Supporting Information

Phosphite in Sedimentary Interstitial Water of Lake Taihu, a Large Eutrophic Shallow Lake in China

Chao Han,[a] Jinju Geng,[a*] Hongqiang Ren,[a] Shixiang Gao,[a] Xianchuan Xie,[b] Xiaorong Wang[a]

[1]State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, 163 Xianlin Avenue, Nanjing 210046, China

[2]Center for Hydroscience Research, Nanjing University, 22 Hankou Road, Nanjing 210093, China

* Corresponding author Jinju Geng   e-mail: jjgeng@nju.edu.cn (J.Geng).

Journal: *Environmental Science and Technology*

This file includes 1 table, 4 figures and 3 detailed informations

S1: Details on the Cleaning and Sampling Protocol
S2: Biochemical and Geochemical Reactions of Phosphite
S3: Secondary Confirmation of the phosphite by 2D-CIC and IC-ESI-MS

Table S1 Physico-chemical Properties of Waters from the Investigated Locations during Four Seasons*

Figure S1 Phosphorus fractions in the investigated locations during four seasons at Lake Taihu
Figure S2 Eh-pH diagram for P species in water. (Pasek, 2008)
Figure S3 2D-CIC chromatogram of phosphite in Lake Taihu bottom water (black line), interstitial water (red line), and spiked water (blue line).
Figure S4 IC-ESI-MS chromatograms of phosphite in Lake Taihu bottom water (black line), interstitial water (red line), and spiked water (blue line).

Table S1 Physico-chemical Properties of Waters from the Investigated Locations during Four Seasons*

	T_w (°C)	pH_w	ORP_w (mV)	Chl-a (μg/L)	DO_w (mg/L)	$EC_w(\times10^2$ μS/cm)	Sal_w (ng/L)
Spring							
DPH	23.88	7.19	100.30	15.30	4.38	7.71	0.39
GHB	22.17	7.81	40.40	4.20	8.99	6.08	0.31
MLB	23.09	7.80	72.90	4.00	9.91	6.39	0.32
ZSB	24.29	7.52	96.10	9.40	7.42	8.16	0.40
SWS	23.49	7.89	125.70	6.10	10.16	5.56	0.28
SS	25.57	7.88	119.80	7.50	9.46	4.63	0.22
DTB	24.43	7.74	117.90	7.30	9.55	5.29	0.26
SCL	25.00	7.82	65.70	5.60	9.13	6.85	0.33
Summer							
DPH	31.33	9.08	-5.50	19.60	11.14	4.01	0.19
GHB	31.74	8.52	112.90	1.10	7.65	5.31	0.25
MLB	31.87	9.48	101.60	5.10	12.64	5.35	0.25
ZSB	31.40	8.70	100.10	19.40	10.77	6.24	0.30
SWS	32.73	8.69	104.20	0.50	7.58	4.81	0.23
SS	32.63	8.46	95.90	0.70	7.88	6.39	0.31
DTB	32.91	8.45	96.10	0.70	7.38	0.00	—
SCL	32.87	8.33	99.20	0.40	7.33	6.32	0.30
Autumn							
DPH	17.91	7.29	51.80	6.60	7.70	8.42	0.42
GHB	17.13	7.60	134.80	10.80	9.65	5.58	0.27
MLB	17.38	7.58	116.20	2.70	9.42	5.00	0.24
ZSB	17.64	7.48	112.00	11.30	8.87	8.42	0.42
SWS	16.38	7.43	101.60	7.10	9.75	5.39	0.26
SS	16.24	7.53	112.30	11.40	9.62	5.22	0.25
DTB	16.33	7.42	92.40	8.10	10.29	5.27	0.26
SCL	16.40	7.80	80.50	3.60	9.53	5.15	0.25
Winter							
DPH	7.05	6.61	106.50	2.60	11.38	8.33	0.41
GHB	5.51	7.53	111.30	5.40	13.08	4.73	0.23
MLB	5.66	7.27	95.70	2.60	14.00	6.95	0.34
ZSB	5.52	7.33	93.90	2.60	13.14	7.63	0.37
SWS	4.67	6.83	59.80	9.70	13.74	5.82	0.28
SS	4.64	6.93	84.80	14.00	13.71	5.35	0.26
DTB	4.82	7.03	75.20	13.70	12.99	5.44	0.26

