Studies of the Ecotoxicological Impact of Pollutants by Using Microscale Community-level Toxicity Tests

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All pollutants either from air or from land will eventually discharge into water system (rivers, streams, seas, underground water ...etc.) through precipitation and surface runoff. All countries have attached great importance to the problem of water pollution.

Part 1. The basic principle of hazard assessment

In resolving the problem of the potential hazard of water pollution, people are anxious to be informed of: (1) how many potential pollutants are discharged into water, when and how ? (2) what will happen to the pollutants in the water physically, chemically and biologically and what effects will they have on individuals (including human being), populations, communities and the entire ecosystems ? Therefore, two scientific judgments should be made on the basis of experimental evidences: the environmental concentration of the pollutant, and its concentration for producing biological effects (two straight lines in Fig. 1). When the estimated concentrations (dotted lines in Fig. 1) come closer to the objective concentration (the straight line), a correct hazard assessment can be made. The water quality is safe only on the condition that the biologically effective concentration is higher than the environmental concentration. If these two concentrations are very close to each other or in the reverse order, it means that hazards will appear or have appeared. In this point of view, the biological monitoring is as important as the chemical monitoring.

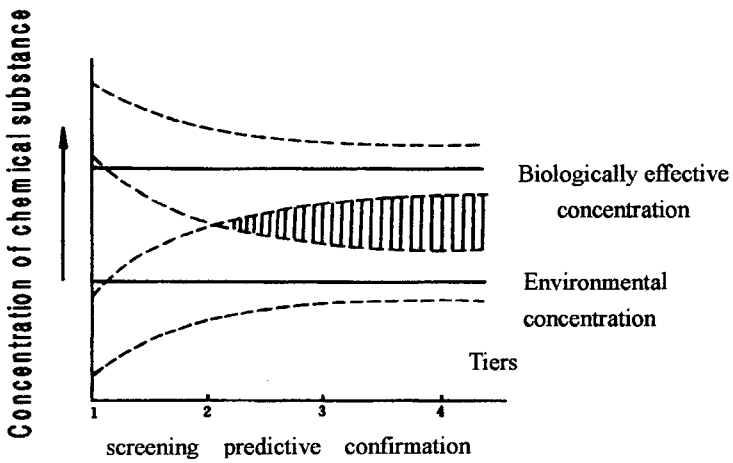

Fig. 1. Relationship between two concentrations of tiered testing in the hazard assessment (Communication with Prof. Cairns)

Part 2. Ecological basis of the microscale community-level toxicity tests

1. Key determinants of ecological risk

When evaluating the ecological risk caused by disposal of chemicals and wastewater, the prevailing model is the single species. Because of the field of ecotoxicology, firstly, scientists are focus on large vertebrates for toxicity testing, such as mice, rates instead of humans. Later, the toxicological test was extended to domesticated species and to some wild species, especially fish. All these toxicity tests were based on single species. The biological organization level is cell – tissue – organ – species – population – community – ecosystem – biosphere. The more high the level, the more precise the toxicity tests. There is no such a species with the same sensitivity to every toxicant. Interactions between species of a community and interactions between communities and their environment are key determinants of ecological risk.

2. Dominant life form in the globe

However, microscale organisms --- bacteria, fungi, algae, protozoans, and small metazoans are the dominant group of organisms on planet both in biomass and metabolic activity. There is a variety of reasons for using the microscale community in toxicological testing. (a) A cosmopolitan distribution of these microscale organisms might provide a good comparison in geographically different regions and countries. (b) Replicability is good or better than tests with larger organisms. (c) Environmental realism based on community level is higher than that based on single species, such as fish.

Part 3. PFU method ---- the best method for the microscale community-level toxicity tests

1. Introduction of the PFU method

(1) What is the PFU method?

