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# Occurrence of carcinogenic illudane glycosides in drinking water wells

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## Abstract

**Background:** Ptaquiloside (PTA), caudatoside (CAU) and ptesculentoside (PTE) are carcinogenic illudane glycosides found in bracken ferns (*Pteridium* spp.) world-wide. The environmentally mobile PTA entails both acute and chronic toxicity. A comparable risk might be associated with the structurally similar CAU and PTE. It is of great health concern if these compounds are present in drinking water, however, it is currently unknown if these compounds can be detected in wells in bracken-dominated regions. This study investigates the presence of PTA, CAU, PTE, and their corresponding hydrolysis products pterosins B (PtB), A (PtA) and G (PtG) in water wells in Denmark, Sweden and Spain. Water samples from a total of 77 deep groundwater wells (40–100 m) and shallow water wells (8–40 m) were collected and preserved in the field, pre-concentrated in the laboratory and analysed by liquid chromatography–mass spectrometry (LC–MS).

**Results:** Deep groundwater wells contained neither illudane glycosides nor their pterosins. However, seven private shallow wells contained at least one of the illudane glycosides and/or pterosins at concentrations up to 0.27  $\mu\text{g L}^{-1}$  (PTA), 0.75  $\mu\text{g L}^{-1}$  (CAU), 0.05  $\mu\text{g L}^{-1}$  (PtB), 0.03  $\mu\text{g L}^{-1}$  (PtA) and 0.28  $\mu\text{g L}^{-1}$  (PtG). This is the first finding of illudane glycosides and pterosins in drinking water wells.

**Conclusions:** Detected concentrations of illudane glycosides in some of the investigated wells exceeded the suggested maximum tolerable concentrations of PTA, although they were used for drinking water purpose. Contaminated wells were shallow with neutral pH and lower electric conductivity compared to deep groundwater wells with no illudane glycosides nor pterosins.

**Keywords:** Caudatoside, Emerging contaminants, Ptaquiloside, Ptesculentoside, Pterosins, Water quality

## Background

Anthropogenic chemicals, such as pesticides and pharmaceuticals, are of high concern as water contaminants because of their persistence and toxicity [1, 2]. However, living organisms can also produce hazardous substances, which are both mobile, persistent and toxic [3]. Plants synthesise a broad variety of toxic chemical compounds, e.g. as a mean of protection against pathogens and herbivores [4]. Such plant-produced compounds are typically

not harmful to the plants themselves but can adversely affect human or animal health through exposure via air, soil, water or food. By their physicochemical properties, many of them can be compared to the environmentally persistent and mobile organic compounds (PMOCs), which are often not easily removed from water due to their high water solubility and having half-lives of weeks to months in the environment [5]. Plant-produced toxins have a potential to pose a higher risk to water quality than anthropogenic chemicals because of their continuous production and comparatively high concentration in plants [6]. Nonetheless, they are still an unexplored group of emerging contaminants and we currently lack the tools to monitor, regulate and remove them.

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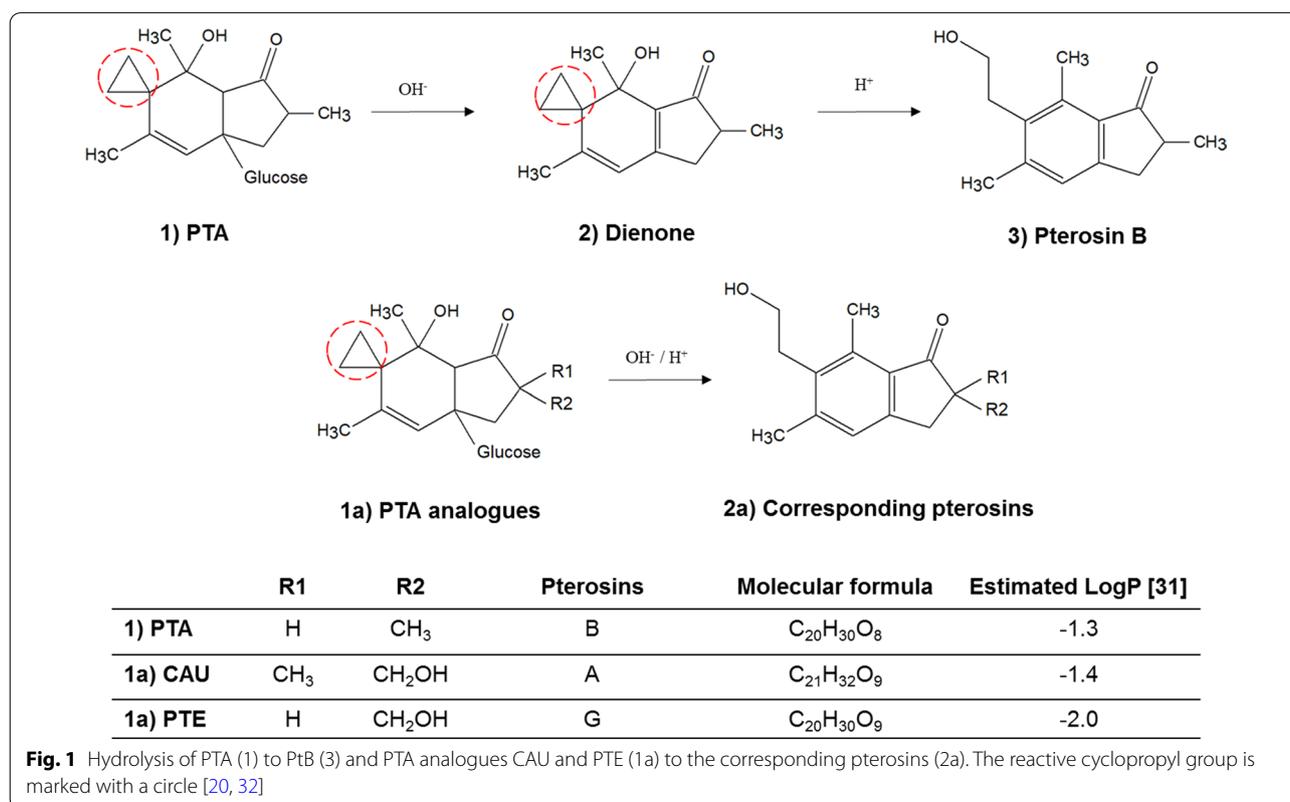
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One of the best-studied phytotoxins with PMOC characteristics under environmental conditions is the carcinogen ptaquiloside (PTA) from bracken fern. Bracken (*Pteridium* spp.) is globally widespread and is highly abundant in many parts of the world. It has an extremely high rate of reproduction and re-growth due to its extensive rhizomes and can rapidly adapt to new ecological conditions [6–9]. Bracken is acutely toxic and classified as possibly carcinogenic towards humans by the International Agency of Research on Cancer/WHO [10, 11]. Intake of toxins takes place via bracken-based food in some countries such as Korea, China and Japan. In addition, contamination of drinking water is proposed as a vector for human exposure [12].