*Note for Table S1: The data in this table reports water quality including water temperature (T_W), water pH (pH_w), water redox potential (ORP_w), chlorophyll-a (Chl-a), dissolved oxygen (DO_w), water electronic conductivity (EC_w) and water salinity (Sal_w). All these date were measured in the field using portable multi-parameter water quality instrument YSI6600 (YSI, USA).

Phosphorus fractions in water samples including total phosphorus (TP_w) and total soluble phosphorus (TSP_w), and total phosphorus in sediments(TP_s) shown in Figure S1a,b,c were measured by ascorbic acid-molybdate blue molybdate.
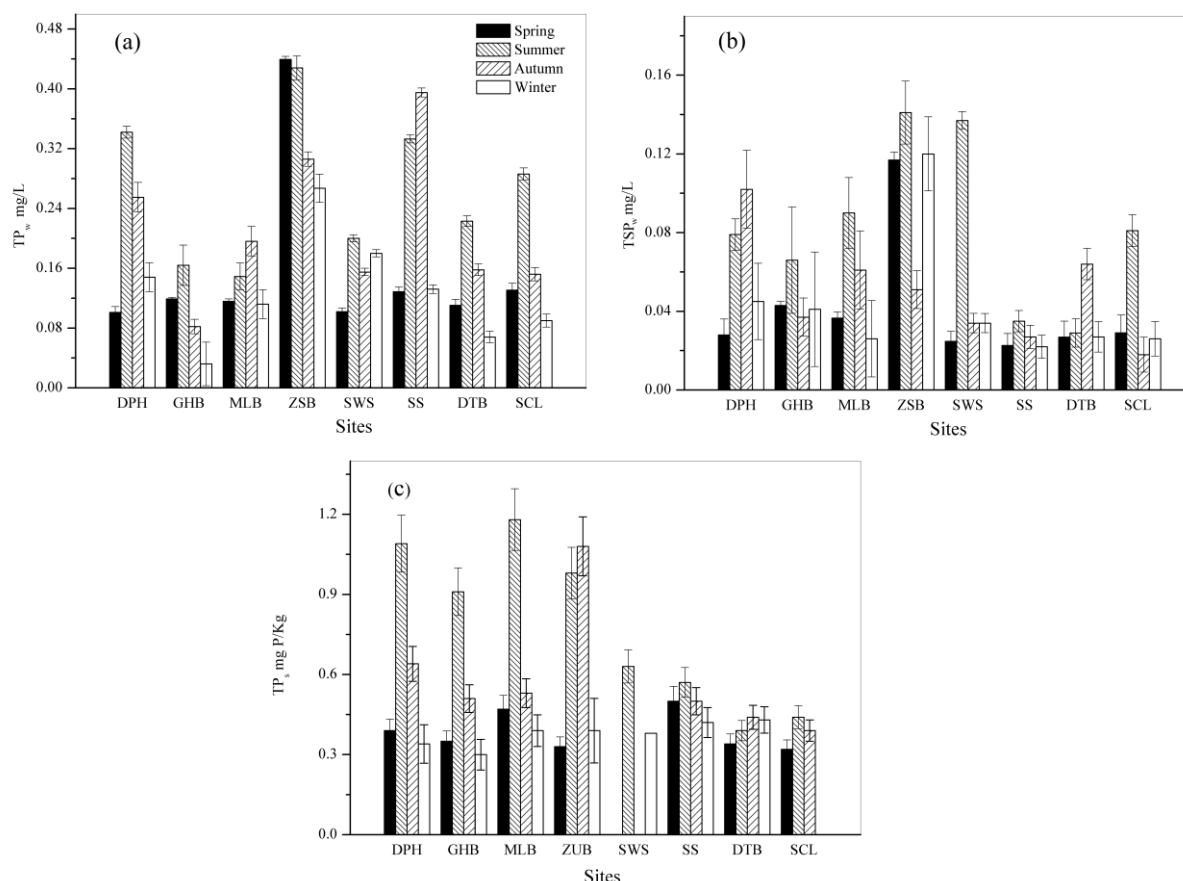
Figure S1 Phosphorus fractions in the investigated locations during four seasons at Lake Taihu

