

RESEARCH ARTICLE

DETERMINATION OF LC₅₀ OF PHENOLIC COMPOUNDS (PHENOL & M-CRESOL) FOR A FISH, *LABEO ROHITA*

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Received: 23 February, 2019/ Revision: 28 February, 2019/ Accepted: 8 March, 2019

ABSTRACT: Water pollution has become a global problem as various pollutants like heavy metals and toxic chemicals are discharged without prior treatment into the water. With the development of industrial production, a large volume of wastewater containing phenols was discharged into the aquatic environment. Moreover, chemical leakage further increased the emission of phenols into aquatic systems. Phenol and its methylated derivative (cresols) were selected due to their extensive use in industry and ecotoxicity to freshwater and marine organisms. This study focused on the ecotoxicity of phenol and m-cresol on aquatic systems. This paper emphasizes on the determination of 96hr LC₅₀ value of phenol & m-cresol for the fish, *Labeo rohita*. The acute toxicity test was performed according to the standard methods in APHA and the value was calculated by probit analysis. The fish specimens were acclimatized in the laboratory conditions for 15 days. The stock solution of phenol & m-cresol was prepared and the fish fingerlings were treated with various concentrations ranging from 1 mg/l to 50 mg/l for 96 hours. The results showed that the median lethal concentration (LC₅₀) of phenol & m-cresol for the fish, *Labeo rohita* is 3212 & 2957 mg/l. The susceptibility of *Labeo rohita* to the lethal effect of phenol & m-cresol were dependent on duration as well as on concentration. The mortality of the fishes is directly proportional to the concentration

KEYWORDS: Phenol, Cresols Ecotoxicity, Biodegradation, Acute Toxicity, *Labeo rohita*, 96hr LC50.

INTRODUCTION:

Water pollution has become a global problem as various pollutants like heavy metals and toxic chemicals are discharged without prior treatment into the water bodies most commonly in developing countries. These toxic chemical due to their properties like long half life period, bioaccumulation, biomagnifications in the food chain and non-biodegradability are hazardous to the aquatic organisms and their consumers which on being exposed to these toxic chemical can suffer from

immense health problems and risk of life¹⁻². Fishes have direct economic importance and are quite sensitive to the wide array of pollutants discharged in the aquatic ecosystems. Fishes are widely used to assess water quality of aquatic ecosystems because they serve as pollution bioindicators³. Fish may concentrate large quantities of toxic chemical from polluted aquatic environments⁴. The toxic chemical concentration in the body of fish depends upon feeding habits, trophic status, food availability,

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physico-chemical properties of water, metabolic rate of animal and toxicity of toxic chemical⁵⁻⁶. Labeo rohita is a commercial fish and widely preferred as edible fish in India. It is very important to evaluate edible organisms like Labeo rohita from toxicity point of view as health of human being is directly associated with it. During the present course of investigation acute toxicity tests of Phenolic compounds to determine 96 hr LC50 have been conducted on Labeo rohita, a fish having high nutritional value as well as it serves as a good pollution indicator⁷⁻⁹.

Phenol, a colorless-to-white solid with a characteristic odour, is the simplest monatomic phenol. Cresols, methylated derivatives of phenol, are composed of three isomers [meta-(m-), ortho-(o), and para-(p-)]. Phenolic compounds are extensively used as materials for organic synthesis and also used in different industries, such as pesticides, dyes, coatings, and oil refining (ASTDR, 2008a, 2008b; Michałowicz and Duda, 2007) and hence, they are ubiquitous in the wastewater from these industries (Jiang et al., 2006; Saravanan et al., 2008; Surkatti and El-Naas, 2017)^{1-2,7-8}.

Moreover, leakage accidents further increased the possibility of the emission of phenols into the environment. Accidental spills of phenol, which happened in the Port of Gothenburg (Sweden) and Xin'an River (China), caused leakage of a significant amounts of phenol, posing a threat to water quality and aquatic systems (China Chemical Safety Association, 2011; HELCOM, 2002). Due to their high water-solubility (phenol, 8.28g/100mL; cresols, 2.15–2.60g/100mL), phenol and cresols can persistent a high concentration in aquatic environments (Wei et al., 2016). Water pollution in China is mainly caused by discharged wastewater from dozens of types of industrial pollution source (Zhou et al., 1991). Phenol and m-cresol, as priority pollutants of many industrial point sources, such as oil processing industry, insecticide factory, and chemical trades, have been named on the list of priority pollutants (68 substances) held by the China Environmental Priority Monitoring Research Group (Zhou et al., 1989). Currently, phenol has been named on a list of the top 20 chemicals likely to pose the highest risk of being involved in an HNS (hazardous and noxious substance) incident held by International Maritime Organization (IMO) (ITOPF, 2011).

