Impact of Selected Heavy Metal (Cd, Pb, Cu and Zn) Concentration on Histological Changes of 2 Species of Estuarine Catfish (Small, Medium and Large Size) *Arius thalassinus* and *Plotosus anguillaris*

D.O.I - 10.51201/Jusst12678
http://doi.org/10.51201/Jusst12678

* Ahmed, A., Ismail, A., Omar, H., Zulkifli, S., Yusof, S. and Rahman, F.
Department of Biological Science, Faculty of Science, Universiti Putra Malaysia, 43400 UPM

Serdang, Selangor, Malaysia

Abstract

Aquatic systems are exposed to a vast number of pollutants that are mainly contained in effluents discharged from industries, sewage treatment plants, and drainage from urban and agricultural areas. This study was conducted to establish baseline information of selected heavy metals (Pb, Cd, Zn, and Cu) in the sediment of two rivers (Kuala Gula and Sepang) and histopathological changes using catfish species (Arius thalassinus and Plotosusanguillaris) as an animal bioindicator. Heavy metals concentration was determined by using atomic absorption spectrophotometer (AAS). The histological changes in gills and liver were detected microscopically and evaluated with criteria of scoring in each organ with emphasis on the histological alterations in these organs. The results showed the highest values of metals concentration were in large fish of Arius thalassinus and lowest concentration while in smallPlotosusanguillaris. Tissues damage is also more obvious in larger fish than smaller fish. Concentrations of Cu in the fish gills and livers were below the maximum permissible limit, however, Zn, Pb and Cd exceeded the permissible limit with means metal concentrations in the livers of two species catfish (µg/g-1d.w.). Livers were higher than the gills with metal concentration of Arius thalassinus Zn (233.8) >Pb (63.1)> Cu (12.5) µg/g-1 d.w. Cd (1.35). However, gills register lowest of accumulations these metals in Plotosusanguillaris with means Zn (94.5) > Pb (16.3) >Cu (7.3) > Cd (0.93) µg/g-1 d.w. respectively in small catfish. Several histopathological alterations, tissue damage was more prominent in fishes with the highest heavy metal content where tissue damage was more obvious in larger fish than smaller fish. Tissue damage in the liver is more prominent than in the gill. Damages observed includes increasing monomicrophages cells, vacuolar degeneration and necrosis of the liver, proliferation in the epithelium of gill filaments and fusion of secondary lamellae, severe degenerative and necrosis in the studied tissues of both fish as a result of the accumulated metals. This Study concluded that histopathological changes could be used as a good biomarker for pollution. Higher metal content in fish is coincides with severe tissue damage in larger fish. Hence histopathology can be used as a tool to indicate the impact of heavy metal in fish.

Keywords:*Heavy metals, Arius thalassinus and Plotosusanguillaris, Kuala Gula, Sepang*

sediment, Histopathology Lesions

1. INTRODUCTION

Heavy metals accumulation in the aquatic environment resulted from regular exchanges between the atmosphere, sediments and water [1, 2]. Natural and anthropogenic sources remain the largest contributors of heavy metals deposit to the aquatic system [3, 4, 5]. Sediments remain the main sink of heavy metals and other chemical pollutants in the aquatic environment. Hence, the heavy metals are the hazardous inorganic and organic pollutants and the level of their concentrations in the aquatic organisms' body are clear indication of environmental pollution in the coastal area [6, 7]. In Malaysia, several organisms have been proposed as bio-indicators, such as *Perna viridis*[8], *Telescopium telescopium*[9], oysters *Isognomon alatus*[10], *Nerita lineata*[3], *Dotilla myctiroides*[2], gastropods (*Chicoreus capucinus*) [11], Catfish (*Hexanematichthys sagor*) and Green Mussel (*Perna viridis*) [12], marine fish [13], Java medaka (*Oryzias javanicus*) [14] and Swamp eels (*Monopterus albus*)[15]. The response of these bioindicators are mentioned in the form of their changes (biochemical, physiological or behavioral) due to exposure and the bioavailability of pollutants that exist in the surrounding environment.

Fish is among the aquatic recipient of heavy metals and organic pollutants, thus impacting negatively on their biological functions as a result of a defensive mechanism through tissues and other vital organs. Fish is an important protein supply for Malaysians [15]. Several organs and tissues of fish like gills and liver [16, 17], kidney [18] and muscle [8] were reported to have been altered due to heavy metals contamination through histological investigation. Essential metals such as Cu, Zn have normal physiological regulatory functions but may bioaccumulate and reach toxic levels. Non-essential metals Pb &Cd are usually potent toxins and their bioaccumulation in tissues leads to intoxication; decreased fertility, tissue damage, and dysfunction of a variety of organs. Heavy metals are non-biodegradable and once discharged into water bodies, they can either be adsorbed on sediment particles or accumulated in aquatic organisms. Fish may absorb dissolved elements and heavy metals from surrounding water and food, which may accumulate in various tissues in significant amounts and often eliciting toxicological effects at critical targets.

Concentrations of metals even in waters in which those metals are below the limit of detection in routine water samples, therefore, fish might provide a better material for detecting metals contaminating the aquatic ecosystems. Intensive studies were conducted on the levels of heavy metals in different water bodies.

Bioaccumulation of metals may lead to a high mortality rate or cause many biochemical and histological alterations in the survived fish. Histopathological changes are utilized as measures of the effects of numerous anthropogenic contaminants on organisms, and can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. These histopathological biomarkers are closely related to other biomarkers [19] of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism [20]. Histological changes associated with heavy metals in fish have been studied by many authors {21, 22].

Heavy metals can be accumulated in different parts of the aquatic organisms and magnified concentration of heavy metals increases due to escalation in bioaccumulation and biomagnification through food chains [12]. On the other hand, no histopathological studies have been carried out on the catfish of Kuala Gula and Sepang River. Therefore, this study was conducted to determine the levels of some metals (Zn, Cu, Pb and Cd) in sediment and different body size tissues (liver and gills) of *Arius thalassinus* and *Plotosus anguillaris* during 2015-2017. In addition, the impact of such metals on the histological structures of tissues of both fish was investigated.

2. MATERIALS AND METHOD

2.1 Study Area

Arius thalassinus and *Plotosusanguillaris* were collected from Kuala Gula at (N 41^{o}816'90''- N 4^{o}563$^/$750 to E 1011^{o}85432^0"- E 100^{o}28$^/$47.89"), Perak, and Sepang Besar(2607885N, 101.703299^o" E to 26077302N, 101.7027612 E), Malaka rivers Peninsular Malaysia. Human activities involved at the nearby rivers include activities related to fishing, the land area surrounding the mangrove area is utilized as oil palm plantations. The major sources of contamination were from domestic effluents, Pig farms, sewage, and anthropogenic inputs of pesticides and fertilizers used in aquaculture and farming activities.

