第 22 卷第 11 期 2002 年 11 月 | 生 态 学 报 ACTA ECOLOGICA SINICA | Vol. 22, No. 11 Nov., 2002

菲、芘、1,2,4-三氯苯对土壤高等植物根伸长抑制的生态毒性效应

宋玉芳[1],周启星[1],许华夏[1],任丽萍[1],宋雪英[1],龚 平[2]

(1. 中国科学院沈阳应用生态研究所陆地生态过程重点实验室,沈阳 110016;2. 加拿大国家研究院生物技术研究所,蒙特利尔,奎北克 H4P 2R2)

摘要:测定了草甸棕壤条件下,菲、芘、1,2,4-三氯苯对高等植物(小麦、白菜、西红柿)根伸长抑制率以及复合污染毒性效应。结果表明,菲、芘、1,2,4-三氯苯浓度与植物根伸长抑制率呈显著线性或对数相关($p=0.05$)。3 种化学品对植物根伸长抑制的强弱顺序为 1,2,4-三氯苯>菲>芘。这与 3 种化学品的水中溶解度大小显著相关。小麦是 3 种供试植物中对有机污染物最敏感植物。菲、芘、1,2,4-三氯苯复合污染主表现为协同作用。

关键词:土壤污染;有机污染物;根伸长抑制率;复合污染

Eco-toxicological Effects of Phenanthrene, Pyrene and 1,2,4-Trichlorobenzene in Soils on the Inhibition of Root Elongation of Higher Plants

SONG Yu-Fang[1], ZHOU Qi-Xing[1], XU Hua-Xia[1], REN Li-Ping[1], SONG Xue-Ying[1], GONG Ping[2] (1. *Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang* 110016, *China*; 2. *Biotechnology Research Institute. National Research Council of Canada*, 6100 *Royalmount Avenue, Montreal, Quebec* H4P 2R2, *Canada*). ***Acta Ecologica Sinica*, 2002, 22(11): 1945～1950.**

Abstract: Ecological bioassays using higher plants were included in a test battery because vegetation is the dominant biological component of terrestrial ecosystem, and they could reflect the toxicity of hazardous chemicals in soils, therefore providing an alternative method for the evaluation of toxic effects such as adverse effects of a bioavailable fraction of pollutants in a complex soil matrix. It is well known that a reduction in toxicity of a complex mixture could not be measured by parent compound disappearance. In addition, bioassays could compensate expenses of chemical analyze and its difficulties in detecting the total amount of bioavailable toxicants due to the lower concentration, lower solubility or insolubility in chemical extracting.

Seed germination test adopted in the OECD guideline suggested two indexes as the endpoints (seed germination and root elongation). The main advantage of the seed germination test as an additional endpoint is the possibility of giving a more fast and cost-effective results based on them, which can be very useful for several applications including identification of phytotoxicity threshold of target pollutants, evaluating contaminated soils and bioremediation sites. Available reports are most based on the early growth stage as the endpoint with recommended species for toxicity test by the US EPA and OECD and ISO to evaluate the toxicity of the toxicants, only a few concerned with seed germination test using root

基金项目:中国科学院知识创新资助项目(KZCX2-401);国家自然科学基金资助项目(20077029);国家重点基础研究发展规划资助项目(G1999011808)

收稿日期:2001-11-17;修订日期:2002-05-21

作者简介:宋玉芳(19),女,辽宁沈阳人,博士,副研究员。从事污染生态学研究。

elongation as the endpoint. Since toxicity can be specific to plant species, it is thus important to use a variety of species from different families for each evaluation. Polycyclic aromatic hydrocarbons (PAHs) and organic chlorides are two groups of priority pollutants listed in US EPA and were well known to be toxic, carcinogenic and mutagenic to a wide range of organisms, whereas less is known of the phytotoxicity threshold of these pollutants. The sensitivity of plants to the PAHs and organic chlorides and tolerance responses of different plant species to them was unknown.

