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Indole and quinolizidine alkaloids from blue lupin leach to agricultural drainage water

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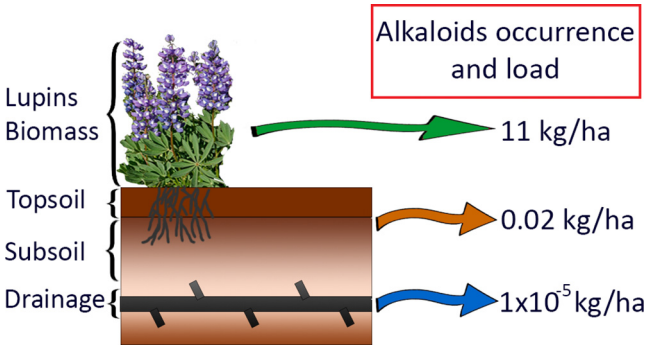
GRAPHICAL ABSTRACT



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ABSTRACT

Phytotoxins are produced in plants including agricultural crops. Lupins and other plants of the *Fabaceae* family produce toxic alkaloids. These alkaloids have been studied in food and feed, however, the environmental fate of alkaloids produced by cultivated lupins is largely unknown. Therefore, we conducted an agricultural field experiment to investigate the occurrence of indole and quinolizidine alkaloids in lupin plant tissues, soil, soil pore water and in drainage water. During the field experiment, alkaloids were regularly quantified (median concentrations) in lupin (13–8.7 × 10^3 ng/g dry weight (dw)), and topsoils at depth 0–5 cm (0.1–10 ng/g dw), and depth 15–30 cm (0.2–8.5 ng/g dw), soil pore water (0.2–7.5 ng/L) and drainage water samples (0.4–18 ng/L). Lupanine was the dominant alkaloid in all collected samples. Cumulative amounts of alkaloids emitted via drainage water were around 0.1–11 mg/ha for individual alkaloids over one growing season. The total cumulative amount of alkaloid in drainage water was 14 mg/ha, which is a very small amount compared to the mass of alkaloid in the lupin biomass (11 kg/ha) and soil (0.02 kg/ha). Nearly half of the alkaloids were exported in the drainage water during high flow events, indicating that alkaloids transport preferentially via macropores. These findings indicate that drainage from lupin cultivated areas contribute to surface water contamination. The environmental and ecotoxicological relevance of alkaloids as newly identified aquatic micropollutants in areas with agricultural activities have yet to be assessed.

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1. Introduction

Lupins (*Lupinus* L.) belong to Fabaceae (*Leguminosae*), a large family including important food and feed crops (Gresta et al., 2010). Lupin seeds are rich in protein, which make them an alternative crop to e.g. soybeans (EFSA, 2011; Lopez-Bellido and Fuente, 1986; Lucas et al., 2015; Sujak et al., 2006). In Europe, white (*Lupinus albus* L.), narrow-leaved or blue (*Lupinus angustifolius* L.) and yellow (*Lupinus luteus* L.) lupin are included in the European Union Novel Food Catalogue for food and feed purposes (European Commission, 2008). Lupins are of recent agricultural interest as a crop and green manure, and used to control soil erosion (Gremigni et al., 2001; Lopez-Bellido and Fuente, 1986) and fixate nitrogen in soil from the air (Lopez-Bellido and Fuente, 1986; Shu et al., 2007).

Plants in the genus *Lupinus* contain toxic alkaloids, especially indole (IA) and quinolizidine alkaloids (QA), Table 1 (Aniszewski et al., 2001). Indole alkaloids are bicyclic compounds, where a five-membered nitrogen containing pyrrole ring is fused to a six-membered benzene ring (El-Sayed and Verpoorte, 2007; Hamid et al., 2017). Quinolizidine alkaloids have quinolizidine as a core structure that consists of two fused six-membered rings with a nitrogen atom at the bridgehead (Boschin et al., 2008; EFSA CONTAM Panel, 2019). In the plants, alkaloids act as a nitrogen reserve and confer resistance towards pathogens and herbivores (Mason and Singer, 2015; Wink, 1993; Wink, 2019). Plant contents of alkaloids can differ considerably within the same species (Boschin and Resta, 2013; Wink et al., 1995). In the genus *Lupinus*, alkaloids are present in high concentrations, i.e. >1 mg/kg dry weight (dw). In addition, the distribution and concentration of individual alkaloids in the plant change with geographical location, year, and soil characteristics (Annicchiarico et al., 2014; Boschin et al., 2008; Calabrò et al., 2015; Jansen et al., 2015). The terms 'sweet' and 'bitter' lupins have been used to refer to the alkaloid content; sweet is for lupins with low alkaloid content ranging from zero to 500 mg/kg dw seeds, whereas bitter is used for lupins with alkaloid contents exceeding 500 mg/kg dw (Carvajal-Larenas et al., 2016; Pilegaard and Gry, 2008; Sbihi et al., 2013).

The main known exposure to lupin alkaloids takes place via direct consumption of lupin seeds and alkaloid-containing foods. Toxification by alkaloids depends on the amounts consumed. Therefore, the health authorities of United Kingdom, France, Australia, and New Zealand recommend a limit of 200 mg/kg dw for the total amount of alkaloids in lupin flours and derived products (Australia New Zealand Food Authority, 2001; ACNFP Annual Report, 1996; Direction Générale de la Santé, 1998; Pilegaard and Gry, 2008). The occurrence of alkaloids in the aquatic and terrestrial environment has not yet been studied to any great extent (Hama, 2020). Release and environmental occurrence for a number of phytotoxins have been shown already, e.g. artemisinin (Herrmann et al., 2013; Jessing et al., 2013), glycoalkaloids (α-solanine and α-chaconine) (Jensen et al., 2009), isoflavones (Hoerger et al., 2009; Hoerger et al., 2011), and mycotoxins (Madden and Stahr, 1993). Both current and future lupin varieties are likely to release considerable amounts of alkaloids from leaves and roots to the environment (Mons et al., 2013). Lupin alkaloids are highly soluble substances (3–32 g/L at 25 °C) (Kalberlah et al., 2014), and are quite persistent in the environment and thereby fall in the category of persistent and mobile organic compounds (PMOCs). Their half-life in natural water ranges from 36 to 60 days (Table 1), similar to many other persistent, mobile, and toxic substances originating from plants (Günthardt et al., 2018). Alkaloids have been found in soil pore water (at soil depth of 10–70 cm) at concentrations up to 450 ng/L (Hama and Strobel, 2020), and the alkaloids gramine and sparteine have been detected in surface water during suspect/non-target screening in Switzerland (Günthardt et al., 2020; Kisielius et al., 2020) and Denmark (Nanusha et al., 2021), respectively. In fact, alkaloids have been detected in soil of lupin fields and soybeans, six months after harvest (Hama et al., 2021; Hama and Strobel, 2020). Therefore, alkaloids should be included in environmental monitoring and risk assessment, as their concentration exceeded the threshold of toxicological concern for drinking water and their presence in water might contribute to complex mixture toxicities (Griffiths et al., 2021; Parmaki et al., 2018), that could threaten water quality, aquatic ecosystems and human health.

