J. Int. Environmental Application & Science, Vol. 11(4): 331-345 (2016)

Acute and Chronic Effects of Water Soluble Fraction WSF of Diesel Fuel on Common Carp (*Cyprinus carpio* L. 1758)

Ayat Munaf Hameed*, Ahmed J. M. Al-Azawi

Department of Biology, College of Science, Baghdad University, Baghdad, IRAQ

Received August 16, 2016; Accepted November 28, 2016

Abstract: This study aimed to determine the acute and chronic toxicity of water soluble fraction WSF of diesel fuel on common carp *Cyprinus carpio*. The median lethal concentrations (LC_{50}) in *C. carpio* were 0.005993, 0.005972, 0.005942 and 0.005931% for exposure period of 24, 48, 72 and 96 hours respectively. Behavioural changes were studied during acute toxicity period, and were abnormal movement, swimming near to water surface of aquaria, lose of body balance and suddenly movement with jump. These changes have increased as concentration of diesel fuel and duration of exposure increased. The chronic toxic effects of water soluble fraction of diesel fuel on *C. carpio* were determined by using three concentrations (0.00039%, 0.00059% and 0.0011%) with eight fishes for six weeks. Histological changes in liver, gill, kidney and muscles were assessed after 2, 4 and 6 weeks of chronic exposure. Histological changes in liver were cellular swelling, degeneration of hepatocytes, necrosis, cellular atrophy and nuclear pyknosis. In gill, these changes were congestion, necrosis, cellular swelling, thickened of primary and secondary lamella. In the kidney, observed changes were glomerular degeneration, dilation of Bowman's space, tubular degeneration and necrosis. While in skeletal muscles, the changes were atrophy and necrosis of muscle fiber and fragmented of myofibrils. These histological changes have found to be more severe when exposure time and concentration of water soluble fraction WSF of diesel fuel increased.

Keywords: Cyprinus carpio, *LC_{50}, WSF Diesel fuel, behavior, Histological changes.*

Introduction

The aquatic environments are contaminated with innumerable of organic and non-organic pollutants (Sen & Kirikbakan, 2009) of domestic waste, industrial, agricultural and mining industrial origin (Lenartova *et al*., 1997). These pollutants not only affect the integrity of the ecosystem and also affect the physiological functions of the animals and even human being consumers (Sen & Kirikbakan, 2009; Perez-Lopez *et al*., 2002).

The accidental release of hazardous materials such as chemical solvents and petroleum into the aquatic environment has become the focus of increasing regulatory and public concern due to the adverse effects of such materials on human health and the environment (Bourodimos & Carvoumis, 1990). As a result of rapid industrialization and urbanization, increasing quantities of man-produced pollutants which include petroleum hydrocarbons have been discharged into environment. When these pollutants go into water bodies, they form direct or indirect impacts on the biota of aquatic ecosystem (Kakkar *et al*., 2011). The leach of engine fuel, such as of diesel oil, from underground bulk storage tanks is often causing groundwater contaminations and such contaminants may end into river and to other aquatic ecosystems and subsequently creating serious threats (Pacheco & Santos 2001a). Only some reports have been published about the effects of diesel oil exposure on morphological and physiological parameters of freshwater fishes (Zhang *et al*., 2003).

The acute spill and especially the intense release of diesel oil is probably a very harmful to the fish community, since those cellular changes in a long time may prompt a irreversible alteration, with a deterioration of the respiratory and also to osmoregulatory system. This may lead to the wide spread of disease and even death of the animals (Furia, 2004). The soluble fraction of diesel oil contains many polycyclic aromatic hydrocarbons (PAH) that cause fish lesion in their liver and gill (Simonato *et al*., 2008).

Dissolution is one of the fundamental mass transfer processes that occur when oil is spilled on water. Although the fraction of oil that dissolved into water is relatively small when compared to the total mass of the oil and this fraction that intimately contacts the aquatic organisms and thus is

Corresponding: E-Mail: ayat_munaf17@yahoo.com; Tel: 009647709787184;

considered more important to determinate of the oil toxicity (Mark, 1992). Soluble petroleum hydrocarbons have the ability for absorption by organisms and to concentrate in their tissues to about 10 to 100 times higher than in the waters (Ramachandran *et al.*, 2006).

The common carp (*Cyprinus carpio* L.) belongs to Cyprinidae, which is considered as the largest freshwater teleost family (Nelson, 1994). This fish species as higher organisms in the food chain of aquatic ecosystem and also foodstuff that frequently consumed by human being has been considered a bioindicator species in waterways (Oruc & Uner, 2004). The common carp (*C. carpio* L.) is probably the oldest and most extensively cultured fish species in the world (Nelson, 1994). *C. carpio* is account to the world's second uppermost farmed fish production, fundamentally from polyculture in Asia (Milstein, 1992).

Toxicity test considered as an important analytical tool for estimation impacts of the chemical agents on living organisms during standardized conditions. In order to be useful for comparisons between chemical agents to reveal acute or chronic effects by using lethal or sublethal concentrations, respectively (Rand & Petrocelli, 1985). The tests are designed to provide dose-response information, expressed as percent effluent concentration that is lethal to 50% of the tested organisms (LC50) within the prescribed period of time (24-96 h), or the highest effluent concentration in which survival is not significantly different from the control (EPA, 2002).

Histological study is a rapid method for detection of pollutants effects on a various tissues of fish and has been extensively used to determine the deleterious impacts of hydrocarbons pollutants (Moreira *et al.*, 2014).

This study aims to investigate the toxic effects of water soluble fraction of diesel fuel on Common carp *C. carpio* by determination of 96 hours LC_{50} values and observing the behavioural changes of common carp exposed to different concentrations of diesel fuel. Also aims to determine the histological changes in organs liver, gill, kidney and skeletal muscles after 2, 4 and 6 weeks of chronic exposure to sublethal concentrations of WSF of diesel fuel.

Material and Methods

Preparation of water soluble fraction of water soluble fraction (WSF)

Diesel fuel used in the study was obtained from Midland Refineries Company (M.R.C) Al-Durah refinery in southern Baghdad. The preparation of water soluble fraction (WSF) of diesel was conducted according to the method suggested by previous studies (Anderson, 1974 and Orlu & Ogbalu, 2013), by added 1 part of diesel fuel on 9 parts of distilled water in bottle and stirring with a magnetic stirrer for 20 hours at room temperature. The bottle was capped to minimize evaporation of more volatile oil hydrocarbons. The stirring speed was adjusted so that the vortex did not extend more than 25% of the distance to the bottom of the container. After mixing of 20 hours, the oil and water phases were allowed to separate for 1 to 6 hours before the water phase was siphoned off, and used in the study. Different concentrations of the WSF of diesel fuel were prepared by diluting the stock (WSF) with de-chlorinated tap water which using in acclimazation of *C. carpio* in order to obtain experimental concentrations for diesel

Acclimatization of fish

Samples of *C. carpio* fish of 40 -60 gm weight used in current work collected from Al- Madaan Hatcheries in south of Baghdad and transferred by plastic tanks to the environment and pollution laboratory. Fish samples were acclimated to the laboratory conditions for 14 days before commencing the experiment at temperature of 21-25 °C and pH of 6.5-8. Fish samples were placed into glass aquaria with diminution of 70×40×70 cm contain 40 L of de-chlorinated tap water which left for about 72 hours (to elimination of chlorine). Fishes were fed daily with dried commercial fish food. Water aquaria were aerated by air pump (aerator) continuously.

Acute toxicity

The acute toxicity test was carried out by using five lethal concentrations and determining the LC_{50} (lethal concentrations for 50% of fishes) (Soorenal *et al.*, 2011). Acute toxicity test of *C. carpio* was performed according to OECD (1993) and carried out for a period of 96 hours. Fish samples were divided into six groups of eight fishes in each group. Fish samples were divided into six aquaria, the first one is control group and other five aquaria were subjected to toxicity test concentrations with

three replications. Acute toxic effect of diesel fuel on the fishes was determined by use of Finney Probit Analysis (Finney, 1971). The concentrations that used in acute toxicity test were 0.0078%, 0.0085%, 0.0087%, 0.0098% and 0.0108%.

Behaviour changes

The behaviour of examined samples is considered as a highly sensitive indicator to environmental changes (Kasumyan, 2001). The fishes behaviour was assessed via movement, body balance, respiration movement and neural response compared to control group (without treatment) and recorded the behaviour changes during acute test from 24 hr. to 96 hr. of exposure period.

Safe concentration

Fishes were exposed to three concentrations of WSF of diesel fuel, which were 1/15th, 1/10th and 1/5th of the 96h LC50 value (Al-Sawafi & Yan, 2013). These three concentrations were used at chronic toxicity.

Chronic toxicity

Three groups of fishes were used in each aquarium and subjected to 0.00039%, 0.00059% and 0.0011% concentrations for six weeks, in addition to control group without treatment. Water was replaced every 48 hours to remove the wastes. Fishes were feed regularly once a day (FAO, 1987).

