been replacing organochlorines and organophosphates in India for controlling pests to increase agricultural productivity [7]. Deltamethrin and cypermethrin are commonly used synthetic pyrethroids in agriculture for controlling pests. Being type 2 (α-cyano) synthetic pyrethroids, these two pyrethroids are more potently neurotoxic than the non-cyano type 1 [6]. In the present study, deltamethrin residues were only recorded in the muscle tissue of *Anabas testudineus* collected from Loktak Lake during the pre-monsoon season ($p \leq 0.05$) at a concentration of 0.009±5.77E-05*μg g^{-1} (Table 1) which was below the maximum residual limit (MRL) of 0.02 mg kg^{-1} in poultry offal [36]. Notwithstanding this, it may be noted that deltamethrin could disrupt enzyme activities and reduce glycogen and protein levels in fish *Anabas testudineus* at a concentration (0.0007 mg l^{-1}) one order of magnitudelower than that recorded in the present study (0.009 μg g^{-1}) [44]. On the other hand, cypermethrin residues were detected in the muscle tissues of *Channa punctatus* and *Anabas testudineus* collected from Nambol and Nambul Rivers ($p \leq 0.05$) during post- and pre-monsoon seasons (Table 1) at concentrations of0.004±0.0001* μg g^{-1} and 0.002±5.77E-05* μg g^{-1}, respectively, while the WHO-FAO MRL is0.05 mg kg^{-1} in edible mammalian offal, and 0.01 mg kg^{-1} in eggs. Deltamethrin and cypermethrin residues were not detected in the two fish species collected from the control or reference site (Table 1). Saqib et al. [45] reported the detection of deltamethrin residue in 7 samples of muscles, liver and fat tissue of the three species of *Labeo* found in two Lakes in Pakistan. Jabeen et al. [20] reported the presence of deltamethrin residues in muscles of fish *Cyprinus carpio* and *Channa marulius* with concentrations ranging from 0.051–0.839 μg g^{-1}, and cypermethrin residues in fish *Channa marulius* (0.141–0.174 μg g^{-1}). These values are much higher than those detected in the present study. Mahboob et al. [43] reported that the concentration of deltamethrin in the muscle tissues of *Catla catla* in river Ravi was higher than the permissible limits for fish set by international agencies and it posed a potential risk to the aquatic organisms and ultimately to human health. Meanwhile, high concentrations of cypermethrin residues were also detected by Vryzas et al. [42] in three trophic levels including fish where its peak concentrations were observed just after high rainfall during pesticide application.In the present study, the detection of cypermethrin and deltamethrin residues in the muscle tissues of the two fish species *Channa punctatus* and *Anabas testudineus* ($p \leq 0.05$) at concentrations far below their maximum residual limits indicates that these two pesticides are yet to become a serious threat to the Loktak ecosystem.

CONCLUSION

The present study revealed that most of the pesticide residues were detected in Loktak Lake in the post-monsoon season. This may be due to the continuous inflow of pesticide-contaminated agricultural runoff to the Lake via its feeder streams and rivers, and this inflow increased during the monsoon, leading to the detection of higher pesticide values in fish tissue during post-monsoon. Among the feeder rivers, more pesticide residues were detected in Nambol River than in the other two, because this river passes through a larger stretch of agricultural areas compared to Nambul and Moirang Rivers. Among the five pesticide residuesdetected, only dichlorvos exceeded the maximum residue limit (MRL) prescribed by the *Codex Alimentarius* for animal tissues, suggesting possible health risks to its consumers including predatory fish, migratory as well as resident waterfowl, and humans. The rest of the compounds were within the MRL laid down for eggs and animal tissues revealing low risk to the consumers of these fish. Nevertheless, pesticides even at sub-lethal levels are known to cause adverse effects on fish, besides posing human health risks on long-term exposure,especially because the inhabitants of the study area are regular consumers of fish and dried fish products. Hence, the concerned authorities and regulatory agencies both at the state and central (federal) levels in cooperation with local non-government organizations should make efforts to create awareness through print and electronic media so that people may realize the hazardous effects of pesticide residues to humans due to bioaccumulation and bio-magnification through the food chain. Moreover, the concerned authorities may promote organic farming and implement integrated pest management measures in the catchment area of the lake and introduce a continuous monitoring program in order to provide pesticide-free environment in the Loktak Lake, which is a "wetland of international importance" (Ramsar Site) and therefore, deserves all possible measures for improvement of its water quality and protection of its biodiversity.

