ASSESSMENT OF SOME SELECTED HEAVY METALS IN WATER, SEDIMENT AND FISHES OF GEIDAM RIVER, GEIDAM LOCAL GOVERNMENT AREA, YOBE STATE, NIGERIA

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ABSTRACT
The aim of this study was to assess heavy metal concentration (Arsenic, Chromium,Cadmium, Copper and Zinc) in sediments, water and two selected fish species Cat fish (Clarius gariepinus) and Tilapia (Oreochromis niloticus) in Geidam river. The samples were collected from February to August, 2020 at five different sampling sites. The heavy metals concentrations were determined using atomic absorption spectrophotometry and the results obtained for water and sediments samples ranged from 0.000mg/kg to 0.405mg/kg and 0.011mg/g to 2.600ml/g for Arsenic Chromium,Cadmium, Copper and Zinc respectively and fish had concentrations of 0.000mg/g to 0.930m/g and the data was analyzed using one way analysis of variance and shows there were statistically significant differences in metal concentrations among the sampling sites and sampling months in sediment. Significant differences accepted at $p \leq 0.05$. All the heavy metals detected were found to exceed the limits stipulated by FAO/WHO which could be due to inflow of domestic waste and industrial effluent into the river. The results show the need for an ecosystem approach towards sustainable management of reservoirs. This will curb aquatic pollution which is a public health threat to people consuming aquatic resources contaminated with heavy metals

Keywords: *Heavy metal, Contamination, River, Sediment, Fish, Water*

INTRODUCTION
One of the major environmental problems is the pollution of aquatic ecosystem by heavy metals. This is because, heavy metals cannot be degraded but they are deposited, assimilated or incorporated in water, sediments and aquatic biota

causing heavy metal pollution (Malik *et al*., 2010).This bioaccumulation of heavy metals in living organisms and biomagnifications describes the processes and path- ways of pollutants from one trophic level to another.

Various fish species are mostly used as bio-indicators of heavy metals contamination (Svobodova *et al*., 2004). For instance, Farkas *et al*. (2000) reasoned that, the concentrations of heavy metals in organs of fish show that the aquatic environment is polluted. Pollution of the aquatic environment by inorganic chemicals has been considered a major threat to the aquatic organisms including fishes. The agricultural drainage water containing pesticides and fertilizers and effluents of industrial activities and runoffs in addition to sewage effluents supply the water bodies and sediment with huge quantities of inorganic anions and heavy metals (ECDC, 2002).

Bioaccumulation of heavy metals is dangerous to human health. Lead, cadmium, cobalt, nickel, and mercury can affect the formation of blood cells. The build-up of heavy metals can cause malfunctions in the liver, kidneys, the circulatory system (responsible for the circulation of blood throughout the body), and the movement of nerve signals. Some heavy metals may also play a role in the development of various cancers (Encyclopedia of Water Science, 2005).

The fate of heavy metals introduced by human activities into aquatic ecosystems have recently become the subject of wide spread concern, since beyond the tolerable limits they become toxic (WHO 1971, Pocock, *et al*., 1994, Koller, et *al*., 2004). The persistent nature of heavy metals in the environment, coupled with their non-degradability has made them priority pollutants, as they account for over 143,000 deaths yearly (WHO, 2015).

Geidam river water body is one of a major sources of fish to many people in Geidam and its environs. However, due to rapid urbanization and industrialization, these water bodies may contain contaminants including heavy metals. These heavy metals are capable of causing great health concerns to people who ingest them due to their non-degradable nature, and ability to accumulate through trophic levels.Thus, determination of harmful and toxic substances in biota gives direct information on the significance of pollution in the aquatic environment (Hugget, *et al*., 1973). Hence, investigating the concentrations of certain heavy metals in fish collected from Geidam river, Yobe State, Nigeria is imperative. The analysis of these heavy metals is therefore justified to provide a form of precautionary information on the consumption of water, flora and fauna from the river. It should also provide a basis to sensitize concerned authorities, such as NEMA (National Environmental Management Authority) towards management of discharge into these water bodies.Hence, this study is aimed at assessing the bioaccumulation of heavy metals (Zn, Cd, Cu and Pb) in different fish species from Geidam River, Yobe State and to determine whether the levels of heavy metals (if present) are above the recommended limit by WHO.

