

Supplementary Material

Improvement of *on-site* sensor for simultaneous determination of phosphate, silicic acid, nitrate and nitrite in seawater

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Table S1. Detailed Procedure for measurements during the analyzer deployment with the number of ports as mentioned in Figure 2.

		Step	Port open	Linear movement ^a		Operation & Description	
		1	a	3	4500 (r ^b)		Draw up the cleaning solution into the syringe and all detectors, and set the light intensity for blank measurement.
			b	8	4500 (i ^c)		
			c	3	4500 (r)		
			d	15	4500 (i)		
			e	3	4500 (r)		
			f	16	4500 (i)		
		2	4	4500 (i)		Initialize the syringe motor and detector, align the switching valve and ensure that the syringe plunger is fully retracted.	
PO ₄ ³⁻ Calibration	Blank	3	a	7	4500 (r)	2x flushing	Washing the syringe and the PO ₄ ³⁻ detector with deionized water
			b	8	4500 (i)		
		4	a	7	2700 (r)		Draw up the blank and set the light intensity for the reference (I ^R , V ^R)
			b	4	1350 (i)		
			c	8	1350 (i)		
		5	a	8	720 (r)		Withdraw the blank from the flow cell and add R1 (Molybdate mixed reagent) and R2 (Ascorbic Acid)
	b		9	180 (r)			
	c		10	180 (r)			
	6	a	8	450 (i)	4x mixing	Mixing the reagent with the blank and inject the reaction mixture inside the detector and waiting for 3 minutes for color development and set the light intensity for the measurement (V, I)	
				450 (r)			
		b	4	180 (i)			
	c	8	900 (i)				
	7	a	7	4500 (r)	2x flushing		Washing the syringe and the PO ₄ ³⁻ detector with deionized water
b		8	4500 (i)				
8	a	13(14, 2) ^d	4500 (r)				

		9	b	8	4500 (i)	2x flushing	Washing the syringe and the PO_4^{3-} detector with STD1 (STD2, STD3)	
			a	13(14, 2)	2700 (r)		Draw up STD1(STD2, STD3) and set the light intensity for the reference (I^R, V^R)	
			b	4	1350 (i)			
		c	8	1350 (i)				
		10	a	8	720 (r)		Withdraw STD1 from the flow cell and add R1 and R2	
			b	9	180 (r)			
			c	10	180 (r)			
		11	a	8	450 (i)	180 (i)	4x mixing	Mixing the reagent with STD1 and inject the reaction mixture inside the detector and waiting for 3 minutes for color development and set the light intensity for the measurement (V, I)
					450 (r)			
			b	4	180 (i)			
		c	8	900 (i)				
washing		12	a	3	4500 (r)		Draw up the cleaning solution into the syringe and all detectors, and set the light intensity for blank measurement.	
			b	8	4500 (i)			
			c	3	4500 (r)			
			d	15	4500 (i)			
			e	3	4500 (r)			
			f	16	4500 (i)			
$\Sigma (\text{NO}_3^- + \text{NO}_2^-)$ Calibration	Blank	13	4	4500 (i)		Initialize the syringe motor and detector, align the switching valve and ensure that the syringe plunger is fully retracted.		
		14	a	7	4500 (r)	2x flushing	Washing the syringe and the PO_4^{3-} detector with deionized water	
			b	8	4500 (i)			
		15	a	7	4500 (r)	2x flushing	Washing the syringe and the $\Sigma (\text{NO}_3^- + \text{NO}_2^-)$ detector with deionized water	
			b	16	4500 (i)			
		16	a	7	2700 (r)		Draw up the blank and set the light intensity for the reference (I^R, V^R)	
			b	4	700 (i)			
			c	16	2000 (i)			
		17	a	16	1440 (r)		Withdraw the blank from the flow cell and add mixed reagent (Griess reagent + VCl_3)	
			b	5	360 (r)			
		18	16	450 (i)	4x mixing	Mixing the blank with the reagent		
		450 (r)						
19	8	1800 (i)		Waiting 30 minutes into PO_4^{3-} detector to allow NO_3^- reduction during incubation with VCl_3 reagent at maximum temperature ($\sim 50^\circ\text{C}$).				
20	a	8	1800 (r)		Withdraw the reaction mixture from the PO_4^{3-} detector, waiting 2 min. inside the syringe housing to cool the mixture and open the $\Sigma (\text{NO}_3^- + \text{NO}_2^-)$ detector, inject the mixture and set light intensity for the measurement (V, I)			
	b	4	225 (i)					
	c	16	1575(i)					

Standard 1, 2, 3	21	a	7	4500 (r)	2x flushing	Washing the syringe and the PO_4^{3-} detector with deionized water	
		b	8	4500 (i)			
	22	a	7	4500 (r)	2x flushing	Washing the syringe and the $\Sigma (\text{NO}_3^- + \text{NO}_2^-)$ detector with deionized water	
		b	16	4500 (i)			
	23	a	13(14, 2)	4500 (r)	2x flushing	Washing the syringe and the $\Sigma (\text{NO}_3^- + \text{NO}_2^-)$ detector with STD1 (STD2, STD3)	
		b	16	4500 (i)			
	24	a	13(14, 2)	2700 (r)		Draw up STD1 (STD2, STD3) and set the light intensity for the reference (I^R, V^R)	
		b	4	700 (i)			
		c	16	2000 (i)			
	25	a	16	1440 (r)		Withdraw STD1 (STD2, STD3) from the flow cell and add mixed reagent (Griess reagent + VCl_3)	
		b	5	360 (r)			
	26		16	450 (i)	4x mixing	Mixing STD1 (STD2, STD3) with the reagent	
				450 (r)			
27		8	1800 (i)		Waiting 30 minutes into PO_4^{3-} detector to allow NO_3^- reduction during incubation with VCl_3 reagent at maximum temperature ($\sim 50^\circ\text{C}$).		
28	a	8	1800 (r)		Withdraw the reaction mixture from the PO_4^{3-} detector, waiting 2 min. inside the syringe housing to cool the mixture and open the $\Sigma (\text{NO}_3^- + \text{NO}_2^-)$ detector and inject the mixture and set light intensity for the measurement (V, I)		
	b	4	225 (i)				
	c	16	1575(i)				
washing	29	a	3	4500 (r)		Draw up the cleaning solution into the syringe and all detectors, and set the light intensity for blank measurement.	
		b	8	4500 (i)			
		c	3	4500 (r)			
		d	15	4500 (i)			
		e	3	4500 (r)			
		f	16	4500 (i)			
H_4SiO_4 Calibration	Blank	30	4	4500 (i)		Initialize the syringe motor and detector, align the switching valve and ensure that the syringe plunger is fully retracted.	
		31	a	7	4500 (r)	2x flushing	Washing the syringe and the H_4SiO_4 detector with deionized water
			b	15	4500 (i)		
		32	a	7	2700 (r)		Draw up the blank and set the light intensity for the reference (I^R, V^R)
			b	4	1350 (i)		
			c	15	1350 (i)		
		33	a	15	225 (r)		Withdraw the blank from the flow cell and add R1(molybdic acid)
			b	11	225 (r)		
		34		15	300 (i)	4x mixing	Mixing the blank and R1 and waiting 3 min for complexation
300 (r)							
35	a	12	225 (r)				

Standard 1, 2, 3		b	10	225 (r)		Add R2 (Oxalic acid) and R3 (Ascorbic Acid) to the reaction mixture	
	36		15	450 (i)	4x mixing	Mixing the blank and the reagents	
				450 (r)			
	37	a	4	225 (i)	Inject the reaction mixture into the H ₄ SiO ₄ detector and set the light intensity for measurement (<i>V</i> , <i>I</i>)		
		b	15	675 (i)			
	38	a	7	4500 (r)	2x flushing	Washing the syringe and the H ₄ SiO ₄ detector with deionized water	
		b	15	4500 (i)			
	39	a	13(14, 2)	4500 (r)	2x flushing	Washing the syringe and the H ₄ SiO ₄ detector with STD1 (STD2, STD3)	
		b	15	4500 (i)			
	40	a	13(14, 2)	2700 (r)	Draw up STD1 (STD2, STD3) and set the light intensity for the reference (<i>I^R</i> , <i>V^R</i>)		
		b	4	1350 (i)			
		c	15	1350 (i)			
	41	a	15	225 (r)	Withdraw STD1 (STD2, STD3) from the flow cell add R1(molybdic acid)		
		b	11	225 (r)			
	42		15	300 (i)	4x mixing	Mixing STD1 (STD2, STD3) and R1 and waiting 3 min for complexation	
				300 (r)			
	43	a	12	225 (r)	Add R2 (Oxalic acid) and R3 (Ascorbic Acid) to the reaction mixture		
		b	10	225 (r)			
	44		15	450 (i)	4x mixing	Mixing STD1 (STD2, STD3) and the reagents	
				450 (r)			
45	a	4	225 (i)	Inject the reaction mixture into the H ₄ SiO ₄ detector and set the light intensity for measurement (<i>V</i> , <i>I</i>)			
	b	15	675 (i)				
washing	46	a	3	4500 (r)	Draw up the cleaning solution into the syringe and all detectors, and set the light intensity for blank measurement.		
		b	8	4500 (i)			
		c	3	4500 (r)			
		d	15	4500 (i)			
		e	3	4500 (r)			
		f	16	4500 (i)			
CRM	PO ₄ ³⁻ determination	47	4	4500 (i)	Initialize the syringe motor and detector, align the switching valve and ensure that the syringe plunger is fully retracted.		
		48	a	7	4500 (r)	2x flushing	Washing the syringe and the PO ₄ ³⁻ detector with deionized water
			b	8	4500 (i)		
		49		6	4500 (r)	2x flushing	Washing the syringe and the PO ₄ ³⁻ detector with CRM
				8	4500 (i)		
		50	a	6	2700 (r)	Draw up CRM and set the light intensity for the reference (<i>I^R</i> , <i>V^R</i>)	
			b	4	1350 (i)		
			c	8	1350 (i)		
		51	a	8	720 (r)	Withdraw CRM from the flow cell and add R1 (Molybdate mixed reagent) and R2 (Ascorbic Acid)	
			b	9	180 (r)		

