Research Article

Human Health Risk Assessment of Accumulation of Heavy Metals via Consumption of Marine Fish Collected from Local Vendors of Olavakkode Fish Market, Palakkad, India

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ABSTRACT

Worldwide increase in consumption of fishes as food has immense therapeutic and nutritional benefits. But heavy metal contamination due to urban and industrial waste discharge, agricultural runoff etc., of marine water ecosystem is globally rises concern and cause huge health risk to human via their consumption. So an attempt was made in the present investigation to monitor heavy metals concentration namely Zn, Fe, Cu, Pb, Cd and Cr in two selected marine fishes such as *Sardina pilchardus* and *Rastrelliger kanagurta* collected from Olavakkode fish market, Palakkad, Kerala. The study revealed significant metal concentration (µg/g/wet weight) in fish collected from Olavakkode fish market were showed that the mean concentrations on selected essential heavy metals in the fish muscle were in the following order: Iron> Zinc> Copper> and non-essential heavy metals were in the following order: Lead> Cadmium > Chromium. The analysed heavy metals were noted to be maximum in *Sardina pilchardus* and minimum in *Rastrelliger kanagurta*. Since chemical contamination of food is considered of the most significant sources of human health risks. The health risk assessment on fish consumer, the local inhabitants of Olavakkode who regularly consume fish by any mode was assessed as it will slow accumulate these metals inside them which later will lead to death. Based on the risk factor daily intake of fish was assessed from a 10 batch of adults and children for a period of 12 months and risk assessment was carried out in adult (70Kg) and children (15Kg).

Keywords: Heavy metals, *Rastrelliger kanagurta*, *Sardina pilchardus*, Risk assessment.

INTRODUCTION

The home of countless living organisms, the marine ecosystem with commendable natural resources occupying in different ecological niches with aesthetic beauty are recently getting polluted due to the influx of pollutant without treatment into it. In addition, the increasing human activities, influx of effluents from industry, settlements and agriculture have also released of heavy metals into the hydrosphere[1]. Moreover, these influxed wastes carry enormous level of toxicants especially the heavy metals have the tendency to accumulate into the basic food chain and move up through the higher trophic level and results in negative impact on the marine resources thus causing economic loss. Thus, the marine ecosystems are being threatened by the discharge of untreated sewage wastes and industrial effluents which ultimately affects the sustainability of living resources and public health.

Fishes, the top most member of food chain when exposed to elevated metal levels can absorb the bioavailable metals like cadmium (Cd), nickel (Ni), lead (Pb), and mercury (Hg)) directly from an aquatic environment[2] and regulate their concentrations through bioaccumulation in its tissues to some extent[3]. Early researchers[4-6] have found evidence that supports the biomagnification of some heavy metals with trophic levels in marine and freshwater ecosystems. Atwell *et al.*[7] too stated that bioaccumulation and biomagnification can cause heavy metal burdens in the muscle tissues of fish to increase. Excessive levels of heavy metals in turn could harm aquatic organisms, causing the escalation of heavy metal levels in fish tissues to dangerous levels[8]. This is true, where changes to the natural environment threaten many species that have developed over millions of years and consumption of these polluted seafoods especially fish could potentially result in serious food poisoning among the general public.

Since ancient time onwards Asian and European countries utilized marine organisms as a source of polysaccharides for food, nutraceuticals and pharmaceutical purpose. Seafood, including various species of fish, crustaceans, molluscs, and echinoderms, are excellent sources of protein, fat, vitamins, and minerals; and are popular due to their delicacy with high nutritive value. Ojewola and Annah[9], stated that fish contains most important nutritional components and serve as a source of energy for human beings. Among seafoods, fish is a favourite foodstuff for the majority of societies because it is one of the cheapest sources of animal protein and with additional essential nutrients which meet the nutritional demand of human diets[10]. They are also increasingly marketed for their health benefits to consumers. In addition, majority of the nutritionists recommend human beings to consume fish every day because it is a vitamin and mineral rich food for young as well as old age people[11,12]. The nutritional value of fish meat comprises the contents of moisture, dry matter, protein, lipids, vitamins, minerals plus the caloric value of the fish which is excellent for growth and development of human body and prevents several nutritional deficiency diseases[13].