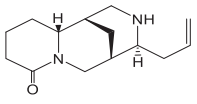
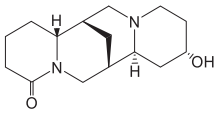
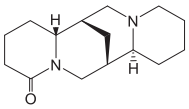
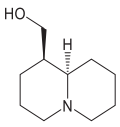
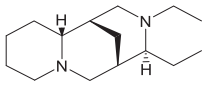
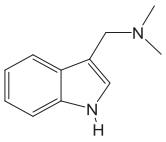
In summary, alkaloids from lupin and grain legume cropped fields are expected to reach the aquatic environment. To test this hypothesis, we cultivated narrow-leaved or blue lupin for one growing season at an artificially drained experimental field equipped with suction cups for sampling soil solution. During this period, alkaloids were regularly monitored in plant tissues, soils, soil pore water, and drainage water. The mobility of alkaloids was evaluated in the soil using apparent soil to water phase distribution coefficients, as well as alkaloid load estimated at the field scale. To the best of our knowledge, this is the first study reporting production and emission of alkaloids from lupins to the soil and water at field scale.

2. Materials and methods

2.1. Field site description

Lupin was cultivated in an experimental field located at the research station Agroscope Reckenholz, North of Zurich, Switzerland (47°25′74″N, 8°30′85″E). The field has been characterized in previous studies (Schenzel et al., 2012; Wettstein et al., 2016); the soil is classified as a Gleyic Cambisol (Table 2) (FAO, 2014). The experimental field covers an area of 100 × 22 m

Table 1
Physical-chemical properties of selected alkaloids quantified in the study.[a]

	Quinolizidine alkaloid					Indole alkaloid
	Angustifoline	Hydroxylupanine	Lupanine	Lupinine	Sparteine	Gramine
Molecular structure						
CAS number	550-43-6	15358-48-2	550-90-3	486-70-4	90-39-1	87-52-5
Nominal mass [Da]	234.17	264.18	248.19	169.15	234.21	174.12
Molecular formula	$C_{14}H_{22}N_2O$	$C_{15}H_{24}N_2O_2$	$C_{15}H_{24}N_2O$	$C_{10}H_{19}NO$	$C_{15}H_{26}N_2$	$C_{11}H_{14}N_2$
Water solubility [g/L][b]	9.9	21.8	8.1	13.6	3.0	32.2
log D_{ow} (at pH 7)[b]	2.8	2.0	2.3	2.2	3.3	2.1
pK_a[c]	10.3	8.8	9.4	9.4, 15.4	12	7.9
Half-life in natural waters [days][b]	36	38	38	38	60	38

[a] Abbreviation: CAS = chemical abstracts service, da = Dalton, g/L = gram per liter, D_{ow} = octanol − water partition coefficient, and pKa = acid dissociation constant at logarithmic scale.
[b] Data from EPISuite (US EPA, 2017).
[c] Data from ACD/Labs Percepta Platform, 2016.

Table 2
Parameters of the soil in the study field at Agroscope, North of Zürich, Switzerland (47°25′74″N, 8°30′85″E).[a]

Soils	Soil depth (cm)	pH_{CaCl2}	pH_{H2O}	C_{org} [%][b]	Clay [%][c]	Silt [%][c]	Sand [%][c]
Topsoil I	0-5	6.6	7.1	2.0	22	33	45
Topsoil II	15–30	6.4	7.1	1.7	22	33	45

[a] Abbreviations: cm = centimeter, $CaCl_2$ = 0.01 M calcium chloride, C_{org} = organic carbon content, and μm = micrometer.
[b] C_{org} = organic carbon content.
[c] Particle size: clay <2 μm, silt 2–20 μm, sand >20 μm.

(0.22 ha) with a gentle slope of 1–2° from northeast to southwest. The field is artificially drained at a depth of 80–90 cm, by two long and two short drain pipe branches, which individually connect to a main plastic drainage tube with a diameter of 15 cm (Fig. S1 in the Supporting Information (SI)). Drainage from the entire field is diverted into a single sampling duct, where discharged drainage water was measured and sampled flow proportionally using flow meters and automated samplers (7612 ISCO with a 730-bubbler module, both from Teledyne Isco Inc., Lincoln, NE) as described in more details below. Precipitation data were gathered by the meteorological station (Reckenholz 443 m above sea level, 47°25′40″N, 8°31′04″E, MeteoSwiss, approximately 300 m from the field site) in 10 min intervals. Precipitation data for the period of the study are presented in Fig. S2 in the SI.

2.2. Lupin cultivation

The field was ploughed at medium depth (20–25 cm) on 14th April 2019, and thereafter harrowed. The field was sowed on 15th April 2019 with blue or narrow-leaved lupin (*Lupinus angustifolius* L. Primadonna) seeds from DLF (Denmark) inoculated with bacterium of *Bradyrhizobium lupini* strain (DSV Frø, Denmark). The seedbed rows were parallel and separated by a distance of 16 cm. The seeding density was approximately 110 seeds/m^2, with seeds placed at depth of 4 cm. After seeding and installation of all equipment, the field was irrigated with 20 mm of water to ensure optimum germination conditions. To reduce the amount of weeds in the field, the herbicide fluazifop-p-butyl was applied once (2 L/ha) on 27th May 2019.

