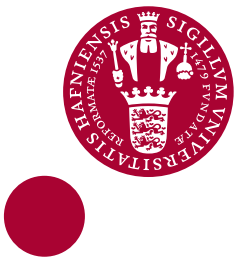

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Natural alkaloids from narrow-leaf and yellow lupins transfer to soil and soil solution in agricultural fields

Jawameer R. Hama* and Bjarne W. Strobel

Abstract

Background: Lupin is a promising legume crop, belongs to the *Fabaceae* (or *Leguminosae*) family. Lupin production for traditional and functional foods or animal feed is limited, due to the content of toxic quinolizidine (QA)s and indole alkaloids (IA)s. These compounds may not only pose a risk to humans and animals through food consumption, but may also affect soil and aquatic ecosystems. Field experiments were conducted to study the alkaloids content in both narrow-leaved or blue (*L. angustifolius*) and yellow (*L. luteus*) lupin plant tissue during a full growing season and understand the environmental fate of alkaloids in soil and water. Suction cups were used to collect soil pore water (soil solution) at four depths: 10, 25, 50 and 70 cm. A full protocol for sample preparation and UPLC–MS/MS quantification of alkaloids in plant, soil and water was developed.

Results: During the field experiments the alkaloids in the plant tissues increased, at the harvest stage the content was highest with 21.4 and 24.6 mg/kg dry weight (dw) for blue and yellow lupin, respectively. In soil, alkaloids quantified during the growing season (max concentration was 1.3×10^2 µg/kg dw) and even detected after harvest (0.2 µg/kg dw). In soil pore water samples, alkaloids were not detected during summer, but the concentrations increased to 9.8×10^2 and 1.5×10^3 ng/L for blue and yellow, respectively, in September when autumn precipitation began.

Conclusions: The results show the amount of alkaloids transferred from plant tissue into soil and soil pore water estimated to be on average 0.016% and 0.005% in soil and soil pore water, respectively. Alkaloids leached from topsoil to subsoil layers; the concentrations decline with soil depth. This study demonstrates that alkaloids are mobile compounds in the soil environments, thus lupin production may affect soil or aquatic ecosystems, and reduce water quality.

Keywords: Alkaloids, PMOC, Natural toxin, Lupins (*Lupinus* spp.), Environment, Suction cups, Leaching, UVCB

Background

Lupins (*Lupinus* spp.) belong to *Fabaceae* (*Leguminosae*), a large family including important food and feed crops [1]. Lupin seeds are rich in protein, which make them an interesting alternative crop, for e.g. soybeans [2–5]. For food and feed purposes four lupin species are cultivated on a commercial scale: white (*L. albus*), narrow-leaved lupin or blue (*L. angustifolius*), yellow (*L. luteus*) and Andean or south American (*L. mutabilis*) lupin, [1, 5–7]. Only the latter one is excluded in the European Union Novel Food Catalogue [8]. The annual world production of lupin seeds exceeds 1 million tons [5, 9]. Recent agricultural interest is on lupin as a crop and green manure, and for control of soil erosion [4, 10]. In addition, it can reduce the use of fertilizers due to the ability to fix nitrogen from the air [4, 11].

All plants of the genus Lupinus contain alkaloids, especially quinolizidine (QA) and indole alkaloids (IA) [12]. QAs are a broad group of secondary metabolites of which more than 170 QAs have been identified in different

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Lupinus species [13–15]. They all have quinolizidine as a core structure that consists of two fused 6-membered rings with a nitrogen atom at the bridgehead [16, 17]. IAs are bicyclic compounds, where a five-membered nitrogen-containing pyrrole ring fused to six-membered benzene ring. The nitrogen atom in the pyrrole ring gives rise to the basic properties [18, 19]. This group of alkaloids is very large and diverse, contains more than 3000 compounds [20]. Angustifoline, cytisine, hydroxylupanine, lupanine, lupinine, matrine and sparteine are example of QAs, and gramine is IA found in *Lupinus* species. Alkaloids act as a nitrogen reserve and confer resistance toward pathogens and herbivores [21–23]. The amount of alkaloids can differ considerably within the same species [15, 24]. In addition, the distribution and concentration of individual alkaloids change with geographical location and year and soil characteristics [16, 25–27], and fall in the Environmental Protection Agency category called Unknown or variable composition, complex reaction products or of biological materials (UVCBs) [28]. Alkaloids occur in all parts of lupin species, generally the highest amounts are in the seeds [15]. The term 'sweet' and 'bitter' lupin have been used regarding the alkaloids content, sweet is for lupins with low alkaloid content from no QAs to 500 mg/kg dw, whereas bitter lupin is used for higher alkaloids content [6, 29, 30].

Alkaloids and in particular sparteine and lupanine show moderate acute toxicity in mammals, the former being the most toxic with LD_{50} values for mice were 220 and 410 mg/kg body weight for sparteine and lupanine, respectively [29, 31, 32]. The exposure to lupin alkaloids largely depends on the amounts consumed through diet, therefore the focus has been on making products safer for consumption. Using sweet varieties does not necessarily safe-guard humans, as cross-pollination from more bitter lupins can occur, and wild or ornamental lupins are found throughout many parts of the world. The total amount of alkaloids in lupin flours and derived products is limited for safety to 0.2 g/kg dw [29] by the health authorities of United Kingdom [33], France [34], Australia and New Zealand [35].

