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Review Article

Toxicity Effects of Fish Histopathology on Copper Accumulation

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ABSTRACT

Copper is a significant trace element necessary for the normal growth and metabolism of living organisms. However, this element may become very dangerous if used beyond its limit, turning into continuous metal compounds with the ability to accumulate in water and cause imbalance to the biological system. Aquaculture activities can also be affected due to the increase in environmental pollution. Copper is observed with the ability to cause some deleterious effects on fish by its toxicity, which can be evaluated from the molecular and structural level of the organism. This is because fish is one of the aquatic organisms that are able to accumulate heavy metals in their tissue. Generally, this accumulation is influenced by several factors namely, metal concentration, time of exposure, ways of metal uptake, environmental condition (water temperature, pH) and intrinsic factors (fish age, size). Different organs of fish show different affinity to copper accumulation. Therefore, this review was conducted with the purpose of investigating the harmful effects of copper on fish as a result of the accumulation of copper in fish organs and the histopathological alteration encountered in fish.

Keywords: Bioaccumulation, copper contamination, environmental pollution, fish, histopathological effects, toxicity

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INTRODUCTION

Heavy metal pollution in rivers has been observed as a serious concern as it is increasing steadily throughout the world each year. This is due to the release of pollutants from the various sources of industrial, agricultural and mining waste such as leaching of mineral and soil erosion as well as anthropogenic activities either directly or indirectly into the aquatic system. This has resulted in disruption to ecological balance of different systems (Joshi, 2011). Heavy metal pollution is unsafe for all living organisms, including aquatic organisms and humans. Aquatic systems are specifically more sensitive to heavy metal pollution, and the level of such metals in aquatic environments due to anthropogenic sources is rising (Ashraf et al., 2012).

Fish is an aquatic organism of high economic value that responds to environmental changes. Thus, it is extremely suitable to be utilised as an indicator for pollution studies. Moreover, fish is a good bioaccumulator as it has the optimum size for analysis and a long lifespan and is easily obtained in large quantity to be sampled for accumulated metals (Ashraf et al., 2012; Batvari et al., 2008). Bioaccumulation is a process where chemicals infiltrate an organism either through exposure to a contaminated medium or through consumption of food containing the chemicals (Perera et al., 2015). Bioaccumulation in fish usually occurs when it is exposed to chemical pollutants, including heavy metals, especially copper. Once the chemical pollutants enter the fish's body, it can damage and weaken the mechanism concerned, which leads to physiological, pathological and biochemical alterations. In addition, copper in a toxic form might serve as a stressor agent for fish that can inhibit several biological functions and cause some histopathological alterations (Sabullah et al., 2014). In conjunction with this statement, Monteiro et al. (2012) also mentioned that copper toxicity may disrupt biochemical functions and cellular morphology.

Hence, it is essential that the levels of a contaminant are determined and the water quality criteria are analysed to produce an accurate conclusion on pollutant exposure in fish. Biological measurement, also known as a biomarker, is one of the best steps for evaluating the presence of pollutant exposure and its impact on the cells of fish. This is because the abnormalities caused by copper in fish may result in cellular and histological changes. These histopathological alterations are then used to indicate the condition of the environment and represent time-integrated endogenous and exogenous impact on the organism stemming from alterations at the lower level of biological organisation (Paulo et al., 2012). Histopathological changes in animal tissue especially fish are powerful indicators for prior exposure of aquatic environmental stressors. Besides that, histopathology can give the net result of adverse biochemical and physiological changes in an organism as it allows the

identification of specific target organs, cells and organelles infected *in vivo*. According to Reddy (2012) and Hinton and Lauren (1990), histopathology is often the easiest method of assessing both short- and long-term toxic effects for field assessment. Abubakar et al. (2014) further added that the advantage of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows the changes on specific target organs to be tested.

In recent years, chemical biomonitoring and histopathology have often been combined with the evaluation of biomarkers representing early indicators of biological effects. Driven by the increase in environmental pollution, the utilisation of fish as a biomarker has attracted a growing interest in pollution studies; thus, the need to develop physiological, histopathological and biochemical biomarkers that are able to indicate stress on organisms exposed to toxicants in the environment has also become urgent. Therefore, this review is focussed on the adverse effects of copper on bioaccumulation and on histopathological studies with the aim of elucidating the most pronounced alteration induced by toxicants on aquatic organisms and their environment.

Copper

Copper is a very toxic metal that is often considered poisonous even at low concentration, but is highly demanded by industry. Apparently, the demand for copper continues to increase annually as it is used in water pipelines, intelligent houses and buildings, electrical motors, power lines, electrical appliances, healthcare, environment-related industries, computers and communication devices. According to AQM COPPER INC, the outlook for copper is greatly focussed in China, where copper consumption is increasing as a result of overall economic growth. This has further increased the use of copper in industry, while increasing copper contamination of the environment. Besides industrial use, copper also plays a vital role in the metabolic function of organisms such as vertebrate and invertebrate animals (Ajani & Akpoilih, 2010; Bambang et al., 1995), plants (Ahsan et al., 2007) and both prokaryotes and eukaryotes (Balamurugan & Schaffner, 2006). In fact, it is also an essential micronutrient required for body metabolism.

There are various functions demonstrated by copper in every organism where its importance in the metabolic processes and cellular biochemistry includes its vital role in cellular respiration. Copper also acts as a catalytic co-factor for at least 12 major proteins (Bambang et al., 1995) and 30 different enzymes (Ajani & Akpoilih, 2010) responsible for countless metabolic processes required to sustain life. Some examples of these enzymes are those indispensable in cellular activities for signal transduction and cell regulation such as superoxidase dismutase (for protection against free radicals), cytochrome *c* oxidase (mitochondrial electron transport chain), tyrosinase (for

pigmentation), peptidylglycine alpha-amidating mono-oxygenase (neuropeptide and peptide hormone processing), lysyl oxidase (collagen maturation), dopamine B-hydroxylase and monoamine oxidase (Ajani & Akpoilih, 2010; Balamurugan & Schaffner, 2006). Besides that, copper in the form of copper sulphate is also important for the aquatic environment as it can be utilised to control algae and kill slugs and snails in irrigation water systems and municipal water treatment systems and it is used in therapeutic chemicals for various ectoparasitic and bacterial infections (Sabullah et al., 2014; Shuhaimi-Othman et al., 2010; Wani et al., 2013).

Copper Toxicity and Distribution in Living Organisms

In terms of toxicity, both copper excess and copper deficiency could disrupt healthy metabolic function by creating mineral imbalance in metabolic processes. Thus, having the proper level and ratio of minerals is crucial for good health. However, Ajani and Akpoilih (2010) found that copper is a heavy metal with a density greater than 5 g/cm^3, causing it to be categorised as a toxic and poisonous heavy metal in relatively high concentrations. In support of this, Hellawell (1986) stated that copper is a metal that is highly toxic after mercury according to its position in the periodic table as shown in Figure 1. Generally, the toxicity of copper occurs when copper enters the cells and binds to proteins and nucleic acids within the cells, disrupting normal cellular function. Copper is able to shift between Cu^{2+} and Cu^{1+} oxidation states within the cells, and this action allows it to precipitate in the Fenton reaction to form free radicals like the highly destructive hydroxyl radical (Balamurugan & Schaffner, 2006). Copper is a non-biodegradable compound that cannot be degraded once it begins cellular functions. However, it can be easily assimilated and bio-accumulated in the organs and cellular functions (Ajani & Akpoilih, 2010).

