Current Trends in Natural Sciences Vol. 3, Issue 6, pp. 82-87, 2014

Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

EFFECT OF PHENOL INTOXICATION ON SOME PHYSIOLOGICAL PARAMETERS OF *PERCA FLUVIATILIS* AND *PELOPHYLAX RIDIBUNDUS*

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Abstract
A significant interest to phenols and their derivatives is connected with the fact that these compounds play an important role in human industrial activity and are widely spread in our environment. In the present paper it was investigated the action of one aquatic pollutant - phenol - under two concentration (25 and 50 mg/l water) on some physiological parameters (energy metabolism, respiratory rate, number of erythrocytes and leukocytes, blood glucose level, survival) on perch (Perca fluviatilis) and marsh frog (Pelophylax ridibundus). The choice of these species is based on the fact that they are widely spread and can be well preserved in laboratory conditions. They are also important in the aquatic ecosystems and last, but not least, they are sensitive to various toxic actions. Phenol produces, after two weeks of immersion, a significant decrease of respiratory rhythm, glycaemia values and the number of erythrocytes. Exposure of perch to phenol action produces oxygen consumption decrease after two weeks of intoxication. In the concentration of 50 mg/l, the phenol produces, after two weeks of immersion, a significant increase of the number of leukocytes.

Keywords: perch, marsh frog, phenol, physiological parameters

1. INTRODUCTION

In present is a growing concern about the effects of waterborne contaminants that pose unacceptable risks to wildlife that use aquatic habitats. Phenol and phenolic compounds are ubiquitous pollutants which come to the natural water resources from the effluents of a variety of chemical industrial (Fleeger et al., 2003). Consequently, aquatic organisms including fish and frog are subjected to these pollutants. Phenol and its derivatives induces toxic effect for fish: they induce genotoxic, carcinogenic, immunotoxic, hematological and physiological effects (Assem et al., 1992; Taysse et al., 1995; Tsutsui et al., 1997) and have a high bioaccumulation rate along the food chain due to its lipophilicity. Phenol affects the central nervous system causing paralysis and interferes with the respiratory system resulting in asphyxia in fish (Gupta and Srivastava, 1984).

Monohydric phenols constitute one of the main pollutants present in phenolic wastes and affect aquatic life adversely both by their direct toxicity to fish and the heavy demand for dissolved oxygen (Ravichandran and Anantharaj, 1984).

The ability to conjugate phenols with either glucuronic or sulphuric acid seems to be completely lacking in fish. Doses of phenols as low as 0.5 mg/kg are often fatal; fish placed in solutions of 10 ppm of phenols absorbed enough of the phenols through tile gills to be toxic in 4-8 h, whereas frogs

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were found to excrete 90-95% of a 5 mg dose of phenols as conjugated compounds within 48 h after administration (Gupta and Srivastava, 1984).
The present study was undertaken to determine the toxicity of phenol by conducting 14-day semi-static tests in perch and marsh frog.