2.2 Sampling

Thirty samples of each species of estuarine catfish namely, *Arius thalassinus* and *Plotosus anguillaris* of three different sizes (Table 1). *Chrysochir aureus* was chosen as a reference for normal tissues of this fish for histopathological study and test fish were obtained from fishermen using fishing net in both location from Kuala Gula and Sepang Besar respectively from 2015 to 2017. The samples were put in clean labelled polyethylene plastic bags, kept in an icebox and transported to the laboratory. Fishes were grouped according to length and weight on the same day and dissected with a clean stainless steel dissecting, tissues (gill and liver) were removed and put in 10% formalin for heavy metals analysis and histopathological observations. Fifteen sediment samples were collected from each location (Kuala Gula and Sepang Besar) using a plastic scoop at the top of 3 to 5 cm of surface sediments from two locations. All samples were kept in clean-labeled polyethylene bags, stored in an ice chest, and taken to the laboratory. The samples were frozen at -20°C for further analysis.

Table 1.Total Length and Weight in Catfish Fish from Kuala Gula and Sepang Besar (Mean & SD)

Location	Species	N	Groups	Length (Cm)	Weight (g)
Kuala Gula	*Arius thalassinus*	10	Small	14.6±0.34	44.6±4.7
		10	Medium	18.84±0.14	57.3±2.4
		10	Large	21.04±0.7	175.9±11.1
Sepang Besar	*Plotosusanguillaris*	10	Small	13.5±0.50	20.0±2.5
		10	Medium	17.7±0.60	38.2±4.1
		10	Large	20.0±0.5	109.4±9.5

2.3 Determination of heavy metals

2.3.1 Sediment

Sediment samples were dried at 60^{0}C in the oven until reaching constant dry weight. Dried samples were crushed and sieved through a 0.63 mm stainless steel sieve. The aqua-regia digestion method was used according to the procedure of [23] and [24]. About 1g of ground dried sediment was digested in a 10 mL mixture (4:1) of nitric acid and perchloric acid.

Digestion was carried out using digestion block with an initial temperature of 40^0C for 1hr and then the
temperature was increased to 140 ^{0}C for the 3hr using digestion block. All digested samples were cooled at room temperature and then made up to 40 ml volume with double-distilled water and were subsequently filtered through filter paper. The samples were filtered through whatman No.1 filter papers and the filtrate was stored for analyses of heavy metals with (AAS). After filtration, the samples were determined for Zn, Cu, Cd, and Pb. Heavy metals in the samples were analyzed using Atomic Absorption Spectrophotometer (AAS) (Perkin-Elmer model 800). The data after analysis were presented as µg/g dry weight basis. For the accuracy of the results, the r2 of the calibration curve was in the range of 0.995-0.998.

2.3.2 Fish tissues

All the samples of catfish *Arius thalassinus* and *Plotosusanguillaris*were dissected with a clean stainless steel dissecting, tissues (gill and liver) were removed from the digestion of sample follows [18], about 0.5g was taken from each and 10 ml of HNO3 (AnalaR Grade, BDH69 %) was added, The samples were heated at 40°C for 1 hour and later increased to 140°C for 3 hours, After that samples were allowed to cool to room temperature and were diluted with distilled water (DW) to a fixed volume (40 ml). The samples were then filtered using filter papers (Whatman No.1) and the filtrates were stored until a metal determination was carried out.

Determination of heavy metals (Zn, Cu, Pb, and Cd) were conducted using an air acetylene flame atomic absorption spectrophotometer (AAS, Perkin-Elmer Model Analyst 800, Shelton, CT, USA). For the accuracy of analytical procedures certified Reference (µg /g dry weight ±SD) (PACS-2) in sediment and Standard Reference Material (SRM2976) for Zn, Cu, Cd, and Pb (Table 2).

Table 2. Observed and Certified Reference (µg /g dry weight±SD) (PACS-2) in Sediment and Standard Reference Material (SRM2976) for Zn, Cu, Cd and Pb

Element	PACS-2 SRM2976	Certified value (a) (µg /g)	Measured value (b) (µg /g)	Percentage of recovery (b/a)%
Zn	PACS-2	364±23.0	294.0±0.70	81.0 %
	SRM2976	137 ± 13.0	129.00±1.4	94.0 %
Cu	PACS-2	310±0.15	281.4±4.8	91.0 %
	SRM2976	4.02±0.33	3.75±0.35	93.0 %
Pb	PACS-2	183.00±8.0	179.5±0.71	98.0 %
	SRM2976	1.19 ± 0.18	1.27±0.01	106.0 %
Cd	PACS-2	2.11±0.15	2.7±0.01	127.0 %
	SRM2976	0.82 ± 0.16	0.69±0.02	84.0 %

Remark: SD: Standard deviation

2.4 Bioaccumulation Factor (BAF)

The bioaccumulation factor (BAF) is the ratio between the accumulated concentration of a given pollutant in any organ and its dissolved concentration in water or sediment and it was calculated using the following equation reported by [25]:

$$\text{Bioaccumulation factor (BAF)} = \frac{\text{pollutant concentration in fish organ } (\mu g/g)}{\text{Concentration of pollutant in the Sediment } (\mu g/g)}$$

2.5 Histopathological Studies

Specimen of gills and liver fixed in 10% neutral-buffered formalin, dehydrated, embedded in paraffin wax and sectioned at 4-5µm then stained with haematoxylin and eosin and examined microscopically [25]. Reference fishes *Chrysochir aureus*, all the tissues were recorded and scored by examination under light microscope (Leica, Germany) at x5, x10, x20 and x40 objectives. Criteria of lesion scoring for gills and livers tissues is shown table 3.

Table 3. Criteria of Lesion Scoring For Gills and Livers Tissues with ModificationFollows [30]

Score	Grade	Detected lesions in gills	Detected lesions in livers
0	Normal	No lesion detected	No lesion detected
1	Mild	Hyperplasia	Vacuolization
2	Mild moderate	Hypertrophy	Hyperplasia & Hypertrophy
3	More moderate	Congestion	Congestion
4	Mild severe	Degeneration of filaments	Degeneration of hepatocytes
5	Severe	Necrosis	Necrosis of hepatocytes

2.6 Statistical Analyses

The data were analyzed using the non-parametric procedure of Statistical Package of Social Science (IBM SPSS) package (IBM Corp. I BM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Pearson’s correlation coefficient was utilized to establish relationships between heavy metal concentration and size (length and weight). Statistical significance was set at $P<0.05$. Non-parametric analyses using ranked value were used to indicate the expression in different species, different sizes, and different organs. $P<0.05$ was considered statistically significant. The rank-based analysis offers alternative methods to interpret complex multi-parameter data that does not require the data to be normally distributed and in the presence of outliers. With this approach, the effect of outliers can be minimized, plus any problem of skewness is avoided because all rank is equally distanced from each other. The main aim of the present study is to observe the trend and relationship between different parameters, their overall interactions withvariables. Therefore, if the data are handled non-parametrically, those issues can be elucidated clearly.

3. RESULT AND DISCUSSION

3.1 Concentration of Heavy Metals in Sediment

Heavy metals concentration in the sediment samples fron Kuala Gula and Sepang rivers respectively were presented in the descending order as Zn > Pb > Cu > Cd in (Table 4). The total of essential heavy metal in the sediment of Kuala Gula was marginally higher by 6.2 µg/g than Sepang Besar. Similarly, the total of non-essential heavy metal from Kuala Gula was marginally higher than Sepang Besar by 9.5µg/g.

