ScienSage
Journal of Advanced Scientific Research
Available online through http://www.sciensage.info
ISSN
0976-9595
Research Article

TOXIC METALS ACCUMULATION AND HISTOLOGICAL EXAMINATION IN HEART AND KIDNEY TISSUES OF *DEVARIO AEQUIPINNATUS* FROM THE RIVER TAMIRAPARANI

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ABSTRACT
In the present investigation, the bioaccumulation of toxic metals (Pb, Cd, Cr) concentration and histological evaluation in heart and kidney tissues of were estimated in *Devario aequipinnatus*, which were caught from the four sampling stations of river Tamiraparani, Tamilnadu, India. The results revealed that Pb, Cd and Cd concentrations in *Devario aequipinnatus* tissues were considerably lower than at river Tamiraparani sites. Pb concentration was greatest in the kidney (0.16 µg/g dry wt.), Cr content was virtually same in the kidney (0.14 µg/g dry wt.) and in the heart (0.13 µg/g dry wt.), and Cr content was mostly enriched in the kidney (0.14 µg/g dry wt.). However, the results indicated significant variations in the three examined metal levels between the species, as well as unique histological changes in *D. aequipinnatus* heart and kidney tissues. Despite this, toxic metal accumulation with histological analysis indicated the most substantial differences in the Tirunelveli area. Furthermore, it appears that the vast majority of them are extremely harmful rather than favorable for fish species. This study found that fish products can accumulate metals and carry them on to humans, mostly through food, resulting in chronic or acute illnesses.

Keywords: Bioaccumulation, *Devario aequipinnatus*, Histology analysis, Toxic metals.

1. INTRODUCTION
Toxic metals have an effect when excretory, metabolic, storage, and detoxifying processes are unable to counteract intake [1], resulting in physiological and histopathological alterations [2]. However, the heavy metals enter a fish's organs primarily by adsorbent and permeability; the rate of accumulation is a function of uptake and meeting diverse rates [3]. Non-essential metals, in addition to being poisonous and persistent, are bioaccumulated and internally controlled by several mechanisms such as influential respiration and retention [4]. Significant changes in non-essential toxic metals concentrations have indeed been documented between organs and fish species within the same aquatic environment [5, 6].
Moreover, toxic metal levels have been established to be elevated in regions experiencing increased settlement, transportation, and agricultural activity [7]. Heavy metals, on the other hand, are poisonous to all living species at large quantities due to their toxicity, persistence, and proclivity to accumulate in water, sediments, and tissues [8]. Eventhough, fish is also a major source of food for the general human population; nevertheless, fish from freshwater bodies receiving industrial effluents have been documented to be unsafe for human eating due to excessive tissue levels of several heavy metals [9, 10]. In the polluted water, particularly heavy metal pollution is known to produce physiological and biochemical changes in fish. These changes, if severe or prolonged, may result in structural changes in other organ systems [11].
Fish species are extremely sensitive to changes in its external environment, such as an increase in pollutants. The fish health can reflect and provide an indicator of the freshwater ecosystems [12]. Before, major changes in fish behavior or appearance can be recognized; the harmful consequences of pollution may be visible at the cellular or tissue level [13]. As a consequence, an attempt has been made in the contemporary study is to determine the bioaccumulation of toxic metals contents and the extent of the ensuing damages in the physical features of heart and kidney of *Devario aequipinnatus* from the four sampling sites of river Tamiraparani.

2. MATERIAL AND METHODS

2.1. Study area

River Tamiraparani originates from the famous Agastyarkoodam peak of Podhigai hills (1,725m above sea level) River meanders through Tirunelveli and Tuticorin districts of Tamilnadu state of Southern India. Stretches of study sites were chosen beginning with Papanasam (S1), Ambasamudram (S2), Cheranmahadevi (S3), and Tirunelveli (S4). The river Tamiraparani, which is between the latitude range of N 08°42'31.23" to 08°43'57.7" and the longitude range of E 77°22'05.81" to 77°43'03.1" (altitude range of 267m-45m), is drastically polluted by the actual impact of anthropogenic activities such as disposable of domestic waste, industrial waste drain, and other man-made activities. These kinds of activities pollute the east flowing river Tamiraparani's study sites.