2. MATERIALS AND METHODS

The study was performed with the approval of the local Committee of Bioethics according to the Romanian law 205/2004 art.7, 18, 22 and regulation number 143/400/2002 for care and use of animals for research purposes.
A total number of sixty healthy adult perch (with an average initial body weight of 18± 0.5 g) and marsh frogs (male and female with an average initial body weight of 28±2.3 g) were used in the study. The animals were captured in spring (April-May 2013) from the surrounding lakes of the city Pitești (South Romania) and were kept in laboratory conditions in glass aquariums and aquaterrarios filled with dechlorinated tap water at a constant temperature (18±2°C) and 12 h light-dark conditions for ten days to test their health and accommodate them for the experiment. Oxygen supply was maintained in each aquarium using an electric aerator pumps. The water was changed daily to avoid the accumulation of toxic substances and the animals were fed *"ad libitum"*. Both the experimental and control fish and frogs were not fed 24 h before or during the experiments, in order to avoid the intervention of this factor (Picoş and Năstăsescu, 1988).
The animals was divided into three groups, each group included ten fish/frogs: groups I and II exposed to 25 and 50 mg/L of phenol respectively, group III was kept without phenol (served as the control). Technical grade of phenol (C_6H_5OH), M.W. 94.11 with melting point of 40.9 °C was obtained from Borealis AG; the stock solution was prepared by dissolving phenol in distilled water (solvent). The solutions in the aquariums and aquaterrarios were changed every 24 hours using semi-static tests. The exposed animals were kept under proper observation during the period of experiment for any external clinical abnormalities.
The following physiological parameters were investigated: the lifespan, the energetic metabolism (only in fish), the breathing frequency, the number of erythrocytes and leukocytes and the concentration of plasma glucose. The oxygen consumption and the respiratory rate being established under standard conditions.
The energetic metabolism, expressed by the o dose in the water was established by using the Winkler chemical method) (Picoş and Năstăsescu, 1988). The oxygen consumption and the respiratory rate were determined for intervals of 24, 48, 72, 96, 168 and 336 hours (where allowed by the surviving animals).
Blood specimens were withdrawn from the frogs by cardiac puncture after chloroform anesthesia; the blood was sampled in fish from the caudal artery using the method recommended by Picoş and Năstăsescu (1988). The number of red and white blood cells was determined using Thoma counting chambers, following the method described by Picoş and Năstăsescu (1988). Collection of blood samples were done at the end of experiment. The blood glucose concentration was determined by means of Accutrend®GCT using a drop of blood in a very short time.
The results were interpreted statistically using SPSS 13.0 program for Windows, in accordance with the specialized studies. The independent t-test for the significance threshold $p<0.05$ was used to compare between two variables.

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3. RESULTS AND DISCUSSIONS

Changes in breathing frequency of perch and marsh frog exposed to phenol are shown in Figure 1.

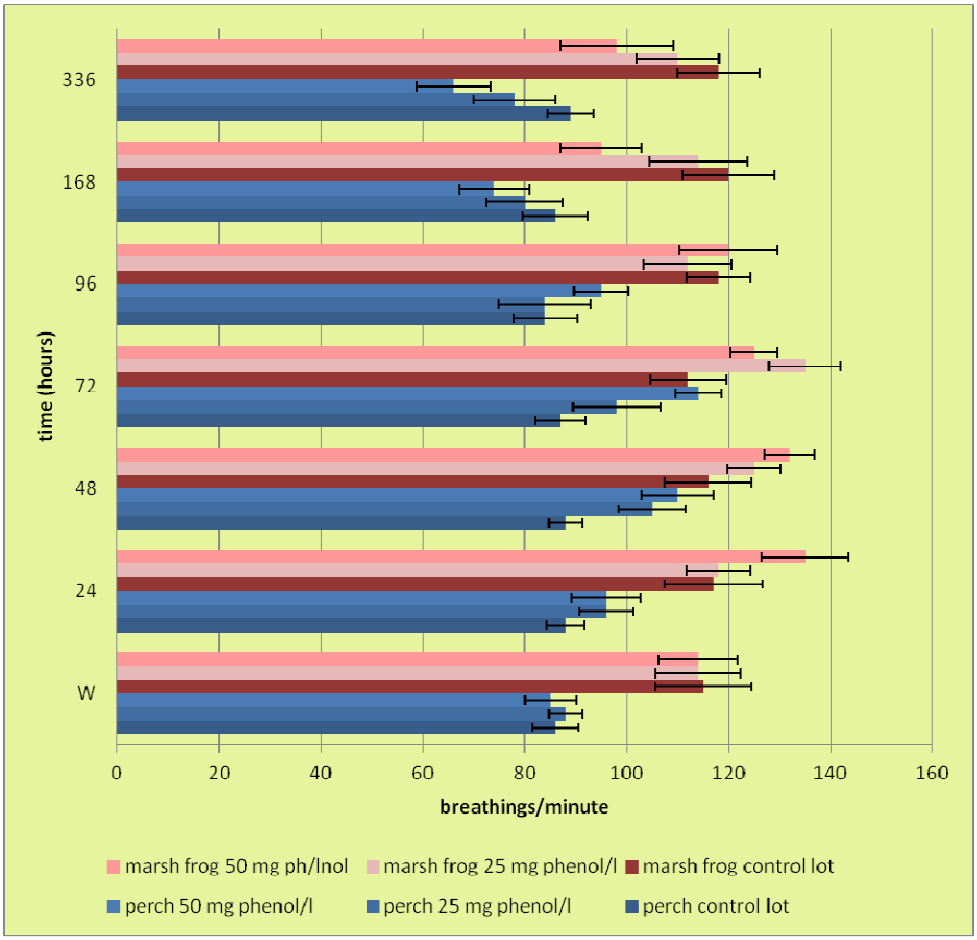

Figure 1. The influence of phenol upon breathing frequency of perch and marsh frog

Respiratory rate increases in the first phase (after 72 hours of exposure) both perch and the marsh frogs; later physiological average values of this physiological index decline gradually, so that at the end of the experiment the animals breathing movements are significantly slower compared with control groups (excepting frogs exposed to 25 mg phenol/l water).