Ayeni (2014) concluded that indiscriminate disposal of wastewaters into the aquatic environment and abrogation of the responsibility to manage industrial wastewater before point of discharge caused higher level concentrations of phenol in water systems. As for seawater, the concentrations of volatile phenols (including phenol and cresols) in Maoming onshore fishery (China) were greater than 10µg/L, and thus were classified as a Class IV pollutant according to Sea Water Quality Standard (SEBC, 1997). Moreover, accidental leakages of phenol and cresols, to sea or a river, may give rise to a significant increase of phenol and/or cresols concentrations in aquatic systems, causing high toxicity of phenols to aquatic organisms²⁻⁵.

The physico-chemical properties of pollutants determine their behaviour upon entering the water (ITOPF, 2011). The Standard European Behaviour Classification (SEBC) codes enshrined a set of criteria for theoretical behaviour according to physico-chemical properties (i.e. physical state, density, solubility in water, and vapour pressure). Sink solids (density>seawater) with a solubility of 10% (or less) and sink liquids (density>seawater) with a solubility of 0.1–5% are classified as sinker (S) and sinker/dissolver (SD) by SEBC, respectively (Le Floch et al., 2012).

The physico-chemical properties of phenol and cresols were listed in Table 1: the properties of phenol, o-cresol, and p-cresol (with a density of 1.03–1.05g/cm³, and a solubility of between 2.15 and 8.28g per 100mL of water) indicate that they will, in theory, behave as S (ATSDR, 2008a, 2008b); however, m-cresol and mixed cresol (with a density of 1.03–1.038g/cm³ and a solubility of 2.27–2.59g per 100mL of water) will behave as SD (ATSDR, 2008b; CAFÉ database, 2017). Phenol is not expected to volatilize or be absorbed by sediments and suspended particulates in the aquatic environment (ATSDR, 2008a). Biodegradation and indirect reactions with photochemically produced hydroxyl radicals and peroxy radicals are expected to be important transport mechanisms (ATSDR, 2008a). For cresols, volatilisation may be the dominant process (ATSDR, 2008b). Yet, these pathways may be influenced by environmental factors in situ¹¹⁻¹⁵.

The results of micro cosmo experiments conducted with seawater under simulated marine conditions

showed that volatilization was the dominant-attenuation type for phenol, o-cresol, and p-cresol, and photolysis dominated for m-cresol, with half-lives of 4.3–7.9 days for phenol and three isomers of cresol (Wang et al., 2017). The Henry's Law constant for m-cresol is the lowest (8.6×10^{-7} atm·m³/mol) (Lyman et al., 1990), which indicates that the rate of evaporation of m-cresol inferior to the others. Experimental bio concentration factors (BCFs) of 0.28–39 indicated that phenol and the isomers of cresol would not bioconcentrate in aquatic organisms (ATSDR, 2008a, 2008b; NCBI, 2017); however, sound evidence could be concluded, from this review, that the effects of phenol and cresols showed high acute toxicity to aquatic systems (see below). Hence, the toxicity of phenol and cresols to aquatic organisms is more likely to be a direct effect rather than via their transfer through the food chain (Rocha et al., 2016)¹⁶⁻²¹.

Table.1 Physic-chemical properties of phenol and cresols.26-27 Sources: ATSDR (2008a, 2008b) and CAFÉ database (2017).70-72

Chemical name	CAS No	Physical state	Density (g/cm ³)	Solubility in water (g/100mL)
Phenol	108-95-2	Solid	1.05	8.28
m-cresol	108-39-4	Liquid	1.03	2.27
o-cresol	95-48-7	Solid	1.05	2.60
p-cresol	106-44-5	Solid	1.03	2.15
Mixed cresol	1319-77-3	Liquid	1.030–1.038	2.59

The bioaccumulation of the highly toxic Phenolic compounds has been observed in various tissues of the fishes like scales, bones, gills, kidneys and liver¹³.The toxic effects of various chemicals may hinder the physiological and metabolic functions, rate of growth, reproductive efficacy and ultimately

causes mortality in fishes¹⁴. Toxicity tests have been performed on fishes to evaluate the effect of toxicants on various aquatic organisms under laboratory conditions. To assess susceptibility and survival potential of the test organisms 96 hr LC50 tests of some particular toxicants have been conducted. The fingerling stage of fish is more reliable to conduct toxicity test of various waterborne toxicants^{15, 16}.