In 1969, Prof. Cairns first used the PFU (polyurethane foam unit) to collect microbiota. PFU is an artificial substrate of three dimensions and can be immersed in water in any time and at any place. The Diameter of pores of PFU is about 150μm. According to the size, the plankton in water can be divided into six groups: picoplankton (0.2 –2μm), nannoplankton (2 - 20μm), microplankton (20 – 200 μm), mesoplankton (o.5 – 1mm), macroplankton (> 5mm), megaloplankton (>1cm) (Porter et. al., 1985). Only the first three parts of plankton can immigrate into PFU. A bundle of PFUs is tied to a stone and thrown them into water. After a definite exposure time, 2 – 4 PFUs are cut off from the bundle and put them into plastic bag separately. The pores and interstices of the PFU are colonized by indigenous microbes. After squeezed out water from the PFU, under the microscope you might discover bacteria, fungi, algae, protozoa and small rotifers with occasional ostracods, cladocerans, gastrotriches, insects, and nematodes. Among them, the motile unicellular protozoa are a particularly effective groups to response the ecological risk, because it encompasses primary producers, decomposers, and several levels of consumers.

a. Primary producers ---- photosynthetic autotrophs, such as colored flagellates, color euglenids, *chlamydomonas* spp.

b. Decomposers ---- osmotrophs (saprotrophs), a variety of flagellates take up dissolved organic molecules such as colorless euglenids, *Astasia, Sphenomonas*

c. Consumers at different levels

(a) Bactivores-detrivores, such as most of small heterotrophic flagellates (*Bodo celer, Codosiga gracilis, Trepomonas agilis*), small Amoebida (*Amoeba limax, Centropyxis hemisphaerica, Hartmannella* spp. *Vahlkampfia limax)* and ciliates (*Glaucoma scintillans, Holophrya atra, Paramecium caudatium, Spirostomum teres, Vorticella panula)*

(b) Algivores, such as *Anthophysis vegetans* in flagellates, *Amoeba proteus, Polychaos dubium, Centropyxis aculeata, Difflugia globulosa, Euglypha laevis, Trinema complanatum,* in Amoebida, and *Chilodonella algivora, Nassula elegans, Colpoda cucullus, Nassula ornata,,Stentor polymorpus* in ciliates.

(c) Predators, such as *Gymnodinium fungiforme* in Dinoflagellida, a species carnivorous on metazoa; *Acineta tuberosa, Tokophrya infusionum, Didinium nasutum, Lacrymaria olor, Lionotus carinatus, Hemiophrys fusidens* in ciliates, carnivorous on other protozoans and small rotifers. Feeding and special competition among different protozoan species will appear and the protozoan community will constitute a complicated food web. This implies that the microbial organisms respond to the toxicity at community level. 85% of the species of microbial organisms can be retrieved from PFU. Therefore PFU community can reflect the environmental reality.

(2) The objective existence of microbial community

Patrick and Cairns (1967) investigated aquatic organisms in 202 rivers of U.S.A, South America and Canada. They found that the species composition of algae, protozoa, insects and fishes in similar environments varied with time and space, but the number of species was rather stable and the distribution in the number of individuals in a species followed certain models. The microbial organisms were not an exception and they constituted an objective community.

(3) The colonization process of protozoa in PFU

Prof. Cairns demonstrated the colonization process of microbiota in the PFU was accordance with the famous MacArthur-Wilson equilibrium model of island biogeography:

$$S_t = S_{eq}(1 - e^{-Gt})$$

Here S_t ---species number at time t; S_{eq} --- species number at equilibrium; G --- colonization rate constant; and $T_{90\%}$ --- the time needed to reach 90% S_{eq}. To consider the effect of environmental pressure (H), Wang et al. (1989) modified the MacArthur-Wilson equilibrium model to use with the PFU method, and derived a more suitable model:

$$S_t = \frac{S_{eq}(1 - e^{-Gt})}{1 + H e^{-Gt}}$$

The four functional parameters (S_{eq}, G, H, $T_{90\%}$) that are used into the evaluation of water quality, are brought forward from the field biological monitoring and the laboratory toxicity tests.

