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Organochlorine pesticide residues and microbiological quality assessment of dried barb, *Puntius sophore* from the north-eastern part of Bangladesh

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Abstract: The present study was carried out at the north-eastern part of Bangladesh to investigate organochlorine pesticide (OCP) residues and microbiological quality of dried barb (*Puntius sophore*). Samples were collected from both producers and retailers from December 2016 to April 2017. A control sample was also prepared with the same raw fish used by the producers in the laboratory to compare the result. Gas Chromatography with Electron Capture Detector (GC-ECD) was used to detect and quantify OCP residues. Around 22 % (6) samples out of 27 were found contaminated with OCP residues. Among these six adulterated samples, four were from retailers and two from producers. Only Aldrin was detected in four samples and rest two samples were detected with both Aldrin + Dieldrin and Aldrin + Endrin. Aldrin was found between 0.332 to 0.967 ppm, Dieldrin 0.762 ppm, and Endrin 0.828 ppm. All these values were much higher than the Maximum Residual Limit (MRL) 0.1 ppm. Aerobic Plate Count (APC) of producer samples were ranged between log5.3 ± 0.02 to log5.4 ± 0.03, log6.2 ± 0.02 to log6.4 ± 0.02 for retailer samples and log5.0 ± 0.03 to log5.2 ± 0.04 for control samples. While fungal count was ranged between log3.2 ± 0.04 to log3.5 ± 0.04, log3.4 ± 0.04 to log3.6 ± 0.03 and log2.2 ± 0.05 to log2.5 ± 0.03 for producer, retailer and control samples respectively. All the producer and retailer samples and one-third of the control samples were found contaminated with *Escherichia coli*. Whereas, *Salmonella* sp. was detected as 13.3% in producer samples, 20% in retailer samples except for the control. In case of *Vibrio* sp., maximum count was found for retailer samples (13.3%), whereas, producer and control samples showed no detection. The finding of the present study revealed the presence of pesticides and poor microbiological quality of dried barb are alarming for the consumers of Bangladesh and which may cause chronic disease and potential long-term risk for human health.

Keywords: Dried fish, *Puntius sophore*, Organochlorine pesticide residues, Gas chromatography, Microbiological quality

1. Introduction

Bangladesh is one of the world's leading fish producing country with a total production of 3.68 million MT in 2014-15. Fish alone supplies about 60% of animal protein intake by the population of Bangladesh [1]. Every year a significant portion of total harvested fish are sun-dried due to high market demand. Moreover, lack of proper storage facilities and unsold from the markets force to

process dried fish. Among other fishery products, dried fish is one of the cheapest and major dietary protein source in Bangladesh [2]. An amount of 3,106 MT dried fish (including salted and dehydrated fish) exported from Bangladesh which is accounted for the 3.71% of total exported fishery products [1].

Unfortunately, there are frequent complaints about the quality of traditionally dried fish as sanitation and hygiene is rarely practiced. The traditional drying of fish is mainly performed by poor and illiterate dried fish producer where fish drying is carried out by spreading fish on the split bamboo mat, concrete floor, raised platform or fish are hanged over the bamboo pole and bar [3]. Additionally, one of the major problem associated with dried fish is an infestation of the products by varieties of insects such as beetles, mites and blowflies [2,4,5]. The extent of damage (loss of soft tissue and weight loss) caused by the larvae and adult insects depends largely on the speed of drying, the size of the fish, and on whether the fish is salted [6]. In Bangladesh, to protect dried fish from infestation and extending storage time producers time and again use organochlorine pesticides like dichlorodiphenyltrichloroethane (DDT), dichlorvos, heptachlor whatever they are getting within their reach [7–10].

Drying of fish causes the gradual death of vegetative bacteria cells but has little effect on bacterial and mold spores due to their resistance to prolonged drying periods and stability when dried [11]. Inadequate protection of fish stored under tropical humid conditions [12] could result in insect infestation [13] and microbial proliferation [11]. The weather of Bangladesh is humid, and the producers do not dry fishes properly in fear of weight loss to make more profit. The quality of salted and sun-dried fishes is adversely affected by the occurrence of microorganisms [14]. Semi-preserved fish have been implicated in fish-borne outbreaks involving *Staphylococcus aureus*, *Escherichia coli*, *Clostridium botulinum*, *Streptococcus faecalis*, *Salmonella, Shigella*, and *Bacillus* sp. 11,15. The occurrence of aflatoxin contamination in dried, salted and smoked fish and fish products has been reported in the Philippines, Thailand, and Indonesia [16], Tanzania [17] and India [18,19]. Determination of microbiological quality of such processed fishes is very important for guarding consumer's health and hygiene [20].

