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Toxic Effects of Phenol on Grey Mullet, *Mugil auratus* Risso

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Phenolic compounds are frequently found as contaminants in surface waters, including marine coastal waters. The potential sources are some effluents from coal, oil and pulp processing industries and municipal sewage plants. Phenols are generally classified as nonspecific metabolic inhibitors, and the main toxic effects are manifested on the nervous system due to the dissolution of lipids, whereas in the circulatory system phenols act as hemolysing agents of the erythrocytes. Depending on the organism tested, the acute toxicity of phenol varies from about 6 to 1800 mg/L and generally the 96-h LC50 values for fish are between 44 and 412 mg phenol/L (Buikema et al. 1979). Fish exposed to higher concentrations are excited, swimming rapidly and become more sensitive to external stimuli (Veselov 1975). Death may occur quickly or it may follow a stage of depressed activity (Lukyanenko 1965). Data about sublethal effects of phenol, particularly to marine organisms are rather scarce. In several fresh water fish species exposed to phenol, the number of erythrocytes and the amount of serum proteins were decreased while lesion of gill filaments with edema and blood infiltration with degenerative changes in liver were also observed (Waluga 1966; Mitrović 1968; Mitrović 1982).

Our investigations concerned the identification of some physiological and biochemical changes in mullet blood as a consequence of exposure to phenol and some observations about the behavior and gross pathology of poisoned fish were also made.

MATERIALS AND METHODS

Grey mullet, Mugil auratus Risso, weight about 125 g length 22-25 cm, were used as test organisms. The experiments were carried out in aerated, 200-L aquarium

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tanks at constant temperature of 12± 0.5°C, salinity 37.5 ± 0.3 °/oo with a continuous flowing sea water system (about 1000 L/d) to which a corresponding quantity of phenol was added with a dosing apparatus. The concentration of phenol in experimental basins was determined using the aminoantypirine method (Ochynski 1960). The number of heterotrophic "phenolic bacteria" in experimental basins was checked by inoculation of Bushnel-Haas media (DIFCO agar) containing 150 mg/L phenol as the only source of organic carbon.

Mullet were exposed to various concentrations of phenol: 0.5, 5.0, 7.5, 10, and 25 mg/L. The exposure time and sampling were determined according to the adopted concentrations. Blood samples were taken by heart puncture and several hematological and biochemical parameters were measured. Hemoglobin (Hb) was determined with the standard cyanmethemoglobin method, hematocrit (Hct) by centrifugation in heparinized microcapillaries and the mean corpuscular hemoglobin concentration (MCHC) was then calculated (Wintrobe 1974). After centrifugation of blood, total Biuret protein (Weichselbaum 1946), total lipids (colorimetric method of Zollner and Kirsch 1962), cholesterol (CHOD-PAP method of Roeschlau et al. 1974) and blood glucose (God-period method of Werner et al. 1970) were measured. The enzyme activity of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase (GOT and GPT colorimetric method of Reitman and Frankel 1957) and lactate dehydrogenase and glutamate dehydrogenase (LDH and GLDH-activated UV method of Bergmeyer and Bernt 1974; Schmidt 1974) were estimated and expressed in International Units (mU/mL).

The behavior of fish in the tanks was frequently checked. After blood sampling fish were sacrificed and inspected macroscopically for the appearance of pathological changes on lips, opercula, fins, gills, liver and gall bladder, kidney and heart.

RESULTS AND DISCUSSION

It was observed that although a constant dosing was established, the actual concentration of phenol during the experiments was decreasing and after a few days it dropped down to 50 % of the initial values. That was due to a rapid proliferation of some "phenolic bacteria", which have been demonstrated by inoculation of water samples from basins containing phenol, into Bushnel-Haas culture media enriched with phenol only. From the basins containing 7.5 mg phenol/L the number of developing bacterial colonies was about 20 times higher than the control - phenol free - basins and they appeared to be the main type of microorganism

population. It was therefore necessary to measure the phenol concentration more frequently (twice a day) and adjust the dosing system in proportion to the phenol deficiency. The proliferation of "phenolic bacteria" was highly disruptive of the course of the experiments but, it also confirmed that moderate concentrations of phenol in the marine environment do not represent a serious problem of pollution. As in fresh waters (Trama 1955; Kristoffersson 1973) groups of heterotrophic bacteria are able to digest phenols, thus preventing their excessive accumulation in the marine environment.

