Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 19(2007) 738–744
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Monitoring bioaccumulation and toxic effects of hexachlorobenzene using the polyurethane foam unit method in the microbial communities of the Fuhe River, Wuhan

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Received 2 July 2006; revised 18 August 2006; accepted 8 November 2006

Abstract

Hexachlorobenzene (HCB) is a chlorinated aromatic hydrocarbon that was widely used for seed dressing in prevention of fungal growth on crops, and also as a component of fireworks, ammunition, and synthetic rubbers. Because of its resistance to degradation and mobility, HCB is widely distributed throughout the environment and is accumulated through food chains in different ecosystems. In this study, a preliminary investigation was carried out on the bioaccumulation and the toxic effects of HCB in the microbial (protozoan in particular) communities in the Fuhe River, Wuhan, a water body receiving industrial wastewaters containing HCB and other pollutants, using the standardized polyurethane foam units (PFU) method. Field samples were taken from eight stations established along the Fuhe River in January and August 2006. The concentration ratios of HCB in microbial communities and in water were 9.66–18.64, and the microbial communities accumulated 13.29–56.88 μg/L of HCB in January and 0.82–10.25 μg/L HCB in August. Correlation analysis showed a negative correlation between the HCB contents in the microbial assemblage, and the number of species and the diversity index of the protozoan communities. This study demonstrated the applicability of the PFU method in monitoring the effects of HCB on the level of microbial communities.

Key words: bioaccumulation; toxicity effect; hexachlorobenzene; PFU method; microbial community; protozoan communities

Introduction

Hexachlorobenzene (HCB) is considered as a "model persistent organic pollutants (POPs)" (Barber *et al*., 2005). It is a chlorinated aromatic hydrocarbon, which was widely used for seed dressing in prevention of fungal growth on crops, and also as a component of fireworks, ammunition, and synthetic rubbers. Restrictions of HCB use initiated in the 1970s have caused a decline of HCB manufacturing. In the past few years, the production of HCB has been an unintentional by-product in the synthesis of chlorinated solvents, aromatics, and pesticides. With its resistance to environmental degradation and mobility, HCB has widely spread throughout the environment (Bailey, 2001; Barber *et al*., 2005). HCB is practically insoluble in water, but is highly lipid-soluble and bioaccumulative (Courtney, 1979; ATSDR, 2002). Studies have focused on bioaccumulation of HCB in higher trophic levels, such as fish, krill, mussels, oysters, and amphipods (Ernst, 1986; Falandysz *et al*., 1994; Weber and Goerke, 1996; Looser *et al*., 2000; Corsolini *et al*., 2001; Cleeman *et al*., 2002; Pa′ez-Osuna *et al*., 2002; Blais *et al*., 2003; Monirith *et al*., 2003; Voorspoels *et al*., 2004). HCB has been reported to be capable of causing a wide range of toxic effects including cancer in animals, hepatotoxicity and porphyria both in humans and animals (Erturk *et al*., 1986; Rozman *et al*., 1986; Kleiman de Pisarev *et al*., 1990; Foster *et al*., 1992; Cabral *et al*., 1996; Alvarez *et al*., 2000). However, few studies on bioaccumulation of HCB in microbial communities have been reported, and the possible toxic effects of HCB on the aquatic microbial communities are still not yet well known.

Freshwater microbial communities mainly comprise bacteria, fungi, algae, protozoa and rotifers. Because HCB is bioaccumulative and that the microorganisms can be predated upon by other zooplankton, benthos and fishes, microbial communities act as important vectors in transporting HCB to higher trophic levels (Wallberg *et al*., 1997; Shen *et al*., 2004; Li *et al*., 2005). In addition, microbial communities can respond rapidly to environmental changes with respect to their species composition and abundance, and these kind of responses have been used to appraise water quality and toxic effects of pollution sources (Shen *et al*., 1986; Xu *et al*., 2002; Li *et al*., 2005).