Σ (NO ₃ ⁻ + NO ₂ ⁻) determination		c	10	180 (r)		
	52	8	450 (i)	4x	Mixing the reagent with CRM and inject the reaction mixture inside the detector and waiting for 3 minutes for color development and set the light intensity for the measurement (V, I)	
			450 (r)			mixing
		4	180 (i)			
		8	900 (i)			
	53	a	7	4500 (r)	2x	Washing the syringe and the PO ₄ ³⁻ detector with deionized water
		b	8	4500 (i)		
	54	a	6	4500 (r)	2x	Washing the syringe and the Σ (NO ₃ ⁻ + NO ₂ ⁻) detector with CRM
		b	16	4500 (i)		
	55	a	6	2700 (r)		Draw up CRM and set the light intensity for the reference (I ^R , V ^R)
		b	4	700 (i)		
		c	16	2000 (i)		
	56	a	16	1440 (r)		Withdraw CRM from the flow cell and add mixed reagent (Griess reagent + VCl ₃)
		b	5	360 (r)		
	57	16	450 (i)	4x	Mixing CRM with the reagent	
			450 (r)			mixing
	58	8	1800 (i)		Waiting 30 minutes into PO ₄ ³⁻ detector to allow NO ₃ ⁻ reduction during incubation with VCl ₃ reagent at maximum temperature (~ 50 °C).	
	59	a	8	1800 (r)		Withdraw the reaction mixture from the PO ₄ ³⁻ detector, waiting 2 min. inside the syringe housing to cool the mixture and open the Σ (NO ₃ ⁻ + NO ₂ ⁻) detector and inject the mixture and set light intensity for the measurement (V, I)
		b	4	225 (i)		
c		16	1575 (i)			
60	a	7	4500 (r)	2x	Washing the syringe and the H ₄ SiO ₄ detector with deionized water	
	b	15	4500 (i)			flushing
61	a	6	4500 (r)	2x	Washing the syringe and the H ₄ SiO ₄ detector with CRM	
	b	15	4500 (i)			flushing
62	a	6	2700 (r)		Draw up CRM and set the light intensity for the reference (I ^R , V ^R)	
	b	4	1350 (i)			
	c	15	1350 (i)			
64	a	15	225 (r)		Withdraw CRM from the flow cell and add R1(molybdc acid)	
	b	11	225 (r)			
65	15	300 (i)	4x	mixing	Mixing CRM and R1 then waiting 3 min for complexation	
		300 (r)				
66	a	12	225 (r)		Add R2 (Oxalic acid) and R3 (Ascorbic Acid) to the reaction mixture	
	b	10	225 (r)			
67	15	450 (i)	4x	mixing	Mixing CRM and the reagents	
		450 (r)				
68	a	4	225 (i)			
H ₄ SiO ₄ determination						

		b	15	675 (i)	Inject the reaction mixture into the H_4SiO_4 detector and set the light intensity for measurement (V, I)		
washing	69	a	3	4500 (r)	Draw up the cleaning solution into the syringe and all detectors, and set the light intensity for blank measurement.		
		b	8	4500 (i)			
		c	3	4500 (r)			
		d	15	4500 (i)			
		e	3	4500 (r)			
		f	16	4500 (i)			
Sea water	PO_4^{3-} determination	70	4	4500 (i)	Initialize the syringe motor and detector, align the switching valve and ensure that the syringe plunger is fully retracted.		
		71	a	1	4500 (r)	6x flushing	Washing the syringe and the PO_4^{3-} detector with seawater
			b	8	4500 (i)		
		72	a	1	2700 (r)	Draw up seawater and set the light intensity for the reference (I^R, V^R)	
			b	4	1350 (i)		
			c	8	1350 (i)		
		73	a	8	720 (r)	Withdraw seawater from the flow cell and add R1 (Molybdate mixed reagent) and R2 (Ascorbic Acid)	
			b	9	180 (r)		
			c	10	180 (r)		
		74	a	8	450 (i)	4x mixing	Mixing the reagent with seawater and inject the reaction mixture inside the detector and waiting for 3 minutes for color development and set the light intensity for the measurement (V, I)
	450 (r)						
	b		4	180 (i)			
	c	8	900 (i)				
	$\Sigma (\text{NO}_3^- + \text{NO}_2^-)$ determination	75	a	1	4500 (r)	6x flushing	Washing the syringe and the $\Sigma (\text{NO}_3^- + \text{NO}_2^-)$ detector with seawater
			b	16	4500 (i)		
		76	a	1	2700 (r)	Draw up seawater and set the light intensity for the reference (I^R, V^R)	
			b	4	700 (i)		
				16	2000 (i)		
		77	a	16	1440 (r)	Withdraw seawater from the flow cell and add mixed reagent (Griess reagent + VCl_3)	
			b	5	360 (r)		
78		16	450 (i)	4x mixing	Mixing seawater with the reagent		
450 (r)							
79		8	1800 (i)	Waiting 30 minutes into PO_4^{3-} detector to allow NO_3^- reduction during incubation with VCl_3 reagent at maximum temperature ($\sim 50^\circ\text{C}$).			
80	a	8	1800 (r)	Withdraw the reaction mixture from the PO_4^{3-} detector, waiting 2 min. inside the syringe housing to cool the mixture and open the $\Sigma (\text{NO}_3^- + \text{NO}_2^-)$ detector and inject the mixture and set light intensity for the measurement (V, I)			
	b	4	225 (i)				
	c	16	1575 (i)				

10 x (measurement loop)

H₄SiO₄ determination	81	a	1	4500 (r)	6x flushing	Washing the syringe and the H ₄ SiO ₄ detector with seawater
		b	15	4500 (i)		
	82	a	1	2700 (r)		Draw up seawater and set the light intensity for the reference (I^R, V^R)
		b	4	1350 (i)		
		c	15	1350 (i)		
	83	a	15	225 (r)		Withdraw seawater from the flow cell and add R1(molybdc acid)
		b	11	225 (r)		
	84		15	300 (i)	4x mixing	Mixing seawater and R1 then waiting 3 min for complexation
				300 (r)		
	85	a	12	225 (r)		Add R2 (Oxalic acid) and R3 (Ascorbic Acid) to the reaction mixture
		b	10	225 (r)		
	86		15	450 (i)	4x mixing	Mixing seawater and the reagents
				450 (r)		
	87	a	4	225 (i)		Inject the reaction mixture into the H ₄ SiO ₄ detector and set the light intensity for measurement (V, I)
b		15	675 (i)			

^a maximum movement of the syringe is 4500 (~2.2 ml)