It is crucial to develop a reliable analytical platform for determining alkaloid contents in grains used for animal or human consumption or minimize the poisoned cases. Gas chromatography–mass spectrometer was the most commonly method employed for both analysing total content and identification of specific alkaloids [36], while few attempts have been made to use ultra performance liquid chromatography–tandem mass spectrometer (UPLC–MS/MS) to analyse selected QAs or in particular matrix [37, 38]. Therefore, a robust analytical method to determine alkaloids in wide range sample types is needed.

Despite alkaloids prevalence, the amount of QAs and IAs naturally emitted and their fate in the environment is largely unknown. Lupin was recently recognized as an emerging environmental pollution source for the toxic alkaloids [39]. Lupin alkaloids transferred to soil through excretion or plant degradation when used as green manure, through plant remains deposited on the fields. There are no measurements of QA and IAs spreading to the environment. The sole measurement of QAs excreted into the rhizosphere is 0.7 mg (2 µmol) per seedling over a period of 39 days [40]. This can be considered as an estimate, because the measurements concern seedlings, not soil. Lupin plants density in the range 14–138 plants/m^2 is optimal for maximum yield, with a mean of 58 plants/m^2 [41]. This plant density, may lead to a very rough estimate that lupin plants may produce approximately 1 mg QAs/m^2 on average daily. The behaviour of lupin alkaloids may be similar to other natural toxins and polar mobile organic compounds (PMOC) originating from plants. Alkaloids transport in the soil depend on soil type and properties of the substance, and the continuous input can cause accumulation over a growing season, like other chemicals such as artemisinin [42, 43], ptaquiloside [44], glycoalkaloids (α-solanine and α-chaconine) [45], isoflavones [46] and mycotoxins [47]. The physical properties of alkaloids (Table 1) however indicate they have medium mobility in soil and leaching cannot be excluded. Therefore, they may cause negative environmental impact on the plant-associated microbiota, soil or aquatic ecosystems. Both current and future lupin varieties are therefore likely to release amounts of alkaloids to the environment.

This study aimed to develop a fast and robust UPLC–MS/MS method to quantify QAs and IAs in environmental samples. The method contains an optimized protocol for suction cup soil water, soil and plant compartment sampling, sample preparation, clean-up and pre-concentration up to 1000 times. Furthermore, field experiments conducted on two lupin species [narrow-leaved or blue (*L. angustifolius*) and yellow (*L. luteus*) lupin] to determine the content of alkaloids in the plant and broaden the knowledge of their environmental fate in soil and soil pore water. To the best of our knowledge, this is the first study attempting to determine the fate of natural alkaloids in the environment and relate to the continuous loading during a full growing season, evaluated at the field scale.

Methods and materials

Chemicals and reagents

Methanol (MeOH) (MS grade), acetonitrile (MeCN) (MS grade), formic acid (FA) (MS grade), ammonium formate (MS grade), acetone (HPLC grade), ammonium hydroxide (NH_4OH) and caffeine (internal standard, for spiking experiments and method validation) were purchased from Sigma-Aldrich (Darmstadt, Germany). Glass fibre (SiO_2, particle size 0.2–0.8 mm) was purchased from Merck (Darmstadt, Germany). Analytical standard of cytisine, gramine, lupinine, matrine and sparteine were purchased from Sigma-Aldrich (Darmstadt, Germany), and angustifoline, lupanine and hydroxylupanine were purchased from Bio-Connect B.V. (Huissen, The Netherlands). Senecionine (internal surrogate standard for recovery) purchased from Phytolab (Vestenbergsgreuth, Germany). Lupin seeds purchased from DLF (Roskilde, Denmark). Suction cup lysimeters purchased from Prenart Equipment ApS (Frederiksberg, Denmark). Oasis cartridge (HLB, MAX, MCX, WCX and WAX) 6 cc, 150 mg sorbent, 30 µm particle size purchased from Waters (Milford, USA). MilliQ water (resistivity 18.2 Mohm cm, TOC less than 1 µg/L) was produced in-house with a type I ultrapure water purification system from ELGA-Veolia LabWater (High Wycombe, UK).

Sample preparation and extraction

The details of the sample preparation and extraction is described in our published paper [48], and summarized below.

Plant and soil extraction

Accurately, 0.1 g plant or 2.5 g soil was weighed into a 25-mL centrifuge tube followed by 10 mL MeOH, then the tube was sonicated (15 min), centrifuged (10 min) at 8000 rpm (2100*g*), and finally the supernatant was collected. This extraction was repeated. After that, 10 mL of MeOH:acetone (85:15 v/v%) solution used for a third extraction. Finally, the three extracts were combined, centrifuged and filtered with a 0.45-µm PTFE membrane filter, and then passed through MCX SPE as explained in "Water extraction" section.

Water extraction

To find the optimal sorbent for solid-phase extraction (SPE), five different Oasis cartridge (HLB, MAX, MCX, WCX and WAX) were tested. Recovery (%) and summarized protocol of all sorbents are listed in Additional file 1: Table S1. Thus, based on the recovery MCX SPE cartridge was the best sorbent for extracting alkaloids. The MCX SPE cartridge was further optimized for efficiency, including pH adjustment of the sample, acid wash solution, eluent volume and sample volume. To evaluate the effect of sample pH on the recovery, five pH solutions (pH: 3.0, 5.5, 7.0, 8.5 and 10) were tested, by adjusting the pH with formic acid and NH_4OH. For acid wash step, formic acid with different concentrations (0.01, 0.05, 0.065, 0.1 and 0.2 mM) were tested. For elution of alkaloids, different volumes (5, 7.5, 10, 15 mL) of different NH_4OH concentrations (5, 7.5, 10 and 12.5%) in MeOH (1:3, 1:2 and 1:1, (v/v)) were tested. Finally, the efficiency of the methods was tested by using different loading volume of 0.25, 0.5, 1, 1.25, 1.5 and 2 L.