PTA is considered the principle compound responsible for both acute and chronic bracken toxicities [13]. PTA is an illudane glycoside with carcinogenic, mutagenic and clastogenic properties and the most studied compound found in bracken [14, 15]. Under alkaline conditions, PTA (1) is converted into an unstable compound, a dienone (2) which subsequently leads to the formation of non-carcinogenic pterosin B (3) (Fig. 1). The dienone (2) is assumed to be the ultimate agent responsible for carcinogenicity with a cyclopropyl group being responsible for reaction with amino acids and DNA, and causing mutations [9, 16, 17]. Other compounds with structural

similarity to PTA have also been found in bracken (PTA analogues) with caudatoside (CAU) and ptesculentoside (PTE) being prevalent [18, 19] (Fig. 1). Although there is only limited knowledge about the toxic properties of these compounds, they share the reactive cyclopropyl group and are expected to show similar reactivity and toxic effects as PTA [20].

PTA is a highly polar compound ( $\log P = -1.3$ ; Fig. 1) and can be easily transferred to the soil by release from living or dead bracken [21, 22]. Due to the high water solubility of PTA and its low sorption to soil, it is prone to leaching from topsoil to surface and groundwater [21]–[23]. Hydrolysis rates of PTA are highly pH dependent, with the lowest degradation rates at neutral to slightly acidic conditions (pH 4.5–6.5) and at low temperatures [13, 21, 24]. PTA is prone to microbial degradation, which makes PTA hydrolysis slow in environments with low microbiological activity (e.g. in groundwater). Hence, PTA presence in groundwater should be governed by pH and temperature and PTA can sustain for months in cold water under neutral to slightly acid conditions [24–26]. PTA has already been detected in different water bodies. Concentrations of  $0.6 \mu\text{g L}^{-1}$  have been reported in spring water wells in Ireland [27], up to  $0.09 \mu\text{g L}^{-1}$  in shallow groundwater (0.1–2.5 m) in Denmark [28], and up to  $2.2 \mu\text{g L}^{-1}$  in stream water in United Kingdom [29].



In contrast to PTA, occurrence of CAU and PTE in the aqueous environment has been much less investigated. A recent study reported the first occurrence of CAU and PTE in surface waters up to 0.07 and 5.3  $\mu\text{g L}^{-1}$ , respectively [30]. This finding suggests that assessments of water toxicity based on PTA only were underestimated. CAU and PTE are slightly more polar compounds and as stable as PTA in surface waters [30].

Recent attempt of modelling PTA fate in plant–soil matrix under Danish/South-Scandinavian conditions indicates that intense precipitation events during cold periods and with a fully developed canopy induce high probability of significant PTA leaching. Furthermore, predicted high concentration of PTA in groundwater is also attributed to macropore transport in soils, sediments and bedrock [33]. Macropores have proven to be important for fast leaching of organic contaminants such as pesticides, bypassing biologically active layers, and thus, resulting in low extent of biodegradation [34]. Leaching of pesticides is pronounced in fractured clayey till soils compared to sandy soils due to rapid well-connected macropores [35]. Hence, transport of organic contaminants from upper soil layers into deep groundwater is frequently observed for a variety of pesticides and herbicides [35–37]. It has never previously been investigated whether illudane glycosides are mobile and stable enough, to reach deep aquifers used for drinking water supply.

In Denmark, groundwater-based drinking water utilities mainly apply deep groundwater abstraction wells, and subsequently, water is treated at waterworks before distribution to the consumers. In contrast to this, in some areas, the consumers rely on private water wells, typically shallower, and the raw water might not be treated prior to consumption. While deep groundwater wells usually abstract the water from confined aquifers, shallow water wells typically abstract water from unconfined aquifers and may be affected by seepage water. Several studies suggest that shallow wells are more prone to contamination by organic contaminants [38, 39]. Pesticides are found in higher concentration in Danish shallow water wells as compared to deep groundwater wells [39]. Therefore, shallow water wells might also be more vulnerable to contamination by illudane glucosides.

It is currently unknown whether the bracken illudane glycosides PTA, CAU and PTE pose a threat to groundwater-based drinking water supplies. Thus, it is important to: (1) investigate if the illudane glycosides are found in wells used for drinking water production, and subsequently, (2) identify the characteristics of vulnerable wells. To do so, we studied the presence of PTA, CAU and PTE and their corresponding pterosins PtB, PtA and PtG in a variety of water wells in bracken-dominated

areas in Denmark, Sweden and Spain, as function of well depth and water chemistry (pH and electric conductivity). This report is the first finding of illudane glycosides and their pterosins in drinking water wells.

