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蚕豆根尖微核技术监测环境污染物的诱变活性

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关键词 蚕豆根尖,微核,矿尘,真菌提取物,诱变性.

化学物质诱发染色体异常已有较多报道,这些物质能诱发生物的遗传突变和癌症。 近20年作为遗传毒物预警系统的生物测试方法发展迅速,其中植物微核监测系统-蚕豆根尖微核技术已引起不少研究者的注目,目前国内外已广泛应用于环境诱变和致癌因子的监测和研究^[1,2]。本文报道笔者应用此技术对矿尘、AFB,和真菌提取物进行微核诱导效应的试验分析结果。说明蚕豆根尖微核技术对不同的遗传毒物具有较高的敏感性,是一种简便而有效的生物监测方法。

一、 实 验

1. 材料

- (1) 蚕豆选用华中师范大学生物系陈光荣 提供的松滋青皮豆 (vicia faba)。
- (2) AFB_1 为 Sigma 精品,诱变剂重铬酸钾 $(K_2Cr_2O_7)$ 为上海化学试剂厂分析纯品,试验时配成 $3.4 \times 10^{-6} mol/L$.
- (3) 分别采集某矿各作业点的矿尘 10 份,分别称重后用蒸馏水配制为 1 mg/ml 的混悬液,静置后取上清液稀释为 1μg/ml 置于 4℃供实验用。
- (4)选取肝癌高发区扶绥县粮食中优势真 菌提取物 15 份,各种真菌提取液的制备见文 献^[3]。

3. 试验方法[1.2]

取松滋青皮豆用蒸馏水浸泡 24 h,置湿棉花发芽 48h;选初生根生长良好的蚕豆 4—5粒,分别放人上述各受试物中浸泡培养 3h,然后用蒸馏水冲洗三次,再置于蒸馏水中修复培养 24h(以上各步骤之温度均为 23—25℃). 然后分别取根固定 18h,用 5mol/L盐酸水解 25min.用 Feulgen 染色。 最后每个受试浓度随机取三条根尖常规制片,每片在高倍镜下观察 1000个间期细胞,分别计算各受试物的 微核细胞个数和微核率(MCN‰)。 实验结果用 MCN率的均值与阴性对照进行 t 检验,从差异的显著性判断受试物的诱变性。 每次实验均用 K₂Cr₂O₂ 作阳性对照和蒸馏水作阴性对照。 重复一次实验。

二、结果

1. 矿尘的诱变活性

10 份矿尘水溶液对蚕豆根尖细胞微核的诱导效应见表 1. 作为阳性对照的 $K_2Cr_2O_7$ 明显地诱发蚕豆根尖细胞微核率的增高,阴性对照的微核率在本底值范围,说明本法结果可靠。当矿尘的浓度为 $1\mu g/ml$ 时,受试的 10 份矿尘样品均能明显诱导蚕豆根尖微核率的增高,

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表 1 矿尘水溶液对蚕豆根尖细胞微核的诱导效应	表 1	矿尘水溶剂	林豆香饭	3.少细胞急	核的诱导效应
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样品号	(µg/ml)	微核细胞个数	$MCN\%(\bar{x}\pm SD)*$	诱 变 性
1	1.0	44(15,13,16)	14.7±1.5*	+
2	1.0	50(17,14,19)	17.5±2.3*	+
3	1.0	55(19,16,20)	18.3±2.1*	+
4	1.0	50(15,19,16)	16.7±2.1*	+
5	1.0	56(18,20,18)	18.7±1.2*	+
6	1.0	59(21,18,20)	19.7±1.5*	+
7	1.0	50(15,19,16)	17.3±1.2*	+
8	1.0	50(20,14,16)	16.7±3.1*	+
.9	1.0	52(21,17,14)	17.3±3.5*	+
10	1.0	44(14,14,16)	14.6±0.6*	+
K ₂ Cr ₂ O,	3.4 × 10 ⁻⁶ mol/L	69(19,20,30)	23.0±5.3*	+
蒸馏水		13(4,5,4)	4.7±1.2	

