Supporting Information for

Impact of agricultural activities on the occurrence of *N*-nitrosamines in an aquatic environment

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Text S1

Well Structure. Unplasticized polyvinyl chloride (UPVC) pipe was used as the main material in the monitoring wells. The diameter of the wells with a depth of 10 m and 25 m is 50 mm. the diameter of the wells with a depth of 50 m is 75 mm. UPVC pipe with a 1 m sliced screen used as the filter pipe was inserted into the drill hole. The annulus was backfilled with clean coarse sand to cover the well screen and then topped with 1 m of bentonite to seal the screen from river water. The remaining fill was with native sediment. Each well was mounted with a concrete apron and sealed with a locked iron cover.[1]

Text S2

Sample Pretreatment and Instrumental Analysis. Each water sample (500 mL) was filtered through a 0.22 μm fiberglass filter paper and then treated with 1 g of sodium bicarbonate ($NaHCO_3$) and 25 g NDMA-d_6 (recovery surrogate) before being extracted through a coconut charcoal cartridge (2 g/6 mL; 80–120 mesh) at a flow rate of < 20 mL/min. The cartridge was conditioned with dichloromethane (6 mL), methanol (12 mL), and ultrapure water (15 mL) before use. The analytes were eluted using dichloromethane (12 mL) and concentrated by using ultra-high-purity nitrogen gas to a final volume of 500 μL. After concentration, 25 ng internal standard NDPA-d_{14} was added to each extract, which was then stored at −20 °C until instrumental analysis. To ensure accurate analysis of the target compounds and compensate for errors due to sample volumes variations and instrument instability, the fixed amount of NDPA-d_{14} was added to each extract before instrument analysis.

Eight *N*-nitrosamines were analyzed using a triple quadrupole mass spectrometer (GC-MS/MS, 7890B-7000C, Agilent) with tandem capillary columns of DB-35 MS (Agilent; 30 m, 0.32 mm i.d., and 0.25 μm film) and HP-5 MS (Agilent; 15 m, 0.25 mm i.d., and 0.25 μm film).[2] These two columns were serially coupled by a zero-dead-volume fitting (the purge union). Electron–ionization mode (70 eV) was used for mass spectrometric ionization. The ion source and transfer line temperatures were set as 230 °C and 280 °C, respectively. The first MRM transition shown for each molecule was used for quantification, while the second transition shown was monitored for confirmation of molecular identification (Table S1).

Text S3

Chemicals and Reagents. All solid reagents ($NaNO_2$, NH_4Cl, $Na_2S_2O_3$, and $NaHCO_3$, AR grade), fiberglass filter paper, coconut charcoal cartridge, *N*-nitrosamine standard products, and two isotope-labeled *N*-nitrosamines (NDMA-d_6 and NDPA-d_{14}) used in the water sampling, pretreatment, and analysis processes were purchased from ANPLE Laboratory Technologies (Shanghai, China), while liquid reagents (dichloromethane and methanol, chromatographic grade) were obtained from Thermo Fisher Scientific (Shanghai, China). Millipore water was produced by a Milli-Q system from Sartorius (Göttingen, Germany).

Text S4

Basic water parameters. NH_4^+ was determined by Nessler reagent spectrophotometry with a detection limit of 0.02 mg/L. In addition, NO_2^- and NO_3^- were detected by UV spectrophotometry, and the detection limits were 0.001 mg/L and 0.08 mg/L, respectively. The other parameters were measured *on-situ* by a water table measuring instrument (HY.SWJ-30, Yuze, China) and a portable water quality measuring instrument (YSI Incorporated, OH, US).

Text S5

MDLs calculation. The method detection limits (MDLs) were calculated by multiplying the standard deviation of seven replicates by the Student's *T* value of 3.14 (one-side *T* distribution for six degrees of freedom at the 99 % confidence level). American Public Association, American Water Works Association, and Water Environment Federation issued this MDL calculation method in the Standard Method 1030 C for the Examination of Water and Wastewater.[3] The resulting MDLs were 0.5 for NDMA, 0.6 for NMEA, 0.9 for NDEA, 1.0 for NDBA, 0.9 for NDPA, 0.7 for NMOR, 1.1 for NPYR, and 0.9 for NPIP ng/L.

