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Relative Sensitivity of Some Selected Aquatic Organisms to Phenol

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In the last 25 years the increase of industrialization process has brought about many ecological problems, which are due to the toxic activities of several compounds (from one to six thousand new compounds are placed on the American market annually) (Boardman and Sherrard 1983). In response to the growing problems in the environment and with the conviction that the potential damage to living societies cannot be ascertained by a single criterion, various biological tests have been developed in the world (Bitton and Dutka 1986). Toxicology started to develop as an independent science in the first years of the 19th century owing to the rapid growth of organic chemistry. Although the possibility of adverse effects of materials on organisms was recognized earlier, not until the 1940s or 1950s short-term acute tests with fishes were being studied (Buikema et al 1982). The fishes have become the most popular test organism because the effects of toxic substances in streams have been most evident on the fishes. Later it was established that many other organisms bearing the important role in the environment live in the waters. Other species of organisms including juveniles began to be used in the toxicological research. Nowadays we propose such great variety of test methods and organisms that the selection of the suitable toxicity test is rather difficult (Baudo 1987).

Hundreds of organisms live in the water, so the choice of the most sensitive one is practically impossible. One or two species of test organisms only are often applied in the determination of the toxic substances or wastewaters. The toxicity test with daphnids is widely used due to its sensitivity to the great part of toxic substances. Baudo (1987) compares the results on cadmium and copper impact on several sorts of organisms from different taxonomic groups. The comparison indicates the most sensitive organism being water flea in the case of copper, but not in case of cadmium.

The purpose of our research was to determine the toxicity of phenol to some aquatic organisms from the groups of bacteria, algae, crustacea and fishes, and to determine the most sensitive kind. We were interested to learn the degree of sensitivity that *Daphnia magna* as the standard test organism in the acute toxicity test (International Organisation for Standardization 1989) will show in comparison with other species. Test organisms have been selected from three basic groups in the food chain (bacteriа - decomposers, algae - producers and crustacea, fish - consumers). Phenol, an organic degradable substance that is a frequent contaminant in wastewaters, has been chosen for the test substance.

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Due to its universal use (production of resins, pesticides, drugs, dyes, oil and fuel additives, etc.) and increased production, very high concentrations of phenol may be found in the wastewaters from petroleum and chemical industries, from pulp and paper mills and wood preservation plants, while lower concentrations are present in municipal wastewaters (Moore and Ramamoorthy 1984).

MATERIALS AND METHODS

The standard phenol solution was prepared according to American standard methods (APHA-AWWA-WPCF 1989). The stock phenol solution was prepared by dissolving the appropriate amount of phenol (analytical grade) in distilled water. Test concentrations were prepared by diluting appropriate aliquots of the stock phenol solution in tap water.

All experiments were conducted with dechlorinated tap water having total hardness 252 mg $CaCO_3$/L, alkalinity 243 mg $CaCO_3$/L and pH 8.5.

As test organisms we have chosen mixed bacterial culture, luminescence bacteria *Photobacterium phosphoreum* (Cohn 1878) Beijerinck 1889, green algae *Scenedesmus quadricauda* (Turp.) Breb. and *Scenedesmus subspicatus* Chodat 1926, crustacea *Daphnia magna* Straus, 1820 and rainbow trout *Oncorhynchus mykiss* Call, 1990.

Test temperature was $20\pm1^{o}C$, with the exception of toxicity test with *Photobacterium phosphoreum* (test temperature was $15\pm0.2^{o}C$).

Toxicity test with mixed bacterial culture was carried out according to Offhaus method (1973). Mixed bacterial culture was obtained from settled municipal wastewater. The biochemical degradation of organic matter (peptone) was determined by measuring the oxygen consumption rate on Sapromat (type B12, Voith, Heidenheim, Germany) and baromat (WTW, BSB-Messgerät, Model 1000, Weilheim, Germany) apparatus. The oxygen consumption for peptone degradation in dilution water and in samples with different concentrations of phenol were compared for 120 hr.

Freeze - dried marine bioluminescent bacteria *Photobacterium phosphoreum* was used as test organism. The luminescence of bacteria was measured on Lumistox apparatus (dr.Lange, Düsseldorf, Germany) before and 30 min after the phenol was added (Bruno Lange 1989; Bruno Lange 1990).