S1: **Details on the Cleaning and Sampling Protocol**

Prior to the sampling campaign, the cylindrical pipes and Peterson grab were cleaned with Decon 90 cleaning agent (phosphate-free) at least twice, then rinsed with running water and ultrapure water, respectively. The first two collections of sediment at each site were discarded, the third collection was preserved for analysis. It was also recommended that containers for the interstitial waters be cleaned overnight with Decon 90 cleaning agent, rinsed with ultrapure water at least twice. As with sample containers, the individual filters (syringe driven filters) must be cleaned prior to use. As for the OnGuard II Ag cartridge and OnGuard II RP cartridge, before application

of the sample, one must flush the OnGuard II RP cartridge first with 5 mL of methanol and then with 10 mL of deionized water, flush the OnGuard II Ag cartridge first with 15 mL deionized water. Then, the OnGuard II Ag cartridge must be flushed with 15 mL of deionized water.

Additionally, we have studied the preserved procedure which has been described in our previous publication (Han et al., 2012). As suggested, we have added a supplementary section about the procedure in the revised manuscript as follows: "The columnar sediments were immediately divided into three layers at 20 cm intervals and stored in black air-sealed plastic bags. Whole sediment samples were centrifuged at 8,000 rmp for 25 min to extract interstitial water by a CT15RT versatile refrigerated centrifuge (China). Interstitial waters were extracted as soon as sediment samples were collected and preserved at 0-4 ˚C in the dark and analyzed within two weeks. Our preliminary investigation has shown that phosphite was stable for at least 14 days under proposed preservation protocols."

S2: Biochemical and Geochemical Reactions of Phosphite

Some possible routes for phosphite production hae been previously reported such as 1) Schreibersite will react with water to form phosphite as a primary product; thus, the likely pathway that led to the production of phosphite is reduction of phosphate into phosphide followed by subsequent alteration of the phosphide to phosphite during extraction process; $2Fe_3P+14H_2O \rightarrow 2H_3PO_3+2F_3O_4+11H_2$ (Pasek et al., 2009); 2) It was demonstrated that among the bacteria, which produce the low redoxpotential in their medium, such bacteria as *Clostridium butyricum* and *Escherichia coli* take part in the phosphate reduction. After one week's incubation of the reductive medium which contained orthophosphate and was inoculated with a small amount of soil, considerable amounts of phosphite and hypophosphite could be detected (Tsubota 1959). In addition, Phosphite can serve as P source for many bacteria, such as strain FiPS-3 isolated from marine sediments, which grew by anaerobic oxidation of phosphite to phosphate while simultaneously reducing sulphate to hydrogen sulphide according to $4HPO_3^{2-}+H^++SO_4^{2-} \rightarrow 4HPO_4^{2-}+ HS^- (\Delta G°'= -364$ kJ/moL per mol sulphate, or 191 kJ per mol phosphite) (Schink and Friedrech, 2000); and 3) Phosphorus can also be released into water during iron corrosion as phosphite and hypophosphite. For instance, in preliminary tests, cast iron filings (0.2% P) were immersed in 10^{-3} M NaOH, and more than 0.5 mg/L P of phosphite was present in water after 21 days (Morton et al., 2005).

