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Published version:

DOI:10.1016/j.ecolind.2013.01.024

APPLICATION OF EUROPEAN BIOMONITORING TECHNIQUES IN CHINA: ARE THEY A USEFUL TOOL?

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ABSTRACT

This paper focuses on the application of various biomonitoring techniques in China. We report a study in the Pearl River Basin (Guangzhou) based on the application of diatom indices as well as a study on the waterways in Wuhan based on evaluation of toxicity (using phytotoxicity, *Daphnia magna* and Microtox^{TM} tests) and the Extended Biotic Index (EBI). Regarding the diatom indices, acceptable results were obtained based on comparison of the chemical water quality level and the European and Japanese indices, despite a lack of taxonomic information. The toxicity tests applied to the Wuhan waterways (Yangtze and Han Rivers) produced interesting results and can be considered to represent a useful tool for water pollution control in this area. Application of the EBI in Wuhan produced results that were contradictory to the toxicological analyses, as there were no indications of toxicity, whereas EBI indicated poor water quality. It can be concluded that in principle, certain European biological indicators can be considered to represent feasible tools to be applied in China. However, further studies will have to be carried out to develop bioindices based on Chinese data sets. The use of bioindices based on macroinvertebrates is limited to less polluted and smaller rivers with a lithic river substratum, whereas diatom indices are also applicable under extreme conditions (e.g., under high pollution loads or in large river streams with sandy riverbed sediments through installing artificial substrates).

Keywords: Pollution; Biomonitoring; Diatom indices; Toxicity; Extended Biotic Index; China.

1. INTRODUCTION

In the last twenty years, China has experienced great industrial and economic development alongside increased pollution in all environmental matrices (air, soil and water). Due to the limited freshwater supply in the world, protecting the integrity of water resources has become one of the most important environmental challenges for the 21st century, including for China (Daughton and Ternes, 1999). The Chinese legislation (GB3838, 2002) provides Environmental Quality Standards for the classification of surface water into 5 categories. These standards are limited to the monitoring of chemical and microbiological parameters. Chemical analysis of the environmental matrix is the most direct approach to reveal the pollution status of the environment. However, the integrated influence and possible toxicity of pollution in organisms and the ecosystem cannot be assessed through chemical analysis (Fernandez et al., 2005). Due to the consistency between selected organisms and their corresponding living space, biomonitoring can directly produce data on the potential effects and actual integrated toxicities of pollutants, reflecting the corresponding degree of deleterious effects in the environment. The ecological relevance and ideal attributes of biological indicators for water quality assessment have been review extensively (Hernando et al., 2005; Zhou et al., 2008). In order to prevent sanitary hazards related to the use of recipient water bodies one of the objectives of the European Community͂s environmental regulations is to reduce the pollution of surface water caused by municipal waste. The European Water Framework Directive (WFD 2000/60, EU, 2000) reminds us of the importance of the multidisciplinary approach based on the analysis of biological indicators combined with the traditional chemical and physical parameters in the evaluation of surface water status (Birk et al., 2012). Although water quality monitoring is usually limited to chemical parameters in China, many biological indicators used elsewhere are likely to be applied for the evaluation of environmental pollution (e.g. use of diatom indices in Vietnam by Duong et al., 2006, 2007; use of fish-based indexes in China by Jia and Chen, 2013; use of biotic indices based on benthic macroinvertebrates in Nepal by Shah and Shah, 2012), or toxicity tests based on standard organisms. The environmental characteristics of the river basins in China are clearly different from European aquatic environmental conditions, but the current biomonitoring approach in European standards is based on "key" species with various tolerances to environmental alterations, allowing the calculation of indices reflecting water quality. Therefore, the question arises of whether European biomonitoring tests and indices can be applied in China. We attempt to provide a response to this question in this paper. We applied different biomonitoring tests (Microtox™, *Daphnia magna*, phytotoxicity) and techniques (Diatom indices and the Extended Biotic index) in two river basins: the Pearl River Basin (Liuxi, Suijiang and Pearl ï main branch- Rivers) and the Yangtze River Basin (Yangtze and Han Rivers).

2. MATERIALS AND METHODS

2.1. Sampling sites.

The two river basins where biomonitoring tools were applied are located in Southern (Pearl River basin) and Southwestern (Yangtze and Han Rivers) Part of China, as described in Figure 1.

2.1.1. The Pearl River basin (sections from Guangzhou/Canton ï Guangdong Province).

The Zhu Jiang, or Pearl River, is Chinaôs third longest river and second largest by volume. The area has a semi-tropical climate (air temperature 14-22°C and 1,200-2,200 mm precipitation) (Changming, 2001). The Pearl River Basin extends over the southern Chinese provinces and northeast part of Vietnam (catchment area 453,690 km²). The Pearl River Delta is a highly industrialised area where water pollution and salt intrusion has become increasingly serious since the last decade (PRWRC, 2001). The study area was located in Guangdong Province, in part of the Pearl River Delta (fig. 1). The Monitoring Study was subdivided into three pilot sites, on three tributary rivers (pilot sites) of the Pearl River in Guangdong Province: the Liuxi He River, the Suijiang River and the Pearl River main branch (Xijiang) in Guangzhou city (Canton). Each of these sites could be characterised by a different type of pressure: Liuxi He River ï low pressure, drinking water protection zone; Suijiang River ï medium pressure, agriculture and small scale handcraft industries; Guangzhou River section ï high pressure, large scale industries and urban pollution. Monitoring stations (in total 27) were positioned at crucial locations within the river systems: at natural locations where human activities were absent, at junctions of tributary rivers, up and down stream of urban areas, close to point pollution sources and at existing hydrological and chemical monitoring stations part of the monitoring network in place of the local water authority.

Water and biological samplings in the Pearl River Basin were carried out by Asconit Consultants, Cemagref (now Irstea) and the local water authority, the Pearl River Water Resource Commission (PRWRC), in May 2007.

- In the Liuxi He River, located in the Conghua district of Guangdong Province, eleven monitoring stations (L1-L11) were selected along a 70 km trajectory. This river is defined as a protected area by local authorities, in which heavy industry is prohibited. Surface water resources are used for drinking water production for Guangzhou city.

- The Suijiang River is located in Huaiji and Guangning county of Zhaoqing city in Guangdong Province and is affected by diffuse pollution (agriculture and small scale handicraft industries). A total of 10 monitoring stations (S1-S10) were situated along a 50 km river stretch. In this pilot site chemical and hydrological monitoring was already in place (at stations S1, S4, S5, S7, S9, S10) and biological and chemical samples were taken at these stations. Additional stations (S2, S3, S6, S8) were introduced, were only biological samples could be taken, but no chemical samples, due to budget restrictions.

- A section of the Pearl River located in Guangzhou City was chosen to evaluate the water quality in a dense urban area with inefficient waste water treatment and very poor water quality. Six monitoring stations (G0-G5) were situated along a 10 km river section. All of them were influenced by sea tides (between 0.5 – 1.0 m). The station (G1) was introduced as an additional station, and as it was not part of the chemical and hydrological monitoring in place (stations G0, G2, G3, G4, G5) only biological samples could be taken, but no chemical samples, due to budget restrictions.

2.1.2. Yangtze and Han Rivers (sections from Wuhan - Hubei Province).

The Yangtze River enters Wuhan city from Liaojiabao in the Hannan District, in the southwestern part of Wuhan. The river flows for 145.5 km in Wuhan and has a width ranging between 1,000-2,000 m. The average annual flow entering the city is approximately 6.49 x 10^{11} m^3.

The Han River enters the city from the Caidian District in the western part of Wuhan and merges with the Yangtze at Longwangmiao. Its length in Wuhan is 62 km, and it has an average width of 300 m. The average annual flow entering the city is approximately 5.54 x 10^{10} m^3.