In the optimized method, the SPE cartridge was conditioned consecutively with 5 mL MeOH and H_2O. Then, acidified (pH 3) samples passed through the cartridge. The cartridge was washed with 5 mL 0.065 mM formic acid. Alkaloids were eluted with 5 mL 50% MeOH (1:1,

Table 1 Physical–chemical properties of selected alkaloids quantified in this study

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	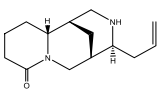	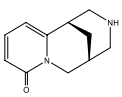	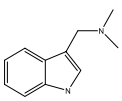	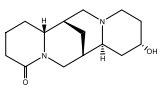	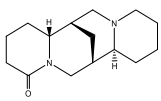	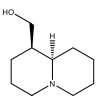	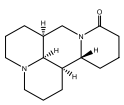	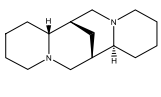
CAS number	550-43-6	485-35-8	87-52-5	15358-48-2	550-90-3	486-70-4	519-02-8	90-39-1
Nominal mass [Da]	234.3	190.2	174.2	264.4	248.4	169.3	248.4	234.4
Molecular formula	$C_{14}H_{22}N_2O_1$	$C_{11}H_{14}N_2O$	$C_{11}H_{14}N_2$	$C_{15}H_{24}N_2O_2$	$C_{15}H_{24}N_2O$	$C_{10}H_{19}N_1O_1$	$C_{15}H_{24}N_2O$	$C_{15}H_{26}N_2$
Water solubility [g/L][a]	9.9	15.1	3.2	21.8	8.1	13.6	0.8	3.0
log Kow[a]	2.2	0.6	1.5	0.2	1.7	1.6	1.7	2.7
pK_a[b]	10.3	10.9	7.9	8.8	9.4	9.4	9.4	12

[a] Calculation with EPISuite v4.0

[b] ACD/Percepta 2016.2

v/v) and 10 mL MeOH:10% NH_4OH (3:1, v/v). The combined eluates dried under a gentle nitrogen flow in a heating block at 40 °C. The dried extract was dissolved in 980 µL 40% MeOH and filtered using 0.2-µm PTFE membrane filter. Finally, the extract was spiked with 20 µL of internal standard (50 µg/L caffeine), prior to analysis.

Site description

The lupin field trials were carried out in an agricultural field in Taastrup, eastern Zealand, Denmark (N 55° 40′ 05.2″ E 12° 18′ 25.2″). Soil characteristics of the experimental field are given in Additional file 1: Table S2. Two species of lupin seeds were cultivated; blue lupin or narrow-leaved (*Lupinus angustifolius L.* Primadonna) and Yellow lupin (*Lupinus luteus L.,* Mister), hereafter called blue and yellow lupin, respectively. Before planting, the seeds were pre-inoculated with a suspension of Bradyrhizobium lupini strain from the commercial product HiStick-Lupin (DSV Frø, Denmark). For each species, a plot (14 × 18 m) of 12 seeding beds was designed, each bed was 1 m wide and 0.5 m space between neighbour beds. In total, 400 seeds seeded per bed in three rows, approximately 28 seeds/m^2 (later 85% germinated). Lupin fields separated and surrounded at all sides by three beds of oat (*Avena nuda L.*) or grass (Fig. 1a). The field trials were carried out from May 2018 to March 2019. Precipitation was measured at the on-site weather station; monthly precipitation is summarized in Additional file 1: Table S3. The fields were irrigated regularly to compensate for very little rain in May–June–July, and weeding was done manually.

Sampling

Sample collection began in May 2018 and continued through March 2019. In total, 10, 18 and 7 samples of plant, soil and soil pore water were collected, respectively. Soil pore water is called water throughout the text. A field plant and soil blank (soil blank 1) collected on 15th May before seeding. During plant growth, sampling (plant and topsoil) every 7–12 days, and after harvest sampling (topsoil and water samples) monthly during rain events. For sampling, 5 plants (the whole plant including roots) and 10–15 g soil (topsoil 1–5 cm away from the plant) were collected in polyethylene plastic bags. The plants were analysed for plant biomass and content of alkaloids. Water was collected in 500-mL amber glass bottles. Bottles were pre-rinsed with MilliQ water prior to the sampling. Water was sampled by using tension lysimeters, which consisted of suction silica porous cup lysimeters, tubes and sampling bottles. A sub-plot (2 × 2 m) for both lupin species and a sub-plot as a designated control site, then four suction cups installed in the soil at four depths 10, 25, 50 and 70 cm (Fig. 1b). Within the same sub-plot, suction cups had 1 m distance. Before installing the suction cups, the hole in the soil was filled with 100 mL of a slurry of silica flour (200 mesh) to establish a good contact between the soil and the ceramic cup wall. The cups were sampled at approximate 60 kPa vacuum applied with a hand pump 24 h prior of sampling. Samples were immediately placed in a cooling storage box for transport to the laboratory. Upon arrival at the laboratory, water samples were filtered with filter paper (Whatman® quantitative-Grade 5) to remove any suspended particles. Soils were sieved on 0.2 mm to remove visible plant roots, coarse sand and gravel. All samples were stored at − 20 °C until extraction, in most cases within 48 h after sampling. In the laboratory, control samples were used to confirm that freezing the samples did not influence the recovery of alkaloids when compared to lab control extracted immediately. Plant dry matter content was measured during the growing season (May through September 2019), using 3 plants after drying for 3 days at 100 °C. For harvesting the fields, about 10 cm of the plants with pods were collected manually and the rest of the plant left on the field. Plants were not harvested within 4 m around the suction cups. During the growing season, control soil (soil blank 2) samples was collected every 2nd month approx. 200 m away on the east side of the field. The control site for installed suction cups located on the east side of the field, where there was no lupin vegetation only grass, water samples (water blank 1) collected monthly corresponding to other suction cups. Plant tissue (plant tissue blank 1) of oat (*Avena nuda L.*) and grass in the plot shelter was collected to test whether they produce QAs and IAs as well.