Table 4.C naeM**oncentration of Zn, Cu, Pb, and Cd sediment (µg/g d.w±SD) from Kuala Gula and Sepang Besar**

Heavy metals	Locations	
	Kuala Gula (G)	Sepang Besar (SB)
Zn	39.20±5.30 (3)	39.90±0.40 (4)
Cu	20.10±4.40 (4)	13.20±1.20 (3)
Total Zn and Cu	59.30±0.70 (4)	53.10±1.60 (3)
Pb	18.90±0.90 (4)	15.20±0.90 (3)
Cd	18.90±0.90 (4)	1.80±0.09 (4)
Total Pb and Cd	20.30±1.00	17.00±0.99
Total of all metals	79.60±10.7 (4)	70.10±2.60 (3)
(Zn+Cu): (Pb+Cd)	59.30:20.30	53.10:17.00

Remark: (Mean of 5 replicate) Value in bracket (rank data). High value gets the highest score.

3.2 Assessment of Bioaccumulation Factor (BAF) Using Non-Parametric Technique

Table 5 show non-parametric results of bioaccumulation factor (BAF) in different tissues of *A. thalassinus* and *P. Anguillaris*. The result demonstrated that liver recorded highest (25.96) cumulative among the organs studied and gill ranked second (22.96).

Table 5 .Non-Parametric Assessment o**f Bioaccumulation Factor (BAF) In Different Tissues of *A. thalassinus* and *P. Anguillaris***

Sediment	Kuala Gula (*A. thalassinus*)			Sepang Besar (*P. anguillaris*)			Cumulative BAF for
BAF	Small	Medium	Large	Small	Medium	Large	each organ
				Zn			
Gills	4.71 (4)	4.78 (5)	5.69 (6)	2.37 (1)	2.47 (2)	2.94 (3)	22.96
Liver	5.24 (4)	5.82 (3)	5.96 (6)	2.95 (1)	2.97 (2)	3.02 (3)	25.96
				Cu			
Gills	0.29 (1)	0.31 (2)	0.37 (3)	0.55 (4)	0.89 (5)	1.07 (6)	3.48
Liver	0.38 (1)	0.41 (2)	0.62 (3)	1.11 (4)	1.23 (5)	1.46 (6)	5.21
				Pb			
Gills	1.33 (3)	2.07 (5)	4.33 (6)	1.07 (1)	1.25 (2)	1.37 (4)	11.42
Liver	1.16 (3)	1.91 (5)	3.34 (6)	0.91 (1)	0.97 (2)	1.19 (4)	9.48
				Cd			
Gills	0.94 (2)	1.04 (3)	1.28 (6)	0.52 (1)	1.06 (4)	1.11 (5)	5.95
Liver	0.79 (3)	0.96 (5.5)	0.96 (5.5)	0.47 (1)	0.54 (2)	0.89 (4)	4.61

Remark: Value in bracket is ranked value. High value gets the highest score.

The accumulation efficiency for any particular pollutant in any fish organ is usually indicated by Bioaccumulation factors. Thus, the studied metals bioaccumulation factors indicate that the higher concentrations of the measured metals in both fish species were derived from aquatic plants, sediment, and water as previously reported [25, 29, 30]. The present study compared heavy metals concentration in *A. thalassinus* and *P. anguillaris* organs with sediments. The results revealed that the bioaccumulation pattern of the heavy metals depends on numerous factors such as different species, different fish size categories, different metals, and different organ/tissues. Based on the findings of this study, Zn is the most bio-accumulated, heavy metals in all organs tested. Pb ranked the second-highest bio-concentrated, followed by Cd and Cu that recorded the least bio-concentrated heavy metals. Similarly, gills has the least tissues/organs bio-concentrate Zn, Cu, Pb, and Cd. However, the liver had a reasonably high bio-centration potential which varies with fish of different size categories and different metals.

Sediment had a higher bioaccumulation factor (BAF) in this study. According to [31], BAF was categorized base on the samples which accumulated metals >1 mg kg-1, and < 1 as hyperaccumulators, accumulator and excluder. Bioaccumulation factor (BAF) of Zn is higher than other heavy metals according to the order Zn> Pb> Cu> Cd. The higher level of Zinc most likely due to the level of agrochemical fertilizers used as Zn is one of the main elements in the composition of such fertilizers. The percentage of Zn allowed in compound fertilizers is 0.18% according to the soil recommended level. Hence, the excessive application of this fertilizer might be responsible for the elevated levels of Zn during the paddy rice seasons [32].

Elevated levels of the pollutants in the sediment might be due to agricultural activities, effluents from boats of fishermen, tourist season and high people’s activities. Furthermore, pollution from aquaculture farms around the rivers may be contributing to an increase in the pollutants in the sediment [4, 33]. Also, farming practices such as the application of a large amount of pesticides and agrochemical fertilizers might cause an increase in pollutants found in the sediment, especially Cd and Pb metal, the presence of which is described as an impurity by [32].

The elevation of BAF in the liver and gill might be attributed to those metals which were being transformed in the liver that could be stored or passed back into the blood for subsequent excretion through kidneys and gills. Livers could also be indicators of the degree of pollution in the ecosystem [34]. Similarly, the presence of Cd and Pb and in the gills of both *A. thalassinus* and *P. anguillaris* is a clear indication that these tissues can accumulate a significant concentration of heavy metals, hence regarded as active metabolic organs. Our results might suggest that the bioaccumulation is due to the loss of homeostatic capacity of both fish exposed to chronic metal exposure. There are variations in metal concentrations in tissues as reported in several studies and this could be related to the capability of inducing metal-binding protein such as metallothionein [35].

Present work has demonstrated that BAF is a good indicator for heavy metals contaminated in water, sediment, and organs of fish. Thus, the concentration of heavy metals in *A. thalassinus* and *P. anguillaris*organs with sediments were compared using parametric and non-parametric procedures and manifestations of their effects.

3.3 Accumulation of Heavy Metals in Gill and Liver Tissues of *Arius thalassinus* and *Plotosus anguillaris*

Accumulation of heavy metals in the gill and liver of two catfish species are shown in table 6. *Arius thalassinus* showed high accumulation in their tissues than *Plotosus anguillaris*. Large fish in both species showed high accumulation than small fish. The liver in both species also exhibited high accumulation of heavy metals (Zn, Cu, Pb, and Cd) larger catfish. Zinc (Zn), Lead (Pb), and Cd exceeded the permissible limit of Malaysia Food regulation in both species catfish while Cu is still low.

