Research Article

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Detection of the herbicide asulam in groundwater: translocation to roots of treated docks (*Rumex*) and exudation to subsoil as a potential input pathway

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Abstract

BACKGROUND: The herbicide asulam is a weakly adsorbing and, thus, mobile substance. However, due to its rapid degradation, leaching to groundwater is not expected based on simulations with soil leaching models used to predict pesticide concentrations in groundwater as part of the authorization process.

RESULTS: Asulam was, nevertheless, frequently detected in samples from 112 groundwater monitoring sites in Switzerland, albeit at low concentrations up to 0.017 μg/L. A potential input pathway for the systemic herbicide includes the processes uptake by leaves of treated weeds, translocation to roots, exudation to subsoil, and leaching to groundwater. In a field trial, we first studied formation and decline of residues in leaves and roots of *Rumex alpinus*. Residues in leaves declined rapidly from ≈100 to ≈3 mg/kg within 3 weeks after treatment. Residues in roots reached a maximum of 6.5 mg/kg after 2 weeks and then declined to similar levels as in leaves. Maximum residues in roots corresponded to ≈2.6% of the applied amount, that is, translocation was rapid and significant. In a greenhouse experiment with *Rumex obtusifolius*, it was shown that asulam is also exuded from roots and found in soil leachate at concentrations of a few μg/L.

CONCLUSION: Transport of asulam to groundwater *via* root exudation may thus be a plausible pathway, in particular, if exudates from deep roots are released into subsoils, where degradation and sorption are minimal. This pathway is currently not considered in leaching models used in the authorization process.

Supporting information may be found in the online version of this article.

Keywords: weed control; plant metabolism of asulam; malonyl asulam; root exudation; groundwater monitoring; systemic herbicide

1 INTRODUCTION

A little-investigated input pathway for pesticides into groundwater involves the processes uptake by leaves of treated plants, translocation to roots, exudation to subsoil, and leaching of exuded substances from subsoil to groundwater. If roots extend into the subsoil, degradation and sorption in topsoil is virtually bypassed. In subsoils, degradation is typically slower and sorption lower than in topsoils due to lower organic carbon contents.[1,2] Consequently, leaching through subsoils is more significant than through topsoils. Such a shortcut pathway *via* plants is, in principle, possible for systemic pesticides, in particular systemic herbicides, for which root exudation has been demonstrated.[3–7] The importance of this pathway was investigated here using the herbicide asulam.

Asulam (Fig. 1) is used for the control of various annual and perennial grasses, broad-leaved weeds, and bracken in meadows, pastures, turf, spinach, sugarcane, non-cropland, ornamental shrubs, flower bulbs, and a number of tropical and subtropical crops. In the European Union, however, authorization was discontinued in 2011 and a new application recently failed due to suspected endocrine disrupting properties and high long-term risk to birds and mammals.[8] In Switzerland (not a EU member state), asulam is still authorized and applied in meadows, pastures, and orchards against docks (*Rumex*) and bracken, but it is also foreseen that authorization will be withdrawn.

In soils, asulam is a mobile substance with Freundlich adsorption coefficients of 15–67 mL/g (normalized for organic carbon, K_{FOC}; sorption is positively correlated with the organic carbon content of soils and negatively with pH, see supporting information).[8]

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[Correction added on 07 August 2025, after first online publication: The Copyright line has been updated]

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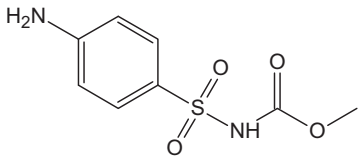
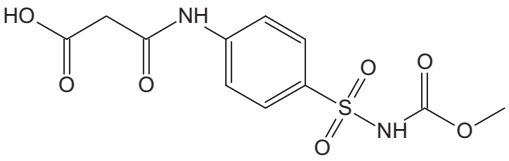


asulam



N-malonyl-asulam

Figure 1. Chemical structure of the herbicide asulam and its plant metabolite N-malonyl-asulam.