MATERIAL AND METHODS:

Chemicals and Reagents used for the study- The chemicals and reagents used for the study were of analytical grade and the diagnostic kits used in the study for biochemical testing were from standard diagnostics company.

Collection and maintenance of test fish - Labeo rohita (20-25g) were collected from the culture ponds of Yamuna river of Delhi region, India and were brought to artificial small ponds/ aquarium in Institute of Transgene Life Sciences,

Dehradun/Lucknow units. Further, these fishes were kept in large tanks where a continuous and gentle flow of fresh water was maintained & fishes were given bath for 2-3 minutes in 0.1% KMnO₄ solution for the prevention of any disease. They were fed on a commercial diet ad libitum and were acclimated in tanks for a month before the experiment.

Experimental design for lethal toxicity study - The values of median lethal concentration (LC50) and median effect concentration (EC50) are usually used to characterize the toxicity of a chemical to a living organism. LC50 determinations were carried out by following semi-static acute toxicity test. For the experiment, 6 fishes were transferred to large experimental tubs, each containing 18 liters of dechlorinated tap water Eight phenol concentrations from 2700 mg l-1 (no mortality) to 3400 mg l-1 (100 % mortality) were chosen for the final 96 hour test to determine the 50 % lethal concentration (LC50) For m-cresol eight concentrations from 2500 mg l-1 (no mortality) to 3200 mg l-1 (100 % mortality) were chosen for the final 96-h test to determine the 50 % lethal concentration (LC50). Fishes transferred to tanks containing no toxicants were utilized as control. Water in the control tanks and water and toxicant in the experimental tanks were renewed daily to remove the debris, taking care to give

minimum disturbance to the fish^{17,19,20}. The fishes were not fed during the entire exposure period. Fishes were checked for mortality at every 24 hours interval. The LC50 levels and 95% confidence limits were calculated using Probit analysis (Finney, 1971)¹⁰. The lethal toxicity experiments were repeated wherever necessary.

RESULTS AND DISCUSSION:

During the present investigation the 96hr LC50 of Phenol & m-cresol for the fish, *Labeo rohita* was found to be 3212 & 2957 mg/l respectively.¹⁷⁻¹⁹⁻²⁰

Table 2: Acute toxicity range of phenol and m-cresol in *L. rohita*

Phenolic Compound (s)	Acute Toxicity Range (mg/l)
Phenol	3212
m-cresol	2957

The relation between the percentage mortality and the concentration of phenolics has been drawn (Table-2).

Figure-1. Shows the regression line between the probit kill of *Labeo rohita* and log concentration of phenolics

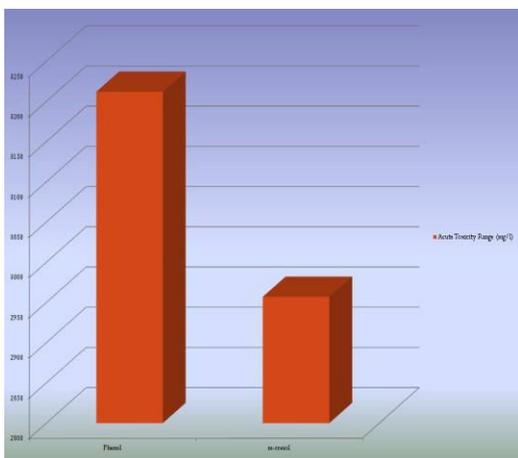


Figure 1: Acute toxicity range (LC50 values) of phenol and m-cresol in *L. rohita*