Individual and combined responses of phenanthrene, pyrene, 1,2,4-trichlorobenzene and the extent of combined toxicity effects were compared by determination of the inhibition rates of phenanthrene, pyrene and 1,2,4-trichlorobenzene on higher plants (wheat, Chinese cabbages and tomatoes) and toxic effects of combined pollution of these chemicals in the meadow brown soils. The root elongation was used as endpoint. Results indicated that there was a significant liner or logarithmic relationship between the concentration of phenanthrene, pyrene and 1,2,4-trichlorobenzene and the inhibition rates of root elongation of plants ($p=0.05$). Inhibition strength of three chemicals on plant elongation was in the sequence: 1,2,4-trichlorobenzene > phenanthrene > pyrene, which was closely related with the water solubility of the chemicals tested. Wheat was the most sensible plant to the organic pollutants. There was a synergism of phenanthrene, pyrene and 1,2,4-trichlorobenzene in the tested soil-plant system.

Key words: soil pollution; organic pollutants; inhibition rate of root elongation; combined pollution

文章编号:1000-0933(2002)11-1945-06 **中图分类号**:X131.3 **文献标识码**:A

高等植物是生态系统中的基本组成部分。一个平衡、稳定的生态系统生产健康、优良的高等植物。反之,一个不稳定或受到外来污染的生态系统,对高等植物的生长可带来不利影响。因此,利用高等植物的生长状况监测土壤污染,是土壤污染诊断的重要方法之一[1~5]。

目前已建立的高等植物毒理试验方法有 3 种[2~4]。如种子发芽试验、根伸长抑制试验及植物早期生长试验。这些实验通过植物在污染条件下根系发育的状况、生物量减少的程度或植物的耐污特性等对污染进行诊断[5~7]。最初,这类试验主要用于纯化学品的毒性检验,但随着土壤污染生态毒理评价的需求,该方法的应用范围已扩展到废物倾倒点,土壤污染现场以及土壤生物修复过程中。有关方面的研究已有较多报道[5~9]。关于有机污染物的植物毒性响应已受到研究者关注,但选用多种植物,进行植物毒性的剂量-效应关系及复合污染效应研究的报道甚少[7]。

菲(3 环)和芘(4 环)属多环芳烃类污染物,是美国环保局优先控制有机污染物黑名单中 16 种多环芳烃中的成员。由于其结构与致癌物苯并(a)芘的相似性,通常被作为模式污染物广泛研究[10~13]。1,2,4-三氯苯属氯苯类有机污染物。与 1,2-二氯苯和 1,4-二氯苯等统属生产苯胺、染料、医药的重要原料及中间体,具有急性及三致毒性的共性。因此,被列入美国、德国、日本及欧共体环保机构所确定的优先污染物黑(灰)名单上[14]。

本文选择草甸棕壤,进行菲、芘、1,2,4-三氯苯对高等植物根伸长抑制率及复合污染生态毒性效应研究。通过植物根伸长受抑制程度,确定不同有机物对不同植物毒性的敏感性,该研究可为筛选土壤有机污染敏感指示植物提供实验科学依据。

1 材料与方法

1.1 供试材料

菲、芘、1,2,4-三氯苯 均为分析纯。供试土壤均为 0~20cm 草甸棕壤土,采自中国科学院沈阳生态实验站。小麦(*Triticum acstivnm*)、白菜(*Brassica pekimensis*)和西红柿(*Lycopersion esculeatum*)种子购自辽宁省农业科学院种子站[15]。

1.2 方法与试验

1.2.1 植物根伸长抑制试验 (1)预备试验 称取 50g 风干土壤于 90mm 直径的玻璃培养皿中,将配制

的有机物丙酮溶液均匀加入培养皿中,放置暗处直至丙酮挥发至干。调节土壤含水量至最大持水量的60%,将种子均匀播种于土壤中,盖好玻璃培养皿,25℃恒温箱暗处培养。对照种子发芽率>65%(本实验发芽率 90%),根长约 20mm(小麦 48h,白菜 49h,西红柿 108h)时结束。确定根伸长抑制率达 10%~50%的浓度区间后,开始正式试验[16]。