Nevertheless, asulam would normally not be expected to leach to groundwater as the compound is rapidly degraded in topsoils with half-lives of 2–11 days (laboratory studies).[8] Predicted environmental concentrations in groundwater (PEC_{gw}), estimated with leaching models using the above substance properties, are below 0.001 µg/L.[8]

However, asulam may be used for the control of *Rumex* (in particular *Rumex obtusifolius* and *Rumex alpinus*), whose roots can grow more than 2 m deep.[9] After uptake by leaves, the systemic herbicide can be translocated to other parts of the plant, including roots.[10–12] After uptake by roots, translocation seems to be less efficient.[12,13] In plants, asulam is metabolized to various conjugates, with N-malonyl-asulam as the predominant transformation product (Fig. 1).[8,12]

The following studies were thus conducted with asulam to evaluate whether a shortcut *via* plants that bypasses the topsoil could be a relevant input pathway into groundwater. We first investigated the uptake of asulam in leaves of *Rumex* and its translocation to roots in a field trial on an alpine pasture. We then studied the root exudation of asulam in a greenhouse experiment. Finally, asulam was analyzed in groundwater samples from 112 monitoring sites in Switzerland.

2 MATERIALS AND METHODS

2.1 Chemicals

Asulam (purity, 96.7%) and desethyl-atrazine-D_6 (97.1%, labeled in the isopropyl-group; used as internal standard for groundwater samples) were purchased from LGC (Augsburg, Germany), asulam-D_3 (98.7%, labeled in methoxy-group; internal standard for soil leachate) from A2S Analytical Standard Solutions (Saint Jean d'Illac, France), N-malonyl-asulam monohydrate (97.6%) from EPP Environmental Centre (Midlothian, UK), the herbicide formulation Asulox (a water soluble concentrate containing 400 g/L of asulam) from Syngenta (Stein, Switzerland), and the adjuvant Break-Thru (containing 765 g/L polyether-modified trisiloxane) from Omya (Oftringen, Switzerland).

2.2 Field trial with *Rumex alpinus*

In order to study the uptake and translocation of asulam to roots under field conditions, we sought out a pasture with a massive *Rumex* infestation, which in Switzerland is primarily found on alpine pastures with *Rumex alpinus*. A suitable pasture was found at Alp Aberen, located at 1090 m altitude in the upper Wägital, Switzerland (47°03′21″N, 8°54′17″ E), with *Rumex alpinus* as the predominant species (Supporitng Information, Fig. S2). At the time of the herbicide application, the various plant species had a leaf surface area of ≈2.3 m^2 per m^2 soil (determined using a LI-3100 C area meter, LI-COR, Lincoln, USA) and a height of ≈10–30 cm.

No herbicide applications had been performed on the experimental plot in the preceding 3 years. Information on herbicide use more than 3 years prior to this field trial is not available. On September 1, 2023, an area of ≈200 m^2 was treated with the herbicide Asulox, using a backpack sprayer (Birchmeier, REC 15, Stetten, Switzerland) with a boom width of 1.2 m and four nozzles (XR TeeJet 11003VS flat fan nozzles, Wheaton, IL). The actual application rate was ≈10% higher (3520 g asulam/ha) than the target application rate (3200 g/ha) due to a slightly higher spray volume (330 L/ha instead of 300 L/ha). For optimal wetting, the adjuvant Break-Thru was added to the spray solution at a concentration of 0.5‰.

To verify the application rate, 10 polystyrene petri dishes (diameter, 13.4 cm) were randomly placed in the field. They were attached to PVC-pipes at a height of ≈30 cm above ground. After 3 days of storage at 4 °C, asulam was extracted from the Petri dishes with a mixture of 3.75 mL of water and 12.5 mL of acidic acetonitrile (containing 1% formic acid). After further dilution with acidic acetonitrile, asulam was quantified by liquid chromatography – tandem mass spectrometry (LC–MS/MS, see section 2.9).