Histological changes

After each of the respective exposure periods 2, 4 and 6 weeks, the liver, gill, kidney and skeletal muscles tissue of *C. carpio* were immediately removed. Tissue sections prepared according to method mentioned by previous study (Junqueria and carneiro, 2003). Samples were fixed with 10% formalin. The fixed tissues were dehydrated with series of up grading ethanol: 70-95% to 100% respectively, cleared in xylene and embedded in paraffin blocks. After blocks have been completely cooled the blocks were trimmed. Then the samples were cut by using a rotary microtome with thickness 4- 5 µm. Tissues sections were stained with Hematoxylin and Eosin [H &E] and tested by light microscope (Olympus, Japan) and photographed.

Statistical Analysis

All experiments were carried out with three replicates. The LC50 values were calculated using probit analysis statistical method. The Microsoft Excel was used to estimate regression equation (Y= mortality percentage; X=log of concentrations) and LC50 was derived from the best-fit line obtained.

Results and Discussion

Acute toxicity

Median lethal concentration LC_{50}:

In acute toxicity test in which five concentrations were used to obtained LC_{50} of WSF diesel fuel on *C. carpio* at 24, 48, 72, and 96 hours. The obtained value of median lethal concentrations were 0.005993%, 0.005972%, 0.005942% and 0.005931% for exposure period 24, 48, 72 and 96 hours respectively (Fig. 1, A.B,C and D) by using the concentrations 0.007830%, 0.008503%, 0.008701%, 0.009887% and 0.010877 %.

According to previous study (Rodrigues *et al*., 2010), it has been reported after 96hr. of exposure to the different WSFs of diesel and gasoline, on marine pejerrey *Odontesthes argentinensis* larvae, the median lethal concentration after 96 h (LC_{50}) of exposure for WSF of diesel and gasoline, which were 13.46% and 5.48%, respectively. Also (Al-Khafagy, 2005) found that the 96h LC_{50} value of WSF gas oil concluded was 2%. In other study, it was reported that the 96hours LC_{50} value of WSF diesel fuel in *O. Niloticus* fingerlings was determined 0.000808% (Dede & Kaglo, 2001). Similar work has found that 96 h static acute toxicity test was also investigated on the juveniles *Clarias gariepinus* (African catfish) and *Clarias anguillaris* (mudfish) on exposure to different concentrations of crude oil polluted water, and after 96 h, probit analysis showed the LC_{50} of crude oil for *C. anguillaris* to be 0.000122% while that of *C. garieinus* was 0.000219% (Awoyinka *et al.*, 2011).

Table 1. Values of median lethal concentration LC_{50} of WSF of diesel fuel on *C. carpio*

Period (hours)	Value of LC_{50} (%)
24	0.005993
48	0.005972
72	0.005942
96	0.005931

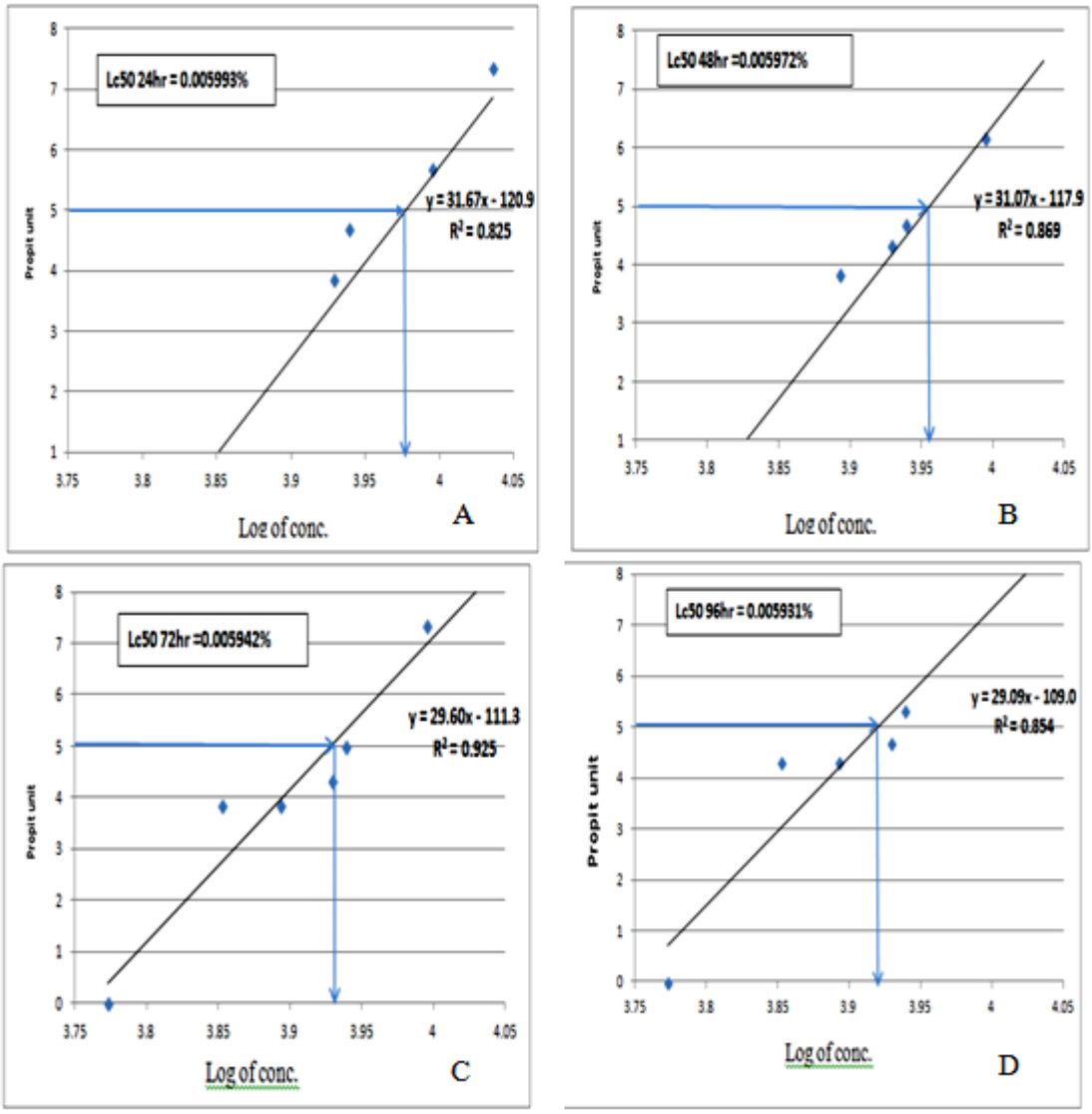

Figure 1. Median lethal concentration value LC_{50} of water soluble fraction of diesel fuel (WSF) through 24, 48, 72 and 96 hr. in *Cyprinus carpio* L. (A, B, C and D)

Behavioural changes:

Behaviour changes considered as sensitive measurement of an organism's response into stresses such as environmental contaminants. Behaviour changes were noticeable and can be found at chemicals concentrations which below to those concentrations cause mortality (LC_{50}), bioassays that are still to apply the behaviour changes in the toxicity testing (Little & Finger, 1990; Gerhardt, 2007). Since behaviour serves as link between ecological and physiological processes, it may be perfect for studying environmental pollutant impacts. Fish are an excellent model in this regard, since many ecologically relevant fish behaviours are easily observed and quantified in a controlled setting (Scott & Sloman, 2004).

In the present study the results showed marked behavioural changes in *C. carpio* as a result of being exposed to different concentrations WSF of diesel fuel during exposure period (96 hours), and

these behavioural changes were increased at higher concentrations, while in the control group not appeared any changes. Results are shown in Table 2.

According to Umejuru (2007) investigation of acute toxicity of water soluble fraction of crude oil on the behaviour of the Juvenile crawfish (*Procambarus clarki*.), and observed anxiety, swimming upside down, loss of balance, excessive mucus secretion and lightening in colour, gathering at the surface for breathing and hitting to the side walls of aquaria.

Table 2. Show the behavioural changes in *C. carpio* during exposure period.

Concentrations	Behavioural changes
Control	The behaviour of fish and their swimming were normal and no mortality recorded
0.007830%	Opercular movement of the fish became faster when compared with a control group and fish swimming were close to the surface.
0.008503%	Disorders and quick movement from one side of the aquaria to another side and then return to the silence state again
0.008701%	Abnormal movement with sudden jumping, swimming close to the surface of water of aquaria.
0.009887%	Fish show nervous and rapid movement directly after adding the pollutant, lose of balance in their swimming.
0.010877%	Fish movement generally more affected, fish become inactive and suddenly rapid movement with jump, lose balance of the body, slow movement of the opercular, weak response to any external stimulus, barrel-rolling fish swimming which evidence to lose body balance, then all fish died

Histological changes

Histopathological biomarkers have been widely used in fish for detection and assessment the effects of exposure to pollutants (Ribeiro *et al*., 2006) the changes detection of liver, gill and kidney organs are normally easier to identify and would serve as warning signs of damage to fish health (Fanta *et al.*, 2003).

