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# Fate of Toxic Potato Glycoalkaloids in a Potato Field

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The toxic glycoalkaloids, α-solanine and α-chaconine, are present in all parts of the potato plant and are possibly transferred to the terrestrial environment. The amounts of glycoalkaloids in plant, soil, and groundwater were followed in a potato field to investigate their distribution and fate during the season. The amount of glycoalkaloids in the plants was up to 25 kg/ha during maturity and decreased to below 0.63 kg/ha during plant senescence. The glycoalkaloids were detected in the upper soil (up to 0.6 kg/ha); this amount accounted only for a minor fraction of the amount present in the plants. Maximum glycoalkaloid concentration of 2.8 mg/kg dry weight soil was detected in September. Dissipation during winter appeared to be slow because glycoalkaloids were still present in the soil in March. No traces of glycoalkaloids were detected in the groundwater (detection limit 0.2 μg/L). From these results, the leaching potential of the glycoalkaloids is evaluated to be small.

**KEYWORDS:** ***Solanum tuberosum*** **L.; soil; groundwater; α-solanine; α-chaconine; natural toxin**

## INTRODUCTION

The interest in natural toxins produced by fungi or plants and their fate in the terrestrial environment has grown for some years. Several classes of compounds can be released from plants (*1*, *2*), and the presence of various plant toxins in soil has been confirmed (*3*, *4*). Two main reasons for the increasing interest are a concern for a possible effect of the toxins on soil organisms and the risk of leaching to surface or groundwater, thereby leading to a possible contamination of drinking water resources.

Among the plant toxins are the glycoalkaloids, α-solanine, and α-chaconine, produced by the potato plant. These two glycoalkaloids share a common steroidal aglycone, solanidine, to which a variable trisaccharide is attached (**Figure 1**). The compounds have been studied intensively; the main focus being on the tubers due to their widespread use for food consumption. The content in tubers is related to size (*5*), variety (*6*), and environmental conditions (*7*−*9*). Glycoalkaloids are present in all parts of the potato plant, the higher concentrations being found in the metabolically active plant parts such as flowers, young leaves, and sprouts, with levels much higher than those in the tubers (*10*, *11*). The glycoalkaloids are possibly a part of the plant defense system (*12*, *13*), and besides humans, they are shown to be toxic toward fungi, snails, and insects (*12*, *14*−*17*). The estimated human lethal dose by oral intake is 3−6 mg of potato glycoalkaloids per kilogram of body weight (*16*). The two main toxic effects of glycoalkaloids comprise inhibition of the two cholinesterases, acetyl- and butyryl-cholinesterase, and cell disruption caused by a complex formation with the cell membrane (*18*). Potato pathogens are more tolerant toward the glycoalkaloids, e.g., some pathogens transform the glycoalkaloids into less toxic metabolites (*19*), where one or more of the sugar units are detached, thereby forming the corresponding β-compounds (two sugar units), γ-compounds (one sugar unit), or solanidine (no sugar units) (*15*, *20*, *21*). A recent study of glycoalkaloid degradation in groundwater reported the formation of the same compounds (unpublished information), and enzymes in parts of the potato plant are also able to convert the glycoalkaloids into some of these transformation products (*22*−*24*). The focus in this study is on α-solanine and α-chaconine, as they are the two glycoalkaloids produced in the potato plant, and as in their overall toxicity, they are the most toxic compounds in comparison to their β- and γ-compounds and solanidine (*9*, *25*).

Glycoalkaloids have been proposed to be able to leach from potato tubers or from plant residues left in the field after harvest (*26*, *27*); however, a transfer of glycoalkaloids from the plant to the soil has not been proven yet. Fields used for potato growing are usually sandy soils with low water holding capacity. The fields are irrigated regularly, and a possible transfer route is leaching from the growing leaves during irrigating or rain events. Plant residues and some tubers are left lying on the soil floor after harvest of the tubers; as they disintegrate, they also may possibly cause transfer to the soil. Sandy soils are vulnerable to leaching due to their low sorption capacity; hence,