2.3 Leaf and root samples

Leaf samples were taken from *Rumex* plants shortly before (in the following denoted as '-0') and ≈0.1, 3, 6, 10, 14, 20, 27, 39, and 52 days after herbicide application (an example of a *Rumex* plant from day −0 is shown in Supporting Information, Fig. S3). For that, 640–1330 g of leaves were collected (days −0 to 27). On days 39 and 52, *Rumex* leaves already showed substantial herbicide damage and only 550 and 290 g of leaves were sampled, respectively. However, on these sampling days, also newly emerging leaves were collected (120 and 70 g, respectively). At all sampling times, additional leaves (≈75 g) were collected to determine the dry weight. All plant samples were packed into polyethylene Ziplock bags. On day 73, the pasture was snow-covered so that no leaves could be collected (only roots, see below). In the following spring, 243 days after application, only a few leaves had developed. No leaf samples were taken, only roots.

Root samples were dug out of the top 20 cm soil (940–2270 g) on days −0, ≈0.1, 3, 6, 10, 14, 20, 27, 39, 52, 73, and 243. Only large roots were sampled (thickness, > ≈ 0.5 cm) since smaller roots were quite fragile and difficult to handle.

The approximate leaf and root biomass of *Rumex* plants was determined on day −0 in an area of exactly 1 m^2. We aimed at collecting the plant material quantitatively, which was quite reliable for leaves (860 g/m^2), but more difficult for roots (≈1410 g/m^2) and, consequently, associated with a high uncertainty.

2.4 Homogenization of plant samples

Upon arrival at the lab, roots were washed with tap water to remove any soil and cut into slices of ≈0.5–1 cm. Roots and leaves were then stored separately in a −45 °C freezer until homogenization within 0–7 days, using a Retsch GM 300 knife mill (Haan, Germany; after addition of 50 mL liquid nitrogen, 500 rpm clockwise 2 × 5 s, 1500 rpm clockwise 3 × 7 s, 4000 rpm counterclockwise 2 × 20 s). A 200 g portion of the homogenate was

transferred to a HDPE container with PE lid and stored at −20 °C until extraction. Aliquots of 10 g were used for dry weight measurements of the homogenates on the day of extraction. For that, homogenate was dried during ≈8 h (80 °C and 50 mbar).

2.5 Extraction of asulam and N-malonyl-asulam

Asulam and N-malonyl-asulam were extracted from leaves and roots of *Rumex* according to a protocol developed for chicory plants and roots,[14] with minor modifications. In a 50 mL polypropylene Falcon tube, 3 g of plant homogenate were suspended in 30 mL water/acetonitrile/formic acid (80/20/0.2; in the original protocol, 10 g were extracted with 100 mL solvent). The suspension was then processed during 5 min with a stand-disperser at maximum speed (polytron PT 10–35, Kinematica, Kriens, Switzerland). After 30–60 min, the tube was centrifuged (4500 rcf, 10 min). For LC–MS/MS analysis, the supernatant was diluted with the extractant (dilution factor up to 1000, depending on the residue level).

2.6 LC–MS/MS analysis – plant extracts

Asulam and N-malonyl-asulam in plant extracts were analyzed by LC–MS/MS. The instrument was configured with an autosampler (PAL RTC, CTC Analytics, Zwingen, Switzerland, with sample trays cooled to 4 °C), two HPLC pumps with degasser (Exion LC, Sciex, Framingham, MA), and a triple quadrupole mass spectrometer (QTRAP 6500+, with turbo ion spray source, Sciex).