The characteristics of water resources in Wuhan are that there is limited local water production, a large quantity of inflowing water, an uneven distribution of water resources and frequent floods and droughts. River water can be abstracted for various usages (e.g., supplying drinking and industrial water). However, heavy rainfalls cause serious flooding every year. In 2007, the Yangtze and Han rivers reached Grade III based on China's Environmental Quality Standards for Surface Water (GB 3838, 2002). In January and June of 2008, two sampling campaigns were carried out. A total of 5 monitoring stations were selected along each river (Yangtze: Y1 to Y5, and Han: H1 to H5, Fig. 1), for collection of water and sediment samples. To achieve the appropriate application of biomonitoring and its promotion in Wuhan, the sampling points were selected in full consideration of the following issues: sewage outflows, major industrial enterprises, major human activities, the water source of major water plants and prerequisites for biomonitoring along both rivers.

2.2. Physicochemical analyses

At each monitoring station where biological samples were collected, measurements of pH, temperature, conductivity (Hydrolab Data Sonde) and dissolved oxygen (iodimetry method) were performed simultaneously.

In the Pearl River basin, water samples were collected at the 22 stations monitored by the PRWRC for further chemical analyses in April and May 2007. At the 5 stations that were not part of the annual monitoring programme of the PRWRC (i.e. Suijiang: S2, S3, S6, S8, and Guangzhou: G1), no further chemical analysis was carried out.

Water samples were analysed for suspended solids (filtration), Chemical Oxygen Demand (COD) (dichromate method) and Biochemical Oxygen Demand (BOD_5) (dilution and seeding method), chloride (ion chromatography method), ammonium (spectrophotometric method), nitrate (ion chromatography method), total phosphorus (spectrophotometric method), silicon (colorimetric method), sodium and heavy metals (lead, zinc, mercury, hexavalent chromium Cr^{6+}, cadmium, nickel) (atomic absorption spectrophotometric method).

In the Yangtze and Han Rivers, water samples were collected for measurements of BOD_5 (dilution and seeding method), the permanganate index (titrimetric method), COD (dichromate method), chloride, sulphate, nitrite, nitrate, phosphate (ion chromatography method), mineral oils (infrared photometric method) and trihalomethanes (headspace gas chromatography method). Sediments were also sampled for the determination of heavy metals (atomic absorption spectrophotometric method), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) (gas chromatography method and high-efficient liquid chromatography method).

All the parameters were analysed as reported in the Chinese reference methods (GB3838-2002).

The chemical monitoring results were then attributed to five water quality classes following the categories of the Chinese environmental quality standard for surface water (GB3838, 2002) (Tab. 1).

2.3. Biological analyses and indices

The choice of the biological analyses performed depended on the local environmental conditions. In the Pearl River basin, diatoms were ultimately considered to be the most appropriate bioindicator, as the application of standardised methods was possible for all river types (shallow/deep rivers; good/poor water quality) and in line with previous positive results obtained from the diatoms studies in Vietnam (Coste and Pateron, 2004; Duong et al., 2006, 2007). In the Yangtze and Han Rivers, we used bioindicators complementary and simple to apply: *Vibrio fischeri*, *Daphnia magna* and the Extended Biotic Index.

2.3.1. Diatoms

Diatoms were sampled in the 27 sites of the Pearl River basin, following the European standard NF EN 13946 (2003). At each monitoring station, five to ten stones were selected at a bright lotic location in the middle of the river transect. If natural inert substrates were absent, diatom samples were taken from other types of hard substrates, concrete bridge pylons or paved river banks, or from artificial substrates which were immersed *in situ* 3 weeks prior to sample collection. Diatom samples were scraped from the substrates and preserved with a formaldehyde solution at pH 7 (final concentration between 1 and 4%).

The physical parameters pH, conductivity, oxygen and temperature were measured on site, and chemical samples were collected simultaneously.

Diatom microscope slides were then prepared according to the European standard NF EN 13946 (2003); in brief, organic matter is removed from the sample with hydrogen peroxide, and the sample rinsed with distilled water, to allow the microscopic observation of the siliceous cell walls of diatoms. Finally the clean samples were mounted in a high refractive index medium (Naphrax ®, RI =1.74).

Diatom counts and identification followed the European standard NF EN 14407 (2004). Diatom counting was performed by scanning successive fields using a light microscope (Leica DMRB photomicroscope, Wetzlar, Germany) at a X1000 magnification. According to this standard, all valves must be taken into account, including broken valves (when more than 2/3 of the valves are present, to avoid double counting of individuals) if they can be identified. Identifications were made at the species level using European floras (Krammer and Lange-Bertalot, 1986-1991) and monographs (Diatoms of Europe series), or older ones dealing with Asian microflora (Hustedt, 1937; Houk, 1992; Qi, 1995; Kobayasi, 1997). After counting at least 400 valves, the results were expressed based on the relative abundance of each taxon.

Diatom indices were calculated from the results of the diatom counts, using Omnidia software (Lecointe et al., 1993 and 1999). Three diatom indices were studied: the two French indices IPS (Indice de Polluosensibilité, or Specific Pollusensitivity Index; Coste *in* Cemagref, 1982) and IBD (Indice Biologique Diatomées, or Biological Diatom Index; Coste et al., 2009) and one Japanese diatom index: the Watanabe Index (WAT; Watanabe et al., 1988). IBD and IPS were selected because of their routine use for river biomonitoring purposes in Europe (Kelly et al., 2009). The WAT index was expected to be valuable for organic pollution assessment in this geographical area, and likely to account for a higher number of species and/or for more adequate ecological profiles of taxa.

The index values range from 1 (very poor quality) to 20 (very good quality) and allow biological assessment of water quality to be performed.

2.3.2. Macroinvertebrates

The modifications of macroinvertebrates communities living on river ecosystems substrata were analysed, for calculation of the Extended Biotic Index (EBI). This index is used to assess changes caused by pollution elements and environmental alterations, with various sensitivity to change of environmental conditions; the abundance of the macroinvertebrates taxa and their specific sensitivity to pollution is used to determine the ecological quality of aquatic ecosystems by converting EBI values into “quality classes”. The EBI was determined for 7 samples collected from river banks and 10 sediment samples during the pilot study conducted in January 2008. The macroinvertebrates were sampled by a hand rectangular net, preserved in 95% ethanol before identification and EBI calculations, conducted as recommended by APAT (2003).

2.4. Toxicity tests

2.4.1. Phytotoxicity test

The phytotoxicity test measures the decrease (or the absence) of seed germination and of the growth of young roots after a few days of exposure of seeds of selected higher plants to toxicants or to contaminated water, sediment or soils in comparison to the test controls (EPA, 1996). It was conducted with seeds of the dicotyl garden cress *Lepidium sativum* (Phytotox kit seeds TB62, MicroBioTests, Nazareth, Belgium) and was applied to all of the water and sediment samples from the two rivers. Seeds germination and root elongation were measured, and the Germination Index (GI) was calculated using the following formula:

GI = (mean N. of germinated seeds in the sample x mean length of the germinated seeds in the sample) / (mean N. of germinated seeds in the control x mean length of the germinated seeds in the control)

2.4.2. *Daphnia magna* test

The *D. magna* test (OECD, 2004) was applied to the water samples and the aqueous extracts of the sediment samples. It is based on evaluation of the immobilisation of the test organisms in the presence of stress sources compared to a control. Dormant crustacean eggs and a stock solution for preparation of standard freshwater medium were obtained from the commercial test system DaphToxkit FÊ magna (MicroBioTests, Nazareth, Belgium).