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DATA AVAILABILITY STATEMENT

The author confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

USE OF AI FOR WRITING ASSISTANCE

Not declared.

ETHICS

There are no ethical issues with the publication of this manuscript.

REFERENCES

[1] S. S. Anigol, S. B. Neglur, and M. David, "Blood glucose and glycogen levels as indicators of stress in the freshwater fish, Cirrihinus mrigal under Cyphenothrin intoxication," Toxicology International, Vol. 30(1), pp. 51–62, 2023. [CrossRef]

[2] D. B. Barr, and B. Buckley, "Reproductive and Developmental Toxicology," 2nd ed., R. C. Gupta, (Ed.), Academic Press, 2011.

[3] S. M. Barlow, F. M. Sullivan, and R. K. Miller, "Drugs during pregnancy and lactation," 3rd. ed., C. Schaefer, P. Peters, and R. K Miller (Eds.), Academic Press, 2015.

[4] R. C. Gupta, I. R. M. Mukherjee, R. B. Doss, J. K. Malik, and D. Milatovic, "Reproductive and Developmental Toxicology," 2nd ed., R. C. Gupta, Ed.), Academic Press, 2017.

[5] E. L. Amweg, D. P. Weston, J. You, and M. J. Lydy, "Pyrethroid insecticides and sediment toxicity in urban creeks from California and Tennessee," Environmental Science & Technology, Vol. 40, pp. 1700–1706, 2006. [CrossRef]

[6] A. Kaviraj, and A. Gupta, "Biomarkers of type II synthetic pyrethroid pesticides in freshwater fish," Biomarkers of Environmental Pollutants, Vol. 2014, Article 928063, 2014. [CrossRef]

[7] B. K. Das, and S. C. Mukherjee,"Toxicity of cypermethrin in Labeo rohita fingerlings: biochemical, enzymatic and haematological consequences," Comparative Biochemistry and Physiology C: Toxicology & Pharmacology, Vol. 134, pp. 109–121, 2003. [CrossRef]

[8] C. A. Damalas, and I. G. Eleftherohorinos, "Pesticide exposure, safety issues and risk assessment indicators," International Journal of Environmental Research and Public Health, Vol.8, pp. 1402–1419, 2011. [CrossRef]

[9] A. C. Gore, "Organochlorine pesticides directly regulate gonadotropin-releasing hormone gene expression and biosynthesis in the GT1-7 hypothalamic cell line," Molecular and Cellular Endocrinology, Vol. 192, pp. 157–170, 2002. [CrossRef]

[10] L. G. Costa, G. Giordano, M. Guizzetti, and A. Vitalone, "Neurotoxicity of pesticides: a brief review," Frontiers in Bioscience, Vol. 13, pp, 1240–1249, 2008. [CrossRef]

[11] L. Fenster, B. Eskenazi, M. Anderson, and A. Vitalone, "Association of in utero organochlorine pesticide exposure and fetal growth and length of gestation in an agricultural population," Environmental Health Perspectives, Vol. 114, pp. 597–602, 2006. [CrossRef]

[12] R. J. Gilliom, and D. G. Clifton, "Organochlorine pesticide residues in bed sediments of the San Joaquin river, California," Journal of the American Water Resources Association, Vol. 26, pp. 11–24, 1990. [CrossRef]

[13] S. M. Gitahi, D. M. Harper, M. Muchiri, M. P. Tole, and R. N. Ng'ang'a, "Organochlorine and organophosphorus pesticide concentrations in water, sediment, and selected organisms in Lake Naivasha (Kenya)," Hydrobiologia, Vol. 488, pp. 123–128, 2002. [CrossRef]

[14] E. Y. A. Pazou, M. Boko, C. A. M.Van Gestel, H. Ahissou, P. Lalèyè, S. Akpona, B. van Hattum, K. Swart, and N. M. van Straalen, "Organochlorine and organophosphorous pesticide residues in the Ouémé River catchment in the Republic of Bénin," Environment International, Vol. 32, pp. 616–623, 2006. [CrossRef]

[15] S. Gupta, "Loktak lake in Manipur, North east India: major issues in conservation and management of a Ramsar site," Bionano Frontier, pp. 6–10, 2012.

