

Effect of Environmental Pollution by Phenol on Some Physiological Parameters of *Oreochromis niloticus*

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Abstract: Phenolic compounds are common water pollutants and include wide variety of organic chemicals. In Egypt, effluents of oil refineries contain phenol and the receiving waters are therefore subjected to low level of chronic phenol pollution. The present study deal with this problem in fish. The effect of three sublethal concentrations (0.7, 1.4 and 2.8 mg/L representing 1/40, 1/20 and 1/10 LC₅₀, respectively) of phenol were investigated on some physiological parameters of *Oreochromis niloticus* after long term exposure (16 weeks). Results showed that serum tri-iodothyronine (T₃) and thyroxin (T₄) hormones decreased significantly, serum total cholesterol and lipids content significantly increased, genotoxic potential was observed through the increase in number of micronuclei production. Also decrease in growth performance and accumulation of phenol in fish tissues (liver, muscles and gills) were detected. It was concluded that phenol causes a lot of harmful effects to fish and of public health concern. Industrial drainage water must be treated before entering the water resources.

Key words: Phenol • Pollution • *Oreochromis niloticus* • Physiology • Growth • Accumulation

INTRODUCTION

Phenol and phenolic compounds are ubiquitous pollutants which come to the natural water resources from the effluents of a variety of chemical industrial such as cool refineries, phenol manufacturing, pharmaceuticals and industries of resin paint, dyeing, textile wood, petrochemical, pulp mill, etc.. [1-3]. Consequently, aquatic organisms including fish are subjected to these pollutants [4].

Studies related to toxicity should be more concerned with sublethal effects and sublethal studies have been forced due to there is urgent need to find the safe concentration of the pollutants [5]. Phenol and its derivatives induces toxic effect for fish. They induce genotoxic, carcinogenic, immunotoxic, hematological and physiological effects [6-10] and have a high bioaccumulation rate along the food chain due to its lipophilicity. Thus phenol pollution represents a threat against natural environment and also to human health [11]. When phenol is present in the aquatic environment, fish food consumption, mean weight and fertility are significantly reduced [12]. Exposure of fish to different types of pollutants (industrial effluents, pesticides and heavy metals) result several biochemical alterations in

blood parameters. Tr-iodothyronine T₃ and thyroxin T₄ are the most commonly assayed thyroid hormones in serum for monitoring of the influence on protein synthesis and oxygen consumption in all tissues, as these hormones are important for growth and sexual maturation. Thyroid status is a good indicator of growth in fish [13, 14]. Several investigators were interested in the effect of pollutants on the levels of T₃ & T₄ in serum in different fish species [4, 14, 15]. Alteration in circulating levels of total lipids and cholesterol in fish have been related to intoxicants [16], whereas, they generally reflect the state of the animals nutrition, endocrine functions as well as the integrity of the vital organs especially liver and kidney [17]. Genotoxic potency of metabolites was confirmed in various fish species. Micronucleus test is one of the most popular and promising test of environmental genotoxicity. Micronuclei (MN) have been induced in fish exposed to genotoxic substances like crude oil, petroleum refinery and alkyl phenol [18-20]. In Egypt, Effluents of oil refineries contain phenol and the receiving water are therefore subjected to low levels chronic phenol pollution. There are detectable levels of phenol in some industrial and agricultural localities, the maximum level of phenol in water samples was 0.42 ppm and in fish samples 0.009 ppm [21]. *Oreochromis niloticus* fish are of economical

importance and they support the fishing industry of inland and lake water in Egypt [22]. These fish produce high yields and therefore are important source of protein for human consumption. Data on the effect of exposure to phenol are not enough [9,10,21], so the current work was designed to investigate the effect of chronic low dose exposure of *O. niloticus* on thyroid hormones and total cholesterol and lipids. Also, effects on genotoxic potency, growth and bioaccumulation were studied.

MATERIALS AND METHODS

Site of Work: This study was carried out at the barrage fish farm station El-Kanater El-Khyria, Cairo Egypt during January to April 2008.

