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Toxicity of Phenol to Fish and Aquatic Ecosystems

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Phenol and its compounds are ubiquitous water pollutants which come to the natural water resources from the effluents of a variety of chemical industries such as coal refinaries, phenol manufacturing, phermaceuticals, and industries of resin, paint, dyeing, textile, leather, petrochemical, pulpmill etc. (Dauble et al. 1983; Mukherjee et al. 1990; Kumaran, 1993). It has been found that phenol, after entering into the fish body, affects the metabolism (Holmberg et al. 1972), survival and growth (Verma et al. 1980; DeGraeve et al.1980; Becker and Crass 1982; Holcombe et al. 1982; Dauble et al. 1983) and reproductive potential of fish (Dauble et al. 1983; Mukherjee et al. 1990, 1991). Despite the active metabolism and detoxification of phenol in fish (Hoar and Randall 1969), sublethal doses of phenol interfere with various enzyme activities (Mukhejee and Bhattacharya 1977) and can produce many unpredictable changes in fish. However, the informations about the phenol toxicity at ecosystem level is limited.Therefore, there is a need to investigate the phenol toxicity to fish and aquatic ecosystems in detail. The aim of the present study was to evaluate the acute and chronic toxicity of phenol to tilapia (*Oreochromis mossambicus*) and effects of sublethal doses of phenol on some limnological parameters of the aquatic ecosystem.

MATERIALS AND METHODS

Test fish tilapia (*Oreochromis mossambicus*) were procured from local hatcheries and were acclimatized to the test condition for 96-192 hr before their use.

Analytical grade phenol, C_6H_5OH (purity 99%; E. Merck, made in Germany) was used as test chemical.

Static bioassays were conducted in 15L glass aquaria with unchlorinated tap water (PH 7.20 ± 0.10, free CO_2 2.6 ± 0.18 mg/L, DO 5.2 ± 0.50 mg/L, alkalinity 170 ± 7.25 mg/L as $CaCO_3$, hardness 105 ± 6.5 mg/L as $CaCO_3$). Each aquarium contained 10 L of water and 4 adult fish (mean length 86.18 ± 1.97 mm, mean weight 8.89 ± 1.07 g). The aquaria were exposed to 11 phenol concentrations (0, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44 mg/L) in 4 replicates. Test animals were not fed from 2 d prior to the end of the experiments. The test medium was replaced in both control and experimental aquaria. All recommendations for static bioassay given by APHA

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(1980) were strictly followed. Mortality of the test animals was recorded every 24 hr and the 96 hr median lethal concentration (96 hr LC_{50}) with 95% confidence limit of phenol was estimated by probit analysis (Finney 1971). The opercular movements (number per minute per fish) of fish were also recorded every 24 hr during this test to evaluate the effect of phenol on respiratory rate of fish.

Feeding rate of fish was evaluated in a separate 96 hr laboratory bioassay in 15 L glass aquaria with 10 L of water. 12 adult fish (3 per aquarium) were exposed to each sublethal concentration of phenol (0.00, 1.26, 2.39, 2.85 and 4.11 mg/L) in four replicates. Live earthworm pieces were supplied daily as food of fish at 8.00 hr and fish were allowed to feed for 4 hr. The weight of unconsumed food pieces were measured and removed to avoid any decomposition. Amount of food consumed by control fish was considered as 100%.

Chronic toxicity tests were conducted in 60 L earthen vats supplied by a local farm. The vats were conditioned before the experiment by keeping them water filled in outdoor condition for 1 month. It also helped to grow natural food for tilapia in the vat water. Such conditioned vats show minimum percolation of water and have been found suitable for evaluating chronic toxicity of chemicals (Saha and Kaviraj, 1996). Water temperature of the vats ranged from 20°C to 30°C during the experiment. Vats were arranged in 5 blocks each with 4 tanks as per Randomized Block Design (Gomez and Gomez 1984), thereby giving four replicates for each of the five sublethal concentrations of phenol (0, 1.26, 2.39, 2.85 and 4.11 mg/L). Each vat was stocked with 15 fingerlings (mean length 51.67 ± 4.25 mm, mean weight 1.85 ± 0.37 *g)* of *O. mossambicus* followed by the treatment of phenol. 10% of test medium was replaced weekly during experiment. In addition to the natural food, the stocked fish were fed a mixture of rice bran and mustard oil cake (1:1) 6 days a week at the rate of 5% of their body weight.

