

## Physiological and Histopathological Alterations Induced by Phenol Exposure in *Oreochromis aureus* Juveniles

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Received 23 December 2006  
Accepted 22 June 2007

### Abstract

In the present study, the impacts of phenol as an environmental pollutant of the inland water habitat was studied on *Oreochromis aureus* (*O. aureus* juveniles. The fish of both sexes were subjected to three sub-lethal concentrations of phenol (20%, 40% and 80% of LC<sub>50</sub>) for seven days. The hepatosomatic index (HSI) was markedly increased, whereas the gonadosomatic index (GSI) was significantly reduced in the fish subjected to phenol. Some enzymes activities and metabolites were assessed in the liver, gills and muscle. The assayed enzymes are alanine and aspartate amino transferases (ALAT and ASAT) and lactate dehydrogenase (LDH). The measured metabolites are the glucose and total proteins content. It was observed increased enzymes activities in fish exposed to phenol. The total liver protein content was significantly reduced, which indicates tissue proteolysis; i.e., phenol induced protein consumption. Also, the liver glucose and glycogen were reduced. The liver of fish subjected to phenol showed high score of histopathological symptoms as inflammation, central necrosis and cell degeneration. These results indicated that phenol intoxication had antagonistic effects of fish health.

*Key words:* *Oreochromis aureus*, Phenol, Metabolism, Enzymes, Pollution, Histopathology.

### Introduction

Phenol and phenolic compounds are xenobiotics stressful environmental factors to which animals are subjected to, and have become environmental problem due to anthropogenic impact on the environment (Hori *et al.*, 2006). They also are good research models of wide spread xenobiotics (Roche and Boge, 2000). Also, they are commonly present in industrial wastewaters and in non-specific pesticides, herbicides, bactericides and fungicides (Gupta *et al.*, 1983). Mukherjee *et al.* (1990) reported that they are commonly found in the marine habitat and in fish tissues. Phenol induces toxic effects for fish health. They induce genotoxic effect (Jagetia and Aruna, 1997), carcinogenic effect (Tsutsui *et al.*, 1997), and immunotoxic effect (Taysse *et al.*, 1995). Controversy, Stich (1991) reported that phenol may act as free radical scavengers and prevent genetic damage caused by other agents. They have a high bioaccumulation rate along the food chain due to its lipophilicity. Thus phenol pollution presents a threat against natural environment and also to human health (Hori *et al.*, 2006). When the phenol is present in the aquatic environment, fish food consumption, mean weight and fertility are significantly reduced (Saha *et al.*, 1999). For these reasons, phenol intoxication must be taken in consideration in the fish farming systems and also in natural aquatic habitat.

The hepatosomatic index (HSI) of the fish has been used as an indicator of environmental risk (Pinkney *et al.*, 2001; Yang and Baumann, 2006). They found a positive correlation between HSI and

the concentration of polycyclic aromatic hydrocarbons (PAHs) metabolites in fish. On the other hand, Zha *et al.* (2006) pointed out that pentachlorophenol (PCP) is an endocrine disrupting chemical (ECD) for Japanese meaka (*Oryzias latipes*). They also found decreased fecundity and fertility of female fish subjected to PCP. Several authors reported that the phenolic compounds (as PAHs, PCP) are EDCs (Kashiwada *et al.*, 2002; Pait and Nelson, 2003; Tollefsen, 2006; Barse *et al.*, 2006; Martin-Skilton *et al.*, 2006)

Fish metabolism was adversely affected by phenol (Gupta *et al.*, 1983; Abdel-Hameid, 1994). The phenol and its derivatives can alter protein metabolism by altering transamination rate of amino acids by enhancing the activity of aspartate aminotransferase (ASAT, EC 2.6.1.1) and alanine aminotransferase (ALAT, EC 2.6.1.2). Also, the carbohydrate metabolism was affected by phenol by altering the activity of lactate dehydrogenase (LDH, EC 1.1.1.27) thus, affecting the interconversion of lactate into puruvate (Hori *et al.*, 2006). Gupta *et al.* (1983) recorded changed ASAT and ALAT activities in different fish tissues induced by phenolic compounds. The enzymes activities (ASAT or GOT and ALAT or GPT) catalyze the interconversion of amino acids and  $\alpha$ -keto acids by transfer of amino groups. The ASAT catalyzes the transfer of this group from aspartate to  $\alpha$ -ketoglutarate to form glutamate and oxaloacetate, while ALAT catalyzes the transfer of the amino group from alanine to  $\alpha$ -ketoglutarate to form glutamate and puruvate (Moss *et al.*, 1986). The measurement of transaminase activities in serum is

frequently used as a diagnostic tool in human and animals (Bernet *et al.*, 2001; Barse *et al.*, 2006). Damage to the liver, kidney and gills is evident from elevated transaminase activities (Bernet *et al.*, 2001).