2.4.3. MicrotoxÊ test

This test was applied on the water sample and the aqueous extracts of the sediment samples following the procedure described in the MicrotoxÊ manual (1995). The principle of this system is based on evaluation of the decrease of the luminous energy naturally emitted by *Vibrio fischeri* bacteria (Azur Environmental, Carlsbad, CA, USA). Luminescence was measured at time zero and after 5, 15 and 30 minutes and compared to the control. The EC50 values were subsequently converted in toxic units (TU):

$$TU = (1/EC50) \times 100$$

TUs are directly proportional to the toxicity of samples. Each test was analysed using a MicrotoxÊ reference toxicant (phenol) for quality control.

2.5. Processing the data

Co-inertia analysis (Doledec and Chessel, 1994) was used to analyse both the results of the diatom (species abundances) and chemical analyses using R software (Ihaka et al.,

1996) coupled with ADE Software (Thioulouse et al., 1997). This type of analysis provides more efficient correlations than canonical correspondence analysis, according to Ter Braak (1986a and 1986b).

The EBI values for the different sampling stations were assigned using a two-way flow chart in which the vertical line corresponds to the total number of taxa, while the horizontal lines correspond to the presence of taxa ranging from sensitive to resistant organisms (Woodiwiss, 1978; Persone and De Pauw, 1979).

The values obtained from *Daphnia magna* tests were used to calculate EC50, EC20 and EC10 (OECD, 2004; Cao et al., 2009; Pignata et al., 2012) using Probit regression. For all toxicity tests, statistical analyses (Spearman's test, Probit regression analysis and *T*-test) were performed with the statistical package SPSS 17.0 (SPSS for Windows, Chicago, IL, USA) in order to identify the possible interactions between the different parameters taken into account.

3 RESULTS AND DISCUSSION

3.1. Environmental conditions

3.1.1. Water quality

The results of the chemical analyses of the water samples are reported in Table 1.

The chemical water quality, based on the Chinese chemical classification (GB 3838, 2002) in the Suijiang River indicated a good water quality class for all stations where chemical analyses were carried out (S1, S4, S5, S7, S9 and S10). The monitoring stations in the Guangzhou urban area were characterised as "worse than class V", corresponding to the "very poor water quality class". Anthropogenic impacts were marked, as also observed, to a lower extent, for the sampling stations along the Liuxi River where water quality ranged from good to bad due to the concentration of COD and BOD_5.

In the Yangtze and Han Rivers, all of the stations were classified in Category I, II or III, considering the parameter ñMineral oilsò as equivalent to ñPetroleum oilò. Regarding the concentrations of trihalomethanes, only chloroform was above the detection limit, and it was under the standard limits established in Chinese legislation (chloroform limit = 0.06 mg/L). The measured value of trihalomethane increased in June compared with the first monitoring results, but its level was still within the normal range and did not affect the overall water quality adversely.

Additionally, the measured values of BOD_5 and of dissolved oxygen decreased in June compared with the results of the first pilot study, but this was due to the seasonal period (summer), during which there was higher algal growth than in winter. Most of the other parameters showed no significant change compared with the results of the first pilot study. The results obtained are below the values specified by the Environmental Quality Standards established by European Directive 2000/60/EC.

3.1.2. Sediments

The results of the chemical analyses of the sediment samples are reported in Table 2. If we consider the ANPA (2000) Italian standard and the Canadian standards (CCME, 2002) for freshwater sediment, it can be observed that the concentrations of lead, zinc and cadmium were below the limits of these standards, but the chromium concentrations were two or three fold higher than these standards. Moreover, the mean copper concentration was more than twofold higher in the Han river than in the Yangtze river, and the mean chromium concentration was higher in the Yangtze river sediment in winter. In June, only the mean lead concentration was higher in the sediment samples from the Han river. These differences could be due to the various human activities along the two rivers and to the natural background. In 2005 Wang et al. (2011) investigated the concentrations of heavy metals in 13 surface sediment samples collected in July and another 23 collected in

December from the Yangtze river catchment of Wuhan. They reported 0.98 Cd, 108.00 Cr, 60.03 Cu, 49.19 Pb and 230.39 Zn mg/kg. These results generally were higher than present results but probably the methods used were different (method established by the laboratory vs reference method GB 3838, 2002). The concentrations of PCBs and OCPs were under the detection limits at all of the sampling points. The only PAH detected in January was fluorene, and its concentration was the same at the six positive sampling points. In June, crysene and the benzo(a)pyrene were found at one sampling point (Y2) on the Yangtze river and one sampling point on the Han river (H4). The most polluted site seems to be Y2. In a study addressing the distribution of the PAHs in the Wuhan section of the Yangtze river, Feng et al. (2007) found that the PAHs concentration ranged from 72 to 1206 µg/kg in sediment samples during the low water season, and three, four and five ring PAHs were predominant. Therefore, the concentrations found in the two pilot studies were similar to the concentrations reported in the scientific literature (Feng et al., 2007).

3.2. Diatom Results

3.2.1. Factors driving diatom community structure

More than 400 diatom taxa were identified from the 27 sampling stations. The most abundant species are listed in Supplementary material. Few endemic species and some non-identified species were present as well as abundant tropical species, and cosmopolitan taxa were dominant in polluted areas, as observed in other Chinese basins (Tang et al., 2002, 2004; Wu et al., 2007, 2010). In particular, the following taxa were found:

- *Discostella asterocostata* (Lin, Xie & Cai) Houk et Klee and *Encyonopsis leei* Krammer.
- Numerous tropical or subtropical taxa: *Diadesmis confervacea* Kützing, *Cymbella japonica* Reichelt in Kuntze, *C. tropica* Krammer, *Hydrosera whampoensis* (Schwarz)

Deby, *Achnanthidium convergens* (Kobayasi) Kobayasi and *A. crassum* (Hustedt) Potapova & Ponader, among others.

- Abundant non-identified forms: *Achnanthidium, Caloneis, Cymbella-Encyonema, Gomphonema, Neidium, Nupela* and *Placoneis* species.
- Brackish ï halophilic taxa: *Luticola, Bacillaria* and *Nitzschia clausii* Hantzsch, which increased in downstream sections of the river due to the influence of salty tides and higher pollution loads in urban areas (especially the Guangzhou Pearl River sections).

Co-inertia analysis of the biological and chemical data discriminated three groups, corresponding to the rivers of origin (Fig. 2): the Guangzhou River monitoring stations were characterised by most of the pollution variables (BOD_5, Ptot, NH_4,COD) and salinity parameters (conductivity (Cond), chlorides (Cl) and sodium (Na)) along the negative part of the F1 axis, while along the positive values were grouped the sampling stations from the Liuxi River (bottom right panel) vs. from the Suijiang River (top right panel), where diatom microflora was mainly composed by fresh to slightly brackish water taxa.

The Suijiang River stations (S1 to S10) were mainly distributed along the F2 axis and were correlated with higher pH values, silica content and chromium. These sites are characterised by a group of diatom species known to tolerate to metal pollution (Gold et al., 2002; Morin et al., 2012), such as *Gomphonema lagenula* Kützing (GLGN), *Sellaphora pupula* (Kützing) Mereschkowksy (SPUP), *Gyrosigma obtusatum* (Sullivan & Wormley) Boyer (GYOB), *Luticola mutica* (Kützing) D.G. Mann (LMUT) and *Surirella* species (SURS), probably selected by chromium contamination.

Sampling stations in the lower courses of the rivers (L8, L9, S8, G4, G5) were correlated with warmer temperature conditions and higher nitrate concentrations (NO_3), as usually observed in downstream stretches.

