Effect Of Sublethal Concentrations Of Phenol On Histopathological Profile Of Fish *Ctenopharyngodon Idella* (Valenciennes, 1844)

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Abstract
The aromatic chemical phenol, which is frequently present in home and industrial effluents, is a cause for concern in toxicology around the world. Significant fish harm is seen when it enters aquatic ecosystems. This study used the gills, muscles, and gut of *Ctenopharyngodon idella* as biomarkers to examine the harmful effects of phenol in various organs. Histopathological investigations have shown high degree of pathological lesions. The examination of the gills indicated fusion, epithelial degradation, vacuolation, hyperplasia, and deformity at the tips of secondary lamellae. In the fish's intestine, clumping of adjacent villi made of columnar epithelium, inflammation, and tissue destruction at the epithelial site have all been seen as lesions. Infiltration of leucocytes, atrophy, breaking of muscle fibres, and vacuolation like changes in the muscles was observed.

Keywords: Phenol, Histopathology, Grass carp, Toxicity, Tissue damage

Introduction
Phenol and its compounds are considered as pervasive pollutants which are disposed into the natural water bodies as effluents from various chemical industries like textile, pulp mill, wood, phenol manufacturing, resin, paint, dyes, petrochemical, cool refineries and pharmaceuticals. As a result of this, many aquatic organisms are exposed and affected by these pollutants (Gad and Saad, 2008). Effluents which contain phenol are discharged directly or indirectly into water bodies such as lakes and rivers at a rate of 20-100 cubic meters per metric ton of product, adding to the toxicity of aquatic ecosystem (Gavrilescu, 2008; Petra *et al.*, 2015).
Histopathological alterations in several critical organs such as the liver, kidney, and gills were identified when *Labeo rohita* (a freshwater fish) was exposed to sublethal concentrations of phenol for 8 days (Butchiram *et al.*, 2013). Similarly, Ibrahem (2012) observed histopathological, behavioral and morphological variations in *Clarias gariepinus* (African catfish) when they were exposed to sub lethal concentration of phenol.
Fish gills serve as a key target organ for environmental pollutants in histopathological research because they are in direct contact with water and have a large surface area and high chemical absorption rates (Pandey *et al*., 2008).

Methodology
Collection of fish samples
Fingerlings of ***Ctenopharyngodon idella*** were collected from carp hatchery and training centre, Sherabad, Peshawar, Pakistan .The average length of fingerlings was 5.0cm and average weight of 8.0 gm. These fingerlings were transported in polythene bags to Department of Zoology, University of Peshawar. The fingerlings were placed in big water tube along with the bags in which they were brought. After conditioning the mouth of the bag was opened and the fingerlings were allowed to swim out of the bag.

Experimental design
A total of 52 fish were equally divided in 4 aquaria i.e. 14 fish in control, 12 fish in aquarium 1, 14 fish in aquarium 2 and 12 fish in aquarium 3. One aquarium was designed as Control while remaining 3 was designated as treatment groups. Air pumps were installed in aquaria to provide fresh oxygen. All the aquaria tops were enclosed with nets to avoid jumping out of fish from the media.

The fish were exposed to the treatment for 21 days. During this period the water was changed on daily basis (after each 24 hours) with the renewal of the phenol. The fish were fed 5% of their body weight. The fish behaviour was also accounted during these 21 days.

Histopathological procedure

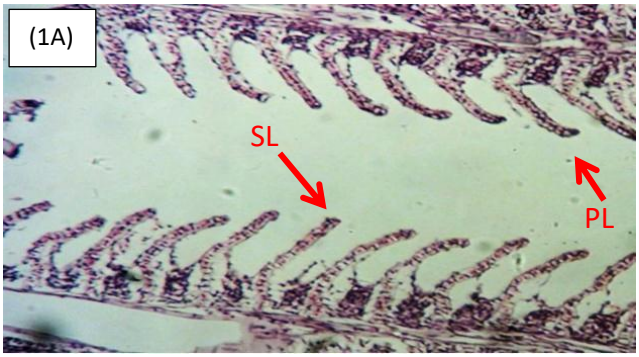
For histopathological study, 3 fish from each aquarium were randomly selected on day 7,14 and 21 and were dissected for isolation of organs including gills, muscles and intestine. Standard histopathological procedure was followed and dissection was done by using sterilized equipments. The isolated organs were fixed in 10% formalin solution. After fixation the tissues were carried out of formalin, cut into smaller pieces and placed in labelled tissue cassettes. After putting the tissues in cassettes they were washed for 10 hours by keeping under water tap. The washed tissues were dehydrated by passing through various grades of alcohol and cleared with xylene and infiltrate in paraffin.

