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Analyses of pesticide residues in water, sediment and fish tissue from river Deomoni flowing through the tea gardens of Terai Region of West Bengal, India

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Abstract
Analyses of chlorpyriphos, ethion and dicofol in the river Deomoni from Terai region of West Bengal revealed mean pesticide residues of chlorpyriphos, dicofol and ethion in water sample as 0.0091 ± 0.0020ppm, 0.0180 ± 0.0071ppm and 0.0892 ± 0.0375 ppm, respectively, in sediments 0.0513 ± 0.0085ppm, 0.0414 ± 0.0045ppm and 0.1271 ± 0.0122ppm and in fish muscles (*Puntius sp.*) 5.0371 ± 1.4236 ppm, 3.7700 ± 0.6391ppm and 2.9599 ± 0.4027ppm, respectively. Analysis of pesticide residues by one way ANOVA for chlorpyriphos, dicofol and ethion in the water and sediments, the sediment and muscles and water and muscles were found to be highly significant ($p<=0.001$) except for dicofol in water and sediment, which is significant at $p<=0.01$. The result indicates that river Deomoni is contaminated by pesticide from the nearby tea gardens which affects the water quality and non target organisms like fishes thereof.

Keywords: Chlorpyriphos, Dicofol, Ethion, Pesticide**,** Residues

1. Introduction
Tea, *Camellia sinensis* (L.) O. Kuntze, an intensively managed perennial monoculture crop, is cultivated on large and small-scale plantations between latitudes 41°N and 16°S. It is grown on over 2.71 million ha in more than 34 countries across Asia, Africa, Latin America and Oceania and produces 3.22 million metric tons of processed tea annually [1]. The tea industry is one of the oldest industries in India and the Indian tea is appreciated worldwide as a health drink for their medicinal properties, unique flavor and aroma [2]. Tea plantations in Eastern India suffer from a large number of pest infestations leading to significant crop loss [3]. Out of 1031 arthropod species associated with tea globally [4] about 300 species of insects have been recorded from India, which includes 167 species from North-East India [3]. West Bengal is one of the tea producing states in India covering a total area of 1.03 million ha including Darjeeling hills, Terai and the Dooars regions and contributes 25.31% of the tea produced in India [5]. Due to infestation of the pests, pathogens and weeds in tea, a variety of pesticides are used to combat these problems [6]. Despite their extremely essential use in agriculture, pesticide residues are found in agriculture and dairy products, food and water causing serious health hazards with regards to environmental pollution and accidental poisoning [7].
Pesticides can reach surface water through runoff from treated plants and soil. Further, the surface water contamination may have an eco-toxicological effect on aquatic flora and fauna as well as on human health, if used for public consumption [8, 9, 10]. Highly polluted sediments adversely affect the ecological functioning of rivers due to their persistence in the environment and long range transport. In India, the concentrations of these pesticides have been detected in almost all segments of the environment due to their extensive use in the past which have shown potential to bio-magnify or accumulate in animal tissues, human blood, adipose tissue and breast milk [11]. Fish take up contaminants directly from the water and/or through their food chain. Generally, the ability of the fishes to metabolize the organochlorine is moderate; therefore, contaminant load in fish is reflective of the state of the pollution in the surrounding environment [12]. The presence of persistent and hydrophobic pesticides in water even at low concentrations, poses a risk to the health of the biota because such residues have a higher affinity for partitioning into sediment and aquatic organisms. Chemicals with long half-life and

