Calibration Procedures for Probes and Atomic Absorption Spectrophotometer (AAS)

Multiparameter Water Quality Probes

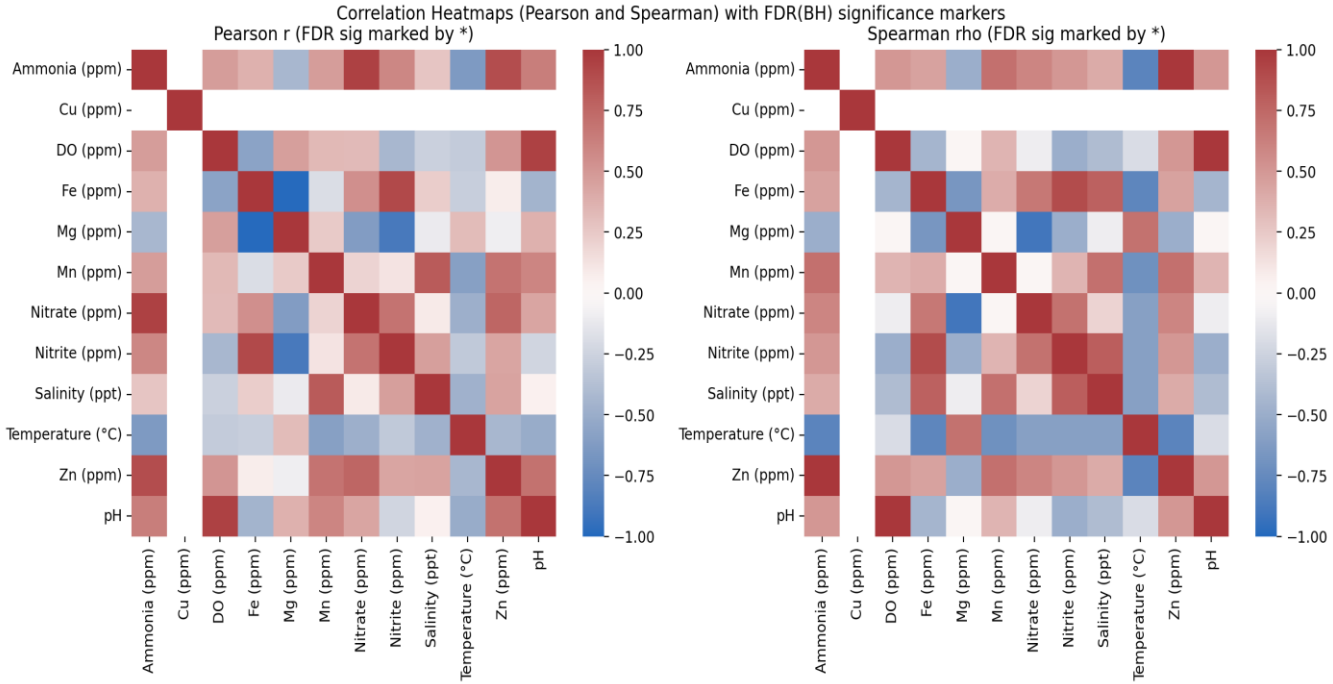
All water quality probes were calibrated prior to sampling to ensure accurate measurements. The dissolved oxygen (DO) probe was calibrated daily using a two-point method: a zero-point calibration with a sodium sulfite (Na_2SO_3) solution to simulate 0% DO, and a saturation-point calibration using air-saturated distilled water at ambient temperature for 100% DO. The pH probe was calibrated using three standard buffer solutions of pH 4.00, 7.00, and 10.00 (Hanna Instruments, USA), with the probe rinsed with distilled water between each buffer to prevent cross-contamination. The salinity/conductivity probe was calibrated using a standard conductivity solution (12.88 mS/cm), and the probe was thoroughly cleaned and air-dried before and after calibration. The temperature probe was verified against a certified NIST-traceable digital thermometer at reference points such as 0 °C (ice water) and approximately 25 °C (room temperature).

Atomic Absorption Spectrophotometer (AAS)

The atomic absorption spectrophotometer (AAS) used was a Thermo Scientific ICE 3000 v1.30 model, equipped with a deuterium background corrector. Stock solutions (1000 mg/L) for Zn, Cu, Mg, Mn, and Fe were sourced from Merck (Germany), and working standards ranging from 0.1 to 5.0 mg/L were prepared through serial dilution using ultrapure deionized water. The AAS was preheated, and lamp alignment was verified before analysis. Specific operating wavelengths were used for each element: Zn at 213.9 nm, Cu at 324.8 nm, Fe at 248.3 nm, Mn at 279.5 nm, and Mg at 285.2 nm. Calibration curves were constructed using at least five standard concentrations, with ultrapure water blanks used to establish zero absorbance. All calibration curves exhibited strong linearity ($R^2 > 0.995$). Instrument drift was monitored after every 10 samples using a mid-range standard. Deuterium background correction was applied throughout to reduce non-specific absorbance. Quality control included the use of known QC standards, with an acceptable variance of ±10% from the mid-range standard. Additionally, duplicate samples and spike recovery tests were performed, with recovery rates maintained within the acceptable range of 80–120%.