[16] S. Samom, "Polluted river in Manipur devastates fish and humans," News Blaze, Sunday, May 4, 2008.

[17] F. Sun, S. S. Wong, G. C. Li, and S. N. Chen, "A preliminary assessment of consumer's exposure to pesticide residues in fisheries products," Chemosphere, Vol. 62, pp. 674–680, 2006. [CrossRef]

[18] A. Khan, and K. Ghosh,"Characterization and identification of gut-associated phytase-producing bacteria in some freshwater fish cultured in ponds," ActaIchthyologica et Piscatoria, Vol. 42, pp. 37–45, 2012. [CrossRef]

[19] B. C.Biswas, and A. K. Panigrahi, "Diversity of exotic fishes and their ecological importance in southwestern part of Bangladesh," International Journal of Innovative Research in Science Engineering and Technology, Vol. 1, pp. 129–131, 2014.

[20] F. Jabeen, A. S. Chaudhry, S. Manzoor, and T. Shaheen, "Examining pyrethroids, carbamates and neonicotenoids in fish, water and sediments from the Indus River for potential health risks," Environmental Monitoring and Assessment, Vol. 187(2), Article 29, 2015. [CrossRef]

[21] S. J. Lehotay, K. Mastovska, A. R. Lightfield, R. A. Gates, "Multi-analyst, multi-matrix performance of the QuEChERS approach for pesticide residues in foods and feeds using HPLC/MS/MS analysis with different calibration techniques," Journal of AOAC International, Vol. 93, pp. 355–367, 2010. [CrossRef]

[22] Y. Cho, N. Matsuoka, and A. Kamiya, "Determination of organophosphorous pesticides in biological samples of acute poisoning by HPLC with diode-array detector," Chemical and Pharmaceutical Bulletin, Vol. 45, pp. 737–740, 1997. [CrossRef]

[23] T. A. Anderson, C. J. Salice, R. A. Erickson, S. T. McMurry, S. B. Cox, and L. M. Smith, "Effects of landuse and precipitation on pesticides and water quality in playa lakes of the southern high plains," Chemosphere, Vol. 92(1), pp. 84–90, 2013. [CrossRef]

[24] E. M. Veljanoska-Sarafiloska, M. Jordanoskiand, and T. Stafilov, "Presence of DDT metabolites in water, sediment, and fish muscle tissue from Lake Prespa, Republic of Macedonia," Journal of Environmental Science and Health- Part B Pesticides, Food Contaminants, and Agricultural Wastes, Vol. 48, pp. 548–558, 2013. [CrossRef]

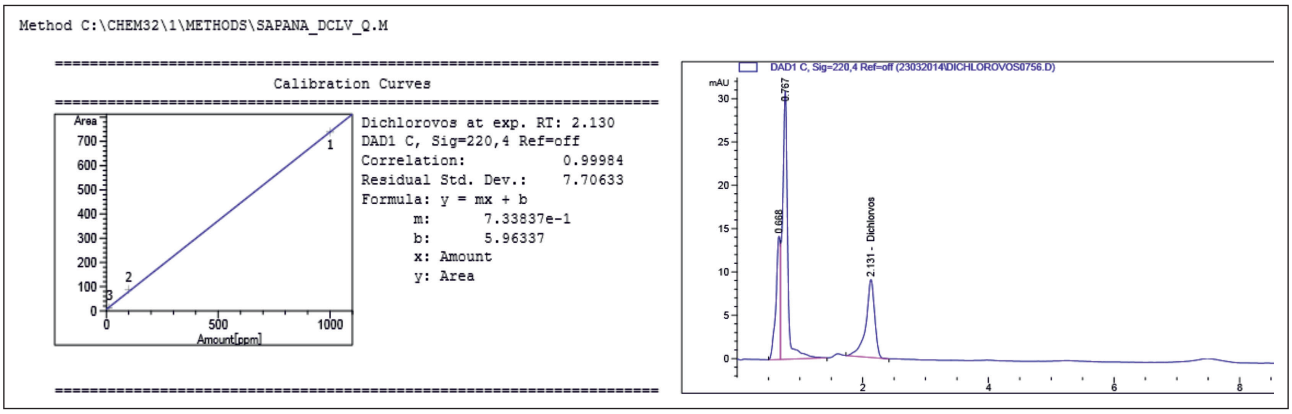
[25] M. Pirsaheb, H. Hossini, F. Asadi, and H. Janjnai," A systematic review on organochlorine and organophosphate pesticides content in water resources," Toxin Reviews, Vol. 36, pp. 210–221, 2016.