Highly toxic					**Decreasing toxicity** ⟶											
Hg																
	Cu	Cd	Au	Ag	Pt											
		Zn														
			Sn	Al	Fe											
				Ni	Fe											
						Ba										
							Mn									
							Co	K	Ca	Sr						
										Mg	Na					

Figure 1. Periodic table on toxicity level of heavy metals (Source: Hellawell, 1986)

Toxicity in Aquatic Organisms

The presence of copper in water or an aquatic environment occurs through several pathways including mining activities, the discharge of industrial and agricultural waste and runoff from mineral deposits. Kamaruzzam et al. (2008) stated that the level of copper in an aquatic environment is caused by the loading and off-loading of fish, cleaning of boats and ships, ballasting, painting and repairing boats, large ships and cargo. These problems may contribute to the contamination of fresh water systems, causing an adverse impact on aquatic organisms as well as on human health (Farombi et al., 2007). It is known that copper cannot be destroyed or degraded through biological degradation and that it has the ability to accumulate in aquatic organisms, especially fish. Thus, it causes toxicants to be deleterious to aquatic environments and consequently, to the humans who depend on aquatic products as a food source.

Copper can easily accumulate in the tissue of aquatic animals, especially fish as they are the final trophic link of the hydro ecosystem (Balambigai & Aruna, 2011; Cepanko et al., 2010). Several studies have been conducted on the accumulation of copper in aquatic organisms, especially fish and crustaceans including shrimp (Balambigai & Aruna, 2011; Bambang et al., 1995; Cunha et al., 2007; Franciscato et al., 2009; Sabullah et al., 2015). According to El-Moselhy et al. (2014), metal bioaccumulation by fish is subsequently accumulated in specific organs and later distributed to different organs such as the liver, kidney, gills, heart, bone, brain and digestive tract. Although copper is an essential micronutrient for fish and other aquatic organisms, it may also become the most toxic to them when it becomes accumulated in their organs. The most toxic form of copper is the cupric ion (Cu^{2+}). Fish and crustaceans have been discovered to be 10 to 100 times more sensitive to the toxicity of copper than mammals (Solomon, 2009).

The effects of copper on aquatic organisms can be directly or indirectly lethal. Considering the fact that the gills of a fish are the organ that is directly in contact with water, they are the first organ to respond to environmental pollution and also the first to be affected by copper. Copper may affect the cardiovascular and nervous system of the fish once it becomes accumulated in the gills since it has the ability to regulate the transport of salt (NaCl) into and out of the fish. Besides disturbing the balance of salt in fish, copper can also reduce sperm and egg production in many fish species including fathead minnows (Solomon, 2009). On top of that, it can affect the glucose metabolism and cellular structure of fish as reported in the study by Sabullah et al. (2014, 2015).

Bioaccumulation of Copper in Fish Tissue

Heavy metals are known to be easily bioaccumulated in fish tissue as fish is a good bioaccumulator (Sabullah et al., 2015). Studies conducted on the bioaccumulation

of heavy metals in living organisms are related to the biomagnification process, which describes the pathway of toxicants from one trophic level to another. Ashraf et al. (2012) and El-Moselhy et al. (2014) stated that the accumulation of metals in fish depends on several factors such as the trophic level, location, feeding behaviour, size, age, duration of exposure to metals and concentration of metals. Various metals are accumulated in the body of fish in different amounts because different metals have different affinity to fish tissue, different uptake and different deposition and excretion rates as recorded in Table 1. Most fish are found at the top of the aquatic food chain and can potentially accumulate a high metal content even in mildly polluted conditions. According to Jezierska and Witeska (2006), the concentration of metal accumulated in the body of fish is usually related inversely to the size and age of the fish. The smallest and the youngest fish are commonly enriched by the accumulated substances compared to larger fish. This is because different species and size of fish contribute to different sensitivity levels toward contaminants. Therefore, metal concentration in fish could be used as an index to estimate the level of pollution especially in aquatic bodies (Akan et al., 2012).

Studies on the bioaccumulation of pollutants by fish are based on these two important reasons. The first reason is to determine the pollutant concentration in fish; this reflects the degree of the environmental pollution, the tolerance limit of the fish species and the effects of the pollutant on the fish. The second reason is to assess the spatial, temporal, speciation trends and transfer processes in the fish species along their food chain (Czedli et al., 2014). However, the first reason was seen to be more important as studies in this field have extended to environmental biomonitoring. Currently, studies predicting toxic effects based on the environment or tissue have been difficult to conduct, whereas many research studies examine the relationship between metal exposure, accumulation and toxicity under laboratory conditions (Annabi et al., 2013; Vijver et al., 2004).

According to the literature, metals that accumulate in fish including copper originate directly from food, water and contaminant residue, and their level of accumulation may reach the concentration of hundreds to thousands of times above the concentration measured in the food, water and sediments (El-Moselhy et al., 2014; Kumar & Prabhahar, 2012). One of the important functions of copper is its use as an algaecide (Ajani & Akpoilih, 2010; Carvalho & Fernandes, 2006), which is globally used to kill algae and to prevent fish from being killed or harmed. However, this application is not safe for all aquatic organisms, especially fish as it can easily enter the fish's body regardless of its amount. It is worth noting that several authors have demonstrated that animal tissue contaminated in the laboratory can accumulate heavy metals in a concentration and contamination period dependent

manner (Annabi et al., 2013; Francis et al., 1984). According to Jezierska and Witeska (2006), environmental factors strongly affect the accumulation of heavy metals in fish because labile metal compounds are the most dangerous to fish. Labile metal compounds have been categorised as a soluble form of heavy metals in water that contributes the most danger to fish. They include various ionic forms with different availability to fish. It has been confirmed by a lot of data from previous research such as studies on the accumulation of various heavy metals in fish organs by Ghosh and Adhikari (2006), Mohammadnabizadeh et al. (2014) and Velma et al. (2009) that most of the heavy metals in water are in labile form.

Copper contamination may be accumulated in fish as they are exposed to copper at high concentrations. Copper is commonly consumed by fish for metabolism functions; however, it becomes toxic if the fish are exposed to a higher concentration for a longer period. The gills are the first organ to accumulate heavy metals at a level higher than the concentration deemed toxic through absorption along the gill surface and gut tract wall (Annabi et al., 2013). The accumulated copper is then distributed and bioaccumulated in the main organs and bodily systems of the fish including the liver, spleen and kidney through the blood. Copper has been reported to become accumulated in the gills of *Cyprinus carpio* in the presence of kaolin particles (Tao et al., 2002). Tao et al. (2002) also stated that the adsorption affinity constant of kaolin for copper at various pH levels has affected the accumulation process in the gill microenvironment due to mucus competition from the copper and the slight increase in water pH. The pH level and mucus binding in the fish gill microenvironment are the most important factors behind the changes in copper speciation. Furthermore, Çoğun and Kargın (2004) have stated that the accumulation of copper in *Oreochromis niloticus* is affected by pH. It was identified that temperature may also affect the accumulation of copper and other heavy metals. This statement is in agreement with that published by Carvalho and Fernandes (2006), who mentioned that the changes in the blood parameter of *Prochilodus scrofa* showed a complex response at high temperature.

In a previous study conducted by Karayakar et al. (2010), it was observed that copper was accumulated more in the liver of *Anguilla* compared to the gills and muscles. A similar observation was also obtained by Rajkowska and Protasowicki (2013), who presented that copper was mostly accumulated in the fish liver compared to other organs (kidney, digestive tract, skin, spleen, gills and muscles). In addition, the result obtained from the study of Al-Yousuf et al. (2000) displayed the same observation in copper accumulation. Meanwhile, Das and Gupta (2013) stated in their study that the liver recorded the highest concentration of copper followed by the gills, kidney, flesh, bones and brain. Hence, based on the data in all these previous studies, it can be concluded that

the liver is the most responsive organ to copper accumulation. This is due to the fact that the metabolism of copper is chiefly controlled by the liver, and that this organ does not only accumulate copper from a medium, but also plays an important role in copper homeostasis (Das & Gupta, 2013).