Part 4. PFU method in People's Republic of China

Most of countries in the world lay particular stress on chemical monitoring at the expense of biological monitoring. The main reason is that the test items and analytical methods of chemical monitoring are relatively standardized, the obtained data is better

in comparison and, analytical persons are easily trained. However, biological monitoring has lack of standard method that can be accepted by people. We considered that the purpose of environmental protection is to protect organisms on which human being relies for existence. If environmental quality is indicated only by chemical monitoring, not by biological monitoring, it is difficult to let people believe.

In 1981 the author visited USA and collaborated with Professor Cairns on the evaluation of water quality using protozoan communities in Cedar Run, a small stream which received wastewater from an electroplating plant as well as sewage treatment plant effluent. (Shen et al. 1986). Since then, the monitoring method based on the PFU microbial communities has received continuous modification, validation, and application over the past 10 years in China. The standard Division of the China Environmental Protection Agency and the Institute of Hydrobiology of the Chinese Academy of Sciences organized training courses in microbial community biomonitoring in 1983, 1984, 1989 and 1990. 151 students from 26 provinces and cities (with the exception of Tibet, Hainan, Qinghai, and Taiwan) attended the courses. They not only studied the basic principle, methodology and systematics, but also proceeded the monitoring in the field and the toxicity test in the lab. The results of all these programs were satisfactory. Finally, in 1990 the China EPA passed the national standard < Water Quility – Microbial monitoring – PFU Method > (GB?T 12990-91). This was the first national standard of biological monitoring established in China for the first time.

1. PFU method can be used in anywhere, at anytime, for any pollutants

In the past decade, numerous tests have provided evidence that this method can be applied to field monitoring of freshwater, industrial wastewater, urban sewage, and various harmful chemicals. The immersed PFUs in water for 1 - 3 days reflect the water quality in 1 - 3 days. Therefore spill from plants can be revealed in time because this method has the property of continuous monitoring.

The industrial wastewater effluents that have been monitored using the PFU method are shown in Table 1 (Shen, 1995).

Table 1. Types of industrial wastewater effluents that have been monitored using the PFU method

Paper mill	Textile mills
Iron-alloy plant	Tobacco factory
Sewage treatment plant	Beverage factory
Electric power plant	Pharmaceutical factory
Oil refinery works	Food processing
Steel mill	Automobile factory
Chemical plant	Stone pit
Water works	Cocking plant
Chemical fertilizer factory	Smeltery
Petrochemical works	Brewery, winery, distillery
Dye factory	
Electroplating (Cyanide, chlorine, sulfides, sodium, cadmium, copper, lead, nickel, zinc)	
Organophosphate pesticide plant (parathion, malathion, dimethaote,	

and benzene hexachloride)

2. Parameters of the PFU method can match with parameters of chemical monitoring method

The project of evaluation of water pollution by using PFU microbial communities supported by the International Development Research Centre, Canada was carried out in the Hanjiang River with 997 km long, the largest tributary of the Yangtze River as well chemical monitoring in 1992-1993 (Shen st al. 1995). 47 sampling stations in the upper, middle and lower reaches of the Hanjiang River and its tributaries were set up. Four biological parameters were included, namely the number of protozoan species, the percentage of phytomastigophora, protozoan diversity index and heterotrophy index. The comprehensive chemical pollution index, Pa and Pb were based on the data of 8 items, NO_3-N, NO_2-N, NH_3-N, COD_{cr}, BOD_5, dissolved oxygen, volatile phenol and cyanide. P from all sampling stations are calculated by using following formula:

$$P = \sum_{i=1}^{n} Pi \quad Pi = Cd / Co,$$

here Pi -- single chemical pollution index; Cd --- concentration of tested chemical parameter in sampling station; Co --- concentration of tested chemical parameter in reference station (for Pa) or upper limit of the concentration of the chemical parameter stipulated in the II grade of standard for surface water issued by China EPA (GB3838-88) (for Pb); n ---- number of control items, here is 8.