2.3. Sampling

Sample collection began 1st April 2019 and continued until 25th August 2019, two weeks after harvest. The field was divided into a sampling matrix with 4 rows and 20 columns, resulting in 80 subplots (5 × 5 m), Fig. S1. Each subplot was further divided into four quarters (2.5 × 2.5 m). To assure a representative sampling of plant and soil over time, the quarters sampled each sampling day were randomly selected at the beginning of the experiment. The sampling points were randomized based on column/row/quarter (Table S4). Each sampling included the collection of plants (whole plant including roots) (n = 10) and 10–15 g soil (topsoil I (at 0–5 cm depth) and topsoil II (at 15–30 cm depth)) (n = 3). Soil samples were collected at a horizontal distance of 5 cm from the lupin plants. Plant materials (manually) and soils (using hand auger) were collected every 7 to 13 days during the growing season and stored in polyethylene bags. The plants were used to determine the plant biomass (n = 10) and alkaloid content (n = 2). A number of weeds developed in the field (11 ± 5 plants/m^2), Table S5. The weed biomass increased considerably from June to the harvest season, even though herbicide was applied. Therefore, weed (1 plant per species, Table S5) samples from the field were collected and analysed to determine whether they contributed as source of alkaloids. Upon arrival at the laboratory, plant (separated into the organs) and soil samples were frozen at −20 °C and stored until processed. All soil samples collected during the field experiment were analysed separately to determine organic carbon content (Table S6). The organic carbon content of the soil had not changed considerably over the last 15 years (Hartmann et al., 2008), despite the field was used continuously for research and agricultural activities.

Soil pore water samples were collected using suction cups (porous polytetrafluoroethylene (PTFE) mixed with silica flour), tubes, and sampling bottles from Prenart Equipment ApS (Frederiksberg, Denmark). Nine suction cups were installed in three designated plots (A, B, and C), positioned in form of an equilateral triangle with a side length of 5 m, as illustrated in Fig. S3. In each edge of the triangles, three suction cups were installed at three different depths (10, 30, and 70 cm; termed soil pore water 10, 30, and 70, respectively), and at least 1 m distance between each suction cup (Fig. S3). A metallic solid pole (diameter of 2.5 cm) was used to make a hole at an inclination of 45 degrees prior installing the suction cups. The hole was then filled with 100 mL aqueous slurry containing 70 g silica flour (75 μm) to establish a good hydraulic between the suction cup and the soil. The suction cells were connected with above ground sampling bottle using polyvinyl chloride tubes (diameter 0.25 cm), and the system vacuumed at approximate 60 hPa with a hand pump 24 h prior of sampling. The details of installing and validating the suction cups follow the procedures in (Hama and Strobel, 2020). Soil pore water samples were immediately transported to the laboratory and kept at −20 °C, and later processed as described in Section S2.2. Note that during July and August no soil pore water was collected by the suction cups.

Drainage water samples were collected from 15th April 2019 until 12th August 2019, at a sampling rate of 1 L for every 200 L from 15th April to 31st May and then changed to 1 L for every 60 L from 1st June to 19th July. Due to instrumental error, from 20th July to 15th August the flow of the drainage water was not recorded, but water samples were still collected for chemical analysis. Water samples were collected in pre-rinsed (with MilliQ) polyethylene plastic bottles (1 L), and transported and stored in the dark at −20 °C within 1–15 h of collection. Every second sample was analysed for alkaloid concentrations. In the field, no lupins or other plants containing any of the targeted alkaloids had been cultivated at least in the past 10 years (Table S7).

2.4. Sample preparation and extraction

In the laboratory at Agroscope, all samples were processed as described in previous studies (Hama and Strobel, 2019; Hama and Strobel, 2020). Briefly, collected lupin plants (all organs) and soil samples were freeze-dried, grinded into fine powder and homogenized prior to extraction. The plant and soil samples were spiked with prochloraz-d4 (100 ng/L), and then extracted three times with 10 mL methanol (MeOH) and sonication (15 min), followed by centrifugation (10 min at 2100*g*). The supernatants (30 mL) were collected and loaded on Oasis® MCX (6 mL, 150 mg sorbent per cartridge from Waters Corporation, Milford, MA) solid phase extraction (SPE) cartridges. In addition, drainage (1 L) and soil pore water (20–555 mL) samples were filtered (glass fibre filters, pore size 1.2 μm, Millipore, Volketswil, Switzerland) by vacuum filtration (Supelco, Bellfonte PA, USA) and spiked with prochloraz-d4 (100 ng/L), then loaded on SPE cartridges. Alkaloids were eluted from the SPE cartridges with MeOH (5 mL) and methanol:ammonium hydroxide (3:1, *v*/v) (10 mL). The eluate was then dried under a gentle stream of nitrogen gas in a heating block at 40 °C, and reconstituted in 500 μL MeOH prior to analysis. The samples were stored at −20 °C prior to, and during shipping to the analytical laboratory (Department of Plant and Environmental Sciences, University of Copenhagen, Denmark). Details on sample extraction, analytical methods and quality control are provided in the SI.

2.5. Data acquisition and analysis

Quantification of alkaloids was performed as described previously (Hama and Strobel, 2019; Hama and Strobel, 2020). Briefly, the samples were analysed on a Waters ACQUITY UPLC coupled with a Xevo Triple

Quadrupole Mass Spectrometer equipped with an electrospray ionization source (Milford, Massachusetts, USA). The separation was performed using a 50 mm × 2.1 mm I.D., 1.7 μm Waters Acquity UPLC HSS C18 column. The mobile phases of eluent A (water and 0.1% formic acid (FA)) and eluent B (MeOH and 0.1% FA) were used in gradient elution mode: 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 6 min before each run, with a total run time of 23 min. Samples of 5 μL were injected onto a pre-heated column at 35 °C, with the eluent flow rate at 0.20 mL/min. All alkaloids were separated in one chromatographic run, followed by positive ionization. The detection was performed with a full scan and multiple reaction monitoring, with ion traces obtained for apex retention time (t_R) ±0.15 min. The following interface parameters were used: source temperature (150 °C), desolvation gas (set to 1000 L/h at 500 °C) and cone gas flow (20 L/h). The optimum capillary voltage was 3.5 kV. The cone voltage ranged from 15 to 40 V and the collision energy ranged from 25 to 45 eV (Table S1). More details on analysis and the LC-MS/MS conditions are provided in the SI. MassLynx 4.1 was used for instrument control and data acquisition. If an analyte was detectable but not quantifiable, its concentration was set equal to its limit of detection (LOD). LODs for all alkaloids were 3–18 [ng/kg], 5–31 [ng/kg], and 0.05–0.3 [ng/L] in whole plant, soil, and water, respectively (Table S2).