In Egypt, extensive use of chemicals in a wide broad of industrial activities such as textile, pesticides, fertilizers, petrochemical, cement, paper and pulp, and food processing. About 50% of industrial activities are localized in Cairo and 40% in Alexandria. The rest is in Delta and Upper Egypt (Barakat, 2004). Most of the industrial activities and human activities are based on River Nile. Therefore, water pollution is generated from disposing the industrial hazardous wastes. The environmental assessment of phenolic compounds in River Nile showed elevated levels of these pollutants at Kafr El Zayat city (El-Gendy *et al.*, 1991). The population of tilapia fish is highly dense in the vicinity of the River Nile. It is also so popular for the Egyptians. Therefore, the objective of the present study is to detect the metabolic and histopathological changes of blue tilapia (*O. aureus*) juveniles induced by exposure to phenol.

## Materials and Methods

### Experimental Groups

Blue tilapia (*O. aureus*) juveniles (Teleostei: Cichlidae) measuring  $8.59 \pm 0.70$  cm in total length and weighing  $10.11 \pm 1.01$  g were gifted from central laboratory for aquaculture research (CLAR) Abassa, Abouhammad, Sharkya, Egypt. The fish were acclimated for one week in large cement aquaria ( $1 \text{ m}^3$ ) in fish biology laboratory, faculty of science, Benha University. Thereafter, the fish were divided into four glass aquaria (40 cm height, 70 cm length and 30 cm width) each containing seven males and seven females. Static experimental system was conducted at a constant water temperature ( $23 \pm 0.5^\circ\text{C}$ ) and normal photoperiod during June 2006. The lethal concentration for 50% of fish for 96-h (96-h  $\text{LC}_{50}$ ) of phenol was found to be 29 mg/L (Abdel-Hameid, 1994). Fish were subjected to three sub-lethal concentrations of phenol (20%, 40% and 80% 96-h  $\text{LC}_{50}$ ) for one week. Those in the fourth aquarium were considered as a control group. Phenol concentrations remained constant throughout the time course of the experiment, it was renewed every 12 h as 30% of phenol has lost by volatilization during this period (Hori *et al.*, 2006). The fish were fed on standard fish diet every 12 h, the remaining food was siphoned. After one week from the beginning of the experiment, the fish were decapitated, weighed and sacrificed. The liver, gills and white epiaxial muscle were isolated from each fish.

### Somatic Indices

Liver, gonad and intestine were weighed and

also the fish were weighed without gut (gutted weight). The somatic indices, hepatosomatic index (HSI), gonadosomatic index (GSI) and intestinosomatic index (ISI) were computed as a ratio of the organ weight to the gutted weight.

### Biochemical Analysis

The fish tissues were homogenized in cold distilled water using glass homogenizer. The tissues homogenates were centrifuged twice (4000 rpm for 5 min). The final supernatants were used for the enzyme assays and metabolites determination. The ASAT and ALAT activities were assayed colorimetrically following the method of Reitman and Frankel (1957). LDH activity was assayed kinetically at 340 nm (Hochachka *et al.*, 1978). The following tissue metabolites were determined by the total protein content with reaction of copper sulphate in alkaline medium (Henry, 1964), glucose with glucose oxidase reaction (Trinder, 1969) and glycogen with alkaline digestion of the tissue (Oser, 1979) followed by enzymatic measurement of glucose.