Table 1. Survival, behavior and gross pathology observations on grey mullet exposed to various concentrations of phenol.

Phenol mg/L	Exposure time	Survival %	Fish behavior, neurotoxic symptoms	Gross pathology observations
0.5	8 d	100	normal	no evident changes
5.0	8 d	100	excited, fast swimming	no evident changes
7.5	8 d	90	excited, sensitive to light, later depressed activity	hemorrhagic opercula and lips, dark liver, swollen gall bladder, anemic kidney
10	5 h	75	loosing equilibrium	inflamed gills, hemorrhagic viscera, coagula in heart cavity
25	1 h	50	convulsions, suffocation and death	damaged and inflamed gills, dark liver and gall bladder

Characteristic neurotoxic symptoms and gross pathology observations in relation to phenol concentration and exposure time are presented in Table 1. Readily after introduction into basins with 5 mg phenol/L, mullet were generally excited: fast swimming and faster branchial ventilation were observed but nearly after 1 day the fish had accommodated. Their behavior was normal and no evident pathological changes on tissues were observed. The first stage of intoxication - excitation followed by a general depression of activity - was noted in fish exposed to 7.5 mg phenol/L. Loss of balance - described by Lukyanenko (1965) as the second

Table 2. Hematological and biochemical values and plasma enzyme activity in mullet exposed to various concentrations of phenol.

Phenol (mg/L)	Con-	0.5	5.0		7.5	10		25	
Exposure time	trol	8 d	4 d	8 d	8 d	5 h		1 h	
Sampling time		8 d	4 d	8 d	8 d	2 d	6 d	1 h	4 d
Hemoglobin (g/100 mL)	8.9 (0.9)	9.1 (0.4)	8.9 (0.6)	10.0 (0.4)	5.7< (0.2)	5.3< (0.1)	4.2< (0.2)	8.8 (0.6)	9.0 (0.7)
Hematocrit (%)	36 (5)	38 (1)	40 (2)	48 (4)	28< (3)	26< (2)	22< (1)	41 (3)	37 (3)
MCHC (%)	24	24	22	22	20	20	19	22	25
Glucose (mg/100 mL)	48 (8)	43 (4)	67> (10)	45 (16)	59> (5)	55 (10)	50 (7)	68> (11)	70> (11)
Lactate (mg/100 mL)	37 (3)	-	-	-	18< (2)	12< (1)	11< (1)	-	-
Total protein (g/100 mL)	3.5 (0.3)	3.8 (0.3)	3.5 (0.2)	4.4> (0.5)	1.3< (0.3)	1.7< (0.3)	1.8< (0.3)	3.8 (0.1)	3.5 (0.6)
Total lipids (mg/100 mL)	1366 (456)	-	-	-	402< (90)	555< (113)	803< (150)	1400 (180)	1004 (365)
Triglycerides (mg/100 mL)	211 (72)	-	-	-	59< (8)	56< (12)	68< (10)	-	-
Cholesterol (mg/100 mL)	180 (28)	-	-	-	72< (12)	78< (15)	94< (18)	160 (12)	165 (16)
G O T (mU/mL)	14 (3)	18 (7)	53> (20)	55> (14)	328> (72)	165> (63)	275> (62)	53> (11)	180> (71)
G P T (mU/mL)	3.0 (0.4)	-	-	-	15> (3.4)	3.2 (0.8)	3.6 (0.2)	-	3.0 (0.4)
L D H (mU/mL)	85 (25)	100 (30)	287> (33)	90 (11)	127 (21)	389> (111)	95 (8)	1335 (635)	328> (110)
G L D H (mU/mL)	60 (5.5)	-	-	-	912> (54)	-	-	-	-

Control group = 8-10 specimens; phenol groups = 5 specimens; (s); significantly higher (>) or lower (<) from control, $p < 0.01$.

stage of intoxication - followed by convulsions and progressive lethality were observed at concentrations of 10 and 25 mg phenol/L. A few hours after introduction into higher phenol concentrations, the gills were evidently damaged and internal hemorrhages with blood coagula in heart cavity, dark liver and gall bladder were observed. Less severe damage involving swollen and dark green gall bladder, dark liver and anemic kidney occurred also in fish surviving 8 days in 7.5 mg phenol/L.

