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ANALYSIS OF HEAVY METAL IN SOME FISH SPECIES FROM RICE-FISH FARM OF WEST TARPET VILLAGE, MAUBIN TOWNSHIP, AYEYARWADY REGION

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Abstract

Rice-fish systems can be broadly defined as the cultivation of rice alongside the simultaneous or rotational presence of naturally occurring fish and other aquatic species that are harvested through fisheries, as well as introduced fish populations that are cultured. Fish species are vital for maintaining ecological health and are also economically important for human consumption. In this study, the seasonal variation of heavy metal concentrations (As , Cd and Pb) which bio-accumulated in muscle tissues of three fish species (*Catla catla, Labeo rohita, Puntius gonionotous*) cultured in the rice-fish farm located at West Tarpet Village, Maubin Township were analyzed. Seasonal variation of metal concentrations in water and soil from rice- fish farm were also investigated during the study period. The concentrations of heavy metals in collected specimens were analyzed in the Universities' Research Center (URC) at University of Yangon. The levels of metal concentrations varied depending on the seasons and the different fish species. The highest concentration of As was found in *Catla catla* in the cold season and Cd in *Puntius gonionotus* in the hot season but the results did not exceed the WHO/FAO maximum permissible limits. Pb concentration of *Catla catla* in the rainy season was found to be the highest and over-established by WHO/FAO. The Cd and Pb concentrations of river water were highest in the hot season and in the rainy season; the results were higher than WHO/FAO. The average concentration of Pb in the rainy season of soil sample was the highest and observed to be beyond the WHO/ FAO limit. Based on the results of the present study, the concentration levels of Pb in soil, water samples and, *Catla catla*, as well as Cd in river water exceeded the acceptable limits recommended by WHO/FAO. Significant relations between As and Cd concentrations in the muscle tissue of the fish species and their size (total length) and (body weight) were observed. According to the results of the transfer factor, all tested metals accumulated in muscle tissues of studied fish species came from their abiotic media. The concentration of heavy metals in muscle tissues of *Labeo rohita* and *Puntius gonionotus* were not exceed the permissible limit of WHO/FAO, 2011 and there was no a risk for the public by eating these species.

Keywords: rice- fish farm, heavy metal, muscle tissue, soil, water, fertilizer, transfer factor

Introduction

Rice-fish farming system experimental trials in Myanmar's Ayeyarwady Delta and Letpadan Township, Tharyarwady District, Bago Region were implemented in 2017. Rice-fish systems are helping the people of Myanmar to increase their income from rice fields and enrich the supply of aquatic products while only having minimum paddy modifications (Kalyoncu *et al*., 2009). Traditionally, farmers and neighboring households in South and Southeast Asian countries caught wild fish from water bodies in and around rice fields for household consumption and as an alternate source of income (Halwart and Gupta 2004; Dey *et a*l. 2013). Not only the adequate supply of carbohydrate, but also the supply of animal protein is significant through rice-fish farming. Fish are rich in micronutrients and vitamins, and thus human nutrition can be greatly improved through fish consumption (Larsen *et al*., 2000; Roos *et al*., 2003). Fish accumulate contaminants directly from water and/or through the food chain. Since fish are at higher trophic levels in aquatic ecosystems, metal concentrations in fish muscle could be several orders of magnitude higher than in the environment, resulting in a cumulative impact of toxic metal accumulation in humans through chronic consumption (Chen *et al*., 2018). The exposures of

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metals through animal-derived food like fish intake are normally higher than plant-derived food, and it would be necessary to clarify the exposure level of heavy metals during the consumption of rice - fish farming system fish, especially for the rice fields that have been polluted. In addition, Soil contamination by heavy metals has been accelerated in many parts of the world in the past few decades, due to anthropogenic activities (Facchinelli *et al.*, 2001; Wei and Yang, 2010). With the increasing threat of heavy metals in soil, the RFS is facing increasing risks (Grant *et al.*, 2008)

Fish acts as the primary source of animal protein within the diet of the Myanmar population; with individual consumption rates estimated at 30 kg per person per year (WorldFish, 2017). Bioaccumulation of heavy metals in fish causes serious threats to human when they are consumed. Heavy metals have attracted attention due to their ubiquity in materials, resistance to biodegradability, and causations of numerous illnesses like gastrointestinal, hematological, hepatic, renal, and neurological problems, as well as carcinogenic effects (Nogawa, K., *et al*, 2017; Rahman, M.S., *et al.*, 2012).