^b r: retract; ^c i: inject

^d port 13: STD1, port 14: STD2, port 2: STD3

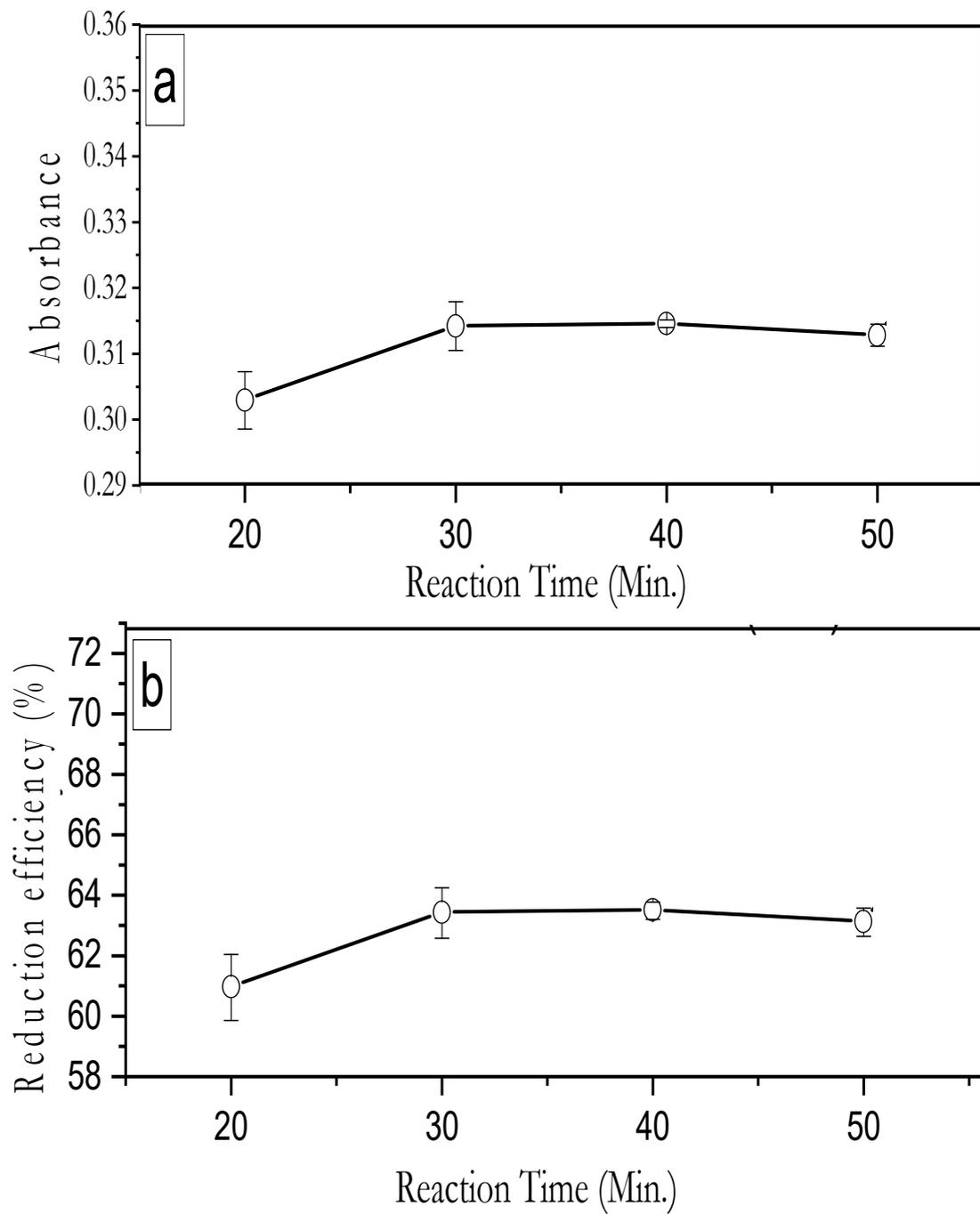
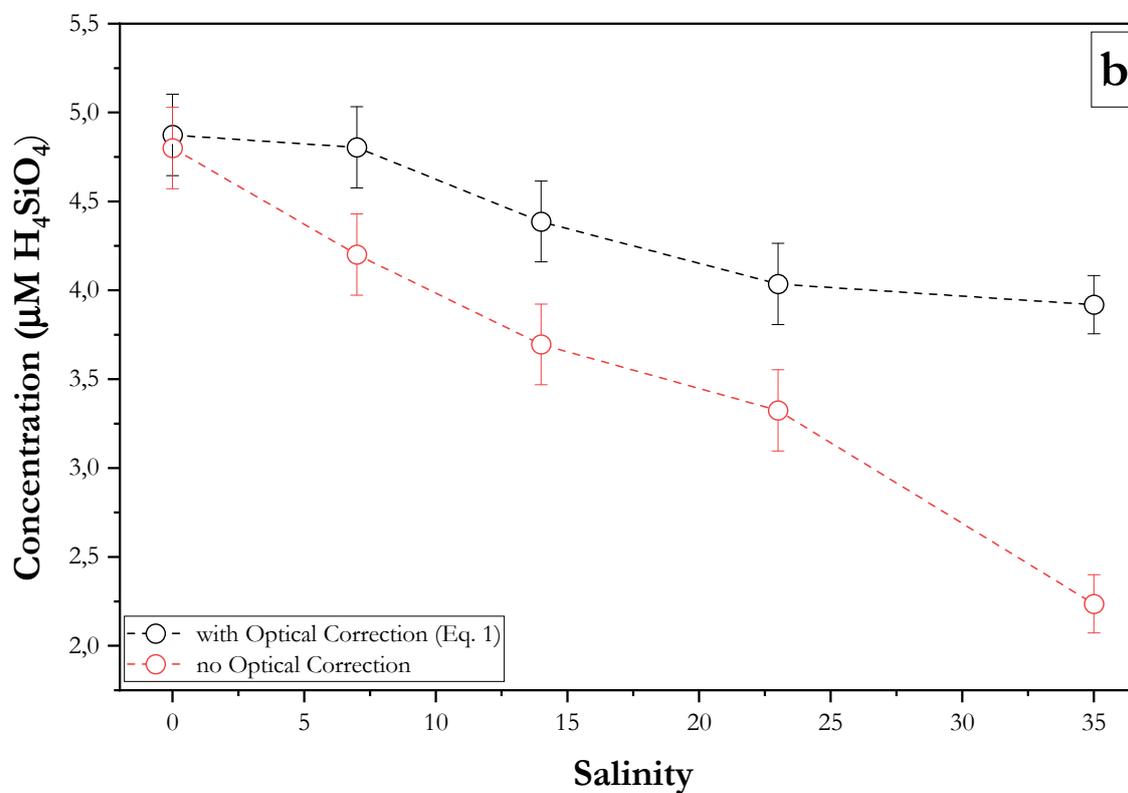
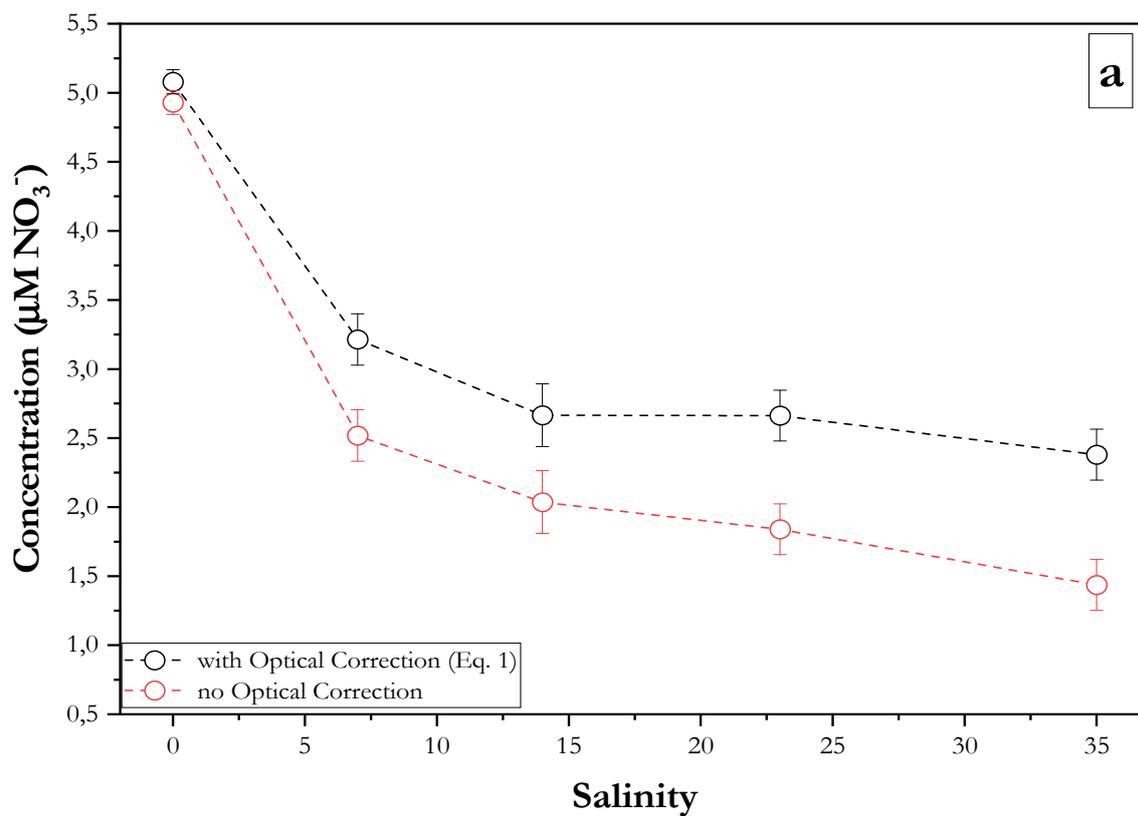


Figure S1. Effect of reaction time in minutes on (a) the absorbance of $10 \mu\text{M NO}_3^-$ and (b) the reduction efficiency (%) which defined as the ratio of the absorbance of $10 \mu\text{M NO}_3^-$ and the absorbance of $10 \mu\text{M NO}_2^-$.



