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Detection of Heavy Metals from Dry Fish and Evaluation of their Toxic Effects on the Swiss Albino Mice Model

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Abstract

Sea-dry fish is a popular protein-containing food in Bangladesh. During the processing of dry fish, it can become contaminated with heavy metals, which is a major health concern for humans. The present investigations were carried out to detect selected heavy metals (Nickel, Chromium, Cadmium, Arsenic, and Lead) in three sea dry fish (Churi, Tuna, and Surma), and to evaluate the toxic potential of the dry fish in Swiss albino mice. The concentrations of heavy metals in three commercially valuable dry fish samples were determined by Atomic Absorption Spectrophotometry (AAS) methods. The toxicity of dry fish was evaluated using a mouse model. The highest levels of Arsenic (As) and Lead (Pb) were detected with 3.07 and 3.33 ppm from dry fishes of *Scomberomorus guttatus* and *Thunnus thynnus*, respectively. The pests of dried fish were highly toxic to mice. Anorexia, depression, diarrhea, and deformities in the structure of the stomach, liver, lungs, and digestive tract were observed in the treated mice. The mice treated with surma fish showed the most toxic effects on mice. In conclusion, the present investigation may help to elucidate the quality of selected dry fish and their effects on humans.

Keywords: Sea Dry Fishes, Heavy Metals, Toxicity, Swiss Albino Mice

Introduction

Fishes are good sources of animal protein and play an important role in the human diet. It provides all the elements necessary for maintaining a healthy body (Dewi *et al*., 2011; Ravichandran *et al*., 2012) [7, 27]. Dry fish is popular for its availability and distinctive flavor and texture. Nowadays, controlled artificial dehydration of fish has been developed in some industrialized countries, enabling fish to be dried regardless of conditions. In any fish-drying process, thermal energy is required to remove water from fish (Paul *et al*., 2018). Fish is a vital component of the daily diet and considered a primary source of animal protein in Bangladesh. Dried marine fish are more popular than freshwater fish because they can be stored and consumed year-round. Dried fish have a high nutrient profile, comprising 80–85% protein. A good quality dried fish contains different types of vitamins such as A, D, E, K, minerals, and can provide 52-73% of protein (Sultana *et al*., 2011) [34]. Dried fish and fish products processed from small fish are rich in vitamins and minerals like iron, calcium, and phosphorus, and contain very low concentrations of cholesterol (Salaudeen *et al*., 2014).

Fish and fisheries are an important part of the life of Bangladeshi people and play a vital role in generating employment, nutrition, earning foreign currency, and other aspects of the economy (Alam, 2002) [2]. Bangladesh has shown steady growth in marine fish production (over 5%) over the last couple of years (DoF, 2018) [8]. The fish and dry fish sectors play an important role in the service, livelihoods, and economic prospects of millions of people worldwide.

Dry fish has numerous health benefits. Dried fish and fish products contain a high amount of animal protein and omega-3 fatty acids. This protein covers the protein requirement of the human body. Several articles on the importance of omega-3 fatty acids for health, especially the cardiovascular system, have been published (Shahidi *et al*., 1998). These fatty acids decrease the risk of stroke and reduce blood pressure. Moreover, fish and dried fish are very important for strengthening the body's immune system and are also required for body growth, maintenance of lean muscle tissues, and active metabolism (Pal *et al*., 2018) [21].

Heavy metals have a high capacity for bioaccumulation and are potentially toxic and non-degradable. The contamination of aquatic environments by heavy metals has become a worldwide problem (Rahman *et al*., 2018). Marine fish are contaminated by the most important sources of heavy metals, including sewage effluents, landfilling associated with coastal infrastructure development, oil spills, petrochemical industries, desalination plants, and mining. Dry fish has been recognized as a good accumulator of heavy metals. In the sea, heavy metals accumulate in marine organisms and sediments, ultimately reaching us through the food chain (Giordano *et al*., 1991) [11]. Both essential and non-essential elements of heavy metals have a particular significance in ecotoxicology, since they are highly persistent and potentially toxic to living organisms (Storelli *et al*., 2008). As Cd, Pb, and Hg are found in dry fish, they are considered the most toxic heavy metals to humans (Govind and Madhuri, 2014) [12]. The major adverse health effects of exposure to these heavy metals, even at low concentrations, are neurotoxicity and carcinogenicity (Jomova and Vaiko, 2010) [15]. Different concentrations of Pb, Cd, Cr, Mn, and Ni have been reported in various species of dried fish, and cytotoxic effects of these heavy metals on animals have been documented (Rakib *et al*., 2021; Samantara *et al*., 2023) [25, 30]. Seafood has high nutritional value and is considered a prime vehicle for pathogenic microorganisms (Das *et al*., 2007) [6]. A wide range of fish spoilage occurs due to the attack of pathogenic bacteria and fungi, which adversely affect the economy in Bangladesh and public health (Dewi *et al*., 2011; Tawfeequa *et al*., 2018) [7, 35].

