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Does the natural carcinogen ptaquiloside degrade readily in groundwater?

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Abstract

Background: Ptaquiloside (PTA) is a natural carcinogen found in bracken ferns. PTA is released from the plants via soil to surface and groundwaters from where humans can be exposed via drinking water. Primary degradation of PTA is due to hydrolysis with formation of pterosin B (PTB). Temperature and pH determine the rate of hydrolysis under pure experimental conditions. To assess the applicability of the experimental model to natural groundwaters, PTA degradation kinetics were examined in a range of natural groundwaters at environmentally relevant conditions.

Results: PTA was quantified by UPLC-MS/MS. Over an 80-day study period, PTA half-lives ranged from 6.5 to 47 days (natural pH; 8.0 °C). The fastest degradation was observed for the most alkaline groundwaters with pH of around 8. Rates of degradation were well predicted using an existing mathematical model for hydrolysis. However, deviations from this model were found, especially at the extremes of the examined pH-range (4.7–8.2). The degree of conversion of PTA to PTB was close to unity around neutral pH. However, at slightly acidic conditions, formation of PTB could only count for 9% of the degraded PTA, indicating formation of other products.

Conclusions: Degradation of PTA in groundwater is determined by pH and temperature, and PTA can prevail for months under slightly acid to neutral pH conditions. The existing laboratory-based model for PTA hydrolysis is generally applicable for groundwaters but needs further validation at high and low pH.

Keywords: Pterosin B, Bracken fern, *Pteridium*, Hydrolysis, Cancer, Drinking water

Background

Ptaquiloside (PTA, Fig. 1) is a naturally occurring illudane glycoside with carcinogenic and mutagenic properties. The group of illudane glycosides comprise PTA and PTA-like compounds like caudatoside, ptesculentoside and hypoloside all sharing the same illudane-type skeleton and a highly reactive cyclopropane moiety responsible for their genotoxicity [1–3]. PTA is the most studied illudane glycoside and is particularly well-studied in bracken ferns (*Pteridium* spp.), which are classified by WHO/IARC as "…possibly carcinogenic to humans" (Group 2B [4]). In addition, ingestion of bracken causes a number of chronic and acute intoxications among farm animals, of which *Bovine Enzootic Haematuria* (urinary bladder cancer among bovines) is widespread in bracken infested areas [1, 2]. Bracken ferns are an abundant and globally-distributed genus, with the exception of areas with extremely cold or dry conditions. Accordingly, the ferns are found on all continents except for Antarctica. Bracken grow as a natural part of forested ecosystems and as a primary species in bush areas. Within agriculture, bracken often appears as a common weed with invasive properties on grasslands, where dense stands can be found covering huge areas [5].

Humans may come in contact with PTA in a number of ways, e.g., by direct ingestion of bracken, inhalation of spores, or via contaminated milk or water [2, 6–9]. PTA is found in all parts of bracken ferns above as well as below the soil surface. It can be released into the

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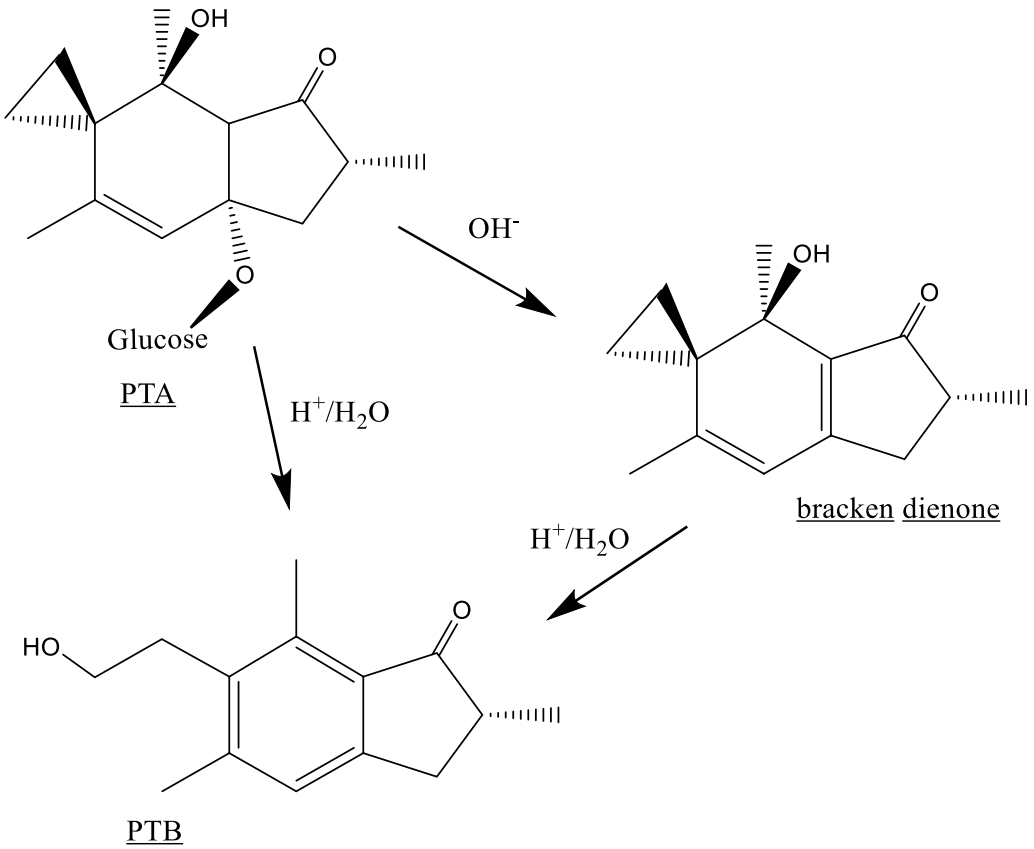