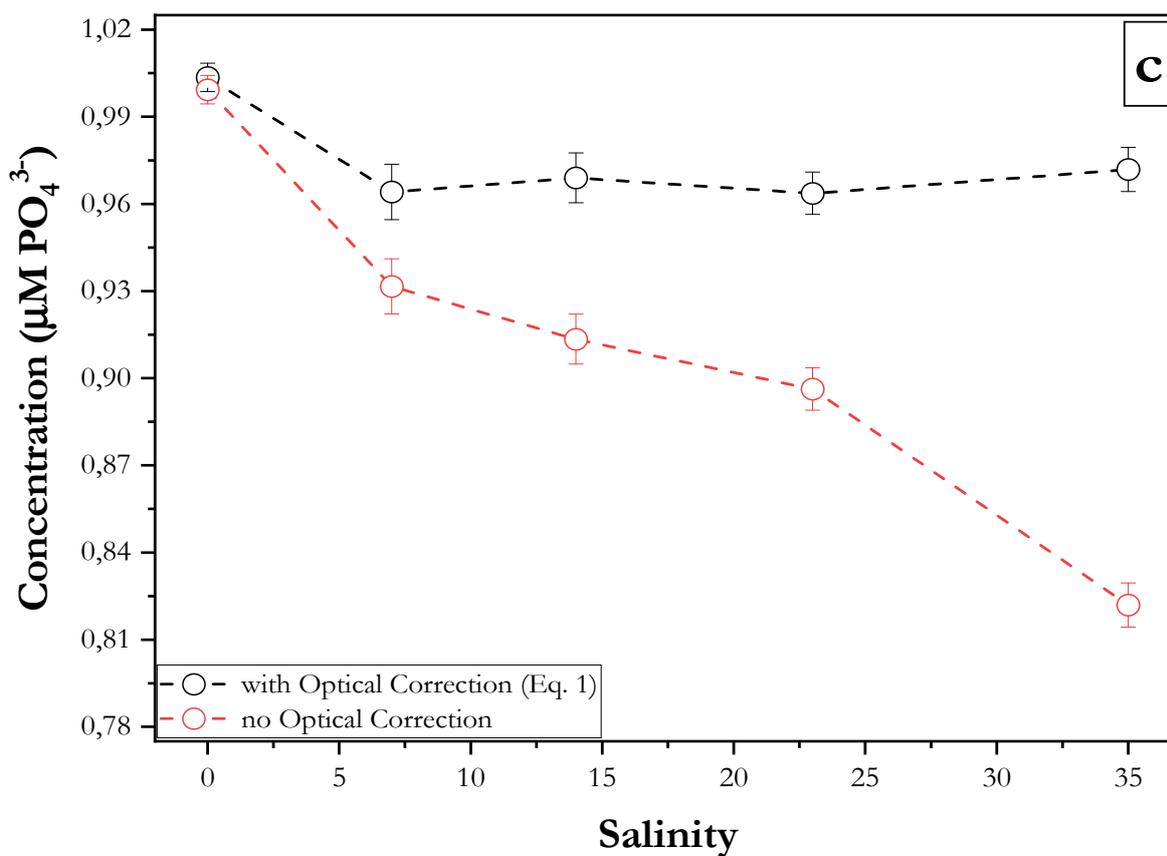


Figure S2. (a) calculated concentrations of 5 μM NO_3^- , (b) calculated concentrations of 5 μM $\text{H}_4\text{O}_4\text{Si}$ and (c) calculated concentrations of 1 μM PO_4^{3-} samples with different salinity (0, 7, 14, 23, 35) based on calibration curves of (0, 1, 5 and 10 μM NO_3^-), (0, 1, 5, 10 μM $\text{H}_4\text{O}_4\text{Si}$) and (0, 0.5, 1, 2 μM PO_4^{3-}), respectively. The raw data were processed using equation 1 (black circles) and the red circles represent the data processed using the traditional Beer's Law equation ($A = -\log_{10}(\frac{I}{I_0})$). Error bar (± 1 SD), $n = 10$.

Table S2. Slopes and intercepts including their standard deviations, t-values and the probabilities for the calibration curves in Figure 6.

		Value	Standard deviation	t-value	Probability
NO_3^-	Int.	0.06876	6.85×10^{-4}	100.42	1.079×10^{-13}
	Slp.	0.00614	9.76×10^{-5}	62.94	4.51×10^{-12}
NO_2^-	Int.	0.04061	0.00228	17.77	1.028×10^{-7}
	Slp.	0.01202	2.94×10^{-4}	40.87	1.414×10^{-10}
PO_4^{3-}	Int.	0.00191	4.88×10^{-4}	3.9	0.00294
	Slp.	0.01211	8.46×10^{-5}	143.1	5×10^{-10}
H_4SiO_4	Int.	-0.00632	0.00351	-1.8	0.10199
	Slp.	0.02377	6.48×10^{-4}	36.65	5.44×10^{-12}

Int.: Intercept, Slp.: Slope.

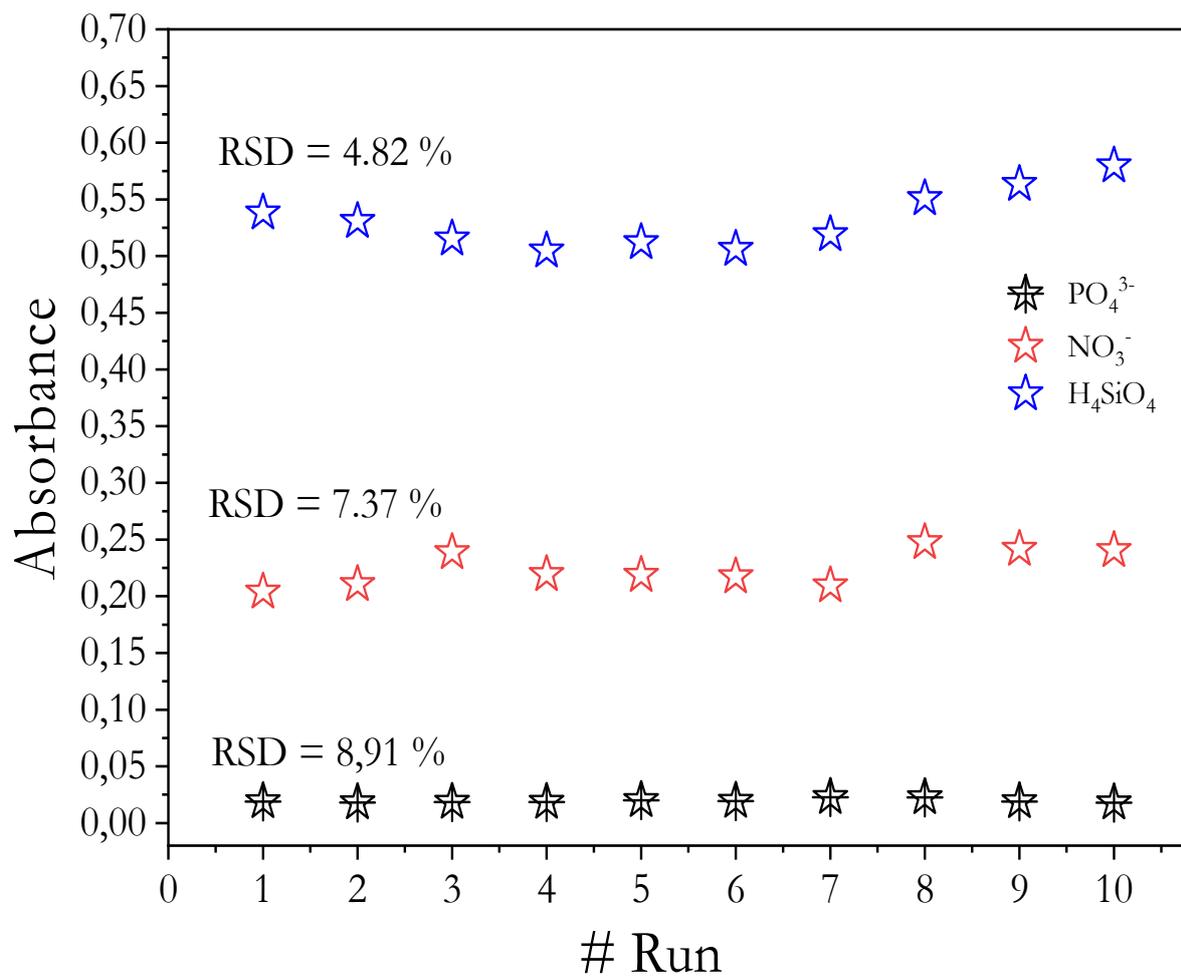


Figure S3. The measured absorbance value of KANSO CRM for nutrients for 6 consecutive runs of PO_4^{3-} , NO_3^- , and $\text{H}_4\text{O}_4\text{Si}$ with RSD (relative standard deviation) value. The Certified value for CRM is $23.7 \pm 0.2 \mu\text{M}$ for NO_3^- , $56.4 \pm 0.5 \mu\text{M}$ for $\text{H}_4\text{O}_4\text{Si}$, and $1.7 \pm 0.02 \mu\text{M}$ for PO_4^{3-} .