Continual exposure of fish to microbes present in the water or during transportation is a contributing factor to fish toxicity (Shabeeb *et al*., 2016) [31]. Fish and fishery products are spoiled by spoilage microorganisms, and contamination by pathogenic microorganisms causes a major problem during the distribution of fish (Parimal *et al*., 2018; Reza *et al*., 2009) [22, 28].

The presence of various pathogenic microbes in dried fish has been reported previously (Mansur *et al*., 2013) [17]. In sun-dried fish, histamine is produced by *E. coli*, *Salmonella*, and *Staphylococcus* species, which also produce histamine residues that contribute to foodborne illness (Kim *et al*., 2003). Pathogens associated with dry fish ruin the quality of dry fish. It causes significant economic losses and hampers public health by causing foodborne illnesses such as diarrhea, vomiting, abdominal cramps, nausea, fever, joint pain, and fatigue (Rahat *et al*., 2024) [24].

Dried fish is an important source of animal protein, and Bangladeshi people eat dried fish frequently. Quality and safety are among the major concerns for the human body. There is insufficient reporting on heavy metal contamination in fresh fish collected from different seas and rivers in Bangladesh. But there is no complete report on dry fish-related toxicity in the mouse model. Therefore, the present research work was designed to determine the selected heavy metals Ni, Cr, Cd, As, and Pb from the dry fish and to evaluate the effects of feeding dry fish to model mice.

Materials and Methods

Dried Fish Samples Collection and Preservation

Three types of dried fish species, viz., Chhuri (*Lepturacanthus savala*), Tuna (*Thunnus thynnus*), and Surma (*Scomberomorus guttatus*) were collected from the local and biggest dried fish market of Cox's Bazar Sea beach area, Bangladesh. The samples were collected separately in sterile, airtight plastic bags to prevent extraneous contamination. The collected samples were then stored at 4°C in the laboratory.

Analysis of Heavy Metal Concentration

Preparation of Reagents

All reagents used for the analysis were analytical grade and consisted of nitric acid (65% v/v) and hydrogen peroxide (30% v/v) (Merk, Darmstadt, Germany). Ni, Cd, Cr, Pd, and As stock solutions were prepared at a concentration of 1000 mg/L by diluting 1 ml of each single-element stock from the combination list to 100 ml with deionized distilled water containing 1% (v/v) nitric acid. The apparatus and chemicals were sterilized by soaking them for approximately 24 h in 10% (v/v) HNO_3, then rinsing them with deionized water prior to the investigations.

Atomic Adsorption Spectroscopy

Flame atomic absorption spectrophotometry is a broadly used method for detecting metals and metalloids in ecological samples. The technique is based on the principle of ground-state metals absorbing light at a specific wavelength. Metal ions in a solution are changed to an atomic state by means of a flame. Light of the suitable wavelength is full, and the amount of light absorbed can be measured against a normal curve. The technique uses absorption spectrometry to regulate the absorption of an analyte in a sample. It requires a standard with known analyte content to determine the relationship between the measured and analyte concentrations and relies on Beer-Lambert's Law (Skoog *et al*., 2005).

The sample is converted into atomic vapor by a process known as atomization. The precision and accuracy of this method depend on the atomization step (Ipeaiyeda *et al*., 2017) [13]. In a continuous atomizer, the sample is fed into the atomizer at a constant rate, yielding a spectral signal that remains constant over time. Atomization procedures of the nonstop type include flame, inductively coupled argon plasma, and direct-current argon plasma. With discrete atomizers, a method quantifies a sample introduced as a plug of liquid or solid. In this case, the spectral signal increases to an extreme and then absorbs radiation from an individual. Wavelengths from an external source; the atoms of lead, chromium, copper, Cadmium, calcium, and magnesium absorb radiation at wavelengths of 217.0 nm, 357.9 nm, 228.8 nm, and 324.0 nm, respectively (Kilic *et al*., 2014) [16]. This technique has been widely employed for elemental analysis across a range of matrices, including soils, water, nuts, wine, and wine products (Narin *et al*., 2000) [19].