Instrumentation

A Waters Acquity UPLC I-Class module was used for chromatographic separation, equipped with a 2.1 × 100 mm Acquity UPLC HSS C18 column, particle size 1.8 µm and Acquity UPLC BEH C18 Column, particle size 1.7 µm (Waters, Milford). Waters Xevo TQD triple quadrupole mass spectrometer was operated with electrospray ionization in positive ion mode. To optimize and improve the sensitivity of the method, the effect of column type, flow rate, gradient ramp, mobile phase composition (MeOH and MeCN) and pH (acid pH = 2.7 and base pH = 8) were tested. The ACQUITY UPLC HSS C18 column had better peak shapes and peak capacity for alkaloids compared to UPLC BEH C18. In the optimized method, 2.5 µL standard or sample was injected in a flow rate of 0.20 mL/min on pre-heated (35 °C) column. Mobile phase of A (water + 0.1% formic acid) and B (MeOH + 0.1% formic acid) used in gradient program: 0−4 min 10% B, 7 min 20% B, 10 min 50% B, 15 min 90% B, 15−17 min 90% B. The column was equilibrated for

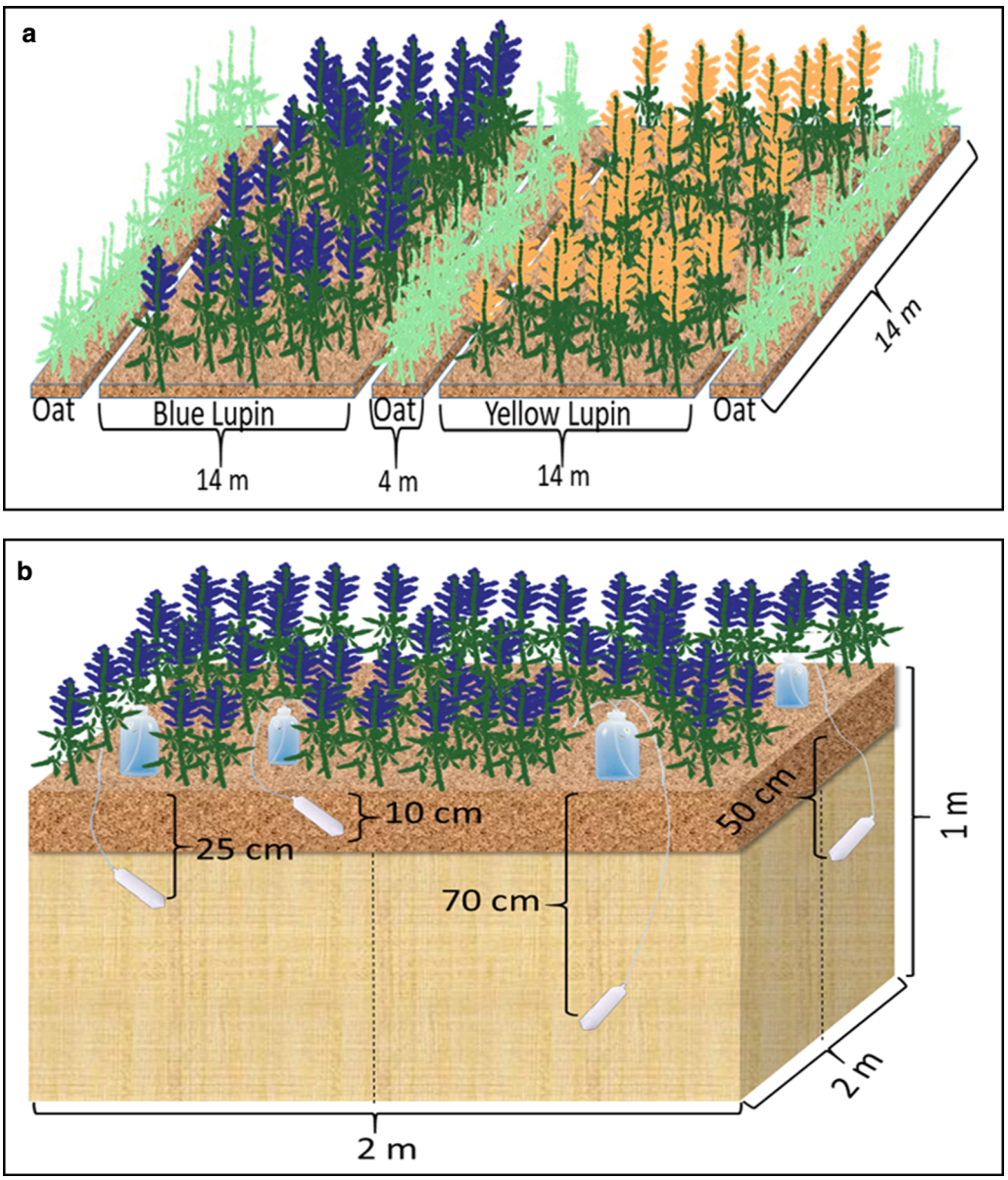

Fig. 1 **a** Experimental field site at Taastrup, Denmark. A plot (14 × 16 m) is cultivated for both blue and yellow lupin, each plot divided into 12 beds (1 m), seeding was 3 rows/bed. The site was sheltered with an oat (*Avena sativa*) or grass belt. **b** Illustration of installed suction cups (pressure-vacuum lysimeter) in designated sub-plot to collect soil pore water, in the blue lupin, yellow lupin and control site

6 min before each run, the total run time was 23 min. Detection was performed with a full scan and multiple reaction monitoring for all alkaloids. The desolvation temperature 600 °C, desolvation gas flow 1000 L/h, and cone gas flow 20 L/h was used. In the MS/MS, the ion traces was obtained for apex retention time (t_R) ± 0.15 min, the corresponding cone voltages (CV) and collision energies (CE) are listed in Table 2. MassLynx™ version 4.1. (Waters, Milford, USA) was used for data acquisition and processing.

Method validation

Method validation was conducted for alkaloids in both soil and water matrix samples (Table 3). Sandy soil from Vejle, sandy loam soil from Taastrup, Denmark, inert glass material and deionized water were used. For quantification, the calibration curves were obtained using an internal calibration curve, using seven standard solutions. Curves were obtained by plotting measured analyte peak areas/internal standard peak area against corresponding analyte concentrations/internal standard concentration