Phenol: Technical grade of phenol (C₆H₅OH), M.W. 94.11 with freezing point of 39.5-41.0°C was obtained from El-Nasr Pharmaceutical Chemical Company. The stock solution was prepared by dissolving phenol in distilled water (solvent).

Experimental Fish: A total number of 180 healthy living specimens of adult *Oreochromis niloticus* with an average initial body weight of 20±0.3 g and an average body length of 12.6±1.5 cm were used in the present study, they were obtained from a Private fish farm free from pollutants at El-Khanater EL-Khyria El Kalyobia province, Egypt. Fish were transported alive in well aerated tanks to the laboratory of fish research station in Al-Kanater Al- Khyria, National Institute of Oceanography and Fisheries. All fish were acclimatized for at least 2 weeks prior to the experiment. During the experimental periods they were fed on artificial diet of small pellet (30% protein). The diet was daily provided at 3% of the body weight. Composition and proximate analysis of experimental diets are shown in Table 1. Fish were kept in clean glass aquaria (50 L) capacity. These aquaria were supplied with dechlorinated tap water. Oxygen supply was maintained in each aquarium using an electric aerator pumps. The water in each aquarium was renewed every 3 days followed by addition of tested pollutant (phenol).

Experimental Design: A static acute toxicity bioassay test was performed according to standard methods [25] to determine the 96 hr. LC₅₀ value of phenol for *Oreochromis niloticus*. LC₅₀ of phenol was found to be (28 mg/L). 1/40, 1/20 and 1/10 LC₅₀ (0.7, 1.4 and 2.8 mg/L) of phenol were selected as sublethal concentrations and used in the present investigation. 12 glass aquaria were used.

Table 1: Composition and proximate analysis of the experimental diets/100g fed to *Oreochromis niloticus*

Ingredients	Weight (g)
Wheat bran	30.00
Yellow corn	20.00
Soybean	20.00
Fish meal	25.00
Corn oil	3.00
Vit. & Min.	2.00
Total	100.00
Dry matter	85.25
Crude protein	29.20
Crude fat	6.15
Crude fiber	2.87
Ash	7.48
NFE	54.30
Gross energy* Kcal/ kg	4459.20
Met. energy** Kcal/kg	3121.40

*Gross energy contents (Kcal/kg) calculate according to [23] using following calorific values: 5.64, 9.44 and 4.11 Kcal/g whole body of protein, fat and carbohydrate, respectively. **Metabolizable energy was calculated from gross energy as 70% reported by [24].

A total number of 180 of *O. niloticus* fish was used and divided into four groups in three replicates, each group included 60 fish. 15 fish / glass aquaria. Groups A, B and C exposed to 0.7, 1.4 and 2.8 mg/L (1/40, 1/20 and 1/10 LC₅₀) of phenol respectively, group D was kept without phenol (served as the control). The exposed fish were kept under proper observation during the period of experiment for any external clinical abnormalities. The experiment lasted for 16 weeks. Collection of blood samples and scarification of fish were done at the end of experiment.

Biochemical Analyses: Blood samples were taken by severance of the caudal peduncle of fish and collected in small vials, left to clot and then centrifuged at 3000 r.p.m for 10 min. to separate serum. Serum samples were used to determine thyroid hormones, total cholesterol and total lipids. Total serum levels of thyroid hormones (T₃ & T₄) were analyzed by enzyme immunoassay test using Biochek kit according to the methods of [26] and expressed as ng/dl for T₃ and ug/dl for T₄. Total serum cholesterol was determined by using Audit Diagnostics kit according to the methods of [27] and total serum lipids was determined according to the methods of [28].

Micronucleus Test: A drop of blood from the caudal vein of *O. niloticus* was obtained and smeared on clean dry slid. The specimen was fixed in ethanol for 5 minutes.

Slides were stained with 10 % Giemsa stained for 10 minutes. Giemsa solution stained the nucleus darker than the cytoplasm and the micronuclei appeared beside the normal nuclei. One thousand erythrocytes were examined for every fish to determine the percentage of cells containing micronuclei [29].

