Heavy Metals Concentration In *Mystus Bleekeri* (Day,1877) And *Hypselobarbus Kolus* (Sykes,1839) From Nira River, Bhor (Maharashtra) India.

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Abstract:
Mystus bleekeri (Day,1877) and Hypselobarbus kolus (Sykes,1839) from the Northern Western Ghats of Maharashtra harbor the riverine system in Maharashtra. Nira river located in Pune district near Bhor, is one of the natural habitats of the fishes under study. Both the fishes are consumed by the local population and are one the source of protein in their dietary constitution. The research was conducted in 2019 to determine the accumulation of heavy metals in several organs, including the liver, kidney, gut, and gills. Atomic Absorption spectroscopy was used to conduct the analysis. Heavy metals such as lead, cadmium, chromium, and copper were detected in the tissues studied in this study. The overall pattern of heavy metal distribution in various tissues is as follows: Pb > Cu > Cr > Cd. The organs with the highest concentrations of heavy metals are the gill > kidney > intestine > liver > muscle. The values obtained are under permissible level advocated by WHO, but the presence of these heavy metals is an alarming situation and would lead to bio accumulation and disturb the physiological function of fishes under study as well as cause adverse effect on human being.

Keywords: Atomic Absorption spectrometer, liver, kidney, intestine, gills,WHO, *Mystus bleekeri* (Day,1877) and *Hypselobarbus kolus* (Sykes,1839).

Introduction:

Due to industrialization and urbanization there has been deteriorating in the physio-chemical status of fresh water bodies throughout the continents. This has caused a treat to numerous aquatic organism, which might become extinct in near future. The study of heavy metals in aquatic organism has received significant consideration due to its bio accumulation in the tissues of aquatic organism [1,2,3]. Aquatic organism can accumulate heavy metals up to 150 folds than the actual concentration present in aquatic ecosystem [4]. The deterioration in water quality is due to addition of sewage waste, industrial waste as well as agricultural run off which enters the water bodies [5,6]. Various heavy metal pollutant can cause lethal effect on the biochemical processes of organism and may lead to disturbance in their physiological process, at the same time bringing about disturbance in the ecological balance along with the imbalance in the diversity [7,8,9].

The consumption of fish as a rich source of protein has increased due to its nutritional value, less amount of animal fat. Fish is rich in vitamins, essential minerals and unsaturated fatty acids [10,11]. As per the recommendation of The American heart Association it is suggested that to make up of the daily requirement of omega-3 fatty acids, one must consumes fish twice in a week. [12]. Various factors namely environment, which includes the physio-chemical properties of water body and biological factors which includes the age, sex, and diet of the species are responsible for the accumulation of heavy metals in fresh water fish.The process of acculturation at time may be passive or selective and could be a result of process of intake and its assimilation, which could be different in any two individual animal depending upon its biological factor [13].The retention of heavy metals in the various tissues of fishes differ as per their natural ecosystem, in case of fresh water ecosystem the fish tries to retain more salts, while in case of marine ecosystem the fishes tend to loose more salt.Due to this the fresh water fishes are assailable to the unfavorable effect of heavy metal pollution [14].

The intake of these heavy metals takes places through gill lamellae, and also through the food consumed which leads to (bio-magnification), liver and kidney also plays a key role in accumulation of these toxins which are usually much higher compared with the environmental concentration of these component in the natural habitat of the organism[15].Various non essential metals namely Cadmium (Cd), and Lead (Pb) plays zero significant role in the normal functioning of the biochemical processes of the organism. At the same time their presence shows intense toxic effect at a low exposure levels showing threat to all biotic form including human beings [16, 17]. The toxic effect finally results in disturbance in the histopathological and physiological processes of the individual [18,19]. Fish is one of the most important protein rich dietary component since it is rich in minerals as well as saturated fatty acids [20].

Numerous research on the accumulation of heavy metals in different tissues of freshwater fish have been conducted during the last decade [21,22]. Fish that inhabit a freshwater habitat with an active supply of industrial effluents have been deemed unfit for human use as food owing to elevated levels of toxins in various tissues [23,24]. These heavy metal can antagonize their useful effects, and their ill effect can be manifested and deteriorate the human health [25].There has been alteration in the physiological activities as well as in the biochemical process of the fish.