As these fresh seafoods are highly perishable product. The supplies of seafoods are unstable and fresh ones can be stored only for a short time. So, assessment of heavy metals in fish from contaminated areas along with the measurement of prime nutritional proximate profiles like protein, carbohydrates, lipids, moisture and ash percentage are often necessary to ensure that they meet the requirements of food regulations and commercial specifications. Moreover, it is also necessary from the public health point of view; to evaluate the potential health risks to human associated with consumption of fish from these contaminated catchments to safeguard of human health. Further from the aquatic environment view point, the present assessment may help to improve our knowledge on the biological status of the aquatic ecosystems as well as to improve our understanding of how the aquatic ecosystem adapts or changes according to the change in surrounding environmental conditions. Thus, the commercial important fishes *Sardina pilchardus* and *Rastrelliger kanagurta* collected from Olavakkode fish market, Palakkad, Kerala was analysed

MATERIALS AND METHODS

Sample collection and Preparation

The study area Olavakkode is a small region 4 km from the Palakkad city, Kerala, India. It lies between 10° 47' 57'' N; 76° 38' 28'' E. Selected experimental fresh fishes EF-1 *(Rastrelliger kanagurta)* and EF-2 *(Sardina pilchardus)* from local market were collected in the early hours of the day individually transferred to clean and unused polythene bags adopting aseptic procedures to minimize secondary contamination and transported to the laboratory in a portable ice chest box with a fish/ice ratio of 1:2 (w/w) then transported within 1 h to the laboratory. Upon arrival, fishes were carefully washed with cooled tap water and degutted, then washed again with tap water to remove blood, slime and unnecessary flesh. Fishes with visible signs of deterioration, injury and disease was discarded.

Wet lab analysis

Proximate analysis

The fish species such as *Rastrelliger kanagurta* and *Sardina pilchardus* collected from the Olavakkode fish market were analysed for the proximate composition. Following parameters like ash and moisture of preserved and unpreserved fish samples were evaluated using standard AOAC[14]. The protein content was determined using the Lowry *et al.*[15]. The total carbohydrate was determined using Hedge and Hofreiter[16]. Fat content was determined using Floch *et al.*[17].

Microbial Analysis

The 10 g of fish sample from EF-1 and EF-2 was weighed aseptically and homogenized with 90 mL of physiological saline solution. Appropriate dilutions were made from the 9.0 mL physiological saline and plated onto nutrient agar plates following Andrews and Hammack[18]. The plates were incubated at room temperature for four days and all colonies were counted and the data was reported as Cfu g^{-1}.

Heavy metal analysis

Captured fishes were weighed using digital weighing balance and values were recorded to nearest grams. The weighed samples were dissected to separate the muscle from the bone body as described by Nabavi *et al.*[19]. The fish muscles were cut into small pieces and transferred into the crucible. The fish muscles were dried under the hot plate till the colour of the fish muscle turned into brown. The dried fish muscle was powdered by the help of mortar and pestle. To this powder 5ml of HCl and 95 ml of HNO_3 was added. This solution was kept overnight. Then the solution was evaporated under the hot plate. This was washed using 100ml distilled water and filtered using a Whatmann's No.40 filter paper. The sample was analysed under an Atomic Absorption Spectrophotometer.

Estimated daily intake of metals (EDI)

Risk assessment in the study was evaluated by considering only the edible part (muscle tissue) to determine the daily intake of metal. The daily intake of metals (EDI) is measured in (mg/kg body weight —weight/ day) was determined by the following[20].

$$\text{EDI (Estimated daily intake)} = \frac{Mc \times IR}{Bw} \times 10^{-3}$$

Mc = Metal concentration in the fish muscle (mg/kg dry weight)

IR = Ingestion rate, which is taken as 55.5 $\times 10^{-3}$ gm/day (Adult);

52.5 $\times 10^{-3}$ gm/day (Children) (UPSEA, 2008)

Bw = An average body weight of Indian Adult is taken as 70kg

An average body weight of Indian children is taken as 15kg.