In the Liuxi and Suijiang Rivers, there was a frequent presence of abnormal forms (up to 1.8%), which is generally linked to high metal pollution (Morin et al., 2012). With the exception of lead (Pb) and chromium (Cr^{6+}) concentrations which were marked at L8- L9, and S2-S6-S7, respectively, most of the investigated heavy metals were not significantly distributed in the three rivers but associated with organic pollution (L9 to L11, G2 and S8). The highest conductivity observed on the Guangzhou River was associated with halophilous diatom assemblages, including *Luticola peguana* (Grunow in Cl. & Moeller) D.G. Mann (LPEG), *L. mutica*, *L. mitigata* (Hustedt) D.G. Mann (LMIT) and *Luticola* sp. (LUTS) and *Actinocyclus normanii* (Gregory ex Greville) Hustedt (ANMN).

3.2.2. Diatom indices vs. water quality and potentialities for biomonitoring applications in the Pearl River basin

The total percentage of taxa taken into account for calculation of IPS was greater than 80%, though it was lower for IBD (average 31%) and WAT (average 29%). The total abundance of the taxa included in the calculations was highest for IPS (99%), followed by IBD (79%) and WAT (39%) and was slightly higher in polluted areas, where cosmopolitan species were often dominant.

Diatom index values (Fig. 3) were generally in concordance with water analysis results, with classes matching with the chemical assessment (similar or adjacent class) in more than 85% of the cases, whatever the index used.

IBD and IPS values from the Liuxi River classified the 6 upstream stations (low pollution) into “good” or “very good” quality classes: L1, L2, L3, L4, L6 and L7, whereas the WAT index would attribute these stations (except for L3) to the “medium” water quality class. . The indices indicated a “medium”, “poor” or “very poor” water quality at L8, L9, L10 and L11; at L8, L10 and L11, there were a high proportion of saprobic taxa (80 to 90%) related to higher organic loads.

The majority of the monitoring stations in Suijiang River indicated a medium (S1, S2, S4, S5, S6, S9 and S10) to good (S3 and S7) biological quality, whereas S8 exhibited poor quality. The diatom communities were characterised by eutrophic taxa, reflecting nutrient-rich environments. Very eutrophic taxa were especially abundant at S8, indicating a very high mineral load. Oligotrophic diatoms were rare, only being found at S2, S3 an S6, confirming a better trophic level.

For the Pearl River main branch in Guangzhou city, all monitoring stations exhibited a poor or very poor water quality class based on application of IPS, IBD and WAT.

However, some limitations can be highlighted from the use of these diatom indices in the Pearl River basin. First, diatom indices were compared to water quality based on punctual chemical analyses. Diatoms are integrative in time of the past conditions. Further investigations would thus be necessary to verify whether uncontrolled pollution discharge appears occasionally, impacting diatom flora, in the sites where chemical status was overestimated, compared to diatom indices values (e.g discordant assessment between chemistry and indices at L7). Second, the indices tested (IBD, IPS and WAT) should be used exclusively for flowing water. For example, the diatom composition in L4, located at a reservoir, was reflected by the presence of planktonic diatom species: for this type of environments we recommend applying bioindicators developed for lake/reservoir monitoring instead. Last, in the sites influenced by sea tides in the Pearl River section in Guangzhou, brackish water species were identified, and the methods used were probably not valid, as they were developed for freshwater conditions.

3.3. Macroivertebrates and EBI evaluation

The sampling points on the Han and Yangtze rivers were defined by YVWEMC (Yangtze Valley Water Environmental Monitoring Center) according to the river environments and human activities, but there were some difficulties during the sampling: the banks were artificial and had no natural vegetation. The substrate features were silt and sand. There was a wide area containing decomposing organic matter, especially algae, this phenomena was observed in 3 of 5 sampling points on the Yangtze river. At some sampling points, we observed refuse such as domestic waste residuals in the water, plastic boxes, shoes and cloths. There were several signs of anaerobiosis. The results are shown in Table 3.

The sampled macroinvertebrates were all very tolerant organisms and belonged to the Mollusca phylum. This was also discussed in an ecological study addressing macrobenthic fauna in East China, in which the most prevalent sampled organisms were Mollusca, representing 34% of the total species present (Liu and Li, 2002). Organisms of the Gastropoda and Bivalva classes were present in some samples from the two rivers. Samples were composed of the *Lymnaeidae* family; the lone *Pulmonate* in the form of a dextrose snail was found in the Y2 sample collected from the bank; and some *Bithyniidae* (*Prosobranchia*) family organisms were observed in the Y2 sediment sample. Sampling point Y2 was the first site after the entry of the Han river into the Yangtze river. We found several of organisms from the *Unionidae* family and few organisms from the *Sphaeriidae* family and *Pisidiidae* family in the Y3, H2, H3 and H4 samples collected from the bank and the Y2 sediment sample. Values of 0 to 3 are considered to be indicative of low tolerance to stress, values of 4 to 6 moderate tolerance and values of 7 to 10 high tolerance (EPA, 1990; APAT, 2003).

Considering the results obtained with respect to macroinvertebrates, a less than positive picture of the water quality and river environment can be observed, especially for the Han river, where we found only bivalves near banks. The value of the EBI is zero using a two-entry table. Therefore, it is attributed to the fifth (worst) water quality level class. When the chemical and EBI results were compared, we observed good chemical water quality and a poor EBI classification. There was a study conducted in the late 1990s on the Yangtze river in Jiangxi Province, approximately 300 km from the Poyang Lake. The authors found species belonging to the Anellida, some Gammaridae, and few Mollusca at five sampling stations on the river. The environmental condition of the river section examined in this study (Wang et al., 2007) is very different from that of the Wuhan area. Specifically, the velocity was very high in the Yangtze Wuhan section during the pilot study, and the seasonal conditions (very cold and frequent snowfall) could limit the presence of macroinvertebrates. The results obtained from application of the EBI in the Wuhan segment of the Yangtze river and the Han river were completely different from the results obtained from the other toxicological analyses. Therefore, we can conclude that EBI is not suitable for evaluation of the environmental condition of these two rivers in the Wuhan section, but it could be used for other parts of the two rivers outside of industrial or anthropic sites.

Further studies concerning the basic ecology of flowing waters in Asia are needed, but the application of EBI in China is hampered by a number of factors, including the lack of knowledge about macroinvertebrate fauna and their tolerance values, especially during their aquatic, immature stages; the scarcity of research programs and formal training opportunities for biomonitoring offered in universities; the shortage of high-quality microscopes and other necessary equipment; and limited government understanding of and support for biomonitoring, the existence of few skilled regulatory staff, and the persistence of old and unusable biomonitoring protocols, as reported by Morse et al. (2007).

3.4. Toxicity tests

3.4.1 Phytotoxicity test

In January, the Germination Index (GI) of the water and sediment samples was higher than the GI of the control, and thus, growth stimulation was observed, rather than a toxic effect due to the nutrients present in the water samples. In June, the water samples of the two rivers and the sediment samples of the Han river did not exhibit toxic effects. The GI of the sediment samples from the Yangtze river was indicative of low toxicity (Fig. 4). This low toxicity could be due to the seasonal conditions of the rivers because in June, the water level is higher than in January, and the water flow velocity is lower, so suspended particles can sediment more easily. Likely for the same reason, there was a significant difference (*t* test, $p < 0.05$) between the mean GI in January and in June.

3.4.2 *Daphnia magna* test

The results of this test are shown in Table 4. In January, three water samples from the Han river (H1W, H2W, H5W) and one water sample from the Yangtze river (Y4W) presented toxic effects, and these effects increased with exposure time, so they could be due to heavy metals or other substances that slowly cause damage to living organisms (Lithner et al., 2012). Moreover, the surface water of the Han river exhibited greater toxic effects on crustaceans, most likely because the human activities along the river, such as the sand quarries, domestic and industrial wastewater and high rate of goods trafficking, had a greater impact than those on the Yangtze river due to the different quantities of water and different dilution rates. In June, none of the samples showed any toxic effects in this test. Comparing the results of the two pilot studies in Wuhan, we found a very low toxicity with the *Daphnia magna* test, and this toxicity was higher during the first pilot study.

