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Effect of monotoxicity and mixtoxicity of nitroaromatics to the green algae, *Scienedesmus obliquus*

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Abstract: In general, many pollutants co-exist in natural aquatic ecosystems. They affect each other and occur different results, such as synergism, addition and antagonism. So the research at mixtoxicity of contaminants to aquatic organism is more important than monotoxicity. In this paper, 50% effectual inhibition concentration 48h EC_{50} values of 6 kinds of nitroaromatic compounds to the green algae, *Scenedesmus obliquus*, were investigated. Mixtoxic experiments, in which any combination of 2,4-DNT and other 6 kinds of compounds, indicate: (1) synergisms between 2,4-DNT and 4-NAn, as well as 4-NAnis, 1,4-DNB; (2) antagonisms between 2,4-DNT and 4-NT, as well as 4-NPh, 4-NCB.

Key words: monotoxicity; mixtoxicity; nitroaromatics; green algae

Introduction

Environmental contaminants may have toxic effects on many different organisms and affect biological processes at cellular, population, community and ecosystem levels of organization. Most recent hazard evaluation programs recommend algae toxicity tests for evaluations of chemicals (Christensen, 1979; Deneer, 1987). Since algae are the primary producers in aquatic ecosystem, their susceptibility to contaminant has been the subject of numerous reports (Fargasova, 1994; Herman, 1991; OECD, 1981; OJEC, 1983). Nitroaromatics are main pollution in Songhua River in Jilin Province of China (Lang, 1998). Because of it, we tested 50% effectual inhibition concentration 48h EC_{50} values of much more nitroaromatic compounds to the algae, *Scenedesmus obliquus* (Liu, 1995). In the natural aquatic ecosystem , there are more than one contaminant at the same time. In other word, generally many pollutants co-exist in water bodies, affect each other and act on the aquatic organism. They maybe that occurs, synergism, addition or antagonism (Marking, 1994). In this paper, 50% effectual inhibition concentration 48h EC_{50} values of monotoxicity and mixtoxicity 2, 4-DNT and other 6 kinds of nitroaromatic compounds were investigated.

1 Materials and methods

Scenedesmus obliquus (CCCFA44) were supplied by the Institute of Hydrobiology, Chinese Academy of Sciences. The Algae Inhibition Test were refered to the method described by OECD and OJEC (OECD, 1981; OJEC, 1983). During the tests, the culture was incubated at 20 ± 1℃, with 4000 lx continuous light which was provided by white fluorescent lamps. The algae were cultivated in an aqueous medium (Lang, 1994). It was supplemented with various concentrations of 7 compounds and mixture of 2,4-DNT with other 6 kinds of 4-NT, 4-NAn, 4-NPh, 1,4-DNB, 4-NCB and 4-Nanis, respectively. Each compound and mixture were tested in five concentrations ranging from 1.17×10^{-5} to 7.32×10^{-5} mol/L. 2,4-DNT concentration was equal to other 6 kinds of compounds. There were four replicates at each concentration and one control. The initial cell concentration in the cultural medium was approximately 1×10^{-4} cells/ml. They were inoculated into 250 ml Erlenmayer flasks with 60 ml cultural medium, pollutants and the algae. The algal growth was monitored by hemocytometer for 48 hours. The data were processed according to the following formulae:

$$\mu = \ln Nt - \ln No/t - to. \quad (1)$$

Where μ was the average specific growth rate (%); No was the cell initial concentration (cell number/ml); Nt was the cell concentration after cultural 48 hours (cell number/ml).

$$I = \mu_{(b)} - \mu_{(tox)}/\mu_{(b)}. \quad (2)$$

where I was the inhibition rate; $\mu_{(b)}$ was the average specific growth rate of adding toxic compounds; $\mu_{(tox)}$ was the average specific growth rate of the control. The percent of reduction in

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average specific growth rate at each test substance concentration compared to that of the control is plotted against the logarithm of the concentration. Their 50% that is mixtoxicity, produced by two or more than two contaminants was evaluated according to the following method: Additive toxicity of chemical mixture (Xiu, 1994):

$$S = A_m/A_1 + B_m/B_1 + C_m/C_1 \cdots\cdots \quad (3)$$

Where S is the semination of additive toxicity of all chemical mixture; A_m, B_m, C_m..... the EC_{50} values of the chemical mixtures; A_1, B_1, C_1..... the EC_{50} values of A, B, C ..... compounds.

$$\text{As } S < 1,\ AI = (1/S) - 1.0, \quad (4)$$

$$S > 1,\ AI = S(-1) + 1.0 . \quad (5)$$

Where AI is the allied index,

As $AI > 0$, the toxicity of chemical mixture was synergism; $AI < 0$, the toxicity of them was antagonism; $AI = 0$, the toxicity of them was addition.

2 Results and discussion

The rank order of the toxicity for *S. obliquus* was 1,4-DNB > 2,4-DNT > 4-NCB > 4-NT > 4-NAnis > 4-NPh > 4-NAn. 50% effectual inhibition concentration 48h of 7 kinds of nitroaromatic compounds and mix compounds of 2,4-DNT and other 6 kinds of compounds are shown in Table 1.

Table 1 The EC_{50} values for 7 kinds of nitroaromatics and the mix compounds

Compounds	EC_{50}, mol/L	Compounds	EC_{50}, mol/L
2,4-DNT	3.47×10^{-5}	2,4-DNT + 4-NT	3.80×10^{-5}
4-NT	1.82×10^{-4}	2,4-DNT + 4-NAn	3.09×10^{-5}
4-NAn	3.98×10^{-4}	2,4-DNT + 4-NPh	4.79×10^{-5}
4-NPh	2.69×10^{-4}	2,4-DNT + 4-NAnis	2.75×10^{-5}
1,4-DNB	1.10×10^{-5}	2,4-DNT + 1,4-DNB	6.31×10^{-6}
4-NCB	1.15×10^{-4}	2,4-DNT + 4-NCB	2.57×10^{-5}
4-Nanis	2.24×10^{-4}		

After compared the monotoxicity and mixtoxicity, the each compound mixed with 2,4-DNT increased the toxicity for the algae. 2,4-DNT toxicity in the mix compound affected by co-exiting compounds was expressed by the allied index (AI). The AI of 2,4-DNT and other 6 kinds of compounds are shown in Table 2. The results indicated that there are synergisms between 2,4-DNT + 4-NAn, 2,4- DNT + 4-Nanis as well as 2,4-DNT + 1,4-DNB. There are antagonisms between 2,4-DNT + 4-NT, 2,4-DNT + 4-NPh and 2,4-DNT + 4-NCB.

Table 2 The allied index (AI) of 2,4-DNT and other 6 kinds of nitroaromatic compounds

Compounds name	AI	Compounds name	AI
2,4-DNT + 4-NT	−0.30	2,4-DNT + 4-NAnis	0.093
2,4-DNT + 4-NAn	0.033	2,4-DNT + 4-DNB	0.32
2,4-DNT + 4-NPh	−0.56	2,4-DNT + 4-NCB	−0.004

Compared the monotoxic and mixtoxic effect (Lang, 1994; 1998; Liu, 1995), we found that it was very complicated when more than one nitroaromatics coexisted in the aquatic ecology system. The mixtoxicity was too different from the monotoxicity while affecting on the algae (Marking, 1994; Xiu, 1994). It will be current significance that the allied toxic effect of low concentration and long-term for the aquatic organism are researched. These can not only apply to determine the environmental safety of nitroaromatics but also forecast and evolute the ecological hazard through the QSAR.

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