pH, free CO_2, total alkalinity, hardness, DO, primary productivity, phytoplankton and zooplankton populations in the test medium were measured at every 15 days interval during the bioassay according to APHA (1980). Fish were sampled at the end of the experiment (90 th day). Length, weight, visceral weight and gonad weight of fish were recorded. Final biomass was used to estimate the yield of fish in each treatment. Formulae used to estimate condition factor (K), gastrosomatic index (GSI), maturity index (Ml) and fecundity were adopted from LeCren (1951) and Bagenal(1978) and are given below :

K = [Body weight (g) / Body length `$(mm)^3$] $\times 10^5$; GSI = [Visceral weight (g) / Body weight (g)] x 100; MI = [Gonad weight (g) / Body weight (g)] x 100; Fecundity = Total number of ripening eggs / female.

All data (except percent mortality) were subjected to analysis of variance and Duncan's multiple range test (Gomez and Gomez, 1984) was used to determine significant differences among means.

Table 1. Mean number of opercular movements of fish during acute toxicity test of phenol.

Concentrations of phenol (mg/L)	Opercular movement/minute / fish at different time intervals			
	24 hr	48 hr	72 hr	
00	103 ^{fM}	102 ^{cdM}	93 ^{abcM}	96 ^{aM}
17	119 ^{eM}	108 ^{abcM}	87 ^{bcdN}	61 ^{eO}
20	128 ^{eM}	107 ^{abcN}	85 ^{cdO}	90 ^{aO}
23	139 ^{dM}	116 ^{abN}	81 ^{dO}	75 ^{cdO}
26	147 ^{cdM}	118 ^{aN}	85 ^{cdO}	86. ^{abO}
29	149 ^{cdM}	102 ^{cdM}	97 ^{abN}	78 ^{bcO}
32	154 ^{bcM}	93 ^{dN}	96 ^{abcN}	70 ^{cdeO}
35	158 ^{bcM}	93 ^{dN}	101 ^{aN}	70 ^{cdeO}
38	164 ^{abM}	107 ^{abcN}	102 ^{aN}	66 ^{deO}
41	172 ^{aM}	106 ^{bcN}	98 ^{aN}	65 ^{deO}

Values within columns indicated by the same small superscript letter (a, b, c, d, e, f) and values within rows indicated by same capital superscript letter (M, N, O) are not significantly different at 5% level determined by Duncan's multiple range test.

RESULTS AND DISCUSSION

The 96 hr LC_{50} value of phenol to tilapia (*Oreochromis mossambicus*) was found 28.49 mg/L with 95% confidence limit ranging in between 25.08 to 32.37 mg/L. Fish exposed to phenol showed excess mucous secretion from skin and gill. Initially opercular movements were also very frequent indicating acute respiratory distress. Mean opercular movement of fish has been shown in table 1. Observation at 24 hr exposure showed that mean opercular movement of the fish increased with the increase of phenol concentration. But subsequent observation at 48, 72 and 96 hr exposure did not show any relation of opercular movement with the concentration of phenol. At these days opercular movemnets were erratic. At higher dose of phenol fish showed acute suffocation in between 72 to 96 hr. They repeatedly came to the surface of water and gulped atmospheric air. Air bubbles were found coming out from the mouth. Some individuals frequently dashed against the walls of the aquarium and finally lost their equilibrium and buoyancy. In the lower concentration of phenol, the effects were less acute.

A single factor ANOVA [$F_{(5, 20)}$, 53.73; $P<0.01$] followed by DMRT showed that as little as 1.26 mg/L phenol significantly reduced the feeding rate of tilapia as compared to control (Table 2). Severe reduction of feeding rate was observed at the higher sublethal concentrations of phenol (2.85 and 4.11 mg/L).