Table S1. Working standards series and correlation coefficient of heavy metals by AAS.

Types of trace elements	Concentration of standard (mgL^{-1})	Correlation coefficient, R^2	Limit of detection (mgL^{-1})	Limit of quantification (mgL^{-1})	Reference Standard
Copper (Cu)	1.0,3.0,5.0	0.9990	0.0045	0.007	Cu 1000 mgL^{-1} (Merck)
Zinc (Zn)	0.5,1.0,1.5	0.9924	0.0033	0.017	Zn 1000 mgL^{-1} (Merck)
Iron (Fe)	1.0,3.0,5.0	0.9980	0.0043	0.03	Fe 1000 mgL^{-1} (Merck)
Magnesium (Mg)	0.5,1.0,1.5	0.9950	0.0022	0.004	Mg 1000 mgL^{-1} (Merck)
Manganese (Mn)	0.5,1.0,1.5	0.9998	0.0016	0.006	Mn 1000 mgL^{-1} (Merck)



Supplementary Figure S1. Correlation heatmaps of water quality parameters and trace metal concentrations. (Left) Pearson correlation coefficients (r). (Right) Spearman rank correlation coefficients (ϱ). Color scale indicates the strength and direction of associations, with red representing positive and blue representing negative correlations. Significance markers (*) indicate correlations that remained significant after adjustment for multiple testing using the Benjamini–Hochberg false discovery rate (FDR-BH) procedure. No correlations were retained at $\alpha = 0.05$ after correction, consistent with the small sample size ($n = 5$).

Supplementary Table S2. Pairwise correlations with multiple-testing adjustments.

Note: * indicates significance at FDR(BH) q < 0.05 (either Pearson or Spearman). Values rounded to 3 decimals.

Abbreviations: r, Pearson correlation; rho, Spearman correlation; CI, 95 percent confidence interval; FDR(BH), Benjamini–Hochberg false discovery rate. Asterisk indicates q < 0.05 after FDR correction.

param_a	param_b	n	pearson_r	pearson_ci95_lo	pearson_ci95_hi	pearson_p_raw	pearson_p_bonf	pearson_p_fdr_bh	spearman_rho	spearman_p_raw	spearman_p_bonf
Temperature (°C)	pH	5	-0.514	-0.961	0.674	0.375	1.000		-0.200	0.747	1.000
Temperature (°C)	Salinity (ppt)	5	-0.473	-0.956	0.702	0.421	1.000		-0.600	0.285	1.000
Temperature (°C)	DO (ppm)	5	-0.303	-0.935	0.791	0.620	1.000		-0.200	0.747	1.000
Temperature (°C)	Ammonia (ppm)	5	-0.636	-0.973	0.561	0.249	1.000		-0.800	0.104	1.000
Temperature (°C)	Nitrite (ppm)	5	-0.320	-0.938	0.783	0.600	1.000		-0.600	0.285	1.000
Temperature (°C)	Nitrate (ppm)	5	-0.487	-0.958	0.693	0.405	1.000		-0.600	0.285	1.000
Temperature (°C)	Cu (ppm)	5									
Temperature (°C)	Zn (ppm)	5	-0.430	-0.951	0.729	0.470	1.000		-0.800	0.104	1.000
Temperature (°C)	Fe (ppm)	5	-0.283	-0.932	0.799	0.644	1.000		-0.783	0.118	1.000
Temperature (°C)	Mg (ppm)	5	0.315	-0.785	0.937	0.605	1.000		0.700	0.188	1.000
Temperature (°C)	Mn (ppm)	5	-0.596	-0.969	0.603	0.289	1.000		-0.707	0.182	1.000
pH	Salinity (ppt)	5	0.048	-0.871	0.892	0.939	1.000		-0.400	0.505	1.000
pH	DO (ppm)	5	0.946	0.386	0.997	0.015	0.985		1.000	<0.001	<0.001
pH	Ammonia (ppm)	5	0.634	-0.564	0.972	0.251	1.000		0.500	0.391	1.000
pH	Nitrite (ppm)	5	-0.234	-0.925	0.817	0.704	1.000		-0.500	0.391	1.000
pH	Nitrate (ppm)	5	0.433	-0.727	0.952	0.467	1.000		-0.100	0.873	1.000
pH	Cu (ppm)	5									
pH	Zn (ppm)	5	0.698	-0.480	0.978	0.190	1.000		0.500	0.391	1.000
pH	Fe (ppm)	5	-0.454	-0.954	0.715	0.443	1.000		-0.447	0.450	1.000
pH	Mg (ppm)	5	0.373	-0.759	0.945	0.536	1.000		0.000	1.000	1.000
pH	Mn (ppm)	5	0.600	-0.600	0.969	0.285	1.000		0.354	0.559	1.000
Salinity (ppt)	DO (ppm)	5	-0.269	-0.930	0.804	0.662	1.000		-0.400	0.505	1.000
Salinity (ppt)	Ammonia (ppm)	5	0.269	-0.804	0.930	0.662	1.000		0.400	0.505	1.000
Salinity (ppt)	Nitrite (ppm)	5	0.467	-0.706	0.956	0.427	1.000		0.800	0.104	1.000
Salinity (ppt)	Nitrate (ppm)	5	0.094	-0.860	0.901	0.881	1.000		0.200	0.747	1.000
Salinity (ppt)	Cu (ppm)	5									
Salinity (ppt)	Zn (ppm)	5	0.440	-0.723	0.952	0.459	1.000		0.400	0.505	1.000
Salinity (ppt)	Fe (ppm)	5	0.230	-0.818	0.925	0.710	1.000		0.783	0.118	1.000
Salinity (ppt)	Mg (ppm)	5	-0.115	-0.905	0.854	0.854	1.000		-0.100	0.873	1.000
Salinity (ppt)	Mn (ppm)	5	0.815	-0.239	0.987	0.093	1.000		0.707	0.182	1.000
DO (ppm)	Ammonia (ppm)	5	0.473	-0.702	0.956	0.421	1.000		0.500	0.391	1.000
DO (ppm)	Nitrite (ppm)	5	-0.431	-0.951	0.728	0.469	1.000		-0.500	0.391	1.000
DO (ppm)	Nitrate (ppm)	5	0.325	-0.781	0.938	0.594	1.000		-0.100	0.873	1.000
DO (ppm)	Cu (ppm)	5									
DO (ppm)	Zn (ppm)	5	0.512	-0.675	0.960	0.378	1.000		0.500	0.391	1.000
DO (ppm)	Fe (ppm)	5	-0.574	-0.967	0.624	0.311	1.000		-0.447	0.450	1.000
DO (ppm)	Mg (ppm)	5	0.466	-0.707	0.955	0.429	1.000		0.000	1.000	1.000