Fig. 1 Hydrolysis of ptaquiloside (PTA) and formation of the main metabolites pterosin B (PTB) and the bracken dienone under alkaline and/or acidic conditions. Other metabolites and reaction intermediates may form depending on reaction conditions. Adapted from [1]

environment from living and decaying plant material by rain. Rain will wash off PTA released from the often-extensive populations of up to hundreds of hectares, which may result in contamination of soils and eventually surface waters and groundwaters. Rain events will result in pulses of PTA entering the environment [10]. However, PTA is also continuously being released from bracken stands into recipients, and baseline levels are encountered in streams in bracken infested areas [10–12]. Recent field based investigations in Denmark, United Kingdom and Ireland have proven the presence of PTA, caudatoside and degradation products in surface and shallow groundwaters in bracken infested areas of up to 2.5 µg/L [10, 12–15]. For humans, it has been shown that consumption of bracken crosiers and/or the mere fact of living in a bracken infested area can result in a relative risk of 1.5 to 5.5 for developing cancers (esophageal/gastric/upper alimentary tract; [1]). According to the guidelines from The Danish Environmental Protection Agency, the tolerable concentration of PTA in drinking water is approximately 0.015 µg/L [16]. Hence, the recent findings of PTA could prove to be problematic, especially if PTA is stable for longer periods in groundwater.

PTA is sensitive to acid and alkaline hydrolysis, resulting in aromatization and formation of non-carcinogenic pterosin B (PTB). Similar pterosins are found as products of hydrolysis from other illudane glycosides. Hydrolysis of PTA can be described by conventional hydrolysis kinetics under pure experimental conditions [17, 18]:

$$k_{\text{obs}} = k_A\left[H^+\right] + k_N + \frac{k_B \times k_W}{[H^+]} \quad (1)$$

where $k_w = 10^{-14.10}$ (22 °C [18]) and the pH-dependent rate constants are $k_A = 25.70\ \text{M}^{-1}\ \text{h}^{-1}$ (acidic conditions), $k_N = 9.49 \times 10^{-4}\ \text{h}^{-1}$ (neutral conditions), and $k_B = 4.83 \times 10^4\ \text{M}\ \text{h}^{-1}$ (alkaline conditions). The rate of hydrolysis has a minimum at pH 5.5 with a window of slow hydrolysis in the pH range 4.5 to 6.5. In addition, clay minerals and low temperatures slow down PTA hydrolysis. Microbial degradation has a pronounced effect on PTA degradation making it most stable in environments with low microbiological activity [17, 19, 20].

Thus, in regions with clay-containing aquifers and/or acid groundwaters in addition to low temperatures and low microbiological activity in aquifers, PTA is expected to hydrolyze slowly. Groundwater may, therefore, be a source of PTA exposure for humans in bracken infested areas. Recently, Skrbic and co-workers (2020) found PTA and caudatoside and pterosins in shallow groundwater wells and proved the hypothesis to be true [14, 21].

The aim of this study was to quantify the stability of PTA under near-natural conditions in Danish groundwaters and to test if the hydrolysis kinetics found in the laboratory (Eq. 1) apply to true groundwaters to gain a better understanding of PTA degradation dynamics and to model PTA dissipation in aquifers with acid to weakly alkaline pH.

Materials and methods

Chemicals

Ammonium acetate, formic acid (MS grade), glacial acetic acid, trifluoroacetic acid, methanol (MS-grade), and polyamide-6 resin were supplied by Sigma-Aldrich (Steinheim, Germany). Sodium hydroxide was from J.T.Baker (Deventer, the Netherlands). Deionized ultrapure water produced by PURELAB Chorus ultrapure water system (ELGA, France) was used throughout.