The economy of the study area is predominantly agricultural, the farmers use chicken manure, combined with NPK fertilizers, other kinds of fertilizers, and pesticides to achieve high yields of their crop which means rice-fish farms could be at risk of heavy metals. Therefore, the risk assessment of heavy metal concentration in fish for human health is important and necessary. Due to above- mentioned reasons, the present study was carried out to assess the content of heavy metals residues that bio-accumulated in muscle tissues of three fish species from rice-fish farm.

Materials and Methods

Study Area

Study area is situated at West Tarpet Village, Maubin Township, Ayeyarwady Region, between 16° 43′ N and 95° 39′ E (Fig. 1 and Plate 1). A total area of 4046.85m^2 of rice - fish farm was chosen as study site.

Study Period

Study period lasted from June 2023 to May 2024.

Collection of Samples

Three fish species (*Catla catla, Labeo rohita, Puntius gonionotus*) were seasonally collected from rice-fish farm. Collected fish specimens were identified followed by Talwar and Jhingran (1991). Water samples were collected seasonally from the rice-fish farm, reservoir, and river. Seasonal sampling was conducted during the rainy (August), and cold (December) and hot (April) seasons of 2023-2024. Fertilizer samples (compound (fertilizer 1) and nitrogenous fertilizers (fertilizer 2)) used in the study area and soil from this area were also collected to assess heavy metal content.

Sample preparation

The collected fish specimens were washed with tap water, and their total length (cm) and body weight (g) of specimens were measured. The muscles were then removed and weighted, dried in an oven at 90˚C until a constant weight was achieved, and stored in airtight containers. Digestion of the samples was carried out according to the dry method by using a furnace. Water samples were filtered through a 0.45µm Whatman filter and analyzed directly by AAS. The soil sample was sun-dried, grounded and sieved to obtain a fine powder. Fertilizer samples were also ground into a fine powder to facilitate further analysis. Digestion of the samples was conducted according to the dry method using a furnace.

Data Analysis

The heavy metal content in the muscles of the collected fish species, as well as in the water, soil, and fertilizers samples were analyzed in triplicate using a Flame Atomic Absorption Spectrometer (FAAS) (Perkin Elmer AA analyst 800 and Winlab-32 software) in Universities' Research Centre (URC) at Yangon University. Seasonal variations in the test results were compared with the maximum permissible limits set by WHO/FAO in 2011 (MPL). The transfer factor (TF) in muscle tissues from the abiotic media, which include water and soil was calculated according to Kalfakakour and Akrida- Demertzi (2000) and Rashed (2001) and Irenosen and Ishmael (2019) as follows:

$$TF = \frac{\text{concentration of metal in fish tissue}}{\text{concentration of metal in abiotic media (water/soil)}}$$

TF value is greater than 1 indicates that there is the bioaccumulation of metals in fish tissue. The relationship between heavy metal concentration (As, Cd and Pb) in muscle tissues and size of fish species were assessed by using the linear regression method (Le Cren, 1951).

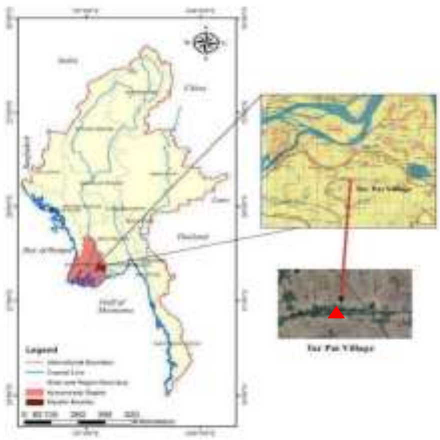

Figure. 1 Mape of study area, West Tarpet village (Source: Google)

Results

Three fish species (*Puntius gonionotus, Catla catla, Labeo rohita*) were collected seasonally from rice - fish farm in West Tarpet Village, Ayeyarwady Region (Fig. 1). The total length and total weight of the collected fish species were presented in table 1. The seasonal variation in the mean concentration of heavy metals (arsenic, cadmium and lead) in different fish samples were presented in fig. 2, 3 and 4 respectively. In the cold season, the highest value of arsenic concentration in muscle tissues of *Catla catla* was 0.00308mg/L (Fig. 2).