### Histopathology

After dissecting the fish, small pieces of the liver were fixed in neutral buffered formalin. Then, it was dehydrated and embedded in paraffin. The liver sections of 4 mm were stained with hematoxylin and eosin. Histopathological activity index (HAI) was scored by examining five fields (at 400x) for each slide. Portal inflammation, central necrosis and degeneration are histopathological signs graded as zero: zero findings; 1: evidence of pathological signs; 2: mild pathological signs; 3: moderate pathological signs and 4: marked pathological signs (Park *et al.*, 2005).

### Statistical Analysis

Statistical analyses of the raw data were computed using SPSS (version 10). The data of this work are presented as mean  $\pm$  standard deviation. Significant differences between the control and experimental groups were computed using two paired student t-test (Pipkin, 1984).

## Results

### The Somatic Indices

The HSI values of the fish subjected to phenol polluted water were generally increased. It was non-significantly differed in male fish subjected to low (20% 96-h  $\text{LC}_{50}$ ) phenol, whereas significant difference was observed in case of females subjected to this phenol level. The medium (40% 96-h  $\text{LC}_{50}$ ) and high (80% 96-h  $\text{LC}_{50}$ ) phenol levels induce significant increase of the HSI of both sexes. Phenol

exposure induces general reduction of GSI of both sexes of blue tilapia juveniles. Non-significant increase was reported for male fish subjected to 20% 96-h LC<sub>50</sub> of phenol, whereas significant reduction was observed in case of male fish subjected to 40% and 80% 96-h LC<sub>50</sub> of phenol. The GSI of the female fish was significantly reduced in all tested groups (Table 1).

The fish exposed to phenol polluted water exhibited hypoactivity and loss of appetite. This is clearly evident in fish subjected the high phenol concentration (80% 96-h LC<sub>50</sub>). This was also supported by the observed reduction of the ISI (Table 1). The ISI was generally reduced after phenol exposure. The data showed non-significant difference in case of fish subjected to 20% 96-h LC<sub>50</sub> of phenol, whereas significant reduction of ISI was reported for both sexes of fish subjected to medium and high phenol levels.

There was a positive correlation between GSI and ISI of both sexes ( $r=0.75$  &  $r=0.607$  for male and female, respectively). The correlation between HSI and GSI was found to be positive for male ( $r=0.23$ )

and negative for female ( $-0.143$ ). The reverse is true for the correlation between HSI and ISI.

### Carbohydrate Metabolism

The glucose content in the liver, gills and muscle of blue tilapia juveniles generally reduced due to phenol exposure (Table 2). The changes in glucose content were found to be concentration-dependent. All tissues have significantly reduced glucose content due to phenol exposure, except the liver of female fish subjected to low phenol level which showed non-significant reduction. Meanwhile, positive correlation was found between tissue glucose content, being positive correlation between the gills and muscle of the female fish ( $r=0.76$   $P < 0.05$ ). On the other hand, the liver glycogen content reduced significantly due to exposure to 40% and 80% 96-h LC<sub>50</sub> of phenol (Table 3). The low phenol concentration induced non-significant alterations of the liver and muscle glycogen content. Conversely, the muscle glycogen content was elevated as a result of exposure to 40% and 80% 96-h LC<sub>50</sub> of phenol.

**Table 1.** Effect of different concentrations of phenol on hepatosomatic index (HSI, %), gonadosomatic index (GSI, %) and intestinosomatic index (ISI, %) of *O. aureus* juveniles

Groups	HSI		GSI		ISI	
	Male	Female	Male	Female	Male	Female
Control	4.07±0.13	4.29±0.25	2.27±0.28	6.19±0.20	18.73±0.41	21.90±0.93
20%96-h LC <sub>50</sub>	4.28±0.57	4.78±0.35*	2.06±0.91	5.66±0.53*	18.58±0.51	21.71±1.13
40%96-h LC <sub>50</sub>	5.23±0.19*	5.47±0.47*	1.38±0.24*	4.49±0.35*	16.38±0.47*	18.55±1.29*
80%96-h LC <sub>50</sub>	5.88±0.35*	6.15±0.33*	1.42±0.16*	3.61±0.17*	14.63±3.23*	16.35±0.95*

All data are mean of seven fishes. Data are expressed as mean± standard deviation. Significant differences are marked by (\*) at  $P < 0.05$ .