Phenol affects the central nervous system causing paralysis and animal respiratory rhythm disorders (McCahon et al., 1990). Increased respiratory rhythm after phenol exposure has also notice in fish (Saha et al., 1999).

The oxygen consumption was found to be significantly influenced by the two concentration of phenol used. As shown in Figure 1, as compared to the values recorded for the control individuals, phenol reduced the energy metabolism in perch, reducing directly proportional to the toxic concentration and duration of exposure.

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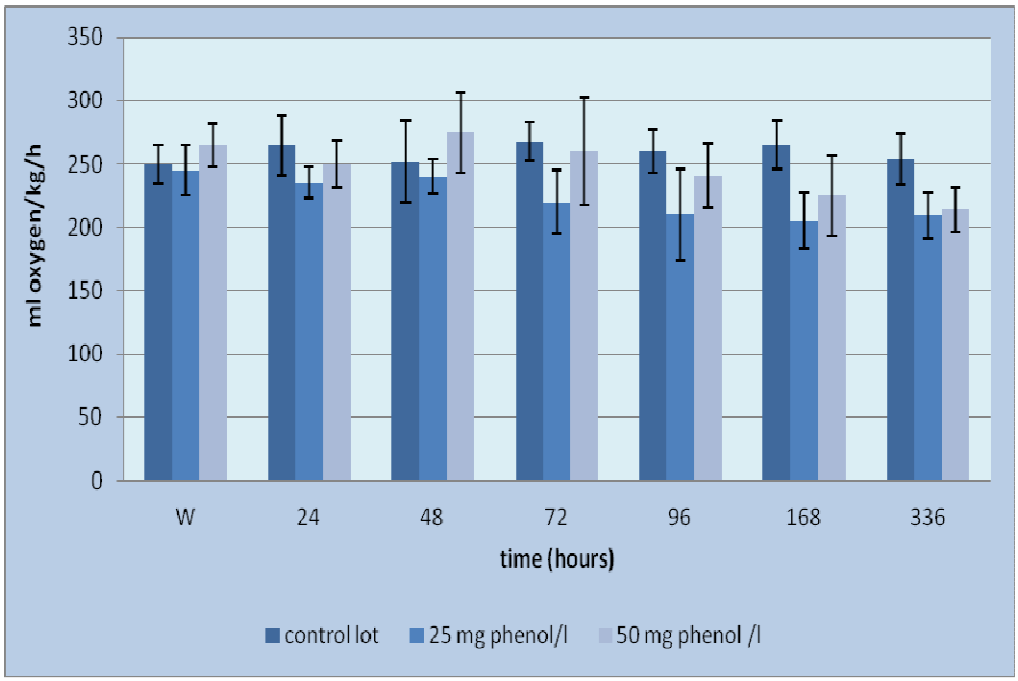

Figure 2. The influence of phenol upon oxygen consumption on perch

For the first 48 and 72 hours after immersion the phenol effect is stimulating oxygen consumption in perch; after this period, effect of energetic metabolism has been inhibiting (80,75% comparative to the control values). The values recorded at the end of the experiments were significantly difference comparing to the control values.

Stimulation of oxygen consumption and respiratory rate in the early hours of the exposure are a first reaction to the main harmful effect of phenol or reducing the amount of dissolved oxygen in water. Reducing oxygen consumption and breathing frequency found by us can be attributed to other effects of phenol reported by different authors.

Various pathological effects, including necrosis of the gills and increased gill mucus production (Reichenbach-Klinke, 1965), destruction of erythrocytes (Waluga, 1966) have been reported in fish following exposure to sub lethal concentrations of phenol.