Sample Analysis

Analysis of the heavy metal content of the samples was performed using a flame atomic absorption spectrophotometer (Shimadzu AA-7000, Japan) with acetylene as the fuel and air as the oxidizer. Processed tasters were introduced into the fuel-rich air-acetylene flame, and metal absorptions were enhanced using standardization curves derived from standard solutions. Each purpose was founded on the normal values of three replicate samples.

Analytical Technique

Trace elements relate to the very small volumes of analyte present in the sample, which needs special instrumental methods to be determined. Not very long ago, trace points were about µg/g. Nowadays, absorptions range from µg/g to ng/g or lower. On the other hand, one element at high concentration in a sample can be considered as a trace in another. The analytical technique used to determine heavy metal levels in all samples was thermo element Solar S4 AAS (International Equipment Trading Ltd, USA). It is a standard laboratory analytical tool for metal analysis, based on the absorption of electromagnetic radiation by atoms. The absorption wavelengths and detection limits for the heavy metals were 211.8 nm and 0.001 ppm for As, 217.0 nm and 0.001 ppm for Pb, 228.8 nm and 0.002 for Cd, 324.7 nm and 0.02 ppm for Ni, and 232.0 nm and 0.01 ppm for Chromium. The key feature is the production of free, ground-state atoms from the sample that pass through the hollow cathode lamp's light beam.

Where A is the absorbance, a is the absorptivity, b is the length of absorption, and c is the concentration of the absorbing species. Beer's Law shows a relation between absorption and concentration of analyte, so calibration of the instrument is needed. Calibration requires establishing a relationship between the signal response and a known set of standards. The standards in atomic absorption spectrometry are a series of aqueous solutions of changing concentrations of the analyte of interest. The real attentiveness of each metal was considered by the formula:

Actual concentration of metal in sample = (mg/kg) × R × dilution factor

Where,

(mg/kg) R = AAS reading of digest.

Dilution factor = volume of the digest used/ weight of the digested sample.

Toxicity Effect Test of Dry Fishes

Ethical Statement

For animal experiments, ethical clearance was obtained from the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC), University of Rajshahi, Rajshahi-6205, Bangladesh, under Memo No: 255(14)320/IAMEBBC/IBSc. All experiments were conducted in compliance with applicable laws, regulations, and ethical guidelines, with strict consideration of animal welfare and efforts to minimize potential harm. All experimental procedures were performed in accordance with the Organization for Economic Co-operation and Development (OECD) guidelines 2008 (OECD, 2008) [20].

Experimental Mice Collection and Rearing

Adult male Swiss albino mice (20–30 g) were procured from the animal rearing facility of the Department of Microbiology, University of Rajshahi. The animals were housed in polypropylene cages under controlled environmental conditions of 25 ± 2 °C, 50–60% relative humidity, and a 12 h light/dark cycle, and acclimatized for 1 week prior to experimentation. Standard pelleted feed and water were supplied throughout the study (Rani *et al.*, 2025).

Preparation of Dried Fish Paste

Dried fish paste was prepared from 3 dried fish samples, viz., *Lepturacanthus savala*, *Thunnus thynnus*, and *Scomberomorus guttatus*, using a mortar and pestle. Double-distilled water was used in this process.

Treatment of Mice

For this test, 24 Swiss Albino mice were divided into 4 groups of 6 each. First, the three groups were used as treatment groups, and 4th one was used as a control. Treatment was administered based on the mice's body weight. The weights of the mice were 23-25g (average weight), corresponding to groups 1, 2, and 3. For treatment, 0.5 g/day *Lepturacanthus savala*, 1 g/day *Thunnus thynnus*, and 0.75 g/day *Scomberomorus guttatus* paste were used for group 1, group 2, and group 3 mice. Treatment was continued for three days. These mice were observed for 11 days, and various symptoms such as diarrhea, anorexia, depression, and movement were observed. After 11 days, one mouse from each of the treated and control groups was randomly selected and dissected.

Toxicity Effect Test

Heavy metals and microorganisms found on dry fish exhibited toxic effects on mice. Various signs and symptoms were observed during the experiment. Internal organ changes were also observed by the dissection of mice.

Behavioral symptoms, including movement, feeding patterns, and facial color and numbers, were observed before and after treatment (Angarone and Snydman, 2019; Zhang *et al.*, 2023) [3, 36]. Moreover, body weights were also measured daily. Sudden weight loss is a sign of diarrhea, which can lead to various health issues and complications (Fang *et al.*, 2023; Qu *et al.*, 2021) [10, 23].