Liver

Liver has a wide range of functions, including detoxification, protein synthesis, and production of biochemicals that necessary for digestion (Maton *et al*., 1993). Normal liver tissues (Figure 2- A) show normal hepatic tissue, central vein, hepatocytes, hepatic sinusoid and nucleus of endothelium.

The histopathological changes in liver of *C. carpio* exposed to different concentrations of diesel fuel for two, four and six weeks (Figures 2, 3, 4) show cellular swelling, hepatocytes atrophy, accumulation of bile pigment, advanced degeneration of hepatocytes, edema, atrophied hepatocytes, nuclear hypertrophy, nuclear kryolysis and pyknosis, necrosis and infiltration of inflammatory cells.

The liver can be considered as target organ and of great significance to fish, since it participates in biotransformation and excretion of xenobiotics. So, the liver can be studied in environmental monitoring because of its high sensitivity to contaminants (Thophon *et al*., 2003). In present study, the longer exposure period with higher concentration (0.0011%) shows infiltration of inflammatory cells and generalized necrosis of liver section that may reflect the damage of organ associated with time and concentration, this is agreement with study of Kakkar *et al*. (2010) reported the liver changes were swelling of hepatocytes, degeneration, necrosis, haemolysis, dilation, congestion and fibrosis of blood sinusoids in *Puntius ticto* when exposed to WSF petrol, in addition these changes were more damages according to dose and time dependents. The shrunk and pyknotic nuclei refer that the cells became hypofunctional and at the end necrosis was extensive in liver of *Corydoras paleatus* fish exposed to sublethal levels of organophosphorus (Fanta *et al*., 2003).

Necrosis of some portions of the liver tissue that was observed possibly resulted from the excessive work required by the fish to get rid of the WSF of diesel fuel from their body during the process of detoxification by the liver (Navaraj & Yasmin, 2012). Importance factor reflects the capacity of the alteration to be reversible following removal of a stressor degenerative alterations (general necrosis) are given as the highest importance factor to be a direct effect of toxicants which are generally irreversible, and their persistence or progression may lead to a fractional or total loss of organ function

(Agamy, 2012). Hinton & Lauren (1990) mention that, although swelling is an integral part of adaptation to cell injury, hepatocyte swelling as a result of toxic injury is rare.

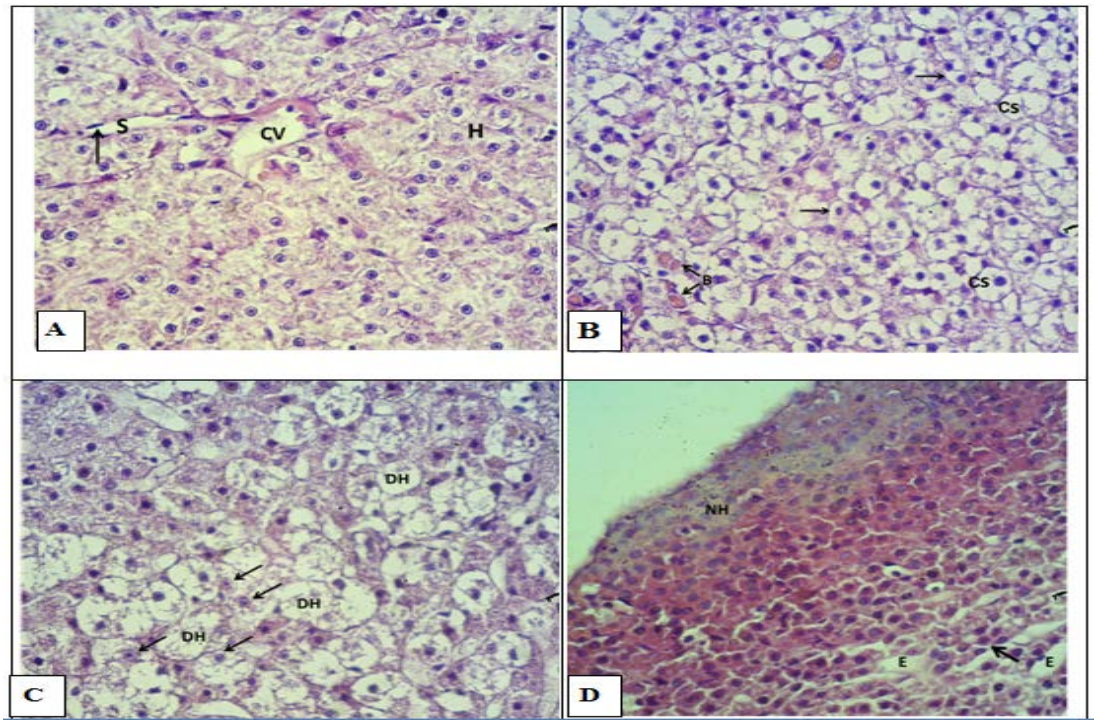

Figure 2. Histological changes of liver of *C. carpio* during 2 weeks [A] Shows normal hepatic tissue, central vein (CV), hepatocytes (H), hepatic sinusoid (S) and nucleus of endothelium (arrow). [B] Liver section treated with 0.00039% shows cellular swelling (S), hepatocytes atrophy (arrows) and accumulation of bile pigment (B). [C] Liver section treated with 0.00059% shows advanced degeneration of hepatocytes (DH) and nuclear pyknosis (Arrows). [D] Liver section treated with 0.0011% shows zone of dead hepatocytes (necrotic hepatocytes), edema (E) and atrophied hepatocytes (arrow). X40 H&E stain.

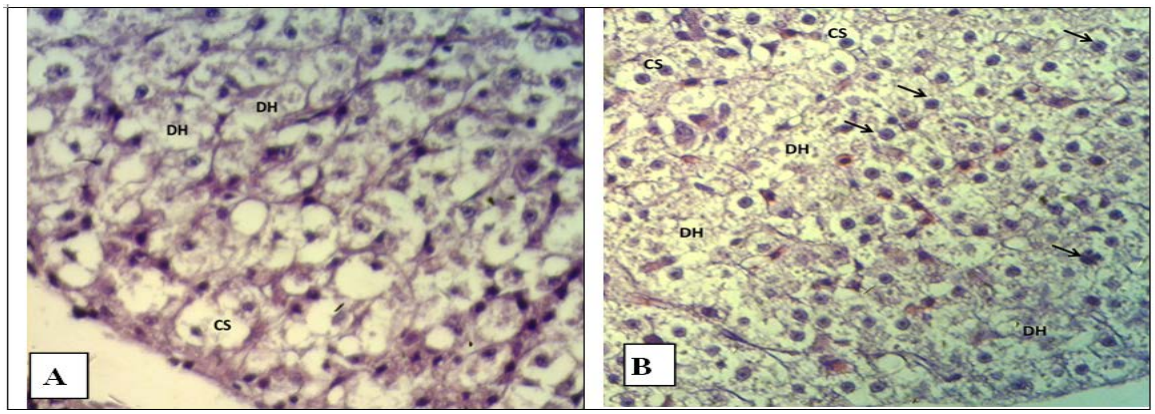

Figure 3: Histological changes of liver of *C. carpio* during 4 weeks [A] Liver section treated with 0.00039% concentration liver: show cellar swelling (SC) and degenerated hepatocytes (DH). [B] Liver section treated with 0.0011% concentration, show cellar swelling (SC), degenerated hepatocytes and nuclear hypertrophy (arrows). X40 (H&E) satin.