The analytes were separated on a Luna C_{18} column (150 × 2 mm, 5 µm particle size, Phenomenex, Torrance, CA, protected by a Gemini pre-column, C_{18}, 4 × 2 mm). LC conditions were as follows: injection volume, 3 µL; gradient elution with the solvents (A) water with 2 mM ammonium formate and 0.1% formic acid and (B) methanol with 0.1% formic acid (initial conditions, 5% B, linear increase to 100% B during 12, 6 min isocratic hold, initial conditions re-established within 1 min, followed by an equilibration time of 3 min); flow, 0.3 mL/min.

The MS was operated in positive mode (ion spray voltage, 4.5 kV, 300 °C) with the following ion transitions: asulam 231 → 156 (collision energy, 15 eV; 231 → 92, 31 eV for confirmation) and N-malonyl-asulam 317 → 242 (17 eV; 317 → 178, 29 eV). Quantification was based on 5-point standard additions to (diluted) extracts, to ensure that matrix effects did not adversely affect analytical results. A typical chromatogram of a root extract is depicted in Supporting Information, Fig. S1. For recovery, reproducibility, and storage stability data, we refer to the supporting information.

2.7 Greenhouse trials on root exudation and leaching of asulam

Root exudation experiments were performed with *Rumex obtusifolius*, since we expected that this *Rumex* species would be easier to cultivate in pots under greenhouse conditions. Furthermore, *Rumex obtusifolius* is more abundant in the groundwater monitoring study area than *Rumex alpinus* (chapter 2.8).

Small plants of *Rumex obtusifolius* (four-leaf stage, length of roots, ≈10 cm) were taken from a nearby field in Wädenswil, Switzerland, end of April 2024 and were transplanted each to 7-L-polypropylene pots (diameter, 21 cm; height, 28 cm) filled with a sandy loam soil (USDA texture classification, 16% clay, 27% silt, 57% sand, 1.7% organic carbon, pH 7.1 (1:2.5 H_2O)). The plants were cultivated in the greenhouse at 12–33 °C (mean, 17.6 °C) and a relative humidity of 26–97% (mean, 71%). Artificial light (high pressure sodium-vapor lamps, 600 W) was turned on during ≈4 h d^{-1}. In the weeks before herbicide application, plants were automatically watered with a mineral fertilizer solution (drip irrigation with Kristalon Red Calcium, 1.5‰). Plants were treated twice against aphids with the insecticide Movento SC (containing 100 g/L of spirotetramate, from Bayer, Zollikofen, Switzerland).

Two months after transplantation, four plants with 10–20 leaves and four plants at flowering stage were treated with asulam at an application rate corresponding to ≈1600 g/ha (two plants with 10–20 leaves were not treated and served as controls). For that, 2 mL of a solution of the herbicide Asulox (containing 2.7 g asulam/L) were applied to the leaves using an acrylic brush (series 600, Royal Talens, Apeldoorn, Netherlands; Supporting Information, Fig. S8). The soil was covered with aluminum foil to prevent any droplets of the application solution from getting directly onto the soil. After application, the pots were placed on polypropylene plant saucers. The pots were watered daily (except during the weekends) with rainwater applied to the soil surface (thus avoiding wash-off from the leaves). The volume (up to 800 mL per day, corresponding up to 24 mm/d) was selected to keep the soils moist with no or only minimal water leaching into the saucers.

Once a week, a sufficient amount of rainwater was used so that at least 40 mL (1.2 mm) of leachate (40–241 mL; typically, ≈100 mL, 3 mm) could be collected ≈1 h after watering. Leachate was withdrawn from the saucers with a polypropylene syringe, with 40 mL being transferred into a Falcon tube (the remaining leachate in the saucers was weighed and then discarded). Following a 10 min centrifugation, the leachate was filtered through a cellulose ester membrane syringe filter (Chromafil MV-45/25, Macherey-Nagel, Düren, Germany) and an 8-mL aliquot was stored at −20 °C until analysis within 32 days. Leachate samples were taken 4, 11, 18, 25, and 32 days after application and at the same sampling times from the control plants. During the experiments, greenhouse conditions were as follows: 13–33 °C (mean, 21.6 °C), 33–98% relative humidity (mean, 73%), ≈3 h d^{-1} artificial light.