The primary purpose of this investigation was to ascertain the concentration of heavy metals, namely Copper (Cu), Cadmium (Cd), lead (Pb), and Chromium (Cr) in following tissues namely livers, gills and muscles of *Mystus bleekeri* (Day,1877) and *Hypselobarbus kolus* (Sykes,1839) caught from the Nira river Pune distrct (Maharashtra) India. This would help in penning down strategies to combat the influx of pollutants in fresh water body as well as to implement remediation for environmental restoration.

Methodology:

Sampling site:

Fifteen individuals of was collected from 2 sampling site the distance between the two sampling sites accounted to 5 kms (Fig:1), located on Nira river (Latitude: 17° 58' 59.99" N, Longitude: 75° 06' 60.00" E). The Nira river originates in India and travels through the state of Maharashtra. It is a tributary of the Bhima river, which runs through the Maharashtra districts of Pune and Solapur. This river originates in Maharashtra's Western Ghats and travels through Bhor Taluka, Shirwal Taluka, Satara District, and Solapur District before meeting the Bhima Basin at Nira Narsingpur.

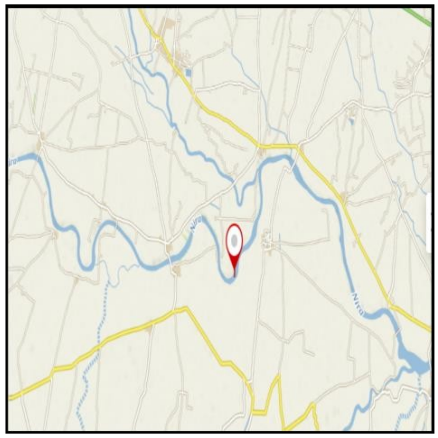

Figure 1. Satellite image of Nira river
(Latitude: 17° 58' 59.99" N, Longitude: 75° 06' 60.00" E)

Method:

Fish were collected from the sample location and transported to the laboratory in an ice box. Fishes were thawed so as to reach room temperature before carrying out the dissection which was was carried out on the same day. Gills, Kidney, Muscle, liver and intestine were separated and were used for the studies. Tissues were well cleansed with double distilled water and wiped dry before being put in a glass beaker and put in an oven set to 60°C for 48 hours. The drying of tissues was carried out till all the water content was evaporated. The tissues were grinded so as to achieve the Pulverization and homogenization as per the method implemented by [26]. After complete drying of tissues they were pulverized to fine dry powder an stored in a properly labeled air tight plastic containers. Each tissue powder sample was weighed using an electronic scale, and a 0.5 gm sample was digested with 6 ml concentrated nitric acid and 1 ml 30% hydrogen peroxide. Throughout the treatment, a microwave digester was used. The digested samples were then filtered through whattman's paper and diluted to 25ml in a volumetric flask with distilled water. The resulting solutions for the various tissue samples under examination were analyzed using a flame atomic absorption spectrophotometer (Perkin-Elmer, Model 2380). Statistical analysis was conducted using SPSS software (version 20). The correlation was estimated with the help of ($p < 0.05$).

Summer (March -May) Station 1.				
Organs	Cu	Cd	Pb	Cr
Gill	0.334 ± 0.000	0.169 ± 0.039	0.631 ± 0.034	0.114 ± 0.002
Liver	0.04 ± 0.001	0.028 ± 0.002	0.064 ± 0.002	0.033 ± 0.001
Kidney	0.073 ± 0.00	0.20 ± 0.014	0.3 ± 0.021	0.041 ± 0.001
Muscle	0.034 ± 0.001	0.040 ± 0.001	0.084 ± 0.002	0.080 ± 0.001
Intestine	0.075 ± 0.014	0.031 ± 0.002	0.044 ± 0.006	0.081 ± 0.002
Winter (Nov -Jan)				
Gill	0.142 ± 0.001	0.120 ± 0.004	0.492 ± 0.035	0.111 ± 0.003
Liver	0.025 ± 0.001	0.021 ± 0.004	0.040 ± 0.002	0.043 ± 0.001
Kidney	0.49 ± 0.001	0.20 ± 0.015	0.21 ± 0.021	0.031 ± 0.001
Muscle	0.093 ± 0.002	0.021 ± 0.001	0.063 ± 0.003	0.090 ± 0.001
Intestine	0.053 ± 0.014	0.023 ± 0.001	0.024 ± 0.003	0.055 ± 0.014