A substantial amount of dried fish are produced per annum in Sylhet, north-eastern part of Bangladesh, because of the availability of raw materials from local water bodies especially from Haors (bowl-shaped wetland ecosystems locally known as *Haor*). Dried barb (*Puntius sophore*) is highly produced and one of most popular dried fish in Sylhet region. To date, however, there is no research found regarding microbiological quality and pesticides residue analysis of this dried fish. The current study, henceforth, aimed to investigate the organochlorine pesticides residue and assess microbiological quality of dried barb collected from north-eastern part of Bangladesh.

2. Results

2.1 Pesticides analysis

Results of the analysis is presented in Table 1, Figure 1 and Figure 2. Organochlorine pesticide residues (OCP) were detected in six samples of dried barb out of 27. Among these six adulterated samples; two from producer and rest of the four from retailer. Aldrin was found in all six adulterated samples. Concentration of Aldrin in producer samples were 0.617 ppm and 0.812 ppm. Aldrin was also detected in two samples of retailer and its concentration was 0.332 ppm and 0.479 ppm. Four samples were found contaminated with Aldrin and remaining two samples were detected with both Aldrin + Dieldrin and Aldrin + Endrin respectively. Highest concentration of Aldrin (0.967 ppm) was found for retailer samples while lowest concentration of Aldrin (0.332 ppm) in case of producer samples. All the detected organochlorine pesticide residue levels were above MRL (0.1 ppm) which was recommended by Australian Pesticides and Veterinary Medicines Authority [21] and FDA [22]. All the control samples were found free of organochlorine pesticides contamination. To best of our knowledge information on Aldrin, Dieldrin and Endrin residue in dried fish was scarce for the present study area and for the Bangladesh too.

Table 1. Concentration of different organochlorine pesticides detected in dried barb collected from the study areas.

Sample source	No of analyzed samples	Adulterated samples			Detected pesticide	Residue-level (ppm)	MRL* (ppm)
		Total	Single pesticide	Multiple pesticides			
Producer	9	2	1	-	Aldrin	0.617	0.1
			1		Aldrin	0.812	
Retailer	9	4	1	-	Aldrin	0.332	
			1		Aldrin	0.479	
			-	1	Aldrin	0.818	
					Dieldrin	0.762	
			-	1	Aldrin	0.967	
					Endrin	0.828	
Control	9	-	-	-	Not detected	-	-

*MRL - Maximum Residue Limit recommended by Australian Pesticides and Veterinary Medicines Authority [21] and FDA [22].

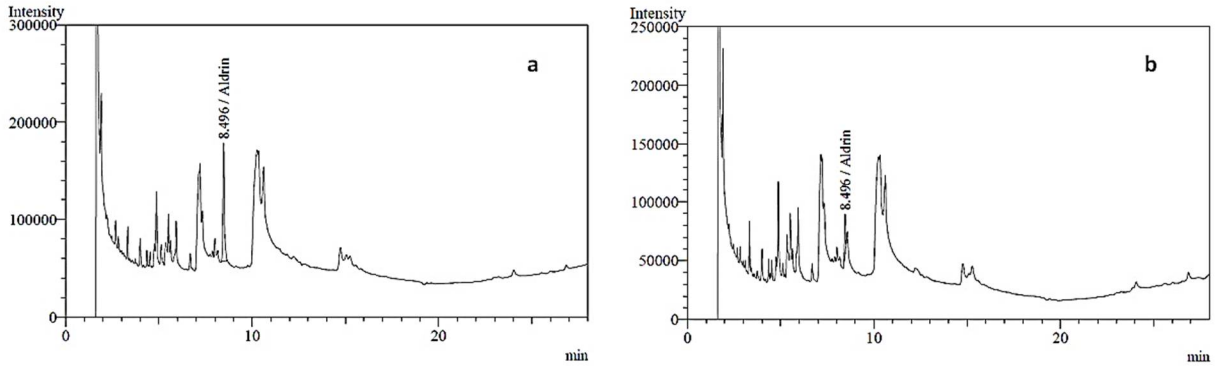

Figure 1. Chromatographs of detected pesticides in dried barb fish from producer (a and b)