(2) 正式试验　在种子根伸长抑制率 10%~50%的浓度(IC-Inhibition Concentration)范围内,设置 6 个浓度,每个浓度 20 粒种子 3 个重复。在与预备试验相同条件下,进行根伸长抑制试验。土壤投加菲、芘和1,2,4-三氯苯后进行土壤样品的提取。实验结束时测定并计算各处理根伸长的平均值及标准偏差[16]。

1.3　有机物测定方法　菲、芘测定采用液相色谱法[17],1,2,4-三氯苯采用气相色谱法[18]。

2　结果与讨论

2.1　菲对高等植物根伸长的抑制效应

以小麦、西红柿、白菜 3 种植物根伸长抑制率对菲浓度作图 1 可见,菲浓度与 3 种植物根伸长抑制率显著相关($p=0.05$,$R^2_{小麦}=0.9203$,$R^2_{白菜}=0.8759$,$R^2_{西红柿}=0.9711$)。其中与小麦和西红柿根伸长抑制率为对数相关,与白菜根伸长抑制率为线性相关。3 种植物对菲毒性的敏感程度不同。引起植物受害的毒性阈值(抑制率>10%)差别较大。例如,小麦、西红柿、白菜根伸长抑制率分别为 9.2%、13%和 9.3%时,菲浓度分别为 20 mg/kg,50mg/kg 和 100mg/kg。菲对 3 种植物根伸长抑制的强弱排序为:小麦>西红柿>白菜。小麦对菲污染的毒性响应最敏感。

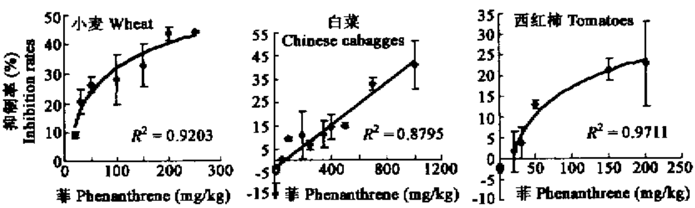

图 1　菲对草甸棕壤中高等植物根伸长抑制率

Fig.1　Inhibition rates of phenanthrene on root elongation of higher plants in meadow brown soils

植物对重金属毒性响应敏感度与有机物明显不同[15]。例如,小麦对重金属毒性最不敏感,但对菲的毒性响应极敏感。这说明,重金属与有机物的植物毒害机制不同。从形态上看,植物在重金属污染胁迫下,除了根伸长受抑制外,无其它受害特征[19]。但在菲污染胁迫下,首先表现出明显的根径变细,即而根长受抑制的特征。这很可能是由于有机污染导致土壤水分和养分传输受阻,进而造成植物生长的生理障碍。

2.2　芘对高等植物根伸长的抑制效应

由图 2 可见,3 种植物根伸长抑制率与芘浓度显著相关($p=0.05$,$R^2_{小麦}=0.9313$,$R^2_{白菜}=0.933$,$R^2_{西红柿}=0.8998$)。但植物受害的毒性阈值(抑制率>10%)浓度明显高于菲。小麦根伸长抑制率为 15%,西红柿和白菜根伸长抑制率 10%时,芘浓度为 80mg/kg,100 mg/kg 和 150 mg/kg 以上。从阈值浓度和剂量-效应曲线关系可见,小麦为最敏感植物,其次为西红柿,再次为白菜。这不仅是因为小麦的毒性阈值低,抑制率 50%的浓度也明显低于其他植物。植物对芘毒性响应的敏感顺序与菲完全一致。但同样浓度的芘对植物的毒害效应不及菲明显。显然是受芘水溶解度(溶解度为 0.03mg/kg)低的限制。由此可见,只有生物可利用态的有机物对植物生长产生最直接的抑制作用。

2.3　1,2,4-三氯苯对高等植物根伸长的抑制效应

由图 3 可见,根伸长抑制率与 1,2,4-三氯苯浓度显著相关($p=0.05$,$R^2_{小麦}=0.9288$,$R^2_{白菜}=0.9166$,$R^2_{西红柿}=0.9414$)。但植物受害毒性阈值(抑制率>10%)浓度明显小于菲和芘。在同样浓度下,1,2,4-三氯苯对植物根伸长抑制效应最强。例如,1,2,4-三氯苯为 2.0mg/kg,小麦根伸长抑制率达 11.4%。此后污染物对植物根伸长的毒性效应随浓度增加呈线性增加,引起小麦根伸长抑制率达 50%的浓度(IC_{50})为