2.2. Collection of Fish Samples

Based among the distribution from the four selected study sites (Papanasam, Ambasamudram, Cheran-mahadevi and across Tirunelveli city), the fishes of *Devario aequipinnatus*, were selected because of its profusion in the four selected study sites which was collected mainly by using mono filamentous gill nets with varying mesh sizes (8 and 12 mm) and drag net. The fish species were recognized flawlessly in laboratory by using the taxonomic keys of Jayaram [14] by referring relevant literatures.

2.3. Analysis of bioaccumulation in fish organs

The tissues of heart and kidney from the selected fishes of *Devario aequipinnatus* were dissected and washed thoroughly with Mili-Q water and placed to dry in an oven at 1000ºC until it reaches the constant weight. After the dry tissues homogenized powdered samples of 0.5 gm were treated with 30% sodium chloride (NaCl) solution and kept for digestion by adding conc. Perchloric acid ($HClO_4$) and conc. Nitric acid (NO_3) for 12hrs. The supernatant was made up to 20ml with double distilled water and stored in polyethylene bottles for analysis [15].

2.4. Histology analysis

The samples were washed in running Mili-Q water and dehydrated by using Isopropyl alcohol in rising concentrations of 60%, 70%, 80%, 90% in each jars and 100% in three jars for 1 hour. The samples were transferred to xylene, a transitional solvent containing two jars each for one hour to harden the tissues. After proper cooling, the embedded samples were sectioned (5μm thick) using Motorize Rotary Microtome of Leica RM 2155 model having an operating voltage of 10ºC-24ºC. The sample sections were stretched with an albumin along with the help of hot distilled water solution and air dried. Dried sections were prepared for light microscopy analysis using standard techniques for H & E (Haematoxylin and Eosin) and DPX (Disterine Polystryne Xylene) [16].

2.5. Statistical analysis

Data from four distinct locations of toxic metals accumulation in heart and kidney tissues of *D. aequipinnatus* were compared. To make the findings more interpretable, PCA was conducted utilizing a correlation matrix among the variables were calculated by using the PAST 2.14 version software [17].

3. RESULTS AND DISCUSSION

3.1. Accumulation of toxic metals in the selected tissues of *D. aequipinnatus*

The toxic metal concentrations of lead (Pb), hexavalent chromium (Cr-VI), and cadmium (Cd) were assessed in two organs of the *D. aequipinnatus* living in four different study sites of river Tamiraparani, including the tissues of heart and kidney. The river Tamiraparani is significant because of its economic and domestic implications at four sites in the Tirunelveli region, which typically indicated lower concentrations from previous reports [18]. In this work, we also noticed patterns in the accumulation of metals at varying concentrations in tissues, which were summarized (Table 1 and Fig 1). From that Pb concentrations in *D. aequipinnatus* were found to be (0.03-0.15 μg/g dry wt.) in the heart and (0.01-0.16 μg/g dry wt.) in the kidney. In the Pb, concentrations were highest within *D. aequipinnatus* heart and kidney organs from Tirunelveli (S4) and Cheranmahadevi (S3), and lowest at Papanasam (S1). Lead accumulates in the tissues of exposed fish species, causing hepatic and renal dysfunction as well as growth retardation [19]. Because it is harmful to the central and peripheral nerve systems, it can induce renal malfunction and brain damage [20].

The concentration of Cd (0.11 μg/g dry wt. in the heart and 0.13 μg/g dry wt. in the kidney) and Cr (0.13 μg/g dry wt. in the heart and 0.14 μg/g dry wt. in the kidney) was found to be high across Tirunelveli (S4), followed by Cheranmahadevi (S3). Cd and Cr were not found in the tissues of *D. aequipinnatus* in the Papanasam (S1) location. Eventhough, cadmium may rapidly bioaccumulated in the lower food chain and bio-concentrate in many organs of fish [21]. Nonetheless, it can accumulate in aquatic species via bio-concentration in the food chain process and eventually endanger human health when consume fish [22]. According to the World Health Organization (WHO), chromium is a potential carcinogen. Several studies have found that chromium compounds can raise the risk of lung cancer [23, 24].