3.4.3 Microtox™ test

The Microtox™ test was applied to all of the water samples and five sediment samples (Y1Sed, Y3Sed, Y5Sed, H2Sed and H5Sed) in January and to all the water and sediment samples obtained in June. All of the samples analysed during the two pilot studies could be considered non-toxic, and the TU values were always under 1. Although the toxicity observed with the Microtox™ test was low in all of the water and sediment samples, the toxic effect of the sediment samples was always higher than the toxic effect of the water samples from the two rivers (Tab. 4).

Even if the toxic effect was never relevant it was lower in the water samples of the second pilot study, and this trend agreed with the one obtained with *Daphnia magna*. Comparing the results of the chemical and toxicological analyses, we found a significant correlation between the toxicity detected using *Daphnia magna* and the chloroform concentrations (r = 0.894, p < 0.01) in January even if in 24-h exposures in a closed vessel, EC50 value for *D. magna* was determined to be 79 mg/L (Kühn et al.,1989; WHO, 2004).

4. CONCLUSION

The aim of this project carried out in the Pearl River and Yangtze basins was to evaluate the feasibility of using already existing biological monitoring methods for rivers in China. Below are summarized, for each of the approaches tested, the main outcomes of this study in terms of suitability of the method (i.e. are the results acceptable) and of possible improvements to be performed for a specific use on the Chinese territory.

In the Pearl River project, the application to Chinese rivers of bioindicators based on diatom assemblages and diatom indices developed in France and Japan demonstrated the applicability of the use of epilithic diatom assemblages for biomonitoring widely in South China, despite the low number of samples collected. The main risk was linked to the presence and abundance of ñnativeòspecies (endemic and/or subtropical), for which ecological requirements were often unknown. However, acceptable results were obtained based on comparison of the chemical water quality status and diatom indices. The Chinese water quality classification standard is different than the French standard and would require more detailed analysis. Further studies are thus necessary to implement biomonitoring in China based on diatom indices with the following aims: 1) Obtaining a better understanding of the taxonomy and ecological requirements of local or ñnativeòtaxa (using a larger dataset of water quality analyses and diatom samples), 2) Elaboration of a Chinese diatom index taking into account endemic microflora and/or particular ecological profiles of species in this biogeographical context, 3) Implementation of a technical identification guide for routine diatom investigations, and 4) Definition of reference conditions according to river types and hydro-ecological ñregionsòfrom a wider area of investigation and intercalibration between the results collected among rivers from different provinces of China.

Regarding the toxicity analysis performed in Wuhan, we detected no or low toxicity for all of the applied bioindicators, confirming the chemical analyses of the water and sediment samples that indicated moderate pollution. The seeds of *Lepidium sativum* were found to be the most sensitive bioindicator tested during the second study period. The Microtox™ test demonstrated very low toxicity in all of the water and sediment samples, especially studying June. In contrast, the crustacean *Daphnia magna* was the most sensitive organism for the January study, and a toxic effect on this organism was detected in three Han River water samples and one Yangtze River water sample. The toxicity tests used in this study demonstrated their applicability in China and highlighted that only a set of bioassays can estimate accurately the effects of toxicants in surface waters as requested by the WFD.

Macroinvertebrates may spend years maturing in a river. Thus, the size and diversity of their population reflect integration of all of the stream conditions that occur during their lifecycles, such as water chemistry, habitat characteristics, pollutant loading, and changes in water flows, temperature or velocity. Certain species are intolerant of pollution and will be absent from streams with degraded water quality or habitat. Biological monitoring adds a significant component to the overall assessment of stream health, but it requires training in proper sampling and identification and adequate interpretation of biological indices. However, in this study, the results of EBI are opposed to the results of the other toxicological tests applied and resulted to be not useful in this situation. Complementary studies including more stations and, above all, an integrated (in time), overview of the water quality, are needed to state on the potential use of EBI in China.

Therefore, European biomonitoring techniques can be suitable for water monitoring in China, but these methods need to be adapted to the different hydro-geological and environmental conditions present in this country. To achieve this goal, further, large scale, research programs on macro-invertebrates have been financed by the EU ï China RBMP (River Basin Management Program) for example. The urgent need of integrating biological monitoring in the national water quality monitoring program is increasingly taken into account by Chinese policy makers, and a National River Health Assessment Program is being implemented, with the objective to carry out regular river basin health assessment by 2015 and to achieve the river health objectives by 2020.

ACKNOWLEDGEMENTS

The Pearl River study was financed by a French FASEP grant and would not have been as successful without the support of Mr. Thomas Clochard and Mr. Alain Berder of the French Ministry of Finance Industry. Special thanks are dedicated to the Ministry of Water Resources of China, particularly to Mr. Li Ge, Department of International Co-operation Science and Technology. Finally, we would like to express our sincere acknowledgement of the Pearl River Water Resource Commission: Mr. Cui Weizhong, Mr. Wu Yadi, Ms. Liu Xinyuan and Dr. Liu Wei. We would like to dedicate special thanks to Dr. Bérengère Laslandes, who proofread the article.

The study in Wuhan was financed by a grant of the European Commission (contract number CN/ASIA PRO ECO II/07 122277). Special thanks go to Mrs. Virginie De Ruyt, Ms. Joy Zang and Ms. Lauren Wang for their help during the project and to Dr. Raffaella Cardente and Dr. Marta Ultre (Ecotox s.r.l. - Italy) for the free rental of the Microtox™.

Special thanks are dedicated to Dr. Giuseppe Rao, Technological Innovation and Industrial Attaché of Embassy of Italy in Beijing.

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FIGURE CAPTIONS

Fig. 1 - Study area and location of the Pearl River basin, and of the Yangtze and Han rivers.

Fig. 2 ï Co-inertia plots of biological and chemical results (Eigenvalues F1: 40.4, F2: 12.2). Distribution of A) sites, B) diatom species, C) physicochemical variables. The analysis is based on the relative abundances of all the species, but only the dominant ones are figured; correspondences between diatom codes and species names are provided in Supplementary material.

Fig. 3 ï Diatom indices and chemical water quality results for the Pearl River.

Fig. 4 ï Results of the phytotoxicity test (mean and SD).

Table 1

Results of the chemical analyses of the water samples and quality status according to the Chinese assessment method (GB 3838, 2002).

