2,4–二硝基甲苯与共存硝基芳烃化合物对斜生栅列藻的联合毒性*

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摘要 为了客观地评价2,4–二硝基甲苯与其它硝基芳烃化合物共存时对藻类的生态毒理学效应,采用OECD藻类生长抑制实验的国际标准方法,研究了2,4–二硝基甲苯与其它6种硝基芳烃化合物共存时,对斜生栅列藻(*Scenedesmus obliquus*)的联合毒性(48 h EC_{50}),同时观察到细胞水平上的中毒症状.结果表明:2,4–二硝基甲苯加对硝基苯胺;2,4–二硝基甲苯加对二硝基苯;2,4–二硝基甲苯加对硝基苯甲醚均表现为协同作用.2,4–二硝基甲苯加对硝基苯酚;2,4–二硝基甲苯加对硝基甲苯;2,4–二硝基甲苯加对硝基氯苯均表现为拮抗作用.细胞水平中毒症状:似亲孢子释放受阻;异形巨细胞出现;细胞核和细胞器解体,原生质解体.通过对中毒症状和毒物浓度相互关系的分析,初步探讨了2,4–二硝基甲苯与其它共存硝基芳烃化合物对斜生栅列藻的联合毒性机制.

关键词 2,4–二硝基甲苯,硝基芳烃化合物,联合毒性,斜生栅列藻,协同作用,拮抗作用.

利用藻类生长抑制实验确定污染物的毒性在80年代逐渐得到公认[1,2],众多文献集中在测定不同种污染物对不同种藻类的单一毒性[2−8].而在自然状态下,多种污染物同时排放而进入水生生态系统,为了客观地评价污染物共存时对藻类的影响,联合毒性的意义尤为重大,并能为进一步的QSAR(定量构效关系)预测化合物的性质和活性提供重要的基础数据.

本研究以斜生栅列藻为试验材料,测定了2,4–二硝基甲苯分别与其它6种硝基芳烃化合物共存时的联合毒性(48 h EC_{50})及联合效应相加指数AI[3],初步探讨了2,4–二硝基甲苯与共存化合物对斜生栅列藻的联合毒性机制,同时观察到显微水平上的中毒症状.

1 实验部分

1.1 试剂与仪器

7种硝基芳烃类化合物(分析纯);

LRH–150G光照培养箱(广东省医疗器械厂);

CX–250型超生清洗器(北京医疗设备厂);

SHA–C水浴恒温振荡器(常州国华仪器厂).

1.2 实验材料与方法

斜生栅列藻(*Scenedesmus obliquus*)购自中国科学院武汉水生生物研究所.

实验方法严格按照OECD藻类阻碍生长实验标准方法[1]进行.

1.3 实验条件

温度:20℃±1℃;酸碱度:pH值调至7.2±0.2;照明:白色日光灯连续照明,平均光照3600—4000lx.

培养液[1,2]配成1000倍贮备液待用.

助剂:1ml/L丙酮溶液.

在光照培养箱中静止培养,每天震荡3次,每次30min.

1.4 实验过程

分别测试2,4–二硝基甲苯及其它6种硝基芳烃化合物对斜生栅列藻的单一毒性,得到48 h半数有效抑制浓度(48 h EC_{50}),以此为依据将2,4–二硝基甲苯分别与6种硝基芳烃化合物等剂量($mol \cdot L^{-1}$)混合,测试各混合物对绿藻的联合毒性,实验设计与测试方法与单一毒性的测试完

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全相同[1,2]. 每种被测混合物共计6组, 含1对照组, 设1平行, 共计12瓶, 依次置于光照培养箱内, 48h 后镜检, 定形群体或单个细胞均以每个细胞作为一个计数单位, 计算出细胞数/ ml.

1.5 实验数据处理

镜检所得数据按如下公式处理:[1,2]

$$\mu = \ln(N_t/N_0)/t - t.$$

$$\text{阻碍率}(\%) = \frac{[\mu(b) - \mu(tox)]}{\mu(b)} \quad (2)$$

式中, N_0为初始细胞数, N_t 为48h 后细胞数, μ(tox) 为加测试化合物组的生长速度, μ(b) 为对照组的生长速度.

根据上述公式, 将浓度与生长速度阻碍率作一元线性回归, 求出 EC_{50}值.