Among them, the PCA analysis indicates that the heart has 97.60% variability, while the kidney has 96.83% variability. Eigen value variability in heart and kidney (2.93% and 2.91%, respectively) is shown in table 1. The principal components retrieved were shown as mean concentration of toxic metals (Pb, Cd and Cr-VI) amongst localities and within the *D. aequipinnatus* of organs at a significant threshold of $P<0.05$ as respectively (Table 2). In general, the correlation matrix of the variables of tissues of heart and kidney showed different capacities for accumulating toxic metals from four different locations of river Tamiraparani were shown in table 2. The variables from different sites of the river Tamiraparani were used to assess the variations of toxic metals in the heart and kidney of *D. aequipinnatus* as represented in Fig 1. Despite that, similar studies were compiled in almost the same riverine environment as metal toxicity proved statistically significant between two locations [5, 8].

Table 1: Toxic metals content in tissues (μg g-1, dry wt.) of *D. aequipinnatus,* and PCA analysis from the samples of river Tamiraparani

Tissues	Toxic metals	Study sites							PCA analysis		
		S1	S2	S3	S4	Min	Max	Mean	Eigenvalue	Variance %	Cumulative %
Heart	Pb	0.03	0.05	0.1	0.15	0.03	0.15	0.08±0.05	2.928	97.604	97.604
	Cd	ND	0.03	0.09	0.11	0.03	0.11	0.08±0.03	0.054	1.812	99.416
	Cr	ND	0.06	0.1	0.13	0.06	0.13	0.10±0.03	0.018	0.584	100.000
Kidney	Pb	0.01	0.08	0.12	0.16	0.01	0.16	0.09±0.06	2.905	96.833	96.833
	Cd	ND	0.05	0.13	0.13	0.05	0.13	0.10±0.04	0.083	2.753	99.585
	Cr	ND	0.09	0.11	0.14	0.09	0.14	0.11±0.02	0.012	0.415	100.000

Table 2: Principal components extracted and Correlation matrix of the variables of toxic metals from the tissues of *D. aequipinnatus*

Toxic metals	Principal components		Correlation matrix variables					
	Heart(n=4)	Kidney(n=4)	Heart(n=4)			Kidney(n=4)		
			Pb	Cd	Cr	Pb	Cd	Cr
Pb	0.984	0.995	1	0.97*	0.95	1	0.96*	0.98*
Cd	0.994	0.974	0.97*	1	0.98*	0.96*	1	0.92
Cr	0.986	0.983	0.95	0.98*	1	0.98*	0.92	1

