Supplementary material

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Content:

Section 1 – Cation effect on the stability of PTA at pH 7.5.

The effect of frequently occurring cations in groundwaters (Fe^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+} and Ni^{2+}) on the stability of PTA were studied under anoxic conditions at slightly alkaline conditions. External quantification of PTA was by HPLC-UV/VIS following a different analytical protocol than the main study. The section summarizes data from two unpublished technical reports from University of Copenhagen [1,2].

Materials and methods

All samples were prepared in a glovebox (COY Lab Products; Grass Lake, Michigan, USA) under anoxic conditions to prevent oxidation of Fe^{2+} in MOPS buffer solutions (3-(N-morpholino)propanesulfonic acid; Sigma-Aldrich, Copenhagen, Denmark). A MOPS buffer control were included (5.30 mM MOPS buffer; pH 7.48), while the effect of the cations were studied in 5.01 mM MOPS at approx. pH 7.48: Ammonium iron(II) sulphate hexahydrate (0.08956 mM Fe^{2+}; pH_{START}= 7.47); Copper (II) sulfate pentahydrate (0.08951 mM Cu^{2+}; pH_{START}= 7.46); Manganese(II) sulfate (0.08958 mM Mn^{2+}; pH_{START}= 7.48); Magnesium sulfate heptahydrate (3.128 mM Mg^{2+}; pH_{START}= 7.47); and Nickel(II) nitrate hexahydrate (0.08969 mM Ni^{2+}; pH_{START}= 7.47). PTA was purified prior to the experiment as described in the main text. PTA_{START} = 20.0 mg L^{-1} PTA. Each batch was divided into at least 42 aliquots of 100 µL kept in the dark at room temperature (T = 22.5°C to 26.0°C). The

vials were sealed with air-tight rubber septa to prevent air from the samples and subsequent oxidation after removal from the glovebox. The pH was measured every two weeks to ensure no changes in the pH of the solution over the course of the experiment.

Quantification of PTA took place on a Agilent 1200 Series HPLC System (Agilent 1100 Series Oven; Agilent Technologies, Santa Clara, California, USA) equipped with a Phenomenex Gemini 3µm C6-Phenyl 110A column (50×2.00mm, 3µm; Værløse, Denmark) kept at 35°C using a corresponding guard column. Separation at isocratic mode for the first 3 min (water-acetonitrile 83:17 v:v); gradient step A 3-4 min (target water-acetonitrile 63:37 v:v); gradient step B 4-7 min (target water-acetonitrile 18:82 v:v) hereafter returning to start conditions. Time of analysis = 14min. Flow = 1.0 ml min^{-1}. Injection volume: 20µl. Performance of the analytical method: Linearity: 0.3-24.7 mg L^{-1} PTA (5.0 mM MOPS buffer; r=0.9995-1.0000, 7 standards). LOD: Conditionally set as smallest concentration of calibration curve. Rt_{PTA} = 0.9-1.0min (quantification); Rt_{PtB} = 4.8-4.9min (qualitative identification of metabolite only). Matrix effects on resolution and Rt: None. Ruggedness of standards (12.4 mg L^{-1} PTA; 5.0 mM MOPS buffer; 30/100hrs; area): 0.06-0.20%. A full calibration curve was produced for each time of sampling with the exception of 30hrs and 100hrs where single standard quantification was applied (12.4 mg L^{-1} PTA; 5.0 mM MOPS buffer).

Degradation of PTA were analyzed assuming first-order degradation kinetics ($[\mathrm{PTA}] = [\mathrm{PTA}]_0 \mathrm{e}^{-\mathrm{kt}}$) using OriginPro® 2020 (OriginLabCorp, MA, USA).

Results and conclusion

The degradation of PTA is shown in Figure S1 and summarized in Table S1. The coefficient of determination (R^2) for the first-order fitting of the degradation data ranged from 0.9516 to 0.9917 in all experiments. Hence, the observed degradation pattern can be explained by first-order degradation kinetics assuming time as the only variable.