In the present study, the seasonal variation of cadmium concentration in study fish species was shown in Figure 3. In the rainy season, the highest average value of cadmium in the muscle tissue of *Puntius gonionotus* was 0.031 mg/L. In the present study, the highest value of lead concentration in the muscle of *Labeo rohita* was 0.334 mg/L in the hot season (Fig. 4).

In the result of the current finding, the metal concentration levels of arsenic, cadmium and lead in water samples for seasonal variations were presented in Figs. 5, 6 and 7 receptively. In the cold season, the highest value of arsenic concentration in the rice-fish farm water was 0.00145mg/L (Fig. 5). Among the water samples, only the river water was observed with the highest concentrations of cadmium (0.038mg/L) and lead (0.295mg/L) in the rainy season (Fig. 6 and 7). In addition, the seasonal variation of metal concentrations in the soil sample was also determined during the study period (Fig. 8). The highest value of arsenic, cadmium and lead concentrations in the soil sample were found 0.00665 mg/L, 0.006mg/L and 0.2mg/L in the rainy season followed by hot season while those were the lowest in cold season. The concentrations of fertilizers used in rice-fish farm were also detected in the study period (Fig.9). The highest values of arsenic, cadmium and lead concentrations in fertilizers were 0.01315mg/l, 0.027mg/l and 0.391mg/l respectively.

Regression coefficient values of the relationship between arsenic and cadmium concentrations in muscle tissue and total length of all studied fish species were found 0.5416 to 0.9998 and 0.1049 to 0.2966. Positive correlation between arsenic and cadmium concentrations and total length and the body sizes of studied fish species were observed. In the present study, linear regression analysis between heavy metal concentrations in muscle tissues and body sizes of fish species was conducted. Regression coefficient values of the relationship between lead concentrations in muscle tissue and total length of all studied fish species were found 0.898 to 0.9983. Linear relation between lead concentration and the body sizes of studied fish species were negatively related (Table 4). Index values of the transfer factor of three heavy metals from water and soil of abiotic-media to muscle tissue of the studied fish species were present in table 5. It is found that the transfer factor of heavy metal in muscle tissue from water and soil was observed to be greater than that of the limitation value of 1.

Table 1 Mean total length (cm) and body weight (g) of studied fish species to test metal concentration

Species	Rainy		Cold		Hot	
	Total length(cm)	Weight (g)	Total length(cm)	Weight(g)	Total length(cm)	Weight(g)
Catla catla (n=10)	6.72 ± 0.59	11.64 ± 1.25	25.23 ± 13.2	325 ± 115.3	28.23 ± 11.25	445 ± 22.15
Labeo rohita (n=10)	10.28 ± 0.74	17.29 ± 4.83	23.2 ± 11.23	300.5 ± 12.35	30.45 ± 12.3	405 ± 26.47
Puntius gonionotus (n=10)	8.37 ± 1	11.83 ± 1.31	25.32 ± 2.19	261 ± 122.38	27 ± 0.7	300.61 ± 43.65

Table 2 Maximum permissible limit of three metals in fish and water samples (WHO/FAO, 2011)

Sr.no	Types of sample	Metals		
		Arsenic	Cadmium	Lead
1	Fish	0.5	0.5	0.3
2	Water	0.01	0.01	0.01