In a preliminary test, recovery of asulam from the polypropylene saucers was determined. For that, asulam was spiked to blank soil leachate to obtain concentrations of 0.1 and 10 µg/L. Aliquots of 75 mL were then kept in saucers in the greenhouse during 1 h. Comparative analysis with respective samples kept in glass bottles yielded recoveries of 90–98%, thus confirming no significant loss of asulam due to sorption to the saucers.

2.8 Groundwater samples

Groundwater samples were received from the water protection authorities of the Cantons of Zurich and Bern, Switzerland. The sampling sites (Fig. 2) are part of the Swiss national groundwater monitoring program.[15] In the Canton of Zurich, two sampling campaigns (denoted as campaign 1 and 2) were carried out in May and November 2022, respectively. In both campaigns, groundwater from 53 wells and 14 springs was sampled. The sampling sites should reflect the main land use and included arable land (17 sites), meadows and pastures (18 sites), urban use (20 sites), forest (10 sites) und other uses (two sites). This (qualitative) information available to the water protection authority is based on the principal land use in the vicinity of the sampling sites.

Only for 18 monitoring sites, so-called contributing recharge areas were identified by the cantonal authorities (defined here as the areas from which ≈90% of the groundwater that reaches a well or spring originates). For these contributing recharge areas (<1 to 13 km^2, Fig. 2), GIS layers were available (accessible in the GIS of the Canton of Zurich,[16] note that some layers have not yet been published), which were used to intersect GIS layers for land use in 2023, that is, meadows, pastures, and orchards, where asulam is authorized (Supporting Information, Table S2).[17]

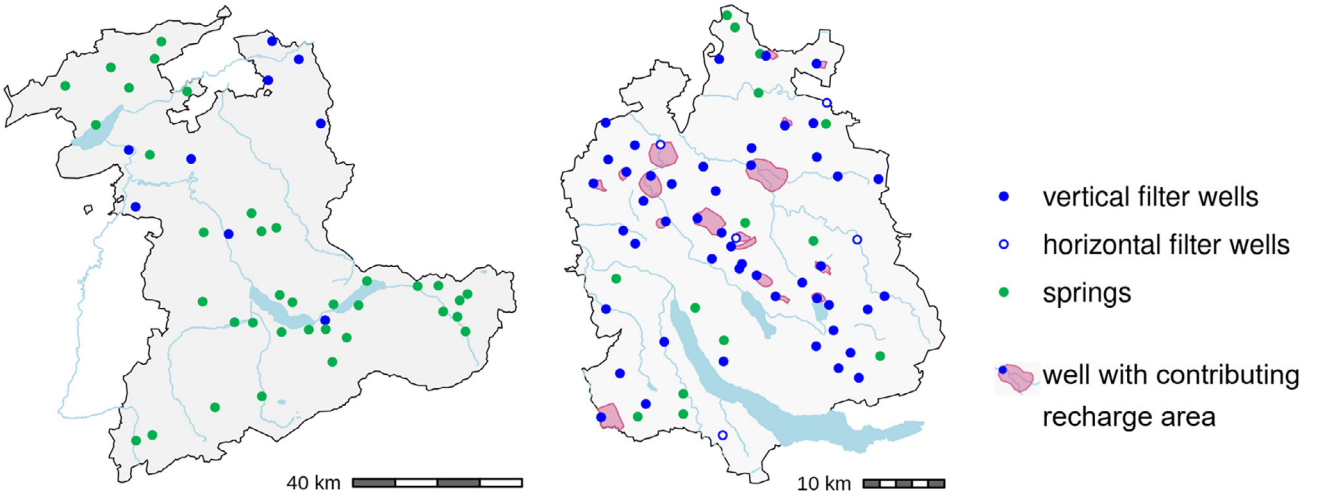


Figure 2. Groundwater monitoring sites in the Cantons of Bern (left) and Zurich (right) with contributing recharge areas for 18 wells in the Canton of Zurich.