^{*} 各样品 MCN 与蒸馏水对照,P<0.01

表 2 AFB, 和真菌提取物的蚕豆根尖细胞微核诱导效应

受试物	浓度 (μg/ml)	微核细胞个数	$MCN\%(\tilde{x}\pm SD)$	P值	诱变性
AFB,	3.2 × 10 ⁻⁷ mol	44(16,15,13)	14.7±1.5	<0.01	+
杂色曲霉	100	79(29,22,28)	26.3±3.8	<0.01	+
棕曲霉	10	55(21,18,16)	18.3±2.5	<0.01	+
构巢曲霉	50	36(13,12,11)	12.0±1.0	< 0.01	+
棒曲霉	50	61(26,17,18)	20.8±4.6	<0.01	+
草长青霉	50	28(5,12,11)	9.3±3.9	>0.05	-
木贼镰刀菌	50	28(8,11,9)	9.3±1.5	>0.05	_
交链孢霉	100	34(12,14,8)	11.3±3.1	< 0.05	+
薛氏曲霉	190	32(8,14,10)	10.7±3.1	>0.05	-
文氏曲霉	100	26(7,9,10)	8.7±1.5	>0.05	-
冰岛青霉	100	29(12,10,7)	9.7±2.5	>0.05	
串珠镰刀菌	100	22(11,3,8)	7.3±4.0	>0.05	-
土曲霉	100	23(10,7,6)	7.7±2.1	>0.05	-
溜曲霉	100	25(6,10,9)	8.3±2.1	>0.05	-
黑曲霉	100	25(8,7,10)	7.5±0.7	>0.05	-
白曲霉	100	28(8,11,9)	9.3±1.5	>0.05	_
K ₂ Cr ₂ O ₇	3.4 × 10-6 mol/L	41(16,14,11)	13.7±2.5	<0.01	+
蒸馏水		20(6,8,6)	6.7±1.2	}	

微核率在 14.6% 至 19.7% 之间,与阴性对照组有明显的差异性 (P < 0.01)。 说明矿尘具有诱变性。

2. AFB₁ 和真菌提取物的微核效应

AFB₁ 和真菌提取物对蚕豆根尖细胞微核 诱导效应见表 2. 当 AFB₁ 的浓度为 3.2 × 10⁻⁷ mol 时对蚕豆根尖细胞微核的形成有明显诱导 作用,15 种真菌提取物中有杂色曲霉、棕曲霉、 构巢曲霉、棒曲霉和交链孢霉等五种能明显诱 发蚕豆根尖细胞微核率的增高,与对照组相比, 微核率有显著性差异 (P < 0.01),说明具有诱变性。

3. AFB₁、5[#] 矿尘和杂色曲霉提取物诱变活性的强度及剂量-效应关系

为进一步观察矿尘、AFB₁和真菌提取物的诱变活性强度及其对蚕豆根尖微核的敏感性,分别测定了不同浓度的 AFB₁、5[#] 矿尘和杂色曲霉提取物对微核效应的影响.结果由图可见,AFB₁、5[#] 矿尘和杂色曲霉提取物在一定 浓度范围内均能明显诱发蚕豆根尖微核率的增高,

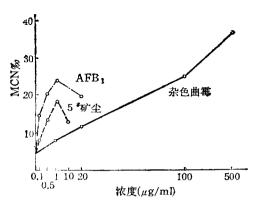


图 1 AFB₁、5^{*}矿尘和杂色曲霉提取物诱导蚕豆根 尖细胞微核的剂量效应关系

当浓度为 1 μg/ml 以下时三者均显示良好的线性剂量-浓度依赖关系;在此浓度之内 AFB₁ 诱导的微核效应大于 5[#] 矿尘而矿尘又大于杂色曲霉提取物,即说明蚕豆根尖细胞微核对 AFB₁ 的敏感性大于矿尘和杂色曲霉提取液。反映了蚕豆根尖细胞微核对不同类型和结构的遗传毒物的不同敏感性。

三、 讨 论

蚕豆根尖微核技术是一种以染色体损伤及 纺锤丝毒性等为测试终点的植物微核监测方 法,目前多见于环境及水体污染的监测。 据 报道此法与动物实验方法之间对环境致突变物 引起的染色体畸变等定性反应的一致性可达 99%以上^[4]。显然此技术无疑是环境遗传毒物 的快速筛选方法之一。