Target hazard quotient (THQ)

Non-carcinogenic risk

To express the potential non-carcinogenic risk of fish samples in this study, the target hazard quotient (THQ) was utilized. The THQ is equal to the ratio of an EDI value to an oral reference dose (RfD, µg per kg per day) value. The RfD was established by the United States Environmental Protection Agency[21]. The THQ for each analyzed metal (Cr, Ni, Cu, Zn, As, Cd, Pb, and Hg) was calculated by using the following formula:

$$THQ = \frac{EDI}{RfD}$$

*EDI= Estimated daily intake

* RfD- Oral Reference Dose

Statistical analysis

Data were analysed using SPSS (Scientific Package of Social Science) version 16.0. The mean, standard deviation (SD) and one-way ANOVA test were performed to compare differences in parameters analysed among selected fish species and a test for the comparison of means ($P < 0.05$).

RESULTS AND DISCUSSIONS

Undernourishment, a dominating quandary, backtrack the development of third world countries primarily, due to the lack of awareness along with awful financial status of middle-class population. India with a significant percentage of men and women are either too lean or too fat because of unhealthy food habits. As per UN estimates 2.1 million Indian children die before reaching the age of 5 every year-4 every min-mostly from preventable infectious diseases. Researchers are in a hurry to find a solution to save our younger ones from the calamity and they are in a search to explore new resources.

To meet the above need of people, fish an available inexpensive and easily digestible animal protein than other source of animal protein can be used. Fish being a major source of essential fatty acids also contains proteins, carbohydrates, lipids, vitamins and trace elements. So, it is extensively consumed worldwide by human as it also provides polyunsaturated fatty acids especially omega 3 fatty acids known to support the good health. As fish are being at the top of aquatic food chain it may concentrate large amount of some heavy metals, which often times endanger public health through consumption of contaminated sea food. So, determination of proximate composition, sensory parameters and heavy metal concentration in highly consumable fishes is very important as far as human health is concerned.

Table 1: The proximate composition (percentage value) of raw flesh muscle of selected commercial important marine fishes at Olavakkode whole sale fish market**.**

Fish Species	Proximate composition (% W/W)				
	Moisture	Protein	Carbohydrate	Lipid	Ash
Rastrelliger kanagurta	$74.54{\pm}0.13^b$	$16.98{\pm}0.35^a$	$0.124{\pm}0.001^a$	$3.50{\pm}0.41^c$	$1.20{\pm}0.09^a$
Sardina pilchardus	$73.79{\pm}0.30^a$	$17.94{\pm}0.66^b$	$0.458{\pm}0.007^a$	$6.17{\pm}0.15^a$	$1.33{\pm}0.14^a$

Table 2: The average amount of microbial colonies (Cfu/ml/gm) in the raw flesh of selected commercial important marine fishes collected from Olavakkode whole sale fish market

Fish Species	No of colonies (Cfu/ml/gm)	
Rastrelliger kanagurta	$192X10^{-4}$	$144X10^{-5}$
Sardina pilchardus	$224\ X10^{-4}$	$204\ X10^{-5}$

Table 3: The average amount of heavy metals (µg/g/wet weight) in the raw flesh of selected commercial important marine fishes.

Fish Species	Heavy metal Concentration (µg/g/wet weight)					
	Zn	Cu	Fe	Pb	Cr	Cd
Rastrelliger kanagurta	$13.9{\pm}0.69^b$	$6.20{\pm}0.24^b$	$9.09{\pm}3.05^b$	$11.6{\pm}0.77^b$	$3.44{\pm}0.18^b$	$6.88{\pm}0.34^b$
Sardina pilchardus	$7.82{\pm}1.02^a$	$3.95{\pm}0.74^a$	$7.10{\pm}1.96^a$	$9.72{\pm}3.05^a$	$2.28{\pm}0.11^a$	$2.29{\pm}0.20^a$

*Each value is the mean of three replicates.