## Materials and methods

### Solvents, chemicals and resins

Acids, bases and buffers (glacial acetic acid, formic and hydrochloric acids, sodium hydroxide and ammonium acetate) were all of analytical grade from Sigma-Aldrich (Germany). LC–MS grade acetonitrile was obtained from Merck Millipore (LiChrosolv hypergrade for LC–MS, Germany) and LC–MS grade methanol was purchased from Honeywell (LC–MS Chromasolv, Germany). MilliQ water (electrical resistivity 18.2 M $\Omega$  cm, TOC less than  $\mu\text{g L}^{-1}$ ) was produced by Sartorius Ultrapure water system (Sartorius Stedim Biotech GmbH, Germany). Polyamide for column chromatography was from Fluka Analytical, Sigma-Aldrich Co (Germany). Oasis MAX (20 cc, 60 mg Sorbent, 30 mm particle size) was purchased from Waters (Milford, USA).

### Preparation of analytical standards

No certified reference materials of PTA, CAU and PTE exist, and therefore, analytical standards were produced in-house. They were prepared from bracken plant material by the method described by Kisielius et al. [40].

### Analytical procedure

Prior to LC–MS analyses, all water samples were pre-concentrated by solid-phase extraction (SPE) by a factor 250. The SPE method used in this study was optimised for PTA and PtB and validated for various groundwater samples [41]. A SPE protocol with more details is provided in Additional file 1: Figure S2 of the supplementary material (SM). Together with each set of SPE samples, positive (for PTA and PtB spiked at concentration of 0.5  $\mu\text{g L}^{-1}$ ) and negative controls (DI water) were assessed at the same time. To determine the efficiency of the SPE method for CAU, PTE, PtA and PtG, 50 mL of MilliQ water was spiked to a concentration of 0.5  $\mu\text{g L}^{-1}$  for each compound. Following the SPE protocol, samples were processed in separate SPE cartridges and analysed by LC–MS ( $n=2$ ). The recovery results for each compound are presented in Table 1.

The samples were analysed using Agilent 1260 Infinity HPLC System equipped with Agilent 6130 Single Quadrupole mass spectrometer by the method described by Kisielius et al. [40]. Chromatographic separation was performed using Agilent Poroshell 120 EC-C18 column and the eluent comprised water (eluent A) and acetonitrile (eluent B), both with 0.1% v/v formic acid at gradient elution mode. The linear range of quantification

**Table 1** Recovery of illudane glycosides and corresponding pterosins in MilliQ spiked samples. OASIS MAX column (60 mg)

| Compounds | Recovery (%) |
|-----------|--------------|
| CAU       | 70 ± 3       |
| PtA       | 95 ± 8       |
| PTE       | 56 ± 5       |
| PtG       | 99 ± 6       |
| PTA       | 85 ± 2       |
| PtB       | 91 ± 3       |

The “±” represents standard deviation ( $n=2$ ). The recovery values for PTA and PtB are from the recent study [41]

was 20–500  $\mu\text{g L}^{-1}$  for PTA and PtB, and 10–250 for the remaining compounds ( $r>0.999$ ). The instrumental limits of detection (LOD) and quantification (LOQ) were: 0.22–0.68  $\mu\text{g L}^{-1}$  (PTA), 0.26–0.78  $\mu\text{g L}^{-1}$  (CAU) and 0.08–0.25  $\mu\text{g L}^{-1}$  (PTE) respectively, and for pterosins, 0.03–0.09  $\mu\text{g L}^{-1}$  (PtB), 0.02–0.05  $\mu\text{g L}^{-1}$  (PtA) and 0.01–0.03  $\mu\text{g L}^{-1}$  (PtG). The LOD of the entire method including pre-concentration and instrumental LOD was  $\leq 0.001 \mu\text{g L}^{-1}$  for all illudane glycosides and pterosins.

For all samples with reported presence of illudane glycosides or pterosins, a qualitative confirmation protocol was used. Three levels of confirmation were applied: (a) retention time of the compounds (always compared with retention time of the standards); (b) spiking test for suspected positive samples (spiked with corresponding compound that was detected, which resulted in appearance of only one larger peak in the chromatogram); (c) the presence of confirmation ions extracted from the total ion chromatogram, e.g.  $[\text{M-glucose-H}_2\text{O} + \text{H}]^+$ ,  $[\text{M-glucose} + \text{H}]^+$ ,  $[\text{M} + \text{Na}]^+$ ,  $[\text{M} + \text{K}]^+$  reported by Kisielius

et al. [40]. All samples with reported presence of illudane glycosides or pterosins were confirmed with spiking test and accurate retention times of the analytes. Furthermore, at least one of the confirmation ions was detected. More details on spiking test are provided in Additional file 1: Figure S1. The concentrations of the compounds are reported as average concentrations measured in duplicates. In case the compound could be quantified in only one duplicate (e.g. below LOQ), the concentration was reported as a *trace*.

#### Identity of illudane glycosides and pterosins in bracken

Bracken plant material was collected from 11 locations during the water sampling campaign to quantify the content of illudane glycosides (Table 2; Fig. 2). From each location, 40 cm tip of a fully matured frond was collected in duplicates. Plant materials were dried in an ordinary plant press and grinded to powder prior to extraction according to Kisielius et al. [40]. Aqueous extracts were passed through a filter vial with 0.2  $\mu\text{m}$  pore size membrane (Syringeless filter device Mini-Uniprep, GE Healthcare Life Sciences UK) prior to LC–MS analyses by which the contents of PTA, CAU and PTE and the pterosins were quantified using in-house produced standards [40].