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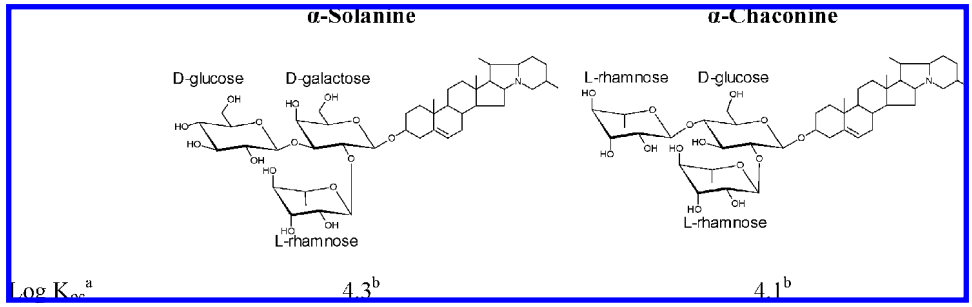


**Figure 1.** Chemical structures of the glycoalkaloids, α-chaconine and α-solanine. For the aglycone, solanidine R = H. [a] $K_{oc}$ organic-soil carbon distribution coefficient. [b] Calculated with EPIwin v3.20 (*38*).

**Table 1.** Selected Properties of the Soil from the Field Location

| | | pH[a] | gravel (>2 mm) (%) | sand (2−0.063 mm) (%) | silt and clay (<0.063 mm) (%) | Org. C[b] (%) | $CEC_7$[c] (cmol (+)/kg) |
|---|---|---|---|---|---|---|---|
| A-horizon | 0−25 cm | 5.7 | 0.5 | 94.7 | 4.8 | 1.8 | 10.5 |
| C-horizon | 60−160 cm | 5.7 | 0.6 | 98.8 | 0.6 | 0.1 | 4.1 |

[a] Measured in 1:2.5 (w/w) soil/MilliQ-water suspension. [b] Measured by combustion (CS-200, LECO Corporation, St. Joseph, MI, USA). [c] Cation exchange capacity at pH 7, measured by the use of the ammonium-acetate method (*39*).

if the glycoalkaloids are transferred to the soil, the low sorption capacity and high irrigation result in a high leaching potential. No studies of potato glycoalkaloids have yet been conducted in the field, while an incubation study at 5 °C showed that glycoalkaloids were detectable in a sandy soil for more than 36 days after spiking (*26*).

The objective of this study was to investigate the glycoalkaloids in a natural environment using a regular sandy potato field. The glycoalkaloid content in the potato plants were followed during the growth season to investigate the possible load of glycoalkaloids to the soil. Similarly, the contents in soil and in groundwater were followed during the growth season and the following autumn. The study should answer if and to what extent the glycoalkaloids are transferred to and present in the soil environment.

## MATERIALS AND METHODS

**Chemicals.** α-Solanine (∼95% purity), α-chaconine (∼95%), and solanidine (∼98%) were purchased from Sigma Aldrich (St. Louis, MO, USA). MilliQ-water was used, and all solvents were of LC grade or better. All chemicals used were of analytical grade or higher.

**Field Location and Sampling.** A typical field for potato growing was selected as the study site. The field is a sandy soil (Typic Fragiorthod (*28*)) located at Fladerne Bæk, Karup in Jutland, Denmark (**Table 1**). Sampling of soil and water was performed during the season 2006−2007. The previous year, the field was used for cropping spring barley. Seed potatoes of the variety Kuras used for starch production were sown in April and emerged in the middle of May, and the potato tubers were harvested by the end of October. The ridge and furrow topography was established at the time of seeding with a furrow ridge furrow equidistance of 70 cm. After harvest, plant remains were left on top of the soil or partly mixed with the soil. Data for precipitation, temperature, and humidity were collected by a climate station at the location with data logging on an hourly basis, and on the basis of these data, the infiltration to groundwater was calculated using the soil−water model SIMPEL (**Figure 2**) (*29*−*31*). The calculation output on a daily basis showed that after a dry winter and spring seasons, late summer and the autumn had periods with rather high infiltration. Furthermore, six times during June−July, irrigation was applied to the potato crop with 20 to 30 mm water.