high solubility in lipids (fats, oils, waxes) will tend to accumulate in fatty tissues. Such lipophilic chemicals easily move into the cells and are sequestered in fat, where they become more persistent [13].
Pesticide residues have been recorded in water and fish samples from lagoons in Ghana, showing that some level of exposure of pesticide could be harmful to human. There are reports of the absence of fish species in Chemu and Korle Lagoons, Ghana due to excessive pesticide contamination [14]. The extent of organochlorine pesticides (OCPs) contamination in Lake Manyas and its tributary rivers and streams in Turkey have also been reported [15]. Likewise, OCP residues have been reported from Malaysia [16], Lake Victoria, Uganda [17], Afyonkarahisar, Turkey [18], Lake Parishan, Iran [19], Lake Naivasha, Kenya [20], Taihu Lake Wetland, China [21], rivers in Northern Nigeria [22], Aqaba Gulf [23], Lake Ohrid, Macedonia/Albania [24], Agboyi Creek, Lagos [25], Tarkwa Bay, Lagos Lagoon [26], Ologe Lagoon, Lagos, Nigeria [27], Densu river basin, Ghana [28], Banten Bay [29], Iraqi Marshland [30], Niger Delta Area of Nigeria [31], Alau Dam, Borno State, North Eastern Nigeria [32], Velke Kozmalovce, Ruzin, and Zemplinska Sirava, Slovakia [33]. Pesticide residues have also been detected in water, sediment and fishes in Manzala Lake and River Nile [34].
The issue of pesticide contamination of major rivers in India is not new. Studies have shown contamination of Ganga River by organochlorine insecticides from different locations in Bihar [35, 36] and Kolkata [37]. Assessment of organochlorine residues in the surface sediments of River Yamuna in Delhi, India has been done [38]. Positive detections of pesticides in fish tissues have also been made from river Gomti, Uttar Pradesh [39] and Lake Kolleru, Andhra Pradesh [40]. The levels of organochlorine pesticide residues have been determined in five freshwater fish species in Punjab [41]. Similarly, OCPs and residues of both DDT and HCH in freshwater fishes in Ludhiana have been studied. The maximum levels of DDT were found to be 3.02 mg/kg [42]. The indiscriminate and injudicious use of OCPs have led to the contamination of water bodies [43,44] resulting in high concentration in aquatic life, especially fish, prawns and oysters [43,45] which may have a larger impact on the aquatic ecosystem with pesticide load leading to deleterious effects in the long run. Residue analysis of endosulfan from tissues of fresh water fish (*Labeo rohita*) was done to determine its bio-accumulation in tissues of *Labeo rohita*, exposed to sub-lethal concentrations 0.06876 μg/L [46].
Pesticides are often considered a quick, easy, and inexpensive solution for controlling weeds and insect pests in agriculture to increase production. However, they have contaminated almost every part of our environment. Pesticide contamination poses significant risks to the environment and the non-target organisms, from beneficial soil microorganisms to insects, plants, fish, birds [47] and human. Northern part of West Bengal is one of the major tea growing areas of India, which has vast stretches of tea plantations, particularly in the areas of Darjeeling, the Terai and Dooars. The average use pattern of chemical pesticides was estimated to be 16.75 kg/ha in the Dooars and Terai and 7.35 kg/ha in Darjeeling [48]. The organophosphates (ethion, chlorpyriphos), organochlorine (dicofol, heptachlor, alpha- endosulfan, beta- endosulfan, endosulfan sulfate) and pyrethroids (cypermethrin and deltamethrin) in the hill and the Dooars region of West Bengal have been reported in an earlier study [6]. Indiscriminate usage of insecticide not only in tea gardens, but also in agricultural fields has resulted in serious problems concerning environmental pollution, both terrestrial and aquatic. Pal *et al.,* (2006) [49] have reviewed degradation and effects of pesticides on soil microbiological parameters and commented that pesticide contamination affects microbial and biochemical aspects of soil quality. Studies by Bishnu *et al.,* (2009) [6] and Pal *et al.,* (2011) [50] have previously shown that contamination of water bodies by use of insecticides in conventionally managed tea gardens is a major concern. Pal *et al.,* (2011) [50] have studied two water bodies flowing adjacent to conventionally managed tea gardens in the Terai region of West Bengal for estimation of the residue level of organochlorine (dicofol), organophosphate (ethion, chlorpyriphos) and synthetic pyrethroid (cypermethrin). The rivers of Terai serves as an important source of water for domestic use, local fishing and watering of adjacent tea plantations. Since most of the tea gardens in the Terai region of West Bengal are conventionally managed, these rivers also serve as the final sink to the pesticide run-offs from adjacent tea gardens. There are scanty reports on pesticide contamination of the water bodies of Terai and Dooars regions of Darjeeling foothills in West Bengal, hence, it is very important to assess the level of pesticide pollution if any. A study on the effect on non-target organisms viz., fish, is also very important as these organisms are very sensitive to changes in hydrological parameters. The present study was undertaken to investigate the residue level of selected organophosphorus and organochlorine pesticides in water, sediment and resident fish present in the water bodies of the selected tea gardens, namely Deomoni, in the Terai of West Bengal, India.