**Correlation is significant at the 0.05 level (2-tailed)*

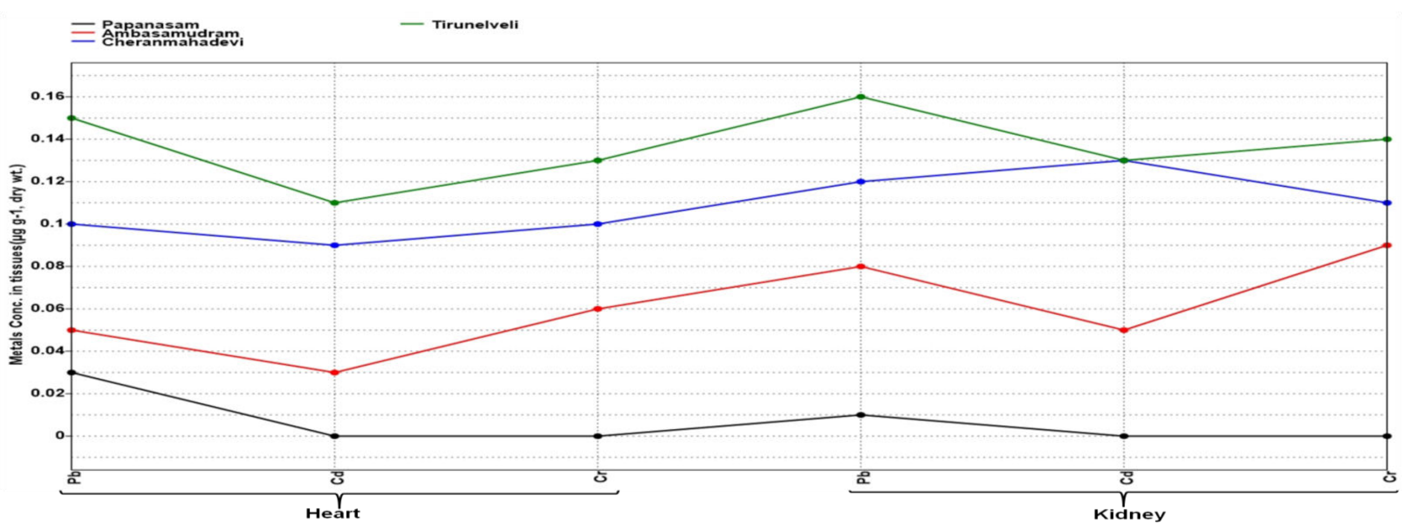
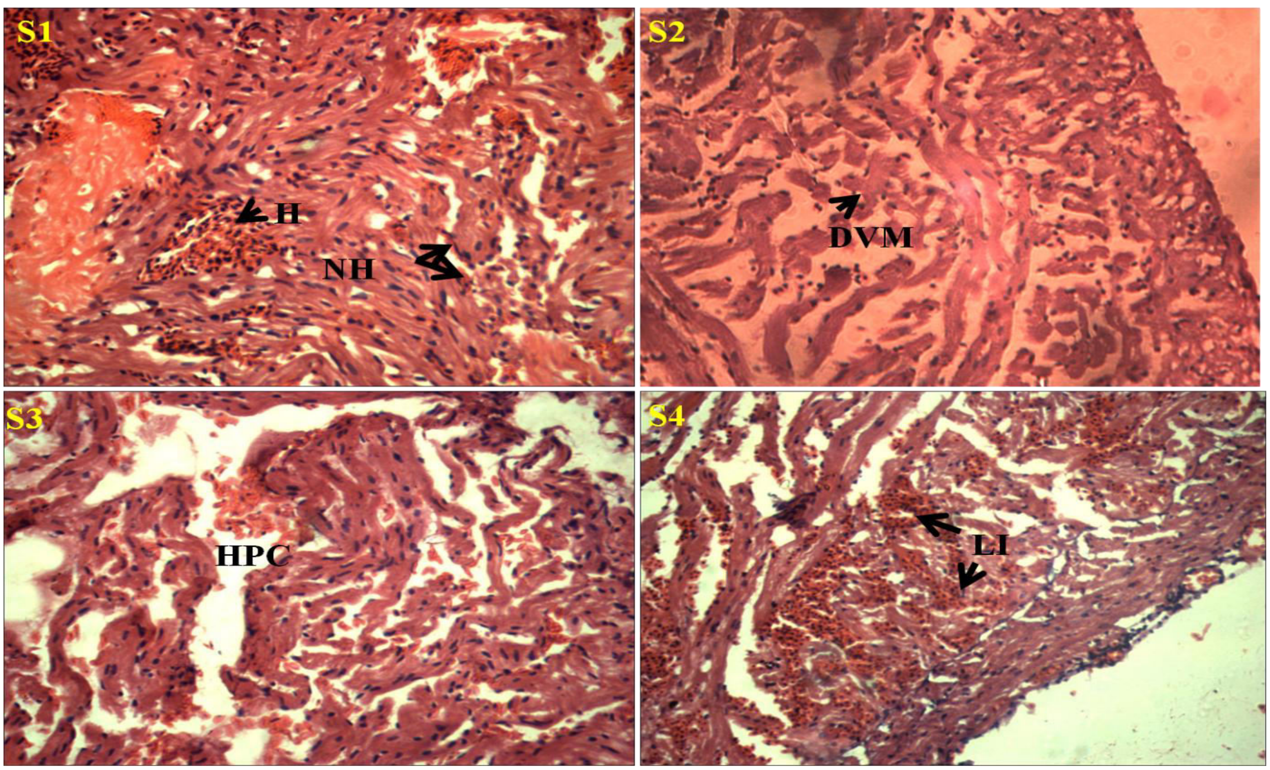