本实验应用蚕豆根尖微核技术对环境污染物的矿尘、 AFB_1 和真菌提取物进行微核诱导效应分析,结果所有的矿尘、 AFB_1 和五种真菌提取物均能明显诱导蚕豆根尖细胞微核率的升高,提示受试样品含有致植物细胞染色体损伤的物质,具有诱变活性。 在剂量关系研究中, AFB_1 的浓度为 $0.1\mu g/ml$, 10 份矿尘水溶液在较低浓度 $(1\mu g/ml)$ 水平即能明显诱导蚕豆根尖细胞微核形成的作用,而当浓度大于 $1\mu g/ml$ 以上时,二者所诱导的微核率随浓度升高而降低,这表明 AFB_1 和矿尘有较强的诱变活性

影响了蚕豆根尖细胞有丝分裂的形成。

鉴定化学致突变和(或)致癌物往往采用一 组不同测试终点的方法进行印证, 在真菌提取 物诱变性的比较研究中, 本实验能诱导微核形 成的5种真菌提取物在 Ames 试验中显示诱 变阳性的有杂色曲霉、构巢曲霉和棕曲霉等三 种,符合率为 66.7%⁶⁵; 在 DNA 重组修复试验 和 SOS 试验中显示阳性的 4 种真菌提取物 (杂 色曲霉、棕曲霉、构巢曲霉和交链孢霉) 在 本实验中均能诱导微核形成的作用,符合率 100%[3,6]。二者有很高的一致性。 此外,本实 验受试物为不同类型和结构、性质的化合物(混 合物),各有特殊的致突变和致癌机理,而这些 环境诱变剂在较低浓度水平均能明显诱导植物 微核的形成和升高, 这表明蚕豆根尖细胞微核 对不同类型的毒物的反应具有较高敏感性,因 此它可以测定不同类别和不同剂量的毒物对生 物的遗传物质损伤。

据研究,致突变性与致癌性高度相关^{CM},致突变物中可能存在致癌危险性。我们的实验结果表明某矿山矿尘和扶绥县真菌提取物具有较强的诱变活性。 文献报告工业矿尘中含有砷、镉、铬等致肿瘤活性的重金属元素,高度的环境污染与恶性肿瘤的发病有关^[8]。另外,AFB₁是强致癌剂,扶绥县肝癌高发与当地居民摄入AFB₁或真菌毒素的协同作用密切联系^[5],10]。本实验结果研究启示矿尘、AFB₁。 和真菌提取物具有较强的诱变活性与流行病学调查研究的一致性与文献报道相符^[6,9],10]。这就为进一步深入研究厂矿职业性致癌和肝癌的环境因素提供了重要线索和可借鉴的又一实验室佐证。

诚然,植物细胞监测系统的结果外推于人时由于植物细胞与人类细胞在生化代谢各方面差距甚远,诱变剂进入靶分子的方式及作用机理很不相同,因此实验研究与流行病学调查的一致性尤为重要。但由于蚕豆根尖微核技术作为遗传毒物的预警系统初筛潜在致癌危险的物质具有经济、快速、准确的特点,如能配合其它测试系统这对环境诱变剂的研究无疑是一种极为有用的手段。

长寿命 BOD 微生物传感器的研究

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摘要 本研究将从淀粉厂活性污泥中分离筛选出的一种性能优良的腊状芽孢杆菌 (Bacillus Cereus) 用高分子 等材料包埋制成薄膜,与氧电极组合成 BOD 传感器。 经对其技术性能进行测试表明,间断使用寿命已达 16 个月以上;对 BOD 标准物质线性响应范围 5-60mg/L;响应时间不大于 8min;测定环境标样平均误差 1%,变异系数 5.1;与 BOD, 经典法同步测定废水样品结果相关性良好。

关键词 BOD 传感器,微生物传感器,BOD 测定,微生物固定化.