References:
Pasek, M.; Block, K. Lighting-induced reduction of phosphorus oxidation state. *Nat. Geosci.* 2009, 2, 553-556.
Tsubota, G. Phosphate reduction in the paddy field I. *Soil and Plant food. 1959,* 5(1),10-15.
Morton, S. C.; Glindemann, D.; Wang, X. R.; Niu, X. J.; Edwards, M. Analysis of

reduced phosphorus in samples of environmental interest. *Environ. Sci. Technol.* 2005**,** 39 (12), 4369-4376.

Schink, B.; Thiemann, V.; Laue, H.; Friedrich, M.W. Desulfotignum phosphitoxidans sp nov., a new marine sulfate reducer that oxidizes phosphite to phosphate. *Arch. Microbiol.*2002, 177, 381-391.
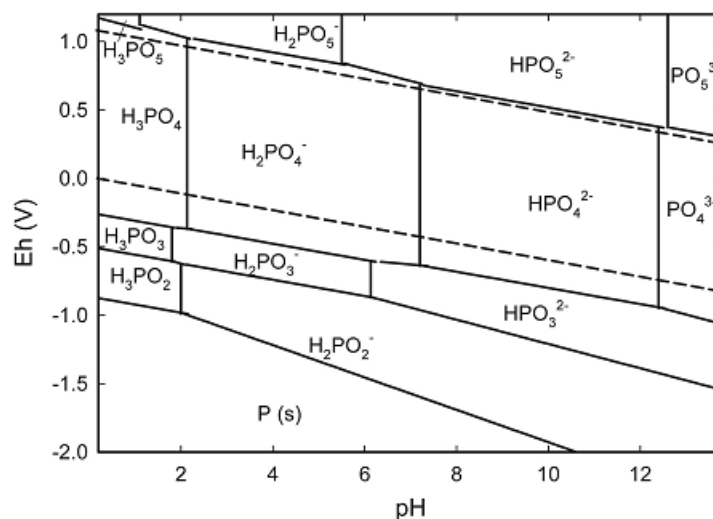

Figure S2 E*h*-pH diagram for P species in water. (Pasek, 2008)

Reference

Pasek, M. Rethinking early Earth phosphorus geochemistry. *P. Natl. Acad. Sci. USA.* 2008, 105 (3), 853–858.

S3: Secondary Confirmation of the phosphite by 2D-CIC and IC-ESI-MS

Demonstration of Method Performance Using 2D-CIC and IC-ESI-MS. Figure S3 shows the separation result obtained using two-dimensional capillary ion chromatography. The 2D-CIC technique, which is based on an ICS system, exhibits excellent matrix elimination and analyte enrichment without complex pretreatment thereby providing a correlation greater than 0.999 for the peak area. An injection volume of 10 μL was used in the first dimension and phosphite in the sample was roughly separated from interferences including hydrogen carbonate (Figure S3-a). The majority of interferences can be excluded on-line with a column-switching technique utilizing 2D-CIC which differs from ICS. In this study, a time span of 20 to 20.8 min (shade area in Figure S3-a) was used to transfer 25 μL effluent containing phosphite and minimized interferences from the first dimension into the MAX 100 capillary concentrator column in the second dimension where the analyte could achieve a further separation and concentration. The peak 1 and 1' are identified as phosphite by matching RT of the spiked sample. Compared to the traditional column, the MAX 100 capillary column is a hydroxide-selective monolithic anion-exchange column for fast