2. Materials and methods

2.1. Study area and sample collection

Sampling sites were selected based on the criterion of easy accessibility, proximity to conventional tea gardens, an abundance of local fish species and perennial nature of the river. Samples were collected at monthly intervals from river Deomoni flowing through the tea gardens and the data were interpolated as seasonal data. Geographical coordinates of the study site is 26.69231N, 88.26339E. Water and sediment samples were collected for a period of two years from March 2013 to February 2015 on a monthly basis from the sampling sites. Water samples were collected in 1L screw cap glass bottles (thoroughly cleaned with soap and rinsed with acetone) from appropriate depth and turbulent midstream positions of water bodies. It was immediately preserved after collection in ice to minimize degradation of pesticide. Samples were stored at 4 ºC until extraction. Sediment samples were taken from positions where a fine-texture substrate deposition takes place. The upper 2cm of the bed sediment was collected with a Teflon coated spoon, stored in aluminium containers at -20 ºC in the laboratory until analysis. *Puntius* sp. was collected for residue analysis and brought to the laboratory in live condition. The residue level in fish tissue was analyzed seasonally. Fish samples were collected once for summer (March to June), monsoon (July to October) and winter (November to February). The fish muscles were used for pesticide residue analysis.

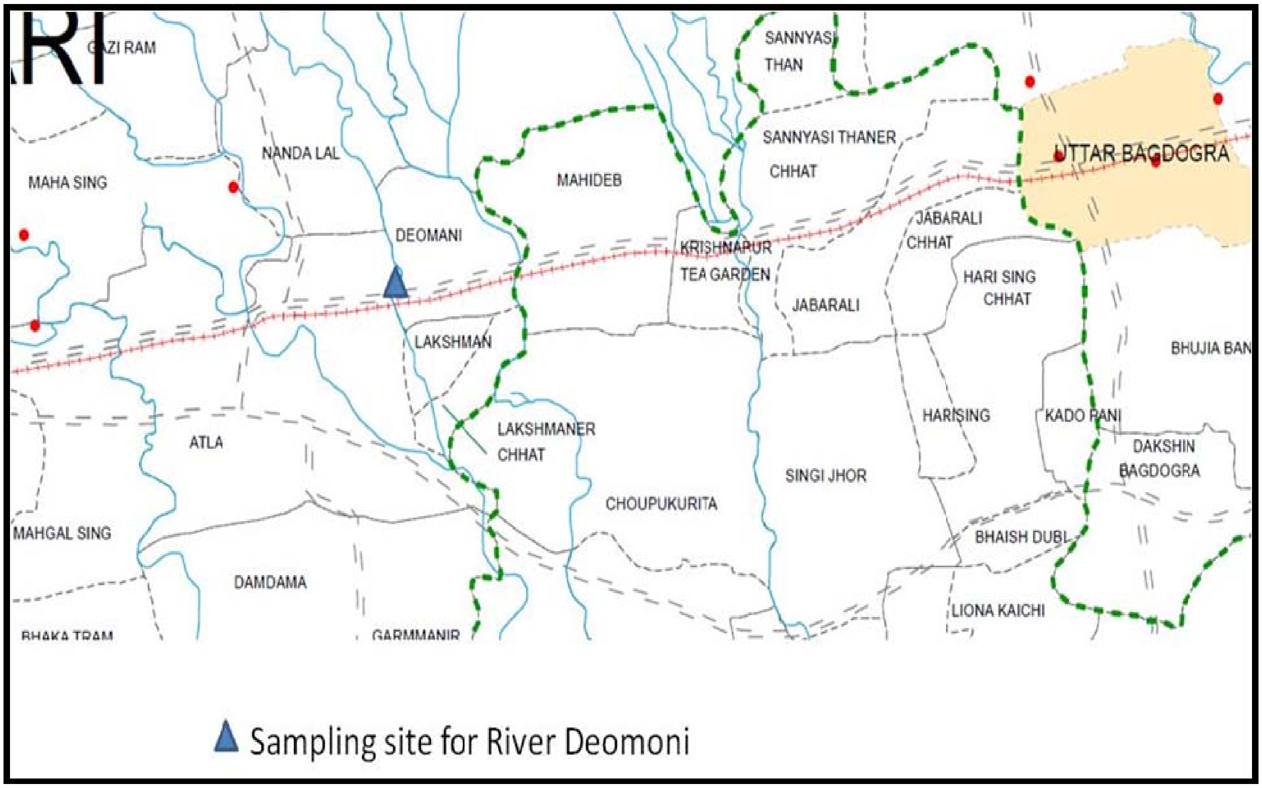

Fig 1: A Map indicating sampling site in perennial river Deomoni *(Not to scale)*

2.2. Extraction of pesticide residue from water and sediment samples

The extraction of pesticide from water and sediment sample was carried out by liquid extraction and cleanup following the method described by Mathur *et al.,* (2003) [51] with minor modifications. Water samples were shaken well and filtered through Whatman filter paper No.1 and about 250mL filtrate was taken in a 500mL separatory funnel and 12.5mL of saturated sodium chloride was added to it. The water sample was partitioned with 100mL of diethyl ether (twice) by shaking the separatory funnel vigorously for 2-3 minutes and releasing the pressure intermittently and the layers were allowed to separate. The two extracts of diethyl ether layers were pooled and concentrated to about 1-2ml followed by a silica gel clean up. Activated silica 10g (2h at 130 ºC) was packed between two layers of sodium sulphate (5g