PTA for use as standards and in experiments was isolated from dried bracken fronds by the method described by Clauson-Kaas et al. [22], based on the work of Rasmussen et al. [20]. In short, an aqueous extract of the bracken fronds was purified on self-packed XAD-2 and ChemElut columns, and further purified using preparative HPLC. The process was followed by an additional purification step using a polyamide column to remove potential degradation products formed during storage. The purity of the PTA was 84% based on quantitative ^{1}H NMR spectroscopy using 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt as internal standard. No contaminants were observed by full-scan MS/UV–VIS spectroscopy of aqueous PTA solutions indicating that the purity below 100% was due to water absorbed by the highly hygroscopic purified PTA product.

Stock solution and calibration standards

PTA stock solutions (61.4–62.5 mg/L) were prepared from isolated PTA in water and stored at −18 °C. Stock solutions were completely thawed at room temperature and shaken in connection with preparation of standards and for use in experiments. A 200 µg/L PTB solution was made by 1:1 conversion of a PTA stock solution following the methods of Aranha et al. and Rasmussen & Pedersen [23, 24]. In brief, an aliquot of a PTA stock was diluted in H_2O, made alkaline with sodium hydroxide, incubated in a water bath at 45 °C for 60 min, then reacted into PTB on addition of trifluoroacetic acid. The solvent composition was adjusted to 40% methanol (v/v) to obtain 200 µg/L PTB. Calibration standards of PTA and PTB in the range 1–40 µg/L were prepared in 40% methanol (v/v) in 2 mL amber LC vials. All standards and samples were kept at −18 °C when not in use which has proved efficient for stability of standards [22].

Collection of groundwater samples

Five groundwaters were chosen to represent the natural variation of deep groundwater chemical composition in Denmark, in particular with respect to pH (Fig. 2). The raw untreated groundwaters were collected from individual wells supplying public waterworks in the Eastern (Zealand) and Western (Jutland) regions of Denmark (except Ermelund, where the abstracted water is mixed from several wells). The temporal variation of the water chemistry is low and to a large extent determined by the aquifer geochemistry. The chemical composition of the waters is shown in Table 1 [25].

The lower pH water types (Agerbaek, Varde, and to some extent Hovborg) are congregated around the area of Western Jutland, which is characterized by sandy aquifer sediments, though the Varde area is also characterized by the presence of some clay. The higher pH water samples (Ermelund and Villingebaek) were taken from the island of Zealand, an area characterized by fine and silty deposits and limestone aquifers. The acid waters are characterized by high contents of dissolved carbon dioxide and higher solubility of metal cations. On the other hand, the non-acid waters have high alkalinity and hence high hardness due to the simultaneous presence of high concentrations of calcium and magnesium [25]. Anoxic conditions may be present in both acid and alkaline groundwaters giving rise to the presence of iron(II).

The Ermelund water has considerably higher levels of most solutes (except carbon dioxide, oxygen, ammonia + ammonium, iron, and nitrate) compared to the other waters, and accordingly a relatively high amount of evaporation residue. The Zealand waters (Ermelund and Villingebaek) also have higher levels of dissolved organic carbon (2.9 and 2.2 mg/L, respectively) and conductivity than their Jutland counterparts. The Varde and Agerbaek water samples have notably higher levels of nitrate. All included waters were raw waters, not yet treated for use.

Raw abstracted water was sampled on two occasions in July 2015. The raw water source was kept running for at least 15 min before collection in double-acid washed 1 L BlueCap glass bottles (Duran® laboratory bottles, Sigma-Aldrich, Denmark). The bottles were filled and rinsed twice with the raw groundwater before being nearly completely filled (to minimize diffusion of air). Two liters of each water were collected. Each bottle was

Fig. 2 Map of Denmark with the five groundwater sampling locations (water plants)

covered in aluminum foil to prevent photochemical reactions during transport and storage. The waters were kept in ice-filled buckets during transport and stored in a dark and cool environment (2–5 °C) until use.

Experimental set-up.

For each PTA hydrolysis experiment, PTA was diluted in 100 mL of groundwater to a starting concentration of about 40 µg/L in 100 mL double-acid washed BlueCap glass bottles (triplicate; Duran® laboratory bottles, Sigma-Aldrich, Denmark). A Control in pure LC–MS grade water was included, also in triplicate (PURELAB Chorus ultrapure water system, ELGA, France). Each bottle was wrapped in aluminum foil to minimize light exposure that could interfere with PTA degradation. The bottles were stored in a dark refrigerator. A temperature logger (EL-USB-2, Lascar Electronics, Erie, USA) took measurements of refrigerator temperature every 10 min for the duration of the experiment. The average temperature was 8.0 ± 1.4 °C (standard deviation), which reproduces the natural temperature level of the groundwaters [25].

