Bull Environ Contam Toxicol (2012) 88:472–476
DOI 10.1007/s00128-011-0498-2

# The Use of a Brine Shrimp (*Artemia salina*) Bioassay to Assess the Water Quality in Hangzhou Section of Beijing-Hangzhou Grand Canal

**Yin Lu · Xiaolu Xu · Tian Li · Yifei Xu · Xu Wu**

Received: 12 April 2011 / Accepted: 12 December 2011 / Published online: 21 December 2011


**Abstract** As physical and chemical tests alone are not sufficient enough for the assessment of potential effects on aquatic organisms, bioassays are required for the integrated evaluation of water pollution. In this study, invertebrate crustacean *Artemia salina* (brine shrimp) was applied as an indicator to assess the water quality of Hangzhou Section of Beijing-Hangzhou Grand Canal. The percentage mortality of brine shrimp was recorded after 24-h exposure to the Canal water. The water samples were collected from five typical sites during October 2008 and April 2009. It exhibited 11% ± 8.3%, 26.7% ± 17%, 31.7% ± 8.5%, 28.0% ± 11.7%, and 4% ± 4.3% percentage mortality for the sample from Tangxi Bridge, Yi Bridge, Gongchen Bridge, Maiyu Bridge, and Gujia Bridge in 2008, respectively. And it exhibited 5.7% ± 4.2%, 10.3% ± 8.2%, 24.3% ± 12.3%, 16.0% ± 12.3%, and 0%, percentage mortality in 2009, respectively. According to the results, a relative improvement in water quality was observed, although the results were not significantly different at the $p < 0.05$ level. It suggested that 24-h *A. salina* exposure trials represent an acceptable bioassay for water toxicity when alternative bioassays were unavailable.

Y. Lu (✉) · X. Xu · X. Wu
College of Biology and Environmental Engineering,
Zhejiang Shuren University, Hangzhou, China
e-mail: luyin@yahoo.cn

T. Li
Modern Education & Technical Center,
Zhejiang Shuren University, Hangzhou, China

Y. Xu
School of Management, Northwestern Polytechnical University,
Xi'an, China

**Keywords** Hangzhou Section of Bejing-Hangzhou Canal · Water quality · Bioassays · Brine shrimp

The Beijing-Hangzhou Grand Canal, which is also called Jing-Hang Canal for short, has a history of 2,400 years. It is not only one of the greatest projects in ancient China but also the longest and largest man-made waterway in the world. It is 16 times larger than the Suez Canal and 33 times larger than the Panama Canal. Historically, this important north–south water route not only facilitated the transport of large amounts of material exchange, but also helped the country's political, economic, and cultural development. It is also known as "the most magnificent ancient works of the world" together with the Great Wall, the pyramids of Egypt, and Buddhist pagoda of India. For thousands of years, the Canal has played an important role in strengthening economic and cultural intercourse between north and south of China and promoting the development of the country's economy. In 2006, it was listed as one of the most important national protected heritage sites, and it has attracted the attention of more and more people.

With a total length of 1,794 km, the Canal interconnects the Yangtze River, Yellow, Huaihe, Haihe, and Qiantang Rivers. It begins in Beijing in the north, flows through Tianjin city, Hubei, Shandong, Jiangsu, and Zhejiang provinces, and end in Hangzhou in the south. In recent years, with industrial development, part of the Canal river has been seriously polluted. A lot of production waste and life sewage were poured into the Canal. The water became stinking, the color of the water was black, and many kinds of garbage were floating on it. The pollution had a serious impact on the lives of people along the coast. What's worse, the ancient buildings were disappearing, and cultural relics have been damaged. However, protecting the Canal is a very difficult task, as it runs through several provinces. The government has accumulated a large number of customary monitoring data (chemical methods) for many years, but absent from the biological evaluation that can measure the effect of pollution content and source to the environment.