**Table 2.** Effect of different concentrations of phenol on glucose content (mg/g fresh tissue) in the tissues of *O. aureus* juveniles

Groups	Liver		Gills		Muscle	
	Male	Female	Male	Female	Male	Female
Control	27.25±0.47	26.52±0.34	2.56±0.20	3.61±0.13	10.57±0.26	9.62±0.28
20%96-h LC <sub>50</sub>	26.12±0.50*	26.10±0.31	2.41±0.17*	3.40±0.17*	9.12±0.26*	7.40±0.36*
40%96-h LC <sub>50</sub>	23.22±0.38*	22.12±0.57*	2.01±0.14*	2.81±0.18*	8.13±0.16*	6.42±0.32*
80%96-h LC <sub>50</sub>	21.92±0.84*	16.34±0.42*	1.11±0.16*	2.01±0.12*	7.10±0.19*	5.52±0.27*

All data are mean of seven fishes. Data are expressed as mean± standard deviation. Significant differences are marked by (\*) at  $P < 0.05$ .

**Table 3.** Effect of different concentrations of phenol on glycogen content (mg glucosyl glucose/g fresh tissue) in the tissues of *O. aureus* juveniles

Groups	Liver		Muscle	
	Male	Female	Male	Female
Control	0.861±0.015	0.832±0.021	0.262±0.031	0.247±0.016
20%96-h LC <sub>50</sub>	0.852±0.025	0.821±0.012	0.252±0.080	0.236±0.09
40%96-h LC <sub>50</sub>	0.712±0.022*	0.621±0.013*	0.202±0.071*	0.213±0.049*
80%96-h LC <sub>50</sub>	0.545±0.013*	0.521±0.014	0.192±0.042*	0.185±0.056*

All data are mean of seven fishes. Data are expressed as mean± standard deviation. Significant differences are marked by (\*) at  $P < 0.05$ .

The activity of LDH in the examined tissues was generally elevated (Table 4). The LDH was necessary to convert lactate into glucose. Significant results were recorded for fish exposed to 40% and 80% 96-h LC<sub>50</sub> of phenol.

### Protein Metabolism

The total protein content in the liver and gills showed reduced levels due to phenol exposure (Table 5). It is a concentration-dependent effect, i.e., the lowest inhibition due to the high phenol concentration. The low phenol level induces non-significant reduced protein level, except the liver of male fish which showed significant reduction. On the contrary, the muscle protein was markedly increased in the fish groups subjected to phenol polluted water. It was found non-significant rises in fish subjected to the low phenol level, whereas significant enhanced protein levels were recorded for fish subjected to the medium and high phenol levels.

It was recorded marked and enhanced ASAT activities in the examined tissues of the fish subjected to all phenol levels (Table 6). The low phenol level

induced non-significant increased enzyme activity, whereas the medium and high phenol levels induced a significant effect.

The ALAT activity in the liver of fish subjected all tested phenol levels showed a marked reduction (Table 7). It significantly differed from the control group, when the fish subjected to 40% and 80% 96-h LC<sub>50</sub> of phenol. The enzyme activity in the gills and muscle was generally increased. The 20% 96-h LC<sub>50</sub> of phenol induced non-significant increased ALAT activity in the gills and muscle, except the enzyme activity in the gills of male fish which showed significant difference. The medium and high phenol levels induced significant rise of enzyme activity in the gills and muscle (Table 7).

### Histopathology

The liver of the control fish showed no histopathological signs (Table 8 and Figure 1). The liver of fish subjected to the low, medium and high phenol levels showed histopathological signs (Figure 2, 3, and 4). These signs include inflammation around

**Table 4.** Effect of different concentrations of phenol on LDH activity (Units/min/g fresh tissue) in the tissues of *O. aureus* juveniles

Groups	Liver		Gills		Muscle	
	Male	Female	Male	Female	Male	Female
Control	1.71±0.41	1.69±0.36	1.50±0.10	1.79±0.13	90.27±1.31	89.21±1.24
20%96-h LC <sub>50</sub>	1.82±0.46	1.86±0.57	1.61±0.21	1.89±0.14	91.62±1.71	90.19±2.14
40%96-h LC <sub>50</sub>	2.51±0.49*	2.31±0.57*	2.59±0.42*	2.79±0.15*	100.12±1.28*	98.12±0.96*
80%96-h LC <sub>50</sub>	3.72±0.52*	2.86±0.76*	3.94±0.23*	4.01±0.24*	118.12±1.21*	119.13±0.64*

All data are mean of seven fishes. Data are expressed as mean± standard deviation. Significant differences are marked by (\*) at P<0.05.