The green algae were cultured in nutrient solution according to Holm Hansen (Bringmann and Kühn 1980) - *Scenedesmus quadricauda* and to Jaworski (Thompson et al 1988) - *Scenedesmus subspicatus* at $20\pm1^{o}C$ with continuous illumination (3000 lux). Ten-day-old algae culture was used in toxicity tests in which the net assimilation and chlorophyll-a were measured. The net assimilation was measured according to method of Peukert (1967) and von Tümpling (1972). The oxygen production of algae in dilution water (control) and in samples with different concentrations of phenol was measured on Warburg apparatus (type V166, B.Braun, Melsungen, Germany). After 24 hr the percentage of inhibition of algae net assimilation was calculated. The chlorophyll-a is proportional to the biomass of algae. After 72 hr of algal growth in the control and in samples with different concentrations of phenol the chlorophyll-a was extracted

in hot ethanol and determined spectrophotometric (Arndt 1982; Kordiš 1988).

The toxicity test with *Daphnia magna* was performed according to ISO standard (International Organisation for Standardization 1989). Daphnids were cultured in 3-L beakers at 20±1°C under the photoperiod of 12-h light: 12-h dark and fed with dry yeast three times a week. The immobility of neonates (less than 24 hr old) in control and in samples with phenol was compared. After 24 hr exposure to phenol the percentages of the daphnids immobility were determined.

The toxicity test with rainbow trout *Oncorhynchus mykiss* was conducted according to Skibba (1982). The fish were obtained from fish-farms Sava and Povodje. Juvenile rainbow trout (length about 10 cm) were acclimated for at least 7 d to test conditions. During acclimatization period the fish were fed daily with commercial food. The test duration was first 96 hr, then it was shortened to 48 hr, because of phenol degradation. Samples in aquaria were slightly aerated because the shortage of dissolved oxygen in water resulting from phenol degradation may cause the fish death. pH and dissolved oxygen were measured daily. The phenol concentrations in samples at the beginning of the test, after 24, 48 and 96 hr (in the initial tests) were determined using spectrophotometric 4-aminoantipyrine method without extraction (APHA-AWWA-WPCF 1989). After 24 and 48 hr the mortality of fish was determined.

For each concentration of phenol and controls in toxicity tests with bacteria and algae at least two replicates were performed, in toxicity tests with daphnids and rainbow trout two replicates of 10 organisms each were carried out. Each type of toxicity test was repeated three times, therefore obtained results are the average of three experiments.

The probit analysis (Statistical Support Staff Computer Sciences Corporation 1988) was used to calculate EC_{10}, EC_{50} (and its 95% confidence limits) and EC_{90} values for bacteria, algae, crustacea and LC_{10}, LC_{50} with 95% confidence limits and LC_{90} for fish.

RESULTS AND DISCUSSION

The results of phenol toxicity to bacteria, algae, daphnids and fish are represented in Tables 1 and 2. The impact of phenol on mixed bacterial culture (Table 1) functions started only in the solutions containing 300 mg phenol/L. Alsop et al (1980) too, had established the impact of phenol on the growth of mixed bacterial culture and observed that the median inhibition is rather high (IC_{50} = 760 mg/L). Low toxicity level of phenol may be explained by the various sensitivities of species in the mixed bacterial culture. Less sensitive bacteria survived in the presence of phenol in the test samples, adapted to it, degraded it and multiplied themselves; the more sensitive species died. Only higher concentrations of phenol were toxic for most of bacteria, and they could not degrade it (Tišler 1987). It is known from the relevant literature that respiration is less sensitive to toxic substances than other physiological functions namely growth, mobility, luminescence (Alsop et al 1980; Dutka et al 1983; Bitton and Dutka 1986). Toxicity tests with pure bacterial culture revealed a rather higher sensitivity to phenol than mixed bacterial culture. The toxicity threshold of phenol to *Pseudomonas fluorescens* was observed at 64 mg/L (Bringmann and Kühn 1980); 15min EC_{50} values for *Photobacterium phosporeum* were 34.3 mg/L (Bitton and Dutka 1986) and 28 mg/L (Qureshi et al 1982). In our study higher

Table 1. Phenol toxicity to bacteria (mixed bacterial culture, *Photobacterium phosphoreum*) and algae (*Scenedesmus quadricauda*,*Scenedesmus subspicatus*)

Species	Mixed bacterial culture	*Photobacterium phosphoreum*	*Scenedesmus quadricauda*	*Scenedesmus subspicatus*	
Phenol concentration (mg/L)	Biochemical degradation inhibition (%)	Luminescence inhibition (%)	Net assimilation inhibition (%)	Net assim. inhib.(%)	Chlor-a inhib. (%)
0	0	0	0	0	0
5	-	-	0	-	-
10	-	-	-	-	21
20	-	26	-	-	-
30	-	40	-	-	-
40	-	46	-	-	-
50	-	58	-	-	-
60	-	60	-	-	-
70	-	60	-	-	-
80	-	66	-	-	-
90	-	69	-	-	-
100	0	-	-	19	27
200	0	-	16	38	43
300	15	-	32	-	-
400	-	-	-	54	-
500	47	-	63	-	-
600	-	-	-	76	-
700	83	-	-	-	-
800	-	-	-	85	-
1000	86	-	96	-	89