Measurement of Body Weight

Before and after treatment, the body weight of mice was measured. In our investigation, we measured body weight for up to 11 days in the treatment and control groups.

Anatomical Observations

After dissection, the liver color and structure of the treated and control groups' mice were compared. Various organs, including the stomachs, hearts, and lungs, were observed in both the treated and control groups of mice.

Statistical Analysis

Data were analyzed using SPSS 20.0 (USA), with means and standard errors expressed as mean ± SEM (n = 6). Figures were prepared using GraphPad Prism 8. Other calculations were performed by Microsoft Excel 2016.

Results

Detection of Heavy Metals

The highest level of As, at 3.07 ppm, was found in Surma, followed by Churi at 2.67 ppm, among the examined species. On the other hand, the highest Pb level was 3.33 ppm in Tuna, followed by 2.63 ppm in Churi. The comparison of heavy metal concentrations for the three selected dried fish is given in Table 1.

Table 1: Concentrations of heavy metals found in Standard Reference Material (Dried Fish) from BCSIR, Dhaka, BDL=Below Detection Level, ppm = parts per million

Heavy Metals (ppm)	Standard Level (ppm)	*Lepturacanthus savala*	*Thunnus thynnus*	*Scomberomorus guttatus*
Arsenic	0.5	2.67	0.95	3.07
Lead (mg/kg)	0.5	2.63	3.33	BDL
Nickel	0.5	BLD	BLD	BLD
Chromium	0.2	BLD	BLD	BLD
Cadmium	0.5	BLD	BLD	BLD

Toxicity Effect Test of Dry Fishes on Mice

Behavioral Observations

All the mice treated with dry fish showed different symptoms, such as depression, anorexia, and slow movement. Mice were suffering from Diarrhea, and a huge amount of watery feces was found. Moreover, abnormal weight loss was also observed for treated mice. On the other hand, no such symptoms or weight loss were observed in the control group.

Measurement of Body Weight

The present investigation showed a dose-dependent pattern for body weight. Before treatment, all mouse groups showed no significant change in body weight. Mice gain weight in a positive pattern. On the other hand, after treatment, the treatment-group mice differed from the control group. Before treatment, the highest body weight was 25.7g on day 3 in group 2, and the lowest was 22.6g on day 1 in group 1. After treatment, the highest body weight was 27.5g on day 11 for the control group, whereas the treatment group 2 had a body weight of 26g on day 11. Details are presented in Figures 1 and 2.

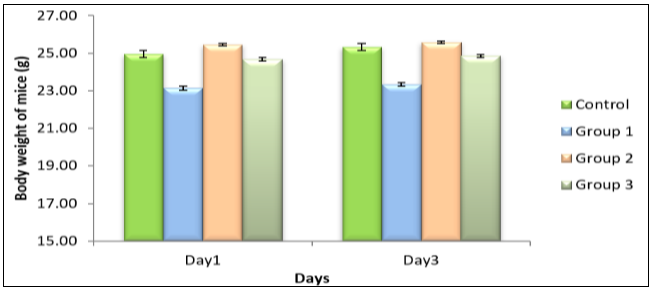

Fig 1: Body weight of mice before treatment

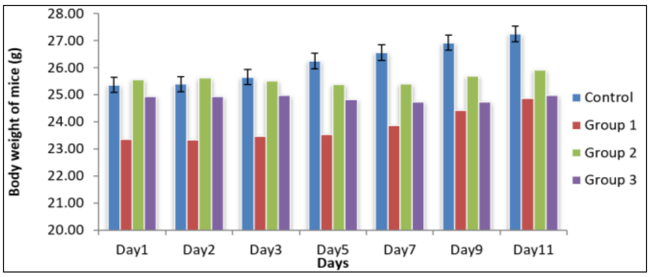

Fig 2: Body weight of mice after treatment from 1 to 11 days

Anatomical Observations

After dissection, the treatment mice group showed abnormality in liver color and structure. The stomachs of mice were larger, and abnormalities were found in the heart and lungs. However, the control group of mice showed a normal-sized stomach, and no abnormality was observed in other organs. Figures 3, 4, and 5 represented details.