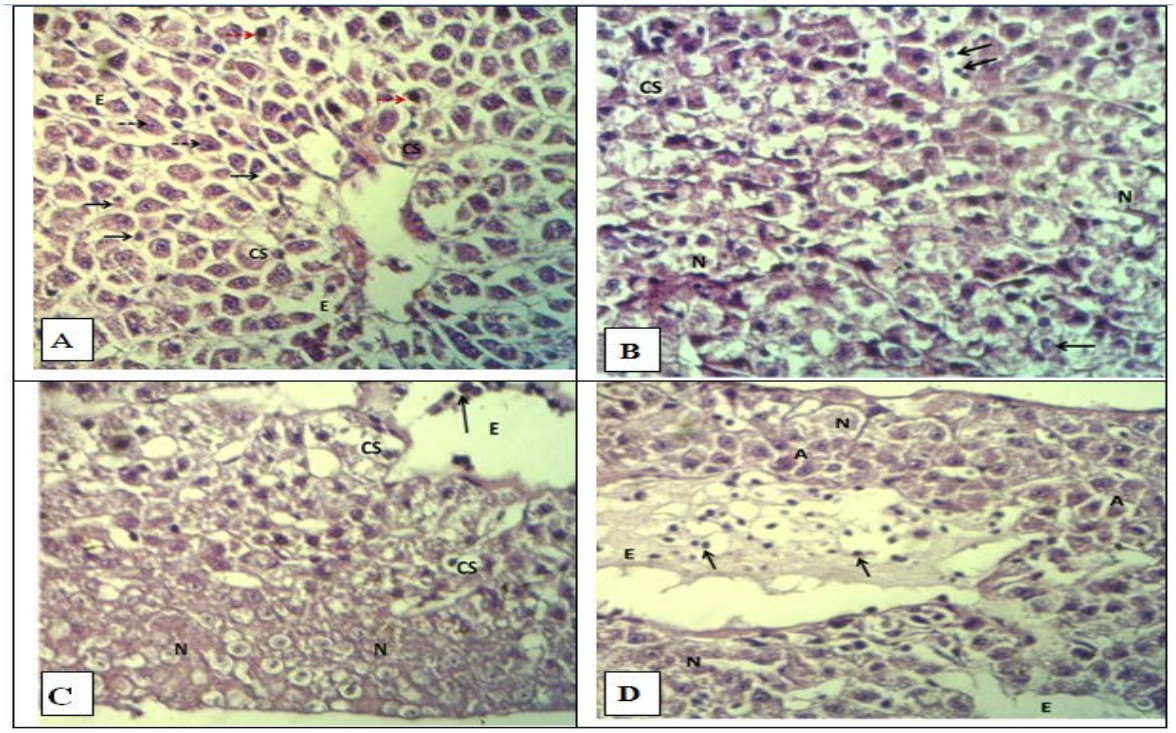

Figure 4. Histological changes of liver of *C. carpio* during 6 weeks [A] Liver section treated with 0.00039% concentration show edema (E), atrophied hepatocytes (black arrow), and nuclear kryolysis (doted arrows) pyknosis (doted red arrows). [B] Liver section treated with 0.00059% concentration show cellular swelling (Cs), necrosis (N) and nuclear pyknosis (Arrows). [C, D] Liver sections treated with 0.0011% concentration show generalized necrosis (N) and cellular swelling (CS), edema (E), infiltration of inflammatory cells (Arrow) and cellular atrophy (A). X40 (H&E) satin.

Gills

They are very important in respiration, acid–base balance, osmoregulation and excretion of nitrogenous wastes in fish (Evans et al., 2005)**.** They include the greatest surface area of the aquatic organisms in contact with external environment. Control group shows normal gill arch, primary lamellae, secondary lamellae, bony support and cartilaginous support. Normal gill tissues (Figure 5-A) show gill arch, primary lamellae, secondary lamellae, bony support and cartilaginous support.

Histopathological changes in *C. carpio* gill during two, four, and six weeks of exposure to different concentrations of WSF diesel fuel (Figures 5, 6 & 7) were, cellular swelling of primary lamellae epithelium and marked atrophy of chloride cells, congestion, cellular hyperplasia and degenerated chloride cells, necrotized cells and chloride cells and edema, while in 0.0011% concentration for six weeks exposure, the findings showed marked thickening of the primary lamellae and loss of secondary once due to sever edema and congestion of blood vessels with generalized necrosis of choroid and epithelial cells. This indicates to at high concentration and increase exposure period lead to more changes.

Gill alterations are dose-time dependent, therefore a greater damage in gill of pompanos fish (*Trachinotus* sp.) occur at highest concentration and long exposure time after exposed to WSF diesel oil, these alterations were hyperplasia of lamellar epithelium, cellular lifting, aneurysms and lamellar fusion these play a role in the defense of the organisms because acts as a barrier that increase distance between toxicant and bloodstream (Furia, 2004).

Gill histopathologic such as lifting, swelling, and hyperplasia of the lamellar epithelium could serve as a defence role, as these alterations enhance the distance across which waterborne irritants must diffuse to reach the bloodstream (Mallatt, 1985)

Thophon et al., (2003) observed that the first sign in the pathology is the edema of the epithelial cell in gills and this is due to epithelium covering the secondary lamella lifting away in continuous sheet from the pillar cell system, thus increasing the diffusion distance from water to a blood.

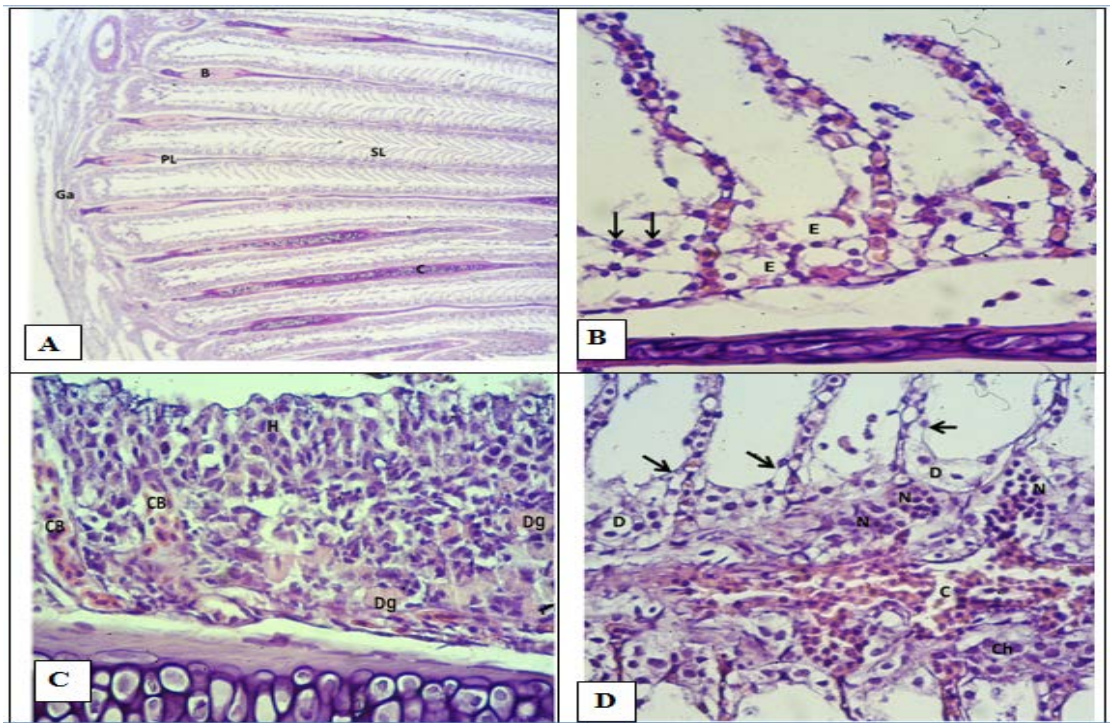

Figure 5. Histological changes of gill of *C. carpio* during 2 weeks [A] Normal gill: shows gill arch (Ga), primary lamellae (PL), secondary lamellae (SL), bony support (B) and cartilaginous support (C), X4. [B] Gill section treated with 0.00039% concentration, shows cellular swelling of primary lamellae epithelium (E) and marked atrophy of chloride cells. X40 (H&E) stain. [C] Gill section treated with 0.00059% concentration, shows: Congestion (BC) , cellular hyperplasia (H) and degenerated chloride cells showed degenerative granules (Dg), X40 (H&E) stain. [D] gill section treated with 0.0011% concentration, shows congestion (C) degenerative changes (D) necrotized cells (N) and chloride cells, X40.

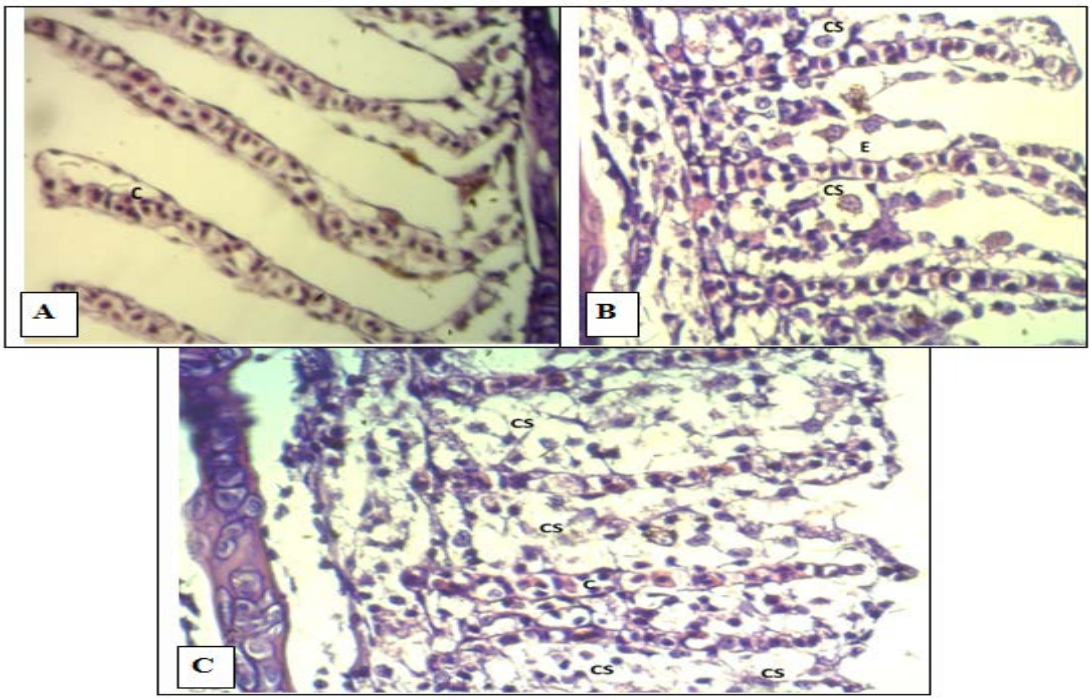

Figure 6. Histological changes of gill of *C. carpio* during 4 weeks [A] Gill section treated with 0.00039% concentration, show congestion (C). [B] Gill section treated with 0.0011% concentration, show cellular swelling (CS) and edema (E). [C] Gill section treated with 0.0011% concentration, show cellular swelling (CS) and congestion(C). X40. (H&E) stain.