Phenol also affected the functioning of the thyroid gland with low levels of thyroid hormone release, as shown in the research of Nahed and Amal in 2008 investigating *Oreochromis niloticus* physiological modifications exposed for 16 weeks in doses of 0.4, 1.4 and 2.8 mg phenol/liter. The authors report, among other things, a significant decrease in serum thyroxin, triiodothyronine.

Hematological and biochemical parameters can be used as standard laboratory tests to determine metabolic disorders caused by the action of xenobiotics (Celik, 2004).

Physiological adaptations to stress in fish causing alterations in blood chemistry standards; hematological parameters are commonly used in fish as secondary stress indicators (Wedemeyer et al., 1990).

The determination of glucose concentration in blood plasma is widely used for the assessment of fish stress (Martınez-Porchas et al., 2009).

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Table 1 shows the changes in the number of erythrocytes, leukocytes and glucose plasma levels of perch and frogs intoxicated for two weeks with phenol.

Table I. Effect of two weeks phenol exposures of RBC, WBC and glucose level in perch and marsh frog

Parameters	Phenol exposure	Perch	Marsh frog
RBC (number of erytrocytes/dL blood)	Control lot	1240211±324211.74	425121±23016.75
	25 mg phenol/l	750000±24321.66	350000±15.124.76
	50 mg phenol/l	625000±15.265.45	320000±14.111.64
WBC (number of leukocytes/dL blood)	Control lot	44000± 8112.56	55000±6325.74
	25 mg phenol/l	49000±4511.66	58000±5542.66
	50 mg phenol/l	52500±6233.44	62000±4621.45
Glucose (mg/dL blood)	Control lot	66± 12.33	46±11.23
	25 mg phenol/l	74±16.56	66±13.55
	50 mg phenol/l	88±11.35	74±11.66

Phenol intoxication is followed by changing some hematological and biochemical parameters, exemplified by increasing the number of leukocytes and glucose level and decreasing the number of erythrocyte. Frogs recorded the strongest hyperglycemic effect.
The data in the literature suggest that the fish subjected to phenol elicit marked changes in carbohydrate metabolism: hyperglycemia after phenol intoxication (26 mg/l) on indian catfish, *Heteropneustes fossilis*, was observed at 3, 6 and 12 h post exposure (Gupta and Srivastava, 1984).
Table 2 shows the survival times on perch and marsh frog during the 14 days of experiments.

Table 2. Lethal effect of phenol on perch and marsh frog

Species	Phenol (mg/l)	**The number of living specimens**					
		Immersion time					
		24 hours	48 hours	72 hours	96 hours	144 hours	336 hours
Perca fluviatilis	0	10	10	10	10	10	10
	25	10	10	10	10	10	8
	50	10	10	8	6	6	4
Pelophylax ridibundus	0	10	10	10	10	10	10
	25	10	10	10	10	10	10
	50	10	10	10	10	8	8

At concentration of 50 mg phenol/ l water, we recorded a low mortality (20 % at the end of the experiment) of marsh frog. There were no mortality during the acute test (96 hours) than the perch exposed to phenol in a concentration of 50 mg/liter of water ; in this experimental variants showed the highest mortality at the end of the experiment (60%).
Obvious behavioral changes we found of fish lots exposed to phenol early hours of installing the experiments, the most important being: frequent lifting the water surface, atmospheric air swallowing, irritability, motor incoordination. Other authors (Saha et al., 1999) reported the following changes in the behavior of the species *Oreochromis mossambicus* exposed to various concentrations of phenol : water surface elevation frequent, swallowing air (air bubbles coming out of the mouth of the fish), some specimens are frequently hitting the edge of the aquarium and finally lost their balance.

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4. CONCLUSIONS

Phenol had an inhibitory effect on the energy metabolism of perch at concentrations of 25 and 50 mg/l water. In the first 48-72 hours after immersion, phenol is stimulating of the breathing frequency of perch and marsh frog; after two weeks phenol caused a significant decrease in the respiratory rhythm of this animals.

Exposure to phenol resulted in significant increase of plasma glucose and number of leukocytes between the experimental and control groups. After two weeks of phenol exposure number of perch and marsh frog erythrocytes decreased.

During acute test (96 hours) mortality was recorded only for perch exposed to 50 mg phenol/l water.

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