The following quality assurance/quality control elements were used: blanks for plant (n = 10), soil (n = 18), and drainage water (n = 16), replicate samples, and surrogate recovery. Blanks for water, drainage water (collected 1st April in the field, i.e. prior to lupin seeding, water blank 1), field (water blank 2), and laboratory blanks (water blank 3) consisted of certified laboratory grade organic free water (i.e. MilliQ water). Blanks for soil consisted of samples taken from the field before cultivation started (soil blank 1), and from the east side of research station Agroscope Reckenholz, North of Zurich, Switzerland (47°25′74″N, 8°30′85″E) (soil blank 2), 0.5 km away from the field. Freeze-dried powder of bracken fern plant (from Humleoreskov, a temperate forest located 60 km west of Copenhagen, Denmark, 55°28′29.7″N, 11°54′26.1″E) and weed samples from the field were used as plant tissue blanks (plant tissue blank 1 and 2, respectively). Concentrations of targeted alkaloids in blank samples (plant, soil, and water) were all below LOD. The average recovery rate of surrogates (senecionine and prochloraz-d4) in the plant, soil, and water samples were over 89%, 86% and 94%, respectively, Table S3. All statistical analyses were conducted at a 95% confidence level using OriginPro software 9.6 (OriginLab Inc., Massachusetts). Statistical outliers were determined as values 1.5 times the interquartile range (IQR) below or above the first or third quartiles, respectively (Vinutha et al., 2018).

The loads of alkaloids in the drainage water were calculated from the quantified concentrations and the drainage water discharge, i.e. the concentrations measured for 1 L samples taken every 200 L was multiplied by 200 L to obtain the total load, assuming constant concentrations over any given sampling interval (see above), given by the ISCO sampler protocol. Alkaloid concentrations in every second ISCO sample was not analysed but calculated as average concentration of alkaloids in the ISCO sample before and after, and multiplied with the water discharge. Apparent soil-water phase distribution coefficient (D_d) and organic carbon-water distribution coefficients (D_{OC}) were estimated for soil depths of both 0–5 cm and 15–30 cm. The D_d (L/kg), was calculated as the ratio of alkaloid concentration in soils to the concentration of alkaloids in soil pore waters, while D_{OC} (L/kg) was calculated as the ratio between D_d and the organic carbon content of the soil (Table S6).

3. Results and discussion

Over the entire period, six out of nine monitored alkaloids were repeatedly detected in all samples, i.e., lupin plant tissue, soil, soil pore water, and drainage water (Table 3). Therefore, results are focused on these alkaloids, which are angustifoline, gramine, hydroxylupanine, lupanine, lupinine, and sparteine.

3.1. Alkaloids in lupin plant tissue

The biomass production of lupin was low (1–5 g fresh weight/plant) during the first 1.5 months of the field experiment. From mid-June the biomass increased dramatically and reached its maximum in mid-July (about 100 g fresh weight/plant) (Fig. S4). Hereafter the plants matured and seeds ripened, and the biomass decreased by 30%, as the plants dried out and lost most of the leaves. Alkaloids were detected in all plant samples from the study site (Table 3, Figs. S5–6). The mean concentration of alkaloids per dry weight of plant biomass increased during the first month of lupin growth, then levelled off until July and increased again towards the harvest season (Fig. S5). Lupanine was detected at the highest concentrations (range and median concentrations are 0.2–738 and 45 μg/g dw, respectively), followed by gramine (0.02–96 and 11 μg/g dw), hydroxylupanine (0.02–3.4 and 0.3 μg/g dw), sparteine (LOD–1.1 and 0.1 μg/g dw), lupinine (LOD–1.5 and 0.1 μg/g dw), and angustifoline (LOD–1.1 and 0.01 μg/g dw), Table 3. The alkaloids found and quantified were confirmed by a parallel suspect screening project in the same field (Liang et al., 2022). Alkaloid concentrations in lupin roots, stems, and leaves were variable, but decreased steadily after flowering in June (Fig. S6). The highest concentration of alkaloids were found in seeds, followed by pods, flowers, leaves, and stems, while the lowest concentrations were found in roots. In fact, lupin tends to transfer alkaloids to the seeds during ripening (Otterbach et al., 2019). The seeds are the reproduction organs of plants, and the alkaloids seem to play an important role in plant defence, and also contribute to nitrogen metabolism (Boschin and Resta, 2013). The alkaloid contents in the seeds were expected to be higher at least by a factor of 2 to 10 compared with literature (Aniszewski et al., 2001; Niwiriska, 2001; Pilegaard and Gry, 2008). The lower content could be due to variation within the same species, soil pH (Jansen et al., 2012), seasonal conditions (Jansen et al., 2009), and the geographical location. The variation in content of pyrrolizidine alkaloids may depend on the exposure to attacks by insects and fungi as seen for other plant generated toxins (Gera Hol et al., 2004; Hol, 2011).

Considering the lupin plant biomass (22.9 g dw per plant at the day of harvest, Fig. S4) and the plant density (60 ± 4 plants per m^2 (n = 10)), the lupin biomass production estimated for the growing season was 14 ton dw/ha. Thus, based on the alkaloid concentrations range listed in Table 3, the highest total alkaloid production in above ground lupin plant material was 11 kg/ha. After harvesting the lupin seeds, half of the plant material (including pods with seeds and half of the stems) was removed, while rest of the stems, leaves, and roots remained in the field as a green manure. Thus, approximately 70% of the total alkaloid content was removed by harvest. Alkaloids may continuously be transferred from the lupin residues into the soil and water (plant residues still contained alkaloids a month after harvest, Table S9). In other studies, much higher alkaloid concentrations (1.4–2.5 mg/g dw total alkaloids) were observed for blue lupins (Aniszewski et al., 2001; Christiansen et al., 1997; Niwiriska, 2001), and also higher plant densities up to 138 plants/m^2 have been reported (French et al., 1994). The observed toxin concentrations in lupins are at the same level as natural toxin concentrations in other plants, e.g. pyrrolizidine alkaloids (ragwort (*Jacobaea vulgaris*), Hama and Strobel, 2021), sesquiterpene (ptaquiloside) (bracken fern (*Pteridium aquilinum*), García-Jorgensen et al., 2021; Rasmussen et al., 2003), glycoalkaloids (potato (α-solanine and α-chaconine), Jensen et al., 2009), and isoflavones (red clover, Hoerger et al., 2009; Hoerger et al., 2011).