All the four parameters of PFU microscale communities correlated to comprehensive chemical pollution index Pa and Pb significantly. Correlation coefficient was 42– 0.98. P-test was 0.03 – 0.0001. But the correlation between the Shannon diversity index of benthic animals and Pa, Pb was not significant. Correlation coefficients were 0.35 – o.49, p-test was 0.10 – 0.07. We considered that this result was because the PFU microscale communities and chemical analysis were sampled from the water surface, but macroinvertebrates were sampled from the bottom of the Hanjaing River. There were 70 species of benthic animals collected mainly Oligochaeta, Mollusca and aquatic insects. They distributed in the bottom and can well reflect pollution situation in interface between sediment and water phase. If in the project the chemical analyses from the water bottom had been carried out and the macroinvertebrate method had been improved, the macroinvertebrates would also become a much better biomonitoring method for evaluating water quality.

3. PFU microscale community toxicity tests can predict safety concentration of chemicals correctly

(1) Procedures of the PFU toxicity test

a. Installing the naturally derived PFUs ---- Epicenter PFUs

A bundle of PFUs is tied to a stone and thrown them into water. The best site is the indigenous clean water of the stream, river, lake, pond, or reservoir where chemical or wastewater will be or has been drained. After a definite exposure time, PFUs are cut off from the bundle and put them into plastic bag separately. The PFUs are then used as Epicenter in the toxicity test.

b. Arranging the microcosm tests

In a typical toxicity test, two replicates of five concentrations of the chemical or wastewater

and a control are tested. There are two types of the microcosm test. One is the static microcosm test and another is the flow-through diluted microcosm (FDM) test.

(a) The static microcosm test

On each side of the tray, 4-5 barren PFUs were placed at equal distant from the center. In the center of the tray was placed the epicenter PFU. Each tray contained 5 liters of filtered and pasteurized test medium, collecting from the field where the epicenter PFU was immersed. (Figure 9-5 in Shen et al., 1990). Each test tray was lighted on a 16 L:8D (16 hours light to 8 hours dark) schedule.

The entire testing system was covered with glass to reduce the possibility of airborne contamination. After a definite exposure time (1,3,6,9,12 days) 2 PFUs were removed from test trays. Squeezed out water from the PFU to a beaker for microscope identification. The squeezed PFU is put to the former place of the tray carefully and marked for never using it again.

(b) The flow-through diluted microcosm (FDM) test

We designed a device can be used simultaneously for two kinds of toxicants with different concentrations. Each concentration, including the control is flowing into a chamber with a uniform velocity. In each chamber the barren PFUs and the epicenter PFU have been placed beforehand.

(3) Validation of PFU microscale toxicity tests

a. Reaching to an unanimity both in field and in lab

Ya-Er Lake is located in the west part of E-Cheng county, Hubei province. It covers about 6ooo Ha. (Now 2000 Ha.) and is one of fish production district in the province. Since 1962 the lake was polluted by wastewater of a chemical plant which produced parathion, malathion, dimethoate and BHC (hexachlorocycohexane). The normal aquatic ecosystem was destroyed. In a survey of 1972, about 40% of fish were abnormal form. The Ya-Er Lake pollution control system was finished in 1976. In a part of Ya-Er Lake, 5 oxidation ponds were constructed and connected in series. The total area is about 187 Ha. And 3 m in depth. The detention time is about 80 days. The effluent from the chemical plant is 70 000 tons per day. All the toxicants were degraded gradually when the effluent was flowing through the 5-oxidation ponds. In 1982 we carried out the examination of the effectiveness of the 5-oxidation pond system by using the PFU method both in field and in lab. The community structural parameters (protozoan species composition, chlorophyll a and bacterial abundance) and functional parameters (protozoan colonization rate, community respiration rate) were estimated. (Fig.2,3,4,5 in Shen et al., 1985) Both structural and functional parameters appeared to be good indicators of stress. According to the degradation of effluent in a series of 5 oxidation ponds (fig 1 in Shen et al., 1985), the bacterial abundance decreased, but the chlorophyll a and community respiration rate increased respectively (fig.5 in Shen et al., 1985). The higher concentration of the toxicants (pond 1,2), the lower was the colonization rate of the protozoan community, the lower concentration of the toxicants (pond 3,4,5), the higher was the colonization curve of the protozoan community (fig.2, 3 in Shen et al., 1985). The results from the field were found to be coincident with that from the laboratory. As to the colonization curve decreased again in the pond 5 in the field, because the pond 5 in the field has been planted fingerling fish, so that protozoans might have been utilized by the fingerling fish. In laboratory tests the colonization curve in pond 5 increased regularly. The results from field and lab are very similar. That means the laboratory results may adequately predict events in natural ecosystems. We hold PFU training courses in 1983, 1984, all the students repeated the field and lab tests and got the same results. They admired that the PFU method is an accurate, economic and