each) and the column was eluted with 100mL acetonitrile. Eluent was collected and concentrated to dryness. Final samples were prepared in acetonitrile (HPLC grade) and analyzed by HPLC LC 20AD, Shimadzu Corp., Japan. For sediments, 10g of sample was taken in a conical flask containing 50mL of diethyl ether and stirred by using a magnetic stirrer for 30min. This step was repeated twice. The extracts were pooled and allowed to dry. The dried samples were cleaned up by silica gel column.

2.3. Extraction of pesticide residue from fish tissue and delipidation

Muscle tissue was weighed and crushed into a fine powder using pestle and mortar containing 4.5 g of anhydrous sodium sulphate. Four grams of the powder was taken in a conical flask with sufficient diethyl ether. The set-up was left to stand for 15 min and the solvent was collected in a beaker. The extraction was repeated twice. The extract was evaporated to dryness in a water bath at 35-40 ºC [52]. The extract from tissue was re-dissolved in 50mL of acetonitrile and kept at -20 ºC for 30 min to freeze the lipids. Most of the lipids were precipitated as pale yellow condensed lump on glassware surface. This extract was immediately filtered through Whatmann filter paper to remove the lipids. The precipitated lipid on the glass surface was redissolved in 50mL of acetonitrile. The filtered extract was concentrated to 1mL followed by silica gel clean up [53].

3. Identification and quantification

High Performance Liquid Chromatography (HPLC; Model LC-20AD), Shimadzu Corp., Japan (ODS C18 (2) column, length-250mm, internal diameter 4.6mm) having UV/ visible detector was used for identification and quantification of pesticides. Samples (20µL) were injected manually with a Rheodyne injector. The detector was connected to the computer for data processing. The working condition of HPLC was binary gradient, mobile phase was acetonitrile, flow rate 1 mL/min, injection volume was 20µL and wavelength of the UV/visible detector was fixed at 229nm, 230nm and 221nm for chlorpyriphos, dicofol and ethion, respectively for the analysis of pesticide residues [54]. The retention time for chlorpyriphos, dicofol and ethion were 4.069 nm, 4.194 nm, 3.771 nm, respectively. Single residue analysis was performed. The compound was identified by comparing its retention time with respect to the technical grade reference standard. The quantification was carried out with the help of calibration curve drawn from chromatographic experiments with standard solution.