The cleared and infiltrate tissues were embedded in paraffin wax. The embedding was done with Automatic Embedding Assembly. After blocks formation thin sections were obtained by slicing through microtome machine. The thin sections were placed in water bath for stretching in order to avoid wrinkles in ribbon. The thin sections were then mounted on clean glass slides using egg albumin as the section adhesive. The mounted slides were kept on hot plate for adhesion of tissue to the slide by melting paraffin at 55^{o}C for one hour. The tissue sections were stained with haematoxylin and eosin through an automatic staining machine by using the staining protocol.

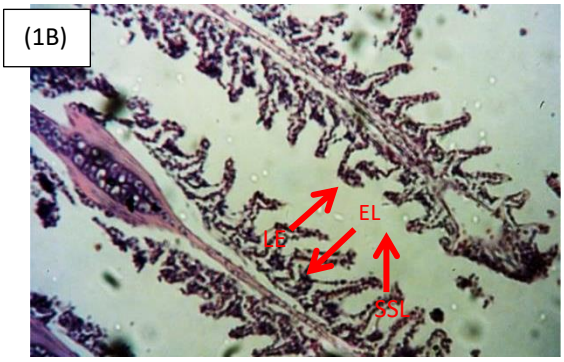
After staining slides were cleaned to remove impurities. The stained section was covered with glass cover slip by applying DPX (Distyrene Plasticizer Xylene) to make them permanent. The slides were observed under microscope, and their images were obtained using camera fitted microscope.

Results

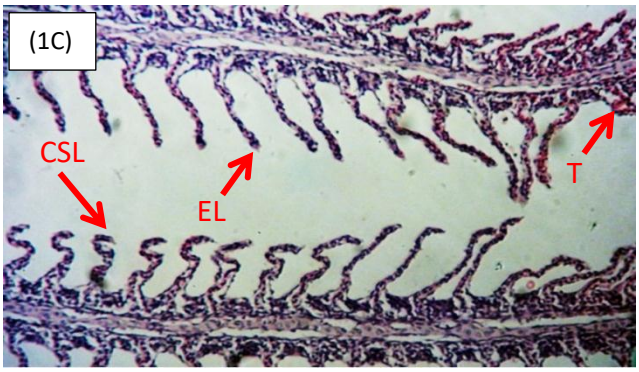
Fish treated to different amounts of phenol (0.37 mg/l, 0.48 mg/l, and 0.57 mg/l) experienced noticeable pathological alterations in their gills compared to controls (Fig. 1A). The changes included; epithelial lifting, loss of epithelium and shortening of secondary lamellae (Fig. 1B). Damages of the gills at concentration (0.48mg/l) were much more extensive with curling of secondary lamellae, epithelial lifting and telangiectasia (Fig. 1C) compared to (Fig. 1B). Also the damages of the gills at concentration (0.57mg/l) were more severe with interlamellar space, curling of lamellae, hyperplasia between interlamellar space, apical fusion of secondary lamellae and apical lifting (Fig. 1D) as compared to all previous concentrations mentioned.\



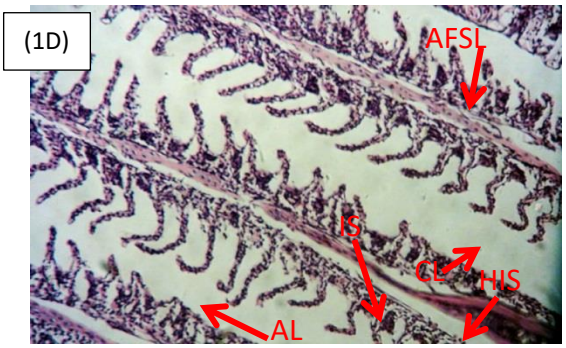
SL-Secondary lamellae
PL-Primary lamellae