The sampling schedule of the stored waters was as follows: 1-day intervals for the first 5 days, then three, five, and approx. 10-day intervals thereafter for a total duration of 80 days (1920 h; all triplicate waters).

A subset of samples collected across the studied groundwaters were analyzed for formation of PTB as well. These were selected at 5 different time points in the experiment (days 13, 19, 49, 69, and 80) from each of the waters, except Villingebaek, where only two samples were included due to undetectable levels of PTA after day 13. The ratio of PTA consumption and PTB (in PTA-equivalents) formation in samples were compared. Each sample was analyzed once in the subset.

Sample preparation and quantification of analytes

A sample of approx. 2 mL was removed from each BlueCap bottle at each sampling time point. One mL of solution was transferred to an LC vial and the pH was measured using a Metrohm microelectrode (triplicate measurement). In a separate 2 mL amber LC vial, 930 µL of sample solution was prepared by dilution with 620 µL methanol to reach a solvent composition of 40% methanol, buffered with 23 µL of 0.3 M ammonium acetate buffer as suggested by Clauson-Kaas et al. [22] and filtered using a fresh RC-syringe filter (0.2 µm,

Table 1 Raw groundwater sampling locations and chemical composition

Location	Villingebaek	Ermelund	Hovborg	Varde	Agerbaek
Coordinates of well	56 °05′ 49.0″ N 12°23′55.6"E	55° 44′ 29.4″N 12° 33′ 04.8″ E	55° 36′ 35.7″ N 8° 56′ 27.1″ E	55° 38′ 09.9″ N 8° 29′ 45.3″ E	55° 36′ 50.7″ N 8° 47′ 08.7″ E
pH	7.8	7.0	5.9	5.8	4.7
Temp (°C)	9.5	11	8.3	8.5	8.1
Conductivity (mS/m)	46	141	12	27	27
Evaporation residue	300	913	87	160	170
Oxygen	< 0.2	0.2	0.7	0.5	3.0
Carbon dioxide	< 2	< 5	30	35	50
Organic carbon	2.2	2.9	0.2	0.5	0.7
Ammonia + ammonium	0.62	0.01	0.08	0.04	0.01
Calcium	64	173	11	19	12
Iron	1.2	< 0.01	2.2	0.58	1.3
Magnesium	10	22	1.5	5.8	7.9
Manganese	0.16	0.52	0.10	0.08	0.07
Phosphorus	0.14	0.05	0.06	0.01	0.02
Potassium	2.6	26	0.92	2	1.8
Sodium	23	82	9.3	22	19
Bicarbonate	250	452	41.2	16.1	4.3
Chloride	22	190	15	34	34
Fluoride	0.32	0.27	0.35	0.12	0.75
Nitrate	< 0.3	3.35	< 0.5	17	32
Nitrite	0.011	0.046	< 0.005	0.011	0.011
Sulfate	12	54	6.9	45	35

Number of digits as reported [25]

Ø = 25 mm) to remove particulates. The filtrates were stored at − 18 °C until quantification. Blank, non-fortified samples, were included in the experiment for quality control.

Water samples were analyzed by UPLC-MS/MS using 10 µL injections (Acquity UPLC I-Class (Waters, Milford); Xevo TQD triple quadrupole mass spectrometer (electrospray ionization; Waters, Milford); Cortecs C18 column (2.1 × 50 mm, particle size 1.6 µm; Waters, Milford) following the method of Clauson-Kaas et al. [22] employing a water–methanol gradient and controlling pH with a low concentration of formic acid (0.01% (v/v)). The flow rate was 0.450 mL/min. PTA was monitored as the ion traces *m/z* 421.1–>241.1 (quantifier) and *m/z* 421.1–>203.1 (qualifier), eluting at 1.40 min; PTB as *m/z* 219.1–>201.1, eluting at 1.80 min (no stable qualifier). PTA and PTB were quantified by external calibration with seven freshly prepared standards in the range 1–40 µg/L PTA and PTA-equivalents, respectively. The limit of quantification was set as the lowest used standard (1 µg/L), with the same quality control considerations as documented earlier [22]. No significant matrix effects were observed following signal/concentration check of the first data point (Fig. 3).

Quality control—pH and microbial activity

Over the course of the 80 day (1920 h; study period, the average pH in four groundwater samples had a noteworthy increase from their starting values (Villingebaek: + 0.40; Ermelund: + 0.53; Hovborg: + 1.06; Varde: + 1.20). This is attributed to venting of carbon dioxide upon opening of the flasks for sampling. However, Agerbaek, with the highest reported content of CO_2 of the waters, had no significant deviation in pH over the course of the study period indicating that acidification reactions such as oxidation of iron(II) may also have taken place.