Growth Parameters and Feeding Efficiency: Body weight (g) of fish were measured biweekly, specific growth rate (SGR) and normalized biomass index (NBI) were calculated according to [30]. Total feed consumed (TFC), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated according to [30].

Residual Analysis of Phenol: Samples from muscles, liver and gills of *O. niloticus* were taken for determination of phenol residues the samples analyzed according to procedure recommended by [31] using atomic absorption spectrophotometer.

Statistical Analysis: Data were subjected to statistically analysis using a one way analysis of variance (ANOVA) followed by Duncan's test according to [32].

RESULTS

Biochemical Parameters: Table 2 represents data indicated that blood parameters of *O. niloticus* were greatly affected by three sublethal concentrations of phenol. Serum T₃ and T₄ decreased to 31.4±2.04 ng/dl and 1.61±0.01 ug/l, respectively at highest concentration of phenol (2.8 mg/L). Total cholesterol and total lipids in serum of *O. niloticus* increased to 310±2.8 mg/dL and

Table 2: Effect of different concentrations of phenol on serum triiodothyronine T₃, thyroxin T₄, total cholesterol and total lipids of *O. niloticus* after 16 weeks of exposure (Mean±SE)

Parameters	Concentrations of phenol mg/L			
	Control	0.7	1.4	2.8
T ₃ ng/dl	76.3±2.11 ^a	54.5±1.4 ^b	43.8±1.3 ^B	31.4±2.04 ^c
T ₄ ug/dl	8.33±0.38 ^a	5.2±0.33 ^b	3.46±0.21 ^c	1.61±0.10 ^d
T. cholesterolmg/dl	165.8±2.4 ^a	200.5±1.5 ^b	283±4.3 ^b	310±2.8 ^c
T. lipids g/100 ml	1.816±0.26 ^a	2.25±0.25 ^b	2.93±0.15 ^B	3.26±0.16 ^c

Number of observation in each mean = 8. Data are represented as Mean±SE. Means with the same latter of the same parameter are not significantly different at P_≥0.05.

Table 3: Mean values and percentage of micronuclei in erythrocyte of *O. niloticus* exposed to different concentrations of phenol M±SE

Conc. of phenol	Total No. of PCEs	Total No. of Mn	Micronucleus	
			M±S.E	%
Control	10000	19	1.9±0.12	0.19
0.7 mg/L	10000	38	3.8±0.41	0.38*
1.4 mg/L	10000	59	5.9±0.95	0.59**
2.8mg/L	10000	87	8.7±1.2	0.87***

Mn: micronuclei PCEs: polychromatic erythrocytes Number of fish =15

* P_≤0.5, ** P_≤0.01 and *** P_≤ 0.001

3.26±0.16 g/ 100 ml with increasing phenol concentrations as compared to the control group which had values of 165.8±2.4 and 1.816±0.26 g/100 ml.

Micronucleus Results: The results of micronuclei in phenol exposed fish were summarized in Table 3. The percentage of micronuclei of examined erythrocytes of

Table 4: Growth parameters of *O. niloticus* exposed to different concentrations of phenol for 16 weeks