EL-Epithelial lifting
LE-Loss of epithelium
SSL- Shortening of secondary lamellae

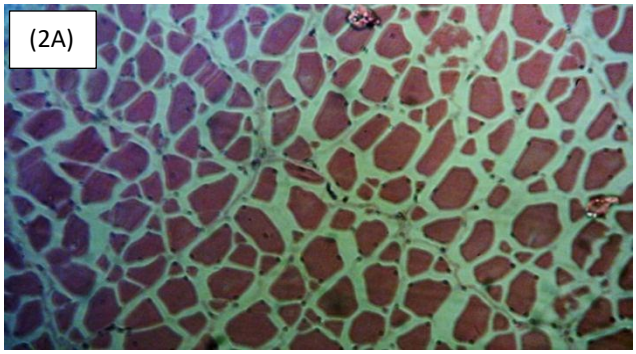


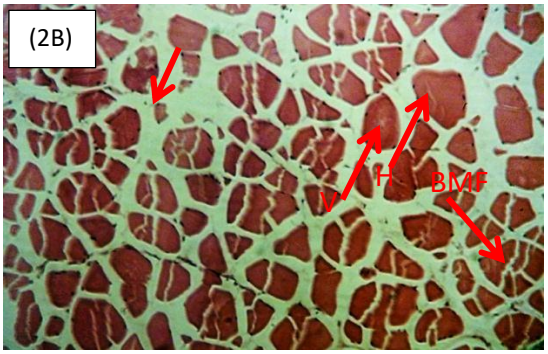
CSL-Curling of secondary lamellae
EL-Epithelial lifting
T-Telangiectasia



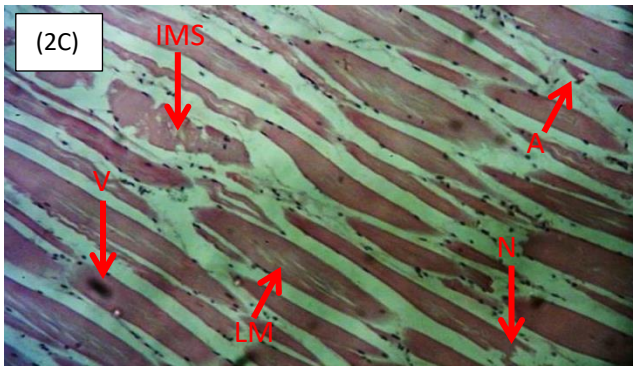
IS-Interlamellar space
CL-Curling of lamellae
HIS-Hyperplasia between interlamellar space
AFSL-Apical fusion of secondary lamellae
AL-Apical lifting

Under concentration (0.37mg/l) exposure, the breaking of muscle fibre, atrophy, hypertrophy and vaculation were obsersved (Fig. 2B) in comparison to control histology (Fig. 2A). Under concentration (0.48mg/l) exposure, loss of myofibril, atrophy, necrosis, vacuolation, intra myofibril spaces were observed (Fig. 2C). Also the damages of the muscle at concentration (0.57mg/L) include infiltration of leucocytes, atrophy, breaking of muscle fibers and vacuolation were observed as mentioned in (Fig. 2D).