The spatial analyses were performed with R[18] using the simple feature package sf.[19]

In the Canton of Bern, one sampling campaign (campaign 3) was carried out in August 2023 including nine wells and 36 springs. These sampling sites were targeted to cover areas with a high proportion of meadows and pastures. The main land use was therefore not necessarily representative for the entire Canton and included arable land (10 sites), meadows and pastures (18 sites), urban use (three sites), and forest (14 sites) (qualitative information according to the water protection authority).

Well screens at the sites with vertical filter wells typically start at a depth of 5 m (lower quartile) and end at 23 m (upper quartile), with extremes of 0.8 and 61 m, and screen lengths are typically 3–10 m.[20,21] After collection, groundwater samples were stored in glass bottles in a −20 °C freezer until analysis.

2.9 LC–MS/MS analysis – groundwater and soil leachate samples

Analysis of asulam in groundwater at trace level and in soil leachate was also performed with LC–MS/MS, with the following modifications of the above-described analytical method for plant extracts. Groundwater samples were fortified with the internal standard desethyl-atrazine-D_6 (addition of 50 µL of a methanolic solution with 100 µg/L to 10 mL of groundwater) and soil leachate with asulam-D_6 (addition of 40 µL of a methanolic solution with 20 µg/L to 8 mL of leachate). To increase sensitivity, we performed an online-enrichment.[22] First, sample volumes of 1 mL were injected into a metal loop. The valves were then switched so that the sample was transferred to an SPE cartridge (two stacked Gemini C_{18} pre-columns, 4 × 3 mm, 5 µm) during 90 s with 0.1% formic acid as mobile phase (flow rate, 1 mL/min). After transfer was complete, the valves were switched to allow elution of the enriched compounds to the analytical column.

LC conditions were as follows: gradient elution with the eluents 1 mM ammonium acetate (adjusted with acetic acid to pH 5.0) and methanol (initial conditions, 0% methanol, linear increase to 100% during 12 min, 6 min isocratic hold, initial conditions re-established within 1 min, followed by an equilibration time of 3 min); flow, 0.3 mL/min. MS conditions were the same as for plant extracts except that additionally, desethyl-atrazine-D_6 was monitored at 196 → 149, 24 eV or asulam-D_6 at 234 → 156, 15 eV.

Quantification was based on peak area ratios relative to the internal standard and in reference to 5-point standard additions to selected groundwater samples or 5-point calibrations in blank soil leachate. Limits of quantification (LOQ) were determined at a signal-to-noise ratio of ≈10 using the primary ion transition, limits of detection (LOD) at a signal-to-noise ratio of ≈3. A typical chromatogram of a groundwater sample is depicted in Supporting Information, Fig. S1.

3 RESULTS AND DISCUSSION

3.1 Field trial with *Rumex alpinus*

Uptake of asulam in *Rumex alpinus* and its translocation to roots was studied in a field trial. The measurements of asulam in the petri dish collectors indicated a correct application rate of 3625 g asulam/ha (103% of the application rate calculated based on the applied spray volume), but a rather high relative standard deviation of 52% (note that an even application was difficult on the bumpy alpine pasture).

After application in September 2023, the plants showed increasing symptoms of chlorosis (Supporting Information, Fig. S4). On day 20, green, yellow, but also some brown leaves were observed, but the mean water content did not yet change notably (80–87%). However, after day 20, pronounced necrosis was observed and the water content decreased to 39% and 46% on days 39 and 52, respectively (Supporting Information, Fig. S6). The newly emerging leaves showed some chlorosis, but a normal water content (88 and 86%, respectively). In the following spring, 243 days after application, asulam treated *Rumex* plants developed only a few leaves with pronounced growth deformations (Supporting Information, Fig. S5).