Text S6

Data analysis and visualization

OriginPro 2021, CorelDRAW X7, and ArcGIS 10.2 were used for graph drawing. Besides, OriginPro 2021 and SPSS were used to conduct the statistical analysis. Among them, Mann–Whitney U test was conducted by SPSS, and Spearman correlation analysis was performed

by OriginPro 2021. Principal component Analysis (PCA) and multiple linear regression analysis (MLRA) were used to evaluate the distribution of the variables.

Text S7

Multiple linear regression analysis

In our study, following principal component analysis (PCA), multiple linear regression analysis (MLRA) was performed using SPSS to apportion the percent contributions of N-fertilizer and pesticide sources in each well. The factor scores of the principal components extracted by PCA representing N-fertilizer and pesticide sources (X_i) and the standardized concentrations of ∑NAs were used as the dependent variable (Y). theses selections can also ensure the noncollinearity of X_i.[4] The intercept was set to zero. The "enter" mode was chosen for including all X_i into the regression.

$$Y = \sum B_i X_i \quad (1)$$

B_i is the regression coefficient for X_i. The B_i were then determined with the 95% confidence limit. The mean percent contribution of X_i (%) to Y can be calculated as:

$$\text{Mean contribution of } X_i = B_i / \sum B_i \quad (2)$$

The contribution of X_i (C_i, ng/L) to ∑NAs in a given groundwater sample can be calculated as:

$$C_i = mean\sum NAs \times \left(\frac{B_i}{\sum_i^n B_i} \right) + B_i \times \sigma_{NAs} \times FS_i \quad (3)$$

where mean∑NAs and σ_{NAs} are the average concentration and standard deviation of ∑NAs in groundwater samples, respectively. n is the number of X_i. FS_i is the factor score for X_i.

Table S1.

Acquisition data for GC-MS/MS analysis of 8 native, 2 labeled *N*-nitrosamines by using MRM mode.

Compounds	MRM transitions (m/z)	
	For quantification (b, c)	For confirmation (b, c)
NDMA-d_6	80–50.1 (20[a], 3[b])	80–48.1 (10, 7)
NDMA	74.0–44.1 (20, 3)	74.0–42.1 (10, 7)
NMEA	88.0–71.0 (50, 3)	88.0–43.0 (50, 5)
NDEA	102.0–85.0 (80, 5)	102.0–56.1 (80, 10)

NDPA-d_{14}	144.0–126.1 (20, 0)	144.0–50.1 (10, 10)
NDPA	130.1–113.0 (20, 0)	130.1–43.0 (10, 10)
NPYR	100.0–70.0 (20, 5)	100.0–55.0 (10, 5)
NMOR	116.0–86.0 (20, 0)	116.0–56.1 (10, 10)
NPIP	114.0–97.0 (50, 5)	114.0–97.0 (20, 5)
NDBA	158.0–141.1 (50, 3)	158.0–99.0 (20, 5)

[a] dwell time (ms), and [b] collision energy (V)

Table S2

Water quality parameters of groundwater and surface water samples.