Sampling was performed within the same 100 $m^2$ of the potato field with intervals of about 1½ months (3rd May, 13th June, 19th July, 6th September, 10th October, 23rd November, 18th January, and 8th March). Plants (above ground material) were collected in total four times (June−October); 8−10 plants (stems and leaves) were collected at each sampling occasion. Died down plant material (debris) lying on

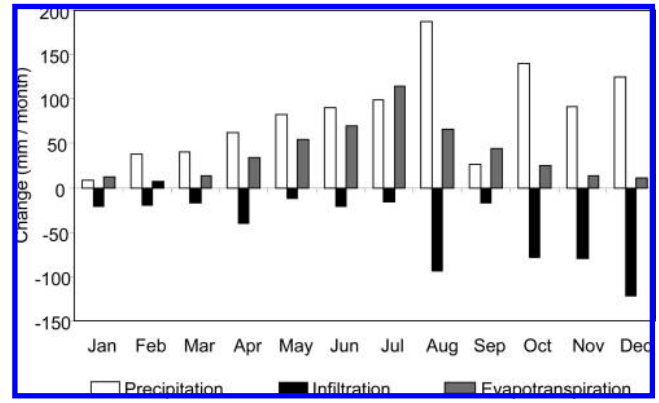


**Figure 2.** Precipitation, infiltration, and evapotranspiration for the field location, Fladerne Bæk, in the year 2006.

the soil surface were collected four times from September to January. Additionally, 22 plants (stems and leaves) were harvested from the potato field in September. These plants were left in a polyethylene tray outside, exposed to the sun, wind, and humidity, but not to rain and leaching, to simulate the natural decay. Five times during a period of 20 days, duplicates were sampled and frozen until further handling, and after the 20 days, the remaining 12 plants were collected as a composite sample. In addition, potato berries were collected in October, and potato tubers left after harvest and exposed to frost were collected in November. Soil samples were taken from the upper approximately 2−3 cm of soil using a steel garden trowel from the ridge and furrow topography covering the seed potatoes; all soil samples were similar in regard to soil properties. Sampling was performed using a transect of approximately 10 m perpendicular to the ridges. Five ridge and five furrow soil samples were analyzed from each sampling occasion from May to March. Groundwater was sampled from 2, 3, and 4 m below the surface in May and June and from 3 and 4 m until November, since the water level was below 2 m. The samples were taken from a well at the end of the transect having 1 m screens and using a peristaltic pump. Groundwater samples were stored in glass bottles. Plant, soil and water samples were frozen (−18 °C) within 24 h after sampling until later treatment.

**Plant Extraction and Preconcentration.** The plant material was freeze-dried and comminuted into pieces (<5 mm). Plant material (0.75−2.00 g) was extracted for 1 h with 30 mL of extraction solution (0.8 M acetic acid, 0.05 M $NaHSO_3$). After centrifugation (10400*g*) for 15 min, an aliquot of the supernatant was applied to a DSC-18, 500 mg, C-18 SPE column (Supelco, Bellefonte, PA, USA). The column was previously conditioned with 3 mL of acetonitrile, followed by 3 mL of clean extraction solution. After application of the extract, the

column was rinsed with 3 mL of 20% acetonitrile, after which the glycoalkaloids were eluted by 2× 0.500−2.00 mL 60% acetonitrile, 0.01 M phosphate buffer ($K_2HPO_4$ and $KH_2PO_4$, pH 7.6). Samples were analyzed by LC-UV. The relative standard deviation (RSD) for the extraction and cleanup procedure in total was determined to be 9−10% by extraction of triplicates.

**Soil Extraction and Preconcentration.** Soil samples were mixed thoroughly after any plant parts had been removed. Extraction was performed in glass tubes by shaking 5 g of wet soil with 10 mL of extraction solution (0.4 M acetic acid, 60% acetonitrile) for 2 h. After centrifugation (1000*g*) for 10 min, the supernatant was diluted with 70 mL of 5 mM HCl before application to the C18 SPE column. The dilution was performed to ensure retention of the glycoalkaloids to the column. The column was previously conditioned by 3 mL of acetonitrile, followed by 3 mL of 5 mM HCl. After application of the diluted extract, the column was rinsed with 3 mL of 20% acetonitrile, and the glycoalkaloids were eluted by 2× 400 μL 60% acetonitrile, 0.01 M phosphate buffer. Samples were analyzed by LC-UV. The method was previously optimized. For triplicate spiked soil samples at an environmental relevant concentration level (1.0 mg α-solanine/kg and 0.4 mg α-chaconine/kg), recoveries of 83 ± 13% and 89 ± 24% were obtained for α-solanine and α-chaconine, respectively.