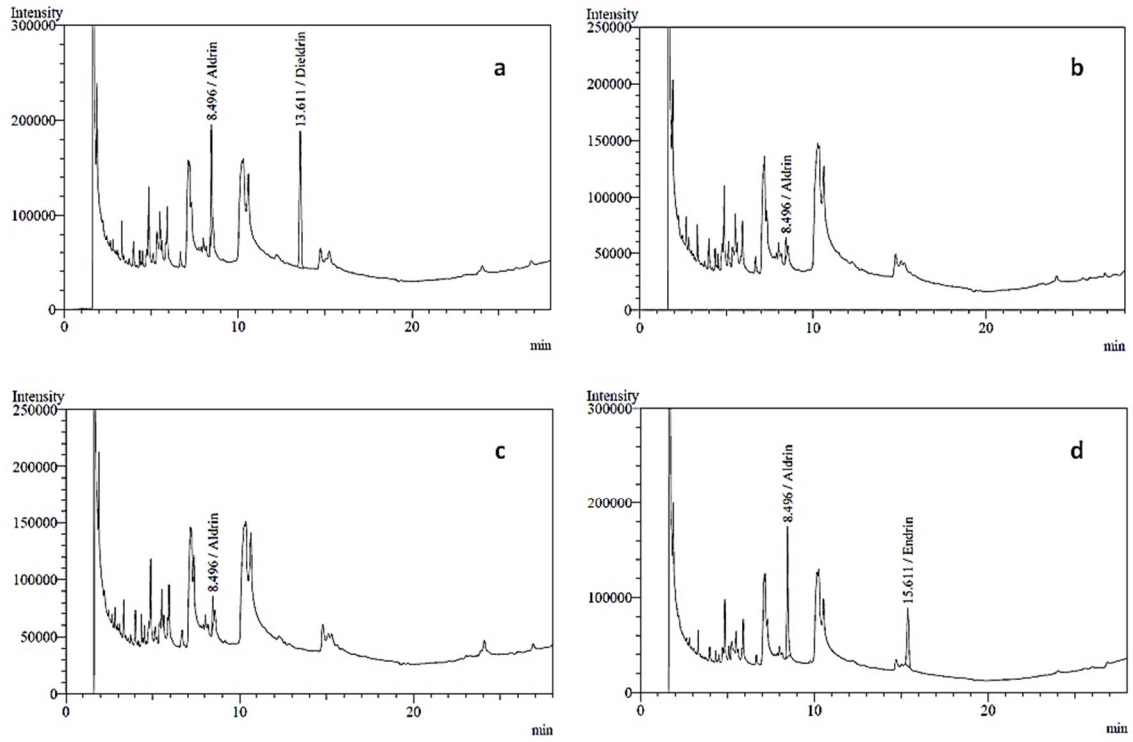

Figure 2. Chromatographs of detected pesticides in dried barb fish from retailer (a, b, c and d).

2.2 Microbiological analysis

2.2.1 Aerobic plate count

Aerobic plate count (APC) of dried barb is presented in Table 2. In every month, APC of retailer sample was found significantly highest ($p<0.05$) among the analyzed samples followed by producer and control. APC of retailer sample measured between log value 6.2 ± 0.02 and 6.4 ± 0.02. Highest APC of dried barb from producer observed log value 5.4 ± 0.03 while lowest was log value 5.3 ± 0.02. Whereas, APC of control sample ranged between log value 5.0 ± 0.03 and 5.2 ± 0.04.

Table 2. APC of dried barb of north-eastern region of Bangladesh from December'16 to April'17.