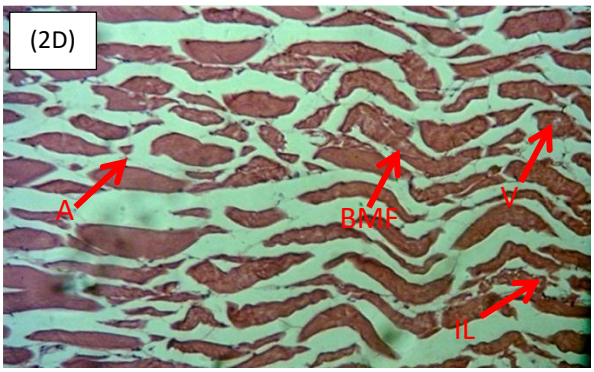




BMF-breaking of muscle fibre
A-Atrophy
H-Hypertrophy
V-vaculation

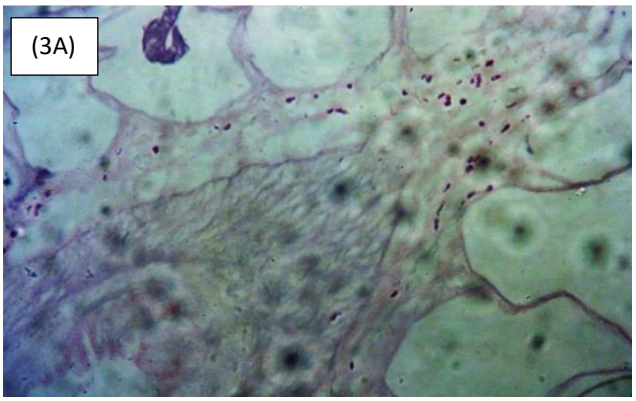


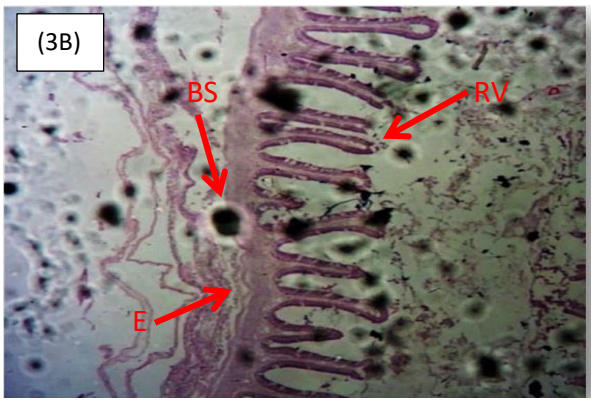
LM-Loss of myofibril
A-Atrophy
N-Necrosis
V-Vaculation
IMS-Intra myofibril space



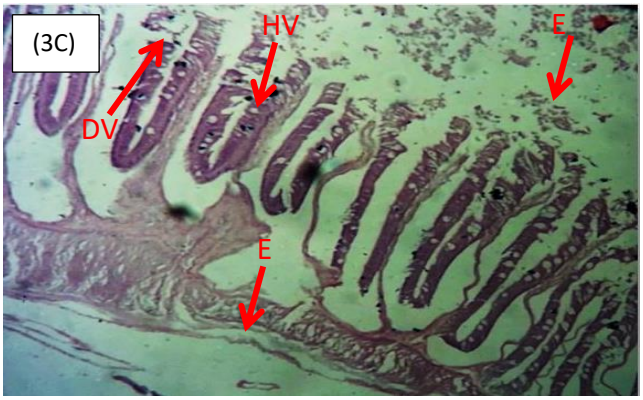
IL-Infilteration of leucocytes
A-Atrophy
BMF-Breaking of muscle fibre
V-Vaculation

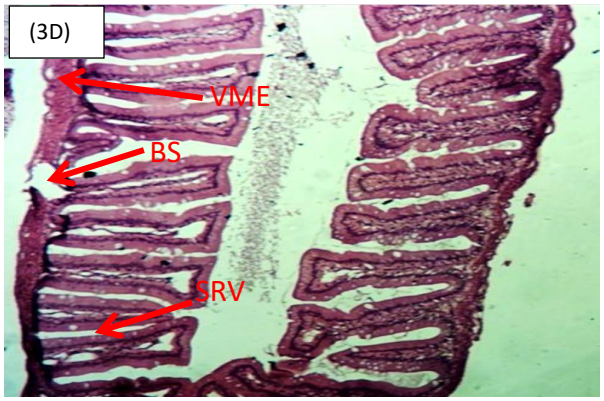
The intestine exposed to (0.37 mg/L) concentration underwent the following histological changes: Breaking of serosa, edema between mucosa and sub mucosa and rupturing of villi (Fig. 3B) as compared to control cells of intestine (Fig. 3A). Under (0.48 mg/L) concentration exposure, the changes observed exudates, degeneration of villi, edema between mucosa and sub mucosa and hypertrophy of villi (Fig. 3C). The changes observed at concentration (0.57mg/L) include, breaking of serosa, shrunken and reduced villi and vacuolation of muscularis externa as mentioned in (Fig. 3D).