MATERIALS AND METHODS

Study Area

The study was conducted in Geidam river, located in Geidam town in Geidam local Government area of Yobe State, about 175 Km from the state capital. The river lies between longitude 8^0 31' to 8^0 45' E and latitude 20^0 13' to 12^0 10' N. It was impounded from the two major tributaries, River Yobe and River kamadugu.

Sampling Sites

During a field reconnaissance, five sampling sites were identified from the river . Sampling was carried out once a month for six month in all the sampling sites in the river . The sampling sites are pointA1-A5

Sampling and Storage of Water

Water sampling was done according to the procedure described by Ndimele and Kumolu-Johnson (2012). Water samples from all five (5) sampling sites were collected at a depth of about 0.3m below water surface into 500 ml plastic bottles. Prior to sampling, the bottles were cleaned with 10% nitric acid and rinsed with distilled water. The bottles were rinsed three times with the dam water at the time of sampling. Samples were then collected by direct immersion of the sampling bottle into the reservoir. Immediately after sample collection, 2 ml nitric acid (AR grade) was added to the water samples. Sample bottles were then labeled to indicate date of sampling and the sampling site. Samples were transported in an ice-box to the laboratory and stored at 4^oC awaiting analysis.

Sampling and Storage of Surface Sediments

Sediment samples were taken from the bottom surface using an Eckman grab according to Osman and Kloas (2010). For each sample, three sediments grabs were randomly taken, and kept in clean polyethylene bags. The polythene bags were then labeled to indicate sampling station and date of sampling. Samples were then stored in ice box for transportation to the laboratory. In the laboratory, the samples were allowed to dry in hot air oven (model 30CG lab oven) and then ground into powder by using porcelain mortar and pestle, further more sieved through a 2mm mesh sieved to removed large particles and stored at – 20^0C until they were processed for heavy metal analysis.

Sampling and Storage of Fish

Tilapia *Oreochromis niloticus* and African Cat fish *Clarias gariepinus* , were obtained from local fisher men at all five sampling sites. A total of 157 Tilapia and 165 Common Carp fish samples were caught during the study period. Fish samples obtained were immediately kept in pre-cleaned polythene bags, sealed, labeled and kept in ice boxes for transportation to the Biological Science Laboratory, Bayero University Kano. In the laboratory, total length (cm) and weight (grams) were recorded. The samples were dissected into gills, liver and muscles followed by oven dry at 105°C for 24 hour and then powdered using motor and pestle.

Digestion of Water Samples for Metal Analysis

Digestion of the water samples were done in triplicates using concentrated nitric acid (Analytical Grade) according to method described by Zhang (2007). Concentrated acid (5 ml) was added to 50 ml of sample water in a 100 ml beaker, and then heated on a hot plate to boil until its volume reduced to 20 ml. Another 5ml of concentrated HNO3 was added and then heated for 10 minutes and allowed to cool. About 5 ml of nitric acid was used to rinse the sides of the beaker and the solution filtered using Whatman 0.42µm filter paper into a 50 ml volumetric flask and topped up to the mark with distilled water. A blank solution was similarly prepared. Heavy metal analysis was done using Varian Atomic Absorption Spectrometer (model 210GP).

Processing and Digestion of fish and Sediments Sample

Fish Tissue and sediment samples were digested using 4G106M (CEM, Matthews, USA) microwave accelerated system. Around 100 g of an oven-dried fish sample and powdered sediment samples were weighed accurately to four decimal places in a Teflon vessel. A total of 6 ml of 65% conc. HNO3 (AR, Sigma) and $H_2O_2$was added and allowed to stand for 15 min in a fume hood for pre-digestion. Then, the Teflon vessel was connected to a microwave digester and digestion was carried out (at 75 °C for 10min then ramped at 10°C per min to 95°C and hold to 30 min). The digested tissue and

Sediment samples were transferred to 50 mL volumetric flasks and made up to the mark with deionized water.

Determination of Heavy metal Contents

Concentration of the Heavy metal (Cadnium Cromium, Copper, Lead and Zinc) present in the sample was determined using AAS (Buck scientific model 210GP) . The Machine was set up at specific wave length of each metal to be determined. Deionized water was aspirated between each analysis. Reading of each metal metal was taken and recorded by taking using the standard calibration curve.

