Organochlorine Pesticide Residues and Assessment of other toxic substances in fresh and sun-dried *Mystus bleekeri* from the Northeastern Part of India

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Abstract

Fresh and sun-dried *Mystus bleekeri* is very popular among the people of Tripura, Northeastern part of India. But sometimes it was observed that fishes are contaminated with several harmful martial likes' salts, dust, organic toxic material including heavy metals, microbes and artificially several toxic chemicals likes pesticides and preservatives. So it is very much essential to determine the presence of heavy metals, pesticides and formalin in both fresh and sun-dried M. bleekeri. Analysis of Formaldehyde, Analysis of Pesticides and Estimation of heavy metals were performed for the analysis. In raw and cooked form of fresh M. bleekeri formaldehyde content was not detectable. But in case of sun-dried M. bleekeri formaldehyde content was below the tolerable levels for human. Zinc, copper and selenium content were found to be higher in the fish samples. On the other hand, Aldrin, Alachlore, o,p′-DDT+ p,p′-DDD were identified as the main organochlorine in the raw and cooked form of sun-dried M. bleekeri.

Keywords: *Mystus bleekeri*, Cooking methods, Formaldehyde, Pesticides, Heavy metals.

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Introduction

Northeastern region of India, the easternmost region of India is very much popular for fisheries in terms of many rivers, streams, flood plain wetlands, lakes, ponds, and large areas under the rice-fish culture system. Rice and fishes are essential ingredients in all the cuisines of north-eastern people from the ancient age. During the unavailability of freshwater fishes, people are habitats with substituted processed fishes like dried, smoked, and fermented fishes, and they are available around the year. Fishes are very highly nutritive food in the human diet. They are the important sources of protein, minerals, polyunsaturated fatty acids (PUFA) especially EicosaPentaenoicacid (EPA) and DocosaHexaenoic acid (DHA). PUFA play an important role to reduce the risk factors associated with several cardiovascular diseases [1]. India is one of the mega biodiversity countries where among 765 freshwater fishes 450 are small indigenous fishes [2]. They serve as one of the richest sources of vitamin A, iron, protein and lipid [3]. But fish may be considered as one of the most perishable among all foodstuffs; therefore, their preservation becomes necessary for future use [4]. Sun-drying is one of the most important traditional methods which have been used for centuries for preserving fish [5].

Literature revealed that during processing, fishes are contaminated with several harmful materials like salt, dust, organic toxic materials including heavy metals, microbes and several toxic chemicals like pesticides and preservatives [6,7]. They possess a great threat to human health. Formaldehyde which is widely used in food industry as antibacterial agent and preservative is carcinogenic to humans [8]. Heavy metals (lead, chromium, mercury, arsenic etc.) are well-known contaminating chemicals which contaminate water and soil [9]. These metals have no beneficial effects on human health. These are generally considered as the most toxic elements for humans and animals. On the other hand, pesticides in fish products may give indication about contamination and possesses a great threat for human health [10]. So the detection of these toxic elements in a food sample is very essential for health.

Mystus bleekeri (*M. bleekeri*) is a small, freshwater catfish maximum length as 15.5 cm and belongs to family Bagridae and order Siluriformes [11]. Among all small sized fishes, it has high economic importance and market value having good taste and nutritional value [12]. It occurs in streams, rivers, side channels, tanks and reservoirs [13]. In Pakistan, it is locally called Tingara and is distributed in Khyber Pakhtunkhwa, Punjab and Sindh [14]. *M. bleekeri* can be consumed both fresh and processed form. Drying is the thermal treatment applying frequently in food industry to preserve of fish and other foods [15]. Drying process involves complete removal of water content from fish and food substances to reduce the action of enzymes, bacteria, yeast [16]. Both fresh and dried *M. bleekeri* is very popular among the people of Tripura. But both fresh and sun-dried *M. bleekeri* cannot be consumed in raw form. Various cooking methods such as boiling, frying, roasting are employed for the preparation of fresh and sun-dried *M. bleekeri*. So the objective of the present experiment was to analyze the level of organoleptic pesticide residues and other toxic materials in raw and cooked form of fresh and sun-dried *M. bleekeri*.