**Table 5.** Effect of different concentrations of phenol on total protein content (mg/g fresh tissue) in the tissues of *O. aureus* juveniles

Groups	Liver		Gills		Muscle	
	Male	Female	Male	Female	Male	Female
Control	83.08±2.42	70.12±2.16	35.12±1.94	30.12±0.86	17.52±0.61	18.52±0.92
20%96-h LC <sub>50</sub>	81.12±1.36*	70.01±2.29	34.12±0.86	30.04±1.02*	18.04±0.97	18.92±0.87
40%96-h LC <sub>50</sub>	70.88±3.69*	56.12±2.32*	26.14±0.79*	27.34±0.87*	20.24±2.4*	19.41±0.43*
80%96-h LC <sub>50</sub>	42.67±1.96*	39.62±1.12*	19.98±0.69*	20.52±0.85*	23.91±0.67*	22.25±0.74*

All data are mean of seven fishes. Data are expressed as mean± standard deviation. Significant differences are marked by (\*) at P<0.05.

**Table 6.** Effect of different concentrations of phenol on ASAT activity (Units/min/g fresh tissue) in the tissues of *O. aureus* juveniles

Groups	Liver		Gills		Muscle	
	Male	Female	Male	Female	Male	Female
Control	41.23±0.81	40.13±0.83	20.12±0.98	21.47±0.81	18.12±0.85	17.16±0.79
20%96-h LC <sub>50</sub>	41.62±0.74	40.82±0.91	20.81±0.91	21.61±0.69	18.51±0.97	17.42±0.69
40%96-h LC <sub>50</sub>	56.24±0.27*	58.14±0.57*	25.23±0.47*	25.72±0.58*	24.38±0.34*	23.96±0.37*
80%96-h LC <sub>50</sub>	76.32±0.28*	80.25±0.96*	36.42±0.29*	37.32±0.23*	32.47±0.59*	31.93±0.55*

All data are mean of seven fishes. Data are expressed as mean± standard deviation. Significant differences are marked by (\*) at P<0.05.

**Table 7.** Effect of different concentrations of phenol on ALAT activity (Units/min/g fresh tissue) in the tissues of *O. aureus* juveniles

Groups	Liver		Gills		Muscle	
	Male	Female	Male	Female	Male	Female
Control	35.23±0.76	33.21±1.12	19.23±0.92	18.39±1.01	16.21±1.23	15.92±0.91
20%96-h LC <sub>50</sub>	35.01±1.21	32.76±0.98	21.10±1.04*	19.01±0.92	16.41±1.47	16.12±0.79
40%96-h LC <sub>50</sub>	28.60±0.89*	27.27±1.92*	24.12±0.71*	23.12±1.91*	23.29±1.27*	24.19±0.95*
80%96-h LC <sub>50</sub>	20.23±1.02*	21.12±2.01*	28.50±0.85*	29.10±2.00*	29.24±1.92*	28.29±0.49*

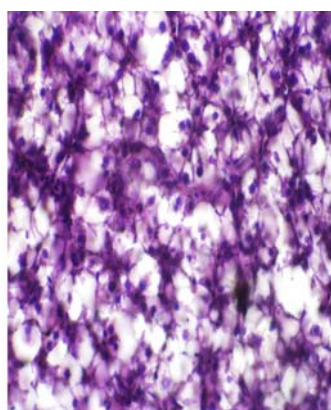
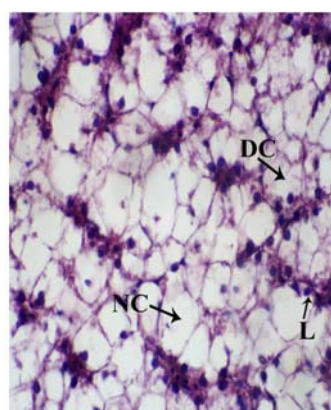
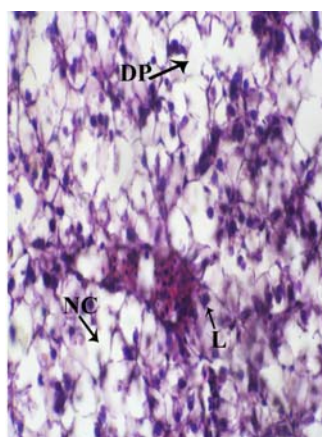
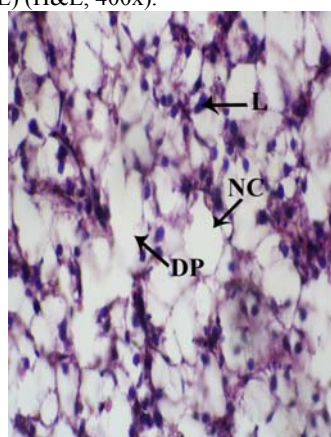
All data are mean of seven fishes. Data are expressed as mean± standard deviation. Significant differences are marked by (\*) at P<0.05.