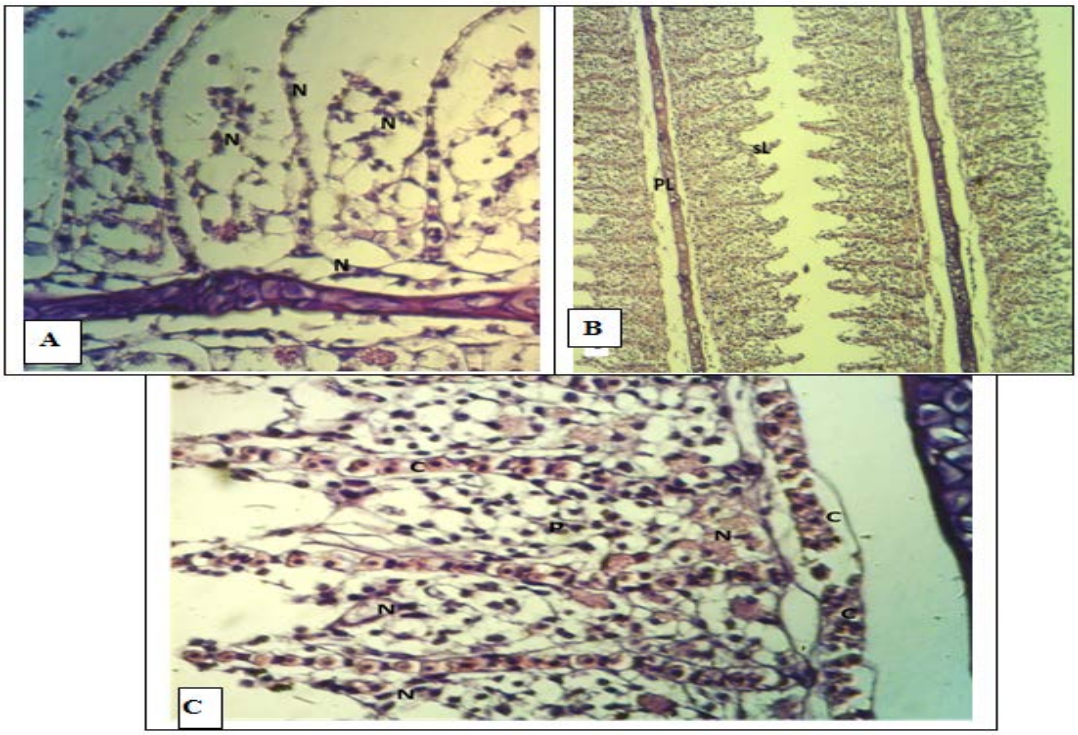

Figure 7. Histological changes of gill of *C. carpio* during 6 weeks [A] Gill section treated with 0.00039% concentration, show severs necrotic cells (N). [B, C] Gill sections treated with 0.0011% concentration, show thickened primary lamella (PL) and secondary lamella (SL), necrosis (N) and congestion (C). X40 (H&E) satin.

Kidney

Exposing fish to the pollutants leads to induce pathological changes in the kidney and liver (Adams et al., 2010**).** Normal kidney tissues (fig. 8- A) show renal tubules, glomerulus and collecting duct.

The histopathological changes in *C. carpio* during two, four and six weeks of exposure (fig. 8, 9, 10, 11) to different concentrations of WSF diesel, the changes were glomerular deterioration, dilation of Bowman's space, tubular occlusion by necrotic tissue, and sever tubular degeneration lead increasing in the diameter of renal tubules, accumulation of edematous fluid, distended glomerulus and Bowman space, aggregation of melanomacrophages, fibrosis, necrotic renal tubules and glomerular degeneration.

In 0.0011% concentration, similar changes in previous concentrations in addition to more necrosis of glomeruli showing deterioration and marked dilation of Bowman's space.

In present study, the main changes in kidney of WSF were tubular degeneration and dilation of Bowman's space, and these findings are in agreement with Haensly et al., (1982) who reported major changes in Pleuronectes platessa collected from two sites in U.K. and France after long term effects of crude oil spill during 1978- 1980. The kidney is one of the first organs to be affected by water contaminants. The necrosis occurred in renal tubules affects the metabolic activities and this promotes metabolic abnormalities in fish (Gabriel, 2007). Gusmao et al., (2012) found that renal changes have been less utilized as biomarkers of environmental pollution than gills and liver, but are also considered as an important biomarkers. In case of exposing to petroleum (WSF), the kidney changes of *Odontesthes argentinensis* were cytoplasmic vacuolation, degeneration, and necrosis in renal tubules, and these changes are related to the presence of toxic substances in the filtrate from the glomerulus.

In the study of Kakkar et al., (2011) that examined chronic toxicity of water soluble fraction of petrol on freshwater *Channa punctatus* has found that the kidney at lower concentration shows damaged blood vessels, necrosis of haemopoitic tissue and severe degenerative and necrotic changes in renal tubules while at higher concentration, it shows rupture of peritoneal lining, widening of tubules, space in the tissue, congestion and lymphocytic infiltration in kidney.

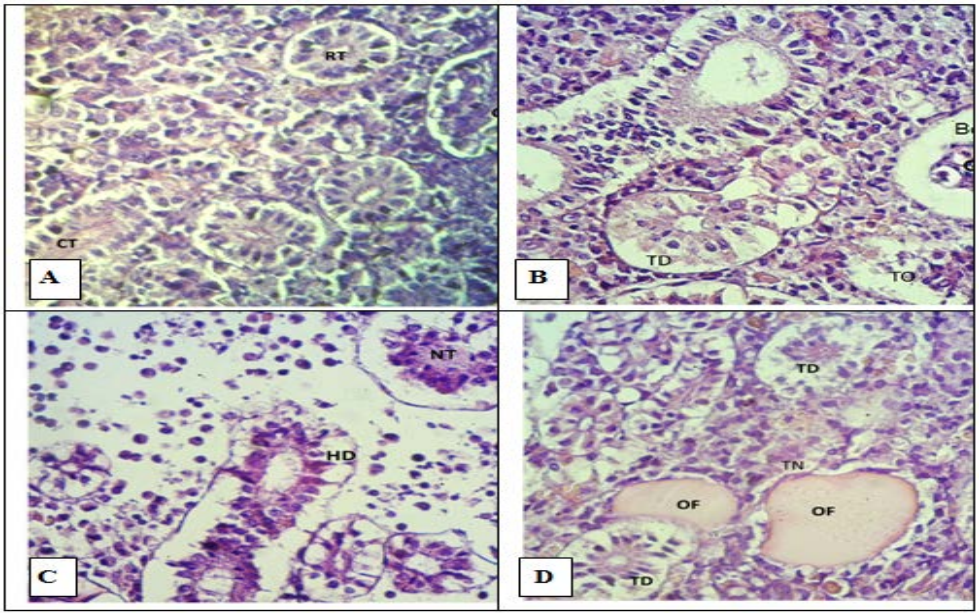

Figure 8. Histological changes of kidney of *C. carpio* during 2 weeks [A] Normal kidney: shows renal tubules (RT), glomerulus (G) and collecting duct CD. [B] Kidney treated with 0.00039% concentration, shows glomerular deterioration (GD), dilation of Bowman's space (Bc), tubular degeneration (TD) and tubular occlusion (TO). [C] Kidney treated with 0.00039% concentration, shows tubular occlusion by necrotic tissue (NT), and sever tubular degeneration lead increasing in the diameter of renal tubules (HD). [D] Kidney section treated with 0.00059% concentration, shows accumulation of edematous fluid (OF), tubular degeneration (TD), and tubular necrosis (TN).