3.2. Alkaloids in soils

In soil samples, only lupanine, hydroxylupanine, and gramine were quantified, while the concentration of lupinine and sparteine were low, between LOD and limit of quantification (LOQ) (Table 3, Fig. S7). Angustifoline was not detected in any soil sample. Overall, the concentration of alkaloids in both topsoil I and topsoil II were similar. In topsoil I at 0–5 cm and topsoil II at 15–30 cm, lupanine showed the highest concentrations of 18–1.8 × 10^4 ng/g dw and 5.4–1.3 × 10^4 ng/g dw, respectively,

Table 3
Concentration of alkaloids[a] in plant tissues, topsoil I, topsoil II, soil pore water (at different depth: 10 cm, 30 cm, and 70 cm), and drainage water. All samples were collected from April to August 2019.[b]

Sample	Alkaloid	Percent detected	Mean ± STD[c]	Median	Min	Max
Plant [μg/g dw]	Angustifoline	67/90 (74%)	0.1 ± 0.3	0.01	det	1.1
	Gramine	90/90 (100%)	17 ± 26	10.7	0.02	96
	Hydroxylupanine	88/90 (98%)	0.8 ± 1.1	0.3	0.02	3.4
	Lupanine	90/90 (100%)	99 ± 203	44.7	0.2	738
	Lupinine	66/90 (73%)	0.2 ± 0.4	0.06	det	1.5
	Sparteine	25/90 (25%)	0.2 ± 0.3	0.1	det	1.1
Topsoil I at 0–5 cm [ng/g dw]	Angustifoline	0/24 (0%)	nd	nd	nd	nd
	Gramine	13/24 (54%)	7.8 ± 9.7	4.9	det	27
	Hydroxylupanine	18/24 (75%)	11 ± 17	5.5	det	59
	Lupanine	18/24 (75%)	2740 ± 4913	485	18	17,864
	Lupinine	4/24 (17%)	det	det	det	det
	Sparteine	12/24 (50%)	det	det	det	det
Topsoil II at 15–30 cm [ng/g dw]	Angustifoline	0/24 (0%)	nd	nd	nd	nd
	Gramine	14/24 (58%)	6.8 ± 7.4	3.8	det	21
	Hydroxylupanine	18/24 (75%)	8.6 ± 11	5.3	det	37
	Lupanine	18/24 (75%)	1989 ± 3904	63	5.4	13,007
	Lupinine	1/24 (4%)	det	det	det	det
	Sparteine	12/24 (50%)	det	det	det	det
Soil pore water 10 cm [ng/L]	Angustifoline	1/9 (11%)	4 ± 4	4	4	4
	Gramine	4/9 (44%)	1 ± 1	0.6	0.1	3
	Hydroxylupanine	9/9 (100%)	0.4 ± 0.4	0.4	det	1
	Lupanine	9/9 (100%)	7 ± 6	6	0.5	17
	Lupinine	9/9 (100%)	0.7 ± 0.5	0.8	0.1	2
	Sparteine	5/9 (56%)	0.3 ± 0.3	0.2	0.1	1
Soil pore water 30 cm [ng/L]	Angustifoline	0/7 (0%)	nd	nd	nd	nd
	Gramine	2/7 (28%)	8 ± 11	8	0.2	15
	Hydroxylupanine	7/7 (71%)	0.4 ± 0.3	0.3	det	1
	Lupanine	7/7 (100%)	33 ± 35	9	0.7	75
	Lupinine	4/7(57%)	0.8 ± 1.1	0.3	0.1	3
	Sparteine	4/7(57%)	0.2 ± 0.1	0.2	0.2	0.3
Soil pore water 70 cm [ng/L]	Angustifoline	2/8(25%)	3 ± 3	3	0.6	4
	Gramine	4/8(50%)	0.8 ± 1	0.4	0.2	3
	Hydroxylupanine	8/8(100%)	0.3 ± 0.3	0.3	det	0.8
	Lupanine	8/8(100%)	24 ± 31	9	0.4	76
	Lupinine	5/8(53%)	0.7 ± 0.6	0.6	0.1	2
	Sparteine	3/8(38%)	0.2 ± 0.1	0.2	0.2	0.4
Drainage water [ng/L]	Angustifoline	6/132 (5%)	1 ± 2	0.9	0.1	6
	Gramine	64/132 (48%)	78 ± 170	2	det	815
	Hydroxylupanine	104/132 (78%)	7 ± 19	0.7	det	95
	Lupanine	110/132 (83%)	52 ± 70	18	0.1	350
	Lupinine	95/132 (71%)	6 ± 16	2	0.1	104
	Sparteine	82/132 (62%)	6 ± 14	0.4	0.1	69

[a] Additional alkaloids (cytisine, matrine, and multiflorine) were monitored during the field experiment, however, were not detected in the sample.
[b] Abbreviations: ng/L = nanogram per liter, ng/g dw = nanogram per gram of dried weight, cm = centimeter, STD = standard deviation, det = detected (i.e., below limit of quantification); nd = not detected (i.e., below LOD).
[c] Mean ± standard deviation.

followed by hydroxylupanine and gramine. The detection frequency of lupanine, hydroxylupanine, and gramine ranged from 54 to 75% (n = 24). The alkaloids detected in the soil were similar to the one in lupin plant tissue, supporting the plant as the source of the alkaloids. With the exception of gramine, the alkaloid concentrations in topsoil I and topsoil II increased when the plant biomass and alkaloids content in the plants increased towards harvest (Fig. S7). This could be due to the inputs from lupin plant litter especially leaves and the alkaloids released (washed off or root exudates) from the plants to surrounding soils. Alkaloids were not detected in the soils collected on 16th July. Total content of alkaloids were between 4.0×10^{-4} and 0.02 kg/ha in topsoil I (soil bulk density 1.5 g/cm^3 (Rai et al., 2017)) (Table 3), while total content of alkaloids in topsoil II were less than 0.01 kg/ha. This is a minor fraction of the 11 kg/ha alkaloids detected in the lupin plant tissues, and thus, the fate is unknown for the majority of alkaloids produced by lupin.

3.3. Alkaloids in soil pore water

In the soil pore water, lupanine was detected in all samples from soil depths of 10 (n = 9), 30 (n = 7), and 70 cm (n = 8), followed by gramine, lupinine, and hydroxylupanine in decreasing frequency (Table 3, Fig. S8). Lupanine was detected in highest concentrations of 33 ± 35 and 24 ± 31 ng/L for soil pore water from 30 and 70 cm depth, respectively, and with four times lower concentration of 7 ± 6 ng/L in 10 cm soil depth. The detection frequencies of all alkaloids were highest in soil pore water from depth of 10 cm (68%), with the highest total concentration in soil pore water from 30 cm (75 ng/L). In all soil pore waters, the concentrations of angustifoline, gramine, hydroxylupanine, lupinine, and sparteine ranged from 0.1 to 15 ng/L, while lupanine ranged from 0.4 to 76 ng/L. Angustifoline, which was not detected in any soil sample, appeared during three sampling times in pore water at concentrations in the range 0.6 to 4 ng/L (Fig. 2). Similar to a previous lupin field experiment conducted in Denmark, lupanine was the most frequently detected alkaloid in soil pore water collected at the depths of 10–70 cm (Hama and Strobel, 2020).