Table 4: Estimated daily intake (EDI) values of metals via consumption of fishes *Rastrelliger kanagurta* and *Sardina pilchardus*

Heavy Metals	EDI (mg/Kg/B.Wt/Day)			
	Rastrelliger kanagurta		*Sardina pilchardus*	
	Adults	Children	Adults	Children
Zn	$6.20{\times}10^{-3}$	$27.37{\times}10^{-3}$	$11.02{\times}10^{-3}$	$48.65{\times}10^{-3}$
Cu	$3.13{\times}10^{-3}$	$13.82{\times}10^{-3}$	$4.02{\times}10^{-3}$	$21.7{\times}10^{-3}$
Fe	$7.21{\times}10^{-3}$	$31.81{\times}10^{-3}$	$5.63{\times}10^{-3}$	$24.85{\times}10^{-3}$
Pb	$13.95{\times}10^{-3}$	$61.6{\times}10^{-3}$	$7.71{\times}10^{-3}$	$34.02{\times}10^{-3}$
Cd	$2.32{\times}10^{-3}$	$10.22{\times}10^{-3}$	$5.45{\times}10^{-3}$	$24.08{\times}10^{-3}$
Cr	$1.81{\times}10^{-3}$	$7.98{\times}10^{-3}$	$2.73{\times}10^{-3}$	$12.04{\times}10^{-3}$

Table 5: The target hazard quotient in adult and children via consumption of selected fish muscle of *Rastrelliger kanagurta* and *Sardina pilchardus*

Heavy Metals	Target Hazard Quotient (THQ)			
	Rastrelliger kanagurta		*Sardina pilchardus*	
	Adults	Children	Adults	Children
Zn	0.02	0.09	0.03	0.16
Cu	0.08	0.3	0.10	0.54
Fe	0.01	0.50	0.01	0.04
Pb	4.65	20.5	2.6	11.34
Cd	4.64	20.4	10.9	48.16
Cr	0.60	2.40	0.91	4.0

Hence the present study has been designed to investigate on two fishes, *Rastrelliger kanagurta* (EF-1) and *Sardina pilchardus* (EF-2) collected from the Olavakkode fish vendors of local fish market which receive fishes from Malappuram and Eranakulam landing centre, Kerala. The average amount of heavy metals (µg/g/wet weight) in the raw flesh of selected commercial important marine fishes such as *Rastrelliger kanagurta & Sardina pilchardus* (EF-1, EF-2) collected from Olavakkode whole sale fish market are represented in the Table 1.

Table 1, shows the mean value of the proximate composition of *Rastrelliger kanagurta* (EF-1) and *Sardina pilchardus* (EF-2). During the present study the moisture content of the sardine and mackerel ranged between 73.79±0.30-74.53±0.13 %. Similar results were found in the studies by Nisa and Asadullah[22] and Ravichandran *et al.*[23]. The percentage of water (Moisture) is good indicator of its relative contents of energy, proteins and lipids. The lower the percentage of water would be greater the lipids and protein contents. The levels of protein content in the two selected fish species of *Rastrelliger kanagurta* (EF-1) and *Sardina pilchardus* (EF-2) calculated were of 16.98±0.35% and 17.94±0.66% respectively. Shaji and Kannan (2013) reported the protein content in sardine was 23.63% and Nisa and Asadullah (2011) reported crude protein in mackerel varied from 16.65% to 20.09%[22]. Ravichandran *et al.*[23] also reported that protein content in sardine and mackerel ranged between 17.04-28.01%.

Lipid content in *Rastrelliger kanagurta* (EF-1) and *Sardina pilchardus* (EF-2) were 3.50±0.41% and 6.17±0.15% respectively. Shaji and Kannan[24] reported that lipid content in sardine, mackerel and anchovy were 3.68±0.17%, 5.03±0.87% and 1.97±0.14% respectively which supports the present study results. Nisa and Asadullah[22] reported fat content of mackerel varied from 3.0% to 12.0% ($p<0.05$), highest in winter. The ash content was 1.20±0.09% in *Rastrelliger kanagurta* (EF-1) and 1.24±0.10 in *Sardina pilchardus* (EF-2). Shaji and Kannan[24] reported that Sardine consist of 3.94% of ash content. The total lipid and ash content of fish vary with the increasing weight or length of the fish; it may also vary with the season and varied habitats[25]. The result in Table.1 showed that the selected fish species *Rastrelliger kanagurta* (EF-1) and *Sardina pilchardus* (EF-2) having poor sources of carbohydrate 0.124±0.001 (EF-1) and 0.458±0.004 (EF-2) in fresh muscles. The relatively low values of carbohydrate could be due to higher values of moisture and a relatively high value of protein content[26]. Gopakumar[27] reported that the, corroborates with the present experimental results with the concept whenever, there is a low percentage of water the lipid and protein content would be high and the energy density also would be high in fishes. From Table .2, the number of bacterial colonies found in the control fish EF-1 was 192X 10-4 cuf g^{-1} and 144X10^{-5} cuf g^{-1} and in EF-2 was 224X10^{-4} cuf $^{-1}$ and 204X10^{-5} cuf g^{-1}. Comparing to *Sardina pilchardus* (EF-2) *Rastrelliger kanagurta* (EF-1) was loaded with less number of bacteria. Fish and fish products are the most important source of protein and it is estimated that more than 30% of fish for human consumption comes from aquaculture[28].