Months	APC (log value)		
	Control	Retailer	Producer
December'16	5.1 ± 0.03^{c}	6.3 ± 0.03^{a}	5.4 ± 0.03^{b}
January'17	5.0 ± 0.03^{c}	6.2 ± 0.02^{a}	5.3 ± 0.03^{b}
February'17	5.1 ± 0.05^{c}	6.3 ± 0.06^{a}	5.3 ± 0.02^{b}
March'17	5.2 ± 0.04^{c}	6.4 ± 0.01^{a}	5.4 ± 0.02^{b}
April'17	5.2 ± 0.01^{c}	6.4 ± 0.02^{a}	5.4 ± 0.01^{b}

Values are means ± SEM, n = 3 per treatment group. Means with the different letters in each row indicates significant differences ($p<0.05$).

2.2.2 Isolation and identification of bacteria

E. coli was found in 100%, 100% and 33.3% of analyzed samples from producer, retailer and control, respectively ($\chi2 = 25.71$, df = 2, P = 0.000). Whereas, *Salmonella* sp. was observed in 13.3% (producer), 20% (retailer) and 0% (control) ($\chi2 = 3.15$, df = 2, P = 0.100). In case of *Vibrio* sp., maximum count was observed for retailer samples, while producer and control samples showed no detection

($\chi 2 = 4.186$, df = 2, P = 0.101) (Table 3). Chi square test suggested that only presence of *E. coli* in dried barb depends on sources ($p<0.05$).

Table 3. Results on qualitative bacterial test for the analyzed samples.

Bacteria	No. of sample analyzed	Positive Percentage			Chi-square value	df	P value
		Producer	Retailer	Control			
E. coli	15	100 (15)	100 (15)	33.3 (5)	25.71	2	0.000
Salmonella sp.		13.3 (2)	20 (3)	0 (0)	3.15	2	0.100
Vibrio sp.		0 (0)	13.3 (2)	0 (0)	4.186	2	0.101

Value in the parentheses indicates the frequency of percentages.

2.2.3 Fungal counts

Fungal count of dried barb collected from producer, retailer, and control during December 2016 to April 2017 is given in Table 4. In every month, among the three sample highest fungal count was determined for dried barb from retailer followed by retailer and control. Though, there were no significant difference observed between retailer and producer sample ($p<0.05$). Fungal count was estimated log value 3.4 ± 0.04 to 3.6 ± 0.03 for retailer sample, log value 3.2 ± 0.04 to 3.5 ± 0.04 for producer and log value 2.2 ± 0.05 to 2.5 ± 0.03.

Table 4. Fungal count of dried barb of north-eastern region of Bangladesh from December'16 to April'17.

Months	Fungal Count (log value)		
	Control	Retailer	Producer
December'16	2.4 ± 0.04^{b}	3.5 ± 0.04^{a}	3.4 ± 0.04^{a}
January'17	2.2 ± 0.05^{b}	3.4 ± 0.04^{a}	3.2 ± 0.04^{a}
February'17	2.3 ± 0.05^{b}	3.5 ± 0.02^{a}	3.3 ± 0.02^{a}
March'17	2.4 ± 0.05^{b}	3.6 ± 0.02^{a}	3.5 ± 0.02^{a}
April'17	2.5 ± 0.03^{b}	3.6 ± 0.03^{a}	3.5 ± 0.04^{a}

Values are means ± SEM, n = 3 per treatment group. Means with the different letters in each row indicates significant differences ($p<0.05$).

3. Discussion

The present study found pesticides in dried barb. Presence of pesticides in dried fish also reported in several others study. Hasan et al. [8] estimated 0.204 ppm and 0.034 ppm DDT in dried barb of Syedpur and Cox's Bazar, respectively. They also reported Dichlorvos was not found in dried barb. Similarly, Chowdhury et al. [23] detected 0.187 ppm and 0.243 ppm DDT in dried *P.. sophore* collected from different markets of Dhaka city. DDT was also found in other dried fishes such as Bombay duck, Ribbon fish [7,8,10,24]. Aldrin was found in dry and wet fish collected from South Patches of Bay of Bengal as (26 to 86 ng g^{-1}) and (17 to 67 ng g^{-1}) respectively [25–27]. These findings reported that dried fish producer or vendors indiscriminately use pesticides in dried fish. However, we did not study the Aldrin and its derivatives residues in wet fish.