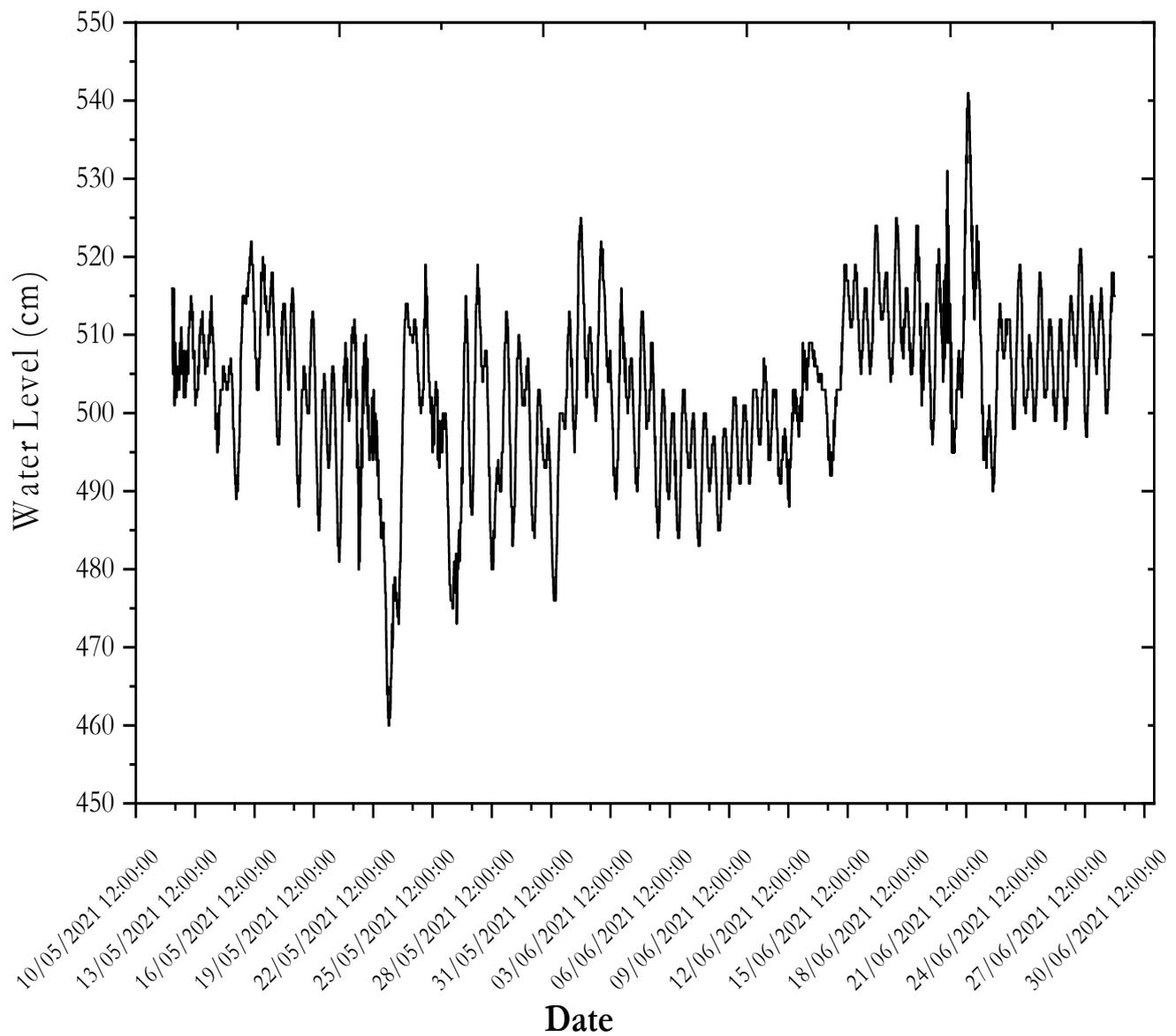


Figure S4. Time series data for the period from May 12 to June 28, 2021, for water level data at the kiel-Holtenau station obtained from the Federal Waterways and Shipping Administration (WSV).