[26] J. C. Brodeur, M. Sanchez, L. Castro, D. E. Rojas, D. Cristos, M. J. Damonte, M. Belén Poliserpi, M. F. D'Andrea, and A. E. Andriulo, "Accumulation of current-use pesticides, cholinesterase inhibition and reduced body condition in juvenile one-sided livebearer fish (Jenynsia multidentata) from the agricultural Pampa region of Argentina," Chemosphere, Vol. 185, pp. 36–46, 2017. [CrossRef]

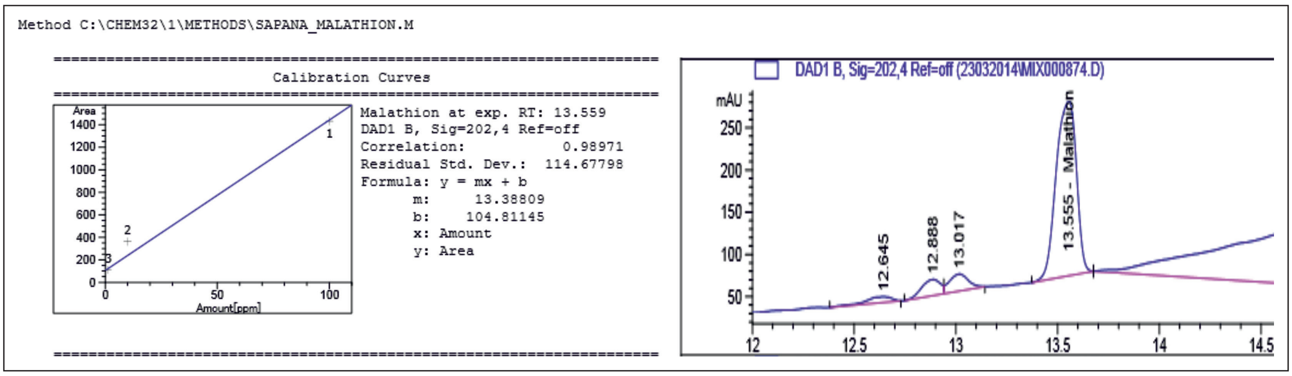
[27] M. Houbraken, V. Habimana, D. Senaeve, E. López-Dávila, and P. Spanoghe "Multi-residue determination and ecological risk assessment of pesticides in the lakes of Rwanda,"Science of the Total Environment, Vol. 576, pp. 888–894, 2017. [CrossRef]