Table 1
The concentration of heavy metal accumulated in different species of fish from different locations

Organs	Fish Species	Level of Accumulation (µg/g)				Locations	References
		Cu	Zn	Pb	Cd		
Muscle	*Plotosus canius*	5.33	2.14	1.28	1.07	Juru River, Penang	Idriss & Ahmad (2015)
	Valamugil cunnesius	6.40	1.45	1.83	1.07		
	Oreochromis niloticus	7.76	1.93	0.98	0.80		
	Anadara granosa	2.10	1.00	0.47	0.46		
	Sillago chodropus	8.26	1.18	2.13	0.64		
	Psammoperca weigiensis	2.98	2.55	0.58	0.80		
	Cynoglosus bilineatus	3.51	1.11	0.58	0.53		
	Megalops cyprinoides	8.83	1.39	1.6	3.2		
	Lobotes surinamensis	6.40	3.89	0.67	1.60		
Gut	*Scylla serrata*	57.06	496.31	2.27	0.13	Penor River, Pahang	Kamaruzzaman et al. (2012)
Liver	*Channa striatus*	5.80	4.70	0.031	-	Pahang, River	Jalal et al. (2014)
Liver	*Epinephelus sp.*	9.60±2.33	59.89±10.02	3.08±0.12	0.86±0.15	Red Sea, Egypt	El-Moselhy et al. (2014)
	Caranx sp.	2.93±0.18	27.30±1.51	0.48±0.11	8.37±0.32		
	Scarus gibbus	0.76±0.13	1.76±0.33	0.14±0.15	0.03±0.02		
	Synodus sp.	4.56±1.22	29.31±2.99	1.00±0.11	0.34±0.24		
	Carangoisdes bajad	3.09±0.61	27.49±0.56	1.64±0.15	0.78±0.04		
Gill	*Tilapia zilli*	2.98	7.15	1.00	0.315	River Benue North-Central Nigeria	Eneji, et al. (2011)
	Clarias gariepinus	2.07	7.05	0.678	0.325		
Intestine	*Tilapia zilli*	5.36	5.66	1.4	0.337		
	Clarias gariepinus	2.26	6.86	0.678	0.333		
Kidney	*Labeo rohita*	0.20–1.02	0.91–1.12	0.53–1.07	0.21–0.64	Upper Lake of Bhopal, India	Malik et al. (2010)
	Ctenopharyngodon idella	0.28 – 1.53	0.92–2.7	0.8–1.31	0.11–0.54		
Liver	*Carassius gibelio*	20.5	27.4	3.1	1.06	Anzali, Iran	Ebrahimpour et al. (2011)
	Esox lucius	22.8	46.5	5.4	1.96		

Histopathological Evaluation

Histopathology is the microscopic evaluation of altered morphology expressing a disease process in an organism, and it is used to display the disease patterns in a population of fish (Osman et al., 2010). According to Liebel et al. (2013), histopathological events can be considered efficient as they can quickly detect water pollution and express the health condition of exposed tissue. Therefore, they are suitable to be used as a biomarker as they give an early response or measurable biological event due to exposure to pollutants (Liebel et al., 2013; Miranda et al., 2008; Ribeiro et al., 2005). Other than that, the main objective of a histopathological study is to observe cellular changes that occur in the target organs of fish. This is because a histopathology study involves cellular biomarkers, which can provide a better indication of the health of an organism and has also been proven to be a cost-effective tool for determining the health of fish population and reflecting the health of the entire aquatic ecosystem (Devi & Mishra, 2013).

Fish are relatively sensitive to the changes that occur in their surroundings, including an increase in pollution. The health of fish may thus reflect and display the health status of a specific aquatic ecosystem. Early toxic effects of pollution are only evident at the cellular or tissue level before significant changes can be identified in the physical appearance and behaviour of the fish. On the other hand, histopathological analysis appears to be a very sensitive parameter crucial in determining the cellular changes occurring in the target organs such as the gills, liver, kidney, brain spleen, gonads and muscles (Al-Balawi et al., 2013; Gaber et al., 2014; Hadi & Ahwan, 2012; Sabullah et al., 2015). Various studies have been conducted using the histopathology of fish organs as a biomarker to indicate water quality. For instance, a study on histopathological effects of the acute toxicity level of copper sulphate on *Puntius conchonius* revealed that metallic salts are capable of producing severe damage in the gills and necrotic changes in the liver and kidney (Pant et al., 1980). A study done by Khabbazi et al. (2015) also stated that copper absorption may result in some alterations on the gills of rainbow trout seen as epithelial hyperthrophy, hyperplasia, lamella fusion, lamella aneurysm and edema. The gills are the first organ to come into contact with waterborne pollutants due to its stable contact with the external environment. Heavy metals accumulated in the gills will affect the respiration and osmoregulation processes, causing cellular damage to gill cells (Maharajan et al., 2016; Pandey et al., 2008). A study by Figueiredo-Fernandes et al. (2007) on *Oreochromis niloticus* treated with copper showed similar abnormalities in the gill tissues including epithelium lifting, interstitial edema, lamellae fusion and lamellae aneurysm. Aneurysm and edema were observed to be clearly related to short-term copper exposure, while lamellae fusion was related to chronic exposure of copper (Khabbazi et al., 2015).

Different organs may show different cellular changes due to the different level of copper accumulation they may be prone to. The liver is one of the most important organs due to its location, function and blood supply, and is associated with the detoxification and biotransformation process (Van der Oost, Beyer, & Vermeulen, 2003). It is also one of the organs most affected by toxicants in the water (Maharajan et al., 2016). At the initial toxicity level, the morphology of parenchyma cells was seen to demonstrate some histopathological alterations such as cytoplasmic vacuolation, dilation and congestion of sinusoid that depend on the concentration of toxicants and time of exposure (Sabullah et al., 2014; Younis et al., 2013). The normal ultrastructure visualisation of parenchyma cells with untreated toxicants showed the normal polygonal shape with the normal form of nuclear envelope, endoplasmic reticulum and spherical shape of mitochondria and cytoplasm. However, treated parenchyma cells usually display the karyorhexis, karyolysis and pyknosis of nucleus with clumping of nuclear chromatin, ruptured plasma membrane, apoptosis, blebbing, cell budding formation, necrosis and lipidosis (Abdel-Moneim & Abdel-Mohsen, 2010; El-Sayyad et al., 2010; Figueiredo-Fernandes et al., 2007; Sabullah et al., 2014). Liver alteration of *Anabas testudineus* has displayed the results of necrosis, vascular haemorrhage, dilated sinusoids and vacuolar degeneration after being treated with copper (Nandan & Kumar, 2014). A similar observation of copper-induced histopathological changes in the liver of Nile tilapia (*Oreochromis niloticus*) was also reported by Abdel-Tawwab (2016). In addition, a study carried out by Udotong and John (2015) also showed similar alterations in the liver by displaying diffuse hepatocyte necrosis. General necrosis, also known as degenerative alterations, was given the highest importance factor because it was considered a direct effect of toxicants. It is generally irreversible, and its persistence or progression may lead to a partial or total loss of organ function (Agamy, 2012). All these alterations may attribute to the direct toxic effect of pollutants on hepatocytes, since the liver is the detoxification site for all types of toxin and chemical (Fatma, 2009).