Bioassays do not measure the cause of the pollution, but they measure directly the polluted water effect on test organisms. The brine shrimp (*Artemia salina* Leach) is routinely used as an test organism for screening in ecotoxicological studies. The organism belongs to the phylum Arthropoda, class Crustacea. Their life cycle begins by hatching of dormant cysts where these cysts are inactive, but once in salt water, they become rehydrated and resume their development (Pelka et al. 2000). These larvae are characterized by common features such as adaptability to wide ranges of salinity (5–250 g/L) and temperature (6–35°C), short life cycle, high adaptability to adverse environmental conditions, high fecundity, bisexual/parthenogenetic reproduction strategy (with nauplii or cysts production), small body size, adaptability to varying nutrient resources as it is a non-selective filter feeder, sensitive to toxic substances, and simple equipment used for the measurements (Persoone and Wells 1987). Accordingly, the intrinsic features of this species turn it into a suitable organism for use in ecotoxicology, guaranteeing reliability, feasibility, and cost-effectiveness in routine and/or research ecotoxicity practices. The aim of this study is to investigate the application of brine shrimp larvae bioassays for the acute toxicity assessment of water quality of Hangzhou Section of Beijing-Hangzhou Grand Canal.

## Materials and Methods

Water samples were collected from five different locations in the area of Hangzhou Section of Beijing-Hangzhou Grand Canal. A schematic diagram of the corresponding sampling points (sites A–E) was shown in Fig. 1. Sampling sites in map were selected in order to cover a range of places, characterized by materials on nature, society, economy, disposal of pollutants, and a different degree of environmental impact from various anthropogenic activities. The Canal ran through the whole city from north to south. In the northernmost part of the section lay the sampling site A (Tangxi Bridge), which communicated with the rest of Jiangsu Section of the Canal at the south boundary. Sites B (Yi Bridge) and C (Gongchen Bridge) were in the commercial port and close to the industrial areas. Sampling site D (Maiyu Bridge) was in the city center with an urban population of approximately 2,218,000 inhabitants, which was supposed to be most polluted due to anthropogenic activities. And the last site E (Gujia Bridge) was in a small bay in the opening of Qiantang River.

To compare the data, water samples had been collected from the same sampling points during October 2008 and April 2009. About 1 L of water samples was collected in glass containers. Sampling was done in shallow waters from piers or docks. The water samples were transferred to the laboratory within 6 h from the sampling time and maintained at 4°C until the bioassay, which was generally performed either the same day or after 24 h.

Hatching procedure followed the one described in ARC-test, standardised short-term toxicity test with *Artemia* nauplii (Vanhaecke and Persoone 1981). Approximately, 1–2 mL of brine shrimp eggs (obtained commercially from Bo Hai, China) was incubated in Ca 10 mL of artificial salt water (25 g NaCl/L oxygen saturated distilled water) in a