The values of D_d (Table S8) and D_{OC} (Fig. 1) showed temporal variation for all alkaloids and soil replicates. In topsoil I, log D_{OC} [L/kg] ranged from 3.3 to 6.7, while the range for topsoil II was 2.3–6.5, where the higher values in the range exceed predicted values (Table S10). In addition, log D_d [L/kg] of hydroxylupanine, lupanine, and sparteine in both topsoil I and topsoil II were in the range of 1.6–4.8. These high log D_{OC} values could be due to non-equilibrium and micro heterogeneity of soil samples, as well as variation of soil organic matter (SOM) in the soil used in this study and in the literature experiments. Furthermore, sorption may not only be attributed to SOM and hence normalization by carbon in SOM

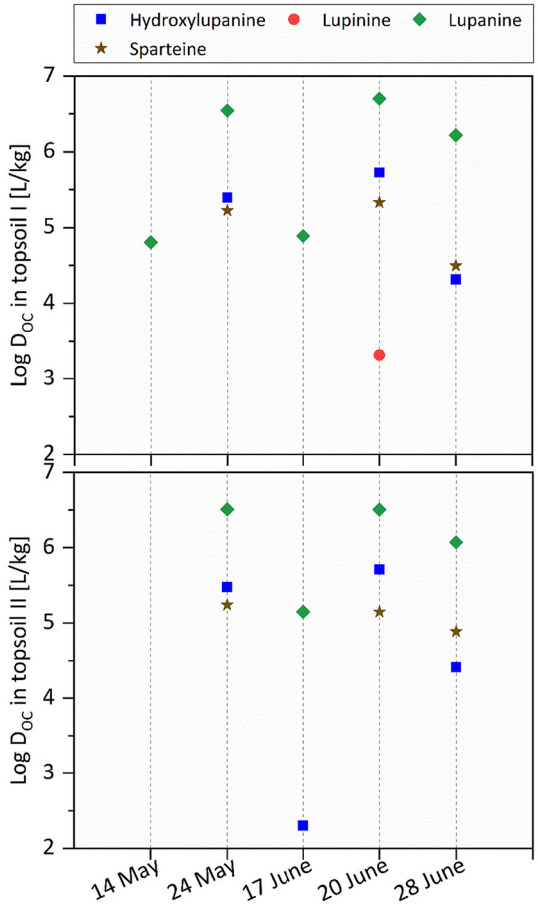


Fig. 1. Log apparent organic carbon-water phase distribution coefficients (D_{OC}) of alkaloids in topsoil I and topsoil II of the lupin field determined for samples collected from April to August 2019. Log D_{OC} were calculated using alkaloid concentrations in topsoil I at 0–5 cm with soil pore water at 10 cm, and topsoil II at 15–30 cm with soil pore water at 30 cm, normalizing with the carbon content in SOM (Table S6).

overestimates the relative importance of SOM among all sorbents present in the soil. More likely, ionic interactions of the positively charged alkaloids to the negatively charged surfaces of soil clay minerals might dominate to the overall sorption. Experimentally determined log D_{OC} of lupanine and gramine were 1.7 and 3.2, at pH 6 for Pahokee peat soil, respectively (Schönsee et al., 2021a). While sorption coefficients of gramine for the clay minerals kaolinite and montmorillonite were 0.21 and 2.01 L/kg (Schönsee et al., 2021b). Higher concentration of alkaloids in soil pore water was documented in a Danish lupin field experiment on a loamy soil with 11% clay. Despite similar plant alkaloids content and lower plant density, the concentration of alkaloids in the soil was lower, as they sorbed less to the soil with lower clay content, and the log D_d of alkaloids ranged from 0.4 to 0.9 L/kg (Hama and Strobel, 2020).

3.4. Alkaloids in drainage water

Drainage water was sampled from April to August 2019 (Fig. 3a), resulting in a total of 132 samples. Lupanine was detected at the highest frequency (83%, n = 132), followed by hydroxylupanine (78%), lupinine (71%), sparteine (62%), gramine (48%), and angustifoline (5%) (Table 3). From the beginning of the field experiment to mid-June, alkaloids were detected in concentrations ranging from the LOD to 100 ng/L, with lupanine as the dominant contributor to the total alkaloid concentration. However, from mid-June to harvest, concentrations rose to a range from LOD to 800 ng/L (Fig. 3), with lupanine, gramine, and hydroxylupanine as the main contributors. Hence, the increase in alkaloid contents *in planta* and in soil is, to a lesser extent, also mirrored in drainage water.

The amount of alkaloids in drainage water was influenced by lupin vegetation, high D_d (Table S8), precipitation and water content in the soil. The soil hydrology played a dominant role as on average 45% of the alkaloids (mg/ha) exported in drainage water leached during rain or irrigation events with flow rate above the 80th percentile (>0.037 L/s) (Fig. 3). This is in line with the event pulses of natural estrogens reported from grassland in the same agricultural field (Rechsteiner et al., 2021). The concentration of all alkaloids found in drainage water mostly peaked with the onset of drainage water flow within 2–15 h after rain and irrigation events. The tailing period of these maximum concentrations ranged from less than a day to a few days, after which the concentrations dropped to below LOQ. During the field experiment, no direct preferential flow measurements were conducted, but preferential flow is considered as a main contributor to pulses of alkaloid transport in drainage water. Previous studies on the same agricultural field identified macropore flow as the main transport process for other organic micropollutants such as estrogens (Rechsteiner et al., 2021), mycotoxins (Hartmann et al., 2008), phytoestrogens (Hoerger et al., 2011), and pesticides (Wettstein et al., 2016), as well as for the conservative tracer bromide (Wettstein et al., 2016).

Overall, higher concentrations of all alkaloids were found in the drainage water compared to soil pore waters collected on the same day (Fig. 2). This could be due to alkaloids being transported by preferential flow through macropores in events taking place over dry soil, which reduces the residence time and hence, minimize the time for sorption to occur and time to interact with the soil matrix. Alternatively, lower concentration in pore water maybe due to uptake by nearby plant roots before leaching further (von Kiparski et al., 2007) and fast microbial degradation occurring at rates faster than alkaloid desorption from the soil. The concentration ranges (0.1–800 ng/L) of alkaloids in the drainage water were far below the very limited toxicity values of *Vibrio fischeri* and *Daphnia magna*. Chemical mixtures and potential long-term exposure effects of alkaloids on specific or non-target soil and water microbial communities are unknown. The only toxicity level reported for alkaloids is an acute toxicity value for lupanine to *Vibrio fischeri* and *Daphnia magna* with EC_{50} (concentration causing immobility of 50% individuals) values at 89 mg/L and 47 mg/L, respectively. In addition, EC_{50} ranges of 28–156 and 6 mg/L have been documented for QAs (Lupanine, Lupinine and Sparteine) and gramine towards *Daphnia magna*, respectively (Griffiths et al., 2021). In areas with small water bodies receiving mainly runoff from large agricultural fields, concentrations of alkaloids are less diluted, therefore alkaloids may contribute more to the total toxicity of the surface water.