Result and Discussion

Determination of formaldehyde content

Fishes are sometimes contaminated with several harmful chemicals like heavy metals and pesticides which are very harmful for human health. Among all the contaminating elements, attention has been paid on formaldehyde which

is carcinogenic to humans as per International Agency for Research on Cancer (IARC) [17]. It is metabolized by normal metabolism process. It is used as preservative for processing food sample especially dried and frozen food [18]. Large doses of formaldehyde can cause minor to serious problems including pain, vomiting, coma and possible death [19]. According to United States Environmental Protection Agency, acceptable daily intake about 0.2 mg/kg body weight [20]. Formaldehyde content (mg/kg) of raw and cooked sample of fresh and sun-dried *M. bleekeri* is presented in Table 1. In raw and cooked sample of fresh *M. bleekeri* formaldehyde was not detectable level. But in sun-dried sample formaldehyde content was 4.14 mg/kg which was reduced after cooking. 1.74 mg/kg in boiled sun-dried *M. bleekeri* and 1.51 mg/kg in fried sample of sun-dried *M. bleekeri*.

Table 1. Formaldehyde content (mg/kg) of raw and cooked form of sample of fresh and sun-dried M. bleekeri

HCHO	***M. bleekeri* (Fresh)**			***M. bleekeri* (Sun-dried)**		
	Raw ± SE	**Boiled ± SE**	**Fried ± SE**	**Raw ± SE**	**Boiled ± SE**	**Fried ± SE**
Formaldehyde	NDL	NDL	NDL	4.14 ± 0.44	1.74 ± 0.07	1.51 ± 0.03

NDL: Not Detectable Level. Five samples were taken to calculate the standard error mean. Significant differences ($p<0.05$) in formaldehyde concentration between raw and cooked sample of fresh and sun-dried *M. bleekeri*.

In the food industry, formaldehyde is used as a preservative. It is also used as an antiseptic solution in very small concentrations. It possesses antibacterial and antifungal properties. It is highly toxic and absorbed by inhalation. It adversely effects respiratory tract and may cause dizziness and suffocation. It is carcinogenic also. Exposure of formaldehyde can cause increased incidence of lung and nasopharyngeal cancer. Therefore, it is necessary to estimate the quantity of formaldehyde present in marketed fish samples. Acceptable daily intake (ADI) of formaldehyde 0.2 mg/kg body weight which has been set by the United States Environmental Protection Agency. The above study revealed that the level of formaldehyde in the fresh *M. bleekeri* was not detectable, and it is below the tolerable levels for humans in the sun-dried sample. During transportation of fish to a long distance, it is very essential to maintain their quality. Formaldehyde is used to maintain the quality of fish unaltered [21]. Concentration of formaldehyde mainly depends on different levels of trimethylamine n-oxide (TMAO) [21]. After breakdown of TMAO, tri-methylamine, di-methylamine and formaldehyde are formed [22]. The major toxic effects caused by the exposure of formaldehyde through inhalation are eye, nose and throat irritation [23]. Damage of gastrointestinal tract was also occurred due to ingestion of formaldehyde [23]. The maximum value of formaldehyde is generally up to 60 mg/kg in fruits and marine fish. However, the average range is 2-50 mg/kg [18].

In the present experiment, level of this toxic element was found to be reduced after cooking process and this may be due to evaporation of formaldehyde during cooking process as it's boiling point is 101˚c [24]. The above study revealed that cooking process had some positive effects on fresh and sun-dried *M. bleekeri* as formaldehyde was found to be degraded through the application of heat.

Determination of pesticide content

Pesticides are substances or mixture of substances used for preventing, destroying and mitigating any pest [25]. Pesticide content (spiking level 5 ng/g) of raw and cooked sample of fresh and sun-dried *M. bleekeri* is represented in Table 2. Alachlor, Carbofuran, Dieldrin, o, p′-DDT+ p, p′-DDD, p, p′-DDT and Hexachlorobenzene was not detectable in raw and cooked form of fresh *M. bleekeri*. All the pesticides content such as Aldrin, Alachlor, Carbofuran, Dieldrin, Endosulfan sulfate, o, p′-DDT+ p, p′-DDD, p, p′-DDT and Hexachlorobenzene were found to be higher in sun-dried *M. bleekeri*. But after cooking the pesticides content was found to be reduced. In the present experiment, marketed sun-dried *M. bleekeri* contained these toxic materials whereas alachlor, carbofuran, dieldrin, o, p′-DDT+ p, p′-DDD, p,p′-DDT and hexachlorobenzene was not detectable in fresh sample of *M. bleekeri*. This may be due to the addition/adulteration of these toxic elements for preservation. But after cooking, this toxic element was found to be reduced because cooking process increase volatilization, hydrolysis or other chemical degradation [26]. On the other hand, it leads to the decomposition by applying heat [27].