in the extracted matrix. Linear regression was performed for each curve. The precision and accuracy of the method were evaluated for intra- and inter-day variations. For intra-day variation, 3 concentration levels 10, 50 and 100 µg/L of alkaloids solution were spiked to matrix samples. Then, the spiked samples were extracted on the same day. For the inter-day variation test, new solutions were prepared in parallel and analysed for 3 consecutive days. Precision was calculated by relative standard deviation and accuracy by the method recovery. Spiked and non-spiked extracts was compared to evaluate the matrix effects (ME) on the analytes. The limit of detection (LOD) and limit of quantification (LOQ) was estimated for each alkaloids from 7 injections of 10 µg/L standards solution as 3 and 10 times the standard deviation (SE) of peak areas divided by the slope of the calibration curve [49]. The method was validated as recommended in SANCO guide [50].

Quality assurance and quality control

Quality assurance/quality control included three field blanks for plant, soil and water, replicate samples, and recovery of a surrogate standard. For water, field (water blank 2) and laboratory blanks (water blank 3) consisted of certified laboratory grade organic free water (i.e. MilliQ water). Soil samples far from the field (Køge, Denmark, N55° 27′ 48.6 and E12° 00′00.1) was used as a field blank (soil blank 3). Freeze-dried powder of bracken fern plant was used for plant tissue blanks (plant tissue blank 2). Concentrations of target QAs and gramine in blank samples (plant, soil and water) were below the LOD (0.03–2.11 ng/L). The average recovery rate of the surrogate (senecionine) in the plant, soil and water samples were 89% ± 8, 86% ± 10 and 94% ± 11, respectively ($n = 3$). The permeability and activity of the suction cups were tested, by soaking the suction cups in a beaker filled with 100 mL of alkaloid standard solutions (1 µg/L stock solution prepared in water), then vacuumed at approximate 60 kPa. The recovery of alkaloids were over 90%, and all water (100 mL) was collected within 3 h.

Results and discussion

The optimized alkaloid extraction method showed result similar to reported methods for plant tissue analysis [37, 38], plus the time-consuming procedure reduced. For cartridge selection, 100 mL of demineralized water spiked with 100 µg/L of QAs and gramine were loaded on the Oasis cartridges. Under these conditions, the recoveries by MCX was highest. The recoveries by MCX sorbent was also optimized for pH 3–10. For all alkaloids, the efficiency of the extraction increased twofold when the pH decreased, as shown in Table 4.

For acid wash step, 0.065 mM of formic acid solution showed better result. During elution, 10 mL solution (10% NH_4OH:MeOH (1:3, v/v)) can simultaneously elute alkaloids with recoveries ranging from 88 to 97%. A volume of the sample was tested, to maximize the sample volume without including any breakthrough. The method can recover all QAs and gramine in 1.0 L sample volume, hereafter the recovery decreased. A breakthrough was observed when the loading volume exceeded 1000 mL, shown in Fig. 2. In addition, the optimized SPE protocol using MCX cartridges increased the recovery of alkaloids during the extraction by 15–30% compared to the strong cation exchanger (benzene sulphonic groups) and reverse phase [51, 52].