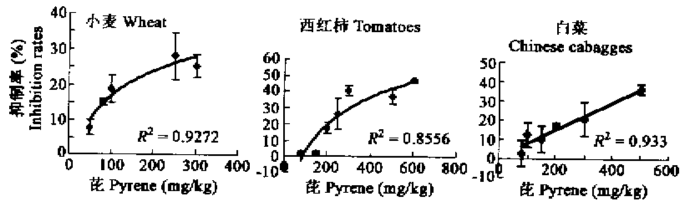

图 2 芘对草甸棕壤中高等植物根伸长抑制率

Fig. 2 Inhibition rates of pyrene on root elongation of higher plants in meadow brown soils

100mg/kg。其他 2 种植物对 1,2,4-三氯苯毒性的响应不及小麦,但仍明显强于对菲和芘毒性的响应。西红柿和白菜根伸长抑制率为 16.6%和 14.9%时，1,2,4-三氯苯为 10 mg/kg 和 20mg/kg。

比较以上 3 组结果可见,植物对有机污染物毒性响应具有一定规律性。小麦对有机污染最为敏感。这不仅表现在小麦受污染的毒性阈值低,同时小麦根抑制率的 IC_{50}浓度也明显低于其他受试植物。从图 1～图 3 可见,3 种供试化学品对植物根伸长抑制率都服从于 1,2,4-三氯苯＞菲＞芘的规律。这充分说明有机污染物在水中的溶解度(1,2,4-三氯苯 49mg/L,菲 1.3mg/L，芘 0.03mg/L)与其生物毒性效应的显著相关性(14),即溶解度越大,对植物的毒性效应越强。这与 Debus[20]等人以 PCP，林丹和 PCB 52 等 3 种有机污染物的生态毒理研究结果相同。

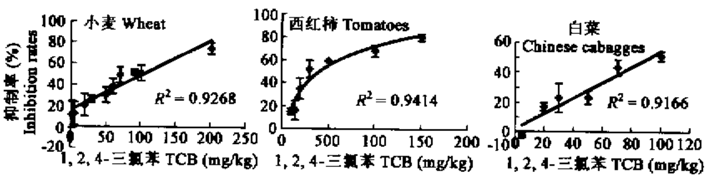

图 3 1,2,4-三氯苯对草甸棕壤中植物根伸长的抑制率

Fig. 3 Inhibition rates of 1,2,4-trichlorobenzene on root elongation of higher plants in meadow brown soils

2.4 有机复合污染对植物根伸长抑制的毒性效应

实验以菲、芘、1，2,4-三氯苯单一污染对小麦根伸长抑制的阈值浓度为复合污染实验的最大浓度。分别进行菲、芘、1，2,4-三氯苯复合污染对小麦、白菜和西红柿根伸长的抑制效应研究表明,复合污染产生明显协同效应。由表 2 可见,小麦根伸长抑制率 12.1%时，菲、芘、1，2,4-三氯苯的浓度（5.0mg/kg，12.5mg/kg,和 0.5mg/kg)比单一污染达到相同抑制率的浓度值(20.0mg/kg，50.0mg/kg 和 2.0mg/kg)

表 1 菲、芘、1,2,4-三氯苯复合污染对草甸棕壤中小麦根伸长抑制率(%)

Table 1 Inhabitation rates of combined pollution of PHE，PY and TCB in the meadow brown soils on the root elongation of wheat

PHE* (Mg/kg)	抑制率 PHE	PY (Mg/kg)	抑制率 PY	TCB (Mg/kg)	抑制率 TCB	抑制率 PHE＋PY＋TCB
2.5	－4.6±3.4	6.3	－14.0±5.8	0.25	－6.7±2.4	0.2±3.7
5.0	－3.1±4.9	12.5	1.7±1.7	0.50	－4.0±4.6	12.1±2.3
10.0	1.1±4.6	25.0	4.4±6.0	1.00	－1.9±3.7	19.7±0.3
20.0	9.2±1.5	50.0	11.2±1.1	2.00	11.4±3.2	20.5±6.1