Table 1. Heavy metal concentrations (µg/g) in several organs of *Mystus bleekeri* (Day, 1877) throughout the summer and winter seasons. Station 1.

Summer (March -May) Station 2.				
Organs	Cu	Cd	Pb	Cr
Gill	0.331± 0.000	0.164 ± 0.039	0.629 ± 0.034	0.111 ± 0.002
Liver	0.04 ± 0.001	0.025 ± 0.002	0.061 ± 0.002	0.030 ± 0.001
Kidney	0.070 ± 0.00	0.21 ± 0.014	0.28 ± 0.021	0.043 ± 0.001
Muscle	0.033 ± 0.001	0.039 ± 0.001	0.081 ± 0.002	0.082 ± 0.001
Intestine	0.077 ± 0.014	0.030 ± 0.002	0.040 ± 0.006	0.078 ± 0.002
Winter (Nov -Jan)				
Gill	0.140 ± 0.001	0.118 ± 0.004	0.490 ± 0.035	0.114 ± 0.003
Liver	0.026 ± 0.001	0.023 ± 0.004	0.039 ± 0.002	0.040 ± 0.001
Kidney	0.074 ± 0.001	0.19 ± 0.015	0.211 ± 0.021	0.029 ± 0.001
Muscle	0.091 ± 0.002	0.022 ± 0.001	0.061 ± 0.003	0.088 ± 0.001
Intestine	0.054 ± 0.014	0.024 ± 0.001	0.020 ± 0.003	0.052 ± 0.014

Table 2. Heavy metal concentrations (µg/g) in several organs of *Mystus bleekeri* (Day 1877) throughout the summer and winter seasons. Station 2.

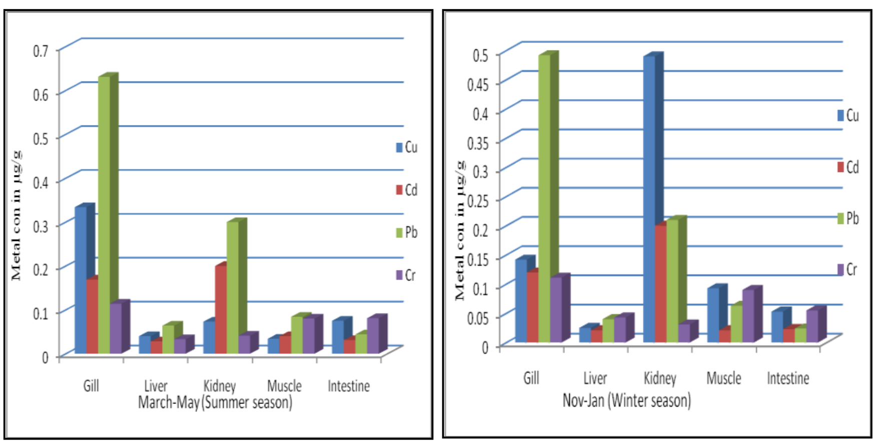

Figure 2. Graphical representation of hazardous metal concentrations (Cd, Cu, Cr, and Pb) in various tissues of *Mystus bleekeri* (Day,1877) during the summer and winter seasons. At ($p < .05$), the values are statistically significant. Station 1.