Reversed-phase derivatized silica LC column is commonly used for QA separation with C18-derivatized columns. The HSS column used here retains and separates smaller, more water-soluble polar organic compounds such as alkaloids better than other C18-type bonded such as the BEH C18 and HILIC columns [53, 54]. The overall performance of the analytical method was comparable with previous methods [7, 37], and the method showed lower LOD for gramine, sparteine, angustifoline, and lupanine. The profile of analysed QAs were similar to analytical methods for suspect screening and non-target

Table 2 Optimized parameters of the method for selected alkaloids: t_R, precursor ion, product ion, CV and CE

Alkaloids	t_R (min)	Precursor ion (m/z)	Product ion (m/z)	CV (V)	CE (eV)
Angustifoline	5.81	235.18	193.13	30	35
Cytisine	3.63	191.11	148.07	20	30
Gramine	5.62	175.12	130.06	15	15
Hydroxylupanine	5.15	265.19	247.18	30	30
Lupanine	6.14	249.19	136.11	30	35
Lupinine	2.98	170.15	152.14	25	30
Matrine	6.02	249.19	148.11	25	35
Sparteine	6.98	235.21	98.09	30	35

Table 3 Performance parameters of the method to quantify selected alkaloids

Alkaloids	Correlation coefficient (R^2)	Sensitivity (slope)[a]	LOD (µg/L)	LOQ (µg/L)	Recovery % ± SE[b]				ME %	
					Soil			Water[c]	Soil	Water
					Sandy soil	Sandy loam soil	Glass fibre			
Angustifoline	0.989	$y = 148x$	0.09	0.20	89 ± 6	79 ± 9	96 ± 7	92 ± 4	11	7
Cytisine	0.997	$y = 74x$	1.20	3.44	92 ± 8	84 ± 9	96 ± 5	90 ± 5	11	8
Gramine	0.990	$y = 33x$	0.02	0.38	90 ± 11	81 ± 9	95 ± 6	94 ± 7	10	12
Hydroxylupanine	0.993	$y = 58x$	0.25	0.64	87 ± 7	72 ± 7	92 ± 5	90 ± 5	14	13
Lupanine	0.986	$y = 69x$	0.03	0.11	88 ± 5	80 ± 8	96 ± 5	95±5	8	5
Lupinine	0.983	$y = 97x$	0.17	0.61	87 ± 9	72 ± 8	93 ± 6	89 ± 6	19	14
Matrine	0.992	$y = 451x$	2.11	4.22	90 ± 6	87 ± 8	93 ± 5	95 ± 7	12	7
Sparteine	0.982	$y = 75x$	0.21	0.73	85 ± 10	77 ± 8	94 ± 8	92 ± 6	14	12

ME matric effect

[a] Average of linear regression of three injections

[b] Average recovery ± standard deviation (SE)

[c] De-mineralized water

analysis, and in addition, the method has a shorter range of linearity and lower LOD [55].

In order to investigate the occurrence of alkaloids in plant, soil and water, field experiments was conducted with two lupin species (blue and yellow). Both species flowered within the same 10 days approximately 50 days after sowing. During the field experiments, lupin biomass was monitored, and increase in biomass was similar for both species (Fig. 3a). Lupin biomass (fresh weight) increased gradually to maximum 139 and 175 g per plant for blue and yellow lupin, respectively. Hereafter the plants matured and seeds ripened, and the biomass deceased with 6–7% as the plants dried out and lost the leaves. Over the entire period of the field investigation, five out of seven QAs and gramine, which were included in the analytical method for plant, soil and water samples, were repeatedly detected (Figs. 3b and 4a, b). The remainder of this paper will focus on those alkaloids: angustifoline, gramine, hydroxylupanine, lupanine, lupinine and sparteine. In plant tissue and soil samples, alkaloids were detected during the growing season and even after the harvest. The total alkaloids concentration in both lupin species increased during growing season, reached the peak at harvest stage with 21.4 and 24.6 mg/kg dw, for blue and yellow lupin, respectively (Fig. 4b). The concentrations of all individual alkaloids in plant tissue are listed in Additional file 1: Table S4. Among plant parts, alkaloids profile showed variations. The highest concentration of alkaloids were found in seeds, followed by pods, flowers, leaves and stems, and lowest concentration in roots. In fact the plant tends to transfer the QAs to the seeds when developed [56, 57]. The seeds are the reproduction organs of plants, and the alkaloids seem to play an important role in plant defense, and also contribute to N metabolism [24]. The concentrations in yellow lupin were slightly higher than in the blue lupin. In the yellow lupin plant tissue samples, lupanine was detected at the highest concentrations (1.2×10^4–1.9×10^4 µg/kg dw, which was 65–77% of total alkaloids), followed by sparteine (12–22% of total alkaloids) and hydroxylupanine (8–12% of total alkaloids). In the blue lupin, QAs were relatively the same, lupanine was detected at the highest concentrations (1.0×10^4–1.4×10^4 µg/kg dw, which was 65–76% of total alkaloids), followed by sparteine (12–23% of total alkaloids) and hydroxylupanine (8–12% of total alkaloids). Based on the lupin plant biomass (24.4 and 29.1 g dw per plant for blue and yellow lupin, respectively) and the plant population density (24 ± 7 ($n = 5$)); the lupin plant production per season was 5.8×10^3 and 6.9×10^3 kg dw/ha for blue and yellow lupin, respectively. Therefore, the highest alkaloids productions by lupin plants from the field were 0.12 and 0.17 kg/ha for blue and yellow lupin, respectively.