**Water Analysis.** The water samples were filtered through a 0.20 μm RC-filter (La-Pha-Pack, Langenwehe, Germany) using a glass syringe before analysis. Previous tests showed no sorption to the filter or the glass syringe. Samples were analyzed by liquid chromatography−mass spectrometry (LC-MS).

**LC-UV Analysis.** LC-UV was used for separation and detection of the glycoalkaloids in the plant and soil extracts. Separation was performed using a Merck Hitachi L-4200 LC (Tokyo, Japan), and detection was performed using UV detection at 202 nm. The compounds were separated using a 250 × 4 mm i.d., 5 μm, Purospher RP-18E column equipped with a similar guard column (Agilent, Santa Clara, CA, USA). Isocratic elution was done by using an eluent of 55% acetonitrile in 0.01 M phosphate buffer ($K_2HPO_4$ and $KH_2PO_4$, pH 7.6) and a flow rate of 1.0 mL/min. The column temperature was kept at 40 °C, and the injection volume varied from 5−80 μL. Retention times of α-solanine and α-chaconine were approximately 9.0 and 11.3 min, respectively. Quantification of α-solanine and α-chaconine were based on external standards, while β-chaconine was quantified as α-solanine as no standard was available. For the determination of the detection limit (LOD), the standard deviation (SD) was estimated by five injections of 58.4 ng of α-solanine and calculated as $LOD = t_{(0.995,\ n = 4)} \times SD = 4.60 \times SD$. LOD was determined to be 12.3 ng, which corresponds to 0.15 mg/L for an 80 μL injection. For the soil extraction, this corresponds to a $LOD_{soil} = 35$ μg/kg DW.

**LC-MS Analysis.** Selected plant and soil samples were analyzed by LC-MS in order to identify unknown peaks originating in the UV chromatogram. Water samples were analyzed by LC-MS to obtain a lower limit of detection. For all LC-MS analysis, separation was performed using a model 2690/2795 LC system (Waters, Milford, MA, USA) and the LC method of Jensen et al. (*27*). The LC system was equipped with a 100 mm × 2.1 mm i.d., 3.5 μm, C18 Xterra column (Waters), and the column temperature was 30 °C. A gradient was used, the two eluents being A (water/acetonitrile, 95:5, v/v, 3 mM ammonium acetate) and B (water/acetonitrile, 5:95, v/v, 3 mM ammonium acetate). Injection volume was 10 μL.

Two different MS instruments were used due to their different capabilities. Screening for unknown metabolites was performed using an LCT orthogonal acceleration time-of-flight (TOF) mass spectrometer equipped with a Z-spray electrospray source (Micromass, Manchester, UK) and the method described in Jensen et al. (*27*). MS data were recorded in the range *m*/*z* 100−1500. Determination of glycoalkaloids in groundwater samples was performed by selected ion monitoring (SIM) using a Quattro Ultima triple quadrupole mass spectrometer equipped with an electrospray ionization probe (Micromass). The following parameters were used: desolvation gas ($N_2$) flow, 800 L/h; cone gas ($N_2$) flow, 80 L/h; capillary voltage, 3 kV; sample cone voltage, 100 V; desolvation temperature and source temperature, 300 and 110 °C, respectively. The ion traces *m*/*z* 852.6 (α-chaconine), *m*/*z* 868.6 (α-solanine), and *m*/*z* 398.4 (solanidine) were recorded using positive ionization. For the determination of LOD, the SD values were estimated by nine injections of 0.53 μg/L (α-solanine), 0.52 μg/L (α-chaconine), and 0.12 μg/L (solanidine). Detection limits were calculated as $LOD = t_{(0.995,\ n = 8)} \times SD = 3.36 \times SD$ and determined to be 0.17 μg/L (α-solanine), 0.14 μg/L (α-chaconine), and 0.03 μg/L (solanidine).