Sample number	Water level (m)	Water temperature (°C)	pH	DO[1] (mg/L)	EC[2] (μs/cm)	ORP[3] (mV)	NO_2^- (mg/L)	NO_3^- (mg/L)	NH_4^+ (mg/L)
GW1-1	1.06	17.6	7.06	2.60	1368	-5.2	0.039	0.283	2.65
GW1-2	2.97	17.2	7.41	9.52	910	-24.8	0.033	0.199	1.40
GW1-3	2.92	17.2	7.28	2.65	709	-17.7	0.021	0.212	2.45
GW2-1	3.12	16.2	7.44	1.20	769	-26.8	0.019	0.186	2.55
GW2-2	0.91	11.8	7.41	0.99	1145	-24.6	0.037	0.159	6.75
GW2-3	3.17	15.4	7.52	1.26	899	-30.9	0.007	0.118	3.85
GW3-1	3.37	16.3	7.6	0.87	860	-35.5	0.003	0.355	2.15
GW3-2	4.05	16.5	7.28	1.31	1014	-17.5	0.003	2.191	3.70
GW3-3	3.53	16.3	7.45	1.86	237	-27.3	0.012	0.133	1.25
GW4-1	0.58	14.7	7.54	2.47	751	-32	0.016	ND	1.35
GW4-2	1.77	16.2	7.46	1.68	632	-27.6	0.021	0.306	0.75
GW4-3	1.84	17.9	6.98	1.57	197.5	-0.9	0.011	0.203	2.45
GW5-1	1.81	17.9	7.00	3.86	939	-1.9	0.051	0.234	2.75
GW5-2	4.42	18.7	7.28	4.17	810	-17.9	0.022	0.453	3.70
GW5-3	4.25	19.1	7.23	3.89	838	-14.7	0.036	0.502	3.60
GW6-1	1.2	15.9	7.37	0.45	892	-22.5	0.043	0.118	0.95
GW6-2	5.91	14.5	7.19	1.31	837	-12.4	0.024	0.429	4.15
GW6-3	3.55	16.2	7.51	3.84	762	-30.6	0.041	0.175	2.75
GW7-1	2.42	14.9	7.00	0.52	1013	-1.8	0.001	0.118	4.00
GW7-2	3.98	16.2	7.38	0.52	1033	-21.9	0.001	0.311	2.10
GW7-3	3.95	14.8	7.11	0.41	1059	-7.8	0.005	0.165	1.30
GW8-2	1.61	17.8	7.24	3.40	293.2	-15.2	0.037	0.325	0.60
GW8-3	1.81	17.8	7.22	3.24	582	-14.5	0.025	0.233	0.70
SW1	NA	10.4	8.08	1.51	500	-61.4	0.009	NA	0.67
SW2	NA	10.2	8.03	1.88	568	-61.2	0.01	NA	0.89
SW3	NA	10.5	7.72	1.60	682	-41.4	0.016	NA	1.28
SW4	NA	10.1	7.64	1.83	602	-27.1	0.005	NA	0.28

DO[1], EC[2], and ORP[3] represent dissolved oxygen, electronic conductivity, and redox potential, respectively; NA: not available; ND: not detected.

Table S3

Compounds	Oral Slope Factor/ $[mg/(kg \times day)]^{-1}$	Drinking water concentration at 10^{-6} Risk Level/ (ng/L)
NDMA	51	0.7
NDEA	150	0.2
NPYR	2.1	20
NDBA	5.4	6

Quantitative estimate of carcinogenic risk from oral exposure.[5]

Table S4

Average *N*-nitrosamine concentrations in difference depths of groundwater wells (ng/L).

	NDMA	NDEA	NMOR	NPYR	NDBA	∑NAs
10 m	7.4	0.9	0.2	2.2	2.3	13
25 m	0.3	0.6	0.2	ND	2.0	3
50 m	2.9	1.1	0.1	ND	0.9	5

ND: not detectable.

Table S5

Sampling adequacy using KMO and Bartlett's test.

KMO Measure of Sampling Adequacy		0.540
Bartlett's test of sphericity	Approx. chi-squre	31.927
	df	10
	Sig.	0.000

Table S6

Profiles of two principle components (PCs) extracted by PCA after the varimax rotation.

	PC1	PC2
NDMA	0.870	—
NDEA	0.509	—
NMOR	—[a]	0.857
NPYR	—	0.917
NDBA	0.880	—
Variance contribution (%)	44.734	26.750

[a]loadings less than 0.5 were not shown.

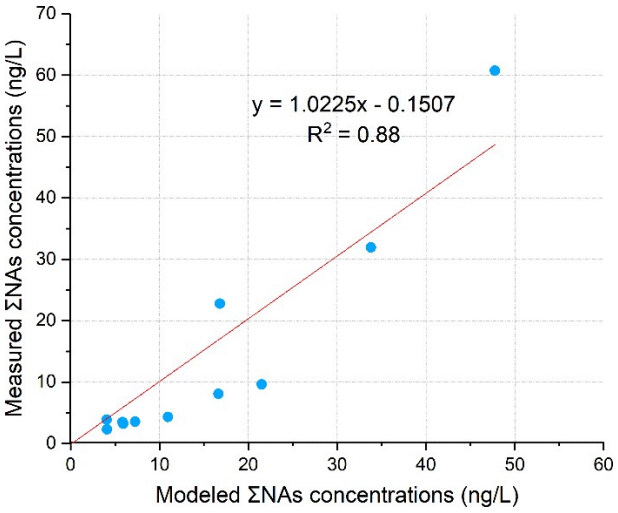

Figure. S1. The linear correlations of modeled ∑NAs (from PCA+MLRA analysis) and measured ∑NAs in the groundwater in JHP.

References

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