The table-3, reveals the significant metal concentration (µg/g/wet weight) in fish collected from Olavakkode fish market were 13.9±0.69 (Zn); 6.20±0.24 (Cu); 9.09±3.05 (Fe); 11.6±0.77 (Pb); 3.44±0.18 (Cr) and 6.88±0.34 (Cd) in *Rastrelliger kanagurta* (EF-1) respectively. In *Sardina pilchardus* (EF-2) the average amount of selected heavy metals recorded were 7.82±1.02 (Zn); 3.95±0.74 (Cu); 7.10±1.96 (Fe); 9.72±3.05 (Pb); 2.28±0.11 (Cr) and 2.29±0.20 (Cd) (Table. 4). In the present study, all the selected heavy metals (both essential and non-essential) are recorded more the permissible limits prescribe by USEPA[29]. Aprill and Sims[30] and Momtaz[31] stated that zinc is fourth among metals of the world in annual consumption behind iron and copper and an essential element for the life of animal and human beings which regulate the physiological mechanisms in most organisms. The previous researchers[32,33] suggested that occurrence of excessive levels of them is regarded as potential hazard which can endanger both animal and human health by causing impairment of growth and reproduction, the clinical signs of Zn toxicosis being reported with symptoms like vomiting, diarrhoea, bloody urine, icterus (yellow mucus membrane), liver failure, kidney failure and anaemia.. Dural *et al.*[34] reported that copper is considered as an essential constituent required in haemoglobin synthesis and in catalysis of metabolic reactions. High copper levels lead to an increase in the rate of free radical formation[35] teratogenicity[36] and chromosomal aberrations[37]. The abundance of Fe in the fish muscle could be associated with the fact that these metals are naturally abundant in Kerala soil and irrespective of the source and the final depositors are aquatic system. However, the occurrence of excessive levels of them is regarded as potential hazards which can endanger both animal and human health[38-40].

Lead is a naturally occurring heavy metal found in all parts of our environment. Cadmium in the environment is consequential from natural sources, whereas remaining 90% is derived from anthropogenic activities[41]. Lead and cadmium are non-essential elements which are accumulated in human tissue and harmful to human health. When accumulates in the human body, it replaces calcium in bones. Lead exposure has been mainly related to retardation of neurobehavioral development[42,43]. The heavy metals, chromium and cadmium specifically were reported to be highly toxic to the aquatic environment. Uptake of chromium and cadmium through food chain in human being may lead to destruction of testicular tissues and red blood cells, kidney damage etc. Thus, the phenomenon of bioaccumulation and bio-magnification intensified with concentration of heavy metals at different tropic levels. In aquatic ecosystem the primary producers absorb the metallic ions, which in turn pass to the consumer level through predictions. Based on the data presented in Table. 4, it can be noted that the maximum daily intake metals were in the order Pb> Fe>Zn>Cu> Cd> Cr. The EDI of the six heavy metals [i.e. three essential and three non-essential] studied is determined based on the assumption of 70-kg body weight per adult person and 15kg/body weight for children and exposure period- a year. The recorded EDI values (per mg/Kg body.wt/ day) of zinc was 6.20×10^{-3} (EF-1) and 11.02×10^{-3}(EF-2) in adult population and 27.37×10^{-3} and 48.65×10^{-3} for children population of metal consumption of fish *Rastrelliger kanagurta* (EF-1) and *Sardina pilchardus* (EF-2) collected from Olavakkode fish vendor market. The estimated intake value of copper was noted to be 3.13×10^{-3}; 4.02×10^{-3}in adult and 13.82×10^{-3} and 21.07×10^{-3} in children those who consume *Rastrelliger kanagurta* (EF-1) and *Sardina pilchardus* (EF-2).