No fish died during the outdoor experiment, but the total yield of fish at the end of experiment was significantly reduced ($P<0.05$) at all the sublethal concentrations of phenol as compared to control. More than 50% reduction of yield was observed at concentrations 2.85 and 4.11 mg/L phenol. Mean length, mean weight, maturity

Table 2. Mean values (± SD) of feeding rate, mean length, mean weight, yield, condition factor (K), gastrosomattc index (GSI), fecundity and maturity index (MI) of fish exposed to sublethal doses of phenol (1.26, 2.39, 2.85 and 4.11 g/L) and control (0.00 mg/L).

Concentrations of phenol (mg/L)	Food consumed (%)	Mean length (mm)	Mean weight (g)	Yield (g/M^2)	K	GSI	Fecundity	MI	
								Male	Female
0.00	100.00 [a]	63.50 [a]	4.65 [a]	251.03 [a]	1.8113 [a]	8.25 [a]	254 [a]	1.00 [a]	8.00 [a]
		(2.21)	(0.12)	(8.91)	(0.21)	(0.36)	(5.85)	(0.07)	(0.50)
1.26	91.53 [b]	60.25 [ab]	4.02 [b]	203.28 [b]	1.84 [a]	8.72 [a]	220 [b]	0.82 [b]	7.78 [a]
		(1.71)	(0.28)	(21.68)	(0.09)	(0.75)	(14.20)	(0.04)	(0.49)
2.39	87.76 [b]	59.63 [b]	3.86 [b]	190.85 [b]	1.82 [a]	8.66 [a]	175 [c]	0.78 [b]	7.64 [a]
		(1.48)	(0.23)	(17.69)	(0.06)	(0.35)	(12.70)	(0.02)	(0.33)
2.85	71.06 [c]	57.69 [bc]	2.90 [c]	117.15 [c]	1.51 [b]	9.97 [b]	135 [d]	0.67 [c]	6.48 [b]
		(1.57)	(0.13)	(10.21)	(0.09)	(0.38)	(11.32)	(0.04)	(0.48)
4.11	63.76 [c]	56.64 [c]	2.61 [c]	95.40 [c]	1.42 [b]	10.51 [b]	116 [e]	0.60 [d]	6.31 [b]
		(1.44)	(0.14)	(10.46)	(0.08)	(0.58)	(17.44)	(0.03)	(0.36)

Values within columns indicated by the same superscript letter (a, b, c, d) are not significantly different at 5% level determined by Duncan's multiple range test.

Table 3. Mean values (± SD) of pH, free CO_2, alkalinity as $CaCO_3$, hardness as $CaCO_3$, DO, primary productivity, phytoplankton and zooplankton populations of phenol treated water (1.26, 2.39, 2.85 and 4.11 (mg/L) and control (0.00 mg/L).

Concentrations of phenol (mg/L)	pH	Free CO_2 (mg/L)	Alkalinity as $CaCO_3$ (mg/L)	Hardness as $CaCO_3$(mg/L)	DO (mg/L)	Primary productivity (mg $C/M^3/hr$)	Phytoplankton (No./L)	Zooplankton (No./L)
0.00	7.65^{a}	2.48^{a}	141.75^{a}	112.75^{a}	7.27^{a}	146.25^{a}	5139^{a}	120^{a}
	(0.06)	(0.09)	(3.50)	(5.56)	(0.50)	(7.50)	(80.93)	(12.97)
1.26	7.77^{a}	2.34^{b}	146.00^{a}	108.75^{a}	7.31^{a}	150.25^{a}	4253^{c}	123^{a}
	0.10	(0.04)	(2.94)	(5.62)	(0.50)	(4.65)	(44.98)	(6.27)
2.39	7.66^{a}	2.44^{bc}	142.50^{a}	109.50^{a}	7.01^{a}	145.50^{ab}	4644^{b}	111^{a}
	(0.07)	(0.06)	(3.70)	(4.20)	(0.24)	(4.80)	(96.41)	(8.77)
2.85	7.60^{a}	2.51^{cd}	133.00^{b}	102.50^{a}	6.51^{b}	137.50^{bc}	4318^{c}	97^{b}
	(0.08)	(0.07)	(2.58)	(5.00)	(0.13)	(5.97)	(52.22)	(6.68)
4.11	7.59^{a}	2.61^{d}	135.25^{b}	103.00^{a}	6.10^{c}	132.00^{c}	3774^{d}	92^{b}
	(0.04)	(0.08)	(3.50)	(5.48)	(0.19)	(3.92)	(31.63)	(7.05)

Values within columns indicated by the same superscript letter (a, b, c, d) are not significantly different at 5% level determined by Duncan's multiple range test.

index and fecundity of fish also significantly decreased ($P<0.05$) in higher sublethal concentrations (2.39, 2.85 and 4.11 mg/L) of phenol compared to control (Table 2).