rapid method.

b. Predicating the safe concentration of chemicals with taxonomic loss

The purpose of ecotoxicology must take these three interrelated forces in consideration: (1) the sustainable use of the planet, (2) the protection of ecosystem services and (3) maintaining natural systems in robust health. Clearly, no any thresholds based on both single species toxicity test and the microscale community toxicity tests are suitable for meeting these three needs. Therefore, we should always rivet our attention on the type of testing that will provide the most useful information for making dicisions.

The most commonly used threshold in ecological toxicology is the LC_{50}, where 50% of the organisms die or affect at a certain concentration of a chemical for a definite exposure time under specified environmental conditions. This threshold is derived from single species laboratory tests low in ecological and environmental realism.

As we have known that the International Organization for Standardization (ISO) issued the LC50 toxicity tests on *Daphnia magna* and zebra fish. On 22 September 1988, the U.S. Senate passed the "Consumer Products Safe Testing Act", which indicated that the acute LD50 (median lethal dose) test using vertebrates is inaccurate, misleading, and unnecessary in products testing. The federal government has thus banned the use of LD50 tests. The reasons are:

(a) Although a toxicity LC50 test using a single species provides useful information about toxicity, it does not provide much information about how the thousands organisms to be affected. The goal of environmental protection is to protect 95% of the species in nature.

(b) Generally speaking, in nature there are a few very sensitive species, a few very tolerant species, and the great majority species are somewhere in between. It is usually described with a log-normal or log-logistic tolerance distribution. The mean and standard deviation of the distribution can be used to extrapolate to a chemical safe concentration (NOEC). This is almost certainly not the case. Species used in laboratory tests are limited and more sensitive organisms make better toxicity test subjects. It is neglected that the great majority species are somewhere between sensitive and tolerant ranges.

Microscale PFU community-level tests might overcome these weaknesses. Each PFU has from 20 to 100 protozoan species. These communities are obtained from nature that has not been exposed to the test chemical. When the loss in taxonomic richness of these communities is plotted over increasing chemical stress, the concentration-response curve reflects a cumulative tolerance distribution. There are some examples.

Example 1: In 1981-2 Prof. John Cairns and I carried out a study on use of protozoan communities to predict environmental effects of pollutants. The study site was Cedar Run, a stream that originates from springs in the town of Blacksburg, Virginia, U.S.A. (fig. 1 in Shen et al.'s paper 1986). Two effluents are discharged into the stream within 3 m of each other. One discharge was an effluent of a sewage treatment plant, and the other was an effluent contained heavy metals from an electroplating plant. Station 1 was a reference station, and stations 9,10 were recovery stations. The chief result is that the number of protozoan species (Y) after three days of colonization was strongly related to total metal concentration (X) for both field and laboratory tests. The formula is: $\mathbf{Y = 50.08 + (-17.06)\log X\ (r = 0.73,\ p < 0.005,\ n = 10)\ (in\ field)}$

$$\mathbf{Y = 56.08 + (-22.45)\log X (r = 0.73,\ p < 0.005,\ n = 10)\ (in\ lab)}$$

Estimates of effect concentrations (EC) for heavy metals were made by inverse prediction from the two linear regressions. (fig 7 in Buikeman's manuscript)

Prediction of Effect Concentrations (EC) for Heavy metals

	EC5 (μg / L)	EC20 (μg / L)
From Lab Test	**18**	**48**
From field	**31**	**51**

This studies has demonstrated that the microscale PFU toxicity test using indigenous protozoan communities can provide accurate predictions of toxic effects in specific receiving systems.