4. Statistical analysis

Statistical analyses were performed using KyPlot version 2.0 beta 15 (32 bit). Data was statistically analyzed using one way analysis of variance (ANOVA), where P>0.05 was considered non -significant. All data are presented as mean ± SE.

5. Results and Discussion

The results of the residue analyses of three commonly used pesticides in the water samples, sediment and fish muscles from river Deomoni are presented in the Tables 1, 2 and 3. Analyses revealed that the mean pesticide residues of chlorpyriphos, dicofol and ethion were 0.0091 ± 0.0020 ppm, 0.0180 ± 0.0071 ppm and 0.0892 ± 0.0375 ppm, respectively in the water sample, whereas in the sediment, the mean concentrations of Chlorpyriphos, Dicofol and Ethion were 0.0513 ± 0.0085 ppm, 0.0414 ± 0.0045 ppm and 0.1271 ± 0.0122 ppm, respectively. The pesticide concentrations varied over the period of two years (Table 1). Mean pesticide residue concentration of Chlorpyriphos, Dicofol and Ethion in fish muscles were found to be 5.0371 ± 1.4236 ppm, 3.7700 ± 0.6391 ppm and 2.9599 ± 0.4027 ppm, respectively. Interestingly, the concentration of chlorpyriphos (11.6709 ± 0.4332 ppm) in fish muscle was comparatively higher in winter (Table 2). Significant differences were found in the water and sediment ($p<=0.001$), sediment and fish muscle ($p<=0.001$) and also water and fish muscle ($p<=0.001$) for pesticide chlorpyriphos. Differences in the water and sediment ($p<=0.01$), sediment and fish muscle ($p<=0.001$) and also water and fish muscle ($p<=0.001$) for pesticide dicofol were highly significant. Significant differences were also observed in the water and sediment ($p<=0.001$), sediment and fish muscle ($p<=0.001$) and also water and fish muscle ($p<=0.001$) for pesticide ethion (Table 3).

Table 1: Seasonal pesticide concentrations in water sample of river Deomoni of Terai region of West Bengal over a period of two years, year 1 (2013-14) and year 2 (2014-15) for three seasons, Summer (March-June), Monsoon (July-October) and Winter (November-February). The mean data of all seasons are given as Mean ± SE where n = 96)

		Seasons	Chlorpyriphos (ppm)	Mean (year 1 and 2)	Dicofol (ppm)	Mean (year 1 and 2)	Ethion ppm)	Mean (year 1 and 2)
Water	Year 1	Summer	0.0382 ± 0.0086	0.0091 ± 0.0020	0.0825 ± 0.0401	0.0180 ± 0.0071	0.0333 ±0.0088	0.0892 ± 0.0375
		Monsoon	0.0124 ± 0.0028		0.0088 ± 0.0014		0.0184 ±0.0066	
		Winter	0.0027 ± 0.0007		0.0055 ± 0.0016		0.0100 ±0.0012	
	Year 2	Summer	0.0007 ± 0.0002		0.0024 ± 0.0002		0.0040 ±0.0006	
		Monsoon	0.0008 ± 0.0003		0.0039 ± 0.0006		0.4634 ±0.2056	
		Winter	0.0000		0.0053 ± 0.0018		0.0063 ± 0.0014	
Sediment	Year 1	Summer	0.1442 ± 0.041	0.0513 ± 0.0085	0.0158 ±0.0053	0.0414 ± 0.0045	0.0859 ± 0.0239	0.1271 ± 0.0122
		Monsoon	0.0530 ±0.0066		0.0396 ±0.0076		0.0651 ± 0.0082	
		Winter	0.0302 ± 0.0085		0.0228 ±0.0045		0.1610 ± 0.0425	
	Year 2	Summer	0.0462 ± 0.0117		0.0735 ±0.0139		0.1835 ± 0.0308	
		Monsoon	0.0309 ± 0.0066		0.0333 ±0.0061		0.0716 ± 0.02466	
		Winter	0.0035 ± 0.0019		0.0638 ±0.0161		0.1959 ± 0.0226	