Polyurethane foam unit (PFU) is an artificial substrate

Project supported by the Key Project of Chinese Ministry of Education (No. 104250). *Corresponding author. E-mail: chenzhulei@263.net.

which can play as a host for the microorganisms in water. When PFU blocks are exposed to water for a certain period of time, most microorganisms in the water such as bacteria, fungi, algae, protozoa and small rotifers can gradually colonize in them and form a microbial community (Cairns *et al*., 1969, 1973). The PFU method was first established by Cairns *et al*. (1969, 1973), and was then standardized by the Environmental Protection Agency of China under the number of GB/T 12990-9 (Water Quality-Microbial Community Biomonitoring-PFU Method) (SBTS and EPA, China, 1992). Shen *et al*. (2004) evaluated the feasibility of using PFU method to biomonitor persistent organic pollutants (POPs) in the environments, and found low concentrations of OCPs and PCBs in the microbial communities of the Gai-Wai ponds and the mangrove of Mai-Po Marshes Nature Reserve, Hong Kong. Later, Wang *et al*. (2005) reported the detection of HCB in microbial communities of the Donghu Lake, Wuhan, using the PFU method, and Li *et al*. (2005) reported a similar work in the Baiyangdian Lake, Hebei Province. The PFU method has also been used to evaluate toxic effects of pollutants (such as detergent, rare earth fertilizer and metal) at community-level (Shen *et al*., 1986, 1999; Xu *et al*., 1994).

The objectives of this study were, using the PFU method, to detect the bioaccumulation of HCB in aquatic microbial communities and its toxic effects on the protozoan communities, from the Fuhe River, Wuhan.

1 Materials and methods

1.1 Sampling sites

The study sites were along the Fuhe River, located in the northwestern Wuhan, which is a tributary of Yangtse River in China (Fig.1). Nutrient-enriched and highly-polluted wastewaters enter the river at two points 500 m apart from each other. The nutrient-enriched wastewater was from a cattle excrement effluent, and the other effluent containing HCB, lipids, disulfide, toluene, and other compounds were from a factory which has been producing additives of plastics and rubber for twenty years. High concentrations of HCB have been detected in the industrial wastewater (170–220 μg/L) and the sediments of wastewater ditch (average concentration was 670.8 mg/kg) (Xie *et al*., 2005a, b; Xie, 2005). Eight sampling stations were set up within this drainage (Fig.1). As a control station, Station 1 was located at the upstream of the first wastewater outlet.

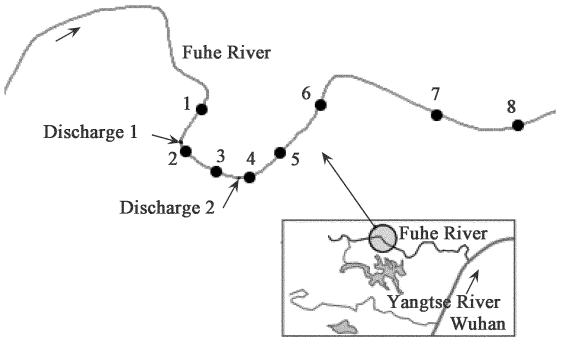

Fig. 1 Sampling stations along the Fuhe River, Wuhan, Hubei, China.

1.2 Collection of PFU microbial community

The size of PFU blocks was about 5 cm × 6.5 cm × 7.5 cm. The aperture of the foam was about 100–150 μm. Microbial communities were collected according to the standard PFU method (GB/T 12990-91, China SBTS and China EPA, 1992). Field study was carried out in 7–10 January 2006 at all the eight stations (Fig.1) and in 21–22 August 2006 at five stations (Station 4, 5, 6, 7, 8). Six PFU blocks were placed at each station and each PFU block was anchored in the river and a microbial community was allowed to colonize on it. PFU blocks were removed after being exposed to water for 1 or 3 d (for river sampling), sealed in a clean plastic bag, and transported to the laboratory. The contents containing microorganism assemblage in PFU blocks were squeezed into clean beakers and aliquoted for light microscopic observations and measurements of HCB. All samples were subjected to microscopic examination within 24 h.