2, 4–二硝基甲苯在混合物中的毒性受共存化合物的影响程度, 可由联合效应相加指数 AI[3]来表示.

$$S = \frac{A_m}{A_1} + \frac{B_m}{B_1} + \frac{C_m}{C_1} + \cdots\cdots$$

式中, S 为所有混合测试物毒性相加的和. A_1、B_1、C_1、…… 为各化合物单一毒性值(EC_{50}), A_m、B_m、C_m…… 为混合后的混合毒性值(EC_{50}).

当 $S \leq 1$, AI= (1/S) – 1.0

当 $S > 1$, AI= S(– 1) + 1.0

式中, AI 为联合效应相加指数.

当 AI> 0时, 毒物间为协同作用.

当 AI< 0时, 毒物间为拮抗作用.

当 AI= 0时, 毒物间为相加作用.

2 实验结果

2.1 单一毒性效应

2, 4–二硝基甲苯及其它6种硝基芳烃化合物对斜生栅列藻的单一毒性(EC_{50}) 见表1. 可以看出其毒 性顺序依次为: 1, 4–DNB> 2, 4–DNT > 4–NCB > 4–NT > 4–NAnis > 4–NPh > 4–NAn.

2.2 联合毒性效应

2, 4–DNT 分别与6种共存化合物混合后对斜生栅列藻的生长阻碍率见表2.

表1 2, 4–二硝基甲苯及6种硝基芳烃化合物对斜生栅列藻的单一毒性[2]

化合物	48h EC50/ mol·L^{-1}
2, 4–二硝基甲苯(2, 4–DNT)	3.47×10^{-5}
对硝基甲苯(4–NT)	1.82×10^{-4}
对硝基苯胺(4–NAn)	3.98×10^{-4}
对硝基苯酚(4–NPh)	2.69×10^{-4}
对二硝基苯(1, 4–DNB)	1.10×10^{-5}
对硝基氯苯(4–NCB)	1.15×10^{-4}
对硝基苯甲醚(4–NAnis)	2.24×10^{-4}

表2 2, 4–DNT 与其它6种共存化合物混合后对斜生栅列藻的影响

2, 4–DNT 初始浓度 / mol·L^{-1}	藻类生长阻碍率/ %					
	2, 4–DNT + 4–NT	2, 4–DNT + 4–NAn	2, 4–DNT + 4–NPh	2, 4–DNT + 4–NAnis	2, 4–DNT + 4–DNB	2, 4–DNT + 4–NCB
7.32×10^{-5}	88.9	78.0	80.6	130.5	93.2	97.3
4.68×10^{-5}	68.8	76.0	39.9	88.7	82.3	85.3
2.93×10^{-5}	45.5	49.8	24.4	53.3	76.1	65.3
1.90×10^{-5}	22.6	26.9	22.1	30.3	65.3	52.4
1.17×10^{-5}	14.3	16.7	12.7	20.7	54.3	12.6

根据表2的数据, 得到联合毒性值(48 h EC_{50}) 及联合效应相加指数见表3.

表3结果表明, 与单一毒性效应相比, 每种共存化合物与2, 4–DNT 混合后, 对斜生栅列藻的毒性均有所加强, 2, 4–DNT 在混合物中的毒性受共存化合物影响的程度可由联合效应相加指数 AI 来表示. 2, 4–DNT + 4–NAn, 2, 4–DNT + 4–NAnis 和2, 4–DNT + 1, 4–DNB, AI 均大于0, 故属协同作用. 2, 4–DNT + 4–NT, 2, 4–DNT + 4–NPh 和2, 4–DNT + 4–NCB, AI 均小于0, 故属拮抗作用.

表3　2, 4–二硝基甲苯与6种共存化合物分别混合后的联合毒性及联合效应相加指数

化合物	48 h EC_{50}/ mol•L^{-1}	AI
2, 4–DNT + 4–NT	3.80×10^{-5}	– 0.30
2, 4–DNT + 4–NAn	3.09×10^{-5}	0.033
2, 4–DNT + 4–NPh	4.79×10^{-5}	– 0.56
2, 4–DNT + 4–NAnis	2.75×10^{-5}	0.093
2, 4–DNT + 4–DNB	6.31×10^{-6}	0.32
2, 4–DNT + 4–NCB	2.57×10^{-5}	– 0.004

2.3　斜生栅列藻的中毒症状

在光学显微镜下(40×10倍)观察到, 在2, 4–DNT 分别与6种共存化合物作用下, 其中毒症状与单一毒性效应所产生的中毒症状[2]既有共同之处, 又有一定的差异(见表4).