Aldrin and dieldrin, once used as an insecticide are the common names of two structurally similar compounds. Exposure of aldrin and dieldrin mainly occurs through contaminated food including fish or shellfish obtained from contaminated rivers, streams etc. Adverse effects of dieldrin are related to its

Table 2. Estimation of pesticide residues: The pesticide values are expressed as spiking level (5ng/g) of raw and cooked form of sample of fresh and sun-dried *M. bleekeri*.

Name of the pesticides	***M. bleekeri* (Fresh)**			***M. bleekeri* (Sun-dried)**		
	Raw ± SE	**Boiled ± SE**	**Fried ± SE**	**Raw ± SE**	**Boiled ± SE**	**Fried ± SE**
Aldrin	55 ± 12	54 ± 13	52 ± 12.5	106 ± 15	105 ± 12	98 ± 11
Alachlor	NDL	NDL	NDL	115 ± 6	96 ± 4	95 ± 5
Carbofuran	NDL	NDL	NDL	26 ± 2	25 ± 2	20 ± 3
Dieldrin	NDL	NDL	NDL	55 ± 1	45 ± 2	42 ± 1
Endosulfan sulfate	5 ± 1	4 ± 3	2 ± 0.5	24 ± 5	18 ± 2	17 ± 1

o,p′-DDT+ p,p′-DDD	NDL	NDL	NDL	115 ± 12	105 ± 9	98 ± 17
p,p′-DDT	NDL	NDL	NDL	66 ± 11	59 ± 13	52 ± 11
Hexachloroben-zene	NDL	NDL	NDL	67 ± 5	65 ± 2	64 ± 6
NDL: Not Detectable Level. Five samples were taken to calculate the standard error mean. Significant differences ($p<0.05$) in formaldehyde concentration between raw and cooked sample of fresh and sun-dried *M. bleekeri*						

concentration in the blood. Aldrin is quickly converted into dieldrin when it is absorbed by an animal. The level of dieldrin in the blood below 105 µg/lit, no adverse effects can be occurred. The main systemic effect of carbofuran poisoning appears to be cholinesterase inhibition. Alachlor, another herbicide was first registered in 1969. Alachlor is absorbed through the gastrointestinal tract of rats and distributed to the blood, spleen, liver, kidney, heart, and, to a lesser extent, eyes, brain, stomach, and ovaries. The LD50 of alachlor is between 1,910 and 2,310 mg/kg in the mouse. But after cooking, this toxic element was found to be reduced because of chemical degradation through the application of heat. So, It could be conclude that cooking method may help to reduce toxic elements in fish.

Determination of heavy metal content

Heavy metals enter the environment by natural and anthropogenic means. The chain of heavy metal contamination follows a cyclic order such as industry, atmosphere, soil, water, foods and human [28]. Arsenic is a metalloid. Its toxic effects depend specially on oxidation state and chemical species. According to FAO/WHO, maximum tolerable level of arsenic intake at 15 µg/kg b.w [29]. Both raw and cooked sample of Sun-dried *M. bleekeri* contained higher amount of arsenic. Mercury is one of the most toxic heavy metals in the environment. Mercury content was found to be 0.061 mg/kg in sun-dried *M. bleekeri* and 0.054 mg/kg in fresh sample of *M. bleekeri* which was reduced after cooking. Cadmium content was found to be 0.31 mg/kg in sun-dried and 0.30 mg/kg in fresh *M. bleekeri*. Copper is essential for maintaining good health. But long term exposure to copper may cause toxic effects on our health such as Wilson's disease [30]. In the present experiment, copper concentration in sun-dried and fresh *M. bleekeri* was 50.10 mg/kg and 51.01 mg/kg. Chromium content was found to be less than 1.0 mg/kg for all raw and cooked sample of fresh and sun-dried *M. bleekeri*. Zinc, another essential nutrient helps for immune system and metabolism function. But high level of zinc can be harmful for body. FAO has set a limit for zinc at 30 mg/kg [31]. The limit of acceptability of zinc was exceeded in both raw and cooked form of fresh and sun-dried *M. bleekeri*. Selenium is an essential trace element required in small amounts by animals and humans. But higher content of Se is toxic and exerts adverse effects on health. In the present experiment, fresh *M. bleekeri* contained 10.80 mg/kg Se and sun-dried sample contained 9.95 mg/kg Se. But cooking had no significant effect on Se content. In the present study, nickel content was found to be within acceptable limit. Lead content was found to be 0.54 mg/kg and 0.52 mg/kg in fresh and sun-dried *M. bleekeri*. But cooking had not such significant effect on lead content of fish sample.