Table 2. Phenol toxicity to daphnids *Daphnia magna* and fish *Oncorhynchus mykiss*

Species	*Daphnia magna*	*Oncorhynchus mykiss*
Phenol conc. (mg/L)	Immobility (%)	Mortality (%)
0	0	0
5	0	-
10	5	10
12	-	33
14	-	54
15	21	76
16	-	92
20	31	100
30	47	-
40	57	-
50	63	-
60	89	-
80	100	-

sensitivity of *Photobacterium phosphoreum* than mixed bacterial culture to phenol was also established (Tables 1 and 3).

The same applies to algae (Table 1) where phenol showed low toxicity level since the phenol concentrations of 100 mg/L inhibited the net assimilation. EC_{50} values for algae (Table 3) are rather high, especially for the net assimilation of *Scenedesmus quadricauda* (24h EC_{50} is 403 mg/L). *Scenedesmus subspicatus* was a more sensitive species to phenol than *S. quadricauda*. Also Shigeoka et al (1988) indicated high EC_{50} values for the alga *Selenastrum capricornutum* of 150 mg/L (4 days) and for the alga *Chlorella vulgaris* 370 mg/L (4 days).

The information on the phenol toxicity on the daphnids *Daphnia magna* is numerous (Verschueren 1977; Rippen 1984; Bringmann and Kühn 1977; Phipps et al 1984). The EC_{50} values obtained (Table 3) match the results as published in the article by authors Qureshi et al (1982).

Phenol effected the rainbow trout within the narrow concentration range, as we achieved 0% - 100% mortality between 10 and 20 mg/L (Table 2). Phenol provoked a considerable death of fish within few hours, and the longer test period did not alter essentially the result. Above mentioned finding is also confirmed in the relevant literature (ASTM 1982). It is stated therein that almost all phenol is absorbed in fishes the first day only after the test has been started (Moore and Ramammoorthy 1984). The chemical analysis of phenol showed that the concentrations in samples (within the range of toxic activity on fishes) amounted to 90 to 100% of initial values in 24 hr. During 24 and 48 hr the degradation of phenol in samples increased, while after 96 hr all phenol degraded. After 48 hr the test water with phenol became turbid owing to phenol degradation. We therefore, shortened the toxicity test with fish to 48 hr.

The values for EC_{10} (LC_{10}), EC_{50} (LC_{50}), EC_{90} (LC_{90}) and 95% confidence limits for EC_{50} and LC_{50} have been calculated (Table 3).

Table 3. Summary of toxicity test results obtained for phenol with some aquatic organisms

Species	Test duration (h)	All values are in mg/L EC_{10}	EC_{50} (95% conf.lim.)	EC_{90}
Mixed bacterial culture	120	280	487 (454-522)	847
Photobacterium phosphoreum	0.5	7.6	44.3 (38.6-50.1)	259
Scenedesmus quadricauda (net assimilation)	24	107	403 (361-466)	968
Scenedesmus subspicatus (net assimilation)	24	68	287 (249-329)	1211
Scenedesmus subspicatus (chlorophyll-a)	72	47	229 (187-279)	1119
Daphnia magna	24	8.8	33.9 (30.0-39.2)	129.9
Oncorhynchus mykiss	48	10.3*	13.1* (11.9-14.1)*	16.6*

* values are for LC_{10}, LC_{50} and LC_{90}

The comparison of the results of the toxicity tests on the phenol influence on test organisms has shown that the concentrations that have already caused its toxic action (EC_{10} and LC_{10}) amounted to 10 mg/L for *Photobacterium phosphoreum*, *Daphnia magna* and *Oncorhynchus mykiss*, while the concentration was higher for mixed bacterial culture and algae *Scenedesmus quadricauda* and *Scenedesmus subspicatus*. Regarding the median lethal (effective) concentration (EC_{50} and LC_{50}), the fish *Oncorhynchus mykiss* showed the highest sensitivity, the daphnids *Daphnia magna* being less sensitive. Lower sensitivity was present in the algae *Scenedesmus quadricauda*, *Scenedesmus subspicatus* and mixed bacterial culture since the median effective concentration amounted to some hundred milligrams of phenol per liter.

We emphasize that the sensitivity of the toxicity test depends not only on the test organism selected, but also on the endpoint selected (death, mobility, etc.), because each toxicity test has its own sensitivity.

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