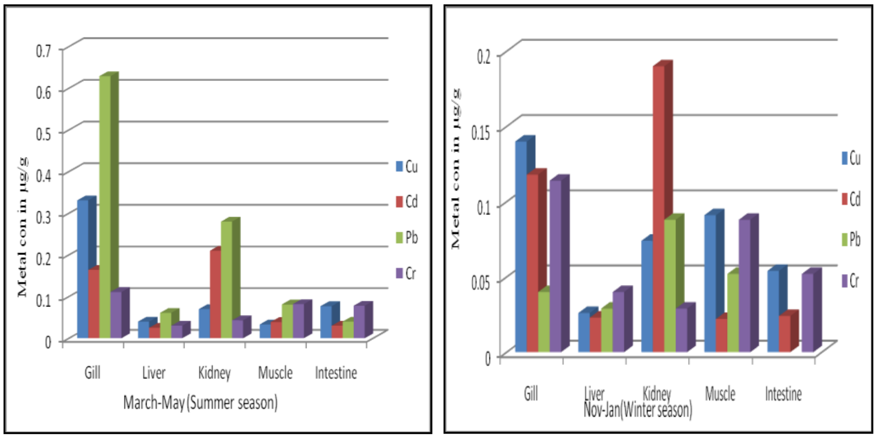

Figure 3. Graphical representation of hazardous metal concentrations (Cd, Cu, Cr, and Pb) in various tissues of *Mystus bleekeri* (Day,1877) during the summer and winter seasons. At ($p < .05$), the values are statistically significant. Station 2.

Summer (March -May) Station 1.				
Organs	Cu	Cd	Pb	Cr
Gill	0.331± 0.000	0.164 ± 0.039	0.629 ± 0.034	0.111 ± 0.002
Liver	0.04 ± 0.001	0.025 ± 0.002	0.061 ± 0.002	0.030 ± 0.001
Kidney	0.070 ± 0.00	0.21 ± 0.014	0.28 ± 0.021	0.043 ± 0.001
Muscle	0.033 ± 0.001	0.039 ± 0.001	0.081 ± 0.002	0.082 ± 0.001
Intestine	0.077 ± 0.014	0.030 ± 0.002	0.040 ± 0.006	0.078 ± 0.002
Winter (Nov -Jan)				
Gill	0.140 ± 0.001	0.118 ± 0.004	0.490 ± 0.035	0.114 ± 0.003
Liver	0.026 ± 0.001	0.023 ± 0.004	0.039 ± 0.002	0.040 ± 0.001
Kidney	0.074 ± 0.001	0.19 ± 0.015	0.211 ± 0.021	0.029 ± 0.001
Muscle	0.091 ± 0.002	0.022 ± 0.001	0.061 ± 0.003	0.088 ± 0.001
Intestine	0.054 ± 0.014	0.024 ± 0.001	0.020 ± 0.003	0.052 ± 0.014

Table 3. Heavy metal concentrations (µg/g) in several organs of *Hypselobarbus kolus* (Sykes,1839) throughout the summer and winter seasons. Station 1.

Summer (March -May) Station 2				
Organs	Cu	Cd	Pb	Cr
Gill	0.025 ± 0.002	0.021 ± 0.001	0.180 ± 0.021	0.165 ± 0.002
Liver	0.090 ± 0.001	0.020 ± 0.014	0.704 ± 0.054	0.314 ± 0.002
Kidney	0.089 ± 0.001	0.051 ± 0.015	0.759± 0.055	0.251 ± 0.002
Muscle	0.031 ± 0.001	0.023 ± 0.001	0.050 ± 0.002	0.046 ± 0.001
Intestine	0.01 ± 0.014	0.06 ± 0.012	0.481 ± 0.035	0.132 ± 0.002
Winter (Nov -Jan)				

Gill	0.016 ± 0.041	0.011 ± 0.001	0.133 ± 0.005	0.155 ± 0.024
Liver	0.053 ± 0.001	0.21 ± 0.003	0.501 ± 0.003	0.214 ± 0.001
Kidney	0.059 ± 0.001	0.020 ± 0.002	0.21 ± 0.022	0.210 ± 0.025
Muscle	0.033 ± 0.005	0.021 ± 0.00	0.034 ± 0.030	0.031 ± 0.002
Intestine	0.03 ± 0.018	0.05 ± 0.012	0.320 ± 0.034	0.112 ± 0.012

Table 4: Heavy metal concentrations (µg/g) in several organs of *Hypselobarbus kolus* (Sykes,1839) throughout the summer and winter seasons. Station 2.