Presence of multiple pesticides (DDT – Heptachlor) in dried Ribbon fish, Bombay duck, Anchovy, Chinese pomfret, Indian salmon was also reported [10,24] which is similar to the findings of the present study. Detection of multiple pesticides in dried fish of retail market revealed that anglers might use more than one pesticides in one dried fish. Another explanation could be drawn that one pesticide might be used by producer and other pesticide might be used by dry fish values chain vendors for long-term preservation of dry fish. At present import and production of 'Dirty Dozen' are banned in Bangladesh [28]. The POPs (Persistent Organochlorine Pesticides) group is known as "Dirty Dozen". The POPs group includes 12 substances, 9 agrochemicals (aldrin, chlordane, DDT, dieldrin, endrin, mirex, heptachlor, hexachlorobenzene, toxaphene), and 3

industrial substances (polychlorinated biphenyls, dioxin, and furans) [29]. Therefore, to speak documentarily, at present there is no legal use of any POPs in Bangladesh. But some old stocks may be available as well as some might be available through other unknown sources [28]. Besides, the use and production of Aldrin, Dieldrin, and Endrin are prohibited or severely restricted by the Stockholm Convention on POPs [30]. The Government of Bangladesh signed the Stockholm Convention on POPs, therefore, the government has taken up the initiative to generate general awareness of consequences of POPs releases and ultimate elimination [28]. The persistence of Endrin in the environment depends highly on local conditions. Some estimates indicate that Endrin can stay in soil for over 10 years [31].

Patterson and Ranjitha [32] stated total plate count seemed to be high in the commercially dried fishes than the experimentally dried fishes which is similar to findings of the present study. Logesh et al. [33] observed highest APC of log value 6.72 ($5.3x10^6$ cfu g^{-1}) in dried fish (*Sardinella longiceps*) of Cuddalore district in India. Similarly, Saritha et al. [18] reported higher bacterial count such as log value 6.32 ($2.13x10^6$ cfu g^{-1}) was observed for the sun-dried fish (*Paraupeneus indicus*). Islam et al. [34] reported bacterial load log value 5.36 ($2.3x10^5$ cfu g^{-1}) in dried barb (*Puntius* sp.) from Natore district, Bangladesh. Variation in APC in different months might be due to the monthly variation of temperature and moisture content in atmosphere [33]. Which is also supported by Lilabati and Vishwanath [20] and Prakash et al. [19] where they reported that there was a direct relationship between the microbial counts and moisture content of the sample.

Patterson and Ranjitha [32] observed higher *E. coli* in commercial dried fish than experimental dried fish which is concurred with findings of the present study. Saritha et al. [18], Prakash et al. [19]and Immaculate et al. [14] mentioned that they found all the analyzed dried fishes contaminated with *E. coli.* The presence of *E. coli* indicates the dried fish samples contaminated with total and fecal coliforms. Fecal contamination in the landing center, washing the catches in polluted water with the disposal of sewage, reused water and improper disposal of fecal materials are the possible sources for coliform contamination in dried fish samples [18].

In recent years, contamination of fish and fishery products with *Salmonella* and *Vibrio* sp. has been reported by many researchers in different parts of India [14,19,33] and Bangladesh [35,36]. Though, Azam et al. [3] and Saritha et al. [18] observed the absence of the spoilage organisms *Vibrio* sp. and *Salmonella* sp. in all the dry fish samples. Incidence of pathogens in the sample of fish market may be attributed to external contamination [37]. In some of the cases, the foodborne illness such as scombroid poisoning is observed in dry fishes mainly due to the chemical agent (histamine) and it is also known as histamine poisoning; *E. coli* is responsible for the production of histamine in the dried fishes [33].

Kumar [38] estimated highest fungal count of log value 4.27 ($1.5x10^4$ cfu g^{-1}) in dried fish collected from market of Southeast coast of India. Likewise, Saritha et al. [18] reported highest fungal count of log value 4.32 ($2.1x10^4$ cfu g^{-1}) in dried fishes of Cuddalore market. The results of this study are paralleled to the report of Patterson and Ranjitha [32] where they observed higher fungal count in the commercially dried fishes than the experimentally dried fishes. The presence of high fungal count in dried fish may be due to post-harvest delay, improper transportation, unhygienic handling and processing during the salting and sun drying process, contaminated working floor, salt and water [18]. Additionally, presence of different types of fungi and bacteria in dried fishes has been reported by several researchers [18,19,33,38]. The fungus *Aspergillus flavus* is responsible for the production of aflatoxin and it is also found that it causes foodborne intoxication which leads to serious health hazards. Hasehm [39] have studied the mycotoxins from the fishes and recorded that *Aspergillus* is the main genus that commonly involved in the production of mycotoxins. Thus, the presences of these fungi are of great significance in view of food safety and quality.