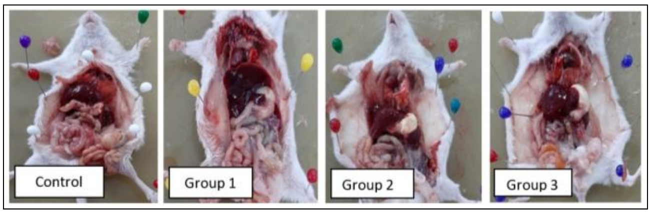

Fig 3: Dissection morphology of the whole mice for the treatment and control groups

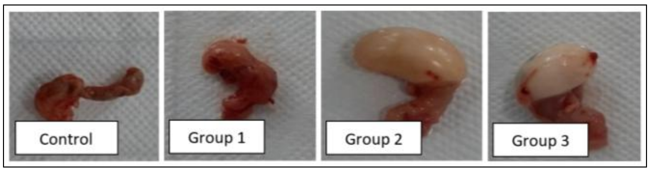

Fig 4: The stomach showed abnormality in the structure of the stomach in the treatment groups

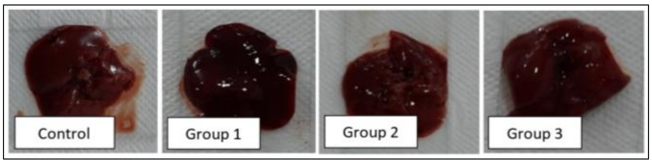

Fig 5: Liver showed abnormality in the color and structure of the liver in the treatment groups

Discussion

Heavy metals such as mercury, lead, cadmium, and arsenic enter the oceans through industrial waste and runoff. These toxic elements accumulate in marine life through bioaccumulation, posing health risks to people who eat sea fish and other foods.

The present investigations were carried out to determine the concentrations of nickel, chromium, cadmium, arsenic, and lead in three dried fish species, namely *Lepturacanthus savala*, *Thunnus thynnus*, and *Scomberomorus guttatus*, and to evaluate the effects of dried fish on mice. This study revealed that as concentrations were highest in *Scomberomorus guttatus* and *Lepturacanthus savala*, at 3.07 and 2.76 ppm, respectively, among the examined species, and comparatively lower in *Thunnus thynnus*, at 0.954 ppm. On the other hand, Pb showed the highest level of concentration for *Thunnus thynnus,* followed by the second highest level of concentration for *Lepturacanthus savala* among the examined species. In a previous study, Billah *et al*. (2023) [4] found As (3.65 ± 0.08 ppm) and Pb (3.29 ± 0.07 ppm) in dried *Channa punctata* fish, compared with standard reference values. This report supports our present study. Jamil *et al.* (2017) [14] reported an excess Pb concentration of 8.281 ppm in sun-dried *Harpodonereu*s, which contrasts with our present findings. Similarly, Canli and Atli (2003) [5] found heavy metals in different fish species from Iskenderun Bay.

In the present study, three selected dry fish were used to assess their toxic effects in mice. Several toxic effects were observed in treated mice, whereas control mice showed none. Anorexia, depression, Diarrhoea, changes in the structure of the stomach, liver, lungs, and digestive tract were observed. The combination of heavy metals (As and Pd) in surma fish showed the most toxic effects in mice. The present study showed that the color and structure of the livers, lungs, and stomachs of mice were altered after treatment with dry fish. Abass (2024) [1] reported that heavy metals have significant cytotoxicity in *Tilapia zillii*. Morcillo *et al*. (2016) [18] reported that heavy metals, including As, Cd, Hg, and Pb, cause toxicity, oxidative stress, and apoptosis in teleost fish. A similar result was observed with the dried fish solution of *C. punctata* in zebrafish embryos, which exhibited significant toxicity and abnormalities (Billah *et al*., 2023) [4].

Conclusion

This research provides new information on heavy metal distributions in three commercially valuable dried fish species collected from the Cox's Bazar Sea beach area, Bangladesh. The metal concentrations, especially arsenic and lead, found in dry fish are at alarming levels compared to the standard levels for fish and fishery products proposed by the Bangladesh Council of Scientific and Industrial Research. Moreover, this study demonstrated that consuming dry fish contaminated with heavy metals has significant toxicity effects on mice. Dry fish processing activities should be carried out with proper care and safe techniques. The authority should take appropriate steps to control heavy metal contamination in the aquatic environment and in dry fish processing. Overall, it can be concluded that there is a potential human health risk in consuming dry fish from the Cox's Bazar Sea beach area.

Author's contributions

MA Momin: Writing – original draft, Visualization, Validation, Methodology, Formal analysis. **K Nahar:** Writing – original draft, Investigation, Validation, Formal analysis. **B Sikdar:** Resources, Validation. **MF Hasan:** Supervision, Investigation, Writing – review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data Availability

Data will be made available on request.

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