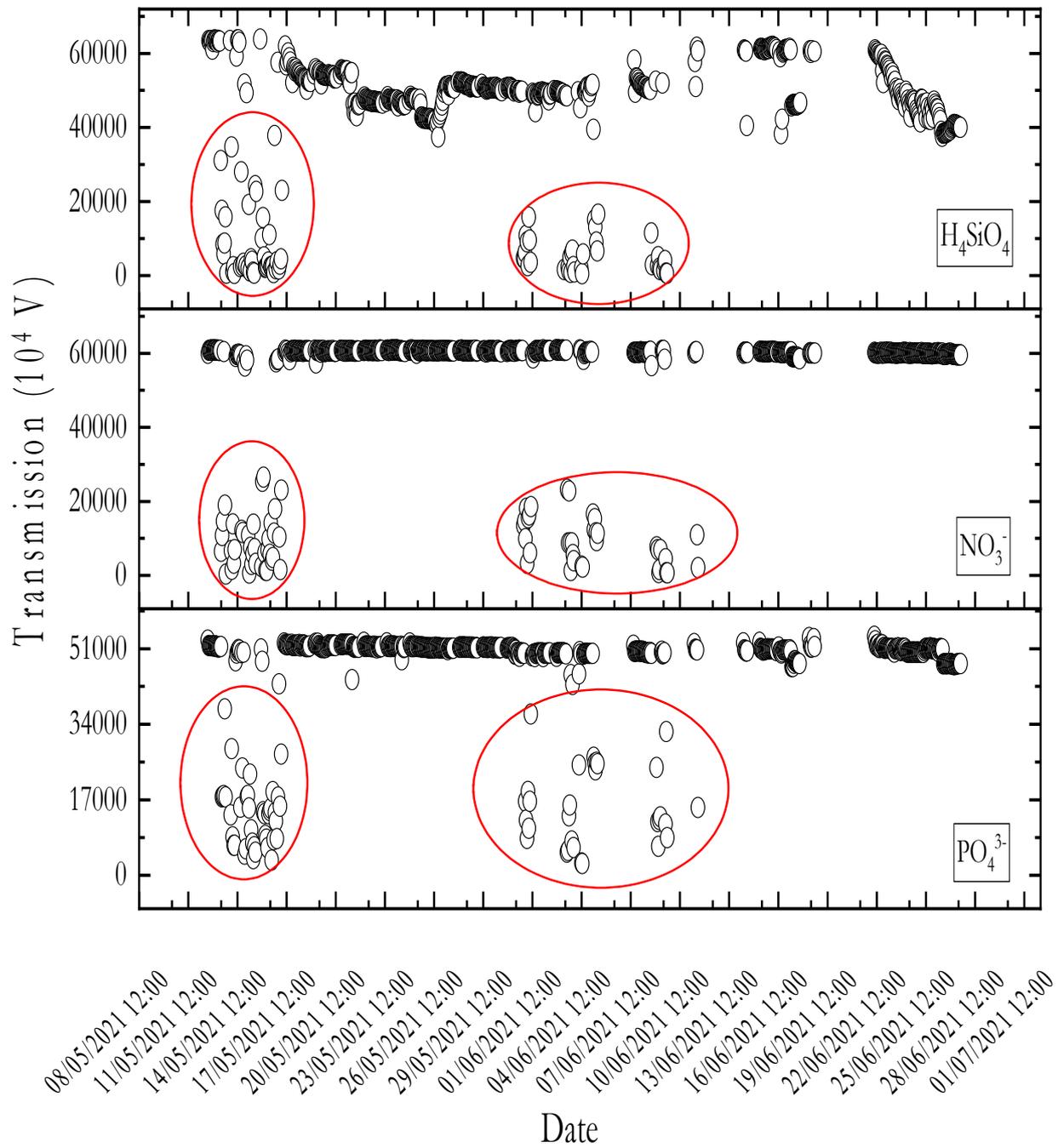
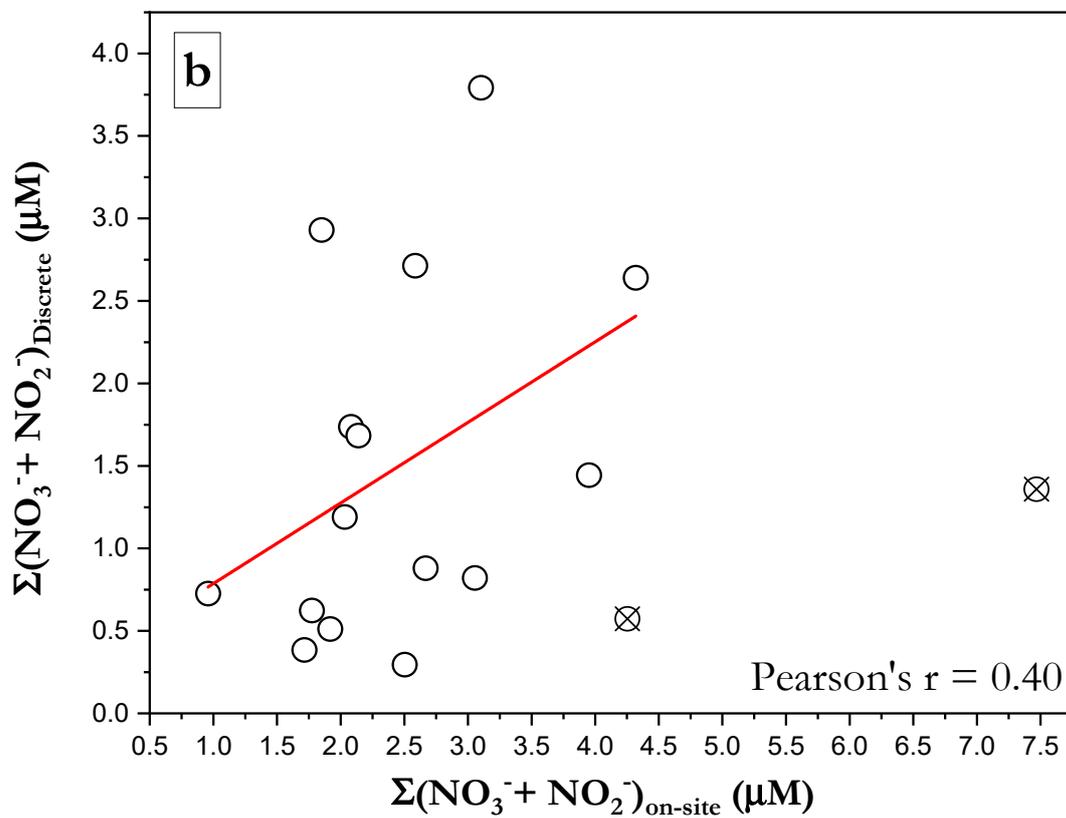
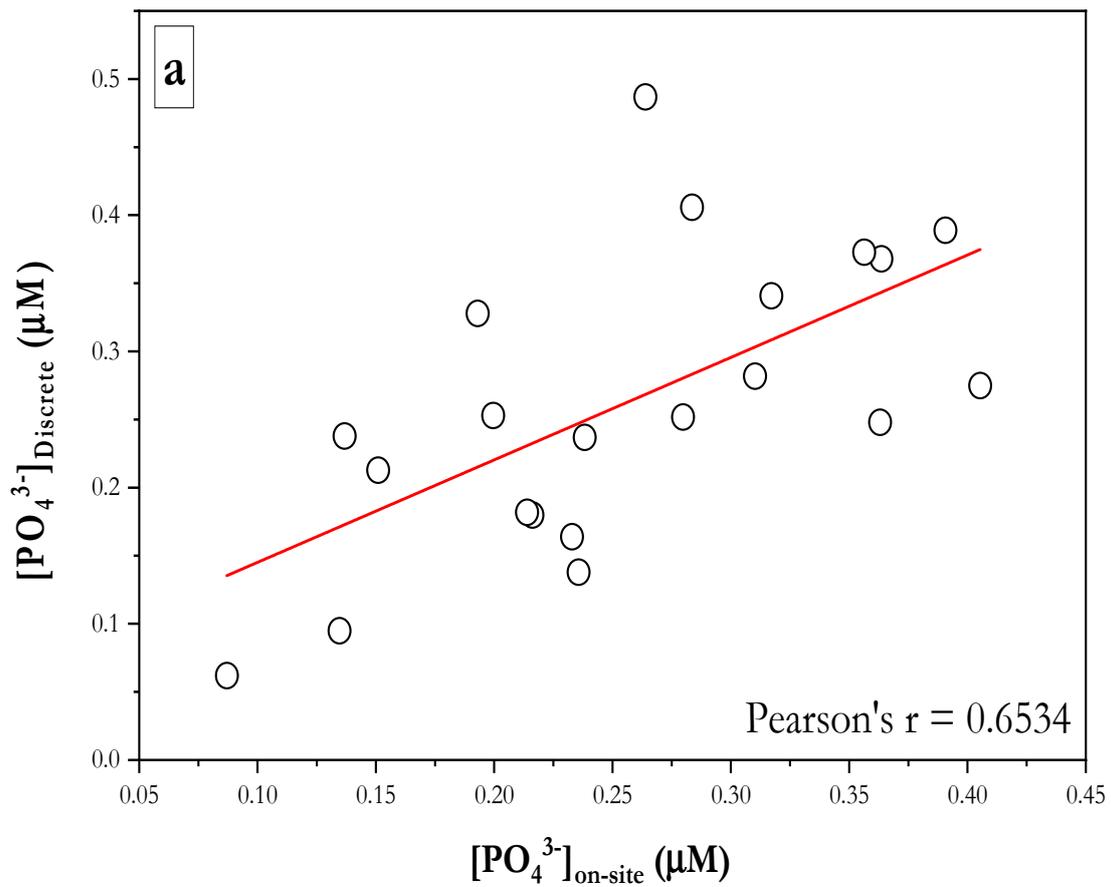


Figure S5. Time series data from May 12 to June 26, 2021, of PO_4^{3-} , NO_3^- , and $\text{H}_4\text{O}_4\text{Si}$ photodiode detector readout; the points in red circles refer to the drop-down of the transmission values due to air bubbles trapped into the flow cell.