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4. Materials and Methods

4.1 Study area and sampling

Dried barb (*P. sopohre*) were collected for a period of five months (December 2016 to April 2017: Peak season for dry fish processing) from dried fish producers and retailers of north-eastern part of Bangladesh (Figure 3). Collected samples were packaged in sterile airtight polythene bags and immediately brought to the laboratory of Fish Processing and Quality Control, Sylhet Agricultural University (SAU), Bangladesh. Besides, one control sample was prepared to compare the results. To prepare control samples, raw barb was collected from the same raw fish lot used by the producers and prepared in laboratory condition after following FAO Code of Practice for fish and fishery products [40]. Pesticides analysis was carried out in the Pesticide Analytical Laboratory of Bangladesh Agricultural Research Institute (BARI), Bangladesh. Microbiological analysis was conducted in the laboratory of Microbiology and Immunology, SAU.

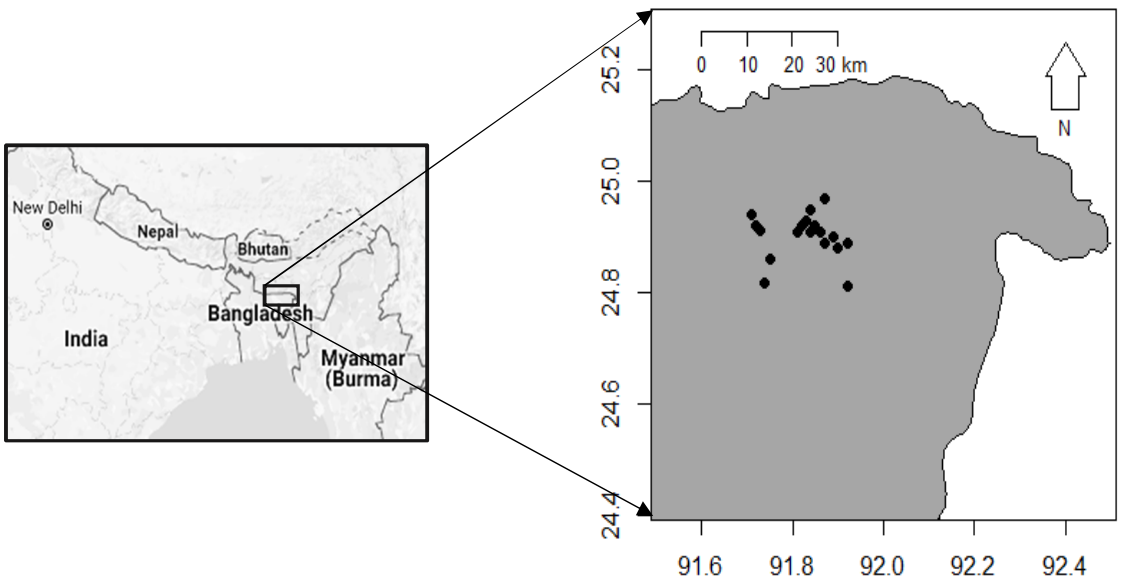

Figure 3. Map of the study area.

4.2 Organochlorine pesticide residue analysis

4.2.1 Sample preparation, extraction, and clean-up

The extractions were carried out according to the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method described by Anastassiades et al. [41] with some necessary modifications. Sample was chopped on a chopping board with a sharp knife and grounded. Ten g of chopped sample was transferred into a 50 ml Teflon centrifuge tube. Then 10 ml of acetonitrile was added into centrifuge tube and agitated well for proper mixing. Again 7.5 g of anhydrous $MgSO_4$ and 1 g of NaCl were added and centrifuge tube was vigorously shaken for 1 minute. Later it was centrifuged at 5,000 rpm for 5 minutes. After centrifugation 2 ml of the supernatant was transferred into an Eppendorf tube containing 100 mg primary secondary amine (PSA), 150 mg $MgSO_4$ and 100 mg of charcoal for clean-up followed by vigorous shaking for 2 minutes. Again, prepared sample extract centrifuged at 10,000 rpm for 5 minutes. Afterward, the sample extract was filtered through a 0.45 µm filter using a syringe and transferred into vial for further analysis in GC.