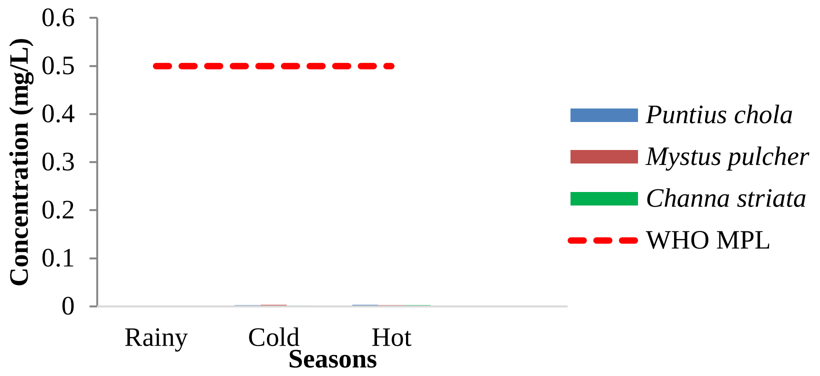

Figure. 2 Seasonal variation of Arsenic concentration in studied fish species

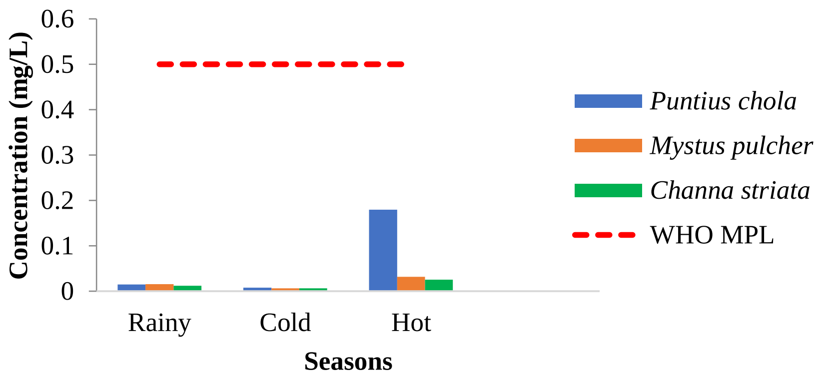

Figure. 3 Seasonal variation of Cadmium concentration in studied fish species

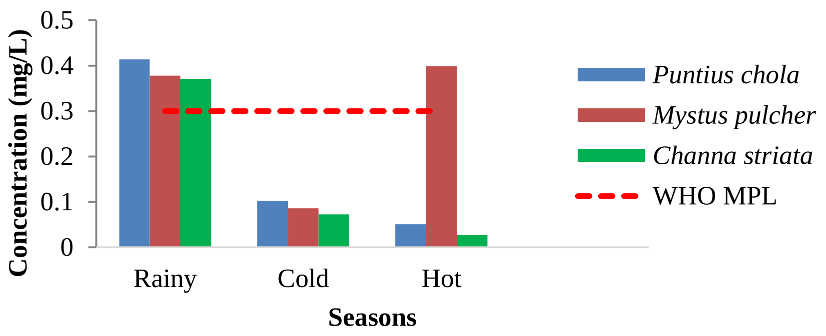

Figure. 4 Seasonal variation of Lead concentration in studied fish species

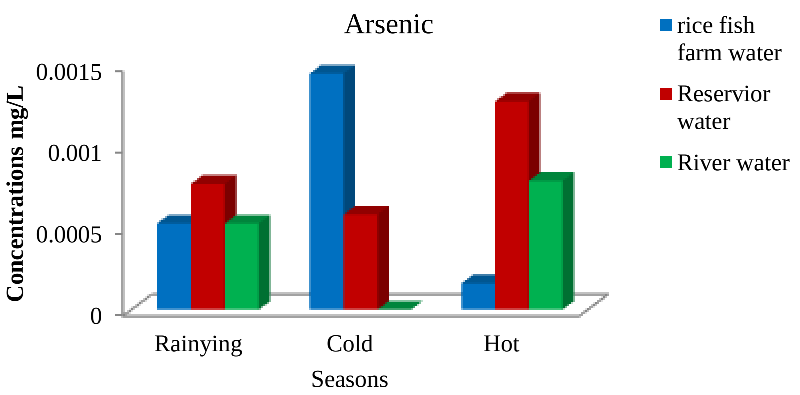

Figure. 5 Seasonal variation of Arsenic concentration in water samples of study area