For the period of lupin growth until harvest, the total amount of alkaloid exported via drainage water was around 11, 1.5, and 1 mg/ha for lupanine, lupinine, and hydroxylupanine respectively, while gramine, sparteine, and angustifoline were about or lower than 0.5 mg/ha, Fig. 3a–g. The accumulated total amount of all alkaloids exported with drainage water was 14 mg/ha. In general, the amounts of alkaloids are underestimated because the capacity of the drainage water flow sampling device was limited to 2.5 L/s, and the drainage efficiency was only around 40% as documented previously (Hartmann et al., 2008; Schenzel et al., 2012; Wettstein et al., 2016) (see Section S3, drainage efficiency in SI). The water from the uphill field diluted the alkaloid concentrations in drainage water by a factor 1.3 to 3 (Wettstein et al., 2016). Thus, the concentrations and amounts of alkaloids exported from the lupin field are likely underestimated. Adding the underestimated amount of alkaloids to the current concentrations will not exceed the acute and chronic environmental values, but will increase the risk to aquatic organism (Griffiths et al., 2021).

The alkaloid compound profile in drainage water was comparable to the profiles for lupin plant, soil, and soil pore water (Table 3, Fig. S9), suggesting that lupin was the source. Lupanine was detected at highest frequency (75–100%), while angustifoline was detected at lowest frequency (0–74%). For all alkaloids, higher detection frequency observed in plant samples compared to the soil, soil pore water, and drainage water samples. In comparison to the relatively high amounts of produced and stored

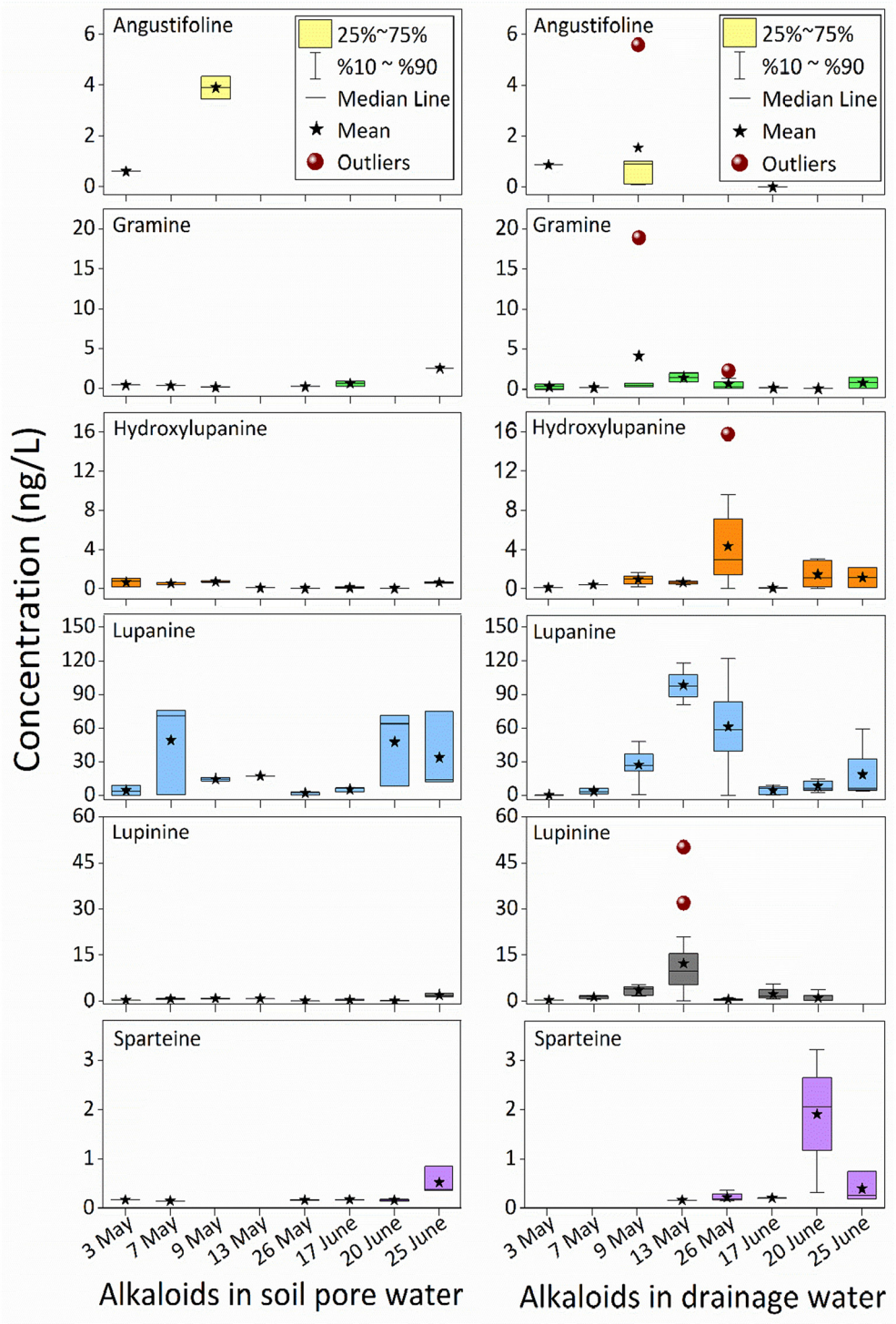


Fig. 2. Alkaloid concentrations in soil pore water (combined) (left) and drainage water (right) at individual sampling days, from lupin field experiment, from April to August 2019. Horizontal black line inside the box, black star, and red dot represent medians, means, and outliers, respectively.

alkaloids in lupin plants and soil (see Sections 3.1 and 3.2), less than 0.0003% was emitted via drainage water. However, drainage water was underestimated, continuous emission of alkaloids after harvest can occur with elution of alkaloids from straw and plant materials left in the field after harvest (Table S9). Furthermore, alkaloids were probably present in protonated form, and thus more water-soluble and mobile ($7 \leq pK_{a1} \leq 12$, Table 1) under the pH conditions in the soil ($6.4 \leq pH \leq 7.1$, Table 1) and drainage water (pH 6.6–7.6). This suggests that most of the alkaloids were not emitted via drainage water, but sorbed to soil (approximately 0.5 kg/ha), and eventually degraded as documented for isoflavones (Hoerger et al., 2011).