Table 6: Mean Concentration of (Zn,Cu,Pb and Cd) in Different Organs and Different Size in Two Species Catfish, *Arius thalassinus* and *Plotosus anguillaris* (Mean ± SD)

Locations	Heavy metals	Species	Small		Medium		Large		Permissible limits Malaysia Food Regulation (1985) µg/g dry weight
			Gill	Liver	Gill	Liver	Gill	Liver	
Kuala Gula	Zn	*A. thalassinus*	184.5±36.4 (1)	205.4±28.5 (3)	187.39±32.6 (2)	228.07±24.7 (5)	223.08 ± 13.5 (4)	233.8±46.2 (6)	100
Sepang Besar		*P. anguillaris*	94.5± 4.5 (1)	117.7 ±2.8 (4)	98.6±7.02 (2)	118.5 ± 4.9 (5)	117.3±1.9 (3)	120.3±2.4 (6)	
Kuala Gula	Cu	*A. thalassinus*	5.9±1.1 (1)	7.6±1.0 (4)	6.3±1.1 (2)	8.2±1.0 (5)	7.5±1.0 (3)	12.5±1.5 (6)	30
Sepang Besar		*P. anguillaris*	7.3±1.5 (1)	14.7±1.2 (4)	11.7±1.2 (2)	16.3±1.8 (5)	14.1±1.8 (3)	19.3±2.7 (6)	
Kuala Gula	Pb	*A. thalassinus*	25.1±8.6 (2)	21.94±8.6 (1)	39.04±2.6 (4)	36.1±6.0 (3)	81.8±9.7 (6)	63.1±7.5 (5)	
Sepang Besar		*P. anguillaris*	16.3±1.6 (2)	13.8±1.00 (1)	19.05±1.2 (5)	14.67±1.5 (3)	20.78±1.6 (6)	18.07±2.2 (4)	2.0
Kuala Gula	Cd	*A. thalassinus*	1.31± 0.04 (2)	1.11± 0.05 (1)	1.45± 0.08 (5)	1.34± 0.13 (3)	1.79± 0.13 (6)	1.35± 0.05 (4)	
Sepang Besar		*P. anguillaris*	0.93±0.05 (2)	0.84±0.04 (1)	1.9±0.07 (5)	0.98±0.03 (3)	2.0±0.25 (6)	1.6±0.06 (4)	1.0

Remark: n=10 specimens of each size for each specie,Values are given in mean ±SD. Value in bracket is rankeddata; the highest value gets the highest score.

3.4 Histopathological Changes

3.4.1 Lesions of Gill

Gill sections of normal (low heavy metal concentration) fish *Chrysochir aureus* (Figure1), showed a normal structure of primary and secondary lamellae. While catfish *Arius thalassinus* recorded higher lesions with severe hyperplasia in the primary and secondary lamellae, lamella fusion, and severe degeneration between filaments (Figure 1). Moreover, multiplication in the epithelium of filaments as well as secondary lamellae was detected with the highest number of gill alterations observed in catfish *Arius thalassinus* from Kuala Gula River comprising almost 90% of the examined tissues compared to catfish *Plotosus anguillaris* collected from Sepang River. On the other hand, the lesions observed in *Plotosus anguillaris* ranged from moderate to severe. The lesions detected were hyperplasia, hypertrophy, degenerations, and necrosis of filaments. Also, congestions were observed between the cells.

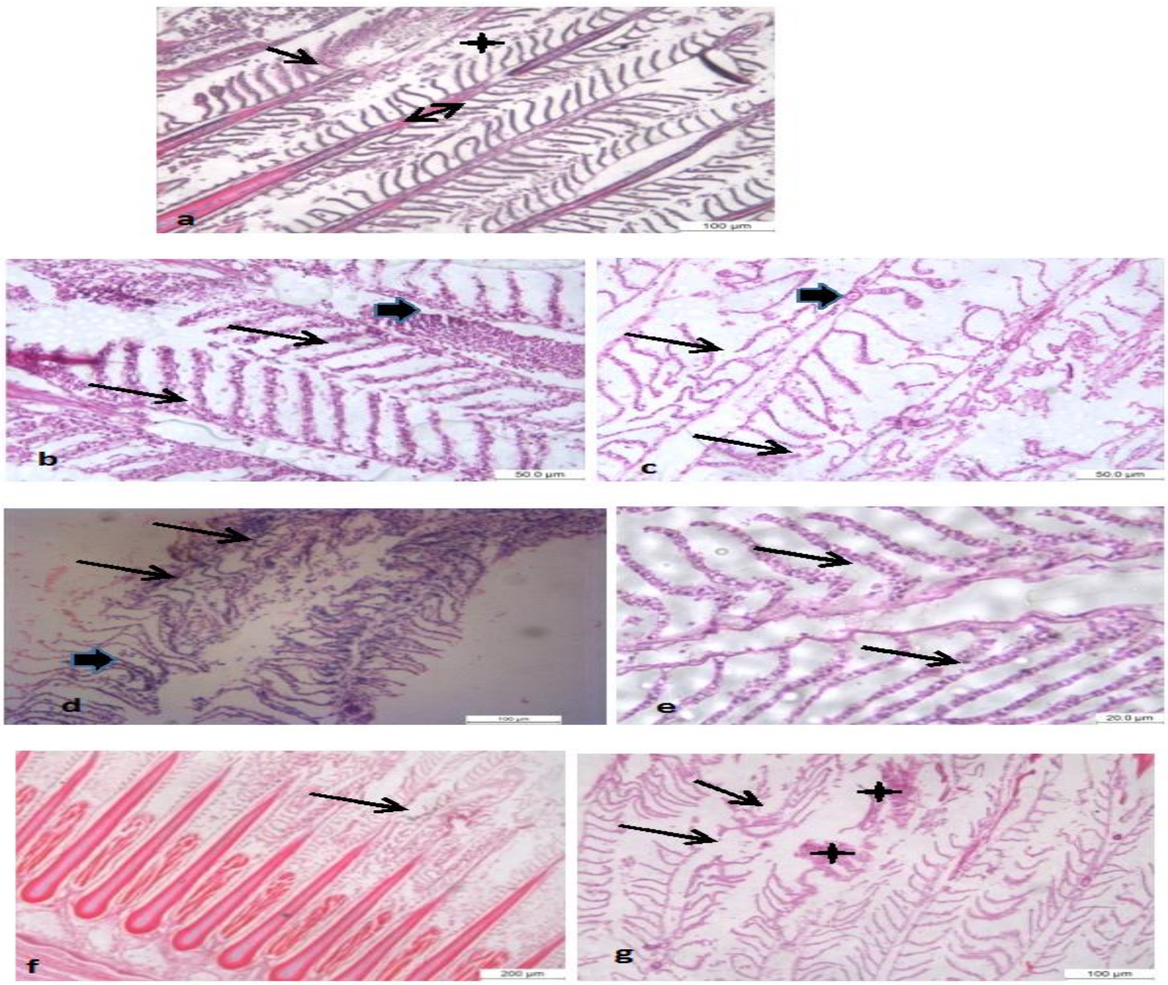

Figure 1. Reference Gill *Chrysochir aureus* (a) Filaments (double arrow), Primary Lamella (star), Secondary Lamella (arrow), (b) Small Catfish *Arius thalassinus*, Hyperplasia (arrows), Lamella Fusion (thick arrow),(c) Medium Catfish *Arius thalassinus*, Hyperplasia (arrows); Lamella Fusion(thick arrow), (d) Large Fish *Arius talassinus*, Proliferation in the Epithelium of Filaments and Secondary Lamellae (Arrows), Severe Degeneration Between Filaments (Thick Arrow). Small Catfish (e) *Plotosus anguillaris*, Hyperplasia (Arrows). Medium Catfish (f) *Plotosus anguillaris*, Slightly Degeneration in Filaments, (G) Large Catfish *Plotosus Anguillaris*, Filaments Degenerations (Arrow); Congestion (Stars), Hyperplasia (Arrow). Scale Bars: (X 100, 20 & 50) µm. HE.