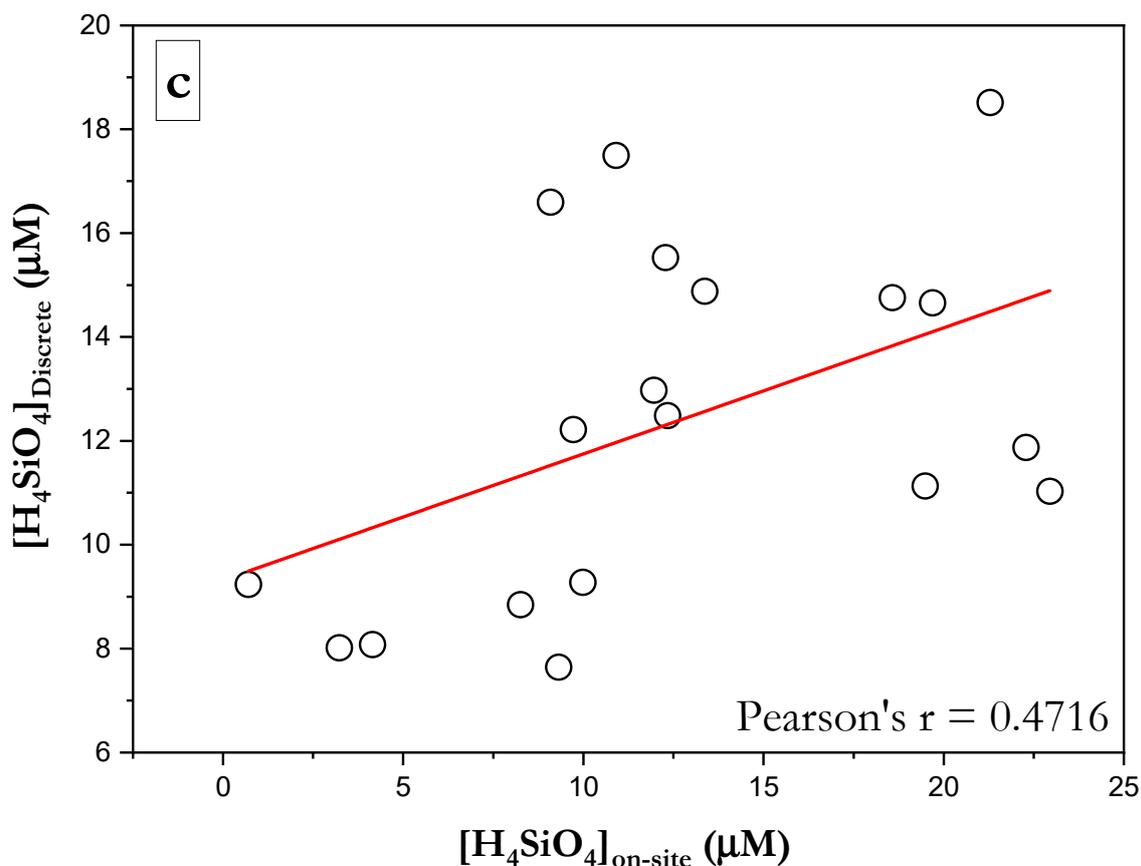
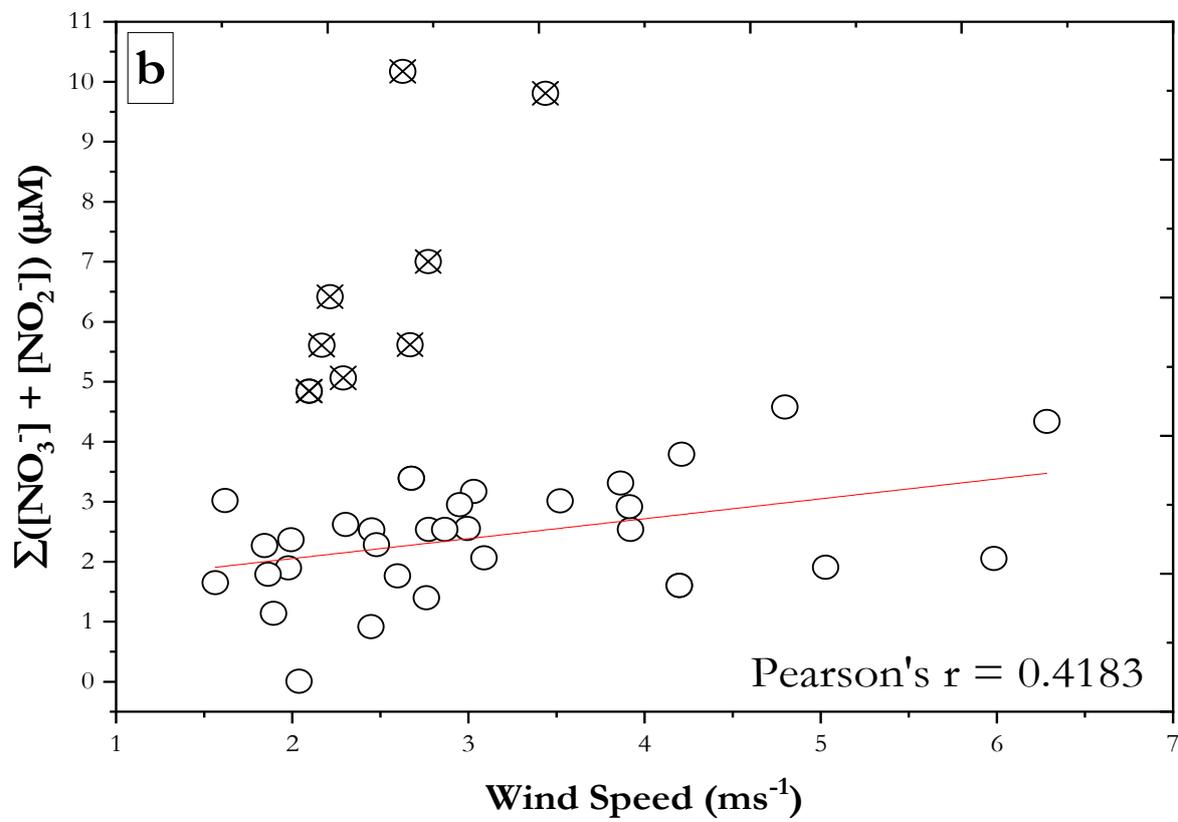
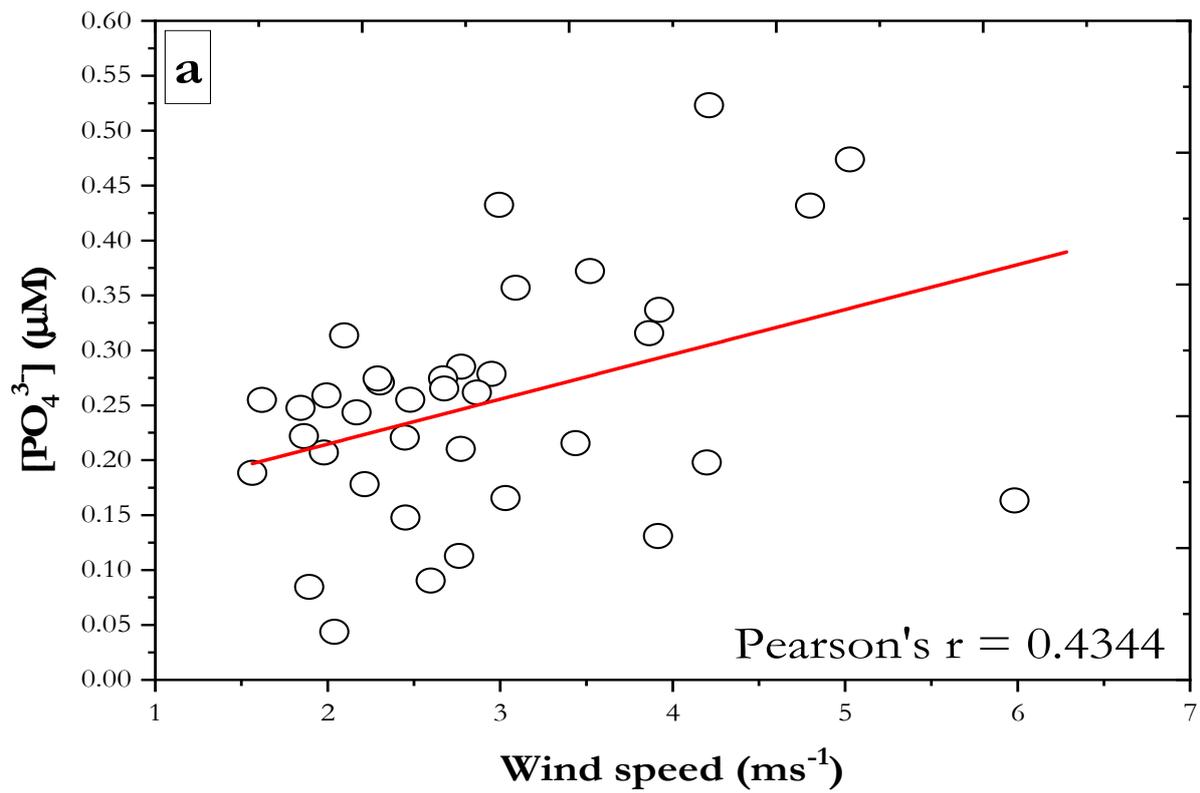
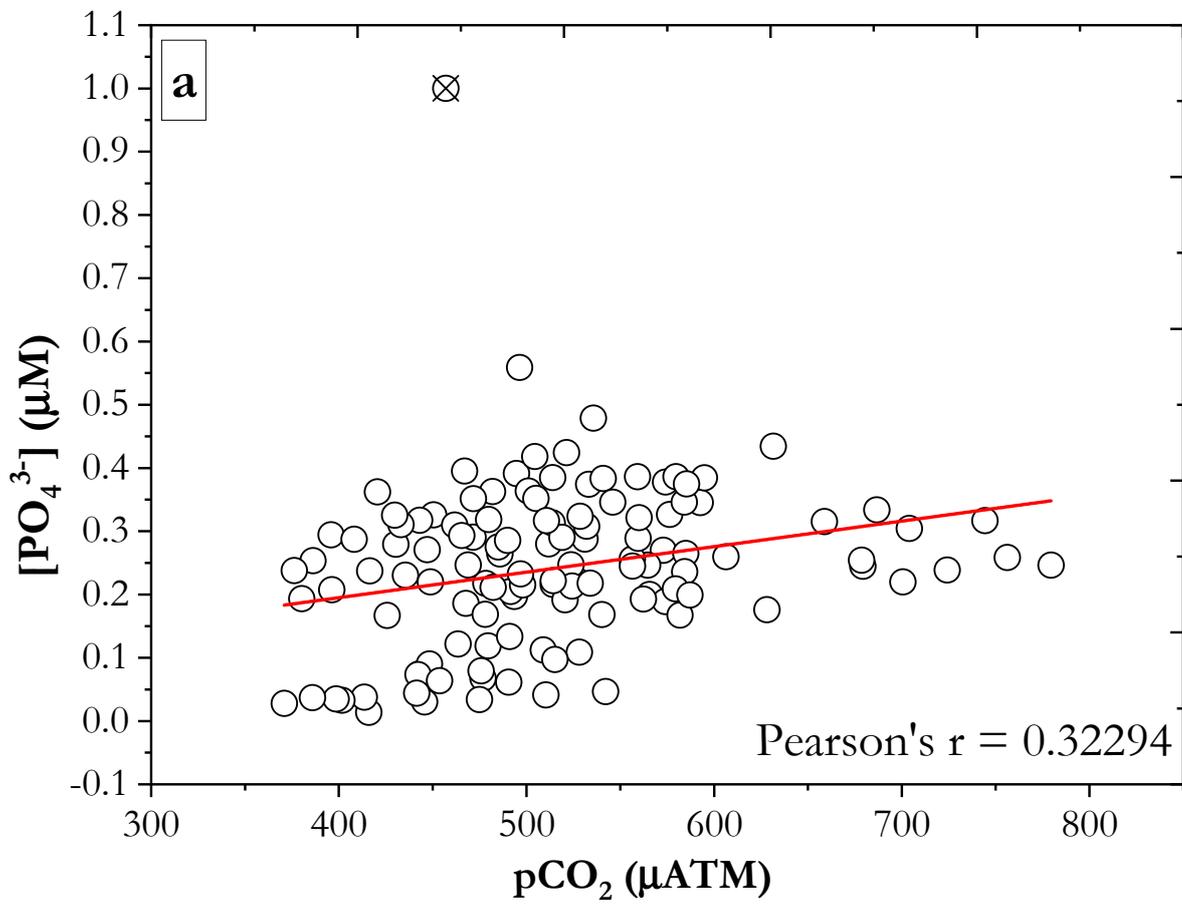
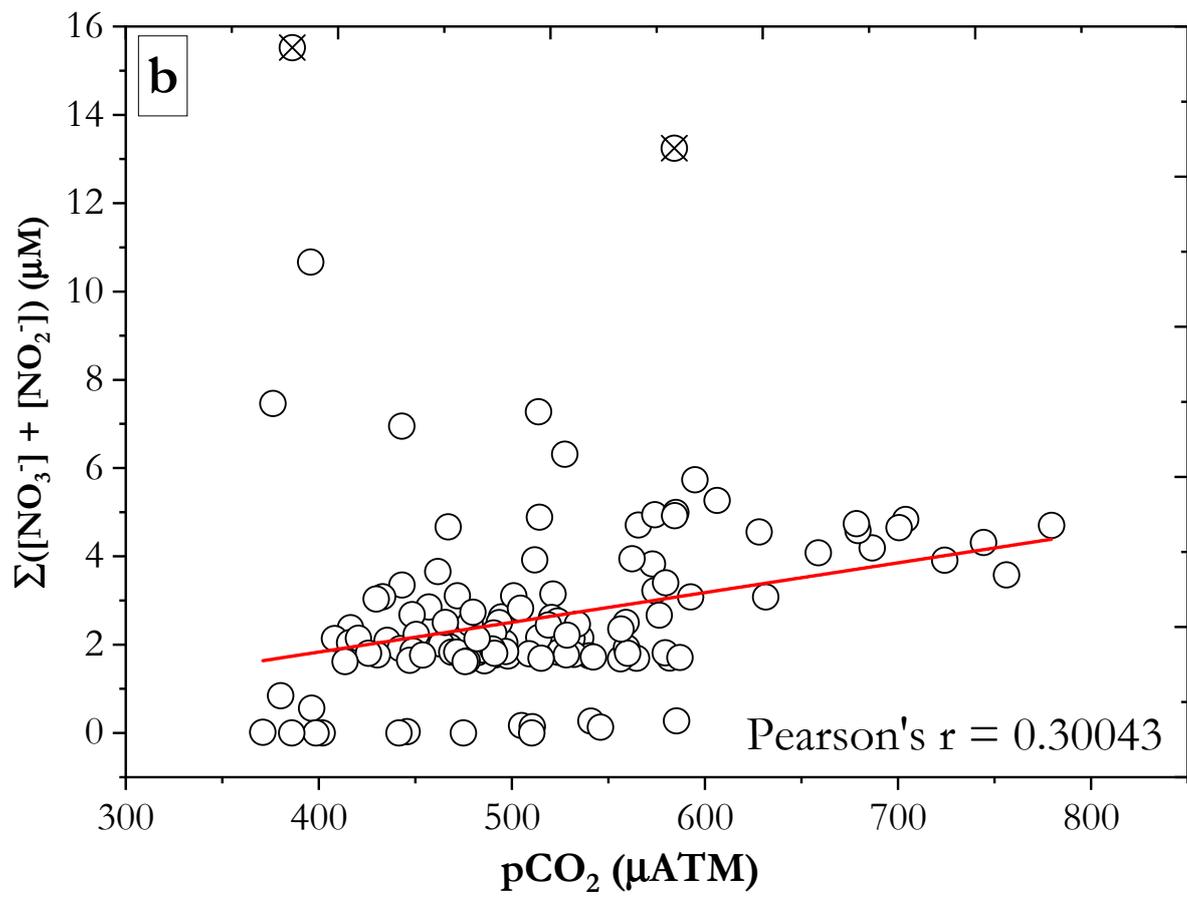