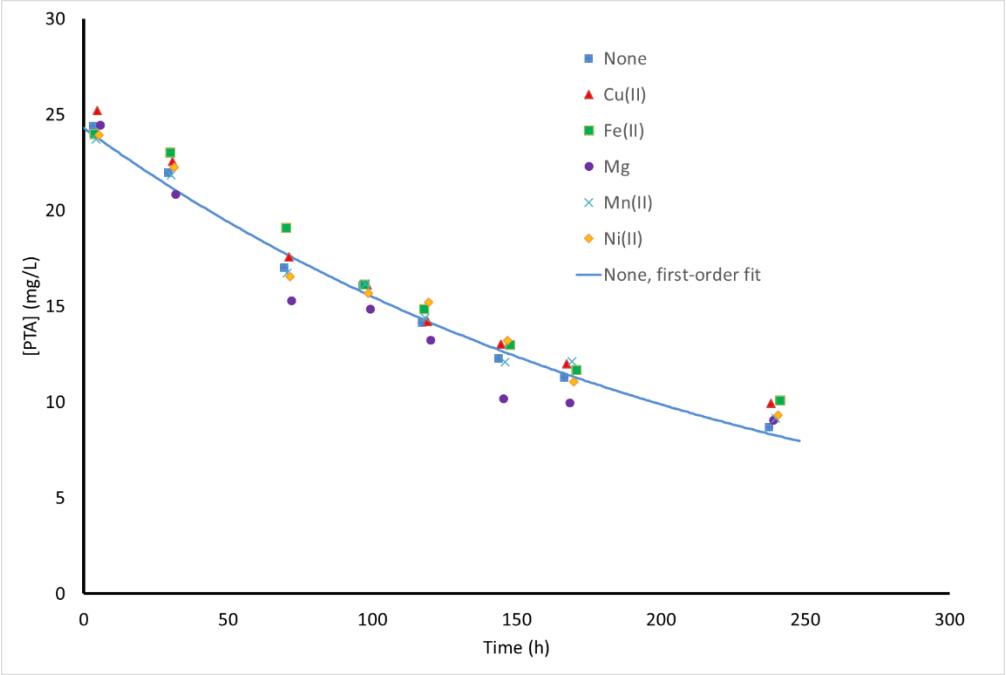

Figure S1. Hydrolysis of ptaquiloside (PTA) and the effect of different cations under anoxic conditions and pH approx. 7.5. First-order fit of unamended MOPS buffered solution inserted.

The rate constants ranged from 0.0042±0.0002 to 0.0052±0.0004. No significant effect of the tested cations was observed at pH 7.5 compared to the control (no cation added), except for Fe(II) having diminutive slower reaction rate.

Table S1 Reaction rate constants. 95%-confidence limits indicated.

Cation	$[M^{2+}]$ (mM)	$[PTA]_0$ (mg/L)	k_{obs} (h^{-1})	R^2
None (control)	0	27.70±0.33	0.0047±0.0002	0.9917
Cu(II)	0.09	25.34±0.51	0.0045±0.0002	0.9807
Fe(II)	0.09	24.95±0.51	0.0042±0.0002	0.9782
Mg	3.13	24.41±0.87	0.0052±0.0004	0.9516
Mn(II)	0.09	24.11±0.48	0.0043±0.0002	0.9803
Ni(II)	0.09	24.56±0.68	0.0044±0.0004	0.9542

In conclusion, the rate of degradation of PTA in sterile anoxic environments is not influenced by the studied metal cations added to the buffered aqueous systems at pH 7.5. Neither the alkaline earth metal cation (Mg^{2+}) as well as the tested transition metal cations had any marked effect on hydrolysis, and temperature and pH remains as the two dominating variables determining the rate of hydrolysis [3].

Section 2 – Effect of ionic strength on the stability of PTA

The effect of ionic strength (IS) on the stability of PTA were studied at pH 3.1 and 6.0 at 8°C. The study used external quantification of PTA by LC-MS following a different analytical protocol than the rest of the studies.

Materials and methods

Decarbonated LC-MS grade water was made immediately prior to the use and used for all solutions. Hydrochloric acid and sodium chloride was of analytical quality (Sigma-Aldrich, Copenhagen, Denmark). PTA was purified in the laboratory according to Kisielius et al. (2020). A stock solution of 915 mg/L PTA dissolved in LCMS-water was used for the experiments. The stock was diluted to obtain a working standard of 2.29 mg/L, used for kinetic experiments. Degradation kinetics was monitored after mixing of 40 µL of the working standard with 960 µL of either 1) 0.100 M NaCl (IS 0.100 M); 2) 0.010 M NaCl (IS 0.010M); 3) 0.001 M NaCl (IS 0.001 M); 4) 0.100 M NaCl, 0.001 M HCl (IS 0.101 M); 5) 0.010 M NaCl, 0.001M HCl (IS 0.011 M); and 6) 0.001 M NaCl, 0.001 M HCl (IS 0.101 M). The resulting starting PTA concentration was 92 µg/L.

The hydrolysis experiment and quantification of PTA took place on an Agilent 1260 Series HPLC System equipped with a 6130 Single Quadrupole Mass Spectrometer (Agilent Technologies, Santa Clara, California, USA) and an Agilent InfinityLab Poroshell 120 column (EC-C18, 2.7 µm, 3.0×50 mm; guard column EC-C18, 2.7 µm, 3.0×5 mm) kept at 35°C. Samples were placed in the autosampler during hydrolysis. Chromatographic separation followed the method of Kisielius et al. (2020; 4) with the following modifications: Runtime adjusted to 7 min; autosampler and column compartment kept at 8 °C.