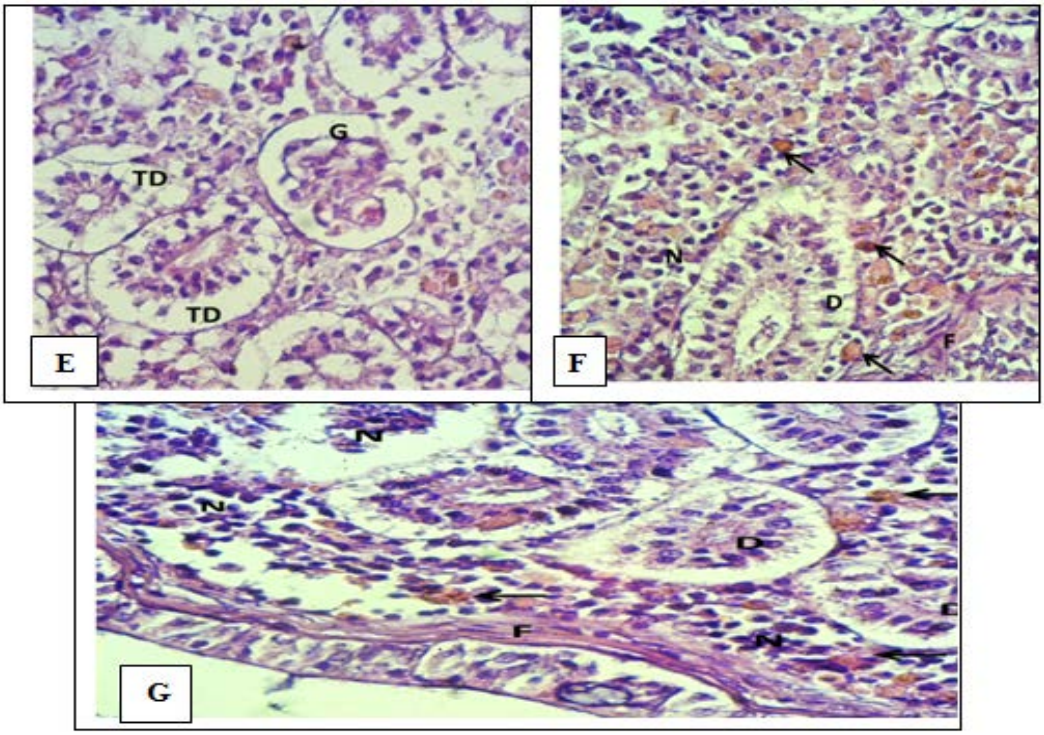

Figure 9. Histological changes of kidney of *C. carpio* during 2 weeks [E] Kidney section treated with 0.00059% concentration, shows distended glomerulus and Bowman space (G), tubular degeneration (TD) and aggregation of melanomacrophages (M). [F] Kidney section treated with 0.0011% concentration, shows degenerated renal tubules (D), fibrosis (F) and aggregation of melanomacrophages (arrows). [G] Kidney section treated with 0.0011% concentration, shows degenerated renal tubules (D) and aggregation of melanomacrophages (arrows) and necrotic renal tubules (N). X40, (H&E) stain.

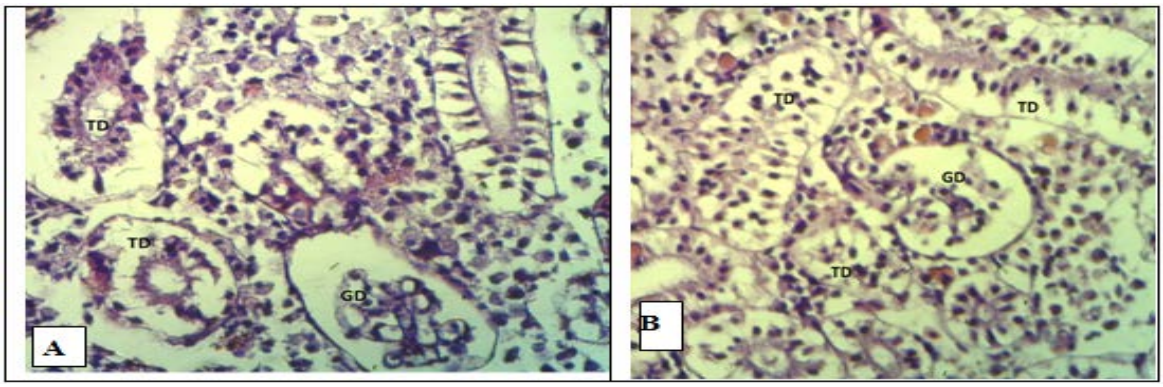

Figure 10. Histological changes of kidney of *C. carpio* during 4 weeks [A] Kidney section treated with 0.00039% concentration, shows tubular degeneration (TD), and glomerular degeneration (GD). [B] Kidney section treated with 0.0011% concentration, shows tubular degeneration (TD), and glomerular degeneration (GD). X40. (H&E) stain.

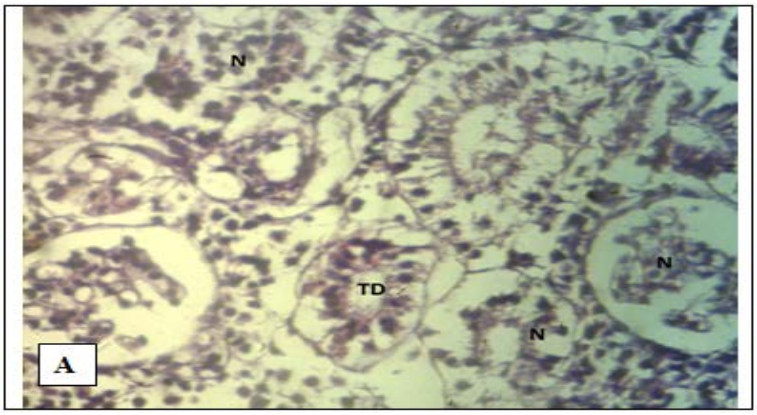

Figure 11. Histological changes of kidney of *C. carpio* during 6 weeks [A] kidney section treated with 0.00039% concentration, shows tubular degeneration (TD) and necrosis (N). X40. (H&E) stain.

Muscles

Fish muscles are commonly analyzed to determine contaminants concentrations and to evaluate the health risks because it is considered as main part consumed by humans (Begüm *et al*. 2005). Normal muscular tissue shows muscle fibers in (fig. 12- A).

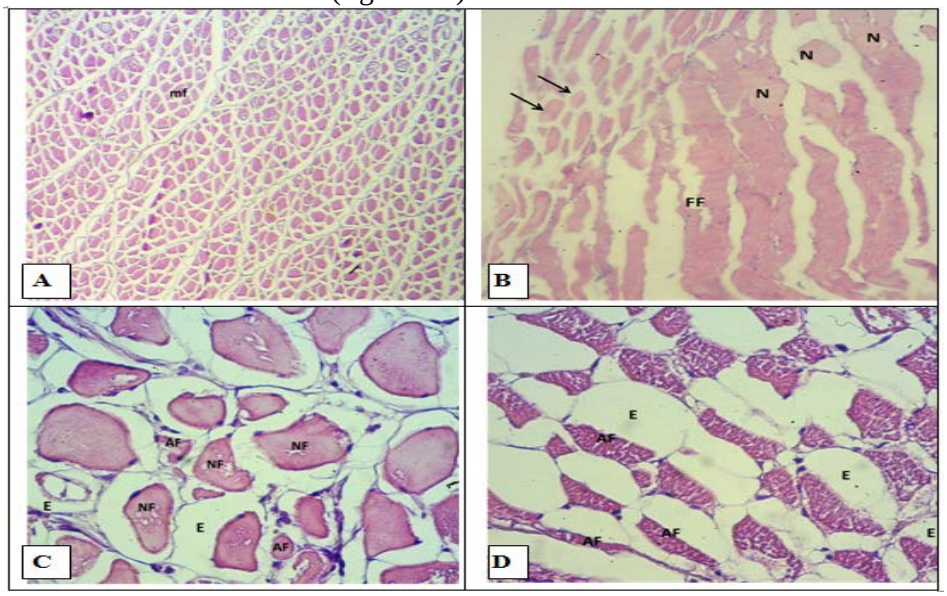

Figure 12. Histological changes of muscles of *C. carpio* during 2 weeks [A] Normal muscular tissue: shows muscle fibers (mf). X10. [B] Muscles section treated with 0.00039% concentration, shows

atrophy of muscle fiber (arrows), necrosis of muscle fibers (N) and fragmented of myofibrils (FF), X10. (H&E) stain. [C] Muscle section treated with 0.00059% concentration, shows necrosis of muscle fiber (NF), atrophied muscle fibers (AF) and edema (E), X40. [D] Muscles section treated with 0.00059% concentration, shows atrophy of muscle fiber (AF) and wide edematous spaces (E), X40 (H&E) stain.

Histopathological changes in *C. carpio* muscles during two, four and six weeks of exposure to different concentrations of WSF diesel fuel (Figures 12, 13 & 14), show atrophy of muscle fiber, necrosis of muscle fibers and fragmentation of myofibrils, edema and wide edematous. In 0.0011% concentration for six weeks, it shows generalized muscular depletion and magnified depleted muscular tissue (Figure 14. B, C).