Changes in various limnological parameters during outdoor bioassay are shown in Table 3. Free CO_2, dissolved oxygen (DO), primary productivity and alkalinity of water significantly varied ($P<0.05$) over control in higher concentration of phenol (2.85 and 4.11 mg/L). Phytoplankton populations significantly reduced from control in all concentrations but zooplanktion populations decreased significantly from control only in phenol concentration 2.85 mg/L or above.

Allthough sufficient literatures are available on the toxicity of phenol to aquatic organisms, exact mechanism of its toxicity is not clearly known. It has been found that phenol enters into the blood circulation of fish from water through gills or mucous epithelium of mouth and finally accumulates into different organs (Verma et al. 1980; Mukherjee et al. 1991) resulting different physiological problems or death depending upon its concentration. Mukherjee et al. (1990) found differences in the rate of uptake and loss of phenol in between *Charma punctatus* and *Cyprinus carpio*. Verma et al. (1980) found 96 hr LC_{5O} values of phenol to *Notopterus notopterus* , *Saccobranchur fossilis* and *Colisa fasciatus* were 12.53, 39.40 and 32.70 mg/L, respectively. The LC_{50} value of phenol to *Oreochromis mossambicus* in the present investigation has been found 28.49 mg/L which is higher than the LC_{so} of phenol to *N. notopterus*, but lower *than* that of *S. fossilis* and *C. fasciatus*. Becker and Crass (1982) reported 10-30% mortality of juvenile fathead minnows after 96 hr exposure to 5.8 - 8.0 mg/L total phenol. The difference in LC_{so} values of phenol for different fish species are possibly due to their differences in sensitivity to the toxicant and differences in uptake rate of the toxicant. The reactions of fish following acute exposure to phenol in the present investigation resemble those observed by Verma et al. (1980). Most of the reactions resulted from respiratory distress. Increased opercular activity of tilapia in the present study confirms that phenol, even in a low dose, interferes with the respiration resulting in a sort of hypoxic stress.

Fish exposed to pentachlorophenol showed reduced weight gain and food gain effrcencies (Webb and Brett 1973). It justifies the observations of present investigation. In 2.85 to 4.11 mg/L phenol concentration tilapia have shown severe 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 sublethal concentrations of phenol. Dauble et al. (1983) also observed reduced growth of fathead minnows exposed chronically to 0.25 mg/L phenol. But DeGraeve et al. (1980) and Holcombe et al. (1982) stated that the lower threshold of chronic effect of phenol on fathead minnows was 2 mg/L. In the present study, in addition to reduced growth, we have also seen reduced fecundity and maturity index of fish as a result of 90 days chronic exposure to phenol (1.26 mg/L). Similar results have been reported by Mukherjee et al. (1991) for *Cyprinus carpio* exposed to the sublethal concentrations of phenol while complete inhibition of spawning of fathead minnows at 1.27 mg/L of total phenol over 21 d exposure has been reported by Dauble et al. (1993).

That 2.85 and 4.11 mg/L phenol is most injurious for aquatic ecosystem is revealed from the marked reduction of dissolved oxygen, primary productivity, phytoplankton and zooplankton populations in these two concentrations. Phytoplankton population is reduced even in the concentration 1.26 mg/L. Reduction of these limnological parameters also produce profound effects on the growth and reproduction of fish.

The present study indicates that phenol produce many adverse effects on fish and aquatic ecosystem when chronically exposed even at a small concentration. Thus to determine safe concentrations of phenol for a species of fish and its habitat chronic toxicity test of at least 90 day duration with sublethal concentration of the toxicant is necessary.

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