Just like the gills and the liver, muscle tissue also comes in close contact with toxicants dissolved in water. Hence, the reactions in histopathology of the muscle are spontaneous. In a study done by Maharajan et al. (2016), the muscle showed progressive damage in its structure such as thickening and separation of muscle bundles with severe intracellular edema. Similar abnormalities were also displayed in the study by Das and Mukherjee (2000), where the separation of muscle bundles was considered an interesting observation; it was believed that copper may induce hyperactivity and excitability in animals, leading to the release of lactic acid and subsequently, muscular fatigue. Another observation by Al-Tamimi et al. (2015)

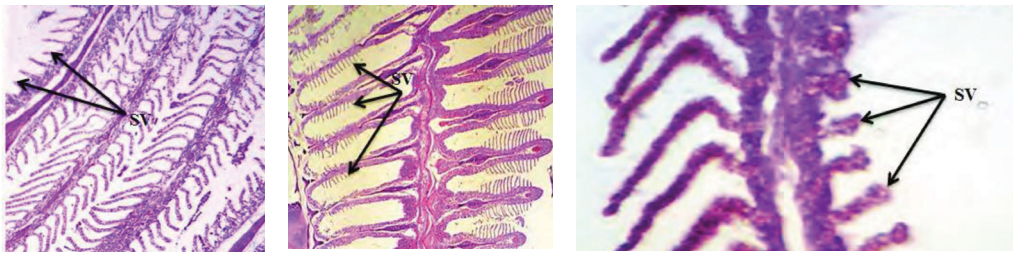
of the muscle tissue of *Cyprinus carpio* recorded hyalinisation, necrosis with mild inflammatory cells infiltration and focal degeneration as abnormalities. However, different toxicants may also give similar alterations, as mentioned by Fatma (2009), who found that the degeneration of muscle bundles with aggregation of inflammatory cells between them and the focal area of necrosis as well as vacuolar degeneration in muscle bundles and atrophy of muscle bundles occurred as fish were exposed to toxicants. The summary of the histopathological alterations of fish affected by copper is shown in Table 2.

Table 2
The histopathological alteration of different fish species affected by copper

Organs	Fish Species	[Cu] (mg/L)	Exposure Period	Histopathological Alteration	References
Liver	*Cyprinus carpio*	1.2	3 weeks	Degenerative and necrosis of hepatocyte cells Mild inflammatory cell infiltration Cholesterol inside the cell	Al-Tamimi et al. (2015) (Figure 5)
			6 weeks	Formation of apoptotic cells	
	Oreochromis niloticus	2.5	21 days	Vacuolation Necrosis Pyknotic nucleus	Figueiredo-Fernandes et al. (2007) (Figure 4)
	Lates calcarifer	6.83	7 days	Cytoplasmic degeneration	Maharajan et al. (2016)
			28 days	Hydropic swelling of hepatocytes Damaged epithelium	
		13.66	7 days	Cytoplasmic vacuolation Hydropic swelling of hepatocytes Blood congestion Nuclear pyknosis	
			28 days	Accumulation of dark granules Blood sinus Nuclear degeneration Cellular necrosis	
Gill	*Ctenopharyngodan idella*	2.5	24 hours	Lamellae epithelium lifting Exude of RBC	Atabati et al. (2015)
			48 hours	Lamellae axis vasodilation Epithelium interstitial edema Proliferation of filamentary epithelium	
			96 hours	Fusion of adjacent lamellae	
		5.0	24 hours	Necrosis	
			48 hours	Necrosis	
			96 hours	Necrosis Epithelium lifting	
	Cyprinus carpio	1.2	3 weeks	Atrophy Congestion of gills short of villi	Al-Tamimi et al. (2015) (Figure 2)
			6 weeks	Shortness of villi	

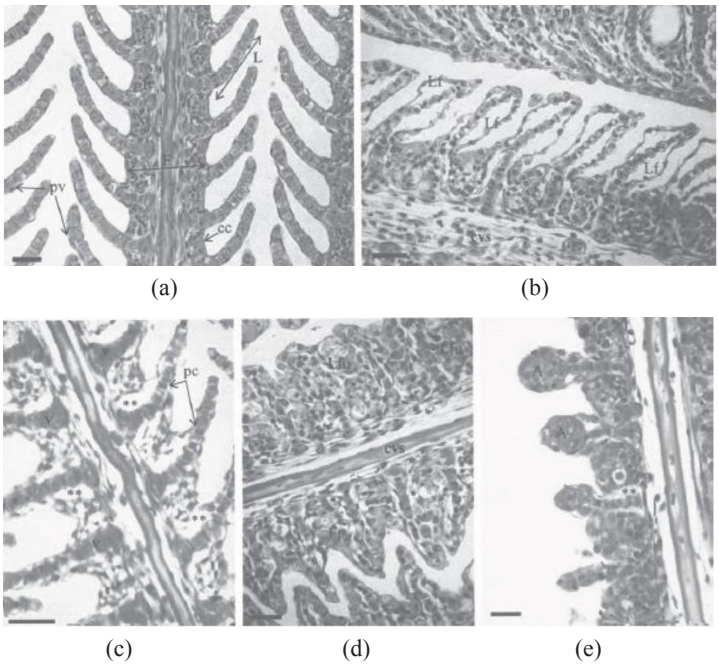
Table 2 (*continue*)

Organs	Fish Species	[Cu] (mg/L)	Exposure Period	Histopathological Alteration	References
	Catla	10.0	96 hours	Hyperplasia Oedema	Bose et al. (2013)
	Oreochromis niloticus	2.5	21 days	Epithelium lifting Lamellae axis vasodilation Epithelium interstitial edema Profileration of filament epithelium Fusion of lamellae Vascular congestion Lamellae aneurysm	Figueiredo-Fernandes et al. (2007) (Figure 3)
	Oncorhynchus mykiss	0.15	7 days	Epithelial hyperthrophy Hyperplasia Lamellae fusion Swollen of mucocytes Lamellae aneurysm Oedema	Khabbazi et al. (2015)
	Lates calcarifer	6.83	7 days	Gap formation in myofibril Inter myofibril space	Maharajan et al. (2016) (Figure 7)
			28 days	Inter myofibril space Muscle oedeme Disintegrated myofibrils	
		13.66	7 days	Disintegrated myofibrils Oedema between muscle fibre	
Muscle			28 days	Muscle degeneration Inter myofibril space Disintegrated myofibril	
	Cyprinus carpio	1.2	3 weeks	Mild hyalinisation of skeletal muscle fibres Loss of interstitial fibres in between the muscle fibre Focal degeneration Necrosis	Al-Tamimi et al. (2015) (Figure 6)
			6 weeks	Normal structure of muscle fibre tissue	



(a) (b) (c)

Figure 2. Histological changes of gills in *Cyprinus carpio* during three-week period (a) Normal; (b) At a concentration of 0.5 mg Cu/L; (c) At a concentration of 0.9 mg Cu/L. (a) It is clear that there were no abnormal changes in the control sample; (b) At a concentration of 0.5 mg/L during three-week period showing presence of congestion (c) with short villi (SV); (c) At a concentration of 0.9 mg/L during three-week period showing short villi (SV). H&E; 400x. (Source: Al-Tamimi et al., 2015)



(a) (b)

(c) (d) (e)

Figure 3. Representative light micrographs of gills in control and copper-treated (B-E, 2.5 mg-l CuSO4, 21 days) tilapia, *Oreochromis niloticus*. (a) Control fish, showing normal appearance of gill filaments (f) and lamellae (L). (b) Gills from exposed fish showing an intense lamellar epithelium lifting (Lf). Note the epithelium proliferation in the above filament (FP). (c) Section of gills with lamellar axis vasodilation (v) and evident epithelium interstitial edema (**) in the filament near the lamellar axis. (d) Proliferation of filamentar epithelium (Fp) with fusion of adjacent lamellae (Lfu). (e) Gill epithelium of treated fish showing vascular congestion or lamellar aneurisms (a). cc=chloride cell, cvs=central venous sinus, fe=filament epithelium, pc=pillar cell, pv=pavement cell. HE, bars=20µm (Source: Figueiredo-Fernandes et al., 2007)