* PHE-菲,PY-芘，TCB-1,2,4-三氯苯。

降低了约 4 倍。以白菜和西红柿进行复合效应实验,其结果与此基本相同(表 3 和表 4)。由此表明,有机复合污染毒性增强，其原因之一为复合污染使土壤中有机污染物的生物可利用性增加,由此导致生物毒性也增加,这与重金属的复合污染效应不同。Ince[8]在研究 Zn、Cu,、Co 和 Cr 两-两元素复合污染毒性研究发

现,复合污染表现为协同作用,但也有时表现为拮抗作用。宋玉芳[19]在进行 4 种重金属复合污染的植物毒性时也发现类似规律。

表 2　菲、芘、1,2,4-三氯苯复合污染对草甸棕壤中白菜根伸长抑制率(%)

Table 2　Inhabitation rates of combined pollution of PHE, PY and TCB in the meadow brown soils on the root elongation of Chinese cabbages

PHE* (Mg/kg)	抑制率 PHE	PY (Mg/kg)	抑制率 PY	TCB (Mg/kg)	抑制率 TCB	抑制率 PHE+PY+TCB
12.5	−7.5±1.1	12.5	−3.1±2.0	1.25	1.4±0.4	4.3±0.5
25.0	0.6±1.6	25.0	1.1±1.6	2.5	2.9±1.4	7.5±2.3
50.0	10.0±4.6	50.0	4.5±0.9	5.0	13.6±2.6	18.3±3.6
100.0	12.0±1.3	100.0	10.3±1.5	10.0	15.8±2.2	35.5±5.1

* PHE-菲,PY-芘, TCB-1,2,4-三氯苯。

表 3　菲、芘、1, 2, 4-三氯苯复合污染对草甸棕壤中西红柿根伸长抑制率(%)

Table 3　Inhabitation rates of combined pollution of PHE, PY and TCB in the meadow brown soils on the root elongation of tomatoes

PHE* (Mg/kg)	抑制率 PHE	PY (Mg/kg)	抑制率 PY	TCB (Mg/kg)	抑制率 TCB	抑制率 PHE+PY+TCB
6.25	−1.3±1.6	200	−4.1±2.2	1.25	−2.1±1.7	−3.4±2.2
12.5	7.3±2.2	100	5.8±0.0	2.5	−1.5±1.8	10.4±2.2
25.0	8.7±2.9	50	9.1±2.7	5.0	13.0±0.0	18.1±4.3
50.0	13.0±5.5	25	17.9±3.6	10.0	14.4±3.2	25.0±4.4

* PHE-菲,PY-芘,TCB-1,2,4-三氯苯。

3　结论

(1) 以植物根伸长抑制率为指标,研究污染物毒性与植物响应的剂量-效应关系,进而发现对污染具有敏感指示作用的植物,该研究可为土壤污染毒理诊断提供有效的生态学方法。

(2) 不同植物对有机污染物的毒性响应具有明显差异,这为敏感植物种的筛选提供了可能。研究结果显示植物根伸长抑制率与污染物的水中溶解度显著相关,说明有效态形式的污染物才能对植物造成直接毒害,是植物毒性作用显著增强的重要影响因子。

(3) 从菲、芘、1,2,4-三氯苯复合污染产生明显的协同效应可以判断,有机复合污染将使污染物的毒性效应增强。

参考文献

[1] Greene J C, Bartels G L, Warren-Hicks W J. Protocols for short-term toxicity screening of hazardous waste sites. US Environmental Protection Agency. EPA/600/3-88/029,1998.

[2] International Organization for Standardization (ISO). Soil quality-Determination of the effects of pollutants on soil flora. Part 1: method for the measurement of inhibition of root growth. ISO 11269-1,1993.

[3] International Organization for Standardization (ISO). Soil quality-Determination of the effects of pollutants on soil flora. Part 2: Effects of chemicals on the emergence and growth of higher plants. ISO 11269～2,1993.