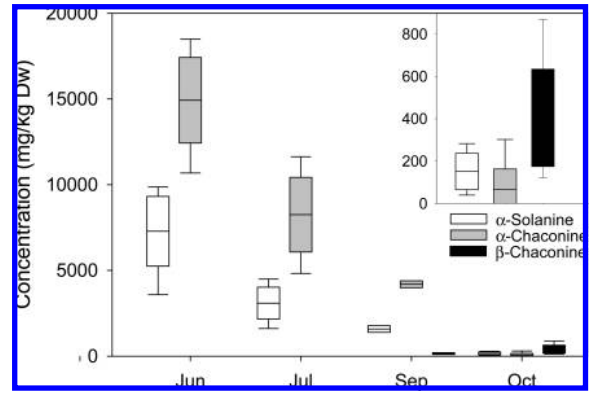


**Figure 3.** Concentration of glycoalkaloids in potato plants during the growth season. The box indicates the mean ± standard deviation, and the whiskers indicate the maximum and minimum concentrations measured. Enlargement of the October concentrations are shown as an insert.

**Table 2.** Glycoalkaloid Concentrations in Various Potato Plant Tissues

| | | α-solanine (mg/kg DW)[a] | α-chaconine (mg/kg DW)[a] | β-chaconine (mg/kg DW)[a] |
|---|---|---|---|---|
| debris | September | 453 (0.7%) | 362 (13%) | 681 (1.6%) |
| debris | October | 48 (28%) | 30 (21%) | 80 (49%) |
| debris | November | <1.1−1.5 | <0.4−0.8 | <1.1−1.5 |
| debris | January | <1.1−1.5 | <0.4−0.8 | <1.1−1.5 |
| berries | October | 1255 (1.7%) | 2640 (2.0%) | ND |
| left tubers | November | 549 (13%) | 783 (20%) | ND |

[a] Deviation in percentage is in parenthese ($n = 2$). DW = dry weight; ND = not determined.

**Data Analysis.** Data analysis was performed by SAS (version 9.1 Win, SAS Institute Inc., Cary, NC, USA). The decaying experiment was analyzed using simple regression analysis. The soil concentrations were analyzed using a one-way ANOVA, between-groups design and a Tukey−Kramer test.

## RESULTS AND DISCUSSION

**Plant Content.** The concentration of glycoalkaloids in the potato plant material decreased during the growth season (**Figure 3**), in spite of the natural variation observed between the individual plant samples. In June, when the plants were 10−25 cm high, a total glycoalkaloid content of 22.2 g/kg dry weight (DW) was detected, while in July (40−65 cm high plants), the concentration was somewhat lower (11.3 g/kg DW). During senescence in September and October, the total concentrations decreased further to 5.8 and 0.2 g/kg DW, respectively. Glycoalkaloids were also detectable in the plant debris in September and October; the concentrations were 2−12 times lower than that in the living plant material collected at the same time (**Figure 3** and **Table 2**). Debris sampled in November and January did not contain any measurable concentrations of glycoalkaloids. The results are in agreement with previous studies, where the highest concentrations were found in very actively growing tissues (*9*), including young leaves compared with old leaves. Similarly, a decrease in leaf concentration was observed during aging of the potato plant (*32*). The concentrations found in this study are in about the same order as leaf concentrations previously reported (*33*, *34*); minor variations

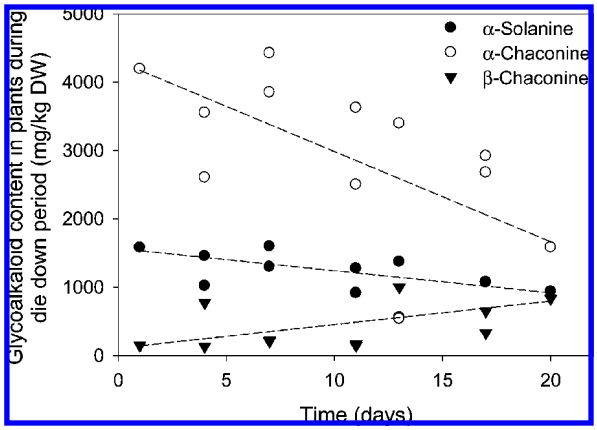


**Figure 4.** Glycoalkaloid concentration in the potato plants during the decaying experiment. Data were fitted using zero order kinetics (dashed lines).

may be explained by differences in age and varieties and by the fact that we analyzed leaves and stem combined.