**Table 8.** Effect of different concentrations of phenol on histopathological activity index (HAI) in the tissues of *O. aureus* juveniles

Groups	Inflammation	Central necrosis	Cell degeneration
Control	0	0	0
20%96-h LC <sub>50</sub>	1.51±0.41	1.21±0.32	1.14±0.41
40%96-h LC <sub>50</sub>	2.75±0.27	2.91±0.34	2.67±0.29
80%96-h LC <sub>50</sub>	3.62±0.41	3.96±0.56	4.21±0.12

All data are mean of seven fishes. Data are expressed as mean± standard deviation. Significant differences are marked by (\*) at P<0.05.

Hepatic injuries were graded as 0= negative finding; 1: evidence of pathological changes; 2: mild pathological changes; 3: moderated pathological changes and 4; marked pathological changes.

**Figure 1.** Photomicrograph of liver of control *O. aureus* juveniles showing normal structure (H&E, 400x).**Figure 2.** Photomicrograph of liver of *O. aureus* juveniles subjected to 20% 96-h LC<sub>50</sub> of phenol showing few necrotic cells (NC), few degenerated cells (DC) and lymphocyte infiltration (L) (H&E, 400x).**Figure 3.** Photomicrograph of liver of *O. aureus* juveniles subjected to 40% 96-h LC<sub>50</sub> of phenol showing degenerated patch (DP) and necrotic cells (NC) and lymphocyte infiltration (L) (H&E, 400x).**Figure 4.** Photomicrograph of liver of *O. aureus* juveniles subjected to 80% 96-h LC<sub>50</sub> of phenol showing more degenerated cells (DC), necrotic cells (NC) and lymphocyte infiltration (L) (H&E, 400x).

portal vein, central necrosis and cell degeneration. Evidence of histopathological signs was recorded in the liver of fish subjected to low phenol level (Table 8 and Figure 2). Histopathological activity index (HAI) was found to be concentration-dependent. The liver of fish subjected to high phenol level recorded the highest HAI (Table 8 and Figure 4), whereas those subjected to low phenol level recorded the lowest HAI (Table 8 and Figure 2).

## Discussion

The aquatic environment of the River Nile subjected to many stressful factors, phenol and phenolic derivatives are one of the serious pollutants that reach the aquatic habitat. For this reason, this work is projected to examine the hazardous effects of phenol on one of the predominating fish species, *O. aureus* juveniles in the River Nile. The body indices are quite general and non-specific, but their low cost, ease and rapidly still make them valuable environmental risk assessment tools (Van der Oost *et al.*, 2003). As the liver is a target for the metabolism in the fish body, the liver index (HSI) is a useful biomarker to detect the hazardous effects of the environmental stressors (Pait and Nelson, 2003). The HSI of *O. aureus* juveniles was elevated due to phenol intoxication. This observed hepatomegaly may partially reflect the enhancement of the liver size due to destructive changes. Barse *et al.* (2006) reported elevated HSI values of *Cyprinus carpio* subjected to 4-*tert*-butylphenol.