表4　单一毒性效应与联合毒性效应中毒症状比较

中毒症状	化合物	
	2, 4–DNT (单一毒性效应)	2, 4–DNT + 共存化合物(联合毒性效应)
似亲孢子释放受阻	有	有
不规则巨细胞出现	无	有
细胞核、细胞器解体	有	有
原生质体解体	有	有

(1) 母细胞内似亲孢子释放受阻, 出现形状异常的不规则巨细胞. 在斜生栅列藻生长阻碍率为70%—100% (见表2) 时, 部分藻胞明显体积巨大, 母细胞内已形成子细胞但释放受阻, 或无似亲孢子的形成, 这时细胞体积为正常藻胞的3—4倍. 这些巨细胞中还出现一些形状异常的不规则巨细胞, 细胞形状由正常的梭形变为球形、逗号形、圆锥形或棒槌形(见图1).

(2) 细胞核和细胞器解体　在斜生栅列藻生长阻碍率为45% —55%时(2, 4–DNT 及混合物浓度、斜生栅列藻生长阻碍率见表2), 斜生栅列藻的部分藻胞体积仅为正常藻胞的1/3. 同时, 细胞核和细胞器解体, 藻胞原生质体呈现类似蓝藻的均匀透明的蓝绿色或淡绿色(见图2a).

(3) 藻胞原生质体解体　当所有被测化合物混合毒物浓度达到使藻生长阻碍率达到50%以上时, 各浓度及不同混合物组均出现部分藻胞原生质体解体现象, 藻胞仅余细胞壁或细胞残体, 而且, 随着混合毒物浓度和毒性的增大, 视野中此类藻胞的比例增加(图2b).

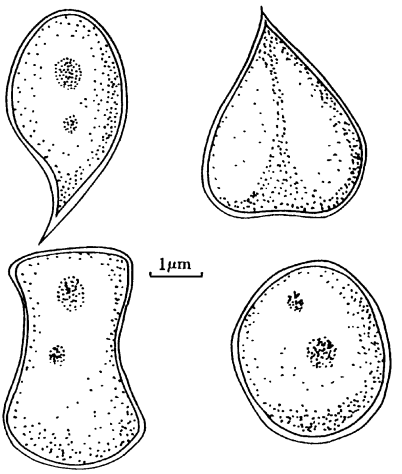

图1　似亲孢子释放受阻及不规则巨细胞(400倍)

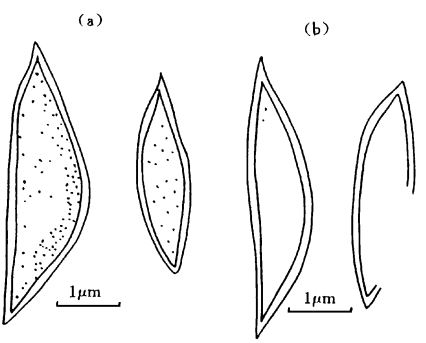

图2　细胞核、细胞器解体(a)和原生质解体(b) (400倍)

3　结论

(1) 与单一毒性相比较, 每种共存化合物与2, 4–二硝基甲苯混合后, 对斜生栅列藻的毒性都有所加强, 2, 4–二硝基甲苯在混合物中的毒性受共存化合物的影响程度可由联合效应相加指数 AI 来表示. 结果表明, 硝基芳烃化合物共存于水体中对斜生栅列藻产生的生态学效应是很复杂的, 与单一毒物的作用结果有很大差异, 因此, 低浓度、长效应联合毒性的深入研究意义尤为重大.

(2) 当混合毒物的浓度达到使藻类生长阻碍率为70% —100%时, 部分藻胞似亲孢子形成

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列出了各物质的 Henry 定律常数.