[28] P. Kaczyński, B Łozowicka, M. Perkowski, and J. Rusilowska, "Multiclass pesticide residue analysis in fish muscle and liver on one-step extraction – cleanup strategy coupled with liquid chromatography tandem mass spectrometry," Ecotoxicology and Environmental Safety, Vol. 138, pp. 179–189, 2017. [CrossRef]
[29] W. Tang, D. Wang, J. Wang, Z. Wu, L. Li, M. Huang, S. Xu, and D. Yan "Pyrethroid pesticide residues in the global environment: an overview," Chemosphere, Vol. 191, pp. 990–1007, 2018. [CrossRef]
[30] A. Deknock, N. D. Troyer, M. Houbraken, L. Dominguez-Granda, I. Nolivos, W. Van Echelpoel, M. A. Eurie Forio, P. Spanoghe, P. Goethals, "Distribution of agricultural pesticides in the freshwater environment of the Guayas river basin (Ecuador)," Science of the Total Environment, Vol. 646, pp. 996–1008, 2019. [CrossRef]
[31] M. Kapsi, C. Tsoutsi, A. Paschalidou, and T. Albanis, "Environmental monitoring and risk assessment of pesticide residues in surface waters of the Louros River (N.W. Greece)," Science of the Total Environment, Vol. 650, pp. 2188–2198, 2019. [CrossRef]
[32] A. M. Taiwo, "A review of environmental and health effects of organochlorine pesticide residues in Africa," Chemosphere, Vol. 220, pp. 1126–1140, 2019. [CrossRef]
[33] C. Olisah, O. O. Okoh, and A. I. Okoh, "Occurrence of organochlorine pesticide residues in biological and environmental matrices in Africa: a two decade review," Heliyon, Vol. 6, Article e03518, 2020. [CrossRef]
[34] R. Mondal, A. Mukherjee, S. Biswas, and R. K. Kole, "GC-MS/MS determination and ecological risk assessment of pesticides in aquatic system: a case study in Hooghly River basin in West Bengal, India," Chemosphere, Vol. 206, pp. 217–230, 2018. [CrossRef]
[35] S. Wang, B. Xiang, and Q, Tang, "Trace determination of dichlorvos in environmental samples by room temperature ionic liquid-based dispersive liquid-phase microextraction combined with HPLC," Journal of Chromatographic Science, pp. 1–7, 2012. [CrossRef]
[36] WHO-FAO. Codex Alimentarius: International Food Standards. World Health Organization and Food and Agriculture Organization of the United Nations. 2016. Available: http://www.fao.org/fao-who-codexalimentarius/standards/pestres/pesticides/en/ Accessed on Apr 19, 2024.
[37] T. E. Horsberg, T. Høy, and I. Nafstad, "Organophosphate poisoning of Atlantic salmon in connection with treatment against salmon lice," Acta Veterinaria Scandinavica, Vol. 30, pp. 385–390, 1989. [CrossRef]
[38] S. R. Amaraneni, and R. R. Pillala,"Concentrations of pesticide residues in tissues of fish from Kolleru Lake in India," Environmental Toxicology, Vol. 16, pp. 550–556, 2001. [CrossRef]
[39] F. Maurano, M. Guida, G. Melluso, and G. Sansone, "Accumulation of pesticide residues in fishes and sediments in the river Sele (South Italy)," Journal of Preventive Medicine and Hygiene, Vol. 38, pp. 3–4, 1997.
[40] A. W. Abu-Qare, and M. B. Abou-Donia, "Simultaneous determination of malathion, permethrin, DEET (N N-diethyl-m-toluamide), and their metabolites in rat plasma and urine using high performance liquid chromatography," Journal of Pharmaceutical and Biomedical Analysis, Vol. 26, pp. 291–299, 2001. [CrossRef]
[41] C. Ensibi, D. Hernández-Moreno, M. P. Míguez Santiyán, M. N. Daly Yahya, F. S. Rodríguez, and M. Pérez-López,"Effects of carbofuran and deltamethrin on acetylcholinesterase activity in brain and muscles of common carp," Environmental Toxicology, Vol. 29, pp. 386–393, 2012. [CrossRef]
[42] Z.Vryzas, C.Alexoudis, G.Vassiliou, K. Galanis, and E. Papadopoulou-Mourkidou, "Determination and aquatic risk assessment of pesticide residues in riparian drainage canals in Northeastern Greece," Ecotoxicology and Environmental Safety, Vol. 74, pp. 174–181, 2011. [CrossRef]
[43] S. Mahboob, F. Niazi, K. Al Ghanim, S. Sultana, F. Al-Misned, and Z. Ahmed, "Health risks associated with pesticide residues in water, sediments and the muscle tissues of Catlacatla at Head Balloki on the River Ravi," Environmental and Monitoring Assessment, Vol. 187, Article 81, 2015. [CrossRef]
[44] M. Sapana Devi, and A. Gupta, "Sublethal toxicity of commercial formulations of deltamethrin and permethrin on selected biochemical constituents and enzyme activities in liver and muscle tissues of Anabas testudineus," Pesticide Biochemistry and Physiology, Vol. 115, pp. 48–52, 2014. [CrossRef]
[45] T. A. Saqib, S. N. Naqvi, P. A. Siddiqui, and M. A. Azmi, "Detection of pesticide residues in muscles, liver and fat of 3 species of Labeo found in Kalri and Haleji Lakes," Journal of Environmental Biology, Vol. 26, pp. 433–438, 2005.