Table 2: Pesticide residue in muscle tissue of *Puntius sp.* collected from the river Deomoni of Terai region. The mean data are given as Mean ± SE where n = 12)

Seasons	Average weight of tissue (g)	Chlorpyriphos (ppm)	Mean (all season)	Dicofol (ppm)	Mean (all season)	Ethion (ppm)	Mean (all season)
Summer	0.663	1.8505 ± 0.1175	5.0371 ± 1.4236	6.1076 ± 0.1491	3.7700 ± 0.6391	3.0132 ± 0.1978	2.9599 ± 0.4027
Monsoon		1.5899 ± 0.1634		4.1763 ± 0.2865		4.5480 ± 0.0173	
Winter		11.6709 ± 0.4332		1.0259 ± 0.0094		1.3184 ± 0.0328	

Table 3: Comparison of the values of three pesticides in the water, sediment and fish muscle from river Deomoni, Terai, Darjeeling district, West Bengal.

Pesticides	Water	Sediment	Muscle
Chlorpyriphos	0.0091 ± 0.0020	0.0513 ± 0.0085 (a***)	5.0371 ± 1.4220 (b***, c***)
Dicofol	0.0180 ± 0.0071	0.0414 ± 0.0045 (a**)	3.7700 ± 0.6389 (b***, c***)
Ethion	0.0124 ± 0.0021	0.1271 ± 0.0122 (a***)	2.9599 ± 0.4022 (b***, c***)
a= relative to water and sediment, b= relative to sediment and fish muscle; c= relative to water and fish muscle. ***= significant at P<=0.001 level; **= significant P<=0.01 level; N.S= Non-significant (P>0.05) level			

The comparison of our results in water and sediments revealed higher concentrations of the three pesticides in fish muscle than in sediment and water, while water had the least concentration. The lower levels of pesticide residues found in the water bodies than in the sediment might be attributed to the fact that the input of pesticides in water is a function of suspended particulate concentrations, where the residues were absorbed and transported, as also commented by Bishnu *et al.*, (2009) [6], varied from season to season, depending on the rainfall events that control the activities of soil erosion and the amounts of suspended particulates, during runoff [55]. The higher levels of pesticides, were found in the sediments than water because sediments are important sinks for various pollutants like pesticides which also play a significant role in the remobilization of contaminants in aquatic systems under favorable conditions and in interactions between water and sediment also commented by Ntow (2005) [7]. Sediments act as secondary contamination source after water in the ecosystem. Sediments are the principal reservoirs of environmental pesticides, representing a source from which residues can be released to the atmosphere, groundwater and living organisms [56]. The higher levels of pesticides found in the fish might be due to the fact that fishes have a greater tendency to accumulate the pesticides in their body due to bioaccumulation [57]. Our results showing higher concentration of three pesticides (Table 3) in sediment than water also corroborate the above fact.