The concentration of 0.5 mg/L did not induce any evident change of the hematological and biochemical values (Table 2), while in fish that were exposed 4 days to 5 mg phenol/L only glucose, LDH and GOT activity in plasma was increased. The LDH increase was of transient character, and after 8 days it returned to the level of the control group. The most obvious alterations were observed in groups exposed 8 days to the sublethal, 7.5 mg/L, as well as in the group exposed only 5 h to the lethal, 10 mg phenol/L, and then transferred to clean sea water and successively sampled after 2 and 6 days. In both groups hemoglobin and hematocrit were decreased, while the MCHC values remained at the same level as the control group. It was supposed that a certain number of erythrocytes had been destroyed and hemoglobin was lost in hemorrhagic organs and tissues as it was confirmed by gross pathology observations. Erythrocyte number decrease was observed also by Halsband and Halsband (1963) and by Waluga (1966) after exposure of trout and sea bream to subtoxic concentrations of phenol. The concentration of glucose in the blood of mullet was not altered, but significant decreases of lactate, total protein, total lipids, tryglicerides and cholesterol were observed. Concurrent with the reduction of protein and lipid concentration, the enzyme activity in the plasma was generally increased and positively correlated with the phenol concentration and exposure time. The enzyme activity increase was observed even when hematological changes were not detected. The highest LDH activity was measured immediately after the exposure of mullet but in a few days it showed a tendency to recover decreasing towards the control values. On the contrary the activity of GOT was increasing even during the recovery period. The originally very low activity of GPT in plasma was increased only after a prolonged exposition (8 days to 7.5 mg phenol/L) and the activity of GLDH was also found to be 15 times higher than the control group. The observed enzyme activity increase in plasma could be explained as a consequence of enzyme leaking from the damaged tissues where they are functionally located as intracellular metabolic enzymes or they can be derived from the changed metabolic

activity to compensate the stress induced by phenol intoxication. The latter explanation is in good agreement with the decreased concentration of total protein and lipids measured in plasma of mullet. The increase of transaminase activity was also observed in pike, Esox lucius (Kristoffersson 1974) in Notopterus notopterus (Verma et al. 1981) and in Leuciscus idus (Tiedge et al. 1986) exposed to phenol. Recently Gluth and Hanke (1983) found a significant decrease of plasma proteins linked with the decrease of cholesterol. It is not easy to understand such a decrease of plasma proteins and lipids in fish because their presence and functional significance in plasma has not yet been clearly explained. As the concentration of lipids decreased at the same time as total protein, the diluton of plasma with water could perhaps be expected. But, there are several pathological processes which can also contribute to the decrease of plasma components: renal damage and excretion by urine, decreased liver protein synthesis, alterations in hepatic blood flow and/or hemorrhages into the peritoneal cavity and intestine.

The highest permissible concentration of phenol in sea water should not exceede 1 µg/L (Buikema et al. 1979), while in polluted coastal waters, the concentration of phenol was found to be in the range of 2-15 µg/L (Baeteman and Vyncke 1979). Like other fish, grey mullet are relatively insensitive to phenol and obvious harmful effects occur only at concentrations of >1 mg/L, an exceptionally high concentration that could be expected in heavy polluted but in space restricted regions. The earliest neurotoxic symptoms were observed at 5 mg phenol/L. The excited phase was rather short, but the changes in plasma were higly significant, particularly regarding the increase of glucose concentration and GOT and LDH activity.

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