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4.2.2 GC analysis

The organochlorine pesticide residues were analyzed by GC-2010, Shimadzu with an Electron Capture Detector (ECD), an auto-injector (Shimadzu, AOC 20i) and GC solution software. The capillary column used in ECD was Rtx-CL, length 30.0 m x ID 0.25 mm x film thickness 0.32 µm. The GC was run under the following conditions: injector temperature: 250ºC; detector temperature 330ºC; oven temperature programme: 260ºC starting from 0 to 180ºC for 0 min and continued at 5ºC min^{-1} to 220ºC held for 12 min and continued at 5ºC min^{-1} to 260ºC; injected sample volume: 1µL; mode of injection: Split; The carrier gas was N_2 with a 77.8 kPa flow rate. Runtime: 28 min. Standards' peak were identified by injecting high concentration of the standard (1 ppm) and the retention time for organochlorine pesticides were determined (

Figure 4). The calibration was done at 5 points (25, 50, 100, 200 and 300 ppb) by composite stock standard solution. GC system was calibrated using external standard technique. Individual standard stock solution (100 mgL^{-1}) was prepared by weighing appropriate amounts of active ingredients in a brown bottle with a Teflon-lined screw cap and dissolving the weighed standard in HPLC grade methanol. Stock standard solution was used to prepare primary dilution standards. An appropriate volume of each individual stock solution was taken in a volumetric flask and mixed the solutions to obtain stock standard solution.

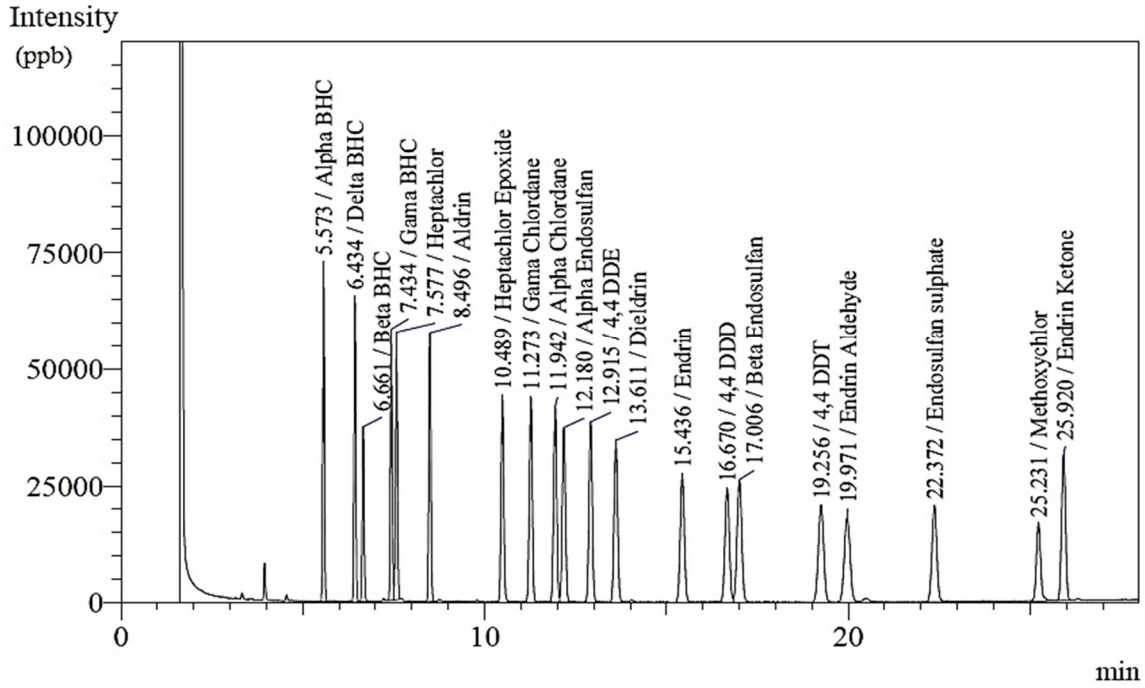

Figure 4. Chromatograms of 20 standard organochlorine pesticide run by GC-ECD.