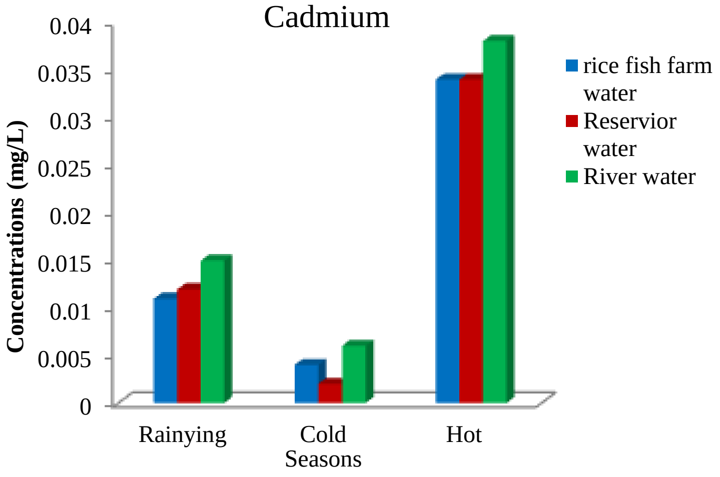

Figure. 6 Seasonal variation of cadmium concentration in water samples of study area

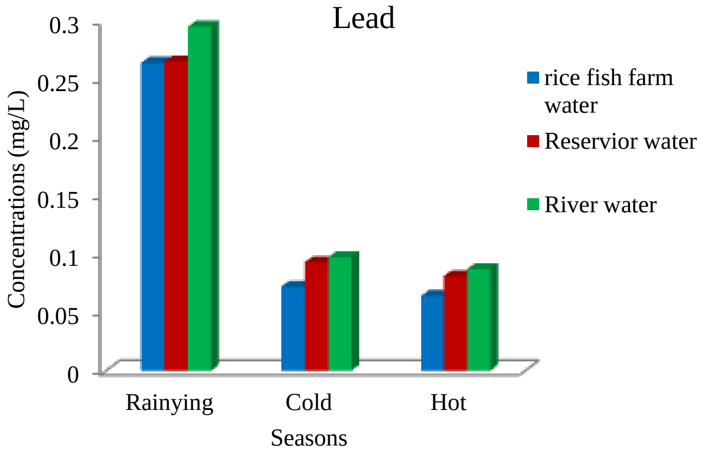

Figure. 7 Seasonal variation of lead concentration in water samples of study area

Table 3 Maximum permissible limit and effects of metals concentration in soil sample

No.	Metals	WHO,2007 MPL	TEC	MEC	PEC
1	Arsenic	0.01	9.8	21.4	33
2	Cadmium	0.01	0.99	3	5
3	Lead	0.05	36	83	130

TEC= Threshold effect concentration, MEC= Midpoint effect concentration,
PEC= Probable effect concentration

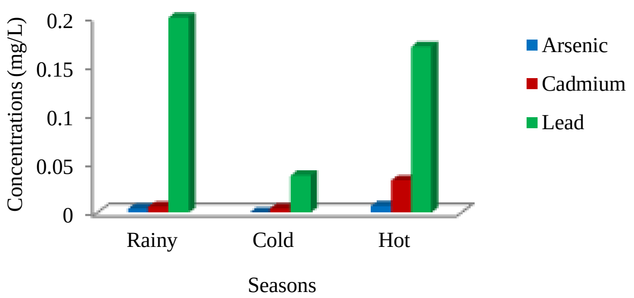

Figure. 8 Seasonal variation of metals concentration in soil samples

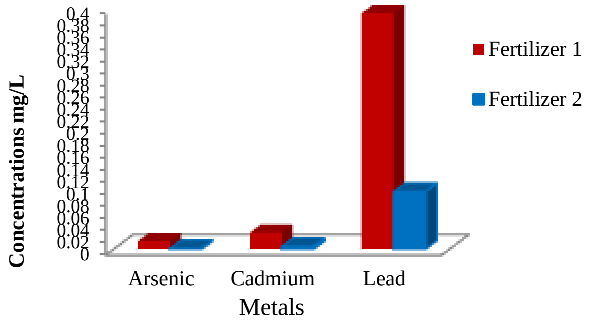

Figure. 9 Concentration of heavy metal in fertilizers

Table 4. Relationship between body length (cm) and body weight (g) of fish and heavy metal concentrations in muscle tissue of studied fish species