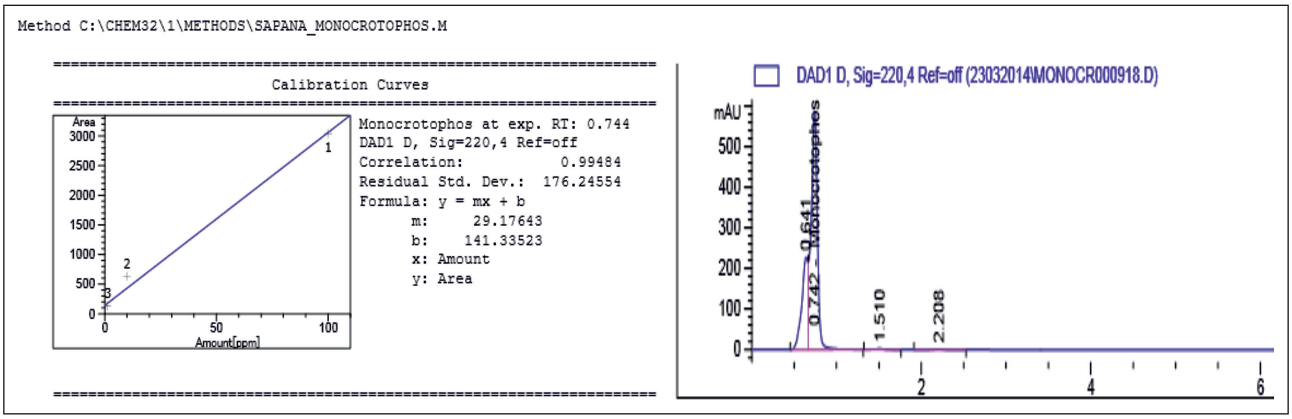
APPENDICES



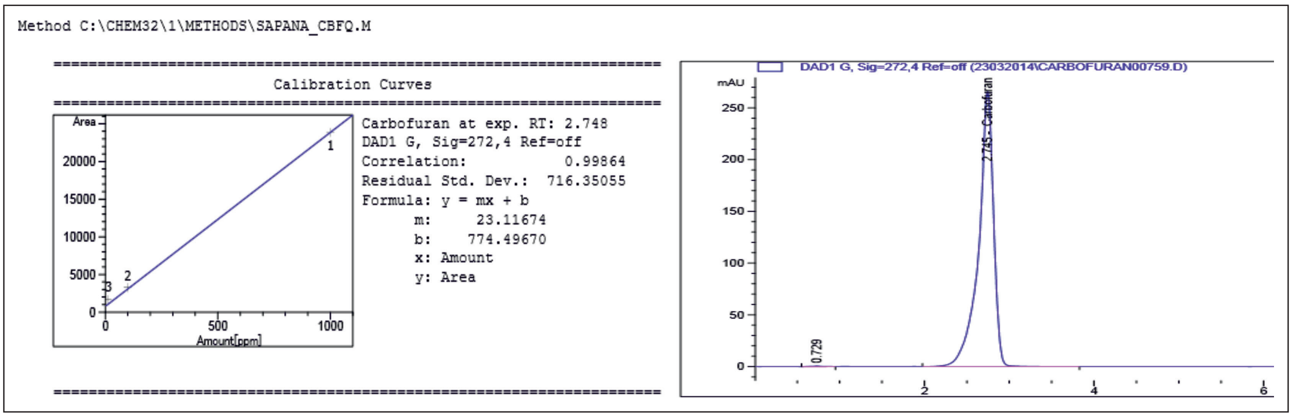
Appendix 1. Standard calibration curves and chromatogram of dichlorvos.