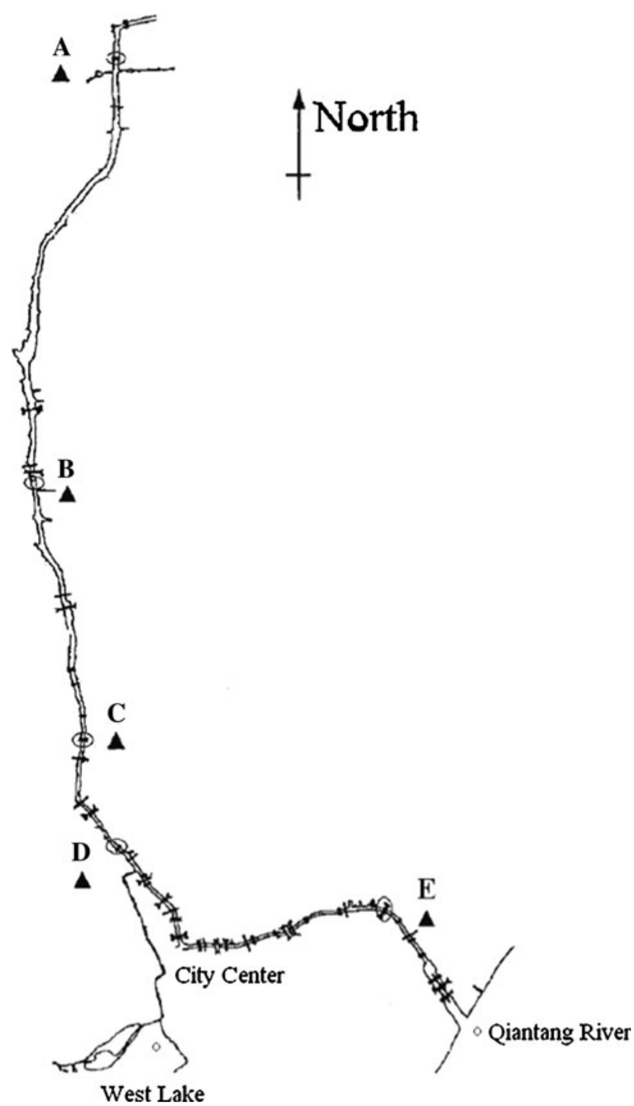


**Fig. 1** Schematic diagram of Hangzhou Section of Beijing-Hangzhou Grand Canal indicating the sites for water samples

petri dish at a temperature of 25°C and constant illumination (60 W lamp). After 48 h from the start of the incubation, all larvae had molted to the instar 2–3 stages, which can swim freely. These instars have, in several papers, been shown to be the most sensitive stage (Sanchez-Fort′un et al. 1996, 1997) and were accordingly used for our experimental bioassay. At this time, the larvae were still living on their own yolk sac and no feeding required during the experimental time.

First, the water samples were adjusted to a salt (NaCl) content of 25 g/L and filter-sterilized (0.22 μm filter). Five replicates were prepared and tested for each sample, and 10 organisms were transferred into each well of a multiwall plate by a Pasteur pipette, containing 5 mL of the test sample saturated by oxygen. This was facilitated by attracting the shrimp to one side of the petri dish with a light source. Analytical-grade potassium dichromate ($K_2Cr_2O_7$, Sigma) was employed as reference toxicant to validate the test procedures. The appropriate media were used as negative controls. The plate was put in the incubator at 25 ± 1°C under direct light. The test end point was death. The number of dead shrimp was recorded after 24-h exposure to the test samples, by using a binocular dissection microscope to count the number of dead species. The tests were considered valid only if the percentage immobility in the control wells did not exceed 10%. There was no feeding and no aeration during the test.

Toxicity results were expressed as percentage mortality adjusted relative to the control, following Abbot's formula:

$$\%\text{Toxicity} = (I_t - I_O)/(1 - I_O) \times 100$$

where It denoted the observed mortality of water samples and $I_O$ represent the natural mortality of negative control. All the analyses were carried out in triplicate, and the results were expressed as the mean ± SD. Statistical analyses were performed by $t$ test. The difference was considered significant if $p < 0.05$.

## Results and Discussion

The water quality is a very important factor for the development of the city, which attracts large number of tourists. The discharge of untreated or partially treated wastewater over a long period and of agricultural wastewater containing fertilizers and pesticides, drained by the rivers, has rendered the entire Canal, and especially the north area where the industrial zone is located. Forty-six manufacturing plants are included here such as food industries, leather tanneries, chrome plating companies, petrochemical refineries, steel manufacturing, and electrolytic manganese dioxide factories, discharging their partially treated effluents to the Canal. The pollutant discharge is estimated as more than $7.9 \times 10^7$ t per year of sewage and $1.5 \times 10^8$ $m^3$/a per year of industrial effluents. Due to the continuous discharge of all these pollutants into the Canal, the concentration of nutrients has increased, while DO shortage below the allowed limits was experienced in some places, especially near the sediment. Typical metal concentrations that have been measured in water samples were shown in Table 1 (A: Tangxi Bridge, B: Yi Bridge, C: Gongchen Bridge, D: Maiyu Bridge, E: Gujia Bridge) (Zhang and Qian 2000). Under these extreme conditions, some species of fishes and other organisms have almost disappeared from this area. A major wastewater treatment project treating municipal effluents has started operation on 2001, while treatment of the whole sewage has been accomplished only recently.

The objective of acute toxicity test is to evaluate the effect of the water samples on the survival of test organism. Because of the correlation between shrimp mortality to pollutant and their potential of inhibiting ceramide synthase (Merrill et al. 1993), which is thought to be a crucial event in the mechanism of action, the assay could be applied to any water samples and might allow conclusions to be drawn about their toxicity relative to pollution level via comparison of the percentage mortality. In this study, the *A. salina* bioassay yielded a 24-h $LC_{50}$ value of 23 mg/L for the reference chemical potassium dichromate. Persoone et al. (1989) previously reported $LC_{50}$ values for potassium dichromate of 27.5 mg/L (25°C and 20‰), and 22.2 mg/L (25°C and 35‰). The effect of Canal water samples collected from the five different sampling sites on the death of brine shrimp was given in Table 2 (A: Tangxi Bridge, B: Yi Bridge, C: Gongchen Bridge, D: Maiyu