In soil, during growing season the total alkaloids concentration was 96.1 and 1.3×10^2 µg/kg dw for blue and yellow lupin, respectively. Maximum alkaloid concentrations were lupanine (76.1 µg/kg dw), followed by sparteine (11.8 µg/kg dw) and hydroxylupanine (9.8 µg/kg dw). Alkaloids detected in the soils at high concentrations correspond to those high in plant tissues as well. In soils, the alkaloids were not detected during the dry period and no irrigation, and increased significantly with irrigation and rain events. In addition, the alkaloid content found in soils was estimated to be 0.015% and 0.017% of the amount found in blue and yellow lupin plants, respectively. In September, alkaloids were still detected with concentrations ranged from 0.17 to 0.21 µg/kg dw for both species (Fig. 4a, b; black stars (*)) when rain events

Table 4 Effect of pH on recovery of alkaloids (%) for 100 mL of water spiked with alkaloids (100 µg/L) loaded on MCX SPE cartridge (150 mg)

Alkaloids	pH of loading solution				
	3	5	7	8.5	10
Angustifoline	89	63	45	41	34
Cytisine	94	81	60	50	45
Gramine	97	86	72	73	79
Hydroxylupanine	96	79	66	37	34
Lupanine	96	86	61	46	30
Lupinine	92	62	49	42	44
Matrine	96	89	71	56	50
Sparteine	89	74	69	44	47

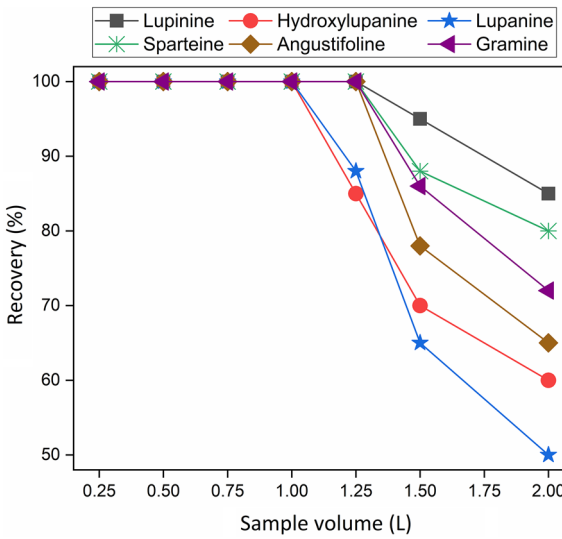

Fig. 2 The effect of sample loading volume on recovery yields of QAs and gramine for optimized SPE method by MCX cartridge (150 mg). A solution of alkaloids (100 µg/L) used for spiking

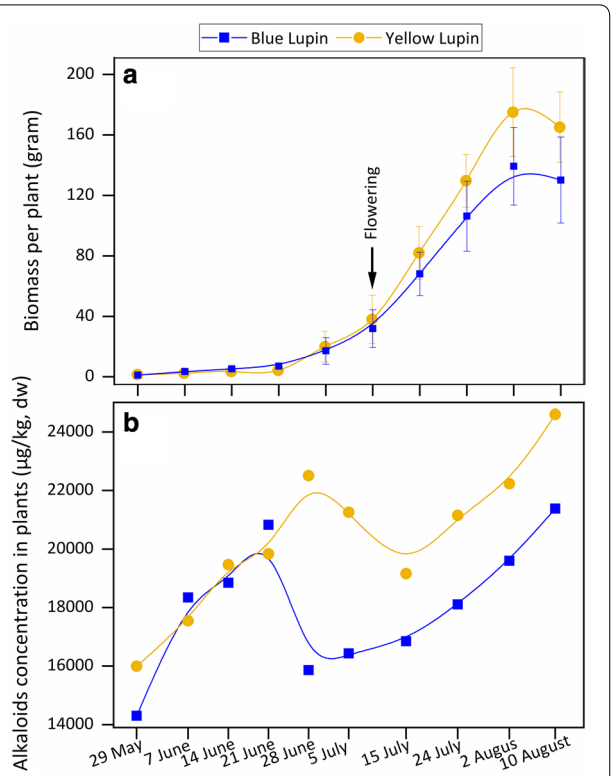

Fig. 3 **a** Plant biomass. Data are given as average ± SE ($n = 3$) and **b** alkaloid contents of lupin species during the field experiments; the line represents smoothed version only

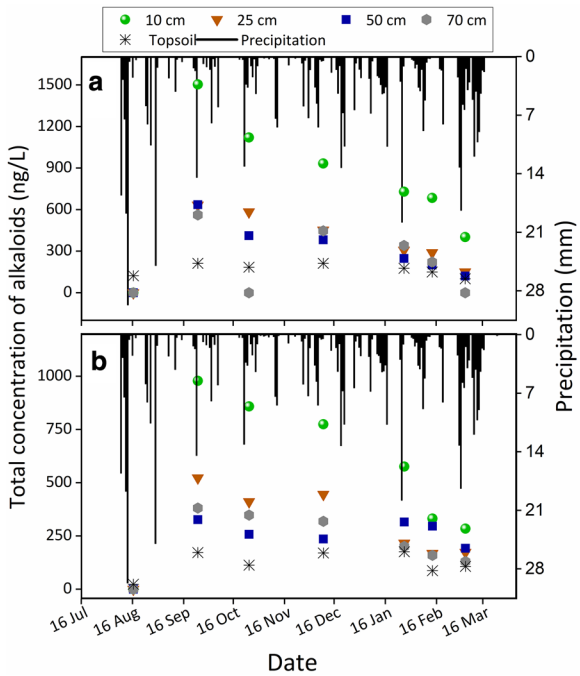

Fig. 4 Alkaloids content in topsoil (ng/kg, dw) and water samples (ng/L) collected by suction cups, in **a** yellow and **b** blue lupin field

occurred. Based on the concentration range in topsoil (5 cm), alkaloids were present in amounts between 1.0 and 1.3 g/ha (considering soil density of 1.5 g/cm^3, bulk density without stones and gravels [58]). This is only a minor fraction of what was produced in lupin plant tissue. The load of alkaloids will increase with increasing plant density in the field [41].