Species	Heavy metals	Length	Remarks	Weight	Remark
		Linear equation		Linear equation	
Catla catla	As	y = 0.0003x-0.0025 R^2 = 0.7207	Positive relation	y = 2E-05x+0.0008 R^2 = 0.8378	Positive relation
	Cd	y = 0.0006x+0.0051 R^2 = 0.1721	Positive relation	y = 4E-05x+0.0065 R^2 = 0.2914	Positive relation
	Pb	y = -0.0142x+0.4283 R^2 = 0.9983	Negative relation	y = - 0.0007x+0.3331 R^2 = 0.9666	Negative relation
Labeo rohita	As	y = 0.0001x+0.001 R^2 = 0.9927	Positive relation	y = 5E-06x-9E-05 R^2 = 0.9998	Positive relation
	Cd	y = 0.0001x+0.0134 R^2 = 0.0413	Positive relation	y = 4E-05x+0.007 R^2 = 0.2106	Positive relation
	Pb	y = -0.0475x+1.5187 R^2 = 0.4432	Negative relation	y = -0.0007x+0.2894 R^2 = 0.9498	Negative relation
Puntius gonionotus	As	y = 9E-05x+0.0007 R^2 = 0.5865	Positive relation	y = 6E-06x+6E-05 R^2 = 0.5416	Positive relation
	Cd	y = 8E-05x+0.0117 R^2 = 0.0782	Positive relation	y = 4E-05x+0.0123 R^2 = 0.1337	Positive relation
	Pb	y = 0.0063x+0.1421 R^2 = 0.8966	Negative relation	y = -0.0009x+0.2885 R^2 = 0.9926	Negative relation

Table 5 The TF of heavy metals from water and soil in muscle tissues of studied fish species in all season

Species	Metals	Rainy		Cold		Hot	
		TF from water	TF from soil	TF from water	TF from soil	TF from water	TF from soil
Catla catla	As	0	0	2.12	0	5.32	0.13
	Cd	0.91	1.67	2.5	2.5	0.74	0.76
	Pb	1.27	1.67	0.88	1.66	0.53	0.2
Labeo rohita	As	0	0	0	0	12.55	0.3
	Cd	0.45	0.83	2.75	2.75	0.65	0.67
	Pb	1.1	1.44	0.54	1.03	0.44	0.16

Species	Metals	Rainy		Cold		Hot	
		TF from water	TF from soil	TF from water	TF from soil	TF from water	TF from soil
Puntinus gonionotus	As	0	0	1.74	0	6.58	0.16
	Cd	0.36	0.67	2.25	2.25	0.91	0.94
	Pb	1.06	1.4	0.71	1.34	0.63	0.24

Discussion

Heavy metals (As, Cd and Pb) in muscle tissue of three fish species (*Catla catla*, *Labeo rohita* and *Puntius gonionotus*) collected from the rice-fish farm in West Tarpet Village of Maubin Township, Ayeyarwady region were analyzed seasonally during the study period. Metal concentrations in the water and soil of the aquatic environment at the study site, as well as those in the fertilizers used in the rice-fish farm, were also assessed.

In the present study, the highest concentration of arsenic (As) was found in *Catla catla* (0.00308 mg/L) among the three species in the cold season. However concentration levels of arsenic were found to be lower than the WHO/FAO maximum permissible limits. Khin Thida Kyaw (2008) and Aye Aye Mu (2011) reported that arsenic concentrations of all studied fish species in all seasons in Daydaye and Hinthada Township, Ayeyarwady region did not exceed the WHO/FAO maximum permissible limit.

In the present study, cadmium (Cd) content was found to be highest in *Puntius gonionotus* (0.031mg/L) in the hot season. But concentration level of this metal was found to be lower than WHO/FAO maximum permissible limits. Yee Yee Win (2021) observed that Cd concentrations of all studied fish species from Thanlwin River Segment were found to be lower than the WHO/FAO maximum permissible limit during all three seasons.