The kinetic experiments were conducted using the autosampler robot in injector programming mode and temperature controlled (8 °C). Samples (three measurements at each timepoint) were injected in random order with a positive control after 7 injections (drift control). The experiment lasted approx. 120hrs. pH was monitored in aliquots made the same way during the duration of the kinetics experiment. The experiment was conducted within the linear range of the method. As no drift was observed, all kinetic calculations were made based on the area of the PTA chromatographic peak.

Degradation of PTA were analyzed assuming first-order degradation kinetics in Microsoft Excel (Microsoft Office 365 ProPlus, Redmond, Washington, USA).

Results and conclusion

The degradation of PTA is shown in Figure S2 and summarized in Table S2. The degradation can be explained by first-order degradation kinetics assuming time as the only variable. The rate constants were approx. 0.0006 h^{-1} at pH 6.0 and approx. 0.0025 at pH 3.1. The coefficient of determination is not so good at pH 6.0 due to the stability of PTA, but the precision of the rate constant is satisfactory (R^2=0.6772-0.9723).

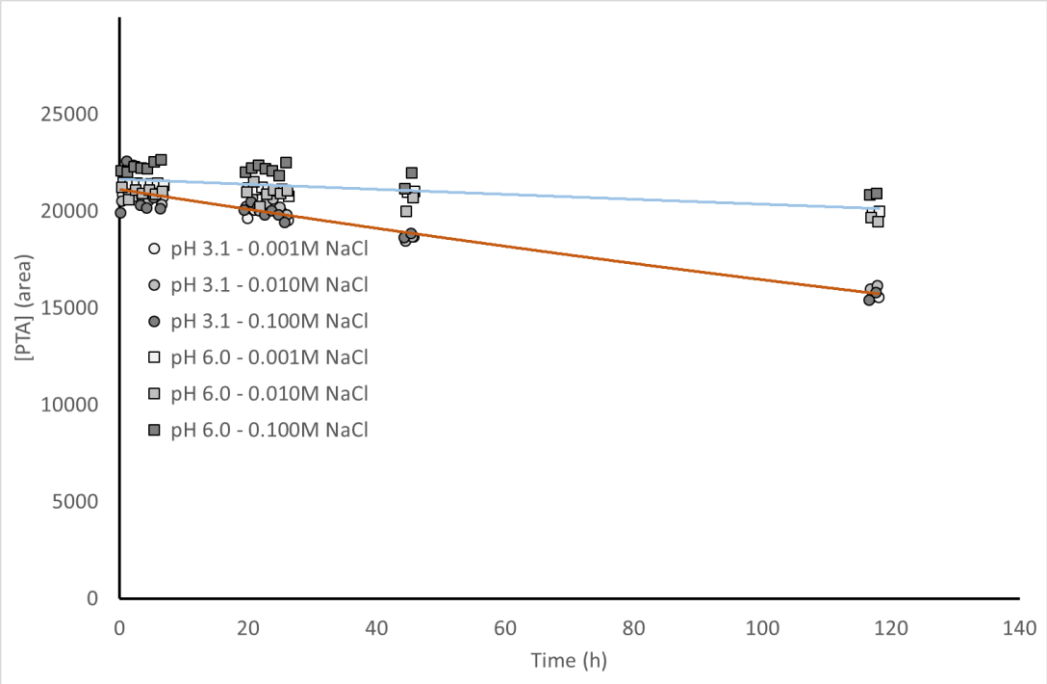

Figure S2. Hydrolysis of PTA at pH 3.1 and 6.0 and the effect of ionic strength on the reaction rate. First-order fit of average inserted (red line = pH 3.1; blue line = pH 6.0).

Table S2 Reaction rate constants. $[PTA]_0$ = 92 µg/L. 95%-confidence limits indicated.

NaCl (M)	pH	[PTA] (area)	k_{obs} (h^{-1})	R^2
0.001	6.04±0.01	21452	0.0006±0.0001	0.9518
0.010	6.05±0.02	21115	0.0006±0.0002	0.6772
0.100	6.04±0.01	22373	0.0006±0.0001	0.7387
0.001	3.11±0.02	21188	0.0026±0.0003	0.9723
0.010	3.10±0.02	21043	0.0023±0.0002	0.9542
0.100	3.08±0.01	21098	0.0026±0.0005	0.8924

No significant effect of the ionic strength on the rate constant was observed at the two pH values at 8 °C. In conclusion, there is no apparent effect of ionic strength within the range from 0.001 to 0.100 M on the rate of PTA degradation.

References

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4. Kisielius V, Lindqvist DN, Thygesen MB, Rodamer M, Hansen HCB, Rasmussen LH (2020). Fast LC-MS quantification of ptesculentoside, caudatoside, ptaquiloside and corresponding pterosins in bracken ferns. J Chrom. B, https://doi.org/10.1016/j.jchromb.2019.121966.