separation of organic acids and inorganic anions, concentrating the analyte species and lowering detection limits by 2-5 orders of magnitude. It can be seen that the use of 2D-CIC system improves the separation for phosphite and also improves the MLD to 0.1 ng P/L (Figure S3-b). The results demonstrate that there were micromolar levels of phosphite in the interstitial water and bottom water (Figure S3-b), which was similar to the ICS detected results.
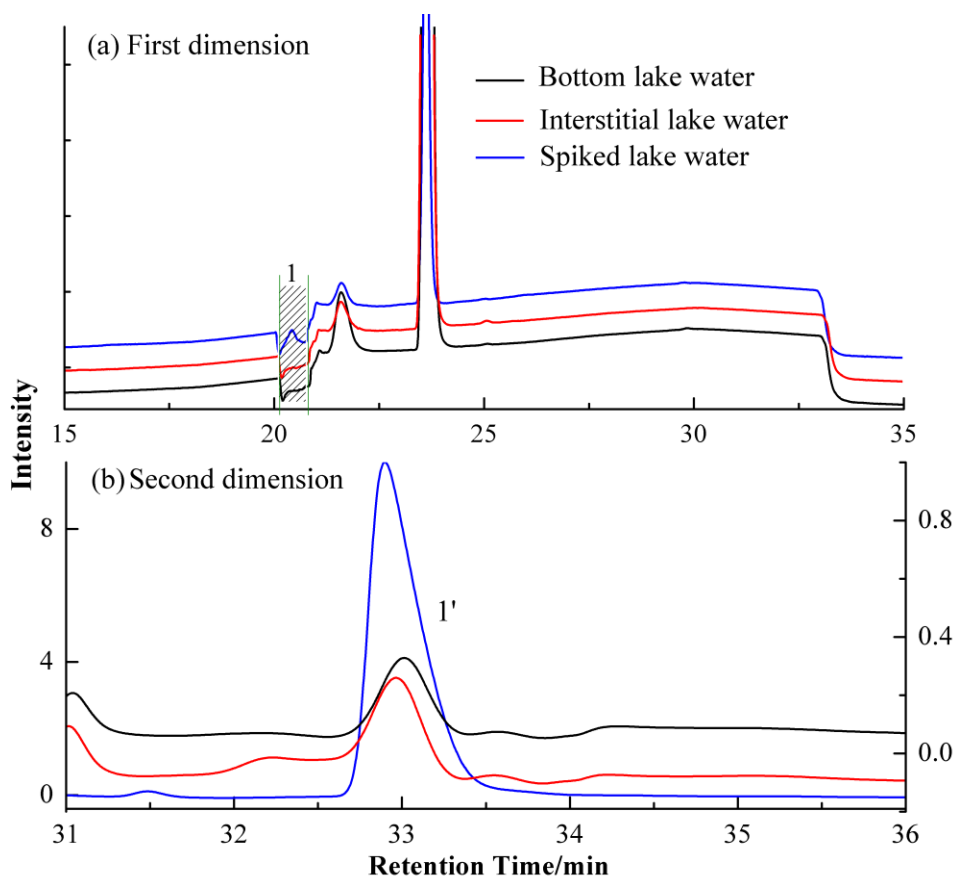

Figure S3. 2D-CIC chromatogram of phosphite in Lake Taihu bottom water (black line), interstitial water (red line), and spiked water (blue line). (a) Detection of phosphite in first dimension. (b) Further detection of phosphite in the second dimension, and data of spiked sample are scaled on the left axis. Both data of interstitial and bottom water were scaled on the right axis.

Figure S4 presents a typical ion chromatography of phosphite in Lake Taihu water samples, which was monitored using a suppressed conductivity detector (Figure S4-a). Single ion monitoring experiments were conducted using mass spectrometer at $m/z = 80.5$-81.5 (Figure S4-b) which corresponds to the single-charged ion of phosphite. Pretreated samples were separated by cation-exchange chromatography in IC, and the suppressed conductivity detector data was collected in the total runtime (40 min). The IC effluent of the time window from 24 to 28 min (shaded areas) was pulsed into the mass spectrometer. The tentative identity of peak 1 (RT = 25.9 min) separated well