[4] Knoke K, Marwood T M, Cassidy M B, *et al*. A comparison of five bioassays to monitor toxicity during bioremediation of pentachlorophenol-contaminated soil. *Water, Air and Soil Pollution*, 1999,**110**:157～169.

[5] Mishra A and Choudhurt M A. Monitoring of phytotoxicity of lead and mercury from germination and early seeding growth indices in two rice cultivators. *Water, Air and Soil Pollution*, 1999,**110**:340～345.

[6] Gong P, Wilke B M and Fleichmann S. Soil based phytotoxicity of 2,4,6,-trinitrotoluence (TNT) to terrestrial higher plants. *Arch. Environ. Contamn. Toxicol.*, 1999, **36**: 152～157.

[7] Gong P, Wilke B M, Strozzi, *et al*. Evaluation and refinement of a continuous seed germination and early seedling

growth test for the use in the Eco-toxicological assessment of soils. *Chemosphre*, 2001, **44**: 491～500.

[8] Ince N H, Dirilgen I G, Tezcanli A G, *et al*. Assessment of toxic interactions of heavy metals in binary mixtures: A statistical approach. *Arch. Environ. Contamn. Toxicol.*, 1999, **36**: 365～372.

[9] Kjaer C, Pedersen N and Elmegaard N. Effects of soil copper on black bindweed (*Fallopia convovulus*) in the laboratory and in the field. *Arch. Environ. Contamn. Toxicol.*, 1998, **35**:14～19.

[10] Heike K and Martin S. A method for analyzing polycyclic aromatic hydrocarbons (PAHs) in plant samples. *Chemosphere*. 1996. **32**(5): 849～854.

[11] Shu Y Y, Lao Robert C. Chiu and Chung H, *et al*. Analysis of polycyclic aromatic hydrocarbons in sediment reference materials by microwave-assisted extraction. *Chemosphere*, 2000, **41**: 1709～1716.

[12] Boldrin B. Degradation of phenanthracene, Floracene and pyrene by a *Mycobacterium* sp. *Appl. Environ. Microbiol.*, 1993, **59**(6):1927～1930.

[13] Stringfellow W T. In: R. E. Hinchee, *et al* eds. Induction of PAH degradation in a phenanthracene-degrading *Pseudomonad*. *Microbial. Proc. Biorem.*, 1995. 83～89.

[14] Jin X C (**金相灿**). *Pollution chemistry of organic compounds-Toxic organic pollution chemistry* (in Chinese). Beijing:Press of Qinghua University, 1990. 55～60.

[15] Song Y F (**宋玉芳**),Xu H X(**许华夏**),Ren L P (**任丽萍**). Eco-toxicity of heavy metals on the seed germination and root elongation of turnip in soils. *Chinese J. of Ecology*(in Chinese) (**生态学杂志**), 2001, **20**(3):4～8.

[16] OECD (Organization for Economic Cooperation and Development). OECD guidelines for testing of chemicals. Paris. France: European Committee, 1984. 208～209.

[17] Song Y F (**宋玉芳**),Ou Z Q (**区自清**) Sun T H (**孙铁珩**). Analysis method for polycyclic aromatic hydrocarbons in soil and plant samples. *Chinese J. of Applied Ecology*(in Chinese) (**应用生态学报**), 1995, **6**(1):92(96.

[18] Zhang H R (**张海荣**). Extraction and 1,2,4-trichlorobenzene for GC determination in water Soil and plant samples. *Chinese J. of Ecology*(in Chinese) (**生态学杂志**), 1996,**15**(4):70～73.

[19] Song Y F (**宋玉芳**),Xu H X(**许华夏**),Ren L P (**任丽萍**). Eco-toxicity effects of heavy metals on the seed germination and root elongation of Chinese cabbages in soils. *Chinese J. of Environmental Science*(in Chinese) (**环境科学**), 2002, **23**(1):103～107.

[20] Debus R and Hund K. Development of analytical methods for the assessment of ecotoxicological relevant soil contamination. *Chemosphere*, 1997, **35**(1/2): 239～261.