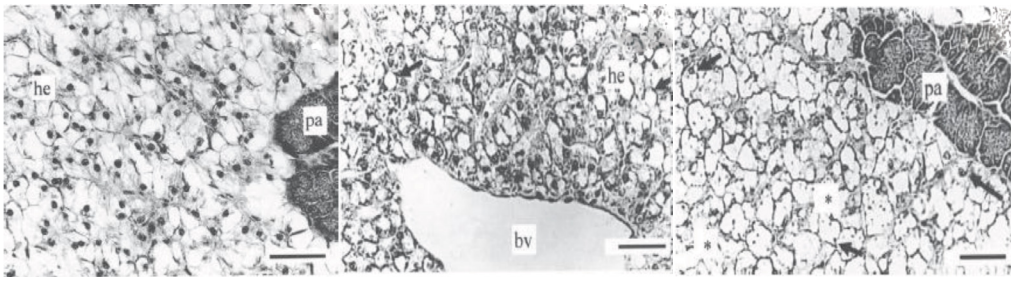


Figure 4. Photomicrographs of Nile tilapia *Oreochromis niloticus* liver tissue. (a) Control group showing hepatocytes (he) and pancreatic area (pa) that corresponds to the acini of exocrine pancreas; (b) Liver of fish exposed to copper (1 mgL-1), showing alterations in hepatocytes and vacuolation (black arrows); bv, blood vessel; (c) Liver of fish exposed to copper (2.5 mgL-1), showing vacuolation (black arrows) and necroses area (*) and picnotic nucleus (black arrow). HE, bars=50µm (Source: Figueiredo-Fernandes et al., 2007)

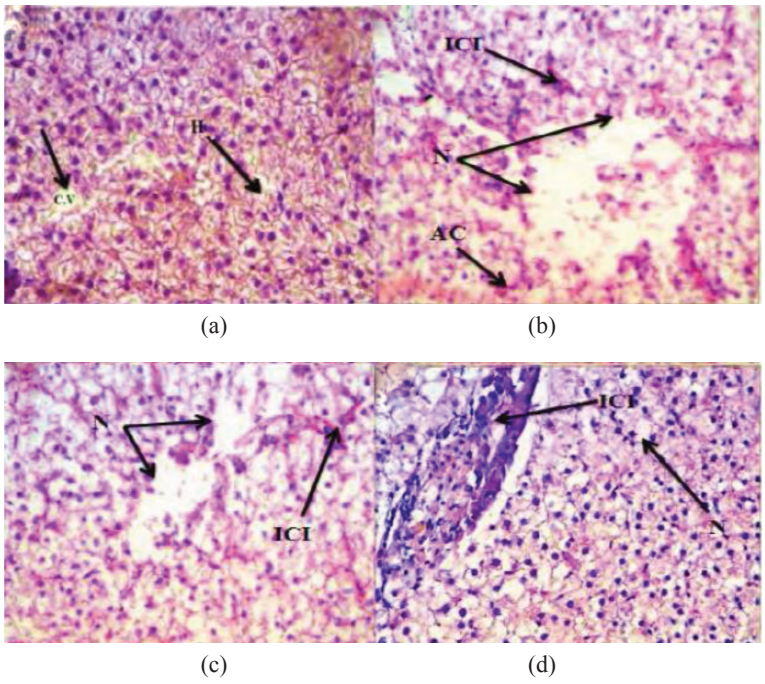
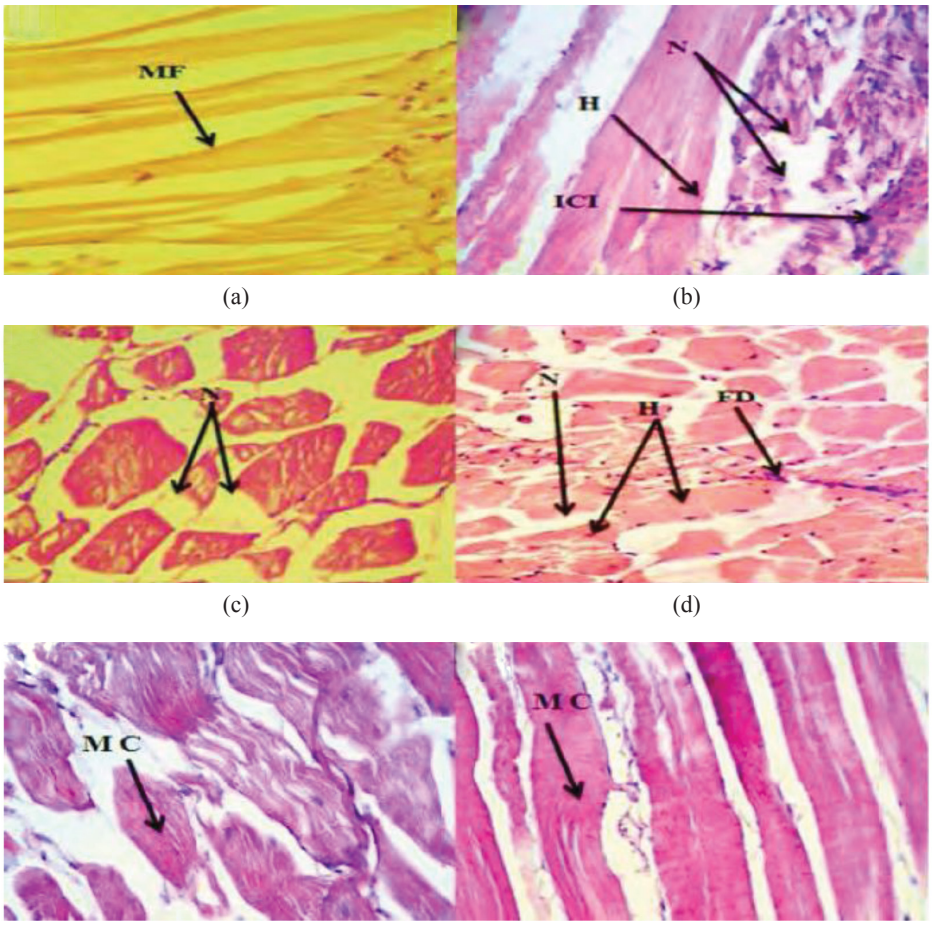


Figure 5. Histological changes of liver in *Cyprinus carpio* during three-week period (a) normal (b) At a concentration of 0.5 mg Cu/L; (c) At a concentration of 0.9 mg Cu/L; (d) At a concentration of 1.2 mg Cu/L. (a) Control liver showing normal histology of hepatocytes (H) with central vein (CV); (b) At a concentration of 0.5 mg/L during three-week period showing presence of necrosis (N) with mild inflammatory cell infiltration (ICI) and accumulation of cholesterol (AC) inside the cell; (c&d) At a concentration of 0.9 mg/L and 1.2 mg/L during three-week period showing necrosis (N) with mild inflammatory cell infiltration (ICI) H&E; 200x (Source: Al-Tamimi et al., 2015)



(a) (b)

(c) (d)

(e) (f)

Figure 6. Histological changes of muscles during three-week period (a) normal (b) At a concentration of 0.5 mg Cu/L; (c) At a concentration of 0.9 mg Cu/L; (D) At a concentration of 1.2 mg Cu/L; (e&f) At a concentration of 0.5, 0.9 and 1.2 mg/L during six-week period. (a) Section of muscles showing normal muscle fibre (MF) with structure; (b) At a concentration of 0.5 mg/L during three-week period showing presence of hyalinisation (H) and necrosis (N) with mild inflammatory cell infiltration (ICI); (c) At a concentration of 0.9 mg/L during three-week period showing necrosis (N); (d) At a concentration of 1.2 mg/L during three-week period showing presence of hyalinisation (H), focal degeneration (FD) and necrosis (N). (e&f) At the concentration of 0.5, 0.9 and 1.2 mg/L during six-week period showing normal structure appearance of muscle fibre tissue H&E; 200x. (Source: Al-Tamimi et al., 2015)