Each of the PTA-spiked groundwaters were tested for microbial growth after 62 days (1490 h; 1×, 10×, 100×, and 1000× dilutions at 30 °C for 24 h; [26]). Only 5 of 60 plates grew any colonies. In total, four plates from the Control exhibited growth (8 and 4 CFUs at no dilution; 5 and 1 CFUs at 10× dilution). One plate from Ermelund had a single CFU at no dilution. All other plates were free of visible microbial growth. Thus, it is anticipated that microbial activity had little to no impact on the degradation of PTA in the stored waters.

Data analysis

The concentration of PTA in the groundwaters is a function of the rate of abiotic hydrolysis plus any other reactions taking place, including microbial degradation. To determine the rate of PTA hydrolysis, a third-degree polynomial function was applied to the observed PTA concentration over time for each groundwater (Eq. 1):

$$[\mathrm{PTA}]_t = at^3 + bt^2 + ct + d \quad (2)$$

where a, b, c and d are constants, t is time and $[\mathrm{PTA}]_t$ is the PTA concentration at time t. Datapoints below the limit of quantification were omitted from analysis. Incremental first-order rate constants ($k_{\mathrm{obs}}^{\mathrm{incr}}$) were calculated for each sampling point as the derivative of the polynomial, divided by the average PTA concentration at that time:

$$k_{\mathrm{obs}}^{\mathrm{incr}} = \frac{[\mathrm{PTA}]_t{}'}{[\mathrm{PTA}]_t}; [\mathrm{PTA}]_t{}' = 3at^2 + 2bt + c \quad (3)$$

where $k_{\mathrm{obs}}^{\mathrm{incr}}$ is the observed incremental rate constant and $[PTA]_t{}'$ is the derived polynomial function of degradation over time (t).

The estimated observed incremental degradation rate constants for the studied groundwaters were compared to the rate constants for hydrolytic degradation in pure aqueous solutions, Eq. 1, using the appropriate pH at the time of sampling in the hydrolysis experiment. In this former study rate-constants were determined at 22 °C. For comparison with the groundwater hydrolysis data obtained at 8 °C, temperature correction is needed to account for temperature dependent changes in reaction kinetics. Hence, the rates at 22 °C have been retransformed to rates at 8 °C using the Arrhenius equation:

$$k_{\mathrm{pred}} = \frac{k_A\left[H^+\right] + k_N + \frac{k_B \times k_W}{\left[H^+\right]}}{\exp\left(\frac{E_A}{R} \times \left(\frac{1}{273.15+T} - \frac{1}{295.15}\right)\right)} \quad (4)$$

where $R = 8.314$ J K^{-1} mol^{-1}, $k_w = 10^{-14.62}$ (8.1 °C; [18]), T = temperature (°C) and the pH-dependent rate constants are as in Eq. 1. The activation energy (E_A) for PTA degradation is assumed to be equivalent to 74.4 kJ mol^{-1} at all pH values (data only available for pH = 4.46 [17]). The resulting k_{pred} is calculated for separate temperatures plotted in Fig. 4.

All statistical analysis was made in Microsoft Excel (Microsoft Office 2016, Redmond, Washington, USA).

Results and discussion

Degradation of PTA in groundwater

Within the 80-day (1920 h) study period, the waters of Villingebaek and Varde both had complete degradation of PTA, while the groundwaters from Ermelund, Hovborg, and Agerbaek had almost complete degradation (Fig. 3). The decrease in PTA concentrations with time could be described by 3rd order polynomium fits (Eq. 1) with $R^2 > 0.98$ throughout the investigated period except for the control which showed no significant degradation of PTA.

The apparent half-life, e.g., the time for half of the initial PTA to degrade, was shortest for the Villingebaek sample with a half-life of 6.5 days (156 h; the remaining half-lives were 18 days (430 h; Ermelund), 39 days (940 h; Hovborg), 39 days (940 h; Varde), and 47 days (1130 h; Agerbaek). A clear effect of pH is seen, with the more alkaline waters showing considerably faster degradation (Figs. 3 and 4). Note also the tendency of higher degradation rates for Hovborg and Varde waters when the pH in these waters increased after about 600 h. The Control showed very little degradation, with a recovery of 93 ± 3% on day 80 (1920 h).

The stability of PTA in the examined groundwater generally fits the model of hydrolysis (Eqs. 1 and 3) rather well as depicted in Fig. 4, where model prediction at different temperatures are shown as function of pH together with the observed data for the groundwaters. The temperature span from 5.5 to 10.5 °C is indicative for the conditions prevalent in Danish aquifers. Looking at the individual waters, then some variation can be seen, e.g., for Villingebaek and Varde, where dissipation of PTA initially follows the model of hydrolysis well until 100 and 813 h, respectively. After these time points, degradation proceeds faster than predicted from Eq. 1, and outliers from the model are observed. These observations could be due to specific interactions between PTA and dissolved organic and/or inorganic species [27]. However, the groundwaters were very low in dissolved organic matter. Furthermore, alkaline earth metal cations and transition metal cations appear to have no effect on hydrolysis rates (Additional file 1: Section 1), and hence the major cations (Ca^{2+}, Mg^{2+}) and Fe^{2+} present in the investigated waters should have no impact on the rates of hydrolysis.