Example 2 Toxicity test of surfactant DBS (Dodecyl Benzene Sulfonate) were carried out at three biological organization levels: species, population and community. Five protozoan species: *Paramecium caudatum, Tetrahymena americanis, T. pigmentosa, T. borealis* 31V WWO, *T. borealis* 311 VM665, and *T. tropicalis* DIV TC89 were selected for acute toxicity test 12h-LC50 at individual level. The species *Tetrahymena americanis* was selected for acute toxicity test 12h-LC50 at population level. The protozoan community collected by the PFUs immersed in center of Donghu Lake for 28 days was used for the flow-through diluted microcosm test at community level. The chief results are as following:

Comparison of the toxic effects of surfactant DBS on Protozoa at different levels (ppm)

	at species level*		at population level*		at community level **	
	LC50	**MATC**	**LC50**	**MATC**	**EC20**	**EC5**
AF 0.1	**7.0**	**0.7**	**17.21**	**1.721**		
AF 0.01	**7.0**	**0.07**	**17.21**	**0.1721**		
					0.51	**0.1681**
China Standard for Drinking Water					**0.3**	
WHO International Standard for Drinking Water					**0.2**	

*** calculated by formula: MATC = LC50 / AF**

**** calculated by the regression formula Y = 29.7281 - 5.6722 X, r=0.9557, p<0.05**

It is nothing to do with species level. At population level, if we selected AF0.01, the MATC closed to the standard. But if we selected AF 0.1, the MATC is far to the standard. According to the community toxicity test, we predicated that to the Donghu Lake, the MATC must be adjusted below 0.5 ppm, below 0.17 even better.

Example 3: The MATC of $CuSO_4$ (copper sulfate) and rare earth fertilizer was estimated by static microcosm toxicity test. China has abundant rare earth ores. Since 1970s rare earth have been widely used in agriculture as fertilizers. As a result the yield of many kinds of crops has been increased. The rare earth fertilizer named "Nongle" contained 38% rare earth elements, including CeO_2 49-51%, La_2O_3 25-30%, Nd_2O_3 15-17%, Pr_6O_{11} 5-6%, Sm_2O_3 < 0.03%. (La - lanthanum, Pr - praseodymium, Sm - samarium, Ce - cerium, Nd - neodymium)

Estimating the Effect Concentration of $CuSO_4$ and Rare Earth Fertilizer at Community level

Chemicals	**Regression formula of Concentration-Response Curve**	**EC5(ppm)**	**EC20 (ppm)**
$CuSO_4$	**$Y = 35.9615\, e^{-2X}+5$, r=0.99**	**0.056**	**0.234**
China Standard for Surface Water		**0.01 - 1.0**	

Rare Earth		EC5(ppm)	EC20(ppm)
Fertilizer	$Y = 9.08 - 0.32\ X,\ p < 0.05$	3.042	12.594

No China Standard for Surface Water

These four examples demonstrated that PFU microcosm toxicity test could be used to predict effects in the field more accurately than single species toxicity tests.

4. **The protozoan species composition in PFU microscale communitites was present in orderliness, not in random**

People want to ask whether species of freshwater microscale communities are randomly aggregated by chance or whether they have a structure that is mathematically describable and can be predicted before one examines the community.