1.3 Structural parameters of the protozoan communities on PFU

We mainly investigated the structural parameters of protozoan communities in this survey. The number of species and the abundance of protozoa were determined with light microscopic observations (Olympus BX60, magnifications of 200× and 400×). The species diversity index was calculated according to the equation (Margalef, 1957):

$$\mathrm{DI} = (S - 1)/\ln N \quad (1)$$

where, DI is the diversity index, S is the number of species and N is the total number of protozoan individuals per 0.1 ml of water squeezed from PFU. Heterotrophic index (HI) is a nontaxonomic parameter of PFU protozoan community and the equation is:

$$\mathrm{HI} = \mathrm{Biomass}/\mathrm{Chlorophyll}\text{-}a \quad (2)$$

where, the ash-free dry weight was used to estimate the total biomass (Shen *et al*., 1986). The measurements of both biomass and chlorophyll-*a* followed the standard methods (APHA, 1980).

1.4 HCB analysis

The PFU samples and water samples underwent a pretreatment following the liquid-liquid extraction method (Shen *et al*., 2004). The 100 ml precipitation aliquot was separated from the whole solution extruded from 3-d PFU (sampling in January), and then was extracted by 20 ml *n*-hexane. The 300 ml solution extruded from 1-d PFU (sampling in August) was extracted by 60 ml *n*-hexane. The 800 ml water sample was extracted by 80 ml *n*-hexane. Glacial acetic acid was added to break the emulsification during the extraction. The extracts were then passed through a small cartridge packed with anhydrous sodium sulfate. Finally, the dry extracts were vaporized on a vacuum rotator followed by the concentration under pure nitrogen gas to 500 μl. At the same time, 0.5 ml aliquots

of 5 μg/ml HCB standard were fortified into 50 ml Milli-Q water. The solution was extracted by 10 ml *n*-hexane in a glass separation funnel. 25 ml glacial acetic acid was added to break the emulsification during the extraction. Both standards and PFU samples underwent the same pretreatment procedure.

HCB identification and quantification in the concentrates were conducted with a gas chromatography equipped with an electron capture detector (GC-ECD) system (Hewlett-Packard 6890, integrator HP 3398A, USA), which was equipped with a HP-1 capillary column (30 m length, 0.32 mm inner diameter, and 0.25 μm film thickness). The operating temperatures of the injector and detector were 250°C and 300°C, respectively. Nitrogen (>99.999%) was used as the carrier gas at a flow rate of 1.5 ml/min. The column was operated under programmed conditions from 150°C initially for 1 min, increasing to 200°C in an increasing rate of 20°C/min with an isothermal period of 10 min at the end. The injection volume was 1 μl.

The results of HCB analysis in samples by GC-ECD were compared with the corresponding standards. The average recoveries of the analytical methods were above 80.5% with standard deviations less than 0.5.

1.5 Environmental factors and statistical analysis

Chemical oxygen demand (COD), biological oxygen demand (BOD_5), NH_4^+-N, total phosphorus contents (TP), temperature, and pH of the eight stations were measured using standard methods (CSEPA, 20021).

The statistical package SPSS 11.5 for Windows (SPSS Inc., Chicago, USA) was used for the correlation analysis between the community indices (*S*, DI and HI) and the concentrations of HCB and other environmental factors (COD, etc.).

2 Results and discussion

2.1 Environmental factors

The environmental factors at the eight stations in January 2006 are listed in Table 1.

Among these variables, temperature in the eight sites varied between 2–4°C, and no significant differences were observed, and pH oscillated around neutral values (7.13–7.78). Station 2 was obviously the highest in COD, BOD_5, NH_4^+-N, and TP concentrations. The cattle excrement effluent upstream of Station 2 has an important contribution to the chemical load (COD, BOD_5, NH_4^+-N, TP). Compared with Station 1, 4, 5, 6 and 8, COD concentrations at Station 3 and 7 were higher. According to the measurements of NH_4^+-N and TP, all the eight stations in Fuhe River were severely eutrophicated.

2.2 Bioaccumulation of HCB in microbial communities

The PFU extrusions from stations and the water samples were analyzed for the concentrations of HCB, which are shown in Table 2 (for January) and Table 3 (for August). HCB was also found in the color PFU block. The highest concentration of HCB in the microbial communities was observed at Station 4 (56.88 μg/L), followed by Station 3, 5, 2, 7, 1, 8 and 6 in order during the dry season (January). In comparison, the concentrations of HCB in the microbial communities in the August were lower.