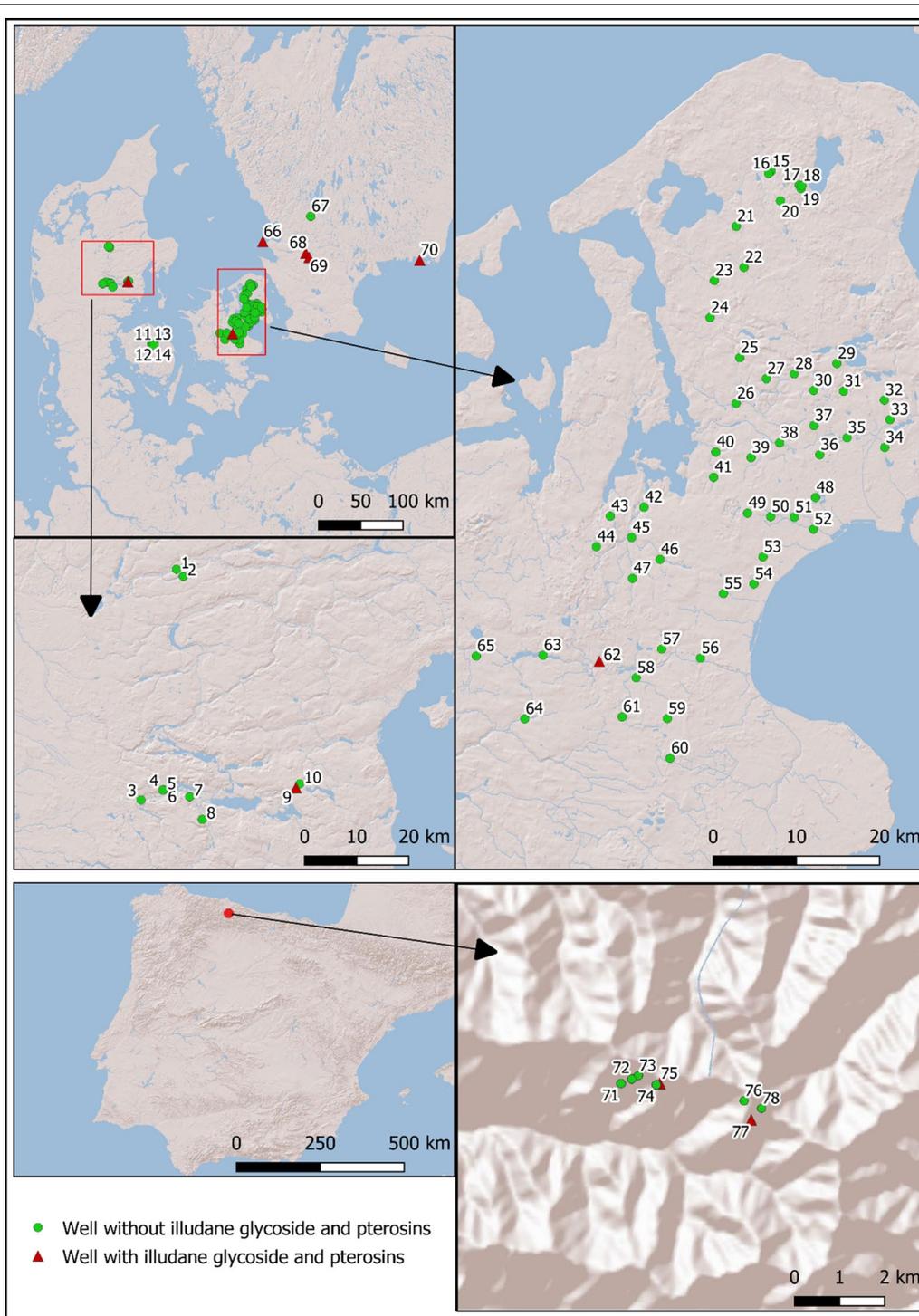
#### Origin of water samples

Water samples from 77 wells in Denmark, Sweden and Spain were collected for analyses of PTA, CAU and PTE and the corresponding pterosins between June and October 2019 (Fig. 2). Water chemistry (pH and EC), well depth and more details on each investigated location are provided in Additional file 1: Table S1. The majority of investigated wells belong to HOFOR utility (44 locations), the largest drinking water utility in Denmark, that

