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Mutagenicity and estrogenicity of raw water and drinking water in an industrialized city in the Yangtze River Delta

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HIGHLIGHTS

- Drinking water from the Yangtze River and Taihu Lake water was monitored from 2010 to 2016.
- Estrogenicity in the raw water and mutagenicity in the finished water were noticed.
- In terms of mutagenicity and estrogenicity, the Yangtze River had poorer water quality.

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ABSTRACT

Public concern was aroused by frequently reported water pollution incidents in Taihu Lake and the Yangtze River. The pollution also caught and sustained the attention of the scientific community. From 2010 to 2016, raw water and drinking water samples were continually collected at Waterworks A and B (Taihu Lake) and Waterworks C (Yangtze River). The non-volatile organic pollutants in the water samples were extracted by solid phase extraction. Ames tests and yeast estrogen screen (YES) assays were conducted to evaluate the respective mutagenic and estrogenic effects. Water samples from the Yangtze River-based Waterworks C possessed higher mutagenicity than those from Taihu Lake-based Waterworks A ($P < 0.001$) and Waterworks B ($P = 0.026$). Water treatment enhanced the direct mutagenicity ($P = 0.022$), and weakened the estrogenicity of the raw water ($P < 0.001$) with a median removal rate of 100%. In fact, very few of the finished samples showed estrogenic activity. Raw water samples from Waterworks A showed weaker estrogenicity than those from Waterworks B ($P = 0.034$) and Waterworks C ($P = 0.006$). In summary, mutagenic effects in drinking water and estrogenic effects in raw water merited sustained attention. The Yangtze River was more seriously polluted by mutagenic and estrogenic chemicals than Taihu Lake was.

1. Introduction

Advances in instrumental analysis techniques have made it possible to detect more and more pollutants in the water environment. However, no matter how advanced these techniques are, it is impossible to monitor all the organic pollutants in water. Considering the many different constituents and the extremely low concentrations of toxic organic pollutants, they are that much more difficult to detect and evaluate (Busch et al., 2016; Caban et al., 2016; Wieczerzak et al., 2016). The latest drinking water quality standards established by the World Health Organization, European Union, United States and China include 172, 48, 84 and 106 items, respectively, and more pollutants are likely to be identified in the future as scientists are discovering new pollutants with potential health hazards. In the field of environmental monitoring, in vitro tests are being rapidly developed. They are able to evaluate the biological effects of mixed trace organic pollutants, such as acute and chronic toxic effects, mutagenicity, estrogenicity, etc. This is very important when conducting risk assessment, without a need for component identification (Di Paolo et al., 2016; Wieczerzak et al., 2016; Teta and Naik, 2017). Mutagenicity and estrogenicity

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of raw water and drinking water are becoming major public health concerns. This is due to the observed toxicities of raw water, and because some of the by-products created during water purification process were identified as mutagenic chemicals (Warren et al., 2015; Manasfi et al., 2016; Praveena et al., 2016; Xiao et al., 2016).

Taihu Lake, located in the Yangtze River Delta, is the third largest freshwater lake in China, supplying daily drinking water for the densely populated cities around it. With the rapid development of the surrounding cities, Taihu Lake is receiving too much wastewater. Yan et al. detected elevated serum vitellogenin in fish collected from Taihu Lake, showing that estrogenic chemicals were disturbing the reproductive and developmental functions of the aquatic animals in Taihu Lake (Yan et al., 2012). Endocrine disruptors and mutagenic chemicals were widely detected in the sediment and water. Some of them exceeded the ecological toxicity threshold concentration (Zhao et al., 2010; Lu et al., 2011). Pollutants concentrated in the sediment make water pollution difficult to control. A variety of measures have been taken to ensure the lake water meets the drinking water quality requirements in China (Schmidt et al., 2016). Several waterworks have applied advanced water treatment technologies. These technologies will be used in all large waterworks, so that the water will be effectively protected in the future.

The Yangtze River in the north is a huge source of water, with a strong self-purification capacity. It is playing an important role in ensuring the safety of the water supply in the cities around Taihu Lake (Qin et al., 2010). Most of the monitoring indicators showed that the Yangtze River's water quality was superior to that of Taihu Lake (Li et al., 2013a,b). However, persistent concerns about the water quality of the Yangtze River have been raised; the cities around Taihu Lake are downstream of the Yangtze River, and the upstream pollutants cannot be removed by the downstream cities (Wang et al., 2016; Xiao et al., 2016; Yi et al., 2016).