Period weeks	Control			Phenol mg/L								
	0			0.7			1.4			2.8		
	FBW	SGR	NBI	FBW	SGR	NBI	FBW	SGR	NBI	FBW	SGR	NBI
2	24 ^a	1.35 ^a	22.5 ^a	23 ^{ab}	0.93 ^b	15 ^b	22 ^b	0.64 ^b	10 ^c	22 ^b	0.64 ^b	10 ^c
4	30 ^a	1.35 ^a	27.5 ^a	27 ^{ab}	1.00 ^b	20 ^b	25 ^b	0.74 ^{bc}	15 ^c	24 ^b	0.61 ^c	10 ^d
6	36 ^a	1.31 ^a	30.0 ^a	31 ^b	0.97 ^b	20 ^b	28 ^{bc}	0.75 ^{bc}	15 ^c	26 ^c	0.58 ^c	10 ^d
8	40 ^a	1.16 ^a	20.0 ^a	34 ^b	0.88 ^b	15 ^b	31 ^{bc}	0.73 ^{bc}	15 ^b	29 ^c	0.62 ^c	15 ^b
10	44 ^a	1.05 ^a	20.0 ^a	3 ^b	0.82 ^b	15 ^b	35 ^{bc}	0.75 ^{bc}	20 ^a	32 ^c	0.62 ^c	15 ^b
12	47 ^a	0.95 ^a	15.0 ^a	40 ^b	0.77 ^{ab}	15 ^a	37 ^{bc}	0.68 ^b	10 ^b	35 ^c	0.62 ^b	15 ^a
14	50 ^a	0.87 ^a	15.0 ^a	44 ^b	0.75 ^{ab}	20 ^b	40 ^{bc}	0.66 ^{bc}	15 ^a	37 ^c	0.59 ^c	10 ^c
16	55 ^a	0.84 ^a	25.0 ^a	47 ^b	0.71 ^b	15 ^b	43 ^{bc}	0.64 ^{bc}	15 ^b	40 ^c	0.58 ^c	15 ^b
		1.1±0.7	21.8±2.1		0.85±0.03	16.8±0.9		0.69±0.01	14.3±1.1		0.60±0.01	12±0.9

FBW: Final body weight (g);SGR: Specific growth rate ; NBL: normalized biomass index

Mean with the same letters in the same row and parameters are not significantly different at P_≥0.05

Table 5: Feeding efficiency of *O. niloticus* exposed to different concentrations of phenol for 16 weeks (M±SE)

Period Weeks	Control			Phenol mg/L																				
	0			0.7			1.4			2.8														
	TFC	FCR	PER	TFC	FCR	PER	TFC	FCR	PER	TFC	FCR	PER												
2	0.14 ^a	1.6 ^a	2.14 ^a	0.13 ^a	2.4 ^b	1.43 ^b	0.12 ^a	3.6 ^c	0.95 ^c	0.12 ^a	3.6 ^c	0.95 ^c												
4	0.18 ^a	1.6 ^a	2.14 ^a	0.14 ^a	2.07 ^b	1.65 ^b	0.13 ^b	2.64 ^b	1.29 ^b	0.11 ^b	3.96 ^c	0.86 ^c												
6	0.22 ^a	1.8 ^a	1.9 ^a	0.16 ^b	2.43 ^b	1.41 ^b	0.14 ^{bc}	3.0 ^c	1.14 ^{bc}	0.11 ^c	4.32 ^d	0.79 ^c												
8	0.26 ^a	3.2 ^a	1.06 ^a	0.18 ^b	3.72 ^a	0.92	0.15 ^{bc}	3.36 ^a	1.02 ^a	0.12 ^c	3.12 ^a	1.09 ^a												
10	0.29 ^a	3.6 ^a	0.95 ^a	0.18 ^a	4.08 ^b	0.84 ^a	0.16 ^b	2.79 ^c	1.23 ^b	0.13 ^b	3.48 ^a	0.98 ^{ab}												
12	0.31 ^a	5.2 ^a	0.65 ^a	0.19 ^b	4.44 ^b	0.77 ^a	0.16 ^{bc}	4.2 ^b	0.54 ^b	0.14 ^c	3.84 ^c	0.89 ^a												
14	0.32 ^a	5.6 ^a	0.61 ^a	0.19 ^b	3.6 ^b	0.95 ^b	0.16 ^{bc}	6.3 ^c	0.77 ^a	0.14 ^c	6.30 ^c	0.54 ^a												
16	0.32 ^a	3.6 ^a	0.95 ^a	0.21 ^a	5.28 ^b	0.65 ^b	0.17 ^b	4.8 ^c	0.71 ^b	0.13 ^b	4.44 ^c	0.77 ^b												
	3.2±0.5			1.3±0.2			3.5±0.4			1.0±0.1			3.9±0.42			0.95±0.09			3.7±0.1			0.85±0.0		