DO (ppm)	Mn (ppm)	5	0.331	-0.779	0.939	0.587	1.000		0.354	0.559	1.000
Ammonia (ppm)	Nitrite (ppm)	5	0.588	-0.612	0.968	0.297	1.000		0.500	0.391	1.000
Ammonia (ppm)	Nitrate (ppm)	5	0.952	0.440	0.997	0.012	0.815		0.600	0.285	1.000
Ammonia (ppm)	Cu (ppm)	5									
Ammonia (ppm)	Zn (ppm)	5	0.885	0.012	0.992	0.046	1.000		1.000	<0.001	<0.001
Ammonia (ppm)	Fe (ppm)	5	0.370	-0.761	0.944	0.540	1.000		0.447	0.450	1.000
Ammonia (ppm)	Mg (ppm)	5	-0.431	-0.951	0.728	0.469	1.000		-0.500	0.391	1.000
Ammonia (ppm)	Mn (ppm)	5	0.469	-0.705	0.956	0.426	1.000		0.707	0.182	1.000
Nitrite (ppm)	Nitrate (ppm)	5	0.693	-0.486	0.978	0.194	1.000		0.700	0.188	1.000
Nitrite (ppm)	Cu (ppm)	5									
Nitrite (ppm)	Zn (ppm)	5	0.432	-0.727	0.952	0.467	1.000		0.500	0.391	1.000
Nitrite (ppm)	Fe (ppm)	5	0.911	0.147	0.994	0.031	1.000		0.894	0.041	1.000
Nitrite (ppm)	Mg (ppm)	5	-0.879	-0.992	0.015	0.050	1.000		-0.500	0.391	1.000
Nitrite (ppm)	Mn (ppm)	5	0.123	-0.852	0.907	0.844	1.000		0.354	0.559	1.000
Nitrate (ppm)	Cu (ppm)	5									
Nitrate (ppm)	Zn (ppm)	5	0.761	-0.368	0.983	0.135	1.000		0.600	0.285	1.000
Nitrate (ppm)	Fe (ppm)	5	0.546	-0.649	0.964	0.341	1.000		0.671	0.215	1.000
Nitrate (ppm)	Mg (ppm)	5	-0.617	-0.971	0.582	0.267	1.000		-0.900	0.037	1.000
Nitrate (ppm)	Mn (ppm)	5	0.197	-0.830	0.919	0.751	1.000		0.000	1.000	1.000
Cu (ppm)	Zn (ppm)	5									
Cu (ppm)	Fe (ppm)	5									
Cu (ppm)	Mg (ppm)	5									
Cu (ppm)	Mn (ppm)	5									
Zn (ppm)	Fe (ppm)	5	0.072	-0.865	0.897	0.908	1.000		0.447	0.450	1.000
Zn (ppm)	Mg (ppm)	5	-0.089	-0.901	0.861	0.887	1.000		-0.500	0.391	1.000
Zn (ppm)	Mn (ppm)	5	0.691	-0.490	0.977	0.197	1.000		0.707	0.182	1.000
Fe (ppm)	Mg (ppm)	5	-0.987	-0.999	-0.816	0.002	0.111		-0.671	0.215	1.000
Fe (ppm)	Mn (ppm)	5	-0.195	-0.919	0.830	0.753	1.000		0.395	0.510	1.000
Mg (ppm)	Mn (ppm)	5	0.249	-0.811	0.928	0.686	1.000		0.000	1.000	1.000