In leaves and roots collected shortly before application, residues of asulam and the plant metabolite N-malonyl-asulam were <0.02 mg/kg. In leaves collected ≈2 h after application, residues of 100 mg/kg asulam were determined, which is less than the theoretical maximum residue level of ≈400 mg/kg (assuming 100% interception of asulam by *Rumex* leaves, and considering an application rate of 352 mg/m^2 and a mass of *Rumex* leaves of 860 g/m^2, determined for the day −0 sample; residue levels are based on fresh weight, unless otherwise stated). However, *Rumex* was not the only plant species growing on the pasture, that is, asulam

was also intercepted by other plants (*e.g.*, *Petasites sp.*) or reached directly the soil surface (Supporting Information, Fig. S2). Furthermore, some asulam may already have been metabolized within the first hours, before the leaves were frozen. First rainfall was registered 11 days after application, that is, wash-off from leaves can be excluded[10] (precipitation and temperature data are shown in Supporting Information, Fig. S6).

Residues in leaves declined rapidly from 100 to 18 mg/kg on day 3 and to 3.3 mg/kg on day 20, followed by a slower decline to 2.9 mg/kg on day 52 (Fig. 3). The slower decline is partly explained by the decreasing water content of the dying leaves (residues on a dry weight basis steadily decreased from 553 mg/kg on day 0 to 4.7 mg/kg on day 52, Supporting Information, Fig. S7). On sampling days 37 and 52, also newly emerging leaves were collected. The detection of asulam in these leaves (2.5 and 2.4 mg/kg, respectively; and 21 and 18 mg/kg on a dry weight basis) confirms that the compound is translocated to growing points in the plant.[10,12]

Asulam was also rapidly translocated to the roots of *Rumex*, which is consistent with its properties as a systemic compound and previous findings with *Rumex* grown in pots under greenhouse conditions.[10] In the field trial, already ≈2 h after application, the compound was detectable in roots at a concentration of 0.27 mg/kg. Note that translocation of soluble compounds in the phloem is a fast process with velocities of 0.3–1.5 m/h.[23] Residues in the roots then further increased to 6.5 mg/kg on day 14, and thus reached a similar level as in the leaves (Fig. 3). Thereafter, residues slowly declined to 1.5 mg/kg on day 73 and 0.53 mg/kg in the following spring on day 243.

Residues of up to 6.5 mg/kg in roots correspond to a depot of ≈9 mg/m^2 soil (based on a root biomass of ≈1.4 kg/m^2), or 2.6% of the applied amount (352 mg/m^2). Translocation of asulam to roots is thus not only a rapid, but also a significant process. For comparison, in a previous study on uptake and translocation of asulam in *Rumex obtusifolius* under greenhouse conditions, ≈10% of the applied radioactivity was present in the roots after 9 days (but it is unclear, how much could be attributed to the active substance).[10]