Degradation of PTA in the Control (ultra-pure water) differs significantly from the model prediction. A consistent high recovery in the experiment stands in contrast to the > 50% degradation predicted by Eq. 1. A similar phenomenon was observed previously for analytical PTA standards and stock. We exclude stabilization of PTA in the Control due to admixed byproducts from the extracted PTA used for spiking as the purity of the extract was high. A buffer was used to control pH in the hydrolysis study of Ayala-Luis et al. [17], which may indicate that PTA hydrolysis is dependent on ionic strength and possibly specific effects with the buffer medium in addition to pH—effects which have been reported for

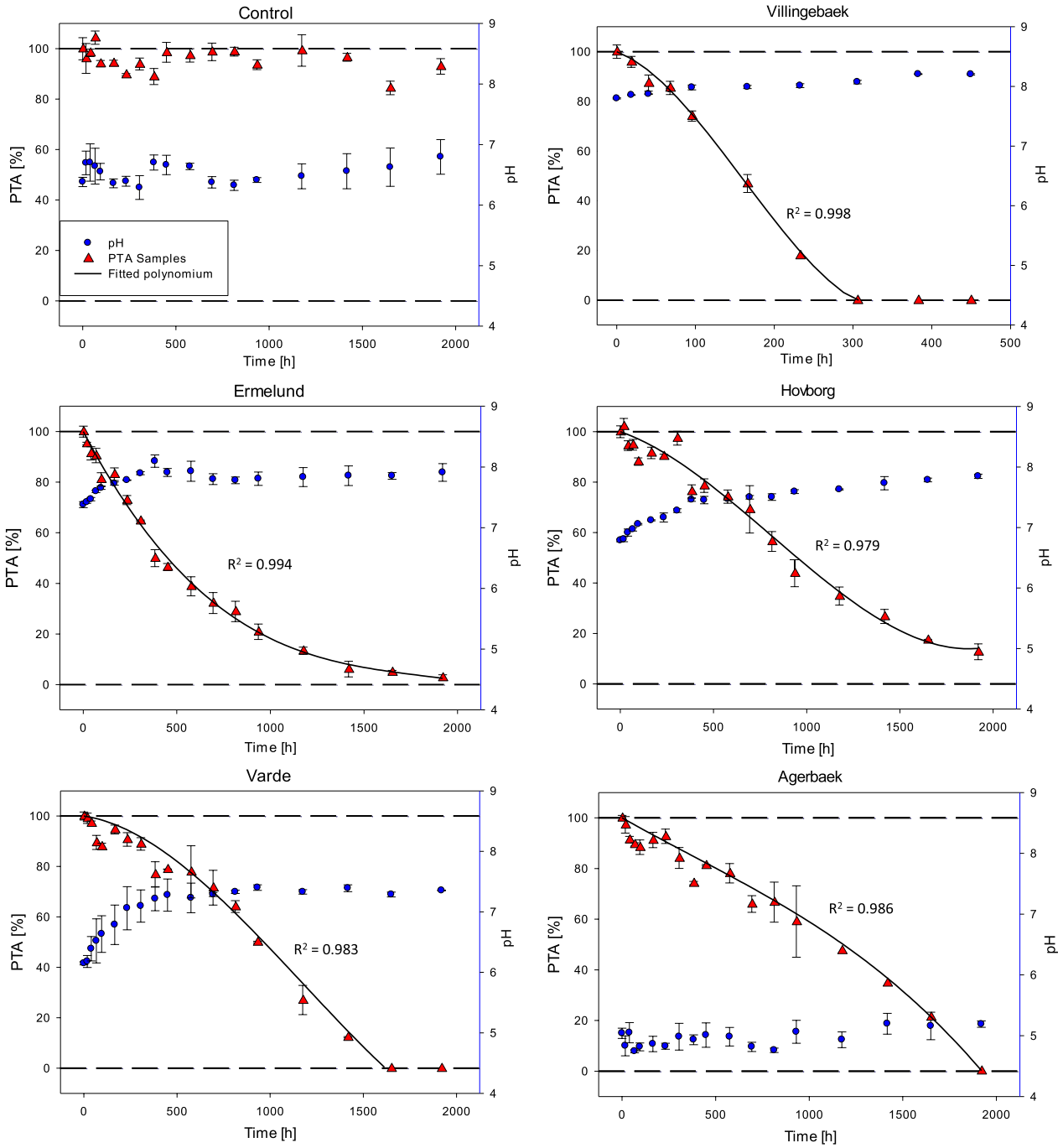

Fig. 3 Relative PTA concentration vs. time in PTA-groundwater hydrolysis experiments (red triangles) and the corresponding fits with a 3rd degree polynomium (Eq. 2; goodness of fit R^2), exept from Control, where no significant degradation took place. Development in pH (blue). Note the short time scale for Villingebaek (fast disappearance of PTA). Error bars show the standard deviation ($n = 3$)