**Table 1** Correlation coefficient (R) and average contents of the organic matter and heavy metals (monitored from 1990 till 1996)

| Site | Item (mg/L) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | OM | Hg | Cr | Pb | Cu | Ni | Cd | Zn | Mn |
| A | 4.76 | 0.311 | 103 | 85.3 | 84.5 | 38.9 | 1.683 | 1,111.0 | 6.56 |
| B | 7.01 | 0.506 | 120 | 128 | 177 | 52.1 | 2.415 | 1,768.4 | 565 |
| C | 10.11 | 0.958 | 131 | 143.4 | 329 | 61.1 | 4.471 | 1,097.3 | 540 |
| D | 12.22 | 1.336 | 117 | 127 | 151 | 43 | 0.835 | 657.2 | 490 |
| E | 1.62 | 0.114 | 77.1 | 21 | 29 | 38.3 | 0.782 | 399.4 | 352 |
| R | – | 0.9784 | 0.7638 | 0.7174 | 0.6591 | 0.3678 | 0.3152 | 0.1546 | −0.1823 |

*OM* organic material

**Table 2** Toxicity assessment (%) of Hangzhou Section of Beijing-Hangzhou Grand Canal with brine shrimp as a test organism

| Site | Toxicity results (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | October 2008 | | | | April 2009 | | | |
| | Aver. | Min. | Max. | SD | Aver. | Min. | Max. | SD |
| A | 11.0 | 0 | 20.0 | 8.3 | 5.7 | 0 | 10.0 | 4.2 |
| B | 26.7 | 10.0 | 50.0 | 17 | 10.3 | 0 | 20.0 | 8.2 |
| C | 31.7 | 20.0 | 40.0 | 8.5 | 24.3 | 10.0 | 40.0 | 12.3 |
| D | 28.0 | 0 | 40.0 | 11.7 | 16.0 | 0 | 30.0 | 12.3 |
| E | 4.0 | 0 | 10.0 | 4.3 | 0 | 0 | 0 | 0 |

Bridge, E: Gujia Bridge). As it was presented in this table, toxicity of natural water was generally low, except for the sampling site B, C, and D collected from the area close to the industrial location and city center. The results of toxicity assessment were similar with the published data (Table 1) on the evaluation of water pollution about the concentrations of organic matter and heavy metals in same sites between year 1990 and 1996. When comparing the bioassay results of two tests performed at two time periods (Fig. 2), a relative improvement in water quality was observed between October 2008 and April 2009, although the results were not significantly different at the $p < 0.05$ level. One possible reason for this improvement was the sewerage cutoff and drawing river water to wash out sewerage, which began on 1998. Equivalent standard pollution load method was applied to assess the sewage resources. Eighty sewage sources were ensured as the main sewage sources, and COD, $BOD_5$, and $NH_3$–N were illuminated as the main pollution factors (Zheng et al. 2005). It was advised that sewage interception should be carried to cut the most sewage sources. The improvement in water quality was relatively clear in midland area (sampling site B, C, and D). This result could be attributed to the extension of the capacity of Hangzhou city municipal wastewater treatment plant. Another possible explanation was the fact that rainfall height in Hangzhou was generally high in the period from March to June, but very low from July to November. Therefore, dilution of toxicants was lower in early autumn (October) than in spring (April), as well as potential toxicity of water was considered to be higher. On the other hand, water toxicity of site E was generally very low, which could be attributed to the location moving towards the open of Qiantang River.

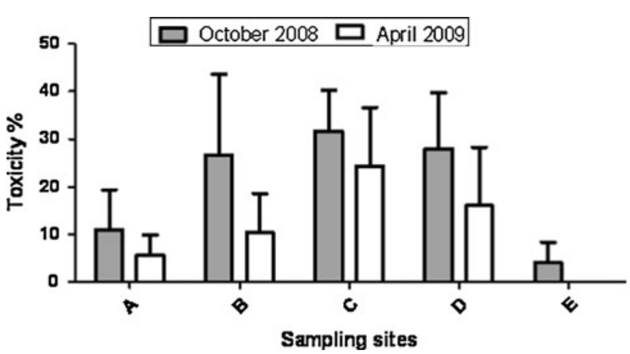


**Fig. 2** Comparisons of water toxicity between October 2008 and April 2009 in Hangzhou Section of Beijing-Hangzhou Grand Canal, using brine shrimp as a test organism