	Liuxi River	Suijiang River	Pearl River	Yangtze River		Han River	
Parameters mean (SD)	May 2007	May 2007	May 2007	January* 2008	June* 2008	January* 2008	June* 2008
T °C	26.43 (3.73)	26.00 (0.51)	27.65 (0.53)	5.95 (0.33)	25.25 (0.19)	2.50 (0.08)	25.92 (0.54)
pH	7.39 (0.27)	7.40 (0.17)	7.35 (0.16)	7.97 (0.02)	7.92 (0.04)	7.97 (0.01)	7.99 (0.04)
Conductivity µS/cm	104.38 (48.39)	99.27 (38.01)	435.67 (196.29)	362.00 (2.62)	307.87 (17.01)	355.60 (1.52)	358.20 (1.64)
Dissolved Oxygen mg/L	7.82 (0.85)	7.0 (0.31)	3.00 (2.20)	11.34 (0.15)	6.27 (0.14)	12.82 (0.08)	6.92 (0.15)
BOD_5 mg/L	1.75 (1.29)	1.16 (0.41)	4.72 (2.33)	1.56 (0.30)	0.73 (0.13)	2.26 (0.16)	1.34 (0.24)
Permanganate Index mg/L	N.D.[§]	N.D.[§]	N.D.[§]	2.56 (0.15)	2.61 (0.10)	3.26 (0.20)	3.15 (0.11)
COD mg/L	15.19 (5.40)	8.48 (3.63)	15.27 (10.01)	< 10	< 10	10.95 (0.64)	<10
Chloride mg/L	7.46 (8.09)	3.07 (0.88)	41.58 (30.45)	15.3 (0.08)	11.37 (0.09)	10.68 (0.13)	10.96 (0.43)
Sulphate mg/L	N.D.[§]	N.D.[§]	N.D.[§]	42.60 (0.21)	32.54 (0.41)	37.96 (0.09)	38.32 (0.72)
Ammonia mg/L	0.24 (0.20)	0.23 (0.04)	3.79 (1.95)	N.D.[§]	N.D.[§]	N.D.[§]	N.D.[§]
Nitrate mg/L	0.68 (0.43)	0.95 (0.24)	1.35 (0.23)	1.60 (0.01)	1.79 (0.01)	1.46 (0.05)	1.22 (0.06)
Nitrite mg/L	N.D.[§]	N.D.[§]	N.D.[§]	0.02 (0.01)	0.01 (0.00)	0.04 (0.01)	0.04 (0.01)
Total Phosphorus µg/L	0.05 (0.03)	31.43 (3.78)	0.21 (0.10)	N.D.[§]	N.D.[§]	N.D.[§]	N.D.[§]
Phosphate mg/L	N.D.[§]	N.D.[§]	N.D.[§]	0.08 (0.01)	0.06 (0.01)	0.05 (0.01)	0.04 (0.01)
Mineral oil mg/L	N.D.[§]	N.D.[§]	N.D.[§]	0.02 (0.01)	0.03 (0.01)	0.02 (0.00)	< 0.01
Chloroform µg/L	N.D.[§]	N.D.[§]	N.D.[§]	0.08 (0.01)	0.22 (0.08)	0.22 (0.19)	0.22 (0.02)
Monobromodichloromethane µg/L	N.D.[§]	N.D.[§]	N.D.[§]	< 0.06	< 0.06	< 0.06	< 0.06
Dibromochloromethane µg/L	N.D.[§]	N.D.[§]	N.D.[§]	< 0.07	< 0.07	< 0.07	< 0.07
Bromoform µg/L	N.D.[§]	N.D.[§]	N.D.[§]	< 0.06	< 0.06	< 0.06	< 0.06
Lead µg/L	13.00	13.00	11.50 (0.71)	N.D.[§]	N.D.[§]	N.D.[§]	N.D.[§]
Zinc mg/L	< 0.05	< 0.05	< 0.05	N.D.[§]	N.D.[§]	N.D.[§]	N.D.[§]
Cadmium µg/L	< 1.00	< 1.00	< 1.00	N.D.[§]	N.D.[§]	N.D.[§]	N.D.[§]
Mercury µg/L	< 0.04	< 0.04	< 0.04	N.D.[§]	N.D.[§]	N.D.[§]	N.D.[§]

Chromium µg/L		4.50 (1.41)	11.83 (1.47)	4.00	N.D.[§]	N.D.[§]	N.D.[§]	N.D.[§]
Nickel mg/L		0.01	< 0.01	0.02 (0.01)	N.D.[§]	N.D.[§]	N.D.[§]	N.D.[§]
Sodium mg/L		8.61 (7.75)	5.42 (3.38)	43.72 (27.40)	N.D.[§]	N.D.[§]	N.D.[§]	N.D.[§]
Chemical status	Good	L1,2,3,5,6	S1,4,5,7,9,10					
	Medium	L4,7,10			Y1,2,3,4,5	Y1,2,3,4,5	H1,2,3,4,5	H1,2,3,4,5
	Bad	L8,9,11						
	Very bad.			G1,2,3,4,5				

*January 2008: first pilot study; June 2008: second pilot study

[§]N.D. = Not determined

Table 2

Results of the chemical analyses of the sediment samples from the Han and Yangtze Rivers.

	January		June	
Parameters	Yangtze River mean (SD)	Han River mean (SD)	Yangtze River mean (SD)	Han River mean (SD)
Copper mg/kg	8.28 (3.44)	20.04 (12.92)	9.36 (1.51)	11.35 (1.14)
Lead mg/kg	14.51 (5.46)	17.30 (5.30)	23.71 (1.10)	33.25 (6.01)
Zinc mg/kg	20.86 (3.26)	26.18 (8.38)	61.76 (5.45)	62.11 (1.11)
Cadmium mg/kg	0.42 (0.28)	0.42 (0.10)	0.08 (0.01)	0.09 (0.01)
Chromium mg/kg	85.68 (27.54)	69.70 (15.39)	69.33 (6.89)	70.28 (4.04)
PCBs µg/kg	< 0.08	< 0.08	< 0.08	< 0.08
OCPs µg/kg	< 0.08	< 0.08	< 0.08	< 0.08
Naphthalene µg/kg	< 0.08	< 0.08	< 0.08	< 0.08
Acenaphthylene µg/kg	< 0.08	< 0.08	< 0.08	< 0.08
Acenaphthene µg/kg	< 0.08	< 0.08	< 0.08	< 0.08
Fluorene µg/kg	0.02	0.02	< 0.02	< 0.02
Fluorantene µg/kg	< 0.08	< 0.08	< 0.08	< 0.08
Pyrene µg/kg	< 0.06	< 0.06	< 0.06	< 0.06
Crysene µg/kg	0.03	< 0.015	0.03	< 0.015
Benzo(b)fluorantene µg/kg	< 0.04	< 0.04	< 0.04	< 0.04
Benzo(k)fluorantene µg/kg	< 0.04	< 0.04	< 0.04	< 0.04
Benzo(a)pyrene µg/kg	0.06	0.02	0.06	0.02
Indeno(1,2,3-cd)pyrene µg/kg	< 0.035	< 0.035	< 0.035	< 0.035
Dibenzo(a,h)anthracene µg/kg	< 0.045	< 0.045	< 0.045	< 0.045
Benzo(g,h,i)pyrene µg/kg	< 0.02	< 0.02	< 0.02	< 0.02

Table 3

EBI results and water quality classes in Wuhan.

Sample	Family benthos	Number of organisms	Tolerance (1-10 range)	EBI values/ Quality class*
Y2[b]	Lymnaeidae	2	7 (high tolerant)	0/V
Y3[b]	Unionidae	1	7 (high tolerant)	0/V
Y4[b]	None found	-	-	0/V
H1[b]	None found	-	-	0/V
H2[b]	Pisidiidae	1	7 (high tolerant)	0/V
	Unionidae	11		
H3[b]	Unionidae	12	7 (high tolerant)	0/V
H4[b]	Pisidiidae	2	7 (high tolerant)	0/V
	Unionidae	10		
Y1[s]	None found	-	7 (high tolerant)	0/V
Y2[s]	Pisidiidae	10	7 (high tolerant)	0/V
	Sphaeriidae	2		
	Bithyniidae	3		
Y3[s]	None found	-	-	0/V
Y4[s]	None found	-	-	0/V
Y5[s]	None found	-	-	0/V
H1[s]	None found	-	-	0/V
H2[s]	None found	-	-	0/V
H3[s]	None found	-	-	0/V
H4[s]	None found	-	-	0/V
H5[s]	None found	-	-	0/V

[b] sample collected from the bank
[s] sediment sample
* Quality class V corresponds to an extremely polluted and impaired environment (Lucadamo et al., 2008).