Fig. 1: Graphical plot represented as toxic metals content in tissues of *D. aequipinnatus* from the four study sites of river Tamiraparani

3.2. Histological examination in tissues of *D. aequipinnatus*

3.2.1. Histology of Heart

The current study's findings revealed distinct histological alterations in D. aequipinnatus heart and kidney tissues, as shown in figs. 2 and 3. The histology sample of heart in *D. aequipinnatus* at the Papanasam (S1) location revealed substantial differences in Nuclear Hypertrophy (NH) and severe Haemorrhages (H). However, the histological sample from the Ambasamudram (S2) location showed significant alterations in myocardial tissues, indicating the Degeneration in Ventricular Myocardium (DVM). The predominant alterations in blood transportation at the Cheranmahadevi (S3) site were hypotonicity with poor circulation (HPC). The main alterations in the white blood corpuscles of Leukocyte Infiltration (LI) the Tirunelveli (S4) site were seen in fig. 2. Although the fish heart is not generally considered a major target organ for toxicant exposure, numerous investigations have revealed histological lesions in cardiac tissue, particularly with relation to metal pollution [25]. Muscular necrosis and inflammation of the cardiac muscle, endocardium, or epicardium are two distinguishable histological abnormalities in the heart [26]. Inflammation of the heart muscle is distinguished by an abnormal accumulation of leucocytes and lymphocytes in the gaps between muscle fibers [6].



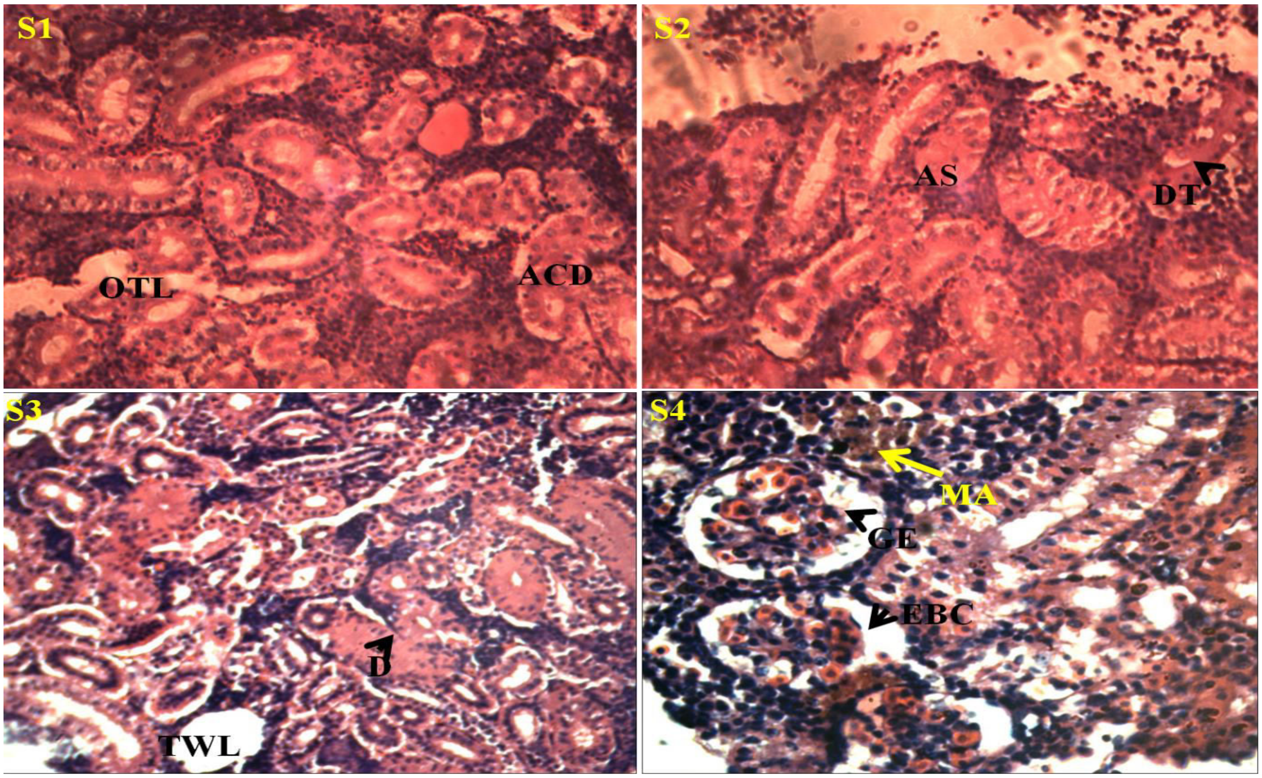
H- Haemorrhage; NH- Nuclear Hypertrophy; DVM- Degeneration in Ventricular Myocardium; HPC- Hypotonicity with Poor Circulation; LI- Leukocyte Infiltration (40X-H&E - Bar = 25 µm)