In this investigation, the lead (Pb) concentration of *Catla catla* (0.334 mg/L) in the rainy season was found to be the highest and it showed over the recommended maximum permissible limit established by WHO/FAO. Cho Cho Thin (2017) revealed that lead concentration in the muscle tissue of herbivorous, carnivorous, and omnivorous fish was highest during the rainy season in the Ayeyarwady River segment of the Salay environs, which aligns with the findings of the present study. Kargin (1996) reported that multiple factors, including season and the physical and chemical properties of water, can significantly influence metal accumulation in various fish tissues.

The concentration of lead was found to be higher in water, fertilizer and soil samples in the present study. Similarly, the valuesobserved for lead concentration was the highest in fish species *Catla catla* in the present study. Ming Ho (2005) stated that food is one of the major sources of lead exposure; the others are air and drinking water. Plant food may be contaminated with lead through its uptake from ambient and soil; animals may then ingest the lead-contaminated vegetation. Therefore, lead concentrations in muscle tissues of the herbivores, *Catla catla* may transfer from water, fertilizer and soil due to bioaccumulation and may attain higher concentrations in such manners.

In all detected heavy metals, the concentrations of Pb in all studied fish species were found to be highest in the rainy season in this investigation. Lead is a non-essential and toxic metal that is released into the aquatic environment through the use of fertilizers (Pascoe and Mattery, 1997). In the study area, the farmer would use fertilizers to obtain high yields for their crops. In the rainy season, the concentration of heavy metals from fertilizers may come from the paddy fields and accumulate in the river and aquatic fish species. Arsenic (As) concentrations in rice-fish farm water (0.00145mg/L) showed the highest in the cold season. But the result did not exceed the maximum permissible limit established by WHO/FAO. Seinn Moh Moh Paing, (2019) observed that arsenic concentrations in the water of Panhlaing River segment of Nyaung Done township were the highest in the cold season, which is similar to the present study's findings as well.

In this study, cadmium (Cd) concentration of river water (0.038mg/L) was highest in the hot season and the result was higher than the WHO/FAO maximum permissible limit. Lead (Pb) concentration of river water (0.295mg/L) was highest in the rainy season and followed by reservoir water (0.265 mg /L) and rice fish farm water (0.264 mg/L) and the obtained results were higher than the WHO/ FAO maximum permissible limit. Cogun *et a*l., (2006) stated that industrial discharge and agricultural runoff are forms of human activities that are released into rivers caused into water heavy metal pollution in water seasonally. In the present study, average concentration values of arsenic (0.00665mg/L) in the hot season, and cadmium (0.006 mg/L) in the rainy season of soil samples were the highest but did not exceed the permissible limit. Lead concentration (0.2 mg/L) in rainy season of the soil sample was the highest and observed to be beyond the WHO/ FAO maximum permissible limit.

According to the recorded data, all metals concentrations (As, Cd and Pb) in the sediment during the study period were observed to be lower than the "threshold effect concentration (TEC)", midpoint effect concentration (MEC)" and probable effect concentration (PEC)" (MacDonald *et al*., 2000). This is in agreement with the previous finding by Cho Cho Thin *et al*., (2022) described that Fe, Zn, Pb, Cd and As concentrations in the sediment of Kyet Mauk Taung Dam were observed lower than TEC, MEC and PEC.

In the present study, the concentration of lead (Pb) in compound fertilizer was observed to be the highest at 0.391 mg/L, followed by cadmium at 0.027 mg/L and arsenic at 0.01315 mg/L, respectively. In Nitrogenous fertilizer, the lead concentration (0.095mg/L), cadmium (0.004mg/L) and arsenic (0.00657mg/L) were detected. Edogbo *et al*., (2020) observed that organic fertilizers may also contain a different concentration of heavy metals applied to fishponds and soil. Base on the all recorded data of the present study, the concentration levels of Pb in soil, water samples and *Catla catla*, as well as Cd in river water exceeded the acceptable limits recommended by WHO/FAO.