Sladeck (1973) and Minoru Sudzuki (1991) gave a protozoan list with saprobic index of each species. According to the Kolkwitz-Marrson (1909), the saprobic system of water quality was divided into 5 groups: clean, oligosaprobic, α- mesosaprobic, β-mesosaprobic, and polysaprobic. Sladeck suggested that the saprobic valeny of the 5 groups for each species is 0, 1, 2, 3, and 4 respectively. Based on the distribution recorded on literature or author's experience, the saprobic valency of each species is varied. For example, *Loxodes rostrum* appeared only in β-mesosaprobic, and α-mesosaprobic water. Based on the frequency of this species, the total saprobic valency 10 is diveded into 4 (β-mesosaprobic) and 6 (α-mesosaprobic). The frequency is highest in the α-mesosaprobic, the saprobic index = 3 (the saprobic valency of α- mesosaprobic) – 0.2 (10% of the β-mesosaprobic) = 2.8. A list of saprobic index in 668 protozoan species was showed (Sladeck and Sudzuki, 1991). However, these indices were not directly based on physical and chemical characteristics, although some indices had a significant correlation with chemical characteristics such as BOD (Chutter, 1972; Sladecek and Tucek, 1975; Hilsenhoff, 1977). We developed a method to create correlation between biological data and chemical parameters. The species pollution value (SPV)for each species was calculated as geometric means of the comprehensive chemical pollution index Pb for each sampling station where the species was present and then dividing by the number of stations.

$$SPV = \frac{\sum_{i=1}^{n} (\ln Pb)\, i}{n_{st.}}$$

The PFU community pollution value (CPV) for each sampling station was calculated as the sum of the pollution values of all species present are divided by the number of species.

$$CPV = \frac{\sum_{i=1}^{n} SPV\, i}{n_{sp}}$$

The IDRC project included two stages: (1) physico-chemical and biological (bacteria, microbial, and macroinvertebrate) monitoring at 42 sampling stations of the Hangjiang River and (2) studies of restoration processes of polluted areas in the Hangjiang River. SPV and CPV of these two stages were calculated. The relationships between CPV and Pb for the two stages were:

for the Ist stage:

$CPV = 1.5 + 0.200\ \ln Pb,\quad r^2 = 0.812,\quad n=42,\quad p<0.00001$

for the 2nd stage:

$CPV = 2.8 + 0.267\ \ln Pb$, $r^2 = 0.978$, $n = 12$, $p<0.00001$

To test the applicability of this method, the CPV of the 2nd stage was calculated based on the SPV of the Ist stage, and the correlation cofficient is $r^2 = 0.858$, n=12. Similarly, the CPV of the Ist stage was calculated based on the SPV of the 2nd stage, and the correlation cofficient is $r^2 = 0.376$, $n = 42$.

Since the SPV of the species identified in both stages were not the same, we combined all 486 protozoan species of the two stages and the lnPb of all sampling stations. The relationship between the comprehensive pollution index Pb and the CPV of all the sampling stations was

$CPV = 2.03231 + 0.200429\ \ln Pb$, $r^2 = 0.847$, $n = 54$, $p < 0.00001$.

There is no doubt that the higher the CPV, the higher the degree of water pollution. Based on the CPV, the classification of water pollution degrees was proposed.

CPV	Pollution status of water
<2.45	Unpolluted or clean water generally suitable for drinking
2.45 – 2.85	Slightly polluted water.
2.85 – 3.15	Moderately polluted water.
3.15 – 3.45	Heavily polluted water.
>3.45	Severely polluted water

The list of protozoan SPV and the range of CPV had been checked for the other water body, such as water system of Changde City in Wunan Province, Donghu Lake of Wuhan City in Hubei Province and in the Torrent Stirone River of Italy (Madoni and Ghetti, 1981). Madoni and Ghetti found that the water quality in different station of the River showed a series of saprobic system from Oligo- to polysaprobic by using chemical monitoring method, but the water quality of the River concentrated mostly on α- mesosaprobic by using the Sladecek's saprobic index. In this paper the protozoan species limited only ciliates and the samples were collected by planktonic net , not the PFU method. We calculated CPV of ciliates with our list of SPV, even the spots look scattered, but the correlation is still good. The p-test is less than 0.03.

Ecotoxicology is the study of the adverse effects of materials on ecological systems. Any methods, including standard methods, still need to be improved. Ecological toxicity testing will be needed more in the future than it is today.

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