In general, HCB may be adsorbed on solid surfaces in water bodies (Barber *et al*., 2005), such as aqueous particles. Because of abundant bioparticles in the water and their high ratios of the surface area to the particle volume, microbial communities can accumulate HCB rather quickly. HCB may accumulate in microorganisms by several ways: adsorption at the surface of the cell, passive diffusion, active transport throughout the membrane, and

Table 1 Physico-chemical parameters of eight sampling locations in Fuhe River (January 2006)

Sampling station	1	2	3	4	5	6	7	8
COD (mg/L)	24.69	111.67	53.16	37.22	35.95	37.79	45.09	25.70
BOD_5 (mg/L)	20.8	77.2	33.2	12	13.8	19.4	27.4	18.2
NH_4^+-N (mg/L)	4.30	17.62	6.50	5.20	5.26	5.14	5.40	4.82
TP (mg/L)	1.49	5.69	1.25	1.52	1.80	1.43	1.84	1.73
pH	7.13	7.40	7.78	7.62	7.31	7.53	7.38	7.58

Table 2 Concentrations of HCB in the eight sampling locations in Fuhe River (January 2006) Unit: μg/L

Sampling station	1	2	3	4	5	6	7	8
PFU extrusions	14.81	19.99	22.92	56.88	20.48	13.29	15.52	14.19
PFU blank				0.03				

0.01 μg/L is the detection limit.

Table 3 Concentrations of HCB in five sampling locations in Fuhe River (August 2006) Unit: μg/L

Sampling station	1	2	3	4	5	6	7	8
PFU extrusions	-	-	-	10.25	1.17	0.84	0.86	0.82
Water	-	-	-	0.55	0.092	0.087	0.07	0.05
Concentration ratio	-	-	-	18.64	12.72	9.66	12.29	16.40

0.01 μg/L is the detection limit.

intake to the cell with food and water during the formation of digestive vacuoles. Wang *et al*. (2005) reported the accumulation of HCB in the microbial community of Donghu Lake, Wuhan, and the concentration of HCB was 2.13 μg/L. Li *et al*. (2005) found that there were 1.11–4.14 μg/L HCB contents in the microbial communities of 4 sampling stations in Baiyangdian Lake, Baoding, Hebei Province. Compared to previous studies, higher concentrations of HCB were observed in microbial communities in present survey. Microbial communities have an accumulation capacity of up to 13.29–56.88 μg/L HCB. It is worth noticing that the concentrations of HCB in the PFU extrusion water were significantly higher than that in the corresponding ambient water. The concentration ratios of HCB in microbial communities and in water ranged from 9.66–18.64 (Table 3).

Because microbial communities are at low trophic level in aquatic systems, and because they constitute an important biomass available for the higher predators, HCB-polluted microbial communities can transfer these contaminants to higher trophic level animals through the food chains. Studies have been focused on bioaccumulation of HCB in higher trophic levels, such as krill, mussels, oysters, amphipods, fish and cormorant (Ernst, 1986; Falandysz *et al*., 1994; Weber and Goerke, 1996; Looser *et al*., 2000; Corsolini *et al*., 2001; Cleeman *et al*., 2002; Pa′ez-Osuna *et al*., 2002; Blais *et al*., 2003; Monirith *et al*., 2003; Voorspoels *et al*., 2004; Barber *et al*., 2005). Compared to the microbial communities, higher concentration ratios of HCB were observed in higher trophic levels.

2.3 Structural parameters of protozoan communities in PFUs

In total, 104 species (45 autotrophic flagellates, 23 heterotrophic flagellates, 7 sarcodinas, and 29 ciliates) were identified at the eight stations. The lowest number of protozoa species was found at Station 4 (3 species), while the highest number was found at Station 2 (51 species). The following species were observed at more than five stations: *Cyclidium glaucoma*, *Paramecium caudatum*, *Glaucoma macrostoma*, *Tetrahymena priformis*, *Chilodonella cucullulus*, *Difflugia globulosa*, *Bodo* sp., *Eudorina elegans*, *Polytoma uvella*, *Chlamydomonas snowiae*, *Trachelomonas volvocina*, *Euglena geniculate*, and *Euglena viridis*.