The heavy metal Pb and Fe recorded in adult population who consume *Rastrelliger kanagurta* (EF-1) was 7.21×10^{-3} and 13.95×10^{-3} and those who consumed *Sardina pilchardus* (EF-2) it was recorded 5.63×10^{-3}; 7.71×10^{-3}. The estimated intake of heavy metal Pb was 31.81×10^{-3}; 24.85×10^{-3} and Fe in children of 15 kg/b.wt/day was 61.6×10^{-3} and 34.02×10^{-3}. The estimated intake of cadmium among the estimated adults with average weight of 70kg was 2.32×10^{-3} and 5.45×10^{-3} and chromium was 1.81×10^{-3} and 2.73×10^{-3} who consume *Rastrelliger kanagurta* (EF-1) and *Sardina pilchardus* (EF-2) while in children of 15kg/b.wt/day it was recorded 10.22×10^{-3} (Cd); 7.98×10^{-3}(Cr) who consumed *Rastrelliger kanagurta* (EF-1) and 24.08×10^{-3} (Cd); 12.04×10^{-3} (Cr) who consumed *Sardina pilchardus* (EF-2). With comparison to USEDA (2012) and USEDA (2017), oral reference dose (mg/Kg-1/day-1) for essential metals like Zn-3.0×10^{1}; Cu-4.0×10^{-2}; Fe-7.0×10^{-1} and non-essential metals Pb-3.0×10^{-3}; Cd-5.0×10^{-4} and Cr-3.0×10^{-3} all the estimated daily intake of metals via consumption of selected commercial important fishes were note to be above the recommendable levels. The target hazard quotient (THQ) of metals through muscle consumption for adults and children are shown in Table. 5. THQ values of CU, Zn and Fe for both age classes (Adults and Children) considered were all below 1. Therefore, the daily intake for these metals derived from a real meal at levels of assumed exposure were not likely to cause any adverse effects during their life time. However, THQ of Cd, Cr and Pb in both adults and children in some level of assumed exposure were >1 indicating the presence of health risk that mean there is a potential risk of developing chronic systemic effects.

Therefore, more attention should be paid to the monitoring of Cd and Pb exposure in west coast region in future which might pose potential health risk. Among the population the range was as follows children > adult. This could be due to the difference in the average weight and life span. In this study, some of the levels of heavy metals detected in muscle tissue have been found to be above the limits for fish recommended by the Food and Agriculture Organization/World Health Organization (FAO/WHO) and the European Union (EU)[19]. This study shows that some metal concentrations (Fe and Pb) are higher than the acceptable values for human consumption as determined by various health organizations. As a result, since these fish are used in the human diet, the results obtained indicate that attention should be taken regarding intake, especially for benthic fish. Potential for high risk is possible due to increasing heavy metal levels in catchment point region of the Kerala District.

Thus, the present analysis on human health risk assessment estimated on the estimated daily intake proved that all the selected heavy metals for analysis of HRI were above stipulated limits recommended by USEPA[21]. The extensive persistence of heavy metals infectivity in bioaccumulation and biomagnification are a serious threat of the food chain[44] and these heavy metals will automatically transfer into the body while consumed this seafood by humans. Due to the increasing environmental pressure on the marine ecosystem of Kerala district, a regular and on-going monitoring of trace metal levels in marine organisms is necessary to prevent any deleterious effect on the human population. Thus, it may be concluded that the artisanal mining activity along the coast and the excessive use of agro-chemicals within the catchments of the near the landing centre are deteriorating the quality of fish sold at vendor market.

CONCLUSION

In this study, some of the levels of heavy metals detected in muscle tissue have been found to be above the limits for fish recommended by the Food and Agriculture Organization/World Health Organization (FAO/WHO) and the European Union (EU/FAO). The study shows that some metal concentrations (Fe and Pb) are higher than the acceptable values for human consumption as determined by various health organizations. As a result, since these fish are used in the human diet, the results obtained indicate that attention should be taken regarding intake, especially for benthic fish. Potential for high risk is possible due to increasing heavy metal levels in catchment point region of the Kerala District. The present analysis on human health risk assessment estimated on the estimated daily intake proved that all the selected heavy metals for analysis of HRI were above stipulated limits recommended by USEPA.

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