Data Analysis

Data analysis was done using a computerized statistical programme (SPSS 17.0). The data were subjected to one way analysis of variance (ANOVA) and significant differences accepted at $p \leq 0.05$ (Zar, 2001). Descriptive statistics for all collected data were also obtained using Microsoft excel.

Results

The results of the average concentration of heavy metals in Clarias sampled in Geidam river are shown in Table 1. The bioaccumulation of heavy metals showed variation in different body parts of the fish. Zinc (Zn) was found to be highest in the liver at a concentration of 113.99 ppm and lowest in the flesh (36.68 ppm). Cadmium (Cd) was only found in the liver at a concentration of 1.68 ppm, and not present in the gills, and flesh of all the samples of fishes examined. Copper (Cu) was present in significantly high amount in the liver (145.50 ppm) and small amounts in flesh (0.09 ppm) and gills (0.32 ppm). Lead (Pb) on the other hand, was only found in the gills, with an average concentration of 21.45 ppm. Non of the fish parts contain Chromium (Cr) and Arsenic (As), however, the result revealed the distribution of heavy metals in Clarias fish species to follow the order Zn > Cu > Pb > Cd, while the distribution of heavy metals in the investigated parts showed the following order: Liver > Gills > Flesh.

The average concentration of heavy metals from Tilapia fish is also presented in Table 1, where the bioaccumulation of heavy metals shows variation in different body parts of the fish. Zn was found to be highest in the liver with an average concentration of 69.69 ppm, and lowest in the flesh (34.5 ppm). Cadmium was not found in all organs of the fish, except in the gills where it was found at a low concentration of 0.12 ppm. Cu was found in highest amount in the liver at a concentration of 1.74 ppm, with its lowest amount seen in flesh (0.08 ppm). Pb was found only in the liver in small amounts, with an average concentration of 0.95 ppm. Cr and As were only found in the gills of Tilapia fish with a concentrations of 0.041 and 0.003, respectively. The result further revealed the distribution of heavy metals in Tilapia of Wasai Reservoir to follow the order Zn > Cu > Pb > Cd > Cr > As, while the distribution of heavy metals in the investigated parts showed the following order: Gills > Liver > Flesh.

By comparing the concentration of zinc in fish samples from Wasai reservoir, using independent sample t-test, it was found out that, there is no significant difference ($p>0.05$) in the bioaccumulation of this metal in the flesh, and liver at p-values of 0.675 and 0.300, respectively. However, there was a level of significant difference in the Zn present within the gills at a p-value of 0.032 ($p<0.05$). However, the bioaccumulation of Cd in fish gills and liver from the water body was not significant ($p >0.05$).

On the other hand, by comparing the bioaccumulation of Cu between the two species of fish, there was no significant difference in the concentration of the metal in the flesh at p-values of 0.941 and 0.423 respectively. The only significant difference in the bioaccumulation of Cu between the two species is in the liver, with a p-value of 0.001 ($p<0.05$). There was also no significant difference in the concentration of Cu in the gills ($p=0.138$) between the two species. There was also no significant difference in the bioaccumulation of Pb in the liver and gills of fish between the two fish species at p-values of 0.423 in each, respectively ($p>0.05$).

The overall concentration of the different metals in fish samples from the two different species, irrespective of the body parts is presented in Table 2. There was no significant difference in the overall concentration of all metals in Clarias and Tilapia fish samples ($p>0.05$) at p-values of 0.650 (Zn), 0.370 (Cd), 0.155 (Cu), and 0.356 (Pb), respectively.

Table 3 shows the seasonal comparative analysis of heavy metals concentration in Geidam river along with the permissible limits from various sampling sites. In Wasai, the concentration of zinc was higher during the rainy season (0.38 mg/L) than the dry season (0.06mg/L). Both values were found to be within the permissible limit of WHO (5 mg/L) and FMH (3 mg/L). However, the 0.38 mg/L zinc concentration during the rainy season was higher than the maximum permissible limit of USEPA (0.12 mg/L). For Cadmium in Wasai, the concentration was also found to be higher during the rainy season than the dry at 0.06 mg/L and 0.05 mg/L, respectively. However, both values were above the maximum permissible limit of 0.001mg/L set aside by the WHO. Copper on the other hand showed higher concentrations in the dry season (0.92 mg/L) than the rainy season (0.42 mg/L) with both values are within the permissible limits of WHO, USEPA, and FMH. Lead concentration followed similar pattern with zinc and cadmium having higher concentration during the rainy season (0.08 mg/L) than the dry (0.06 mg/L). However, both values were above the permissible limits set aside by WHO (0.05 mg/L) and FMH (0.01 mg/L). Chromium showed higher concentration in the dry season (0.08 mg/L) than rainy (0.06 mg/L), and both values were found to be above the maximum permissible limits of WHO (0.05 mg/L) and FMH (0.05 mg/L), but within the limits of USEPA (0.1 mg/L).