In the present study, linear regression analysis between heavy metal concentrations in muscle tissues and body sizes of fish species was conducted. Significant relations between As and Cd (positive relation) concentrations in muscle tissues of all studied fish species and their body size (body length and body weight) were observed. Cho Cho Thin (2017) observed that the relationship between As concentration in muscle tissues of some carnivorous and omnivorous fishes and their body size were significant. Yee Yee Win (2021) stated that the length and weight of *Polynemus paradiseus* and As concentration were positively correlated in the study site. The result showed that significant decline in lead (Pb) concentrations with the increase in size in all studied fish species. Many researchers reported that Pb concentration were inversely proportional to the total length and body weight of fish (De Wet *et a*l., 1994; Canil and Atli., 2003. None of the samples recorded increasing Pb levels with increasing body weight as reported by Farkas *et al*., (2003) and Namin *et al*., (2001). Liang *et al*. (1999) studied metal (Zn, Cu, Cd, Cr, Pb, Ni) concentrations in various polycultured fish species and found that metals in fish viscera were negatively correlated (except Zn in viscera of common carp). One explanation for the negative relationships between metal concentrations and size found in this study may be the difference in metabolic activity between younger and older fish. The net accumulation of heavy metals in an organism is a result of the difference between uptake and depuration and this is the most important factor in metal accumulation. The dilution of tissue metal concentrations due to growth and/or lower metabolic activity in older individuals may not be seen if metal concentrations in water are higher than the capacity of these factors. Body size has been shown to have a potentially strong effect on concentrations in muscle tissues and organs (Canli and Atli, 2003; Farkas *et al*., 2003; Yi and Zhang, 2012; Noël *et al*., 2013).

Rased (2001) reported that when the calculated transfer factor was higher than 1 it means that fishes bioaccumulate metals from the environment in all cases (eg. water, soil and plants). In the present study, the transfer factor (TF) of lead from water to muscle tissue of all studied fish species was observed in the rainy season and cadmium from soil in *Catla catla*. the lead from soil environment to muscle tissues of *Catla catla*, *Labeo rohita* and *Puntius gonionotus* was observed in the rainy season. In the cold season, the transfer factor of arsenic from water environment to muscle tissue of *Catla catla* and *Puntius gonionotus* was observed. Next, the transfer factors of Cd from the water environment to muscle tissues of all studied fish species were observed. And the transfer factor of Cd and Pb from the soil to muscle tissues of all fish species was also observed in the cold season. In this investigation, the transfer factor of As from water environment to muscle tissues of all studied fish species was found in the hot season. The enrichment of heavy metals in the environment can result from both anthropogenic activities and natural processes (Forstner and Whitman, 1979; Nriagu, 1989; Veena *et al*., 1997). In this study, most of transfer factor for water to fish and soil to fish showed greater than 1. This means that the fish undergo bioaccumulation of these tested metals from the abiotic media (water, soil and fertilizers) (Kalfakakour and Akrida- Demertzi, 2000). According to the index value of transfer factors, it may be assumed that Pb concentration in muscle tissues of *Catla catla* came from their abiotic media. The result revealed that the lead concentrations in *Catla catla* in studied fish species were higher than the permissible limit. However, other fish species from the study area were generally safe for human consumption, although the bioaccumulation of metals in fish tissue was slightly elevated. Therefore, regular monitoring of heavy metal levels in fish is necessary, and the public should be made aware of the environmental damage caused by human activities.

Conclusion

In the present study, the concentrations of heavy metals (As and Cd) in all examined fish species were found to be below the maximum permissible limits set by WHO/FAO in all three seasons. The concentration of heavy metal (Pb) in *Catla catla* was found to exceed the maximum permissible limit set by WHO/FAO during the rainy season, likely due to agricultural runoff of fertilizers and pesticides. Significant relations between As and Cd concentration in the muscle tissue of the fish species and their size (total length) and (body weight) were also observed. The results revealed that the concentration of Pb in all water samples, as well as in soil and the concentration of Cd in river water from the study area during the study period were higher than the maximum permissible limits set by WHO/FAO. All the study fish species, except for *Catla catla* from the study site, were below the maximum permissible limits set by WHO/FAO (2011) and were generally considered safe for human consumption. According to the results of the transfer factors, heavy metals accumulated in different tissues of the studied fish species primarily came from water and soil, and the level of bioaccumulation was found to be slightly elevated.

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