The results of this study reported several pathological lesions in the gills which differed significantly according to the sizes of the fishes at $p<0.05$. Besides, the lesions recorded positive correlations with Zn, Cu, Pb, and Cd as well as with the body size of the two species of the catfish.

Generally, the gills of *Arius thalassnius* collected from Kuala Gula River indicated more severe damages when compared with *Plotosus anguillaris* from Sepang River. Large fish showed more severe damages to gills tissues because this fish accumulated high concentration of heavy metals. This finding is in agreement with[36] who reported a positive relationship between age and size with Zn and Cu accumulation in fish tissues. The results suggested that non-essential (Pb and Cd) heavy metals inflicted more damages than essential heavy metals (Zn and Cu), due to its high toxicity. The presence of pollutants such as (Zn, Cu, Pb, and Cd) in the aquatic environment exerts its effect at a cellular or molecular level which results in significant changes in biochemical responses and for monitoring of aquatic environment[21].

The histopathological changes of the gills might be due to hypoxic, respiratory failure difficulties with ionic and acid-base stability when the fish gets contaminated with heavy metals [28]. The reason attributed to the severe damage among *Arius thalassnius* catfishes could be due to the high concentrations of heavy metals in the gills.

Among the results from this study, the mean concentration of the Zn, Pb, and Cd in the gills of the small, medium, and large catfishes of *Arius thalassnius* exceeded the permissible limit of Malaysian Food Regulation (1985). The reasons is because *Arius thalassnius* being relatively bigger can accumulate high heavy metals than *Plotosus anguillaris*. Most studies [37] reported that more pollution results in more damages to fish tissues. Contrastingly, the concentration of the Zn in the small, medium, and large *Plotosus anguillaris* was low and didn't exceeded the permissible limit ofMalaysian Food Regulation (1985), except large fish, which showed slight increase in Zn, which explained the mild lesions reported among the gills of *Plotosus anguillaris* catfishes.

Based on the higher levels of metal bioaccumulation in the fish species, it could be unsafe for human consumption. Fish are at the higher level of the food chain and therefore, may biomagnify toxicants from the food they consume [28]. Increase in the lesions from mild in small fish to severe in medium and large fish, the alterations were hyperplasia and could be a defence response of the circulatory system against pollutants. Such lesions would expand the width of water–blood barricade and reduce the oxygen uptake. Lamella fusion usually resulted in a decrease in free gas exchange thus affecting the general health of fish [28]. Degenerations and necrosis of primary, secondary lamellae and filaments were earlier reported by[25]. Authman and Abbas [26] have reported that continuous accumulation of heavy metals in the tissues leads to the death of fish [21].

The result of this study indicated that Cu accumulation is still low in both species of the catfishes, and this might explain the mild lesions detected in the different sizes of fishes gills. Moreover, the Cu concentration was under the permissible limit of Malaysian Food Regulation (1985).

3.4.2 Lesions of Liver

The liver of reference fish *Chrysochir aureus* (low heavy metal content) collected from Sepang River showed normal structure with compactly arranged hepatocytes (Figure 2). On the otherhand, the liver of *Arius thalassinus* fishes showed varying degree of lesions that ranging from mild to severe and the lesions in *Plotosusanguillaris* fishes were less than those in *Arius thalassinus*.

Most of the lesions comprised of vacuolization, hyperplasia, hypertrophy, congestion, degeneration, and alteration of the hepatocytes, together with the infiltration of the cells. The current study showed an increase in melano macrophage cells in both *Arius thalassinus* and *Plotosusanguillaris* (Figure 2).

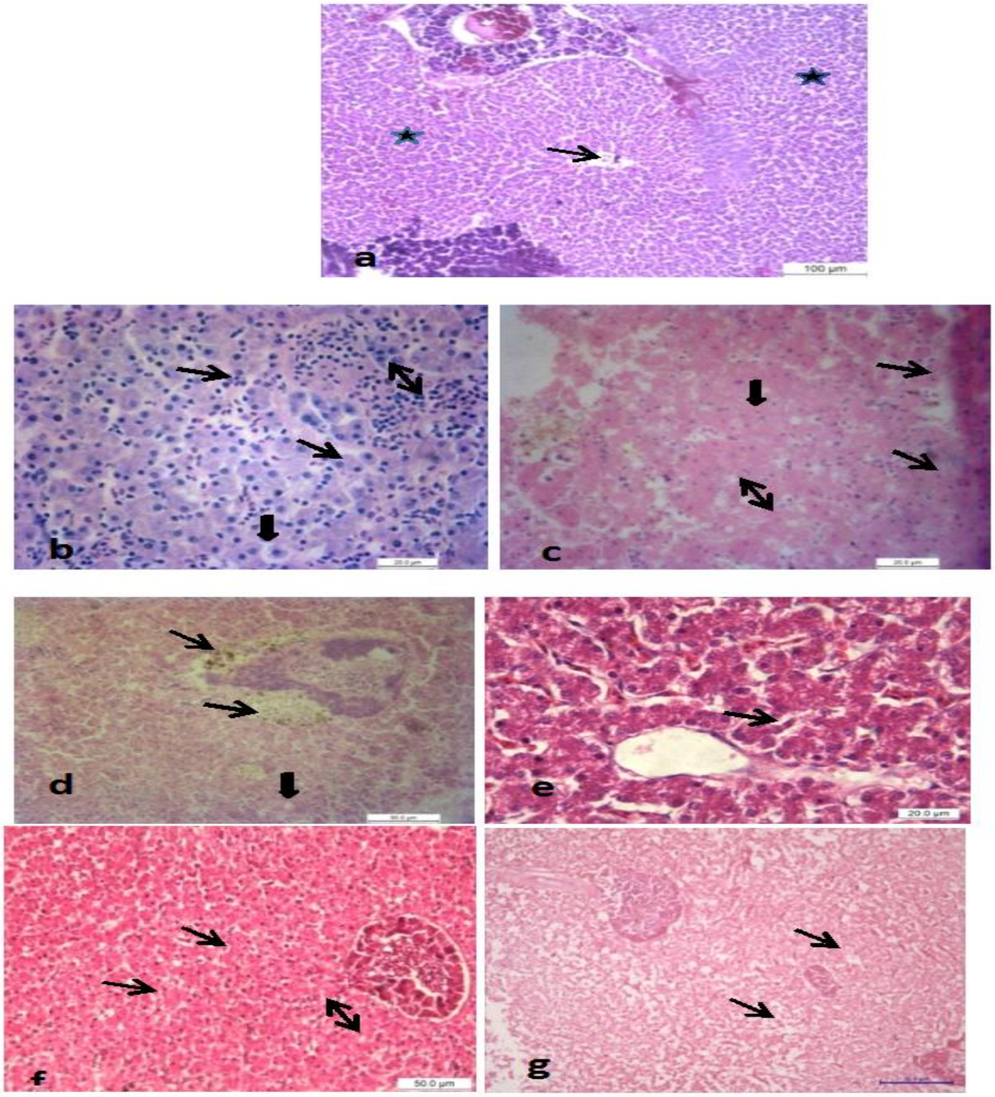

Figure 2. Liver of Normal Catfish (a) Hepatocytes in Liver Normal Fish (Stars) Central Vain (Arrow), (b) Small Catfish *Arius thalassinus*, Pycnotic Nuclei (Arrow), Inflammatory Cell Infiltration (Double Arrow) and Hepatocellular Necrosis (Thick Arrow). (c) Medium Catfish *Arius thalassinus*, Indicates Cytoplasmic Eosoniphila (Thick Arrow), Hepatocytes Cellular Degeneration (Arrows), Necrosis (Double Arrows), (d) Large Catfish, Melano Macrophage Centers (Arrows), Necrosis (Thick arrows). (e) Lesion of Small Catfish *Plotosus anguillaris*, Degeneration of Heptocyts (arrow), (f) Medium Catfish *Plotosus anguillaris*, Hepatocytes Degeneration (Arrows), Necrosis (Thick arrow), (g) Large Fish Necrosis (Arrows). Scale Bars: (100X, X50 & 20) µm. HE.