Earlier studies with pesticide residues in some rivers of Dooars and Terai of West Bengal have already shown that the tea, soil and water bodies are contaminated with pesticides used in the conventionally managed tea gardens of the region [6, 50]. Bishnu *et al.*, (2009) [6] have shown the mean concentration of pesticides in water as 0.0005 ppm (ethion), 0.0036ppm (dicofol) in dry season (April), while chlorpyriphos was not detected. Our study showed mean concentrations of 0.0326 ppm (chlorpyriphos), 0.1534 ppm (dicofol) and 0.0128 ppm (ethion) in April which are comparatively higher than their results. Pal *et al.*, (2011) [50] have shown the presence of dicofol and ethion in the river Deomoni during summer and the concentrations were found to be 0.0086 ppm and 0.0053 ppm in water and 0.4844 ppm and 0.0012 ppm in sediment samples, respectively. Chlorpyriphos was not included in the study of Pal *et al.*, (2011) [50]. Our study showed the presence of chlorpyriphos (0.0194 ppm), dicofol (0.0424 ppm) and ethion (0.0186 ppm) in water samples and in sediments it was found to be 0.0952ppm (chlorpyriphos), 0.0446ppm (dicofol), and 0.1346ppm (ethion) over the period of two years which were comparatively higher indicating that these pesticides are overused in the management of pests in tea gardens that comes to the water bodies through runoffs. Studies on pesticide in river, lake, fish and sediments have been a major environmental focus especially during the last decade [15, 18, 19, 21, 32]. The total organochlorine pesticide level in sediment samples ranged from 0.15771 to 0.30766 ppm in pre-monsoon, 0.19586 to 0.57774 ppm in monsoon and 0.3069 to 0.84445 ppm in the post-monsoon season [38]. Our study reported the showed total concentrations of pesticides in pre- monsoon as 0.2744ppm, monsoon 0.1466ppm and post monsoon 0.2384ppm in sediments which is comparable to the results of Pandey *et al.*, (2011) [38]. Pesticide contamination of water bodies in other parts of the world has been shown by different workers. Erkmen *et al.*, (2013) [15] have shown total OCPs concentrations in water and sediments which ranged from 0.0014 to 0.0086 ppm and 0.0170 to 0.0391 ppm in Lake Manyas, Turkey. The total average pesticide residues in water samples from the four lagoons, Chemu, Korle, Fosu and Etsii are 2.6384 mg/L, 0.4992 mg/L, 0.3045 mg/L and 1.3629 mg/L respectively [14].

Fish samples can be considered as one of the most significant indicators in fresh water systems for the estimation of pesticide contamination [7]. The Organochlorine concentration in water and sediments ranged from below detection limit (BDL): BDL to 0.0136 ppm and 0.00052 to 0.45 ppm whilst the mean OCPs in the fish ranged from 0.00264 ppm to 0.066 ppm, respectively [57] which is slightly lower than the concentrations found in our study. The higher level of pesticides in dry season in fish generally may be ascribed to increased feeding on insect larvae, coarse vegetable matters and sediment-associated particles, that had accumulated the pesticides over time. There is a greater possible bioaccumulation tendency in the fish species during the dry season [57]. In the present study also pesticide residues in fish tissue were relatively higher in the dry season which corroborates Ogunfowokan *et al.*, (2012) [57]. The total average pesticide residues obtained in fish samples from Fosu and Etsii Lagoon in Ghana were 0.0155 ppm and 0.0088 ppm, respectively [14]. The levels of OCPs in common carp (0.021 ppm), Rohu (0.019 ppm), Grass carp (0.019 ppm), Catla (0.017 ppm), Silver carp (0.017 ppm), *Channa punctatus* (0.00966 ± 0.0056 ppm) have been studied in India by different workers [39, 41]. Presence of very high levels of pesticide residue in fish (*Puntius sp.*) ranging from 2.9599 to 5.0371ppm is higher than their results which indicate extensive bioaccumulation. The concentration of pesticides was found to be higher than 0.0001mg/kg limit for individual pesticide in drinking water directive 80/778/EEC and also higher than the default MRL level for pesticides (0.01ppm) for substances that are not included in any of the annexes in European Union (EU) regulations like fish tissue. Results showed the higher concentration of pesticide residues in the tissue and sediment than water. Some physicochemical parameters viz. pH may have direct influence on the solubility of these pesticides in river water [36].

6. Conclusion

The present study shows that Deomoni, one of the major rivers of the Terai region, is contaminated by organochlorine and organophosphate pesticides. From the above discussion, it is very much clear that the order and concentration of these pesticides in water and sediment of the river Deomoni varied in the water, sediment and fish tissues. The contamination of river water by pesticide residues from runoffs have greater impact on the aquatic ecosystem which may lead to deleterious effects on the aquatic organisms and humans dependent on the water supply from the river. Effective use of pesticides and maintenance of water quality presents a greater challenge and is the need of the hour. From the results obtained through the entire course of the study, it can be concluded that the river flowing through the tea gardens in the Terai region of West Bengal is continuously contaminated with pesticide runoff from the nearby tea gardens located in the region. This affects the quality of the water flowing in the rivers and also affects the non - target organisms like fishes thereof.

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