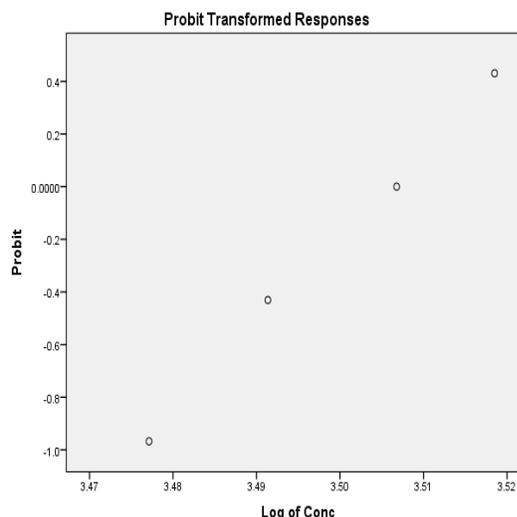


Figure 2: Regression line between the probit kill of *Labeo rohita* and log concentration of Phenol

Table: 2 Model summary of phenol relation with mortality

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics			Durbin-Watson		
					R Square	F Change	df1			
1	.961 ^a	.924	.905	89.410	.924	48.379	1	4	.002	1.929

a. Predictors: (Constant), mortality
 b. Dependent Variable: Dose

Table: 3 ANOVA Model summary of phenol relation with mortality

Model	Sum of Squares	Df	Mean Square	F	Sig.
1	386743.543	1	386743.543	48.379	.002 ^b
Residual	31976.457	4	7994.114		
Total	418720.000	5			

a. Dependent Variable: Dose
 b. Predictors: (Constant), mortality

Table : 4 Coefficient Model summary of phenol relation with mortality

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	2708.686	61.389		44.123	.000
1 mortality	128.743	18.510	.961	6.955	.002

a. Dependent Variable: Dose

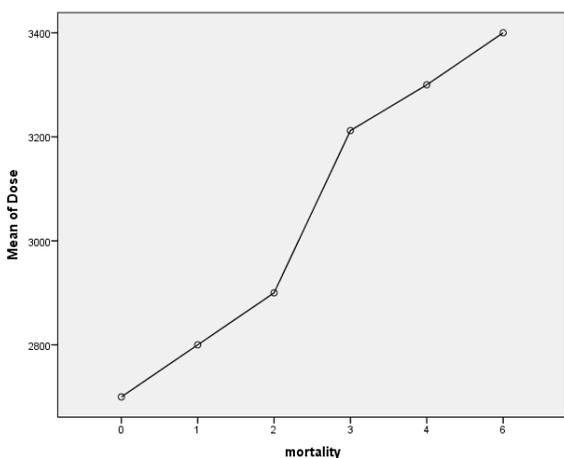


Figure : 3 Model summary of phenol relation with mortality

Carp (*Cirrhinus mrigala*) was the most sensitive freshwater organism with a 96h-LC50 of 1.555mg/L for phenol (Verma et al., 1984). Mortality of the freshwater fish *Oncorhynchus mykiss* was increased to 50% after a 96-h exposure to 3.88mg/L m-cresol (Saglam and Ural, 2005). Among marine organisms, the opossum shrimp (*Archaeomysis kokuboi*) was the most susceptible to phenol, with a 96h-LC50 of 0.26mg/L (Kim and Chin, 1995). Pink salmon (*Oncorhynchus gorbuscha*) was the most sensitive to p-cresol in marine systems, with a 96h-LC50 of 3.36mg/L (Korn et al., 1985). LC50 or EC50 values for all organisms ranged from 0.26 to 1204.6mg/L (a 4633-fold range) for phenol, 3.88–196.78mg/L (a 51fold range) for m-cresol, 5.0–167.49mg/L (a33-fold range) for o-cresol, 1.4–110.66mg/L (a 79-fold range) for p-cresol, and 10-> 100mg/L (a>10-fold range) for mixed cresol.

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According to the analysis of compiled data, the m-isomer of cresol was considerably less toxic than either the p-cresol or o-cresol for all of the organisms exposed to three isomers of cresol. This was inconsistent with the viewpoint of Benville and Korn (1977). The reason may be that the toxicity to an organism depends on the chemical structure and the bioavailability to the test organisms. The toxicity of the mixture is far more complex than a single substance. Synergistic and antagonistic effects are more likely to occur in mixture with two or three compounds (Backhaus, 2014). However, any certain conclusion could not be given according to the available study (Table 5) for the lack of toxicity data of individual cresol and mixed cresol to the same organisms.