The results of this study showed that the histological changes in the liver of *Arius thalassinus* showed the most severe lesions compared to those in the liver of *Plotosus anguillaris*, which ranged from mild to moderate. This coincide with the concentration of Zinc (Zn) in both species which was highest and exceeding the permissible limit of Malaysian Food Regulation (1985).

Therefore, it could be concluded that the concentrations of Zn caused histological alterations in the livers of the exposed fishes which suggests that *Arius thalassnius* and *Plotosus anguillaris* to be used as a biomarker for Zn exposure . All the damages and lesions in the liver of the fishes due to Zn in this study were in agreement with many previous studies in the literature [26]. Although Zinc (Zn) is an essential metal and played major role in biological roles like cell structure, enzyme activities, protein, and carbohydrate metabolism but high concentration of Zn in the aquatic environment may lead to its toxic effect, which resulted in the alteration of the liver and gills morphology [27]. This explained the presence of lesions in the liver of both species in the present study, as high concentration of Zn was reported in the water of the river. However, Zn is generally regarded as one of the less hazardous metals but the presence of other metals in nature such as Cd makes Zn frequently hazardous[27].

Vacuolization of the hepatocytes, following lipid dystrophies, happened most regularly. While lipid build-up might be usual physiological loading, it may also be an instrument for protection against lipo soluble pollutants. The presence of eosinophilic hepatocellular degeneration in highly damaged livers is one of the most conspicuous alteration patterns observed. Bearing in mind their correlation to necrosis, eosinophilic forms may be signals of severe cirrhosis [28].Necrosis is greatly linked to oxidative stress in which lipid peroxidation is a perfect cause of membrane bilayer vulnerability. Pollutants (pesticides and heavy metals) are often associated with increased free radical concentrations within the cytosol. These oxidative forms may increase programmed cell death or disturbed cell homeostasis and cellular necrosis[28].High damages caused in these tissues are due to Pb and Cd effects.

Copper (Cu) is a necessary trace metal, micronutrient for cellular metabolism in living creatures, and considered a key component of metabolic enzymes. However, a high concentration of Cu is extremely toxic to intracellular mechanisms. A plentiful element forms a natural mineral that has many functions. Even though Cu is a trace element, but a toxic effect can occur in humans through fishes that accumulate Cu in their liver via ambient exposure.

It should be noted, that the low environmental levels of Cu display definite attraction to accrue in the fish's liver[38] that on prolonged exposure, can cause a toxic effect to human [39, 40].

The current study also suggested that Melano-macrophage centers increase in size due to their association with chronic inflammatory lesions or frequency in conditions of environmental stress. Due to this, they have been suggested as dependable biomarkers for water quality in terms of pollution[41].

3.5 Correlation Between Tissues Damage and Heavy Metals Concentration in Tissue

A positive relationship between damages of tissues and body size of two species of catfish was shown in Table 7.

Table 7.Correlation Between Histopathological Damages of Gill and Liver in Different Size of Catfishes *Arius thalassinus* and *Plotosus anguillaris*, (n=5 for each size) and Heavy Metals from Kuala Gula and Sepang Besar Rivers

Lesions of	length	weight	Zn	Cu	Pb	Cd
Arius thalassinus						
Gill	0.39**	0.29*	0.31**	0.33**	0.55**	0.48**
Liver	0.58**	0.48**	0.28*	0.42**	0.50**	0.52**
Plotosusanguillaris						
Gill	0.45**	0.30*	0.53**	0.40**	0.63**	0.51**
Liver	0.52**	0.27*	0.50**	0.51**	0.57**	0.64**

*Correlation is significant at the 0.05 level (2-tailed).
******Correlation is significant at the 0.01 level (2-tailed).

Among the results from this study, all the lesions of the organs in all the different sizes of the fishes, positively correlated with the heavy metals (Zn, Cu, Pb & Cd). Liver of both catfish species showed mild correlation with length, r=0.58 and 0.52 respectivelythan gill r= 0.39 and 0.45. For *Arius thalassinus*, a mild and positive correlation was detected between the gills and Pb (r=0.55) and liver (r=0.52), while the liver and Cd are moderately correlated (r=0.52). On the other hand, for *Plotosus anguillaris*, the positive and mild correlation was in the liver with Cd (r=0.64) but the gill showed weak correlation (r=0.51). The weak relationship detected was between gills of the fishes and Cu (r=0.40). All the correlations were statically significant at 0.01 & 0.05.

Results from the present studies have established a significant relationship between Pb and body size in *A. thalassinus* and *P. anguillaris* Previous findings have reported that Pb is mostly introduced into the water bodies through the disposal of used batteries, discharge of sewage, vehicle exhaust, and agricultural runoff from fields that use sewage sludge as fertilizers. Fish usually accumulated Pb indirectly from the sediments and contaminated water traced to high activities of fishing boats which always discharge to the river. A positive correlation was also established between fish length and Cd. Development of land-based aquaculture, increase of mangrove reclamation activity, and increase of multiple anthropogenic activities in Kuala Gula coastal area, could be one of the important reasons for the increase in metals level in the area as reported by several studies (Rahman et al., 2017). Thus, the possible reasons for the high relationship between heavy metals and body size of *A. thalassinus* compared to *P. anguillaris* recorded in this work.

The results showed that heavy metals level significantly correlated with damages organs in the different sized of two catfish species (P<0.01 and P<0.05) respectively. These findings suggested that the body size (length and weight) is very important factor in accumulation of heavy metals in different damaged tissues of different catfish species. These finding are in agreement with previous study [5,42].

4. CONCLUSION

Histopathological changes are a good biomarker to visually observed the degrees of damages and dangers to the fish and humans. In addition, this damage in tissues correlates with the concentration of heavy metal in tissues. Non-essential heavy metals exhibited higher correlation with organs than essential heavy metals. Hence, causing more damage to the tissues than essential heavy metals.

Arius thalassnius showed more damage than *Plotosus anguillaris* due to its size more then difference in species. The large-sized fish exhibited serious damage to their tissues; the scoring techniques differentiate and demonstrate the effect of heavy metals in various organs, species, fish, size, and location more clearly than conventional statistic . The liver showed more damage than the gill.