用微生物传感器法测定废水 BOD,与BOD, 经典法、库仑法、差压法、活性污泥法等比较,具 有快速、简便、适于废水处理工程控制监测等优 点。对该方法的研究进展已有综述^[1,2],主要存 在两个问题,一是传感器寿命短,最长为两个 月^[3];二是多用夹层法固定微生物^[1-5],微生物 层松散,易流失,且只能一次性使用。虽然也有 的用单一材料包埋制膜^[1,6],但膜强度差,也是 一次性使用。

本研究筛选出的微生物菌株性能优良,用混合材料在常温下包埋,制成机械强度、韧性和透气性均较理想的微生物膜。膜的制作方法简便,同一膜可多次更换使用,间断使用寿命已超过16个月以上。

一、传感器制备及实验方法

- (一) 仪器及主要试剂
- 1. 仪器
- (1) 由极谱式溶氧测定仪改制的 BOD 速测仪实验样机;
 - (2) 3066 型记录仪
 - 2. 试剂

- (1) 磷酸盐 缓 冲 溶 液 (0.05 mol/L, pH 7.2), 由 KH₂PO₄ 和 Na₂HPO₄ (均为分析纯) 配制;
- (2) BOD 标准溶液 称取葡萄糖 (分析 纯)、谷氨酸(生化试剂)各 0.250g, 于 500ml 容量瓶中用磷酸盐缓冲溶液定容 (下称 GGA 标 液);
- (3) 环境标准样品 中国环境监测总站水质标样 (BOD₅-3200205, GSBZ50002-88)。准确移取 10ml, 用蒸馏水稀释至 250ml, 其保证值为 110.9mg/L,不准确度±5.4。
 - (二) 传感器制备
 - 1. 菌种及培养
- (1) 菌种 从淀粉厂活性污泥中分离筛选得到。 经鉴定,为腊状芽孢杆菌(Bacillus Cereus)。
- (2) 液体培养基 牛肉膏 2%,蛋白胨 1%,淀粉 2%,加适量磷酸盐缓冲溶液配成营养液。 将营养液与城市污水按 1:1 体 积 比混合,调节至 pH 7.0。

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Abstracts

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well as the operation conditions of the pond.

Key words: waste water, stabilization pond, anaerobic, biodegradation, benthic deposit.

Synthesis of 1-Hydroxypyrene and Its Identific. ation. Ma Jiang zhao (Beijing Municipal Research Institute of Environmental Protection, Beijing 100037): Chin. J. Environ. Sci., 13(4), 1992, pp.27—30

A procedure for the preparation of 1-hydroxypyrene from pyrene was described. Its mass spectrum, nuclear magnetic resonance, infrared, ultraviolet and fluorescence spectra were determined. The results show that the compound thus prepared is a very pure product.

Key words: 1-hydroxypyrene, pyrene.

Research on Leaching Dynamics of Dimethypo in Soils. Li Deping. Jin wei (Institute of Soil Science, Academia Sinica, Nanjing 210008) Chin. J. Environ. Sci., 13(4), 1992, pp.31-34

With reference to OECD-Chemicals Testing Guidelines, laboratory experiments were carried out to examine the leaching dynamics of dimethypo in soils. The results suggest that dimethypo possesses a strong tendency of leaching. Thus, most of the pesticide can leach through the soil columns. With the continuous addition of leaching water, all the pesticide in soil columns can leach out. Soil properties did not show great influence on leaching, while flow rate and temperature exerted remarkable effect on leaching dynamics of dimethypo.

Key words: dimethypo, leaching test, dynamics.

Study on the Degradation of Hydrazine Hydrate
in River Water. Sun Hong et al. (Environmental
Protection Monitoring Station of Benxi 118000):Chin.

J. Environ. Sci., 13(4), 1992, pp. 35-39

Labo ratory studies and monitoring practice in a river demonstrate that the degradation of hydrazine hydrate can be described by the first-order reaction dynamics. Its degradation rate was found to be dependent on water temperature, microorganism, concentration of dissolved oxygen, pH and other conditions. The effect of temperature on the degradation rate could be expressed with the formula: $K_T = K_{20}$. $\theta^{(T-20)}$ Calculation with computer shows that when pH=6, then $K_{20} = 0.028$ 1/h and $\theta = 1.045$, when pH=8, then $K_{20} = 0.021$ 1/h, $\theta = 1.036$. The degradation coefficient of hydrazine hydrate measured in the experiment carried out in the river was equal to 0.3001/h. **Key words:** hydrazine hydrate, degradation, first-order reastion dynamies

Oxidation Dephenolization of Waste Water Catalyzed by Horseradish Peroxidase Hu Longxing et al. (Shanghai University of Technology 200072): Chin. J. Environ. Sci., 13(4), 1992, pp 40-44

The results of the study on oxidation dephenolization of waste water catalyzed by horseradish peroxidase show that for the treatment of waste waters containing only one of the three phenols (phenol,o-chlorophenol and o-aminophenol), the highest pheanol removal efficiency can be reached at pH values around 7. It was also found that at low (4°C) and high (40°C) temperatures, the removal efficiencies reduced

by about 10%, and in the treatment of waste water containing more than one phenols, there existed synergistic "ffect among various phenols resulting in phenol removal efficiencies as high as 95%.