BS-Breaking of serosa
E-Edema between mucosa and sub mucosa
RV-Rupturing of villi





E-Exudates
DV-Degeneration of villi
E-Edema between mucosa and sub mucosa
HV-Hypertrophy of villi

BS-Breaking of serosa
SRV-Shrunken and reduced villi
VME-Vaculation of muscularis externa

Discussion

Significant pathological alterations, such as epithelial hyperplasia with lamellar fusion and epithelial hypertrophy, were observed in the gills of fish exposed to phenol, as well as severe degeneration of the primary and secondary gill lamellae and lamellar disorganization. These observations are similar to Butchiram *et al.* (2012). According to Tilak *et al*. (2006), phenol is acutely poisonous to *Catla catla*. We found similar effects on *Ctenopharyngodon idella*, a freshwater cyprinid, in which the epithelial layer of the gills swelled in case of mild damage, and detached, in cases of severe damage.

Given that phenol is rapidly metabolised by fish tissues (Solem *et al.*, 2003). The primary target of the contaminants is thought to be the fish's gills, which are involved in many vital processes like respiration, osmoregulation, and excretion. These organs are in constant touch with the outside environment and are very sensitive to changes in the water's quality (Mazon *et al.*, 2002; Poleksic and Mitrovic-Tutundzic, 1994; Fernandes and Mazon, 2003).

Respiratory issues may have caused fish to gulp air while swimming at the water's surface. Lamellar fusion and hyperplasia were symptoms of serious gill injury. Defence mechanisms include changes like epithelial lifting, hyperplasia, and hypertrophy of the epithelial cells, as well as partial fusion of some secondary lamellae. By increasing the distance between the blood and the external environment, these modifications serve as a barrier against the introduction of pollutants (Butchiram *et al.*, 2009; Akaishi *et al.*, 2004; Fernandes and Mazon, 2003).

These modifications were non-specific and could have been caused by various pollutants. The oxygen uptake is compromised because of the greater distance created by epithelial lifting between water and blood. Additionally, hyperplasia may lessen the gills' secretory and excretory activities (Tilak *et al.*, 2006). Gill filaments' epithelial membrane, secondary lamellae, and, in extreme situations, the epithelial wall, were all enlarged. Asphyxia was most likely the cause of death and was likely brought on by the disintegration of the gill structure. The extreme reactions was observed in deadly groups, with tissue destruction that in some cases would have resulted in death very quickly due to the accompanying drastic reduction in respiratory surface. The various muscle cell types that make up muscular tissues and transmit and produce power, including skeletal, cardiac, and smooth muscle cells. The striated muscles make up between 40% and 75% of the weight of an adult fish (Carani *et al*., 2008).

The majority of a fish's edible portions come from its skeletal muscles, and striated muscle also includes muscles involved in fin movement (Hibiya, 1982). Degenerative features such muscle atrophy, fractured myofibrils, swelling sarcolemma, and sarcoplasmic reticulum have been characterised as indications of exposure to toxins like pesticides and metals. Muscle injuries are potentially inducible markers of environmental contamination (Pearse, 1985).

In the present study various effects of phenol on the histology of fish muscles including infiltration of leucocytes, atrophy, breaking of muscle fibres, and vacuolation were observed as similar to the study of Pearse (1985).

Edema between mucosa and sub mucosa and rupturing of villi, breaking of serosa, shrunken and reduced villi and vacuolation of muscularis externa in the intestine of fish was observed in present study is almost similar to the study of Hossein (1986), who assessed that intestine in mild damages, the goblet cells of the epithelial layer were less abundant and in some cases were completely destroyed.

Conclusion

Present study revealed that phenol, if present even in sub lethal concentration, have toxic effects on aquatic fauna especially fishes. Histopathological studies revealed that modifications occur in the structural patterns of gills, intestine

and muscles. These histological alterations may be considered as a helpful tool for bio monitoring of various toxicants in the aquatic environment. It is recommended that use of phenolic compounds be restricted in industries and effluents be treated before release into the environment.

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