from adjacent peaks in Figure 4-a, and was identified as phosphite by matching RT of the spiked sample. Chromatogram in Figure 4-b contains a single and well-defined peak 1' eluted at 26.2 min at m/z = 80.5-81.5. The spiked samples were used to determine that phosphite elutes at 26.2 min in ESI-MS. It has been verified that the peak RT in ESI-MS is always 0.3 min later than that in IC due to the time interval between the two detectors. The peak 1' observed in Figure 4-b has a RT and a m/z in agreement with the assignment of peak 1 to phosphite, and the addition of 1 μM phosphite to water sample resulted in an increase in the peak 1', indicating that the observed single ion at m/z = 80.5-81.5 is phosphite. Results from 2D-CIC and IC-ESI-MS support the conclusion that phosphite is present in Lake Taihu and agree with the ICS data.
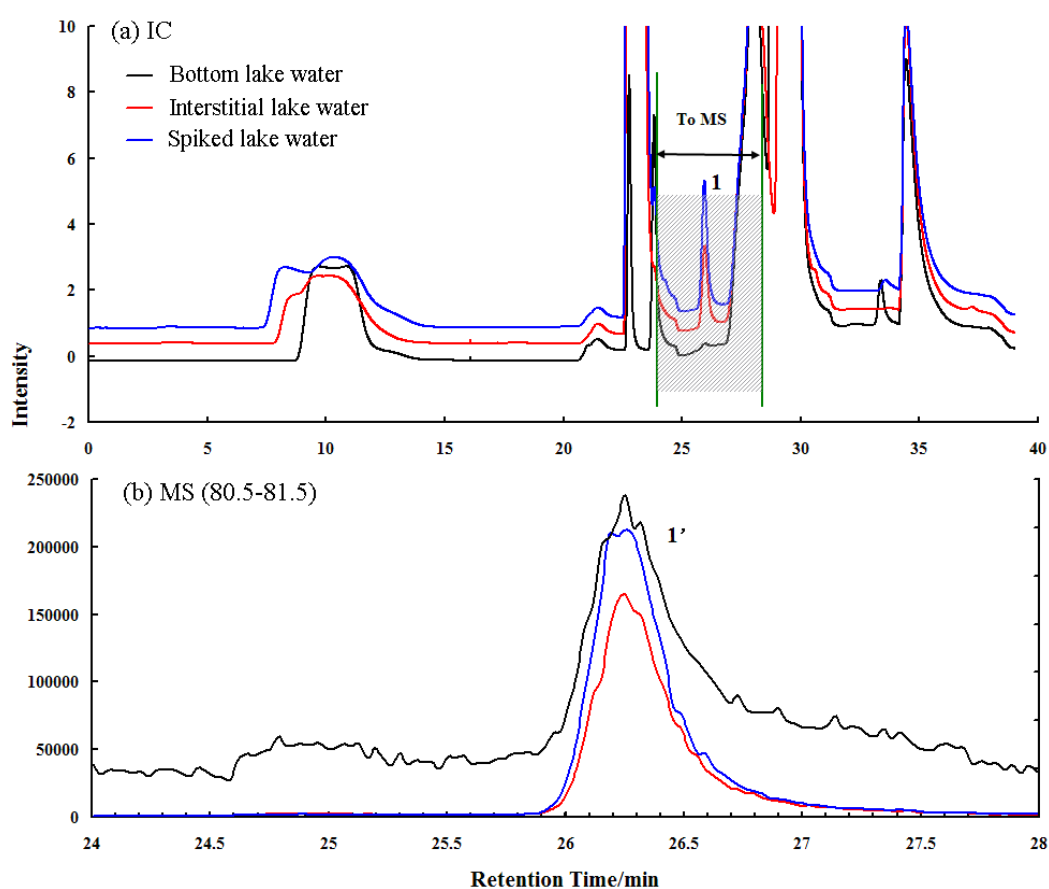

Figure S4. IC-ESI-MS chromatograms of phosphite in Lake Taihu bottom water (black line), interstitial water (red line), and spiked water (blue line). (a) Phosphite was analyzed with suppressed conductivity detector. (b) Phosphite was detected by electrospray mass spectrometry single ion monitoring experiments at m/z = 80.5-81.5, and ESI-MS data of bottom water are scaled on the right axis. Both ESI-MS data of interstitial and spiked water were scaled on the left axis.