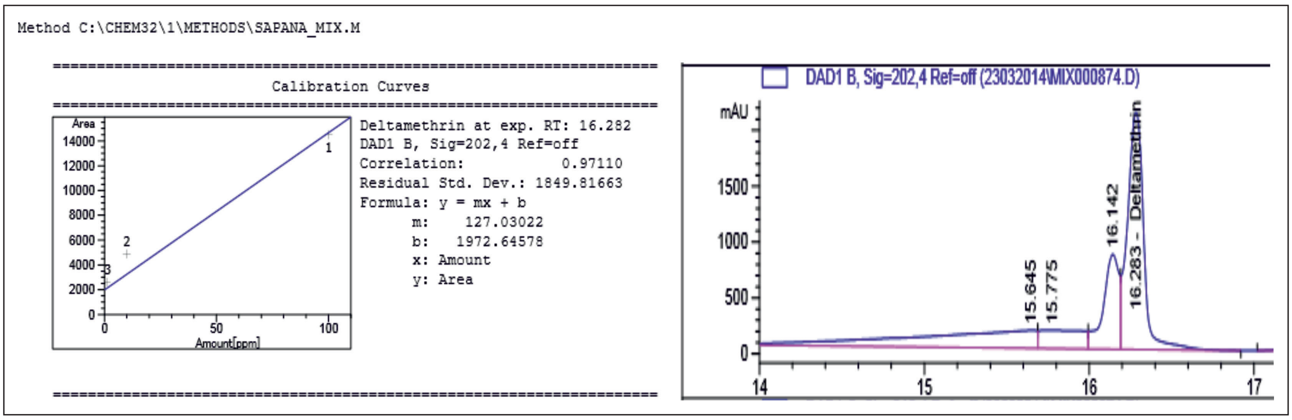
Appendix 2. Standard calibration curves and chromatogram of malathion.



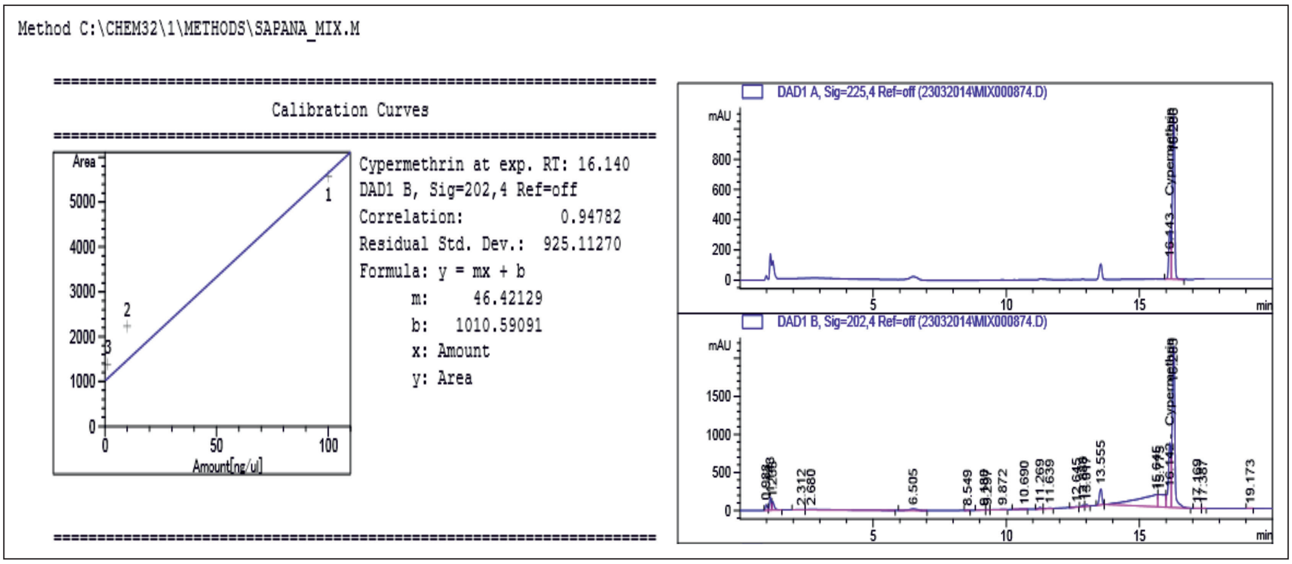
Appendix 3. Standard calibration curves and chromatogram of monocrotophos.



Appendix 4. Standard calibration curves and chromatogram of carbofuran.



Appendix 5. Standard calibration curves and chromatogram of deltamethrin.



Appendix 6. Standard calibration curves and chromatogram of cypermethrin.