TRC: Total feed consumed (kg), FCR: Feed conversion ratio, PER: Protein efficiency

Mean with the same letters in the same row and parameters are not significantly different at P≥0.05

Table 6: Residue of phenol (ppm) in *O. niloticus* tissues after 16 week of exposure (M±SE)

Conc. of phenol ppm	Muscles	Gills	Liver
0.7	1.18±0.003	2.06±0.20	6.5±0.70
1.4	2.34±0.05	3.80±0.05	10.8±0.70
2.8	3.62±0.06	5.0±0.04	18.3±0.92

control *O. niloticus* was 0.19% and the mean number was 1.9±0.12. There was a significant increase of micronuclei production in phenol exposed fish after 16 weeks of exposure to 0.7, 1.4 and 2.8 mg/l compared to the control group, where the mean values were 3.8±0.41, 5.9±0.95 and 8.7±1.2, respectively.

Effect of Phenol on Fish Growth Rate: Statistical analysis showed that the tested fish exposed to various sublethal concentrations of phenol had significant low in FBW, SGR and NBI compared to the control group Table 4.

Effect of Phenol on Feeding Efficiency: Feed utilization which include, total feed consumed (TFC), feed conversion ratio (FCR) and protein efficiency ratio (PER) of *O. niloticus* were also affected by different concentrations of phenol. TFC and PER ratio decreased below levels of control fish. TFC of *O. niloticus* increased above the optimal value of the control with the exposure to phenol Table 5.

Residue of Phenol in Fish Tissues: The present results showed that phenol was accumulated in fish tissues (muscle, gills and liver) after 16 weeks of exposure to different concentrations Table 6. The concentration of

phenol in muscle, gills and liver of *O. niloticus* increased with increasing phenol concentrations, maximum value (18.3±0.92 ppm) was recorded in the liver and minimum value (3.62±0.06 ppm) was recorded in the muscle. The concentration of phenol in the following order muscle < gills < liver

DISCUSSION

The control values of serum T₃ (76.3±2.11 ng/dl) and T₄ (8.33±0.38 ug/dl) of *O. niloticus* were found to be within the same range of other freshwater fishes reported by [4,14,15]. In the present study serum T₃ and T₄ levels of *O. niloticus* exposed to high concentration of phenol significantly decreased compared to the control groups. The observed decrease in serum T₃ and T₄ in the present study may be due to increased deiodination and biliary excretion of thyroid hormones which increase rate of T₃ and T₄ elimination from the blood as reported by [33] or may be attributed to direct effect of thyroid hormones on the production of ATP in the mitochondria as a result of continuous demand and need of fish body to ATP for the production of energy to overcome the stress by phenol [34]. As reported by [35] the decrease in T₃ levels may be resulting in or inducing toxic goiter which is manifested by increased metabolic reactions or may be secondary to a pituitary insufficiency. [36] observed reduced thyroid stimulated hormone TSH in serum, as well as in the pituitary of *H. Fossilis* exposed to cythion and hexadrin and suggested that thyroid dysfunction is probably caused by reduced TSH out put. The observed decrease in T₃ and T₄ of the studied fish is in agreement with that recorded in *salmo gairdneri* exposed to mirex and PCB

[37]; in *Clarias gariepinus* collected from different polluted site [38]; in *H. Fossilis* exposed to cythion and hexadrein [36] and in *O. niloticus* exposed to butataf herpicides [4].

The concentration of total cholesterol and total lipids in serum and tissues of fish have been reported to be moderately sensitive to environmental pollutant but the direction of change in these parameters seems to be dependent on many factors, such as the types of contaminant, the concentration, mode of its action, duration of exposure and fish species [39].

The control values of serum total cholesterol 165.8 ± 2.4 mg/dl and serum total lipids 1.816 ± 0.26 g/100 ml of *O. niloticus* in the present study are within the same range of other freshwater fish reported by [40-42]. Fish exposed to phenol exhibited marked hypercholesterolemia and hyperlipidemia, serum total cholesterol and total lipids of *O. niloticus* increased significant after 16 weeks of exposure compared to the control groups.