Heavy metals are naturally accumulated in the environment likes streams, lakes, and oceans from various natural source and human contaminants or effluents and are also released to the environment through many types of human activity. Naturally they can be accumulated in aquatic animals including fishes. In

Table 3. Heavy metal content (mg/kg) of raw and cooked form of sample of fresh and sun-dried M. bleekeri

Heavy metals	**M. bleekeri (Fresh)**			**M. bleekeri (Sun-dried)**		
	Raw ± SE	**Boiled ± SE**	**Fried ± SE**	**Raw ± SE**	**Boiled ± SE**	**Fried ± SE**
Total arsenic (µg/g)	4.07 ± 0.002	4.02 ± 0.005	3.98 ± 0.005	27.02 ± 0.005	27.00 ± 0.005	26.87 ± 0.002
Inorganic arsenic (µg/g)	0.048 ± 0.0002	0.042 ± 0.0002	0.043 ± 0.0005	0.281 ± 0.0002	0.280 ± 0.0002	0.241 ± 0.0005
Mercury (mg/kg)	0.054 ± 0.0005	0.052 ± 0.0005	0.051 ± 0.0002	0.061 ± 0.0005	0.061 ± 0.0005	0.060 ± 0.0002
Lead (mg/kg)	0.54 ± .002	0.51 ± 0.002	0.50 ± 0.005	0.52 ± 0.006	0.51 ± 0.002	0.50 ± 0.005
Cadmiun (mg/kg)	0.30 ± 0.005	0.30 ± 0.005	0.30 ± 0.005	0.31 ± 0.005	0.30 ± 0.002	0.30 ± 0.005
Chromium (mg/kg)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Copper (mg/kg)	51.01 ± 0.005	50.00 ± 0.006	50.00 ± 0.002	50.10 ± 0.005	50.00 ± 0.005	50.00 ± 0.005
Nickel (mg/kg)	1.80 ± 0.005	1.79 ± 0.006	1.78 ± 0.005	1.81 ± 0.002	1.80 ± 0.005	1.80 ± 0.005
Selenium (mg/kg)	10.80 ± 0.002	10.20 ± 0.002	10.21 ± 0.005	9.95 ± 0.005	9.95 ± 0.002	9.94 ± 0.002
Zinc (mg/kg)	110.0 ± 0.251	109.9 ± 0.251	109.8 ± 0.032	102.5 ± 0.066	102.3 ± 0.066	102.2 ± 0.065
Significant differences is $p<0.05$ in heavy metals concentration between raw and cooked samples of *M. bleekeri.*						

our study, we have estimated the heavy metals content in fish samples raw and cooked forms. Among heavy metals, copper, zinc and selenium levels were found to be higher in both fresh and sun-dried *M. bleekeri* but cooking had no significant effects on heavy metal content.

Materials and Methods

Sample preparation and cooking

Fresh and sun-dried sample of *M. bleekeri* was taken from Battala, a local market in Tripura. After collection, fish samples were cleaned to remove dust particles. The fresh samples were washed properly and internal organs were removed. In case of fresh sample, the main purpose was to obtain maximum amount of flesh portion and for this purpose they were cut along the backbone. The flesh portion was cut into small pieces and prepared for different cooking procedure. Boiling and frying process was applied for the above experiment. In the case of a processed fish sample *M. bleekeri*, they were washed with water and cut into small pieces for the further cooking process. Fresh and sun-dried *M. bleekeri* was then boiled for about 20 minutes. Deep-frying of all the samples was done using vegetable oil for about 15 minutes, and the temperature was 240ºc.