In the water samples, the alkaloids concentration ranged from below the LOD (0.03–0.25 ng/L) in summer to 9.8×10^2–1.5×10^3 ng/L in September (Fig. 4a, b), the quick increase was due to the rain events that started to occur in mid-August. In water samples, the highest concentration of individual alkaloids detected were lupanine, sparteine and hydroxylupanine that correlate with the plant and soil concentrations. The alkaloid concentrations exceeded those observed during summer and pre-harvest season. However, the concentration gradually decreased from September to the end of March; alkaloids could be degraded, adsorbed in the soil, and transported off the site. Alkaloids were ubiquitous in all water samples once rainfall had commenced and the total amount was estimated being 4.6×10^{-3} to 6.1×10^{-3}% of the alkaloids present in blue and yellow lupin, respectively, that leaches to soil pore water. The time interval (24 h) for sample collection from the suction cups was sufficient to collect maximum 235 mL of soil pore water, with vacuuming the system at approximate 60 kPa [59, 60].

In the fields, seasonal leaching dynamics observed for alkaloids. In winter, their concentrations were higher and decreased slowly, while in summer their concentrations was lower (Fig. 4). The alkaloid concentrations in water varied over winter suggesting other driving factors beyond degradation or rainfall. This study shows the importance of rain events as the main driving factor for transporting alkaloids from plants to soil and water. To the best of our knowledge, this is the first study on lupin documenting alkaloid concentrations in soil and water and providing an estimation based on release data.

Alkaloids continuously transferred into the soil and water, even though the concentration declined toward the next growing season. Blank samples: plant tissue blank 1 (oat (*Avena nuda L.*) and grass), plant tissue blank 2 (bracken fern—procedural blank), soil blank 1 (from the field before seeding), soil blank 2 (collected 200 m away from the east side of the field—no lupin vegetation), soil blank 3 (collected in Køge, 35 km away from the field), water blank 1 (from suction cups that installed, where no lupin vegetation), water blank 2 (field water blank, certified laboratory grade organic free water (i.e. MilliQ water) collected on the field) and water blank 3

(laboratory procedural blank, certified laboratory grade organic free water (i.e. MilliQ water) were analysed free from alkaloids or their concentrations were below the LOD (0.03–0.25 ng/L). In addition, the blank samples prove that lupin is the only source for alkaloids in both soil and waters collected for this study.

Environmental relevance

The concentration levels of alkaloids found here in the soil and water, can cause suppression of grass weeds [61], and inhibit seed germination for some species [40]. The environmental and ecotoxicity data are rather limited, however, the only toxicity level reported in the literature that comes close to the maximum soil and water concentration of QAs is an acute toxicity value for to *V. fischeri* and *D. magna* (EC50 values of 89 mg/L and 47 mg/L, respectively) [62]. In addition, chemical mixture and potential long-term exposure effects on non-target soil and water microbial communities are unknown [63].

Conclusions

Alkaloids were monitored in lupin plant tissue, soil and water samples during an agricultural field experiments from May 2018 to March 2019. During the growing season, alkaloid contents in the plant increased and being the highest at the harvest stage (21.4–24.6 mg/kg dw), after harvest they were presented in soil ($96 - 1.3 \times 10^2$ µg/kg) and water (from below the LOD up to 1.5×10^3 ng/L). The dominant alkaloids in all samples were lupanine, sparteine and hydroxylupanine. Overall, the results show that the primary proposed route of lupin alkaloids is leaching, however only a very small percentage of alkaloids are detected later in the soil and water to what is present in the plant, which is estimated to be 0.016% and 0.005% in soil and water, respectively. In soil, the highest alkaloid concentrations are found in September after harvest; therefore, the major transfer of alkaloids from the plants to the soil layers most likely occurs during plant deterioration in the autumn. Alkaloids are detected in the soil and water for several months after harvest, which shows their stability during the winter months. Alkaloids formation in monoculture lupin fields can lead to potential environmental consequence, as these compounds are toxic, thus may affect the soil microbial community and upper groundwater. Details of the environmental fate of the lupin alkaloids will be of great help in developing effective strategies to protect local water resources.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12302-020-00405-7.

Additional file 1: Table S1. The recovery (%) and protocols for extracting alkaloids using Oasis SPE cartridges, performed by loading 100 mL of de-water spiked with alkaloids (100 µg/L) on cartridges (150 mg sorbent). **Table S2.** Soil characteristics of the experimental field. **Table S3.** Precipitation data collected for the experimental field. **Table S4.** Concentrations (µg/kg, dw) of individual alkaloids in blue and yellow lupin plants during the growing season.

Abbreviations

QA: Quinolizidine alkaloid; IA: Indole alkaloid; dw: Dry weight; UVCBs: Unknown or variable composition, complex reaction products or of biological materials; PMOC: Polar mobile organic compound; UPLC–MS/MS: Ultra performance liquid chromatography–tandem mass spectrometer; MeOH: Methanol; MeCN: Acetonitrile; SPE: Solid-phase extraction; LOD: Limit of detection; LOQ: Limit of quantification.

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Authors' contributions

JH: conceptualization, methodology, investigation, writing—original draft. BS: conceptualization, methodology, writing—review and editing, funding acquisition. All 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 article.

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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