Key words: phenols, waste water treatment, horseradish peroxidase.

A Study on Modification of the Surface of Coal Ash. Lü Yaojiao, Zhang Jishuang (Hunan University 410082): Chin. J. Environ. Sci., 13(4) 1992, pp.45-47 The modification of the surface of coel ash was achieved by treating the ash with six kinds of surfactants including H-NA, H-R through dry or wet processes of activation. Experiment results indicate that the number of hydroxyl group on the ash surface decreased while corresponding groups of activator increased resulting in the improvement of its property of dispersion in organic medium. The properties of processed PVC and rubber samples with the activated coal ash as filler were greatly improved compared with those products with untreated ash as filler. All the technical targets of the new products, except for the decrease of abrasive wear of rubber, which needs to be further improved, reached or surpass the relevant standards. No doubt, thtis is a new path for making use of the regenerated resource with the advantages of lowering the cost of composite material and protecting the environment.

Key words: coal ash, surface modification, composite material.

Taxonomic System for Environmental Science. Wang Huijun, Chen Jingsheng (Center of Environmental Science, Peking University, Beijing 100871): Chin. J. Environ. Sci., 13(4), 1992, pp. 48-51

A new and more reasonable taxonomic standard for environmental science is introduced based on the systematic analysis of the synthetic, integrate and inter-disciplinary character of the science and the forms and extent of the synthesis and integration. According to the new standard, environmental science can be regarded as an organic entirety composed of three levels of interrelated discipline groups.

Key words: taxonomic system, environmental science. Application of Enzyme Immunoassay in Pesticide Analysis Li Zhixiang, et al. (Institute of Agroenvironmental Protection Tian Jing 300191): Chin. J. Environ. Sci., 13(4), 1992, pp. 51-55

This paper introduces briefly the technique of Enzyme Immunoassay and its application in pesticide analysis. The basic types, principles, procedures of development and the prospect of future application and further advancement of Enzyme Immunoassay were discussed.

Key words: pesticides, enzyme immunoassay.

Application of Micronuclues Test in Vicia Faba Root Tips in the Rapid Detection of Mutagenic Environmental Pollutants. Ruan Cuicai, Liang Yu. an, Liu Jinling et al. (Guangxi Cancer Institute, Na. nning 530027): Chin. J. Environ. Sci., 13(4), 1992, pp. 56—59

Micronucleus test in vicia faba root tips was performed for

Abstracts

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10 mine dust tamples. AFB 1 and 15 fungal extracts. The results showed that 10 (100%) mine dust, AFB1 and 5(33.3%) fungal extracts significantly increased the frequency of micronucleated cells in vicia faba root tips with a clear dose-effect relationship suggesting that these samples had mutagenic activity and the relevant environmental factors might be responsible for the incidence of lung cancer in he mine area and liver cancer in Fu-shui county. Thus micronucleus test in vicia faba root tips proved to be an effective method in the research of environmental mutagens.

Key words: vicia faba root tips, micronucleus, mine dust, fungal extracts, mutagenicity.

Study on a Long Life BOD Microbial Sensor. Sun Yusheng, Liu Xianmei et al. (Hebei Institute of Che. mical Technology and Light Industry, Shijiazhuang 010050): Chen. J. Environ. Sci., 13(4), 1992, pp. 59-63 A microbial sensor consisting of thin membrane which was prepared by using Bacillus cereus cells (isolated from active sludge) immobilized in synthetic polymers and oxygen electrode combined with the sensor were used for the estimation of BOD. The lifetime of the sensor was determined to be more than 16 months. A linear relationship was observed between the relative current decrease and the level of BOD of the standard solution (GGA) in a range of 5-60mg/L. The response time of the biosensor is less than 8 minutes and the average relative error is 1% when an environmental standard sample was used to test the senson, BOD values determined with the sensor were identical to those determined with the convential method for the same water samples.