Over the years, with the help of local water quality monitoring organizations and CDCs (Centers for Disease Control and Prevention), we have been committed to assessing the raw water and drinking water of the cities around Taihu Lake by Ames test and YES (yeast estrogen screen) assay. This research explores (a) the occurrence of mutagenic and estrogenic activities of raw water and their drinking water in the past few years; and (b) the effect of water treatment for mutagenic and estrogenic activities. This leads to the question: which provides better drinking water, the Yangtze River or Taihu Lake?

2. Materials and methods

2.1. Water sampling and solid phase extraction

Sampling campaigns were conducted in 2010 (October), 2011 (March and August), 2012 (March), 2013 (January, March, June, August, October and December), 2014 (January, March, June, August and November), 2015 (January, March and September) and 2016 (January) in a city in the Yangtze River Delta. Two Taihu Lake raw water samples and one Yangtze River raw water sample, and their subsequent drinking water, were studied. Only one sample was collected at each sampling point. There were 19 sampling campaigns, but only samples from 8 campaigns were tested for mutagenic effects mainly because it was difficult to collect 60 L of water. Waterworks A (including A1 and A2, which used the same Taihu Lake raw water and pretreatment procedures, but used different plants to further purified the water) and Waterworks B which purified raw Taihu Lake water from a different intake, while Waterworks C treated the Yangtze River water. Waterworks A and B use advanced water treatment processes, including ozonation, biological aerated filter, coagulation and sedimentation, sand filtration, activated carbon filtration, polyvinylidene fluoride (PVDF) member filtration, and chlorination. Waterworks C use conventional water treatment processes, including coagulation and sedimentation, sand filtration, and chlorination.

Water samples were temporarily collected in stainless steel buckets (60 L, for Ames test) and amber glass bottles (4 L, for YES assay) before the extraction of nonvolatile organic chemicals from the water. 60 L of water was extracted by a column filled with 40 mL of XAD-2 resin at a speed of 40 mL of water per minute. The XAD-2 resin was in turn cleaned by methanol, acetone, dichloromethane and hexane using the Soxhlet extraction method to ensure the XAD-2 resin was free of organic pollutants. 4 L of water was extracted by a C18 cartridge (6 mL, 1 g) which was preconditioned with 5 mL of methanol and 5 mL of deionized water, respectively, before use, at the speed of 4 mL of water per minute. The columns and C18 cartridges were brought back to the lab for further processing.

The columns were dried in a vacuum freezing dryer overnight before eluting. Organic chemicals extracted by the XAD-2 resin were then eluted by 160 mL of acetone and hexane (v:v, 3:1) and 80 mL of dichloromethane. The eluent was dried in a rotary evaporator and then the extracts were dissolved in 3 mL of dimethyl sulphoxide (DMSO) to make non-volatile organic compounds in 2 L of water dissolved by 0.1 mL of DMSO solution.

The C18 cartridges were dried in a vacuum freezing dryer for 2 h before eluting. 2 mL of methanol, dichloromethane, and hexane were in turn added into the cartridges and collected in a glass tube. The eluent was dried by a gentle nitrogen stream with a water bath temperature of 40 °C, and then re-dissolved in 1.6 mL of anhydrous alcohol to achieve an enrichment factor of 2500.

2.2. Ames test

Salmonella typhimurium strains TA98 were used to evaluate the frame-shift mutation of water samples. The strains used in the experiment were provided by Prof. B. N. Ames (University of California, Berkley, USA) with normal characteristics. 2 mL molten top agar with biotin and trace of histidine in a tube was added with 0.1 mL of extracts and 0.1 mL of bacterial suspension with and without 0.1 mL of S9 (a microsomal enzyme metabolic activation mixture). The S9 fraction was prepared from rat liver which was induced by Aroclor 1254 and was used to simulate human metabolism. The top agar was then fully shaken and poured into a glucose minimal agar plate. The negative solvent control was conducted by replacing the extracts with DMSO. Each sample was checked three times and the median number of colonies on the three plates was recorded after 48 h of incubation in an environment of 37 °C. A two-fold increase in the mutation ratio (MR, colonies on test plate/colonies on DMSO plate) was considered a positive mutagenic response (Mortelmans and Zeiger, 2000). Ames test (−S9) and Ames test (+S9) were to evaluate the direct and indirect mutagenicity, respectively.

2.3. YES assay

YES assays were used to compare the estrogenic effects of water samples and 17β-estradiol, the positive control, to acquire the 17β-estradiol equivalent (EEQ) values of water samples. The recombinant yeast for the test had been transfected with the human estrogen receptor (hER) gene, which contained an expression plasmid carrying promoter sequences and the reporter genelac-Z (β-galactosidase). YES assays were conducted in a 96-well plate with a positive control row (1500−0.37 ng/L), a blank control row, and six samples rows (125−0.03 mL) where two samples were measured three times in parallel. Samples were added to the first well of each