The structural parameters of protozoan communities in PFU in the eight stations in January 2006 were significantly different (Table 4). The lowest number of species at Station 4 coincided with the highest concentration of HCB (Table 2), indicating the toxic effects of HCB on the species composition of the microbial assemblage. The numbers of species from the three main groups (autotrophic flagellate, sarcodina, and ciliate) were negatively affected by HCB wastewater in the river. Heterotrophic flagellates, sarcodinas and ciliates were sensitive to HCB and none of them were observed at Station 4. Compared to other stations, Stations 1, 2, 7 were characterized by higher numbers of species (Table 4). The highest concentrations of COD, NH_4^+-N and TP were observed at Station 2, where, however, the number of species was not negatively affected by the high loading of nutrients and organics.

Among the PFU protozoan communities at the eight stations, the diversity index values at Station 1, 2, 7, 8 were high (exceeded 4). The lowest value (0.24) was recorded at Station 4, which was regarded as the most polluted site in the survey. It must be noted that species number and diversity at Station 2 were not heavily affected by the cattle excrement load. The cattle excrement effluent seems to be helpful in the development of microbial community, because it contains abundance of bacteria, suspended particles, and dissolved organic matter which were important food resources for heterotrophic flagellate, sarcodina, and ciliate.

HI is a nontaxonomic parameter of PFU microbial community. Field studies have shown that HI correlated well with changes in microbial communities (Shen *et al*., 1986, 1996). The higher the HI, the worse is the water quality. HI is analogous to the autotrophic index but uses ash-free dry weight to estimate total biomass. It has been proposed that an autotrophic index for field studies of between 50 and 200 would be normal (APHA, 1980). High HI values (>600) were detected at all stations, and this indicated that the water quality was severely polluted. The results coincided well with the assessments of chemical parameters. HI increased dramatically at Station 2 and 4 due to pollution stress, and return to control values at Station 7.

Table 4 Number of protozoan species in the eight sampling stations in Fuhe River (January, 2006)

Station number	1	2	3	4	5	6	7	8
Autotrophic flagellata	24	23	14	3	9	11	22	19
Heterotrophic flagellata	10	10	10	0	7	7	12	7
Sarcodina	3	5	2	0	2	1	3	2
Ciliata	12	13	6	0	3	6	13	17
Total (S)	49	51	32	3	21	25	50	45
Diversity index (DI)	4.50	4.64	2.94	0.24	1.93	2.23	4.53	4.07
Heterotrophic index (HI)	876.8	1111.1	1094.2	1396.5	1351.8	1179.3	626.0	746.5

Table 5 Number of protozoa species in five sampling stations in Fuhe River (August, 2006)

Station number	1	2	3	4	5	6	7	8
Total (S)	-	-	-	46	45	51	59	58
Diversity index (DI)	-	-	-	4.50	4.69	5.27	5.94	5.91

Structure parameters of PFU protozoan community of five stations in August are shown in Table 5. The lowest number of species and diversity index were at Station 4 and followed by Station 5, 6, 7 and 8 in order.

2.4 Toxicity effects of HCB reflected in the structural indices of protozoan communities

The PFU method has been attested to be a useful tool for the bioassessment of water quality and the structural indices of PFU microbial community (e. g. DI and HI) have been verified to correlate well with water quality (Shen *et al*., 1990; Chung *et al*., 1999). The present study, to the best of our knowledge, is the first report combining the PFU method and the structural indices to study the toxicity effect of HCB on the freshwater microbial community. The above-mentioned chemical factory has been discharging wastewater with high levels of HCB (170–220 μg/L) into the Fuhe River for about twenty years and the microbial communities in the river thus have been exposed chronically to these high levels of HCB, which may have caused significant changes in the structure of microbial community. Therefore, a Pearson correlation analysis (Table 6) was applied to the environmental factors (data in Table 1 and HCB data in Table 2) and the structural indices (S, DI and HI data in Table 4).