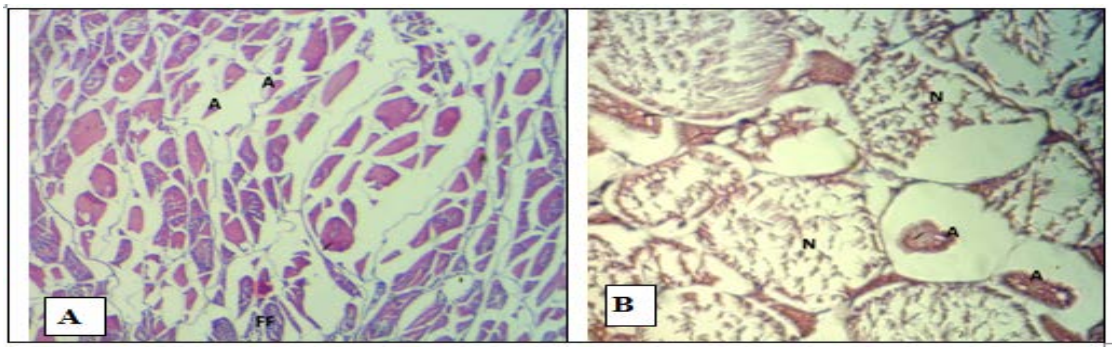

Figure 13. Histological changes of muscles of *C. carpio* during 4 weeks [A] Muscles section treated with 0.00039% concentration, show atrophy of muscle fiber (A), and fragmented of myofibrils (FF), X10. (H&E) stain. [B] (A) Muscles section treated with 0.0011% concentration, show atrophy of muscle fiber (A), and necrosis of fibers (N), X14. (H&E) stain.

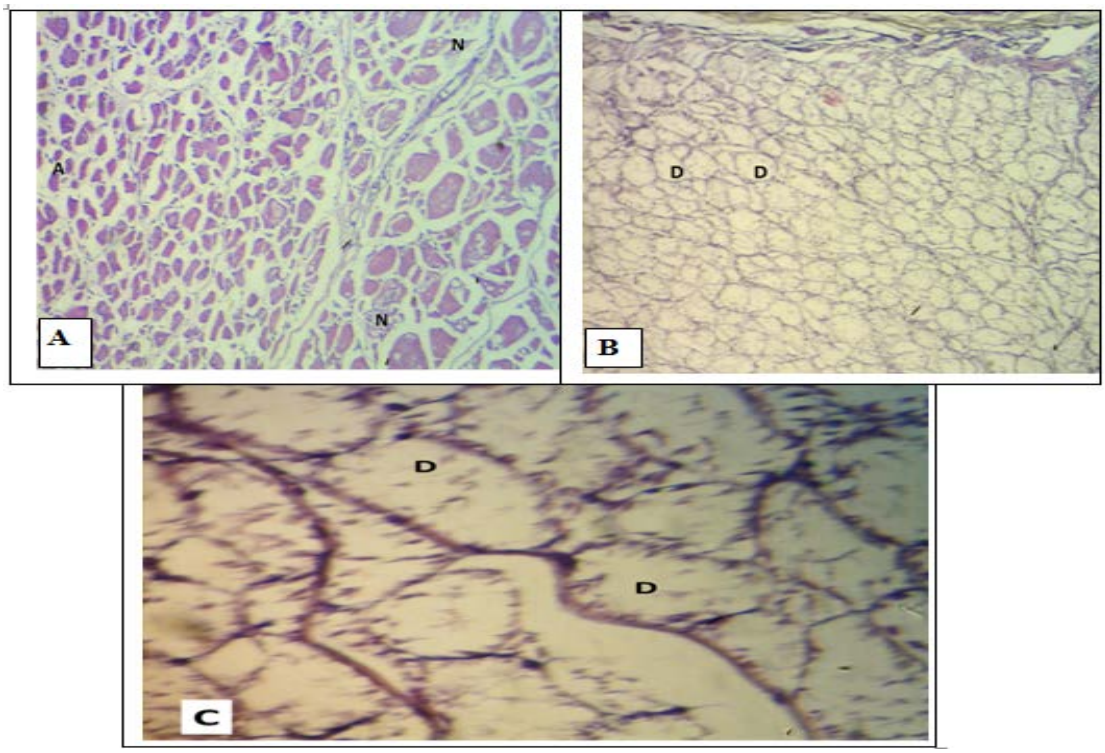

Figure 14. Histological changes of muscles of *C. carpio* during 6 weeks [A] Muscles section treated with 0.00039% concentration, shows necrosis of muscle fibers (N) and atrophy (A). X10. (H&E) stain. [B] Muscle section treated with0.00059% concentration, shows generalized muscular depletion (D). X10. [C] muscle section treated with 0.00059% concentration, shows magnified depleted muscular tissue (D). X40. (H&E) stain.

In study of Kazempoor et al., (2015), it has been found that the pathological lesions in muscles have increased after 16 day exposure to 16% WSF of Iranian crude oil in yellow fin sea bream fish due to infiltration of inflammatory cells and low degenerative disturbance of peripheral muscle fiber and might be as result of accumulated of oil derivatives in muscle tissue. The skeletal muscle of *Siganus canaliculatus and Epinephelus morio* were collected from different sites at Jeddah and Yanbou coast revealed minimal lesions which reflected in minimal effect of total petroleum hydrocarbon on the muscles, slight edema, hyalinization and rarely necrosis of some muscle fibers, were seen in addition few to focal aggregation of round cells among degenerated or necrotic muscle fibers (Afifi et al., 2014). Muscles fiber degeneration associated with food intake polluted with hydrocarbons due to the food intake consider as main way of hydrocarbons entry to fish body (Khan, 1995).

Conclusion

In present study the results explain the toxic effects of water soluble fraction of diesel fuel on *C. carpio*. The mortality of *C. carpio* increased with increaseing concentrations of WSF diesel fuel. *C. carpio* exposed to WSF diesel fuel induced behavioural response and histological alterations which dependent on the concentrations and the exposure time. Sublethal concentrations of WSF diesel fuel lead to damage in liver, gill, kidney and skeletal muscles. Therefore, leaching amount of diesel to aquatic environment result in effects on aquatic organisms and edible species which lead to effects on human health.

References

Adams DH, Sonne C, Basu N, Dietz R, Nam DH, Leifsson PS, Jensen AL, (2010) Mercury contamination in spotted seatrout, Cynoscion nebulosus: an assessment of liver, kidney, blood, and nervous system health. *Sci. Total Environ.*, **408**, 5808–5816.

Afifi M, Alkaladi A, Abu-Zinada O, Zahed NZ, (2014) Histopathological and ultrastractural alterations in skin, gills, liver and muscle of *Siganus canaliculatus and Epinephelus morio* caught from Jeddah and Yanbu coast as bio-indicators of oil hydrocarbons pollution. *Life Sci. J.*, **11**, 880-888.

Agamy E, (2012) Histopathological liver alterations in juvenile rabbit fish (*Siganus canaliculatus*) exposed to light Arabian crude oil, dispersed oil and dispersant. *Ecotoxicol. Environ. Saf.*, **75**, 171-179.

Al-Khafagy TYF, (2005) Oil pollution in the Tigris River water (Baghdad) and the impact of toxic gas oil in some vital and pathological indicators of Common Carp *Cyprinus carpio* L. Physiology. Ph.D. thesis, Baghdad University pp.118. (In Arabic).

Al-Sawafi AG, Yan Y, (2013) Bioconcentration and antioxidant status responces in Zebrafish (Danio rerio) under atrazine exposure. *Int. J. Chem. Eng. Appl*., **4**, 204-208.

Anderson JW, Neff JM, Cox BA, Tatem HE, Hightower GM, (1974) Characteristics of dispersion and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustacean and fish. *Mar. Biol*., **27**, 75- 88.

Awoyinka OA, Atulomah E, Atulomah NOS, (2011) Comparative effects of crude oil on juveniles *Clarias gariepinus* and *Clarias anguillaris*. *Int. J. Fish. Aquac*., **3**, 239-243.

Begüm A, Amin MN, Kaneco S, Ohta K, (2005) Selected elemental composition of the muscle tissue of three species of fish, *Tilapia nilotica, Cirrhina mrigala and Clarius batrachus*, from the fresh water Dhanmondi Lake in Bangladesh. *Food Chem*., **93**, 439-443.

Bourodimos EL, Carviomis C, (1990) Oil transport management and marine pollution control: oil spill prevention. In: *Environmental Problems and Solutions: Greenhouse Effect, Acid Rain, Pollution, Hemisphere*, New York, pp. 399-411.

Dede EB, Kaglo HD, (2001) Aqua-toxicological effects of water soluble fractions (WSF) of diesel fuel on *O. Niloticus* Fingerlings. J. Appl. Sci. Environ. Mgt., 5, 93-96.

Evans DH, Piermarini PM, Choe KP, (2005) The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation and excretion of nitrogenous waste. *Physiol. Rev*., **85**, 97-177.

Fanta E, Rios FS, Romao S, Vianna ACC, Freiberger S, (2003) Histopathology of the fish Corydoras paleatus contaminated with sublethal levels of organophosphorus in water and food. *Ecotoxicol. Environ. Saf.*, **54**, 119- 130.

FAO Food and Agriculture Organization (1987) Manual of methods in aquatic environment research, part 10-Short-term static bioassay. FAO Fish. Tech. pp. 247- 262.