表4 有机物挥发速率常数与 Henry 定律常数

挥发程度	物质名称	挥发速率常数/ d^{-1}	Henry 定律常数[3] / $atm \cdot m^3 \cdot mol^{-1}$
强挥发物质	乙苯	0.041	8.7×10^{3}
	甲苯	0.029	6.6×10^{3}
	二甲苯	0.027	5.1×10^{3}
	呋喃	0.030	5.4×10^{3}
	萘	0.018	1.15×10^{3}
	吡啶	0.035	7.0×10^{3}
	吡咯	0.045	9.3×10^{3}
	联苯	0.019	1.5×10^{3}
中等程度挥发物质	苯酚	8.36×10^{-4}	3.97×10^{-7}
	甲基苯酚	9.87×10^{-4}	2.16×10^{-6}
	二甲基苯酚	8.54×10^{-4}	$< 1\times10^{-6}$
	吲哚	9.43×10^{-4}	$< 1\times10^{-6}$
	喹啉	9.79×10^{-4}	9.35×10^{-7}
	异喹啉	9.21×10^{-4}	9.86×10^{-7}

从表4可知:

(1) 不同挥发程度的物质其挥发速率常数 K_v 差别很大, 在0.018—045d^{-1}之间, 而中等程度挥发物质与强挥发物质 K_v 值相差2个数量级.

(2) 在本研究中易挥发物质的挥发速率常数 K_v 与 Henry 常数之间具有良好的线性相关关系, 对它们进行线性分析, 得如下方程:

$$K_v = 2.906\times10^{3}H + 0.0146 \quad (2)$$

($r = 0.987$)

式中, K_v: 挥发速率常数(d^{-1}); H: Henry 定律常数($atm \cdot m^3 \cdot mol^{-1}$).

4 结论

(1) 对焦化废水中24种有机物质进行空曝试验, 在曝气吹脱条件下, 各种有机物表现出不同的挥发特性.

(2) 有11种有机物, 包括乙苯、苯、吡咯、联苯等比较容易挥发, 它们的挥发速率常数 K_v 与 Henry 常数间具有良好的线性关系, 线性相关方程为 $K_v = 2.906\times10^{-3}H + 0.0146$, 挥发作用在有机物的去除中起很重要的作用.

(3) 苯酚、喹林等12种物质具有中等程度的挥发性; 间苯二酚在曝气吹脱条件下几乎不挥发.

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和释放受阻, 部分藻胞巨大且形状不规则. 和2, 4-二硝基甲苯等硝基芳烃类化合物对斜生栅列藻单一毒性[2]相比, 混合毒物在较高浓度下不仅阻碍斜生栅列藻的繁殖, 而且改变藻胞的形状.

(3) 当藻类生长阻碍率为45%—50%时, 混合毒物使斜生栅列藻部分藻胞的细胞核和细胞器解体, 这与2, 4-二硝基甲苯对斜生栅列藻单一毒性相比, 混合毒物的初始浓度均低于2, 4-二硝基甲苯的初始浓度. 且呈协同作用的混合毒性物的视野中, 斜生栅列藻呈现此中毒症状的藻胞占45%—50%; 呈拮抗作用的混合毒物视野中, 呈现此中毒症状的藻胞占30%左右. 可见混合毒性效应比单一毒性效应更为复杂.

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northern China. The maximum value of N_2O emissions from forest and grassland soil is about 23$\mu g/(m^2 \cdot h)$. A high rate of N_2O loss is observed when the temperature is lower than 15℃. The maximum value of N_2O negative emissions as high as 18.984 $\mu g/(m^2 \cdot h)$ has been measured.

Key words: N_2O, O_3, emission flux, source, sink, exchange between atmosphere and territory.

Ultrafiltration Membrane Bioreactor for Domestic Wastewater Treatment and Its Hydraulic Behavior. Xing Chuanhong, QianYi, (State Key Laboratory of Environmental Simulation and Pollution Control, Dept. of Environ. Eng., Tsinghua University, Beijing, 100084), Tardieu Eric (CIRSEE-Lyonnaise des Eaux, 38, rue du President Wilson, F78230 Le Pecq.): *Chin. J. Environ. Sci.*, **18** (5), 1997, pp. 19—22

It is proven that Ultrafiltration Membrane BioReactor (UMBR) applied to domestic wastewater treatment, under conditions of hydraulic retention time 5h, sludge retention time 30d, membrane surface velocity 4m/s and membrane flux 75L/($m^2 \cdot h$), is technically feasible and reliable during several weeks. Removal rate of COD, NH_3-N, and turbidity of the systme are equal to or higher than 98%, 97% and 98%, SS and E. coli., 100%. The effluent quality is always better than the quality standard for reuse issued by the Ministry of Construction in China. Furthermore, the hydraulic behavior of UMBR is concisely discussed. The hydraulic boundary layer is about 185—5.9μm thick, and the mass transfer boundary layer, 18.5—0.59μm when the typical Reynolds number is 4×10^3—2×10^5.