Much of attention has been focused on the endocrine disrupting chemicals (EDCs) on fish species (Celius *et al.*, 1999; Barse *et al.*, 2006). Lye *et al.* (1997) recorded apparent endocrine disruption in the wild fish populations in a number of locations. The EDCs enhance fish estrogenic activity so that the level of vitellogenin (VTG) production in the liver was increased (Barse *et al.*, 2006). As the phenolic derivatives are a group of EDCs (Barakat, 2004; Barse *et al.*, 2006; Tollefsen, 2006). Furthermore, nonylphenol induces sex reversal in Japanese medaka, *Oryzias latipes* (Gray and Metcalfe, 1997). So it may be possible that phenol stimulates VTG production in the liver and thus HSI was increased. In spite of no clear data, this is indicating that phenol is one of EDCs. To elucidate this effect, more works are required to cover this point. It could possibly mention that the enhancement of HSI may reflect reduced body weight. This result is in agreement with those reported by Pait and Nelson (2003) and Diniz *et al.* (2005). The reduction of ISI observed in the present study may support the reduction of the total body weight as a result of reduced appetite that induced by phenol pollution.

Similarly, the reduction of GSI of both sexes of blue tilapia subjected to phenol may possibly reflect the reduction of the gonad mass. Same result was reported for carp (*Cyprinus carpio*) subjected to 4-*tert*-butylphenol (Barse *et al.*, 2006). They also added

that the reduction of the spaces in interstitium, disrupted lumen and spermatozoa necrosis results in a reduction of testicular mass.

Carbohydrates are usually the first energy source particularly in case of stress. Therefore, exposure to the pollutants causes reductions in muscle and liver glycogen content. Dange' (1986) reported that phenol (96-h LC<sub>50</sub>) can bring these energy stores down to zero after 96 h of exposure. This runs parallel with the present results as the glucose in the tested organs was reduced due to phenol intoxication. Hori *et al.* (2006) reported same result for *Brycon cephalus* juveniles exposed to phenol. The enhanced LDH activity in fish exposed to phenol observed in this study may reflect the increased rate of conversion of lactate to pyruvate and then to glucose. This result is in agreement with those reported for other fish species exposed to phenol (Hori *et al.*, 2006). The fish liver glycogen was reduced after exposure to phenol. The reduction of hepatic glycogen stores could possibly due to that the hepatic synthesis of detoxifying enzymes requires high energy levels (Begum and Vijayaraghavan, 1995; Hori *et al.*, 2006). Some fish such as *O. mossambicus* exhibit enhanced opercular movements when exposed to phenol, which suggests high oxygen demand (Saha *et al.*, 1999). This may be a causative factor to induce the anaerobic oxidation to release energy by enhanced LDH activity.

The total protein content was reduced in the liver and gills of fish subjected to phenol. This may show that the protein was taken as an alternative source of energy, due to high energy demand that induced by phenol intoxication (Hori *et al.*, 2006). This result is confirmed by the marked rise of ASAT and ALAT activities that reported in the present study. The enhanced activities of transaminases induced tissue proteolysis. This phenomenon is previously recorded for different fish species subjected to phenol (Dange', 1986; Abdel-Hameid, 1994; Barse *et al.*, 2006; Hori *et al.*, 2006). The total protein content in the muscle was elevated. This is concomitant with the metabolic profile that does not indicate a metabolic demand that is able to induce substantial shift from carbohydrate to protein catabolism (Hori *et al.*, 2006). The recorded elevated activities of transaminases in the muscle might have indicated a preliminary protein breakdown to obtain energy. Hori *et al.* (2006) stated that energy mobilization becomes more evident in the liver than the muscle which might only be a complementary strategy. This could possibly explain the elevated protein in the muscle.

It is generally reported that the histopathological biomarkers are useful as indicators of the general health of the fish and are considered as a mirror that reflects of the exposure to a variety of anthropogenic pollutants (Van der Oost *et al.*, 2003). The liver of *O. aureus* juveniles subjected to phenol showed marked histopathological signs which were found to be directly proportional with the concentration of phenol. The high incidence of histopathological score reflects the degenerative effect of phenol on fish liver. This

degenerative effect was reported for carp (*Cyprinus carpio*) subjected to 4-*tert*-butylphenol.

## Conclusion

The present study exhibited that phenol induces liver damage and disturbs the metabolic state of *O. aureus* juveniles. Also, it draws the attention to conduct of research work to test whether the phenol is endocrine disrupting chemical.

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