Finney DJ, (1971) *Probit Analysis*. 3rd Edition, Cambridge University, Press, London. 333.

Furia RR, (2004) Preliminary aspects of water soluble fraction of diesel oil effects on gill histology of *Trachinotus* sp. P. 343-349 In: Sloman, K.; Wood, C. and Mackinlay, D. Behavior, physiology and toxicology interactions in fish. American Fisheries Society, United state. ISBN: 1-894337-42-5.

Gabriel UU, Ezeri GNO, Amakiri EU, (2007) Liver and kidney histopathology: biomakers of No.1 fuel toxicosis in African Catfish, *Clarias gariepinus*. J. Anim. Vet. Adv., 6, 379-384.

Gerhardt A, (2007) Aquatic behavioral ecotoxicology: Prospects and limitations. *Human Ecol. Risk Assess.*, **13**, 481–491.

Gusmao EP, Rodrigues RV, Moreira CB, Romano LA, Sampaio LA, Miranda-Filho KC, (2012) Growth and histopathological effects of chronic exposition of marine Pejerrey Odontesthes argentinensis larvae to petroleum water-soluble fraction (WSF). *AMBIO*., **41**, 456–466.

Haensly WE, Neff JM, Sharp JR, Morris AC, Bedgood MF, Boem PD, (1982) Histopathology of *Pleuronectes platessa* L. from Aber Wrac'h and Aber Benoit, Brittany, France: long-term effects of the *Amoco Cadiz* crude oil spill. J. Fish Dis., 5, 365- 391.

Hinton DE, Lauren OJ, (1990) Liver Structural Alterations Accompanying Chronic Toxicity in Fishes: Potential Biomarkers of Exposure. Biomarkers of Environ- mental Contamination. Lewis Publishers, MI, pp. 12–68.

Junrueira L, Carneiro J, (2003) Basic histology 10th ed. The Mc Graw-Hill companies, Inc, USA.

Kakkar PH, Saxena RM, Joshi M, (2010) Histopathological analysis of liver in *Puntius ticto* exposed to water soluble fraction (WSF) of petrol. *J. Appl. Nat. Sci.*, **2**, 290-292.

Kakkar PH, Saxena RM, Pandey M, (2011) Chronic toxicity of petroleum hydrocarbons on fresh water fish *Channa Punctatus* with special reference to biological parameters. *N Y. Sci. J.*, **4**, 116-120.

Kasumyan AO, (2001) Effects of chemical pollutants on foraging behavior and sensitivity of fish to food stimuli. *J. Ichthyol.*, **41**, 76–87.

Kazempoor R, Asl AHK, Motallebi AA, Alaie E, Marammazi GJ, Roshani A, (2015) Histopathological changes of water soluble fraction of Iranian crude oil in muscle of yellow fin sea bream (Acantopagrus latus). *Int. J. Biosci*., **6**, 451- 459.

Khan RA, (1995) Histopathology in Winter Flounder, *Pleuronectes americanus,* following chronic exposure to crude oil. *Bull. Environ. Contam. Toxicol*., **54**, 297- 301.

Lenartova V, Holovska K, Pedrajas JR, Martinezlara E, Peinado J, Lopezbarea J, (1997) Antioxidant and detoxifying fish enzymes as biomarkers of river pollution. *Biomarker*, **2**, 247-252.

Little EE, Finger SE, (1990) Swimming behavior as an indicator of sublethal toxicity in fish. *Environ. Toxicol. Chem.*, **9**, 13–20.

Mallatt J, (1985) Fish gill structural changes induced by toxicant and other irritants: a statistical review. C*an. J. Fish Sci*., **42**, 630- 648.

Mark B, (1992) *Solubility behavior of petroleum oil in water*. P.O. Box 4472, Station E. Ottawa, Ontario, Pp. 1-33.

Maton A, Jean H, Charles W, Susan J, Maryanna Q, David L, Jill D, (1993) *Human Biology and Health.* Prentice Hall. Englewood Cliffs, New Jersey, USA. Pp. 235-279.

Milstein A, (1992) Ecological aspect of fish species interactions in polyculture ponds. *Hydrobiologia,* **23**, 177-186.

Moreira CB, Rodrigues RV, Romano LA, Gusmão EP, Seyffert BH, Sampaio LA, Miranda- Filho KC, (2014) Genotoxicity and histological alterations in grey mullet. Mugil liza exposed to petroleum water-soluble fraction (PWSF). *Environ. Sci. Pollut. Res. Int.*, **21**, 5565- 5574.

Navaraj PS, Yasmin J, (2012) Toxicological impact of diesel fuel on the vital organs of *Oreochromis mossambicus. J. Chem. Bio. Phy. Sci., Sec. A,* **2**, 1870-1873.

Nelson JS, (1994) *Fishes of the World*. 3rd edition. John Wiley & Inc., NewYork, 600 pp.

OECD Organization for Economic Co-Operation and Development (1993) OECD G*uidelines for Testing of Chemicals*, Test No. 203: Fish Acute Toxicity Test. OECD, Paris, France.

Oliveira Ribeiro CA, Filipack NF, Mela M, Silva PH, Randi MAF, Costa JRA, Pelletier E, (2006) Hematological findings in neotropicalfish Hoplias malabaricus exposed to subchronic and dietary doses of methylmercury, inorganic lead and tributyltin chloride. *Environ. Resour*., **101**, 74–80.

Orlu E E, Ogbalu O K, (2013) Evaluation of the effect of water soluble fraction (Wsf) of bonny light crude oil and sublethal concentrations of Lepidagathis alopecuroides (Vahl) on reproduction in Clarias gariepinus (Burchell 1822). *Int. J. Anim. Veter. Adv*., **5**, 240-244.

Oruc EO, Sevgiler Y, Uner N, (2004) Tissue- specific oxidative stress response in fish exposed to 2,4-D and azinphosmethyl. *Comp. biochem. Physiol*., 137C, 43-51.

Pacheco M, Santos M A, (2001a). biotransformation, endocrine, and genetic response of Anguilla anguilla L. to petroleum distillate products and environmentally contaminated waters. *Ecotoxicol. Environ. Saf.*, **49**, 64-75.

Perez-Lopez M, Novoa-Valinas MC, Nlelgar-Riol MJ, (2002) Glutathione-S-transferase cytosolic isoforms as biomarkers of polychlorinated biphenyl experimental contamination in rainbow trout. *Toxicol. Lett.*, **136**, 97- 106.

Ramachandran SD, Sweezey MJ, Hodson PV, Boudreau M, Courtenay SC, Lee K, King T, Dixon JA, (2006) Influence of salinity and fish species on PAH uptake from Dispersed crude oil. *Mar. Pollut. Bull.* **52**, 1182- 1189.

Rand GM, Petrocelli SR, (1985) Fundamentals of aquatic toxicology: methods and applications. Hemisphere Publishing Corporation, New York.

Rodrigues RV, Miranda-Filho KC, Gusmão EP, Moreira CB, Romano LA, Sampaio LA, (2010) Deleterious effects of water-soluble fraction of petroleum, diesel and gasoline on marine pejerrey Odontesthes argentinensis larvae. S*ci. Total Environ*., **408**, 2054-2059.

Scott GR, Sloman KA, (2004) The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. *Aquat. Toxicol.*, 68, 369–392.

Sen A, Kirikbakan A, (2004) Biochemical characterization and distribution of glutathione-S-transferase in a leaping mullet (*Liza saliens*). *Biochemistry*, **69**, 993-1000.

Simonato JD, Guedes CLB, Martinez CBR, (2008). Biochemical, physiological, and histological changes in the neotropical fish *Prochilodus lineatus* exposed to diesel oil. *Ecotoxicol. Environ. Saf*., **69**, 112:120.

Soorena1 A, Ayoub YJ, Rezvanollah K, Mohammad AY, (2011) Effects of atrazine (herbicide) on blood biochemical indices of grass carp (*Ctenopharhyngoden idella*). *Journal of the Persian Gulf (Marine Science*), **2**, 51-56.

Thophon S, Kruatrachue M, Upatham ES, Pokethitiyook P, Sahaphong S, Jaritkhuan S, (2003) Histopathological alterations of white seabass, *Lates calcarifer,* in acute and subchronic cadmium exposure. *Environ. Pollut.*, **121**, 307– 320.

U.S. EPA United States Environmental Protection agency office of water (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth edition. Pennsylvania Avenue, NW, Washington, DC 20460.

Umejuru O, (2007) Juvenile Crawfish (Procambarus Clarkii) LC_{50} Mortality from south Covisiana crude, Peanut & mineral oil. Ph.d Thesis, Lovisiana state University. BS, Agricultural Science, Rivers, state College of Education, Port Harcourt, Nigeria.

Zhang JF, Shen H, Xu TL, Wang XR, Li WM, Gu YF, (2003) Effect of long-term exposure of low-level diesel oil on the antioxidant defense system of fish. *Bull. Environ. Contam. Toxicol.*, **71**, 234-239.