4. Conclusions

This study documented the transport of alkaloids from lupin plant tissues into soil, soil pore water, and drainage water during an agricultural field experiment for one growing season. Alkaloids concentrations in lupin plant tissue gradually increased, and peaked at 4×10^4 ng/g dw

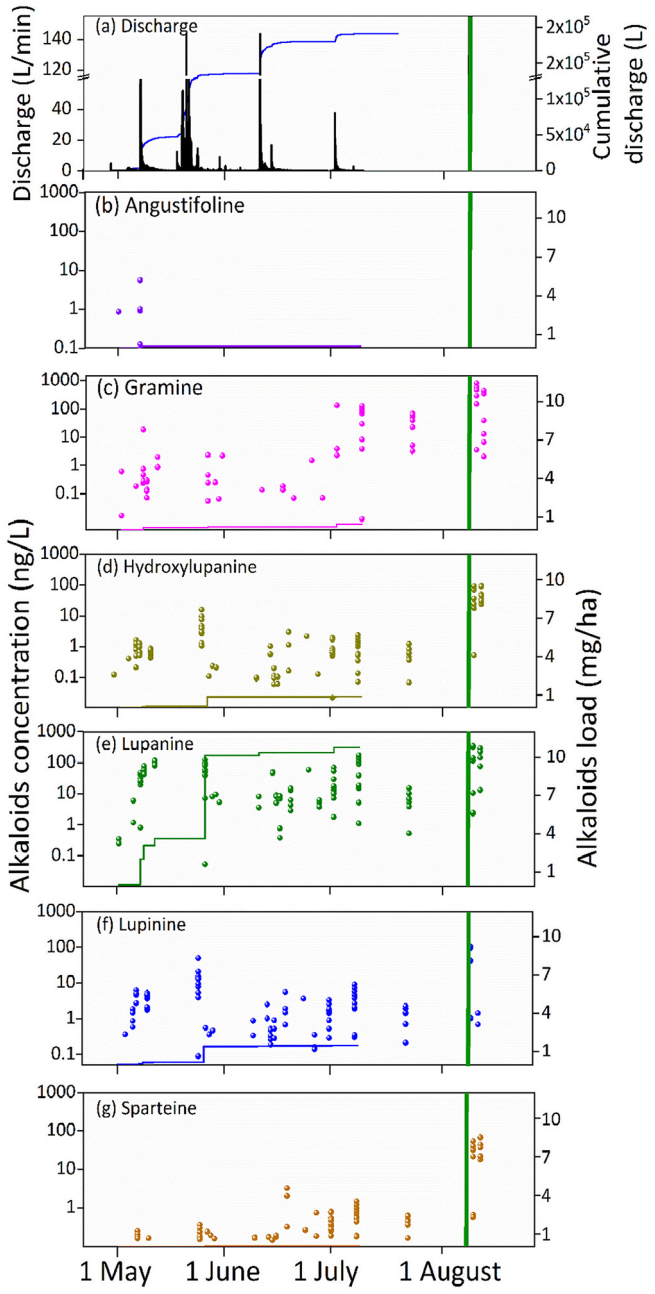


Fig. 3. Occurrence of alkaloids in drainage water from April until August 2019: (a) drainage water discharge (black peaks: water flux [L/min]; blue line: cumulative discharge [L/min]), and the green lines represent the harvest day (9th August); (b) angustifoline; (c) gramine; (d) hydroxylupanine; (e) lupanine; (f) lupinine; (g) sparteine. In panel (b) to (g): circles: alkaloids concentration [ng/L], line: accumulated amount of alkaloids exported (mg/ha). Note that panels b–g have primary y-axis (left) in logarithmic scales.

during the harvest season. Alkaloids were detected in soil, soil pore water, and drainage water throughout the growing season and after harvest, however, made up a very small proportion of the alkaloids in the plant. Overall, the alkaloids profile was similar in the plants, soil, soil pore waters, and drainage waters indicating the plant as the source of the alkaloids. Lupanine was the most detected alkaloid among all collected samples, while angustifoline was the least detected. Alkaloids were transferred to the soil and drainage water, at rates of 0.16% and 0.0003% of the alkaloid contents in the lupin crop. Higher concentrations of alkaloids in drainage water than in soil pore water reflect the fast transport through soil (macropore transport). The majority part of alkaloid mass was either removed during harvest, degraded or retained in the soil, rather than being leached via drainage water. Lupin plant density, alkaloid contents, soil properties, and irrigation/precipitation were the main variables determining the transport of alkaloids from the plant to the fields, eventually resulting in leaching to water bodies. The environmental exposure to natural toxins, in particular alkaloids, needs further exploration. In addition, the fate of alkaloids in lupin plants and transfer from plants to soils needs further investigation.

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CRediT authorship contribution statement

Jawameer R. Hama: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing–original draft. **Daniel Bernardo Garcia Jorgensen:** Conceptualization, Data curation, Investigation, Validation, Writing–review & editing. **Efstathios Diamantopoulos:** Conceptualization, Validation, Writing–review & editing. **Thomas D. Bucheli:** Conceptualization, Investigation, Funding acquisition, Resources, Supervision, Writing–review & editing. **Hans Chr. Bruun Hansen:** Conceptualization, Investigation, Funding acquisition, Resources, Writing–review & editing. **Bjarne W. Strobel:** Conceptualization, Investigation, Funding acquisition, Resources, Supervision, Writing–review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Experimental: Chemicals used; Extraction for Plant, soil, and water samples; LC-MS/MS parameters, description of method validation; Quality Assurance and Quality Control for the experiment: Tables: LC-MS/MS parameters; Method validation; Randomization of the felid; The organic carbon (C_{org}) content of the soil; Crop record of the field; Apparent soil-water phase distribution coefficient D_d of alkaloids; Alkaloid in lupin plant tissue residues; Predicted D_{OC} of alkaloids; Figures: Scheme of the experimental field site at Agroscope; Illustration of installed suction cups in the soil; Daily precipitation; Lupin plant biomass; Alkaloid concentrations in lupin plant tissue, topsoil I and topsoil II; Individual alkaloid concentrations in all plant organs; Individual alkaloid concentrations in topsoil I and topsoil II; Individual alkaloid concentrations in soil pore water at 10 cm, 30 cm and 70 cm; Occurrence of alkaloid in lupin plant tissues, soils, soil pore waters and drainage water. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155283.

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