Key words: ultrafiltration membrane, bioreactor, domestic wastewater, hydraulic behavior, boundary layer thick.

Soil Sensitivity to Acid Deposition in South China Ⅶ. **Cation Leaching and Buffering Mechanism**. Qiu Rongliang et al. (Department of Environmental Science, Zhongshan University, Guagzhou 510275): *Chin. J. Environ. Sci.*, **18**(5), 1997, pp. 23—27

Acidic deposition is considered an environmental problem that may affect the soil's cation-exchange status. This study used column leaching experiments to examine the influences of simulated acid rain with different pH values on base cation leaching of main soil types collected from South China. The results showed that amounts of leaching base cations increased obviously when the pH value of simulated acidic rain was lower than 3.0 or 3.5, while the amounts almost did not differ when pH higher than 3.5. The leaching of Ca^{2+} and Mg^{2+} was affected evidently by the pH value of simulated acidic rain, compared with the leaching of K^+ and Na^+. The H^+ buffering mechanisms which may vary in different simulated pH acidic rain are proposed for main soils studied. The dissolving reaction of salts was the dominant resources of leaching base cations and H^+ buffering when pH value higher than 3.5. The mechanisms of H^+ buffering treated with acid rain at pH 3.5 were the exchangeable cations and sulfate sorption. Dissolved aluminum on the broken edge of clay and aluminum oxides weathering of original and secondary minerals and sorption of sulfate played a grea role on the leaching of soil cations and H^+ buffering when the soils treated with acid rain of pH lower than 3.5.

Key words: simulated rain, base cations, leaching, soil sensitivity.

Studies on Effect of Water Treated by High-Voltage Electrostatic Field on Oxygen Utilization Rating of Activated Sludge. Yang Feng, Kong Jilie, Deng Jiaqi (Dept. of Chem., Fudan University, Shanghai 200433), Xiang Yang, Gao Tingyao (School of Environ. Eng., Tongji University, 200092): *Chin. J. Enwiron. Sci.*, **18**(5), 1997, pp. 28—30

The microorganisms extracted from activated sludge were selected as the sensitive material to make a BOD biosensor for monitoring the metabolic ability of themselves in high-voltage electrostatic treated water (HVETW). The oxygen utilization rating of the microorganisms which operated in such an aqueous media for appropriate time, could be promoted by at least 20%. At the same working high-voltage, this effect depended on how long the water was treated. The water treated for too long a time would lead to the negative effects. At 5000 V, it took about 6.0 h for the microorganisms to obtain the maximum oxygen utilization rating, whereas it did about 4.0 h at 7000 V. This discovery might offer a great potential for improving the new waste water disposal techniques.

Key words: high-voltage static electricity, activated sludge, BOD, microbial sensor.

Mixtoxicity of 2, 4-DNT and 6 Kinds of Nitroaromatic Compounds to the Algae. Liu Jingling, Yuan Xing, Lang Peizhen (Dept. of Environ. Sci., Northeast Normal Uni., Changchun, 130024): *Chin. J. Enwiron. Sci.*, **18** (5), 1997, pp. 31—33

In order to evaluate objectively the ecological effects of 2, 4-DNT mixed with 6 kinds of ni-

troaromatic compounds in the ecosystem, the study on the mixtoxicity of 2, 4-DNT and other 6 kinds of nitroaromatics to the algae (*Scenedesmus obliquus*) was carried out. 48h-EC_{50} of monotoxicity of 7 kinds of compunds and 48h-EC_{50} of mixtoxicity of 2, 4-DNT + 6 kinds of compounds were measured. Results indicated that there are synergisms between, 2, 4-DNT + 4-NAn, 2, 4-DNT + 4-NAnis, and 2, 4-DNT + 1, 4-DNB as well as antagonisms between 2, 4-DNT + 4-NT, 2, 4-DNT + 4-NPh and 2, 4-DNT + 4-NCB. Under the microscope (400 times) 3 kinds of toxic symptoms, the inhibition to the filial spores forming and releasing, the irregular big cells appearing, nucleus and cell organs as well as the protoplast distegrating were observed.