The toxicity of heavy metals in the fishes has been indicated histologically in the current study. Metals certainly bring an early reaction in the fishes as shown by changes both at structural and functional stages of diverse organs comprising enzymatic and genetic effects, thereby upsetting the natural immune system of exposed fishes and/or escalating vulnerability to numerous kinds of infections. Biomarkers can provide extra biologically and environmentally significant detail as a precious instrument for the formation of guidelines for successful environmental management.

Therefore, it can be specified that fish biomarkers are required for observing environmentally generated changes to evaluate the effect of heavy metals on fishes. It is also suggested that handling of all types of wastewaters; sewage and agricultural wastes should be done prior to release into the aquatic environment. Implementation of all articles of laws and regulations concerning the conservation of aquatic settings should be taken into account. Catfish Arius thalassinus accumulated high concentrations of heavy metals in the liver and gills, so we can regard it as a good bio-indicator for pollution assessment in the aquatic environment.

5. ACKNOWLEDGEMENT

The team of this work acknowledged the contribution of Special thanks to Universiti Putra Malaysia for providing sampling and infrastructural support. In addition, we wish to express sincere gratitude to Yemen government and Thamar University for their support.

REFERENCES

[1] S. Z. Zulkifli, F. Mohamat-Yusuff, T. Arai, A. Ismail and N. Miyazaki, "An assessment of selected trace elements in intertidal surface sediments collected from the Peninsular Malaysia''. *Environmental Monitoring and Assessment*, vol. 169, no 1and 4, **(2010)**, 457–472.

[2] S.Z. Zulkifli, F. Mohamat-Yusuff and A. Ismail, Bioaccumulation of selected heavy metals in soldier crabs, *Dotillamyctiroides* (Decapoda: Ocypodidae) from Bagan Lalang, Selangor, Malaysia. *Acta Biol Malays*, vol. 1, **(2012)**, pp. 94-100.

[3] A. Ismail, and R. Ramli, ''Trace metals in sediments and molluscs from an estuary receiving pig farms effluent''. *Environmental Health Perspectives*, vol. 18, no 5, **(1997)**, pp. 509–515.

[4] F. Rahman, A. Ismail and S. Yusof, ''Metals contamination in the foraging area of Milky Stork: evidence of anthropogenic inputs in the aquatic environment of Kuala Gula, Malaysia. *Toxicological and Environmental Chemistry*, vol. 95, no. 9, **(2013)**, pp. 1499-1505.

[5] F. Rahman, A. Ismail, H. Omar and M.Z. Hussin, ''Exposure of the endangered Milky stork population to cadmium and lead via food and water intake in Kuala Gula Bird Sanctuary, Perak, Malaysia''. *Toxicology Reports*, vol. 4, no. 3, **(2017)**, pp. 502–506.

[6] A. Safahieh, F.A. Monikh, A. Savari and A. Doraghi, Heavy metals concentration in Mullet Fish, (*Liza abu*) from petrochemical waste receiving creeks, Musa Estuary (Persian Gulf). *Journal of Environmental Protection*, vol. 2, no. 9, **(2011)**, pp. 1218.

[7] J. Baby, J.S. Raj, E.T. Biby, P. Sankarganesh, M.V. Jeevitha and S.S. Rajan, ''Toxic effect of heavy metals on aquatic environment''. *International Journal of Biological and Chemical Sciences*, vol. 4, no 4, **(2010)**, pp. 940.

[8] C.K. Yap, A. Ismail and S.G. Tan, ''Heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel *Pernaviridis* (Linnaeus) collected from some wild and aquacultural sites in the west coast of Peninsular Malaysia''. *Food Chemistry*, vol. 84, no 4, **(2004)**, pp. 569–575.

[9] A. Ismail and A. Safahieh, ''Copper and zinc in intertidal surface sediment and Telescopium Telescopium from Lukut River, Malaysia''. *Coastal Marine Science*, vol. 29, no. 2, **(2005)**, pp. 111-115.

[10] K. Saed, A. Ismail, H. Omar and M. Kusnan, ''Heavy metal depuration in flat tree oysters (*Isognomonalatus*) under field and laboratory conditions''. *Toxicological and Environmental Chemistry*, vol. 86, no. 3, **(2004)**, pp. 171–179.

[11] F.E. Berandah, Y.C. Kong and A. Ismail, ''Bioaccumulation and Distribution of Heavy Metals (Cd, Cu, Fe, Ni, Pb and Zn) in the Different Tissues of *Chicoreus capucinus* Lamarck (Mollusca: Muricidae) Collected from Sungai Janggut, Kuala Langat, Malaysia''. *Environ Asia*, vol. 3, no. 1, **(2010)**, pp. 65–71.

[12] F. Mohamat-Yusuff, S.Z. Zulkifli, K.W. Cheah and S.Y. Liong, ''Profile of heavy metals level in catfish (*Hexanematichthyssagor*) and green mussel (*Pernaviridis*) from Kong Kong''. *Acta BiologicaMalaysiana*, vol. 4, no. 2, **(2015)**, pp. 46–50.

[13] P. Hajeb, S. Jinap, A. Ismail, A.B. Fatimah, B. Jamilah and M.A Rahim, ''Assessment of mercury level in commonly consumed marine fishes in Malaysia''. *Food Control*, vol. 20, no. 1, (2009), pp. 79–84.

[14] A. Ismail and S. Yusof, ''Effect of mercury and cadmium on early life stages of Java medaka (*Oryziasjavanicus*): A potential tropical test fish''. *Marine Pollution Bulletin*, vol. 63, no. 5–12, **(2011)**, pp. 347–349.

[15] A.Y. Sow, A. Ismail and S.Z. Zulkifli, ''An Assessment of Heavy Metal Bioaccumulation in Asian Swamp Eel, Monopterus albus, During Plowing Stages of a Paddy Cycle''. *Bulletin of Environmental Contamination and Toxicology*, vol. 91, no. 1, **(2013)**, pp. 6–12.

[16] A. Naji, A. Ismail and E. Kamrani, ''Correlation of MT levels in livers and gills with heavy metals in wild Tilapia (*Oreochromis mossambicus*) from the Klang river''. *Bulletin of Environmental Contamination and Toxicology*, vol. 92, no 6, **(2014)**, pp. 674-679.

[17] F.A. Bashir, M. Shuhaimi-othman and A.G. Mazlan, ''Evaluation of trace metal levels in tissues of two commercial fish species in Kapar and Mersing coastal waters, Peninsular Malaysia''. *Journal of Environmental and Public Health*, **(2012)**, pp. 1–10.

[18] A.Y. Sow, A. Ismail and S.Z. Zulkifli, ''Heavy metals uptake by Asian swamp eel, Monopterus albus from paddy fields of Kelantan, Peninsular Malaysia: Preliminary study''. *Tropical Life Sciences Research*, vol. 23, no. 2, **(2012)**, pp. 27–38.

[19] F.C. Jerome, A. Hassan, G.O. Omoniyi-Esan, O.O. Odujoko and A.V. Chukwuka, ''Metal uptake, oxidative stress, and histopathological alterations in gills and hepatopancreas of (Callinectes amnicola) exposed to industrial effluent''. *Ecotoxicology and Environmental Safety*, vol. 39, **(2017)**, pp. 179-193.