Fig. 2: Histological damages observed in the Heart tissues of *Devario aequipinnatus* from the four locations of river Tamiraparani.

3.2.2. Histology of Kidney

The histology sample of kidney in *D. aequipinnatus* at the Papanasam (S1) site showed substantial variations of Acute Cellular Degeneration (ACD) and Occlusion of Tubular Lumen (OTL). Acidophilic Secretion (AS) and Degenerating Tubules (DT) were found to be significantly different in the Ambasamudram (S2) location. However, the histological sample of kidney in *D. aequipinnatus* from Cheranmahadevi (S3) site exhibited substantial differences in Tubules with Widened Lumen (TWL) and Desquamation (D). Significant differences were observed at Tirunelveli (S4) site, which including Extension of Bowman's Capsule (EBC), Glomerular Expansion (GE), and Melanomacrophage Aggregates (MA) were represented in Fig 3. Similar results have also been reported by Mohan *et al.*

revealed that Tubular Lumen, Degenerating Tubules and Desquamation were seen in the gill, liver, heart and kidney of *Dawkinsia tambraparniei* fish species raised in heavy metal-polluted Tamiraparani River [6]. Camargo and Martinez, observed that lead acts directly on the glomerular expansion and the absence of the bowman's space in a neotropical fish caged in an urban stream, as well as tubule cells with hypertrophied nuclei, tubules beginning the regeneration process, occlusion of the tubular lumen, and cloudy swelling degeneration [27].



ACD- Acute Cellular Degeneration; OTL- Occlusion of Tubular Lumen; AS- Acidophilic Secretion; DT- Degenerating Tubules; TWL- Tubules with Widened Lumen; D- Desquamation; MA- Melanomacrophages Aggregates; GE-Glomerular Expansion; EBC- Extension of Bowman's Capsule (40X-H&E - Bar = 25 µm)

Fig. 3: Histological damages observed in the Kidney tissues of *Devario aequipinnatus* from the four locations of river Tamiraparani

4. CONCLUSION

As a result, the findings of the present toxic metals accumulation and histological examinations revealed a direct relationship between toxicity and histo-pathological abnormalities found in many tissues. Furthermore, it appears that the majority of them are harmful rather than adaptive. Fish are thought to be the most important biomonitors in aquatic systems for estimating metal contamination levels. It may acquire metals and transmit them on to humans mostly through food, resulting in chronic or acute illnesses. Based on current research in the Tamiraparani river basin, little is known about the histology and histopathological responses of *D. aequipinnatus* and other fish species, with limited reading on fish histology in field assessments and bio-monitoring.

5. ACKNOWLEDGMENTS

The first author (Mohamed Shahila) is thankful to the authorities of Sri Paramakalyani Centre of Excellence in Environmental Sciences, Manonmaniam Sundaranar University at Alwarkurichi, Tirunelvlei, Tamilnadu for providing facilities and carrying out this work.

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