Key words: mixtoxicity, 2, 4-DNT, nitroaromatic compounds, algae, *Scenedesmus obliquus*.

Study on the Volatile Property of Organics in Coke-Plant Wastewater under the Aerated Stripping Condition. He Miao, Zhang Xiaojian etal. (Dept. of Environ. Eng., Tsinghua University, Beijing 100084): *Chin. J. Environ. Sci.*, **18**(5), 1997, pp. 34—36

A study was conducted to determine the volatile property of organics in the coke-plant wastewater under the condition of aerated stripping. The results show that there are different volatile characteristics among the organics. 11 kinds of compounds such as ethylbenzene and naphthalene are more volatile. The removal rate can get to 20% - 40% after an aeration of 12 hours. Their volatile rate constant K_v have a fairly good linear relation with the Henry constant (H), of which relation equation is $K_v = 2.906 \times 10^3 H + 0.0146$. Volatile effect plays a very important role in the removal process of these organic compounds. 12 compounds like phenol have a medium volatile property. The other compounds like resorcinol have a low volatility.

Key words: volatile property, coke-plant wastewater, aerated stripping, volatile organic compound, volatile rate constant.

Activity, Kinetics and Spatial Variation of Dissolved Alkaline Phosphatase in Lake Donghu. Zhou Yiyong, Li Jianqiu, Chen Xudong et al. (Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan 430072): *Chin. J. Environ. Sci.*, **18**(5), 1997, pp. 37—40

The extra cellular enzyme activities in aquatic environments are of monitoring significance. Dissolved alkaline phosphatase activities (APA_{Diss}) at the littoral outfalls, which receive the domestic waste water were significantly higher than those in pelagic zone in Donghu Lake. APA_{Diss} detected at the outfalls and in pelagic zone showed different relationships with soluble reactive phosphorus.

Key words: dissolved phosphatase, kinetics, characteristics, spatial distribution.

A Study on Bioaccumulation and Biomagnification of BHC and DDT in Baiyangdian Lake Foodweb. Dou Wei, Zhao Zhongxian (Institute of Zoology, Chinese Academy of Sciences, Beijing 100080): *Chin. J. Environ. Sci.*, **18**(5), 1997, pp. 41—43

BHC、DDT and their metabolites were analysed in Duancun area from Baiyangdian Lake ecosystem in June 1995. The content levels of pesticides in water and bottom sediments were relatively lower: in water, the average values of BHC and DDT were 0.3μg/L and 0.1μg/L, respectively; in sediments, the residue level of BHC was same with DDT with a mean concentration of 0.7μg/kg(wet). The organochlorine insecticides content in aquatic organisms was much higher: the average concentration of BHC in hydrophytes, planktons, benthic macroinvertebrates, young-of-the-year *Carassius auratus and* 2-year-old *Ophiocephalus cris-pus* was 19.0, 30.0, 60.9, 17.2 and 110.7 μg/kg, respectively; for DDT, the content sequence was 6.3, 21.0, 37.9, 19.4 and 124.4g/kg, respectively. So the lipophilic nature and low biodegradation rates of BHC and DDT led to the accumulation of these compounds and subsequent magnification of concentration in organisms progressing up the food chain. DDT had a much profound bioaccumulation potential than BHC for the bioconcentration factor of DDT in organisms (63.3- 1244) was well above that of BHC (63- 369). The ratios of BHC isomers monitored in organisms ($\delta > \alpha > \gamma > \beta$) were different from that in water and sediments ($\alpha > \delta > \gamma > \beta$). The predominant DDT derivative was p, p′-DDE. Base upon the observed that the original DDT (P, P′-DDT) was indentified in some specimens, it has been concluded that there was a recent input of DDT to Baiyangdian Lake.

Key words: Baiyangdian Lake ecosystem, BHC, DDT, foodweb, the bioconcentration factor.

Joints Toxicities of Heavy Metals and Pesticides to *Pagrosomus major and Rhabdosargus sarba* Larvae. Dai Jiayin, Zheng Weiyun, Wang Shuhong (Environ. Sci. Res. Centre, Xiamen University, Xiamen 361005): *Chin. J. Environ. Sci.*, **18**(5), 1997, pp. 44—46

The additive index of coeffects was used to study the toxicities of Cu-Mn, Cu-isofenphos-methyl and methamidphos-isofenphos-methyl