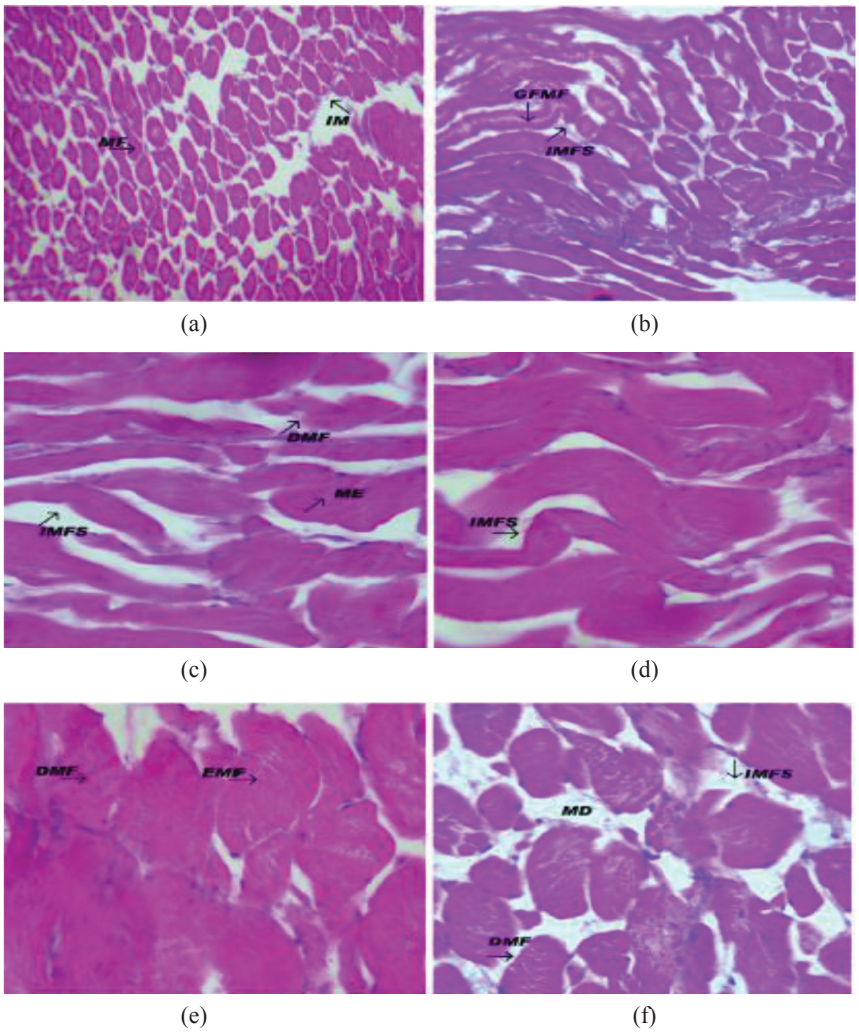


Figure 7. Histological changes of muscle in *L. calcarifer*. Light micrographs of a paraffin section stained with Hematoxylin and Eosin (40). (a) Control; (b) after 7 days of exposure to 6.83 ppm concentration of copper; (c) and (d) after 28 days of exposure to 6.83 ppm concentration of copper; (e) after 7 days of exposure to 13.66 ppm concentration of copper; (f) after 28 days of exposure to 13.66 ppm concentration of copper. Abbreviations used: MF – myofibrils; IM – interstitial materials; GFMF – gap formation in myofibril; IMFS – inter myofibrillar space; DMF – disintegrated myofibrils; EMF – oedema between muscle fibres; MD – muscle degradation; ME – muscle oedema (Source: Maharajan et al., 2016)

CONCLUSION

This study on the accumulation of heavy metals reflected on the degree of contamination in the environment. Besides that, the level of copper contamination in fish was considerably interesting since fish is an important source of copper for the general population. Most of the copper content in fish is highly absorbable in the form of copper sulphate. Acute toxicity and

physiological effects on aquatic organisms following waterborne copper exposure can be altered by several parameters including the concentration of the copper absorbed and the time of exposure. Other studies have also proven that the application of histopathological alteration can be used as a biomarker of copper exposure.

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REFERENCES

Abdel-Moneim, A. M., & Abdel-Mohsen, H. A. (2010). Ultrastructure changes in hepatocytes of catfish *Clarias gariepinus* from Lake Mariut, Egypt. *Journal of Environmental Biology*, *31*(5), 715–720.

Abdel-Tawwab, M. (2016). Effect of feed availability on susceptibility of Nile tilapia, *Oreochromis niloticus* (L.) to environmental zinc toxicity: Growth performance, biochemical response, and zinc bioaccumulation. *Aquaculture*, *464*, 309–315.

Abubakar, M. I., Ezeri, G. N., Omoniyi, I. T., & Akinloye, O. A. (2014). Histopathology of the gills, livers and kidney of *Clarias gariepinus* (Burchell, 1822) exposed to Sniper 1000EC under laboratory conditions. *Journal of Medical and Applied Biosciences*, *6*(1), 44–60.

Agamy, E. (2012). Histopathological changes in the livers of rabbit fish (*Siganus canaliculatus*) following exposure to crude oil and dispersed oil. *Toxicologic Pathology*, *40*(8), 1128–1140.

Ahsan, N., Lee, D. G., Lee, S. H., Kang, K. Y., Lee, J. J., Kim, P. J., … & Lee, B. H. (2007). Excess copper induced physiological and proteomic changes in germinating rice seeds. *Chemosphere*, *67*(6), 1182–1193.

Ajani, E. K., & Akpoilih, B. U. (2010). Effect of chronic dietary copper exposure on haematology and histology of common carp (*Cyprinus carpio* L.). *Journal of Applied Sciences and Environmental Management*, *14*(4), 39–45.

Akan, J. C., Mohmoud, S., Yikala, B. S., & Ogugbuaja, V. O. (2012). Bioaccumulation of some heavy metals in fish samples from River Benue in Vinikilang, Adamawa State, Nigeria. *American Journal of Analytical Chemistry*, *3*(11), 727–736.

Al-Balawi, H. F. A., Al-Akel, A. S., Al-Misned, F., Suliman, E. A. M., Al-Ghanim, K. A., Mahboob, S., & Ahmad, Z. (2013). Effects of sub-lethal exposure of lead acetate on histopathology of gills, liver, kidney and muscle and its accumulation in these organs of *Clarias gariepinus*. *Brazilian Archives of Biology and Technology*, *56*(2), 293–302.

Al-Tamimi, A. H., Al-Azzawi, A. J., & Al-A'dhmi, M. A. (2015). Chronic toxicity assessment of histological changes and micronuclei in fish *Cyprinus carpio* L. after exposed to copper. *American Scientific Research Journal for Engineering, Technology and Sciences*, *13*(1), 194–210.

Al-Yousuf, M. H., El-Shahawi, M. S., & Al-Ghais, S. M. (2000). Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex. *Science of the Total Environment*, *256*(2–3), 87–94.

Annabi, A., Said, K., & Messaoudi, I. (2013). Cadmium: Bioaccumulation, histopathology and detoxifying mechanisms in fish. *American Journal of Research Communication*, *4*(1), 60–79.

Ashraf, M. A., Maah, M. J., & Yusoff, I. (2012). Bioaccumulation of heavy metals in fish species collected from former tin mining catchment. *International Journal of Environmental Research*, *6*(1), 209–218.

Atabati, A., Keykhosravi, A., Askari-Hesni, M., Vatandoost, J., & Motamedi, M. (2015). Effects of copper sulfate on gill histopathology of grass carp (*Ctenopharyngodon idella*). *Iranian Journal of Ichthyology*, *2*(1), 35–42.

Balambigai, N., & Aruna, D. (2011). Impact of copper sulphate, an essential micronutrient on ACh, AChE and Na^+K^+ATPase in various tissues of the fish *Cyprinus carpio* (L.). *Research Journal of Environmental Toxicology*, *5*(2), 141–146.