Key words: BOD sensor, BOD electrode, microbial sensor.

Determination of Nitrate-N and Nitrite-N in Surface Water by Highly Sensitive Spectrophotometric Method. Qiu Xingchu, Zhu Yingquan, (Environmental Science Research Institute of Ganzhou Prefecture, Ganzhou 341000, Jiangxi Province), Li Cuisheng (Forestry Science Research Institute of Ganzhou Prefecture, Ganzhou 341000, Jiangxi Province.): Chin. J. Environ. Sci., 13(4), 1992, pp. 63—66

The conditions of the reaction of nitrate-N and nitrite-N with N-phenylanthranilic acid (N-PAA) were studied in detail and a highly sensitive spectrophotometric method was developed for the determination of trace amount of nitrate-N and nitrite-N in surface water. Maximum absorptions of both the colour products (N-PAA-NO₃-N and N-PAA-NO₂-N) appeared at wavelengths of 560-565 nm and the corresponding molar absorptivities were found to be 1.07×106 (NO₃-N) and 1.77×107 1. mol-1 cm⁻¹ (NO₇-N). Beer's law was obeyed, in a concentration range of 0.03-0.15 µg/ml for NO₇-N and 0.05-0.20 µg/ml for NO₇-N. The interferences of foreign ions were examined and the method has been used to determine NO₂-N and NO₃-N in surface water with satis-

factory results obtained.

Key words: Nitrate-N, nitrite-N, spectrophotometry, N-phenylanthranilic acid, water pollution.

Identification of Spilled Oil at Sea Surface with Fuzzy Maximum Matrix Method. Xu Hengzhen, Li Zongping (Institute of Marine Environmental Protection, SOA, Dalian 116023): Chin. J. Environ. Sci. 13(4), 1992, pp. 67—69

A Medol of fuzzy maximum matrix for the identification of spilled oil at sea surface is established, the concept of fuzzy area of confidence level λ_i for identifying types of oil was introduced. Fuzzy dynamic clustering charts of fourteen common types of oil, two types of weathered oil and oil spill at sea surface are given out. Confidence level λ_i was found to be greatly influenced by weathering. After 30 days weathering, confidence levels for differentiating Ren Qiu Crude oil and 35^{th} heavy diesel oil, from other thirteen types of oil, are in the ranges of 0.99939 $>\lambda_i>0.99725$ and $0.99791>\lambda_i>0.99026$, respectively. The method proved to be able to give more accurate result than the finger-print recognition method.

Key words: fuzzy maximum matrix, fuzzy dynamic clustering chart, oil spills at sea.

Study on Soil Environmental Background Values in Fujian Province. Chen Zhenjin, Chen Chunsiu, Liu Yongqing (Fujian Institute of Environment Protection 350003); Wu Yuduan, Yang Sunkai, Lu Changyi (Institute of Environment Protection, Xiamen University 361005): Chin. J. Environ. Sci., 13(4), 1992, pp. 70—75

With the methods of network and systematic layers, 123 typical sampling sections were selected and soil environmental background values for 61 elements were determined in the whole region of Fujian province. The results show that the soil background values for 15 rare-earh elements in Fujian are higher than those in the country, that the background values for sulphophilic and siderophilic elements in Fujian are also higher compared with those of the same elements in the country and that the background value of Se in the soil of Fujian is 0.55mg/kg, 2.5 times as high as the level of the country. The major factors affecting soil environmental background values in the province are analyzed and suggestions for reasonable development of soil resource proposed. **Key words:** soil environmental background value, earth elements, Fujian province.

Chemical Speciation and Pollution of Heavy Metals in Rivers, Reservoirs and Waste Water in Urumqi Area. Chen Xibao et al. (Institute of Geography, Academia Sinica Beijing 100012): Chin. J. Environ. Sci., 13(4), 1992, pp. 75-81

A research on physico-chemical speciation and characteristics of pollution of heavy metals Cd. Zn, Pb and Cu in certain water bodies in Urumqi area was carried out with a view of developing water resource as well as evaluating water quality in this area. The physico-chemical speciation of the heavy metals in water samples were determined by means