Figure S6. Property-to-property plots for (a) PO_4^{3-} in μM measured on-site with the AutoLAB analyser compared to synchronised PO_4^{3-} in μM measured with the air-segment analyser in the laboratory for discretely collected samples pearson' $r = 0.6534$, $n = 21$, (b) $\Sigma(\text{NO}_3^- + \text{NO}_2^-)$ in μM measured on-site with the AutoLAB analyser compared to synchronised $\Sigma(\text{NO}_3^- + \text{NO}_2^-)$ in μM with the air-segment analyser in the laboratory for discretely collected samples, pearson' $r = 0.4$, $n = 17$, two clear outliers (\times) were removed and (c) H_4SiO_4 in μM measured on-site with the AutoLAB analyser compared to synchronised H_4SiO_4 in μM measured with the air-segment analyser in the laboratory for discretely collected samples, pearson' $r = 0.4716$, $n = 19$.







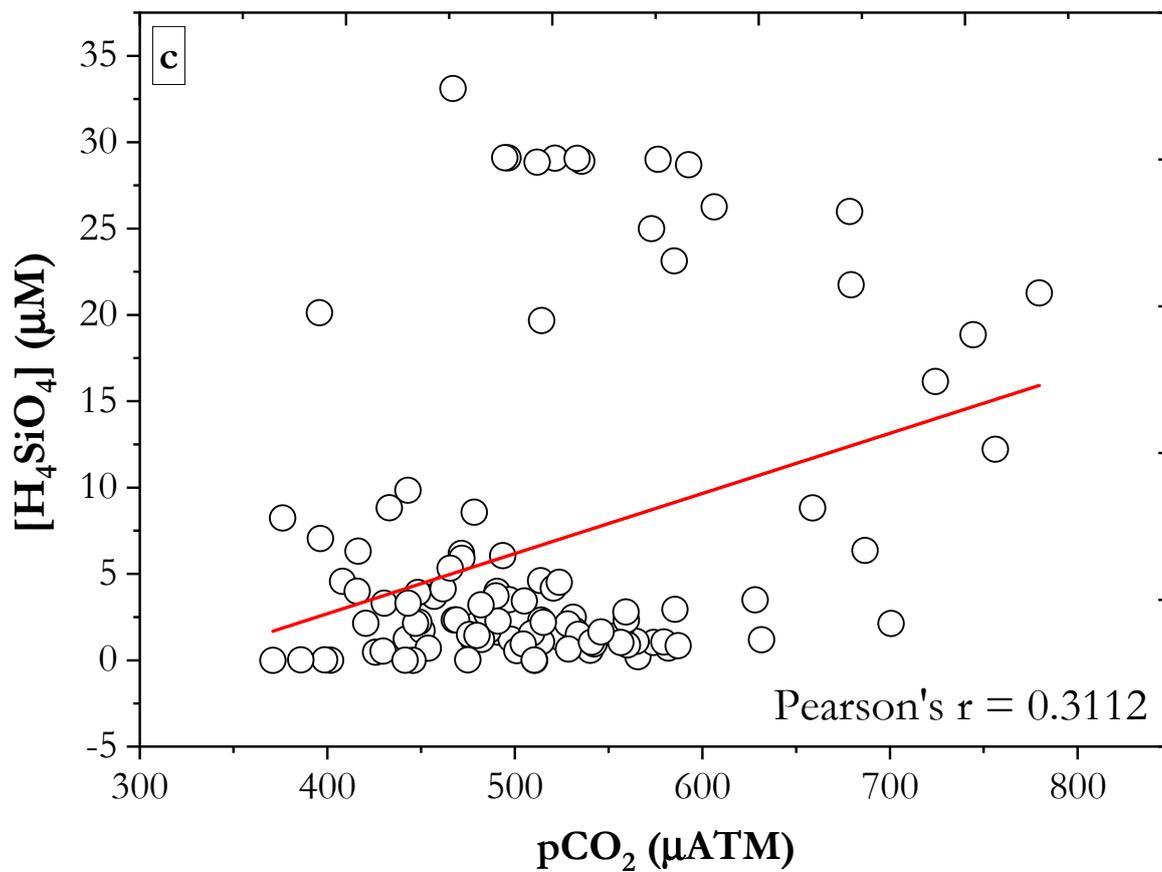


Figure S8. Plot-by-plot plots for the 11-day period from June 4 to June 9 and from June 18 and June 22 to June 27, 2021 for (a) in situ $p\text{CO}_2$ data compared to on-site PO_4^{3-} measured by AutoLab with a unique outlier (\times) was excluded (pearson's $r = 0.32294$, $n = 122$), (b) in situ $p\text{CO}_2$ data compared with on site $\Sigma(\text{NO}_3^- + \text{NO}_2^-)$ measured by AutoLab with two clear outliers (\times) excluded (pearson's $r = 0.30034$, $n = 122$), and (c) in situ $p\text{CO}_2$ data compared with on site H_4SiO_4 measured by AutoLab (pearson's $r = 0.3112$, $n = 108$).