Balamurugan, K., & Schaffner, W. (2006). Copper homeostasis in eukaryotes: Teetering on a tightrope. *Biochimica et Biophysica Acta (BBA) – Molecular Cell Research*, *1763*(7), 737–746.

Bambang, Y., Thuet, P., Charmantier-Daures, M., Trilles, J. P., & Charmantier, G. (1995). Effect of copper on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* bate (Crustacea, Decapoda). *Aquatic Toxicology*, *33*(2), 125–139.

Batvari, B. P. D., Kamala-Kannan, S., Shanthi, K., Krishnamoorthy, R., Lee, K. J., & Jayaprakash, M. (2008). Heavy metals in two fish species (*Carangoidel malabaricus* and *Belone stronglurus*) from Pulicat Lake, North of Chennai, Southeast Coast of India. *Environmental Monitoring and Assessment*, *145*(1–3), 167–175.

Bose, M., Ilavazhahan, M., Tamilselvi, R., & Viswanathan, M. (2013). Effect of heavy metals on the histopathology of gills and brain of fresh water fish Calta calta. *Biomedical and Pharmacology Journal*, *6*(1), 99–105.

Carvalho, C. S., & Fernandes, M. N. (2006). Effect of temperature on copper toxicity and hematological responses in the neotropical fish *Prochilodus scrofa* at low and high pH. *Aquaculture*, *251*(1), 109–117.

Cepanko, V., Idzelis, R. L., Kesminas, V., & Ladygiene, R. (2010). Radiological investigation of roach and perch from some lakes in Lithuania. *Journal of Environmental Engineering and Landscape Management*, *14*(4), 199–205.

Çoğun, H. Y., & Kargın, F. (2004). Effects of pH on the mortality and accumulation of copper in tissues of *Oreochromis niloticus*. *Chemosphere*, *55*(2), 277–282.

Cunha, I., Mangas-Ramirez, E., & Guilhermino, L. (2007). Effects of copper and cadmium on cholinesterase and glutathione S-transferase activities of two marine gastropods (*Monodonta lineata* and *Nucella lapillus*). *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, *145*(4), 648–657.

Czedli, H., Csedreki, L., Sziki, A. G., Jolankai, G., Pataki, B., Hancz, C., … & Nagy, S. A. (2014). Investigation of the bioaccumulation of copper in fish. *Fresenius Environmental Bulletin*, *23*(7), 1547–1552.

Das, B. K., & Mukherjee, S. C. (2000). A histopathological study of carp (*Labeo rohita*) exposed to hexachlorocyclohexane. *Veterinarski Arhiv*, *70*(4), 169–180.

Das, S., & Gupta, A. (2013). Accumulation of copper in different tissues and changes in oxygen consumption rate in Indian Flying Barb, *Esomus danricus* (Hamilton-Buchanan) exposed to sub-lethal concentrations of copper. *Jordan Journal of Biological Sciences*, *6*(1), 21–24.

Devi, Y., & Mishra, A. (2013). Histopathological alterations in gill and liver anotomy of fresh water, air breathing fish *Channa punctatus* after Pesticide Hilban® (Chlorpyrifos) treatment. *Advanced Biomedical Research*, *4*(2), 57–62.

Ebrahimpour, M., Pourkhabbaz, A., Baramaki, R., Babaei, H., & Rezaei, M. (2011). Bioaccumulation of heavy metals in freshwater fish species, Anzali, Iran. *Bulletin of Environmental Contamination and Toxicology*, *87*(4), 386–392.

El-Moselhy, K. M., Othman, A. I., Abd El-Azem, H., & El-Metwally, M. E. A. (2014). Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. *Egyptian Journal of Basic and Applied Sciences*, *1*(2), 97–105.

El-Sayyad, H. I., Zaki, V. H., El-Shebly, A. M., & El-Badry, D. A. (2010). Studies on the effects of bacterial diseases on skin and gill structure of *Clarias gariepinus* in Dakahlia Province, Egypt. *Annals of Biological Research*, *1*(4), 106–118.

Eneji, I. S., Sha'Ato, R., & Annune, P. A. (2011). Bioaccumulation of heavy metals in fish (*Tilapia zilli* and *Clarias gariepinus*) organs from River Benue, North-Central Nigeria. *Pakistan Journal of Analytical and Environmental Chemistry*, *12*(1–2), 25–31.

Farombi, E. O., Adelowo, O. A., & Ajimoko, Y. R. (2007). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African catfish (*Clarias gariepinus*) from Nigeria Ogun River. *International Journal of Environmental Research and Public Health*, *4*(2), 158–165.

Fatma, A. S. M. (2009). Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. *World Journal of Fish and Marine Sciences*, *1*(1), 29–39.

Figueiredo-Fernandes, A., Ferreira-Cardoso, J. V., Garcia-Santos, S., Monteiro, S. M., Carrola, J., Matos, P., & Fontaínhas-Fernandes, A. (2007). Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. *Pesquisa Veterinária Brasileira*, *27*(3), 103–109.

Francis, P. C., Birge, W. J., & Black, J. A. (1984). Effects of cadmium-enriched sediment on fish and amphibian embryo-larval stages. *Ecotoxicology and Environmental Safety*, *8*(4), 378–387.

Franciscato, C., Bueno, T. M., Moraes-Silva, L., Duarte, F. A., Flores, É. M., Dressler, V. L., & Pereira, M. E. (2009). High doses of zinc and copper alter neither cerebral metal levels nor acetylcholinesterase activity of suckling rats. *EXCLI Journal*, *8*, 138–147.

Gaber, H. S., Abbas, W. T., Authman, M. M. N., & Gaber, S. A. (2014). Histological and biochemical studies on some organs of two fish species in Bardawil Lagoon, North Sinai, Egypt. *Global Veterinarian*, *12*(1), 1–11.

Ghosh, L., & Adhikari, S. (2006). Accumulation of heavy metals in freshwater fish – An assessment of toxic interactions with calcium. *American Journal of Food Technology*, *1*(2), 139–148.

Hadi, A., & Ahwan, S. (2012). Histopathological changes in gills, liver and kidney of fresh water fish, *Tilapia zillii*, exposed to aluminum. *International Journal of Pharmacy and Sciences*, *3*(11), 2071–2081.

Hellawell, J. M. (1986). *Biological indicators of freshwater pollution and environmental management*. USA: Elsevier Applied Science Publishers.

Hinton, D. E., & Lauren, J. C. (1990). Integrative histopathology approaches to detecting effects of environmental stress on fishes. *American Fisheries Society Symposium*, *8*, 51–56.

Idriss, A. A., & Ahmad, A. K. (2015). Heavy metal concentrations in fish from Juru River, estimation of the health risk. *Bulletin of Environmental Contamination and Toxicology*, *94*(2), 204–208.

Jalal, K. C. A., Mohd, A. Y., Habab, M., & Kamaruzzaman, B. Y. (2014). Bioaccumulation of selected metals in fresh water Haruan fish (*Channastriatus*) collected from Pahang river basin, Malaysia. *Oriental Journal of Chemistry*, *29*(4), 1553–1558.

Jezierska, B., & Witeska, M. (2006). The metal uptake and accumulation in fish living in polluted waters. In I. Twardowska, H. E. Allen, M. M. Häggblom, & S. Stefaniak (Eds.), *Soil and water pollution monitoring, protection and remediation* (pp. 107–114). Dordrecht: Springer Netherlands. Retrieved from http://link.springer.com/chapter/10.1007/978-1-4020-4728-2_6

Joshi, P. (2011). Impact of zinc sulphate on behavioural responses in the freshwater fish *Clarias batrachus* (Linn.). *Online International Interdisciplinary Research Journal*, *1*(2), 76–82.

Kamaruzzaman, B. Y., John, B. A., Maryam, B. Z., Jalal, K. C. A., & Shahbuddin, S. (2012). Bioaccumulation of heavy metals (Cd, Pb, Cu and Zn) in *Scylla serrata* (Forsskal 1775) collected from Sungai Penor, Pahang, Malaysia. *Pertanika Journal of Tropical Agricultural Science*, *35*(1), 183–190.