**Table 2** Measured content of illudane glycosides and corresponding pterosins in bracken ferns

| Location no | Country of origin | Sampling date | mg g <sup>-1</sup> dry weight |                    |                    |                    |     |     |
|-------------|-------------------|---------------|-------------------------------|--------------------|--------------------|--------------------|-----|-----|
|             |                   |               | PTA                           | PtB                | CAU                | PtA                | PTE | PtG |
| 1           | Denmark           | 08.08.19      | <b>0.05</b> ± 0.01            | <b>0.01</b> ± 0.01 | –                  | –                  | –   | –   |
| 4           | Denmark           | 07.08.19      | <b>1.10</b> ± 0.97            | <b>0.07</b> ± 0.05 | <b>0.12</b> ± 0.05 | <b>0.02</b> ± 0.02 | –   | –   |
| 6           | Denmark           | 07.08.19      | <b>0.13</b> ± 0.01            | <b>0.02</b> ± 0.00 | –                  | <b>0.01</b> ± 0.00 | –   | –   |
| 7           | Denmark           | 07.08.19      | <b>0.74</b> ± 0.23            | <b>0.11</b> ± 0.01 | <b>0.18</b> ± 0.02 | <b>0.11</b> ± 0.01 | –   | –   |
| 8           | Denmark           | 07.08.19      | <b>0.10</b> ± 0.06            | <b>0.03</b> ± 0.02 | <b>0.04</b> ± 0.01 | <b>0.01</b> ± 0.01 | –   | –   |
| 12          | Denmark           | 21.08.19      | <b>3.23</b> ± 1.50            | <b>0.18</b> ± 0.25 | <b>0.37</b> ± 0.19 | <b>0.08</b> ± 0.06 | –   | –   |
| 13          | Denmark           | 21.08.19      | <b>0.63</b> ± 0.07            | <b>0.07</b> ± 0.01 | <b>0.07</b> ± 0.01 | <b>0.01</b> ± 0.01 | –   | –   |
| 17          | Denmark           | 05.08.19      | <b>0.08</b> ± 0.03            | <b>0.01</b> ± 0.00 | –                  | –                  | –   | –   |
| 66          | Sweden            | 13.10.19      | <b>0.24</b> ± 0.12            | –                  | <b>0.42</b> ± 0.07 | <b>0.01</b> ± 0.00 | –   | –   |
| 68          | Sweden            | 01.08.19      | <b>3.19</b> ± 0.18            | <b>0.18</b> ± 0.03 | <b>0.10</b> ± 0.02 | <b>0.01</b> ± 0.01 | –   | –   |
| 69          | Sweden            | 01.08.19      | <b>3.02</b> ± 0.19            | <b>0.45</b> ± 0.05 | <b>0.12</b> ± 0.01 | <b>0.11</b> ± 0.03 | –   | –   |

The “–” represents no detection (< LOD) and “±” represents standard deviation ( $n=2$ ). Average coefficient of variation equals 33%, 37%, 21% and 44% for PTA, PtB, CAU and PtA, respectively. Location numbers correspond to the map in Fig. 2



**Fig. 2** The investigated water wells in Denmark, Sweden and Spain. All sampling locations were associated with adjacently growing bracken ferns. Map is produced by Qgis 3.4 Madeira and background map is ESRI Shaded Relief

is providing drinking water to approximately 1 million customers in the Greater Copenhagen area. The remaining investigated locations were selected based on the

presence of dense bracken ferns in their vicinity, diverse depth of water wells, geographical area and water type. To identify water wells with these features, an advertising

campaign was performed through different public media prior to monitoring. Raw water (with no treatment) was abstracted from single wells. In case of HOFOR wells, mixed groundwater was collected (raw water mixed from several single wells). Only one location (number 9) does not represent a water well but is seepage water collected from a creek (3–4 m deep) just after a rain event. Wells 68 and 69 were sampled on two occasions, summer and fall 2019.

Most of the monitored wells serve for drinking water purpose. The 44 groundwater wells operated by HOFOR utility included in the study were deep groundwater wells (40–100 m). The remaining 33 wells were less than 40 m deep. In the following text, HOFOR wells are classified as deep wells (44) and the others as shallow wells (33).

### Water sample collection

Water samples were collected and preserved in the field according to the method of Skrbic et al. [41]. The preservation protocol was originally developed for PTA and PtB, but was further validated for CAU and PTE and their pterosins in this study. 50 mL groundwater was collected in amber glass bottles, buffered in the field by adding between 0.05 and 1 mL of 0.5 M ammonium acetate adjusted to pH 5 with glacial acid for stabilising the PTA against hydrolysis. Samples were transported to the lab without ice for transportation up to 2 h (otherwise, placed on ice and transported within 4 h) and stored at 4 °C. SPE was performed in the lab within 72 h after sampling. Final samples were stored in vials at –18 °C until LC–MS analyses. Filtration with sterile syringe through 0.45 µm pore size filters was performed only on two occasions, when very shallow/murky water was collected (locations 9 and 62). The samples were collected in duplicates, except for the HOFOR samples that were collected as single samples. The field blanks (DI water) were collected and treated as field samples during transport, preparation and analysis. The samples from Spain were packed with dry ice and delivered to Denmark by express mail (within 2 days).

## Results

### Identity of illudane glycosides and pterosins in bracken

The most commonly found illudane glycoside in bracken plant material was PTA, which was detected at all eleven sampled locations, while at eight locations CAU could also be detected (Table 2). The highest PTA concentration was measured at location 12 (3.23 mg g<sup>-1</sup> dry weight) and the highest CAU content was detected at location 66 (0.42 mg g<sup>-1</sup> dry weight). Neither PTE nor its

degradation product PtG was detected in any of the analysed plant samples.

### Leaching of illudane glycosides after a rain event

The presence of illudane glycosides and the corresponding pterosins was determined in seepage water collected just after a rain event in a bracken-dominated region (location 9). At this location, both PTA (1.27 µg L<sup>-1</sup>) and CAU (0.28 µg L<sup>-1</sup>) and their hydrolysis products were detected (Table 3).

### Water samples

We sampled 77 water wells in Denmark (64), Sweden (5) and Spain (8) representing different geological settings. Neither bracken illudane glycosides (PTA, CAU and PTE) nor their hydrolysis products (PtB, PtA and PtG) were detected in 70 of the investigated wells (91%). However, in seven wells (9%), at least one of the compounds was detected (Fig. 2).

The deep groundwater wells did not contain illudane glycoside or pterosins. All seven wells in which at least one of the compounds was detected were shallow wells (Fig. 3). In terms of geography, one of the shallow wells with the presence of illudane glycosides and/or pterosins was located in Denmark, four in Sweden and two in Spain (Fig. 2). Apart from well 62, all these wells serve for drinking water purpose for humans and/or livestock (Table 3).