Among all these factors (COD, BOD_5, NH_4^+-N, TP, pH, and concentration of HCB), the concentration of HCB was significantly correlated with the two community structural indices: both the number of species (S) and the diversity index (DI) were negatively correlated with the concentration of HCB (S vs. HCB: r=–0.753, p<0.05; DI vs. HCB: r=–0.753, p<0.05, n=8). This result indicates HCB may be one of the most important factors affecting the structure of microbial community. The restoration in the number of species at Station 7 and 8 to the levels equivalent to Station 1 (Table 4) corresponded to the decreased HCB concentrations (15.52 and 14.19 μg/L, respectively), after a self-cleaning process along the three upstream stations (Stations 4, 5, and 6).

To highlight the pollution status as reflected by HI among stations, COD, HCB concentrations and HI are illustrated in Fig.2. It can be seen that the HI increased with COD and HCB concentrations. Station 2 distinguishes itself from other stations by the highest COD concentration (high HI value as well). The nutrient level at Station 4 was lower than that of Station 2 and 3 during the survey. However, HI at Station 4 was still higher than other stations, due to its higher HCB level and chemical pollutants levels. The HI correlated with the HCB concentration at all stations except for Station 2. HI showed to be sensitive to pollution caused either by organic matters or HCB, and it was confirmed that HI increased dramatically at Station 2 and Station 4.

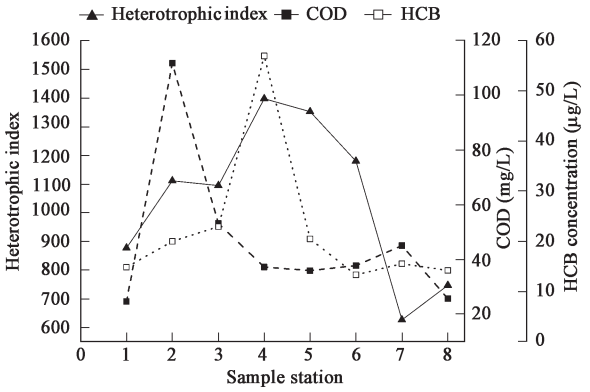

Fig. 2 Relationship between COD, HCB concentration, and herotrophic index after 3 d of PFU anchoring at Stations 1–8 in January 2006.

3 Conclusions

Aquatic microbial communities appear to be at risk of adverse effect from POPs in water. In this study, PFU microbial communities of Fuhe River have an accumulation capacity of up to 13.29–56.88 μg/L of HCB in dry season and 0.82–10.25 μg/L of HCB in flood season. The concentration ratios of HCB in microbial communities and in water were 9.66–18.64. Pearson correlation analysis showed that HCB was inversely correlated with the species number and diversity of protozoan communities. The results provide further evidence that PFU microbial communities could be effectively used in assessment of bioaccumulation and toxic effect of POPs in water.

Table 6 Pearson correlation between microbial community structure parameters and physico-chemical parameters in Fuhe River (January 2006)

		Total (S)	Diversity index (DI)	Heterotrophic index (HI)
HCB	*r*	–0.753	–0.753	0.602
	p	0.031	0.031	0.114
	n	8	8	8
COD	*r*	0.300	0.299	0.137
	p	0.470	0.471	0.746
	n	8	8	8
BOD_5	*r*	0.530	0.530	-0.079
	p	0.177	0.177	0.852
	n	8	8	8
NH_4^+-N	*r*	0.349	0.348	0.122
	p	0.396	0.398	0.774
	n	8	8	8
TP	*r*	0.411	0.409	0.047
	p	0.311	0.314	0.912
	n	8	8	8

The results were considered significant when p<0.05; r is the Pearson's correlation coefficient; p is the significant level; n is the number of sample.

Acknowledgements

The authors are grateful to Dr. Dong Xiang for sampling in field and Dr. Qin-Jie Xie and Ms. Su-Qin Tang for helpful and valuable discussions. Thanks also go to anonymous referees who offered valuable advice on an earlier draft of this manuscript, and to Dr. Ying-zhong Tang for offering valuable advice and improving the English draft.

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