In A1, zinc was higher in the rainy season (0.45 mg/L) than the dry season (0.40 mg/L). These values are within the permissible limits of WHO (5 mg/L) and FMH (3 mg/L), but not that of USEPA (0.12 mg/L). Cadmium was also found to have higher concentration during the rainy season (0.06 mg/L) than the dry season (0.04 mg/L). Just like in A1, the cadmium concentration in A1 was found to be above the maximum permissible limit of 0.001 mg/L set by WHO. For copper, the dry season had a concentration higher than that of the rainy season at 0.92 mg/L and 0.44 mg/L, respectively. These values were all within the permissible limits of WHO (1 mg/L), USEPA (1.3mg/L), and FMH (1mg/L). Lead was also found to have higher concentration during the dry season (0.20 mg/L) than the rainy season (0.06 mg/L). However, both values were above the permissible limits set aside by WHO (0.05 mg/L) and FMH (0.01 mg/L). Chromium was found in abnormally high amounts in both dry and rainy season at values of 0.65 mg/L and 0.73 mg/L respectively. These values were found to be above the permissible limit of WHO (0.05 mg/L), USEPA (0.1 mg/L), and FMH (0.05 mg/l).

In A2, zinc concentration was found to be higher in rainy season (0.90 mg/L) than the dry one (0.06 mg/L). This followed similar pattern with A1and A1. These values are within the permissible limits of WHO (5 mg/L) and FMH (3 mg/L), but not that of USEPA (0.12 mg/L). The concentration of cadmium was found to be the same during the rainy and dry seasons at a value of 0.05 mg/L. Just like in A1 and A2, the cadmium concentration in A3 was found to be above the maximum permissible limits of 0.001 mg/L set by WHO. For copper, the rainy season had a concentration higher than that of the dry season at 0.50 mg/L against 0.22 mg/L, respectively. These values were all within the permissible limits of WHO (1 mg/L), USEPA (1.3mg/L), and FMH (1mg/L). Lead was found to have higher concentration during the rainy season (0.07 mg/L) than the dry season (0.06 mg/L). However, both values were above the permissible limits set aside by WHO (0.05 mg/L) and FMH (0.01 mg/L). Chromium was found in both dry and rainy season at values of 0.05 mg/L and 0.20 mg/L, respectively. The dry season concentration value stood just on the permissible limit mark of 0.05mg/L designated by FHM and WHO. However, the rainy season value is obviously above these limits, and also above the limit put forward by the USEPA (0.1 mg/L).

In A4, the concentration of zinc was higher during the rainy season (0.71 mg/L) than the dry season (0.60 mg/L). Both values were found to be within the permissible limit of WHO (5 mg/L) and FMH (3 mg/L). However, both concentrations were higher than the maximum permissible limit of USEPA at 0.12 mg/L. Cadmium had same concentration for both dry and rainy season at 0.06 mg/L. This value was found to be above the permissible limit of 0.001 mg/L set by the WHO. For copper, the rainy season had a concentration higher than that of the dry season at 0.50 mg/L as against 0.45 mg/L, respectively. However, these values were all within the permissible limits of WHO (1 mg/L), USEPA (1.3mg/L), and FMH (1mg/L). Lead was found in abnormally high amounts during the rainy (0.47 mg/L) and dry season (0.42 mg/L). These values were found to be significantly above the permissible limits set aside by WHO (0.05 mg/L) and FMH (0.01 mg/L). Like lead, chromium was found in abnormally high amounts in both dry and rainy season at values of 0.65 mg/L and 0.70 mg/L, respectively. These values were found to be way above the permissible limit of WHO (0.05 mg/L), USEPA (0.1 mg/L), and FMH (0.05 mg/l).