4.2.3 Analytical quality control

Gas chromatograph equipped with ECD was checked for linearity. Instrumental limit of detection for GC-ECD was 1.0 μgL^{-1} for organochlorine pesticides. An aliquot of dry fish samples which were collected as blank and treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents used with the sample matrix. No analytic peak was detected in the laboratory reagent blank. An aliquot of fortified samples matrix was prepared to which known quantities of the pesticides were added in the laboratory in ppb range. This laboratory fortified matrix was analyzed exactly like the sample. Extraction and clean up were done as mentioned and the recoveries from untreated control samples of dry fish fortified with the analyzed compounds at level of 25 ppb was 96-100% for organochlorine mix. Before to injection of the first sample solution, a

standard solution was injected at least three times to checks the opening conditions and the constancy of the detector signal was checked by injecting serial dilution of organochlorine mix.

4.2.4 Quantification of detected pesticide

Any pesticide detected from the tested samples were identified and quantified by the chromatogram of standard pesticides. Sample results were quantities in ppm automatically by the GC software, which represented the concentration of the final volume injected. From this value, the actual amount of insecticide residues present in the sample was determined by using the following formula:

Amount of pesticide residues = (Conc. of obtained pesticide in injected sample (ppm) × Quantity of final volume (L)) / (Amount of sample taken (Kg))

4.3 Microbiological quality analysis

4.3.1 Aerobic plate count (APC)

Spread plate method [42] was used to estimate total aerobic bacterial counts of dried barb fish. Twenty-five (25) g of sample was suspended in 225 ml Butterfield's buffered phosphate diluent. One milliliter of aliquots of homogenate solution were serially diluted (10^{-1} to 10^{-9}). Then 0.1 ml aliquot was inoculated on triplicate plate count agar (Difco) and incubated 35^0C for 48 hours. Plates containing 30-300 colonies were used to calculate bacterial population results, recorded as colony forming unit of sample.

4.3.2 Fungal count

Sample was prepared in same way mentioned in aerobic plate count method. Sample was inoculated on Sabourad dextrose agar (Himedia) and incubated at 23^0C in inverted position for 5 days. Colonies of fungus were counted as colony forming unit from the plates having 10-100 colonies [43].

4.3.3 Isolation and identification of bacteria

To isolate different bacteria in the samples of dried barb fish, bacterial colonies were divided into different groups according to colony shape, size, elevation, structure, surface, edge, color, and opacity and counted the number of colonies of each recognizable type. With some exceptions, three to five representatives of each colony type were then streaked repeatedly on tryptic soya agar (TSA) plates until pure cultures were obtained. For all populations, an average 5% of primary isolates failed to grow despite repeated attempts at subsequent subculture. Purified cultures were inoculated onto TSA slants and kept at 48^oC to get stock inoculum; then the inoculum were again cultured on agar slants at every 6 weeks [44]. Bacterium was identified according to the criteria described in Bergey's Manual of Determinative Bacteriology [45] up to genus and species level.

4.4 Data analysis

All data were subjected to statistical analysis. One-way ANOVA and post-hoc Tukey's test were used to analyze values of APC and fungal count [46] and the difference within the variables [47]. Values are expressed as mean ± SEM. P values < 0.05 were considered statistically significant. Meanwhile, data from qualitative microbiological tests were analyzed using the chi-square test with the SPSS statistical software (Version 23). Map was generated using "R" (Version 3.2.4).

5. Conclusions

The present study revealed that anglers have been using organochlorine pesticides in dried barb fish without considering health hazards of pesticides. Concentration of the detected pesticides were much higher than recommended MRLs which might cause health problems to the consumer for long time. Besides, microbiological quality of died barb fish was found very poor. The government of

Bangladesh should take all the necessary initiatives to tackle this situation. It can be done by implementing the existing legislation, increasing the awareness about harmful effect of those pesticides and barring the trade of harmful pesticides in market. Besides, the anglers should dry fish properly and package very carefully so that the dry fish cannot absorb moisture from the environment. Further study is also required to reveal the residual limits of other pesticides with more geographical coverage and larger samples size.

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Conflicts of Interest: The authors assert no conflict of interest.

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