Based on the classification criteria raised by the Joint Group of Experts on Scientific Aspects of Marine Environmental Protection (GESAMP) (GESAMP, 2013), L(E)C50 values (mg/L) are divided into seven levels of eco-toxicological hazard:

- non-toxic ($L(E)C50 > 1000$),
- practically non-toxic ($100 < L(E)C50 \leq 1000$),
- slightly toxic ($10 < L(E)C50 \leq 100$),
- moderately toxic ($1 < L(E)C50 \leq 10$),
- highly toxic ($0.1 < L(E)C50 \leq 1$),
- very highly toxic ($0.01 < L(E)C50 \leq 0.1$), and
- Extremely toxic ($L(E)C50 \leq 0.01$).

According to this rating scheme, the LC50 or EC50 values the phenol was slightly toxic to moderately toxic to fish but significantly higher, indicating that the toxicity of phenol to aquatic creatures ranged from non-toxic to high. The toxicity of cresols to aquatic organisms is the same as with phenol. Overall, the toxicity of phenol and the isomers of cresol were at similar level in terms of effect on aquatic life forms.

For aquatic organisms, salinity may affect some aspects of the system (Rocha et al., 2016). Besides, salinity may affect the toxicity of some chemicals for species specificity, altered bioavailability, and physiological phenomena (Rocha et al., 2016). Therefore, the sensitivity of marine organisms to a chemical may be different from that in a freshwater system (Rocha et al., 2016; Duan et al., 2017b). For instance, the 96h-LC50 values of phenol were 27.32–53.64mg/L for marine diatoms.

Apart from salinity, other environmental factors, such as temperature (Oksama and Kristoffersson, 1979; Cowgill et al., 1985), dissolved oxygen (DO) (Gupta et al., 1983a) and hardness of water (Stephenson, 1983), also had an influence on the toxicity of a certain chemical to the same organism. When water temperature increased, the toxic effect of phenol was accelerated (Oksama and Kristoffersson, 1979). The toxicity of phenol decreased with the increase in DO levels due to a decrease in the susceptibility of fresh water fish (*Notopterus notopterus*) (Gupta et al., 1983a). Phenol was 1.3–1.6 times more toxic in soft water than in hard for fresh water shrimp *Gammarus pulex*, while water temperature had no influence on the acute toxicity (Stephenson, 1983). Based on the discussion above, factors like organism species and growth stage, temperature and water quality, should be regulated to allow for comparing toxicity data obtained. In this case, toxicity data obtained from different labs will be well compared.

Hormesis is defined as a life-supporting beneficial effect caused by the cellular responses to single or multiple rounds of (mild) stress (Shushimita et al., 2016; Singh et al., 2012). Significant growth enhancements were observed at lower concentrations of phenol ($\leq 15\text{mg/L}$) for a marine diatom (*Skeletonema costatum*) by the authors (a manuscript in preparation). A similar phenomenon was reported at low concentrations of phenol (about 5–20mg/L) for some species of freshwater microalgae (Tadros et al., 1994). It was found that there was a positive relationship between the mortality and concentration levels; when the concentration level increased, the mortality rate increased as well. However, there was a negative relationship between the mortality time and concentration level; when the concentration level

increased, the mortality time decreased. We employed Finney's probit analysis method of data evaluation for acute toxicity bioassay.

CONCLUSION:

It is concluded that some organisms become sensitive to high concentrations of some Phenolic compounds in the aquatic ecosystem and that causes deleterious effects on them. It helps us to determine the permissible limit of a toxicant in an ecosystem. Acute toxicity test reveals about the health of given aquatic ecosystem and eventually help us to propose policies to protect the aquatic ecosystem. It helps us to evaluate the environmental damage resulting from the pollutants and the establishment of water quality criteria to protect aquatic life

Acknowledgement

Authors are highly thankful to Director, Institute of Transgene Life Sciences (ITLS), Dehradun for making available all the necessary facilities to carry out this piece of research work.

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Cite of article: Gaur V, Mathur A; Determination of lc_{50} of phenolic compounds (phenol & m-cresol) for a fish, *labeo rohita* . Int. J. Med. Lab. Res. 2019, 4(1):55-63

CONFLICT OF INTEREST: Authors declared no conflict of interest

SOURCE OF FINANCIAL SUPPORT: Nil

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