For A5 site, zinc concentration was found to be higher during the dry season (3.32 mg/L) than the rainy season (3.00 mg/L). These values were above the permissible limit of USEPA (0.12 mg/L), and within the limit of WHO (5 mg/L). Cadmium was found in both dry and rainy season at values of 0.07 mg/L and 0.06 mg/L, respectively. Just like in all other sampling sites, the cadmium concentration in A5 was found to be above the maximum permissible limit of 0.001 mg/L set by WHO. For copper, the dry season had a concentration higher than that of the rainy season at 1.50 mg/L and 1.30 mg/L, respectively. These values were not within the permissible limits of WHO (1 mg/L), USEPA (1.3mg/L), and FMH (1mg/L). Lead was found in significantly high amounts during the rainy (0.72 mg/L) and dry season (0.77 mg/L). These values were found to be significantly above the permissible limits set aside by WHO (0.05 mg/L) and FMH (0.01 mg/L). Just like in A3, the chromium in A5 was found in abnormally high amounts in both dry and rainy season at values of 0.75 mg/L and 0.76 mg/L respectively. These values were found to be significantly above the permissible limit of WHO (0.05 mg/L), USEPA (0.1 mg/L), and FMH (0.05 mg/l).

Table 1: Bioaccumulation of Heavy Metals in Clarias and Tilapia fish Species of Geidam River

Heavy Metals	Body Parts	Clarias	Tilapia	p-value
Zn	Flesh	36.68±3.55	34.50±2.73	0.675^{ns}
Liver		113.99±20.24	63.69±30.06	0.300^{ns}
Gills		78.66±3.30	49.91±4.06	0.032*
Cd				
Flesh		0.00±0.00	0.00±0.00	-
Liver		1.68±1.68	0.00±0.00	0.423^{ns}
Gills		0.00±0.00	0.12±0.00	0.423^{ns}
Cu				
Flesh		0.09±0.00	0.08±0.00	0.941ns
Liver		145.50±5.50	1.74±0.00	0.001*
Gills		0.00±0.00	0.45±0.00	0.138ns

Pb			
Flesh	0.00±0.00	0.00±0.00	-
Liver	0.00±0.00	0.95±0.00	0.423^{ns}
Gills	21.45±21.45	0.00±0.00	0.423^{ns}
Cr Flesh	0.00±0.00	0.00±0.00	-
Liver	0.00±0.00	0.00±0.00	-
Gills	0.00±0.00	0.041±0.03	0.42^{ns}
As Flesh	0.00±0.00	0.00±0.00	-
Liver	0.00±0.00	0.00±0.00	-
Gills	0.00±0.00	0.003±0.002	0.42^{ns}

Values are Mean ± SD, ns= not significantly different, * significantly different ($p<0.05$) using independent sample t-test

Table 2: Seasonal Comparative Analysis of Mean Heavy Metals Concentration in Water of Geidam River with the Permissible Limits

Sampling Sites	Season	Zn (mg/L)	Cd (mg/L)	Cu (mg/L)	Pb (mg/L)	Cr (mg/L)	As (mg/L)
A1	Dry Rainy	0.06±0.01 0.38±0.0.1	0.05±0.02 0.06±0.03	0.92±0.53 0.42±0.01	0.06±0.01 0.08±0.03	0.08±0.02 0.06±0.02	0.00 0.00
A2	Dry Rainy	0.40±0.02 0.45±0.10	0.04±0.01 0.06±0.02	0.92±0.20 0.44±0.00	0.20±0.02 0.06±0.01	0.65±0.03 0.73±0.02	0.00 0.00
A3	Dry Rainy	0.06±0.02 0.90±0.02	0.05±0.02 0.05±0.01	0.22±0.02 0.50±0.20	0.06±0.01 0.07±0.01	0.05±0.02 0.20±0.01	0.00 0.00
A4	Dry Rainy	0.60±0.11	0.06±0.02	0.45±0.02	0.47±0.10	0.65±0.03	0.00 0.00

		0.71±0.03	0.06±0.01	0.50±0.03	0.42±0.02	0.70±0.01	
A5	Dry Rainy	3.32±0.60 3.00±0.04	0.07±0.03 0.06±0.01	1.50±0.01 1.30±0.01	0.72±0.03 0.77±0.01	0.75±0.03 0.76±0.02	0.00 0.00
FMH (2007)		3.0	-	1.0	0.01	0.05	-
USEPA (2003)		0.12	-	1.3	0.00	0.1	-
WHO (1993, 2003, 2005)		5.0	0.001	1.0	0.05	0.05	-