The observed increase in serum total cholesterol and total lipids in the present study may be due to one or more of the following reasons (1) increased production by the liver and other tissues. (2) release of cholesterol and other lipids constituents from damaged cell membranes. (3) decrease hepatic excretion of cholesterol (4) thyroid dysfunction and finally blocked conversion of cholesterol to sex steroids as a result of gonad dysfunction [43-46]. The observed increase in total cholesterol and total lipids in serum of *O. niloticus* in the present investigation are in accordance with that recorded in the serum of eels and mullets exposed to endrin and DDT [47]; in *Puntitus conchoniis* exposed to phosphamidon [48]; in *Clarias anguillaris* exposed to cadmium and lannete [41]; in *O. niloticus* exposed to copper [42] and in *Clarias lazera* exposed to mercury and gallant [49].

Genotoxic potency of metabolites was confirmed in various fish species [18-20]. The most popular and promising test of environmental genotoxicity is the micronucleus test. Micronuclei (MN) are produced from chromosome fragments or whole chromosome that lag at cell division due to lack of centromere, damage in centromere or defect in cytokinesis. In tissues with activity dividing cells, micronuclei records reflect action of clastogenic or aneugenic compounds [20].

A micronucleus is a supnumerary nucleus visible under light microscopy in the cytoplasm of the cell [50]. In the present study cytogenic damage was observed in the *O. niloticus* exposed to different concentrations of phenol. High frequency of micronucli in fish erythrocytes exposed to 0.7, 1.4 and 2.8 mg/L of phenol after 16 weeks of exposure compared to the control groups Table 3. This

results are nearly similar to those described by [51-54]. Many studies confirmed the validation of micronucleus test in fish as a monitor for the occurrence of aquatic genotoxic agents through the observation of close association between the frequency of micronucleus and the degree of pollution [55, 56].

Results from this investigation revealed that *O. niloticus* exposed to different sublethal concentrations of phenol for 16 weeks grew significantly less than unexposed fish. This decrease in growth was directly proportional to the phenol concentrations and time of exposure. The inhibition of growth reported in this study may therefore be due to a disturbance of normal metabolism by the phenol [57]. Fish increased their metabolic rates to metabolize and excrete phenol and consequently, allocate more energy to homeostatic maintenance than storage, hence reduction in growth rate occur [58]. The same toxic effect was observed within the work of [59] who reported that sever reduction of feeding, loss of appetite is one of the important reasons behind the reduction of growth and development of fish after chronic exposure to phenol. The inhibition of growth rate of *O. niloticus* exposed to phenol in the present investigation are in agreement with that recorded in coho salmon *Oncorhyncius Kisutch* exposed to naphthalene for 40 days [60]; in *Salmo clarki* exposed to crude oil for 60 days [61]; in *O. massambicus* exposed to phenol [59]; in teleosts exposed to phenol [57]. It is estimated that fish can act as front-line indicators of suspected aquatic pollutants such as organic compounds and may adsorb dissolved pollutants from the surrounding water and food, which may accumulate in various tissues in significant amount and are eliciting toxicological effects at critical targets [62]. However, fish may accumulate significant amount of pollutants even in waters in which those pollutants are below the limit of detection in routine water samples [63]. The present study showed that the concentration of phenol in studies fish organs are in between 3.62 ± 0.01 and 18.3 ± 0.92 ppm and followed sequence of muscle < gills < liver. Liver showed more bioaccumulation than gills and muscle, phenol concentration in fish organs were more than the maximum permissible levels 0.01 ppm [64]. Phenol residue in fish organs generally increase with increasing levels of phenol in water.

CONCLUSION

Phenol should be listed under the highly toxic pollutants to fish even under the sublethal doses where it may cause endocrine dysfunction, liver dysfunction,

genotoxic effect, reduced growth rate and accumulate in significant amount. Therefore, for public health concern, the industrial drainage water should be treated before entering the water resources by removal chemical pollutants through evaporation, distillation, precipitation and ionic exchange units.

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