Although the brine shrimp assay is rather inadequate regarding the elucidation of the mechanism of action, it actually has proven to be a convenient system for monitoring the water quality. The assay is inexpensive, uncomplicated to establish, and simple to handle, not requiring particular equipment or aseptic techniques. However, during determination of water toxicity by brine shrimp, special care should be taken for the effect of salinity; standards of same salinity with the sample have to be prepared. If such factors as temperature, composition and salinity of the medium, and the age of the larvae are considered, a fairly satisfactory repeatability can be achieved. By performing the test in the micro-well scale, rather low toxin amounts are sufficient. These advantages make the brine shrimp assay attractive, despite its limitations in the mechanistic field. However, it should be mentioned that the correlation of water chemical and physical parameters to the bioassay results is difficult to be obtained as the analysis of all potentially toxic compounds is very expensive and time consuming, while sometimes there are no available analytical techniques for the quantification of chemical substances at low concentrations. At this point, in addition, several effects may be developed due to the combined action of the individual components resulting to the increase of the toxicity of a mixture. As a result, bioassay is often not specific enough, but rather gives a value of the total acute toxicity, providing useful clues of the relative toxic potential of water samples. It is supplementary to the chemical analysis, in order to determine the ecological quality of a water body and to evaluate the effect of water discharges to the aquatic life. At the same time, since no single marine organism is sensitive to all potential toxicants, it is essential that a battery of bioassays should be used for a proper assessment of water quality.

**Acknowledgments** The authors of this study express their gratitude to National Natural Science Foundation of Zhejiang Province

(Y5090304), Scientific and Technological Innovation Grant of undergraduates of Zhejiang Province, for financial support of this study.

## References

Merrill AH Jr, van Echten G, Wang E, Sandhoff K (1993) Fumonisin B1 inhibits sphingosine (sphinganine) N-acyltransferase and de novo sphingolipid biosynthesis in cultured neurons in situ. J Biol Chem 268:27299–27306

Pelka M, Danzl C, Distler W, Petschelt A (2000) A new screening test for toxicity testing of dental materials. J Dent 28(5):341–345

Persoone G, Wells P (1987) *Artemia* in aquatic toxicology: a review, morphology, genetics, strain characterization, toxicology. In: Sorgellos P, Bengtson DA, Decleir W, Jaspers E (eds) *Artemia* research and its applications, vol 1. Universa Press, Belgium, pp 271–350

Persoone G, Van de Vel A, Van Steertegem M, De Nayer B (1989) Predictive value of laboratory tests with aquatic invertebrates: influence of experimental conditions. Aquat Toxicol 14(2): 149–166

Sanchez-Fort′un S, Sanz F, Barahona MV (1996) Acute toxicity of several organophosphorous insecticides and protection by cholinergic antagonists and 2-PAMon *Artemia salina* larvae. Arch Environ Contam Toxicol 31(3):391–398

Sanchez-Fortun S, Sanz F, Santa-Maria A, Ros JM, De Vicente ML, Encinas MT, Vinagre E, Barahona MV (1997) Acute sensitivity of three age classes of *Artemia salina* larvae to seven chlorinated solvents. Bull Environ Contam Toxicol 59(30):445–451

Vanhaecke P, Persoone G (1981) Report on an intercalibration exercise on a short-term standard toxicity test with *Artemia* nauplii (ARC-test). In: Leclerc H, Dive D (eds) Les tests de toxicité aigue en milieu aquatique. Les colloques de l'INSERM. Ministère de la Santé, Institut National de la Santé et de la Recherche Médicale, Paris, pp 359–376

Zhang M, Qian TM (2000) Discussion on correlativity between organic matter and heavy metals in sediment of the Grand canal (Hangzhou Section). Environ Pollut Control 22(2):32–44 (in Chinese)

Zheng AB, Wang CH, Ruan JA (2005) The water quality analysis for Beijin-Hangzhou Grand Canal (Hangzhou Section) and countermeasures for pollution. J Shenyang Agric Univ 36(5):631–633 (in Chinese)