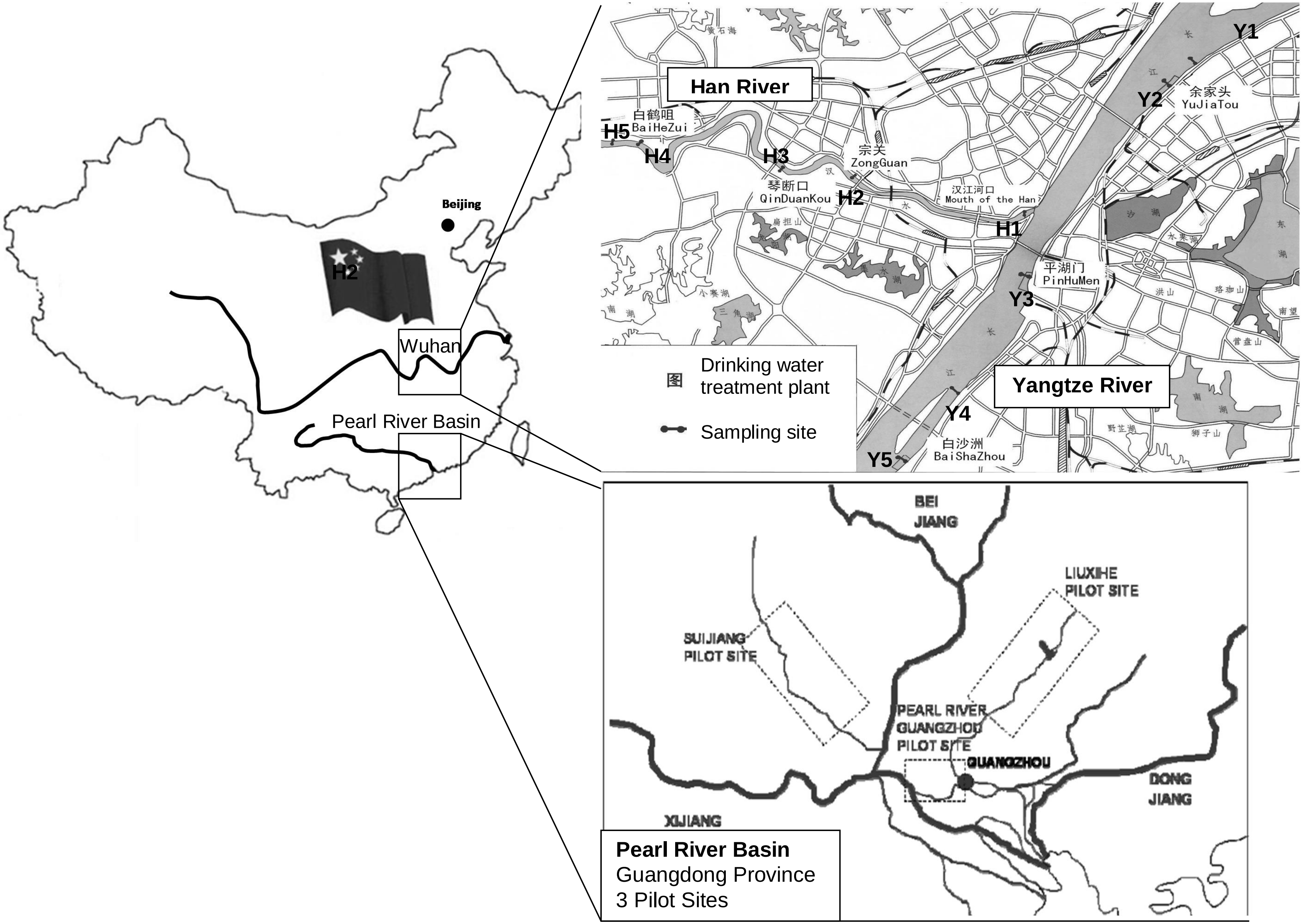
Table 4

Results of the Daphnia magna test and of the Microtox^{TM} test (Toxic Unit TU).

	Daphnia magna test				Microtox^{TM} test	
	January		June		January	June
Sample	TU 24h	TU 48h	TU 24h	TU 48h	TU	TU
H1 water	0.70	1.34	Not toxic	Not toxic	0.45	Not toxic
H2 water	1.35	1.94	Not toxic	Not toxic	0.37	Not toxic
H3 water	N.D.*	N.D.*	Not toxic	Not toxic	0.55	Not toxic
H4 water	N.D.*	N.D.*	Not toxic	Not toxic	0.32	Not toxic
H5 water	1.12	1.63	Not toxic	Not toxic	0.29	Not toxic
Mean (SD)	1.06 (0.33)	1.64 (0.30)			0.40 (0.11)	
Y1 water	Not toxic	Not toxic	Not toxic	Not toxic	0.25	Not toxic
Y2 water	Not toxic	Not toxic	Not toxic	Not toxic	0.42	Not toxic
Y3 water	Not toxic	Not toxic	Not toxic	Not toxic	0.50	Not toxic
Y4 water	Not toxic	0.72	Not toxic	Not toxic	0.34	0.11
Y5 water	Not toxic	Not toxic	Not toxic	Not toxic	0.33	Not toxic
Mean (SD)					0.37 (0.10)	
H1 sediment	N.D.*	N.D.*	Not toxic	Not toxic	N.D.	Not toxic
H2 sediment	Not toxic	Not toxic	Not toxic	Not toxic	0.47	Not toxic
H3 sediment	N.D.*	N.D.*	Not toxic	Not toxic	N.D.	0.44
H4 sediment	N.D.*	N.D.*	Not toxic	Not toxic	N.D.	0.34
H5 sediment	Not toxic	Not toxic	Not toxic	Not toxic	0.84	Not toxic
Mean (SD)					0.65 (0.26)	0.39 (0.07)
Y1 sediment	Not toxic	Not toxic	Not toxic	Not toxic	0.54	0.39
Y2 sediment	N.D.*	N.D.*	Not toxic	Not toxic	N.D.	0.49
Y3 sediment	Not toxic	Not toxic	Not toxic	Not toxic	0.57	0.48
Y4 sediment	N.D.*	N.D.*	Not toxic	Not toxic	N.D.	0.25
Y5 sediment	Not toxic	Not toxic	Not toxic	Not toxic	0.49	0.39
Mean (SD)					0.53 (0.04)	0.40 (0.10)