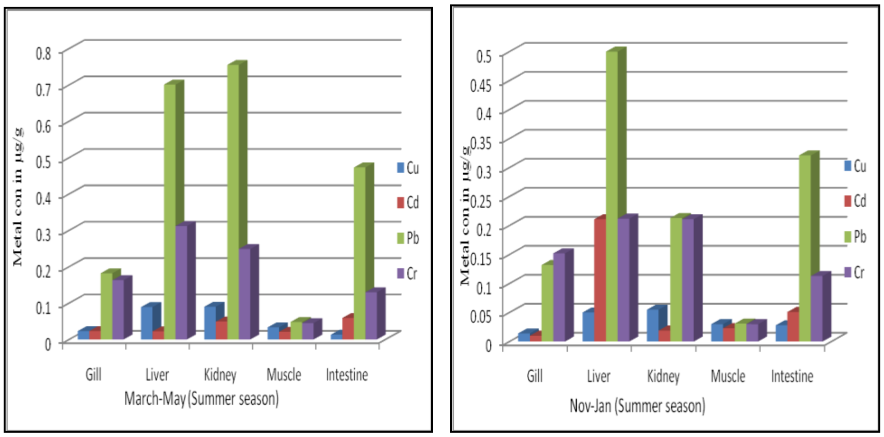

Figure 4. Graphical expression of Metal concentrations (Cd, Cu, Cr, and Pb) in various tissues of *Hypselobarbus kolus* (Sykes,1839) during summer and winter seasons. At these levels, the values are statistically significant. ($p < .05$). Station 1.

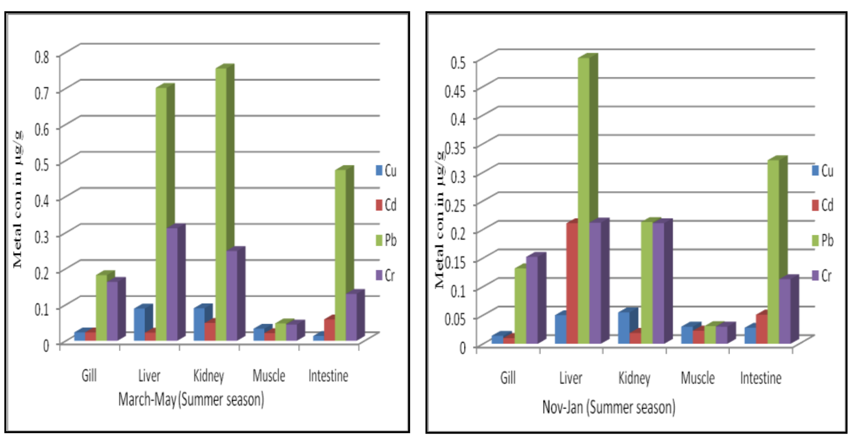

Figure 5. Graphical expression of Concentrations of toxic metals (Cd, Cu, Cr and Pb) in different tissues of *Hypselobarbus kolus* (Sykes,1839) during summer and winter seasons. Values are statistically significant at ($p < .05$). Station 2.

Result & Discussion:

Fish have the potential to store heavy metals in their tissues, which leads to bioaccumulation when these metals reach the human body via fish consumption. These heavy metals cannot be degraded and remain in the ecosystem and cause serious threat to the biotic components [27]. The heavy metals reaches the water bodies through agricultural, industrial run-off and affect the aquatic species.[28,29]. The result in the present studies is tabulated in (Table 1) and (Table 2) for *Mystus bleekeri* (Day 1877) for the two sampling sites for two seasons respectively. While in case of *Hypselobarbus kolus* (Sykes,1839) the results are tabulated in (Table 3) and (Table 4) for two different sampling sites and two different seasons respectively.