Key:
FMH: Federal Ministry of Health
USEPA: United States Environmental Protection Agency
WHO: World Health Organization

Table 3: Comparative Analysis of Heavy Metals Concentration in Sediments of Geidam River with the Permissible Limits

Sampling Sites	Heavy Metals (mg/kg)	Mean± STD	ASV	TRV	USEPA	WHO	WRS
A1	Zn	13.26±6.75	95	110	0.12	3.0	350
	Cd	0.22±0.07	0.30	0.60	-	0.003	-
	Cu	5.85±3.00	45	16	1.3		100
	Pb	14.55±4.33	20	31	0.00		-
	Cr	60.00±5.30	90	26	0.1	0.01	100
	As	1.07±0.40	-	-	-		-
A2	Zn	17.60±4.50	95	110	0.12	3.0	350
	Cd	0.23±0.01	0.30	0.60	-	0.003	-
	Cu	2.80±0.70	45	16	1.3		100
	Pb	12.55±4.40	20	31	0.00		-
	Cr	80.70±7.55	90	26	0.1	0.01	100

	As	0.03±0.00	-	-	-		-
A3	Zn	16.22±6.50	95	110	0.12	3.0	350
	Cd	0.20±0.01	0.30	0.60	-	0.003	-
	Cu	4.56±2.55	45	16	1.3		100
	Pb	14.25±5.12	20	31	0.00		-
	Cr	89.23±9.55	90	26	0.1	0.01	100
	As	0.00	-	-	-		-
A4	Zn	14.30±5.70	95	110	0.12	3.0	350
	Cd	0.25±0.10	0.30	0.60	-	0.003	-
	Cu	9.44±5.11	45	16	1.3		100
	Pb	10.85±5.23	20	31	0.00		-
	Cr	59.77±11.40	90	26	0.1	0.01	100
	As	0.00	-	-	-		-
A5	Zn	19.22±6.67	95	110	0.12	3.0	350
	Cd	0.28±0.04	0.30	0.60	-	0.003	-
	Cu	12.05±5.55	45	16	1.3		100
	Pb	15.50±2.21	20	31	0.00		-
	Cr	65.44±9.55	90	26	0.1	0.01	100
	As	1.15±0.06	-	-	-		-

Key:
ASV: Average Shale Values TRV: Toxicity Reference Values
WHO: World Health Organization WRS: World River System
USEPA: United State Environmental Protection Agency

Discussion

The results of this research are in agreement with results from research carried out by Shareef (2011), which also showed the bioaccumulation of heavy metals to follow the following order Zn> Ni > Cu > Mn > Pb > Cd. The bioaccumulation of the heavy metals showed varying concentrations among the analysed fish samples with Zn having higher values than the others. The result revealed the distribution of heavy metals in Wasai reservoir to follow the order Zn > Cu > Pb > Cd > Cr > As. The concentration of most of the investigated heavy metals in this report were found to be below the maximum permissible limit set by the World Health Organization, WHO. Liver from the fish species was found to contain high zinc and cadmium content that exceeded the limit set by WHO. The fish gills from river Geidam also contained lead levels that were above the maximum permissible limit set by WHO (1990, 1994). The result of this study therefore, unveils the adverse health effects of the people in the study area where they could be exposed to these metals by consuming fish parts shown to contain high levels of the heavy metals. The results of water and sediment samples from Geidam river contained an overall higher concentration of each of the investigated heavy metals. This concentration may probably be due to the high levels of municipal waste it receives from the two tributaries and the voracious feeding habits of fish

Recommendations

1. In view of the importance of fish to diet of Man, it is necessary that regular biological monitoring of the water bodies and fish meant for consumption should be done. Regular monitoring of metallic content of edible materials in the environment such as fish is very important in order to ensure the safety of consumers.
2. Similarly, studies may be performed to check contamination with other toxic heavy metals such as mercury and cobalt in fish, water and sediment from these water bodies to ascertain their bioaccumulative indices.
3. Establishing of suitable standards for fish quality including both fresh and frozen types according to international guidelines is required.

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