From June to September, α-chaconine was the dominant glycoalkaloid in the plant material (leaves and stem), where the ratio of α-chaconine to α-solanine varied from 2.1−2.7. However, during autumn a relative decrease of α-chaconine was observed in this material, where the ratio in October was 0.4, and similar ratios were found in the debris (0.6−0.8). In other plant parts, the berries and the left potato tubers, α-chaconine ratios of 2.1 and 1.4 were found. Typical glycoalkaloid ratios (α-chaconine/α-solanine) from 0.7−2.8 have been published (*6*, *10*, *35*); ratios are reported to vary between tissues and varieties, but in almost all cases, α-chaconine is the dominant glycoalkaloid. The observed change in α-chaconine to α-solanine ratio during autumn indicates that either production or degradation of the two glycoalkaloids do not follow the same rate.

A closer look at the decay process showed that the concentration of both glycoalkaloids decreased significantly during the 20 days of decay (**Figure 4**). Data were fitted using simple zero order kinetics. The decrease of α-chaconine was significantly faster than for α-solanine, when the rates are compared, and half-lives were estimated to 16 ± 2 days and 24 ± 9 days, respectively. In these samples, a third peak appeared in the UV chromatograms ($R_t$ 22.6). By the use of LC-MS-TOF, the peak was determined to have *m*/*z* 706, which corresponds to a β-glycoalkaloid. By comparison of the obtained retention time on LC-MS-TOF to retention times for $\beta_2$-solanine and β-chaconine, the peak was identified as β-chaconine. The concentration of β-chaconine increased significantly during the 20 days of decay; a doubling time was estimated as 3 ± 2 days. β-Chaconine was detected in plant and debris samples from September and October as well (**Figure 3** and **Table 2**).

The decay study showed that both glycoalkaloids were degraded in the plant, as no other dissipation routes were likely since the plants were not exposed to rain. Because of the simultaneously appearance of the α-chaconine transformation product, β-chaconine, this indicates that α-chaconine was transformed to β-chaconine during plant senescence. A similar trend was reported in a previous study, where α-chaconine was converted to β-chaconine after prolonged storage of sprouted potatoes, while α-solanine was unaffected (*9*, *36*). Additionally, other reports of the presence of β-chaconine in tissues from potato roots or tubers exist (*10*, *37*). The transformation is possibly caused by enzymes in plant tissue, as enzymatic transformation of one or both glycoalkaloids in tubers, sprouts, and leaves have been observed previously (*22*−*24*). However, only a relativly small amount of β-chaconine was detected compared to the previous amount of α-chaconine, which may indicate that the produced β-chaconine was rapidly further degraded.

**Soil Content.** Glycoalkaloids were detected in many of the soil samples, but some of the concentrations measured were close to the detection limit ($LOD_{soil}$). The identities of the small peaks observed by LC-UV were confirmed by further analysis by LC-MS for some samples. LC-MS enables a better identification by having a lower detection limit and a higher specificity due to determination by *m*/*z* values. The samples analyzed by LC-MS did all contain both glycoalkaloids, even when no peaks were observed by LC-UV. In order to be able to handle the samples statistically, a concentration level had to be determined for samples where no quantification was possible by LC-UV. It was not reasonable to assign a concentration of 0 μg/kg DW as the LC-MS analysis showed the glycoalkaloids to be present. $LOD_{soil}$ was determined for α-solanine, and as the standard curves for α-solanine and α-chaconine were similar, the same $LOD_{soil}$ was assigned to α-chaconine. For samples below the detection limit, concentrations were set to $1/2 \times LOD_{soil} = 18$ μg/kg DW. This approach affected 19 and 32 of a total of 79 samples for α-solanine and α-chaconine, respectively.