Metals are essential for the organism for its normal physiological process and its normal functioning. When these metals cross the permissible limit it becomes a serious threat to the animal as well as other organism in the food web. The mean value of Copper in the tissue sample of *Mystus bleekeri* (Day, 1877) in the current investigations was between 0.04–0.334 mg/kg in the summer and 0.025–0.142 mg/kg in the winter season, respectively. The mean value of Copper in the tissue sample of *Hypselobarbus kolus* (Sykes,1839) was between 0.03–0.331 mg/kg in the summer and 0.03–0.140 mg/kg in the winter season. Copper has an acceptable amount of 3 g/g, according to WHO guidelines. The value found in the current investigations is larger, but it does not surpass the WHO-recommended allowed threshold. Excess Copper intake may lead to acute stomach pain, diarrhea, fever as well as nausea in human being [30]. The higher value of Copper may be due to commercial activities, as well as industrial run-off in the study area. Excess Copper level causes deformed functioning of liver and kidney [31].

Lead does not fall under the category of essential metals required by the body for its physiological process. Many clinical studies have proved the adverse effect of lead on the nervous system of human, there has been alteration in the social behavior of mammals along with rapid behavioral malfunction [32]. The graphical expression has been depicted in Figure 2 and Figure 3 for *Mystus bleekeri* (Day, 1877) while Figure 4 and Figure 5 depicts the graphical expression for *Hypselobarbus kolus* (Sykes,1839). Lead concentrations in *Mystus bleekeri* (Day, 1877) tissue samples were between 0..031–0.755 mg/kg during the summer and 0.024–0.492 mg/kg during the winter seasons, respectively. In the instance of *Hypselobarbus kolus* (Sykes,1839), the mean lead concentration in tissue samples varied between 0.040–0.629 mg/kg during the summer and 0.034–0.501 mg/kg during the winter season. As per permissible recommendation by WHO, the value of Lead is 2 μg/g. The results obtained are in permissible range, but still it is n alarming signal for the aquatic life.The high level of lead in fishes could be due to the influx of lead from industrial as well as from agricultural discharge [33]. Lead could be found in river beds in the sediments and the bottom dwellers could be directly affected by lead contamination [34].

Cadmium levels in *Mystus bleekeri* (Day,1877) tissue samples were between 0.030–0.164 mg/kg during the summer and 0.020–0.118 mg/kg during the winter season. In the instance of *Hypselobarbus kolus* (Sykes,1839), the mean concentration of cadmium in tissue samples varied between 0.02-0.06 mg/kg during the summer and 0.01–0.21 mg/kg during the winter season. Lead has an acceptable value of 2 g/g, as recommended by the WHO. The concentration of lead is higher in gills than in other tissues, which may be related to its deposition and mobilization [35]. Due to respired CO2, the gills' surface often has a lower pH, which might breakdown lead into a soluble form and therefore readily permeate into the gills [36].

The concentration of Chromium in the various tissues ranged between 0.031-0.111 mg/kg in summer, while in winter the value ranged between 0.031-114 mg/kg for *Mystus bleekeri* (Day,1877). The values obtained for *Hypselobarbus kolus* (Sykes,1839) ranged between 0.046-165 mg/km in summer, while in winter the value ranged between 0.031-155mg/kg. The general pattern of heavy metal distribution in various tissues is as follows: Pb > Cu > Cr > Cd. The organs with the highest concentrations of heavy metals are the gill > kidney > gut > liver > muscle. This pattern persists regardless of the season. The primary organ for gas exchange is the gills, which also serve as a key entrance point for heavy metals [37]. Thus, the gills are a reflection of the amount of heavy metals in the organism's habitat and the degree of degradation in the ecosystem [38,39]. Kidney acts as an cleaning unit hence these heavy metals lodge in the kidney, followed by their retention in intestine.

In conclusion the present studies on both the fish from fresh water is an alarming situation even though the values of heavy metals are in permissible range, the concentration of these heavy metals in water bodies can cause serious biological disturbance as well as the bio accretion of these hefty metals in various tissues can be a biggest threat to human being. Since fishes are looked upon as a rich source of protein component in the dietary constitution. Unless a stick action plan is not penned down and implemented, consumption of fishes from fresh water bodies could be lethal to the life of human in a long run of time.

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