Three shallow wells contained CAU in concentrations up to 0.75 µg L<sup>-1</sup> (Table 3). However, PtA, the degradation product of CAU, occurred in six out of the seven wells, indicating even more frequent presence of CAU. PTA was present in three wells up to 0.27 µg L<sup>-1</sup>, while two wells contained PtB. PTE was not detected in any of the wells, while its degradation product PtG was detected in three of them.

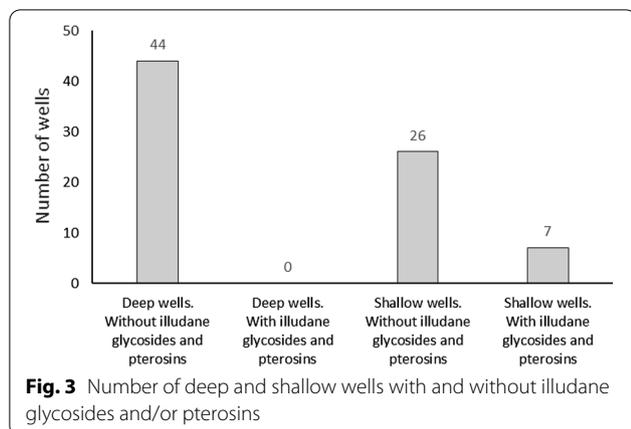
In wells 62, 68 and 70, we detected both CAU and PtA. Similarly, PTA and PtB were detected in well 77. Detection of glycosides and corresponding pterosin in the same well validates the presence of glycosides. Well 62 was very shallow and not used for drinking purpose, but contained CAU and PtA at concentration up to 0.03 µg L<sup>-1</sup>. The presence of PTA has previously been reported for this location [41]. Wells 72 and 77 showed the presence of PtA, PtB and trace levels of PTA. The depth of these wells is unknown.

To include seasonal variations, we sampled wells 68 and 69 both during summer and fall. In the summer, location 68 contained both CAU and PtA, while only PtA was found in the fall. At location 69, PtB and PtA were detected in the summer, but again only PtA appeared in the fall.

**Table 3** Water wells (site number 62–77) and seepage water (site number 9) with the presence of illudane glycosides and/or corresponding pterosins

| Site number                  | 9                      | 62                     | 66                     | 68a                    | 68b                    | 69a                    | 69b                    | 70                      | 75                     | 77                      |
|------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|-------------------------|------------------------|-------------------------|
| Country                      | DK                     | DK                     | SE                     | SE                     | SE                     | SE                     | SE                     | SE                      | ES                     | ES                      |
| Sampling date                | 07.08                  | 05.07                  | 13.10                  | 01.08                  | 13.10                  | 01.08                  | 13.10                  | 15.08                   | 01.10                  | 01.10                   |
| Distance to bracken (m)      | 4                      | 5                      | 1                      | 1                      | 1                      | 3                      | 3                      | 3                       | 2                      | 2                       |
| Well depth (m)               | –                      | 8                      | n.a                    | 40                     | 40                     | 10                     | 10                     | 20                      | n.a                    | n.a                     |
| Water pH                     | 5.9                    | 7                      | 7.1                    | n.a                    | 7.2                    | n.a                    | 6.4                    | 6.8                     | 7.1                    | 7.3                     |
| EC ( $\mu\text{S cm}^{-1}$ ) | 171                    | 320                    | 467                    | n.a                    | 310                    | n.a                    | 121                    | 487                     | 812                    | 737                     |
| Rain event (day b.s.)        | 1                      | 1                      | 1                      | 1                      | 2                      | 1                      | 2                      | n.a                     | n.a                    | n.a                     |
| Soil type [42]               | CS                     | SC                     | BR                     | BR                     | BR                     | n.a                    | n.a                    | n.a                     | SS                     | SS                      |
| Groundwater purpose          | Seep                   | Tech                   | Drink                  | Drink                  | Drink                  | Drink                  | Drink                  | Drink                   | Drink                  | Drink                   |
| PTA ( $\mu\text{g L}^{-1}$ ) | <b>1.27</b> $\pm$ 0.08 | –                      | <b>0.27</b> $\pm$ 0.02 | –                      | –                      | –                      | –                      | –                       | <b>trace</b>           | <b>trace</b>            |
| PtB ( $\mu\text{g L}^{-1}$ ) | <b>0.08</b> $\pm$ 0.03 | –                      | –                      | –                      | –                      | <b>0.05</b> $\pm$ 0.00 | –                      | –                       | –                      | <b>0.003</b> $\pm$ 0.00 |
| CAU ( $\mu\text{g L}^{-1}$ ) | <b>0.28</b> $\pm$ 0.01 | <b>Trace</b>           | –                      | <b>0.59</b> $\pm$ 0.05 | –                      | –                      | –                      | <b>0.75</b> $\pm$ 0.04  | –                      | –                       |
| PtA ( $\mu\text{g L}^{-1}$ ) | <b>0.01</b> $\pm$ 0.01 | <b>0.03</b> $\pm$ 0.00 | <b>0.01</b> $\pm$ 0.05 | <b>0.01</b> $\pm$ 0.01 | <b>0.01</b> $\pm$ 0.01 | <b>0.03</b> $\pm$ 0.01 | <b>0.03</b> $\pm$ 0.00 | <b>0.003</b> $\pm$ 0.00 | <b>0.01</b> $\pm$ 0.00 | –                       |
| PtE ( $\mu\text{g L}^{-1}$ ) | –                      | –                      | –                      | –                      | –                      | –                      | –                      | –                       | –                      | –                       |
| PtG ( $\mu\text{g L}^{-1}$ ) | –                      | –                      | <b>0.28</b> $\pm$ 0.04 | <b>trace</b>           | –                      | –                      | <b>0.02</b> $\pm$ 0.02 | –                       | –                      | –                       |