Estimation of formaldehyde

Sample preparation: Formaldehyde content was determined according to Claeysa et al. 2009 [32]. 5 g of fish samples were taken; blank and spiked formalin was added into the samples. Then added 5 ml of acetonitrile, sonicated for 30 minutes at 25-30˚c, shaken for 30 minutes in a shaking water bath at 150 rpm at normal temperature. Centrifuged in 6000 rpm at 22˚c for 5 minutes, filtered through a Whatman filter paper (90 mm). Approximately 5 ml of the upper layer of the extract was carefully taken. 2.5 ml DNPH solution (dinitrophenylhydrazine) and vortex were added well. Recrystallization of DNPH was carried out by dissolving 10 ml of anhydrous acetonitrile acetate to form a saturated solution. The samples were derivatised by shaking at 150 rpm, at 40˚c for 1 h in a shaking water bath and after incubation the supernatant got filtered by a syringe micro filter (0.45 µm).

Analytical condition of HPLC: A 10 µl sample solution was analyzed with a C-18 column (250 × 4.6 mm, 5 µm) with a 60% methanol solution as mobile phase and detected at 355 nm. The flow rate was 1 ml/min and the operating time was 13 min.

Estimation of pesticides

Samples were homogenized with an Ultra-Turrax T25 homogenizer and stored in a -20˚c freezer. A standard mixture of pesticides at 20 µg/ml in 0.05% formic acid in methyl cyanide was used as a spiking solution for 100 and 50 ng/g spiking levels. A pesticide standard mixture at 500 ng/ml in 0.05% formic acid in methyl cyanide was used for 5 and 1 ng/g spiking levels in the recovery experiments. A mixture of atrazine-d5 and fenthion-d6 at 10 µg/ml was prepared in 0.05% formic acid in methyl cyanide.

Sample preparation: Weigh 10 g of homogenized fish sample into a 50 ml polypropylene centrifuge tube. Add 1000 ng of internal standards (ISTDs), yields 100 ng/ml. Add 10 ml of methyl cyanide and vigorously shake by hand for 30 s. Pour the entire extract including tissue into another 50 ml centrifuge tube with 4 g of MgSO4 (anhydrous) and 1 g of NaCl. Shake vigorously for 1 min and centrifuge the tube for 2 min at 3250 rcf. Take 1 ml of the extract and 150 mg MgSO4. Shake the tube vigorously for 30 s and centrifuge for 2 min at 3250 rcf then transfer 0.5 ml of the fish extract into an auto sampler vial for analysis by using low-pressure Gas Chromatography/ Tandem Mass Spectrometry (LP-GC/MS-MS). For reagent blank sample, use 5 ml of distilled water in place of tissue. The equivalent tissue concentration per sample extract was 1 g/ml [33].

Five replicates of fish samples were prepared for spiking level. Each batch of samples included a reagent blank and a sample blank. Matrix-matched calibration standards at concentration of 0.5, 1, 5, 10, 25, 50, 100, 250 and 400 ng/ml were used to calculate the recoveries of the pesticides. Fenthion-d6 was used as an ISTD for pesticide quantification, and atrazine-d5 was used as a back-up ISTD if needed. Method detection limits were determined as the pesticide's lowest calibrated levels (LCL).

Estimation of heavy metals

0.3-0.5 g of fish tissue was weighed and placed in a Teflon digestion vessel with 7 ml of concentrated (65%) nitric acid and 1 ml of 30% hydrogen peroxide. The sample in the vessel containing concentrated nitric acid was then subjected to a microwave program:

Step I: 25-200˚c for 10 minutes at 1000 W

Step II: 200˚c for 10 minutes at 1000 W

Digests were finally made up with deionized water to 25 ml. Heavy metal content was analyzed by inductively coupled plasma/mass spectrometry (ICP-MS) (Model X series, Winsford-cheshire UK) [34].

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) v. 16.0 for windows (SPSS, SAS Institute Inc. Cary, USA). The data were analyzed to determine the descriptive statistics such as Standard Error of Mean (SEM), Standard Deviation (SD), Statistic Mean, Minimum and Maximum value and Ranges of variables. One way ANOVA and Duncan multiple tests were done to test the significance between two floodplains using 5% level of significance.

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Conflicts of Interest

The authors declare no conflicts of interest.

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