[20] F.A. Mohamed, ''Histopathological studies on (*Tilapia zillii*) and (*Solea vulgaris*) from lake Qarun'', *Egypt. World Journal of Fish and Marine Sciences*, vol. 1, no. 1, **(2009)**, pp. 29–39.

[21] M.M.N. Authman, S.A. Ibrahim, M.A. El-Kasheif and H.S. Gaber, ''Heavy metals pollution and their effects on gills and liver of the nile catfish*Clariasgariepinus*inhabiting El-Rahawy drain, Egypt''. *Global Veterinaria*, vol. 10, no. 2, **(2013)**, pp. 103–115.

[22] S.E. Abalaka, ''Heavy metals bioaccumulation and histopathological changes in *Auchenoglanis occidentalis* fish from Tiga dam, Nigeria''. *Journal of Environmental Health Science and Engineering*, vol. 13, no. 1, **(2015)**, pp. 1–8.

[23] A. Ismail, M.A. Badri and M. Noor Ramlan, ''the background levels of heavy metal concentration in sediments of the west coast of Peninsular Malaysia''. *Science of the Total Environment*, vol. 134, **(1993)**, pp. 315–323.

[24] K. Saed, A. Ismail, H. Omar and M. Kusnan, ''Accumulation of heavy metals (Zn, Cu, Pb, Cd) in flat-tree oysters *Isognomonalatus* exposed to pig farm effluent''. *Toxicological Environmental Chemistry*, vol. 82. No. 1–2, **(2002)**, pp. 45–58.

[25] F. A. S. Mohamed, ''Bioaccumulation of Selected Metals and Histopathological Alterations in Tissues of *Oreochromis niloticus* and *Lates niloticus* from Lake Nasser, Egypt''. *Global Veterinaria*, vol. 2, no. 4, **(2008)**, pp. 205–218.

[26] M. M. N. Authman and H. H. H. Abbas, ''Accumulation and distribution of copper and zinc in both water and some vital tissues of two fish species (*Tilapia zillii* and *Mugil cephalus*) of Lake Qarun, Fayoum Province, Egypt''. *Pakistan Journal of Biological Sciences*, vol. 10, no. 13, **(2007)**, pp. 2106–2122.

[27] A.A. Abdel Warith, E.M. Younis and N.A.O Al Asgah, ‘’Effect of zinc toxicity on liver histology of Nile tilapia, *Oreochromis niloticus’’. Scientific Research and Essays*, vol. 6, no. 17, **(2011)**, pp. 3760–3769.

[28] A.M. Abdel-Moneim, M.A. Al-Kahtani and O.M. Elmenshawy, ‘’Histopathological biomarkers in gills and liver of *Oreochromis niloticus* from polluted wetland environments, Saudi Arabia’’. *Chemosphere*, vol. 88, no. 8, **(2012)**, pp. 1028–1035.

[29] M. Fatima and N. Usmani, ‘’Histopathology and bioaccumulation of heavy metals (Cr, Ni and Pb) in fish (*Channastriatus* and *Heteropneustesfossilis*) tissue: a study for toxicity and ecological impacts – ProQuest’’. *Pakistan J Biol Sci*, vol. 16, no. 5, **(2013)**, pp. 412–420.

[30] Y.S. Saleh and M.A.S Marie, ‘’Assessment of metal contamination in water, sediment, and tissues of *Arius thalassinus* fish from the Red Sea coast of Yemen and the potential human risk assessment’’. *Environ Sci Pollut Res*, vol. 22, **(2015)**, pp. 5481–5490.

[31] M. Rezvani and F. Zaefarian, ‘’Bioaccumulation and translocation factors of cadmium and lead in Aeluropuslittoralis’’. *Australian Journal of Agricultural Engineering*, vol. 2, no. 4, **(2011)**, 114-119.

[32] S. Yin, ‘’Bioaccumulation of heavy metals and Metallothionein effects on reproductive biology of Asian Swamp Eel (*Monopterus Albus*Zuiew, 1793) in paddy fields in Tumpat,Kelantan, Malaysia’’. (PhD Thesis), Universiti Putra Malaysia, **(2016)**.

[33] R.M. Lomoljo, ‘’Diversity of migratory shorebirds and their habitat characteristics in Kuala Gula Bird Sanctuary, Perak, Malaysia’’. (PhD Thesis), Universiti Putra Malaysia, **(2011)**.

[34] F. Yilmaz, ‘’the comparison of heavy metal concentrations (Cd, Cu, Mn, Pb, and Zn) in tissues of three economically important fish (*Anguilla anguilla*, *Mugil cephalus* and *Oreochromis niloticus*) inhabiting Köycegiz Lake-Mugla (Turkey)’’. *Turkish Journal of Science and Technology*, vol. 4, no. 1, (**2009)**, pp. 7–15.

[35] M. Canli and G. Atli, ‘’the relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species’’. *Environmental Pollution*, vol. 121, no. 1, (2003), pp. 129–136.

[36] C. Fernandes, A. Fontaínhas-Fernandes, S.M. Monteiro and M.A. Salgado, ‘’Histopathological Gill Changes in Wild Leaping Grey Mullet (*Liza saliens*) from the Esmoriz- Paramos Coastal Lagoon , Portugal’’. Environmental Toxicology: *An International Journa*l, vol. 22, no. 4, **(2007)**, pp. 443–448.

[37] Y.S. Saleh and M.A.S. Marie, ‘’Use of *Arius thalassinus* fish in a pollution biomonitoring study, applying combined oxidative stress, hematology, biochemical and histopathological biomarkers: A baseline field study. *Marine Pollution Bulletin*, vol. 1-2, no. 106, **(2016)**, pp. 308–322. https://doi.org/10.1016/j.marpolbul.2016.03.030

[38] M.M. Authman, M.S. Zaki, E.A. Khallaf and H.H. Abbas, ‘’Use of Fish as Bio-indicator of the Effects of Heavy Metals Pollution’’. *Journal of Aquaculture Research and Development*, vol. 06, no. 4, **(2015)**, pp. 1-13. https://doi.org/10.4172/2155-9546.1000328

[39] A.M. El-Naggar, S.A. Mahmoud and S.I. Tayel, ‘’Bioaccumulation of Some Heavy Metals and Histopathological Alterations in Liver of *Oreochromis niloticus* in Relation to Water Quality at Different Localities along the River Nile, Egypt’’. *World Journal of Fish and Marine Sciences*, vol. 1, no. 2, **(2009)**, pp. 105–114.

[40] M.M. Zeitoun and E.E. Mehana, ‘’Impact of Water Pollution with Heavy Metals on Fish Health: Overview and Updates’’. *Global Veterinaria*, vol. 12, no. 2, **(2014)**, pp. 219–231.

[41] C. Agius and R.J. Roberts,’’ Review Melano-macrophage centers and their role in fish pathology’’. *Journal of Fish Diseases*, vol. 26, no. 9, **(2003)**, pp. 499–509.

[42] O.E. Atobatele and G.O. Olutona, ‘’Distribution of three non-essential trace metals (Cadmium, Mercury and Lead) in the organs of fish from Aiba Reservoir, Iwo, Nigeria. Toxicology reports, 2, **(2015),** pp. 896–903.