Empty cells represent no detection (< LOD) and “±” represents standard deviation (n=2). Average coefficient of variation equals 8%, 24%, 6%, 15% and 42% for PTA, PtB, CAU, PtA and PtG, respectively  
 DK Denmark, SE Sweden, ES Spain, a summer, b autumn, n.a. = not available, CS clayey sand soil, SC sandy clay soil, BR bedrock, SS sandstone, Drink drinking water, Tech technical water (groundwater not used for drinking water purpose), EC electrical conductivity, day b.s. = before sampling. All dates relate to year 2019



### Characteristics of the water wells in relation to the presence of illudane glycosides

To investigate determining factors for the presence of illudane glycosides in water wells, we analysed three characteristics of the sampled wells: depth, electrical conductivity (EC) and pH. First, for the subset of wells with known depths (59 out of 77 wells), the shallow wells with the presence of illudane glycosides and/or pterosins were on average 19 m deep (three wells with known depths; Table 3). Shallow wells without detected compounds were on average 30 m deep, while deep wells were 40 + m.

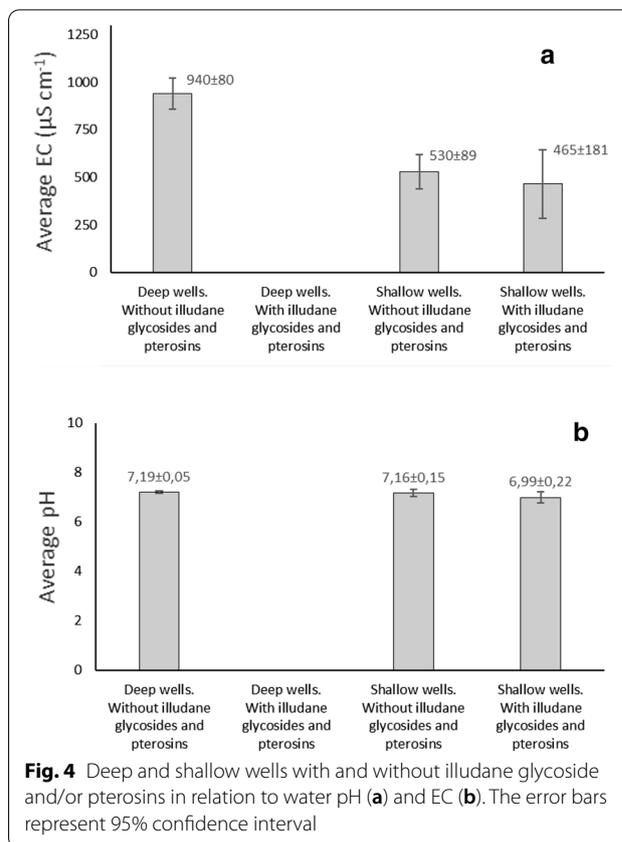
Second, EC in the deep wells were  $940 \pm 80 \mu\text{S cm}^{-1}$ , while the conductivity in the shallow wells were  $516 \pm 79 \mu\text{S cm}^{-1}$ , showing different water chemistry in the deep versus the shallow wells ( $p$  value  $< 0.001$ ) (Fig. 4a). There was no statistically significant difference in-between the shallow wells containing illudane glycosides and/or pterosins ( $465 \pm 181 \mu\text{S cm}^{-1}$ ) and the other shallow wells ( $530 \pm 89 \mu\text{S cm}^{-1}$ ).

There was no statistically significant difference in pH between the different groups of wells all being around pH 7, where illudane glycosides are most stable (Fig. 4b).

## Discussion

### The presence of illudane glycosides in drinking water wells and bracken

We have detected illudane glycosides and their corresponding hydrolysis products in drinking water wells for the first time. Interestingly, CAU occurred more frequently than PTA, even though PTA is considered the most abundant carcinogen in bracken [43, 44]. Based on estimated LogP values and order of chromatographic elution, CAU is observed to be more polar than PTA (Fig. 1) [31, 40], and is, thus, more prone to be mobile in the environment, and potentially end up in water recipients.



In a recent study, concentrations of CAU and PTE in surface water were also found to be similar or higher than PTA [30]. In addition, the same study showed that content of CAU in bracken was higher in Sweden than in Denmark [30], which could explain higher CAU occurrence in wells in Sweden in this study.

We did not detect PTE and PtG in any of the collected bracken plant samples, and in accordance with this, none of the water samples contained PTE. In Northern Europe PTE has only been detected rarely in bracken and in very low concentrations compared to PTA and CAU [30]. Nevertheless, we detected PTE's degradation product PtG in three wells, indicating prior presence of PTE or yet unknown soil transformations. The content of illudane glycosides in bracken is decreasing from June to November while transformations into pterosins occurs [27, 30]. Hence, pterosins might still be present in water sampled in autumn while the glycosides are not present anymore or with contents below LOD. The content of PTE in bracken populations is highly variable and with a random distribution [30]. Hence, plant materials collected for this study might not represent the real PTE occurrence.

Maximum measured illudane glycoside concentrations in drinking water wells in this study were  $0.27 \mu\text{g}$

L<sup>-1</sup> for PTA and 0.75 µg L<sup>-1</sup> for CAU. The maximum estimated tolerable concentration of PTA in drinking water is 0.002 µg L<sup>-1</sup> (based on a model with one cancer incidence per million) [45], and the PTA analogue CAU, is presumably equally toxic [20]. The tolerable concentration were, thus, exceeded with more than a factor of 100 in these specific drinking water samples, indicating a health threat to humans or animals who are supplied with this drinking water.