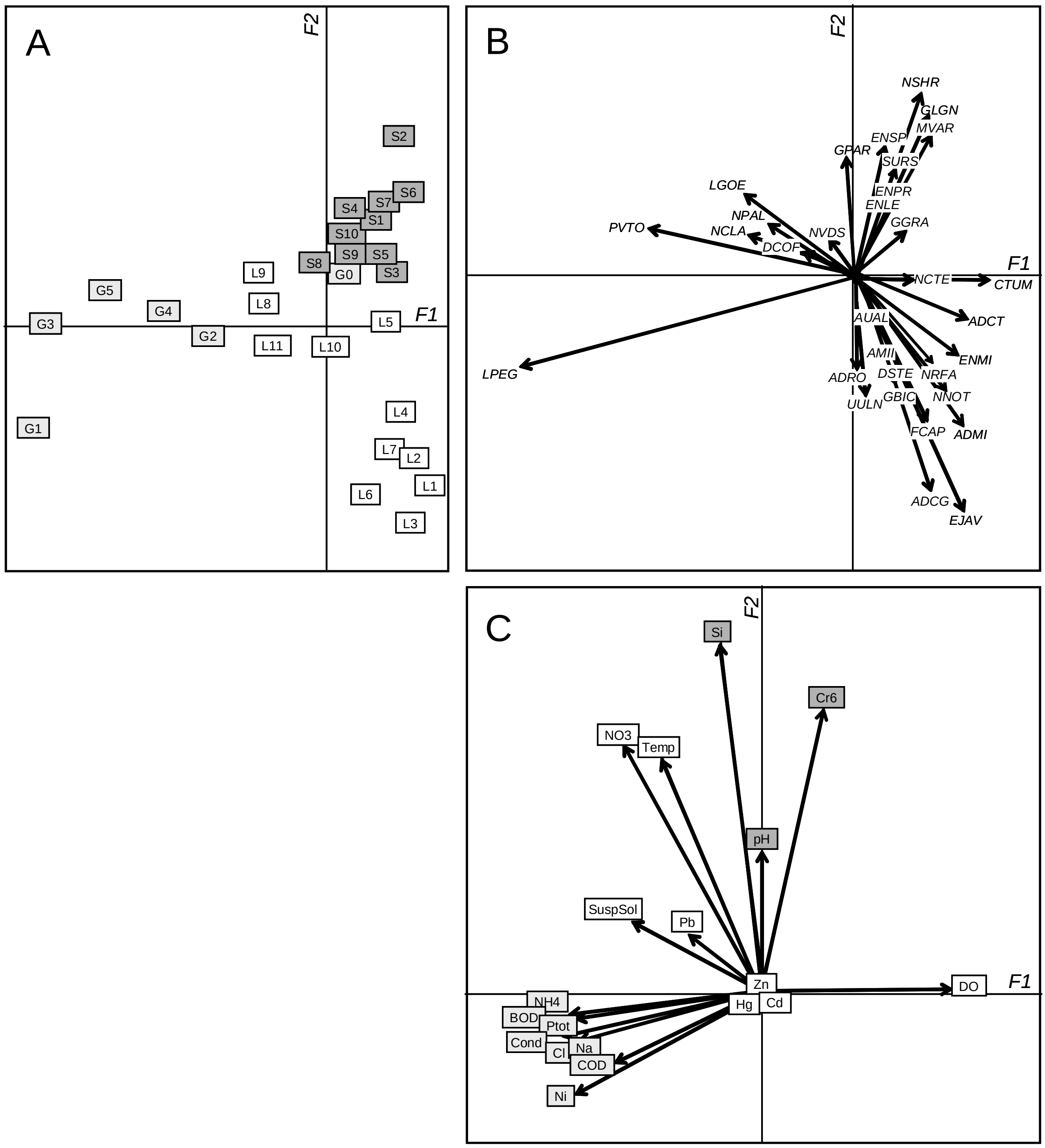
*N.D.= Not determined

Figure 1



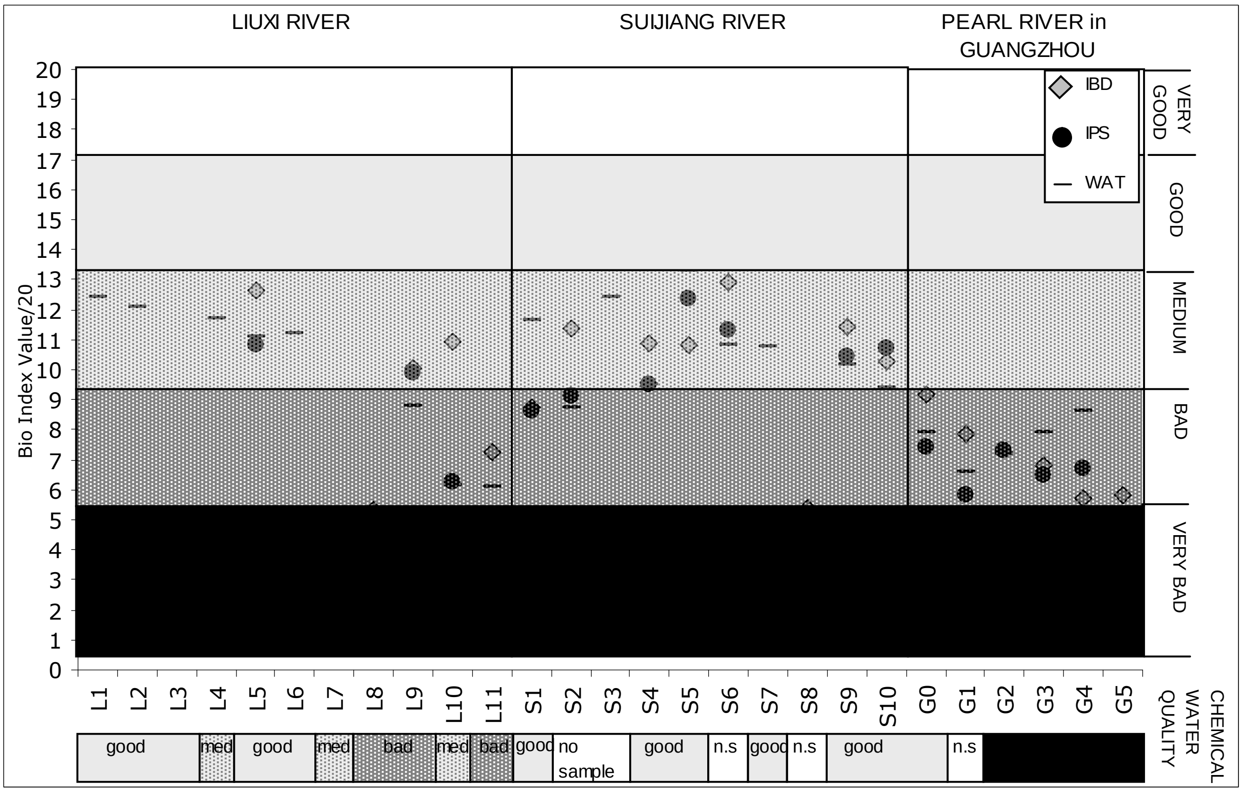
Beijing
H2
Wuhan
Pearl River Basin
Han River
白鹤咀
BaiHeZui
H5
H4
H3
宗关
ZongGuan
琴断口
QinDuanKou
H2
汉江河口
Mouth of the Han
H1
Y1
Y2
余家头
YuJiaTou
平湖门
PinHuMen
Y3
Drinking water treatment plant
Sampling site
Yangtze River
Y4
白沙洲
BaiShaZhou
Y5
BEI JIANG
LIUXIHE PILOT SITE
SUIJIANG PILOT SITE
PEARL RIVER GUANGZHOU PILOT SITE
GUANGZHOU
DONG JIANG
XIJIANG
Pearl River Basin
Guangdong Province
3 Pilot Sites

Figure 2



A
F2
F1
S2
S6
S7
S4
S1
S10
S9
S5
S8
G0
S3
L9
G5
L8
G4
L5
G3
G2
L11
L10
L4
G1
L7
L2
L1
L6
L3
B
NSHR
GLGN
MVAR
ENSP
GPAR
SURS
ENPR
ENLE
GGRA
LGOE
NPAL
PVTO
NCLA
NVDS
DCOF
NCTE
CTUM
AUAL
ADCT
AMII
ENMI
ADRO
DSTE
NRFA
LPEG
UULN
GBIC
NNOT
FCAP
ADMI
ADCG
EJAV
C
Si
Cr6
NO3
Temp
pH
SuspSol
Pb
Zn
DO
NH4
Hg
Cd
BOD
Ptot
Cond
Na
Cl
COD
Ni

Figure 3



LIUXI RIVER
SUIJIANG RIVER
PEARL RIVER in GUANGZHOU
IBD
IPS
WAT
VERY GOOD
GOOD
MEDIUM
BAD
VERY BAD
Bio Index Value/20
L1 L2 L3 L4 L5 L6 L7 L8 L9 L10 L11 S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 G0 G1 G2 G3 G4 G5
good
med
good
med
bad
med
bad
good
no sample
good
n.s
good
n.s
good
n.s
CHEMICAL WATER QUALITY