Glycoalkaloids were detectable in soil in concentrations up to 2780 μg/kg DW, and when the concentrations of α-solanine and α-chaconine are summed up, the concentrations varied a lot within each sampling occasion (**Figure 5**). Comparing the concentrations in the samples from the ridge and the furrow topography covering the seed potatoes, no significant difference was observed; therefore, no differentiation between ridge and furrow samples was done in the following. In May−July, only some samples contained detectable amounts of α-solanine, while all but one sample in the period September−January contained α-solanine. All measured α-solanine concentrations were low in May and June (<150 μg/kg DW), while more variable concentrations were found in July (up to 907 μg/kg DW). The α-solanine concentrations found in September were significantly higher than that in all other months (450−1240 μg/kg DW), and the concentrations found in October and November were significantly higher than the concentrations observed in May and June. In January and March, the α-solanine concentrations leveled off. A similar pattern was observed for α-chaconine, where no α-chaconine or low concentrations were found in May and June (<170 μg/kg DW). In July, up to 700 μg/kg DW was detected, but the concentrations varied widely. α-Chaconine concentrations in September were again significantly higher than in all other months (480−1720 μg/kg DW). Thereafter a faster decline was observed, and the concentrations in October and November did again vary widely. No other compounds were identified from the soil, but as the extraction method was only optimized for the α-compounds, other more hydrophobic compounds may not have been extracted from the soil or released from the column during SPE. Overall, the concentration of α-chaconine in the soil appears to decline faster than α-solanine during autumn and winter, e.g., only four of the 30 samples from the period November to March did not contain measurable α-solanine, while no α-chaconine was detected in 12 of the samples. Hence, not only in the plant material but also in the soil, the degradation of α-chaconine is faster than that for α-solanine.

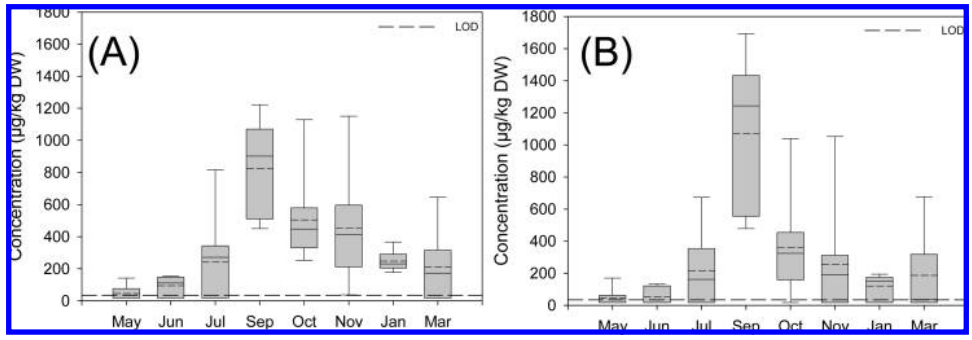


**Figure 5.** Concentrations of the glycoalkaloids in the soil during the season. Detection limits (LOD) are shown as long dashed lines. The boundaries of the box indicate the 25th and the 75th percentile, respectively. The whiskers indicate the 90th and 10th percentiles. In the box, the mean is presented by a solid line and the median by a short dash line. (**A**) α-Solanine. (**B**) α-Chaconine.

**Table 3.** Glycoalkaloid Content in Plant and Soil during the Season

| | α-solanine | | | α-chaconine | | |
|---|---|---|---|---|---|---|
| | plant (g/ha) | left tubers (g/ha) | soil (g/ha) | plant (g/ha) | left tubers (g/ha) | soil (g/ha) |
| May | | | 15 | | | 15 |
| June | 1080 | | 30 | 2030 | | 20 |
| July | 6890 | | 80 | 18310 | | 70 |
| September | 2020 | | 270 | 5360 | | 350 |
| October | 390 | | 170 | 240 | | 120 |
| November | | 90−130 | 150 | | 120−190 | 80 |
| January | | | 80 | | | 40 |
| March | | | 70 | | | 60 |

**Water Content.** All groundwater samples from the six samplings from May to November were analyzed, and neither α-solanine, α-chaconine nor solanidine were detected in any of the samples.