other compounds [28–30]. To test the effect of ionic strength, a small sub-study was made using NaCl as electrolyte (Additional file 1: Section 2). However, no effect of salt concentration was observed in the salt concentration range of 0.001 to 0.100 M for the two pH values tested. Worth to note is also that the hydrolysis rates seen in the ionic strength experiment are consistent with the existing model by Ayala-Luis et al. [17] indicating that specific interactions with buffer medium are non-existing. We cannot exclude that stabilization takes place at very

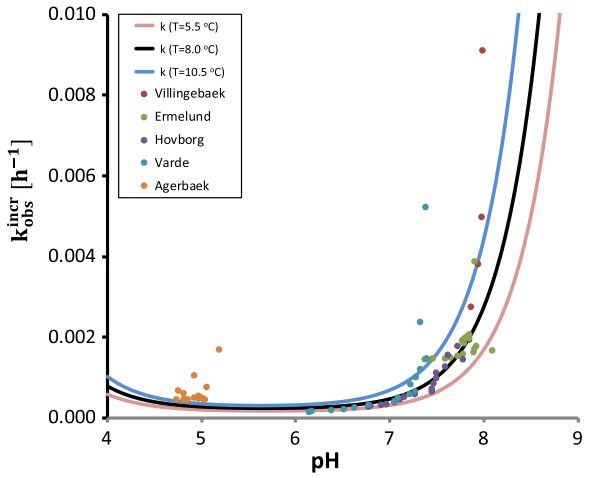

Fig. 4 Observed degradation constant (k_{obs}^{incr}) vs. pH in groundwaters. Dots represent measured data. Curves show predicted degradation rates from Ayala-Luis et al. [17] at $T = 5.5$, 8.0 and 10.5 °C corresponding to experimental conditions. Measurements above the line indicate faster degradation rates than expected and below the line indicate slower degradation rates than expected

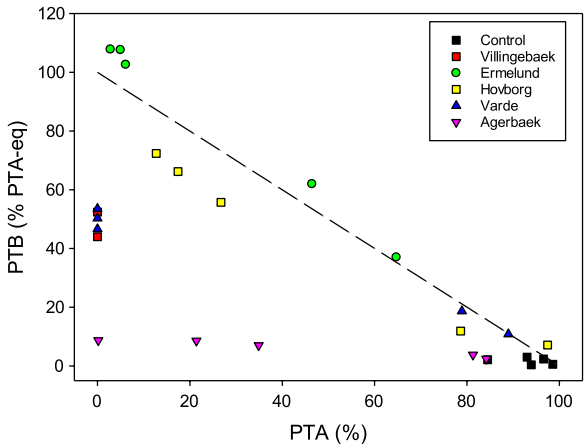

Fig. 5 PTB vs. PTA detected in samples at different time points in the degradation experiment relative to the concentration at start for each of the waters. The solid line marks a 1:1 conversion from PTA to PTB. Relative standard deviation (quality control standards) was < 7%. At Villingebæk, the squares are on top of each other

low salt concentration stabilizing the cyclopropyl group against nucleophilic attack. Thus, the unexpected stability of PTA in ultrapure water is still to be explained. For practical reasons it may be less important as natural waters always have salt concentrations higher than 0.001 M.

Under natural aquifer conditions, minerals such as clays, lime, and quartz will be in contact with the water and the PTA. From soil sorption and stability studies, it is known that PTA is stabilized by clay particles [20]. The present study included no aquifer materials in the reactivity tests. Hence, the observed degradation patterns should be considered a fast tier for PTA degradation. For the aqueous phase itself, then the observed relationship between pH, temperature and k_{obs}^{incr} confirms the existing model for PTA hydrolysis in water, although the model appears less accurate under alkaline conditions and for some of the acid samples. Further elucidation of the reaction kinetics in both the alkaline and acid range is needed. In particular, elucidation of the kinetics in the transition area between the slow neutral hydrolysis and the rapid alkaline reactions.