#### Leaching of illudane glycosides after a rain event—pulse effects

Both PTA (1.27 µg L<sup>-1</sup>) and CAU (0.28 µg L<sup>-1</sup>) as well as their hydrolysis products were detected in fresh seepage water extruding from clayey sand soil in a small creek (location 9). The sampling took place immediately after a rain event [27, 30]. The finding demonstrates the potential of bracken toxins to pass through the topsoil in line with previous findings [27, 29, 41]. Although the study was not designed to cover precipitation-related pulses, it should be noticed that it rained just prior to sampling well 66, 68 and 69 in Sweden, all being positive for illudane glycosides and/or pterosins (Table 3). The presence of fissured bedrock and/or fractured till could facilitate fast transport of CAU to deeper layers and explain CAU presence in a 40 m deep well (location 66 and 68) [33]. Such conditions are expected to be more likely for shallow wells compared to the deep wells.

#### Characteristics of the water wells in relation to the presence of illudane glycosides

Water wells with the presence of illudane glycosides and/or pterosins in this study shared several features. They were mostly privately owned, shallow and with neutral pH and EC lower than the deep wells. Electrical conductivity is typically higher in deep groundwater [46]. We observed significantly lower EC in the shallow wells indicating connection with rain and surface water (i.e. the presence of seepage water). In addition, water in shallow wells (0–40 m) that contain illudane glycosides and/or pterosins had slightly lower average pH in comparison to deep groundwater wells (40–100 m). Lower pH (pH 4.5–6.5) is known to have stabilising effect on PTA hydrolysis [24]. Furthermore, a very short distance to high bracken biomass (< 10 m) and rain event prior to sampling supported illudane glycoside occurrence.

Six out of seven wells with the presence of illudane glycosides serve for drinking water supply. In Denmark, there are 55,000 similar private single-abstraction drinking water wells [47] and it is estimated that that 1 in 10 citizens of the EU receives drinking water from small and local systems, including private wells [48].

These systems are more vulnerable to illudane glycoside contamination and might not have any sort of water treatment.

Illudane glycosides and their hydrolysis products were not detected in deep groundwater wells investigated in this study (40–100 m). This complies with the fact that PTA's half-life in alkaline water is shorter than under neutral-acid conditions, e.g. the half-life of PTA at 8 °C is approx. 100 days at pH 6, 30 days at pH 7 but only 3 days at pH 8 [24], indicating PTA is not sufficiently stable to reach deep groundwater aquifers as travel times from soil surface to deep aquifers are 25–100 years [46]. In contrast, shallow wells investigated in this study showed the presence of illudane glycosides and/or pterosins. Shallow wells are easily affected by younger seepage water that is more vulnerable to illudane glycosides contamination. The presence of cracks or macropores in soil profiles of shallow wells could support fast transport of illudane glycosides in relation to pulses [35, 49]. Similar observations were noticed when leaching of pesticides was investigated in clayey tills with up to 5–6 m deep macropores [50]. In that study, leaching of pesticides into shallow groundwater was noticed and the transport occurred in macropores and was driven by precipitation events [50]. Hence, leaching of illudane glycosides to shallow drinking water wells is possible, it is site specific and likely occurs in pulses.

#### Conclusions

In this study, we detected at least one illudane glycoside or pterosin from bracken fern in seven of the 77 studied wells. Thereby we demonstrated that leaching of illudane glycosides to drinking water wells is possible and could pose a threat to human health. Deep groundwater wells did not contain bracken illudane glycosides, probably due to the long travelling time to these aquifers. However, we detected bracken illudane glycosides in shallow wells, which could be due to intrusion of seepage water. A dense bracken biomass aboveground is a risk factor, especially in relation to rain events.

Six out of seven wells with presence of illudane glycoside were used for drinking water supply. The concentrations in these drinking water wells were 0.27–0.75 µg L<sup>-1</sup>, which violate the maximum estimated tolerable PTA concentration in drinking water by approximately 100–300 fold. In this study, CAU was more frequently detected in drinking water wells than PTA. Hence risk assessments are likely to underestimate water toxicity if they are based on PTA only and should also include CAU and PTE. These results are of great importance for consumers supplied by shallow wells in bracken dominated regions.

## Abbreviations

PTA: Ptaquiloside; CAU: Caudatoside; PTE: Ptesculentoside; PtB: Pterosin B; PtA: Pterosin A; PtG: Pterosin G; PMOC: Persistent and mobile organic compounds; WHO: World Health Organization; DNA: Deoxyribonucleic acid; Kow: Octanol–water partition coefficient; LC–MS: Liquid chromatography–mass spectrometry; SPE: Solid-phase extraction; LOD: Limit of detection; LOQ: Limit of quantification; dw: Dry weight; EC: Electrical conductivity; DK: Denmark; SE: Sweden; ES: Spain; n.a: Not available; CS: Clayey sand soil; SC: Sandy clay soil; BR: Bedrock; SS: Sandstone; Drink: Drinking water; Tech: Technical water; day b.s.: Before sampling; EU: European Union.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12302-021-00486-y>.

**Additional file 1.** Supplementary material (additional tables and figures).

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## Authors' contributions

Conceptualization: NS, A-KP, SCBC, MJH, HCBH and LHR. Investigations: NS, VK and LHR. Data curation: NS and LHR. Visualisation: NS. Funding acquisition: A-KP, HCBH and LHR. Supervision: A-KP, SCBC, MJH, HCBH and LHR. Writing—original draft: NS. Writing—review and editing: NS, A-KP, SCBC, MJH, VK, HCBH and LHR. All the authors read and approved the final manuscript.

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## Availability of data and materials

All data generated or analysed during this study are included in this published article and its additional information file.

## Declarations

### 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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