**Glycoalkaloid Distribution.** To obtain an overview of the amount of glycoalkaloids in the plant material in comparison to the soil, estimates of the amount of glycoalkaloids per hectare were calculated (**Table 3**). Field observations of approximately three potato plants per m and intervals of 0.7 m in between rows have been used for the estimation of the plant glycoalkaloid amount. Soil estimates are based on a soil density of 1.3 g/cm$^3$ and a soil depth of 2.5 cm, as the soil samples were collected from the upper 2−3 cm of soil. The obtained figures are rough estimates due to a high degree of uncertainty in up-scaling. The main observation in **Table 3** is that only a few percent of the glycoalkaloids produced in the plants were found in the upper soil, e.g., the highest amount in soil (September: 0.6 kg glycoalkaloids/ha) equals only 2% of the maximum present in the plants (July: 25 kg glycoalkaloids/ha). The highest amount of glycoalkaloids was found in the plants in July, while the highest amount in soil was detected in September. This discrepancy between the maximum amounts detected in the plant and soil shows that the transfer of glycoalkaloids to the soil was not only a function of the amount of glycoalkaloids in the plants. It probably reflects that the glycoalkaloids were mostly transferred to the soil during the beginning senescence of the plants. At that time, the content in the plant was still relatively high, and disintegration of the leaves and debris formation had started, which may have facilitated the leaching of the glycoalkaloids from the plant material during rain events.

The decrease of glycoalkaloids in the plants was fast during autumn, and only 3% of the amount found in July was still present in October. In comparison, the decrease observed in the soil during autumn was much slower and about 20% of the amount found in September was still detectable in January and March. As the plant content of glycoalkaloids was reduced to zero during autumn, further transfer of glycoalkaloids from the plants to the soil during late autumn and winter does not seem likely. The glycoalkaloids detected in the soil during winter must then have been present in the soil since autumn, indicating a slow dissipation of glycoalkaloids in the soil during winter time. This is consistent with a previous experiment in the laboratory, where only a little dissipation was observed within 36 days of incubation at 5 °C (*26*). No glycoalkaloids were detected in the groundwater in spite of the constant presence of glycoalkaloids in the upper soil during the winter, when no more glycoalkaloids were produced. Possibly, the glycoalkaloids remained sorbed to the soil organic matter in the upper soil because of their relatively high $K_{oc}$ values (**Figure 1**).

Another proposed source for the glycoalkaloids in soil was tubers left after harvest. The concentration in the frost exposed tubers were determined to be 1330 mg/kg DW (390 mg/kg FW) (**Table 2**), which is about twice the upper limit of 200 mg/kg FW for potatoes used for consumption in current guidelines (*9*). The higher concentrations were probably caused by glycoalkaloid increasing conditions in the field after harvest, as they were exposed to sunlight, mechanical damage, or attacks by pests and animals, factors which are all known to stimulate the production of glycoalkaloids. The possible glycoalkaloid mass load from these tubers was about 210−320 g/ha, the calculations based on the number of tubers collected within 5 m$^2$. The observed soil content of glycoalkaloids were two to three times higher and detected in September, before harvest and frost, and therefore, the remaining tubers could not be the primary source of glycoalkaloids in the soil. These tubers may have contributed to the glycoalkaloids content in November because the soil content was about the same order, but probably the main part of the glycoalkaloids in the soil originated from the plants.

Overall, the results indicate that the primary dissipation route for the plant glycoalkaloids is degradation within the plant, as only a very small percentage of the plant glycoalkaloids are detected later in the soil. The highest soil concentrations are found in September; therefore, the major transfer from the plants to the soil most likely appears during plant senescence in autumn, where leaching from the decomposing plant material is the proposed route for transfer to soil. The glycoalkaloids are present in the soil for months, indicating that the degradation is rather slow during the winter months. Nevertheless, no glycoalkaloids were detected in groundwater, which is why the leaching potential is evaluated to be small.

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