Rate of PTA to PTB conversion

Figure 5 shows the detected PTA and PTB in the samples analyzed for both compounds plotted vs. the initial PTA concentration for the waters; PTB is shown as PTA-equivalents for direct comparison. The data set includes samples from days 13 to 80 (312–1920 h). The slightly alkaline waters of Ermelund and Hovborg in this time period follow the 1:1 conversion well, and linear regression of these two combined yields a slope of −1.02 and an intercept of 1.00 ($R^2 = 0.89$). The more pH neutral Varde seemingly starts out similarly to Ermelund and Hovborg but ends with only about 50% conversion of PTA to PTB. At Villingebaek, the most alkaline of the waters, all PTA degraded with a conversion rate to PTB of only 50% indicating other reaction pathways and/or presence of stable reaction intermediates like the bracken dienone which is stable under alkaline conditions (Fig. 1). The slightly acidic Agerbaek water behaves different than any of the other waters, as degradation results in formation of small amounts of PTB (about 9%). The lower extent of PTB formation in Varde and Agerbaek is like what has been observed by Ayala-Luis et al. [17] for the pH ranges of these waters pointing to formation of other reaction products [1]. Intermediates were observed under laboratory conditions in studies of PTA degradation in surface waters and rapid biological sand filters by Kisielius and co-workers and Skrbic et al. in 2020 [15, 31]. PTB is generally assumed to be more stable than PTA but degrades rapidly in soils due to microbial activity [32]. However, PTB as well as other pterosins have been found in a range of surface waters, soil water as well as shallow groundwater [11–15]. The groundwaters included in this study did not exhibit microbial activity. Hence, the lack of PTB in the samples can be ascribed to formation of PTA intermediates that did not react into PTB within the duration of the experiments as indicated by Skrbic et al. [31]. As expected, the Control showed very little PTB, underscoring the stability of PTA in pure water. Taken together, the results indicate that PTB is formed at a near

1:1 conversion ratio from PTA when neutral to alkaline reaction dominates (pH > 6.39). At more acidic conditions, other unclarified reaction pathways seem to be at play, which was also suggested by Ayala Luis et al. [17].

The results from this study indicate that PTA can exist for long time periods in groundwater aquifers. PTA will not exist for long in calcareous aquifers as pH > 8. Similar conditions prevail in acid aquifers. But in aquifers with pH 6–7, and in particular shallow aquifers affected by seepage water and influenced by rain mediated preferential flow patterns and incidents in cooler regions, then PTA may be a drinking water contaminant in areas with bracken dominated land [11, 14, 15, 17].

PTA is also present in other ferns than bracken (e.g., *Cheilanthes*, *Dryopteris*, and *Pteris)*. Hence, PTA distribution in the environment is potentially larger than can be expected when looking at bracken alone. In addition, compounds similar to PTA should be included in groundwater risk assessment due to their observed or potential presence in drinking water resources, e.g., caudatoside and hypoloside [14, 15]. To conclude, contamination of drinking water with PTA and PTA-like compounds from many fern species is plausible and requires further insight.

Conclusions

Based on the experiments presented in this paper, it can be concluded that:

- PTA can be stable in natural groundwaters for months under slightly acid to neutral pH conditions, and pH is a key factor determining PTA stability with PTA being most stable in slightly acidic groundwater.
- The rate of PTA hydrolysis in groundwaters were well predicted by the rate model developed for buffered laboratory waters except for alkaline and some acid waters, where observed PTA hydrolysis were faster than predicted.
- PTA was found to convert to PTB in a 1:1 ratio in most samples, but with significant deviations from this under acid conditions, indicating formation of other reaction intermediates and products.

Hence, PTA can prevail for months under slightly acid to neutral pH conditions. Degradation of PTA in groundwater is determined by pH- and temperature, as explained by the existing model of hydrolysis. The model is generally applicable for groundwaters but needs further validation at high and low pH. Future studies should include a larger variety of groundwaters with larger variation in pH and groundwater chemistry making it possible to test effect of variables using multivariate statistical analyses, incl. effect of environmental PTA concentrations.

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-021-00468-0.

Additional file 1: Section 1. Cation effect on the stability of PTA at pH 7.5. **Section 2**. Effect of ionic strength on the stability of PTA at pH 3.1 and 6.0.

Abbreviations

HPLC: High performance liquid chromatography; IS: Ionic strength; LC: Liquid chromatography; LOD: Limit of determination; LOQ: Limit of quantification; MS: Mass spectrometry; PTA: Ptaquiloside; PTB: Pterosin B.

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Authors' contributions

JSW: conceptualization, methodology, investigation, writing-original draft. FCK: conceptualization, writing-review and editing. DNL: investigation, writing-review. LHR: conceptualization, methodology, investigation, writing-review and editing. FCK: conceptualization, writing-review and editing BWS: conceptualization, methodology, writing-review and editing. HCBH: conceptualization, methodology, investigation, writing-review and editing. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed are available upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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