Kamaruzzam, B. Y., Ong, M. C., & Jalal, K. C. A. (2008). Level of copper, zinc and lead in fishes of Mangabang Telipot River, Terengganu, Malaysia. *Journal of Biological Sciences*, *8*(7), 1181–1186.

Karayakar, F., Cicik, B., Ciftci, N., Karaytug, S., Erdem, C., & Ozcan, A. Y. (2010). Accumulation of copper in liver, gill and muscle tissues of *Anguilla anguilla* (Linnaeus, 1758). *Journal of Animal and Veterinary Advances*, *9*(17), 2271–2274.

Khabbazi, M., Harsij, M., Hedayati, S. A. A., Gerami, M. H., & Ghafari-Farsani, H. (2015). Histopathology of rainbow trout gills after exposure to copper. *Iranian Journal of Ichthyology*, *1*(3), 191–196.

Kumar, P. M., & Prabhahar, C. (2012). Bioaccumulation of heavy metals in fish from waste water: A review. *International Journal of Pharmaceutical and Biological Archive*, *3*(6), 13550–1359.

Liebel, S., Tomotake, M. E. M., & Ribeiro, C. A. O. (2013). Fish histopathology as biomarker to evaluate water quality. *Ecotoxicology and Environmental Contamination*, *8*(2), 09–15.

Maharajan, A., Kitto, M. R., Paruruckumani, P. S., & Ganapiriya, V. (2016). Histopathology biomarker responses in Asian sea bass, *Lates calcarifer* (Bloch) exposed to copper. *The Journal of Basic and Applied Zoology*, *77*, 21–30.

Malik, N., Biswas, A. K., Qureshi, T. A., Borana, K., & Virha, R. (2010). Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. *Environmental Monitoring and Assessment*, *160*(1–4), 267–276.

Miranda, A. L., Roche, H., Randi, M. A. F., Menezes, M. L., & Ribeiro, C. A. (2008). Bioaccumulation of chlorinated pesticides and PCBs in the tropical freshwater fish *Hoplias malabaricus*: Histopathological, physiological, and immunological findings. *Environment International*, *34*(7), 939–949.

Mohammadnabizadeh, S., Pourkhabbaz, A., & Afshari, R. (2014). Analysis and drtermination of trace metals (nickel, cadmium, chromium, and lead) in tissues of *Pampus argenteus* and *Platycephalus indicus* in the Hara Reserve, Iran. *Journal of Toxicology*, *2014*, 1-6. https://doi.org/10.1155/2014/576496

Monteiro, S. M., Oliveira, E., Fontaínhas-Fernandes, A., & Sousa, M. (2012). Effects of sublethal and lethal copper concentrations on the gill epithelium ultrastructure of Nile tilapia, *Oreochromis niloticus*. *Zoological Studies*, *51*(7), 977–987.

Nandan, S., & Kumar, B. G. (2014). Copper toxicity: Haematological and histopathological changes and prophylactic role of vitamin C in the fish, *Anabas testudineus* (Bloch, 1792). *Journal of Zoology Studies*, *1*(3), 4–13.

Osman, A. G. M., Reheem, A.-E., Baset, M. A. E., AbuelFadl, K. Y., & Rab, A. G. G. (2010). Enzymatic and histopathologic biomarkers as indicators of aquatic pollution in fishes. *Natural Science*, *2*(11), 1302–1311.

Pandey, S., Parvez, S., Ansari, R. A., Ali, M., Kaur, M., Hayat, F., … & Raisuddin, S. (2008). Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctata* Bloch. *Chemico-Biological Interactions*, *174*(3), 183–192.

Pant, S. C., Kumar, S., & Khanna, S. S. (1980). Toxicity of copper sulphate and zinc sulphate to fresh water teleost *Puntius conchonius* (Ham.) in hard water. *Comparative Physiology and Ecology*, *5*(3), 146–149.

Paulo, D. V., Fontes, F. M., & Flores-Lopes, F. (2012). Histopathological alterations observed in the liver of *Poecilia vivipara* (Cyprinodontiformes: Poeciliidae) as a tool for the environmental quality assessment of the Cachoeira River, BA. *Brazilian Journal of Biology*, *72*(1), 131–140.

Perera, P. A. C. T., Kodithuwakku, S. P., Sundarabarathy, T. V., & Editrisinghe, U. (2015). Bioaccumulation of cadmium on freshwater fish: An environmental perspective. *Insight Ecology*, *4*(1), 1–12.

Rajkowska, M., & Protasowicki, M. (2013). Distribution of metals (Fe, Mn, Zn, Cu) in fish tissues in two lakes of different trophy in Northwestern Poland. *Environmental Monitoring and Assessment*, *185*(4), 3493–3502.

Reddy, P. B. (2012). Histopathogical studies as potential and direct biomarkers of pollution. *Trends in Life Sciences*, *1*(1), 27–31.

Ribeiro, C. A. O., Vollaire, Y., Sanchez-Chardi, A., & Roche, H. (2005). Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the Eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. *Aquatic Toxicology*, *74*(1), 53–69.

Sabullah, M. K., Ahmad, S. A., Shukor, M. Y., Gansau, A. J., Syed, M. A., Sulaiman, M. R., & Shamaan, N. A. (2015). Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction and proteomics approaches. *International Food Research Journal*, *22*(2), 435–454.

Sabullah, M. K., Shukor, M. Y., Sulaiman, M. R., Shamaan, N. A., Syed, M. A., Khalid, A., & Ahmad, S. A. (2014). The effect of copper on the ultrastructure of *Puntius javanicus hepatocyte*. *Australian Journal of Basic and Applied Science*, *8*(15), 245–51.

Shuhaimi-Othman, M., Nadzifah, Y., Ahmad, A. K., & others. (2010). Toxicity of copper and cadmium to freshwater fishes. *World Academy of Science, Engineering and Technology*, *65*, 869–871.

Solomon, F. (2009). Impacts of copper on aquatic ecosystems and human health. *Environment and Communities, 25*(8), 25-28.

Tao, S., Long, A., Dawson, R. W., Xu, F., Li, B., Cao, J., & Fang, J. (2002). Copper speciation and accumulation in the gill microenvironment of carp (*Cyprinus carpio*) in the presence of kaolin particles. *Archives of Environmental Contamination and Toxicology*, *42*(3), 325–331.

Udotong, J. I., & John, O. U. (2015). Histopathological changes in liver and muscle of tilapia fish from QIRE exposed to concentrations of heavy metals. *World Academy of Science, Engineering and Technology*, *9*(6), 659–662.

Van der Oost, R., Beyer, J., & Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology and Pharmacology*, *13*(2), 57–149.

Velma, V., Vutukuru, S. S., & Tchounwou, P. B. (2009). Ecotoxicology of hexavalent chromium in freshwater fish: A critical review. *Reviews on Environmental Health*, *24*(2), 129–145.

Vijver, M. G., van Gestel, C. A. M., Lanno, R. P., van Straalen, N. M., & Peijnenburg, W. J. G. M. (2004). Internal metal sequestration and its ecotoxicological relevance: A review. *Environmental Science and Technology*, *38*(18), 4705–4712.

Wani, A. A., Sikdar-Bar, M., & Khan, H. A. (2013). Acute toxicity of copper sulphate to African catfish, *Clarias gariepinus*. *GERF Bulletin of Biosciences*, *4*(1), 14–18.

Younis, E. M., Abdel-Warith, A. A., Al-Asgah, N. A., Ebaid, H., & Mubarak, M. (2013). Histological changes in the liver and intestine of Nile tilapia, *Oreochromis niloticus*, exposed to sublethal concentrations of cadmium. *Pakistan Journal of Zoology*, *45*(3), 833–841.