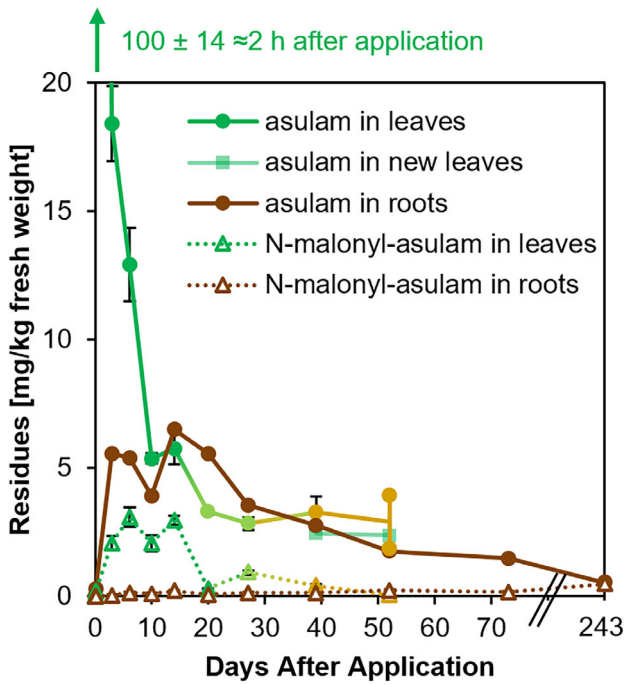


Figure 3. Residues of asulam and N-malonyl-asulam in leaves and roots of *Rumex alpinus* during the field trial on the alpine pasture. Error bars denote the standard deviation of triplicate extractions and analyses. Lines connecting points are only shown for better readability. The changing color for residues in leaves hints at their gradual discoloration.

Residues of N-malonyl-asulam in leaves of *Rumex* reached a maximum of 3.1 mg/kg on day 6 and then decreased to 0.05 mg/kg on day 52 (Fig. 3). Residues in roots were low, but slowly increased to a maximum of 0.47 mg/kg on day 243. For comparison, in an outdoor study conducted with spinach in Switzerland (early post-emergence application of 2400 g asulam/ha), residues of asulam declined even faster from 172 mg/kg on day 0 to 4.0 mg/kg on day 7,[12] and N-malonyl-asulam reached a higher maximum of 9.2 mg/kg on day 7. In leaves of *Rumex*, in contrast, residues of N-malonyl-asulam never exceeded those of asulam (Fig. 3). In *Rumex* (which is susceptible to asulam treatment), residues of asulam apparently remained sufficiently high for a significant impact on plant health. Tolerance of other weeds and crops such as spinach is due to faster metabolic inactivation.[12]

3.2 Transfer of asulam to soil leachate after application to leaves of *Rumex obtusifolius*

Following translocation of asulam to roots, the compound may, in principle, be exuded into the soil, or it may be released slowly after decomposition of the roots. Root exudation is expected to be the faster process that is also easier to study experimentally. For that, a greenhouse experiment was conducted with *Rumex obtusifolius* grown in pots filled with a sandy loam soil.

In leachate samples from untreated plants, asulam concentrations were ≤0.02 µg/L at all sampling times. In leachate from treated plants, asulam was already detected in the first samples taken on day 4 at concentrations of 0.3–7.3 µg/L (average concentration, 3.4 µg/L). Concentrations then tended to decrease to 0.4–2.5 µg/L (1.0 µg/L) by day 32 (Fig. 4). Concentrations displayed a high variability between the eight replicates for a number of possible reasons. On sampling days, we tried to water the plants in such a way that a similar amount of excess leachate water could be collected in the saucers, but with the sandy loam soil, this was difficult to achieve experimentally. Differences in the physiological state of the plants or root depths may also have contributed to the large differences in concentration observed in the soil leachate. Consequently, no difference was found between the two plant growth stages tested (see statistical analysis in supporting information). The plant metabolite N-malonyl-asulam was not found in soil leachate (<0.02 µg/L).

Asulam yields in soil leachate were calculated from the measured concentrations and the leachate volumes. The mean cumulative yield per plant increased continuously during the experiment to 1.2 µg by day 32 (Fig. 4), which represented only ≈0.2‰ of the applied amount per plant (5440 µg).

For many other systemic herbicides, a few percent of the dose applied to leaves were found in root exudates. For example, up to 16% of glyphosate applied to leaves of quinoa was exuded to the soil within 8 days.[5] In studies with a sterilized sand, up to 3.5% of triasulfuron and 0.9% of diclofop-methyl applied to leaves of ryegrass was exuded within 5 and 20 days, respectively.[4] In another study, 4% of nicosulfuron applied to leaves of Johnsongrass was exuded to a sterilized sandy soil within 30 days.[3]

The experimental design of the aforementioned and most other root exudation studies, however, differed from ours. Many studies were performed with ^{14}C-labeled compounds, but in some cases, only total radioactive residues were determined in the exudate or soil (e.g., ref. 24). It is thus not always clear, to what extent the

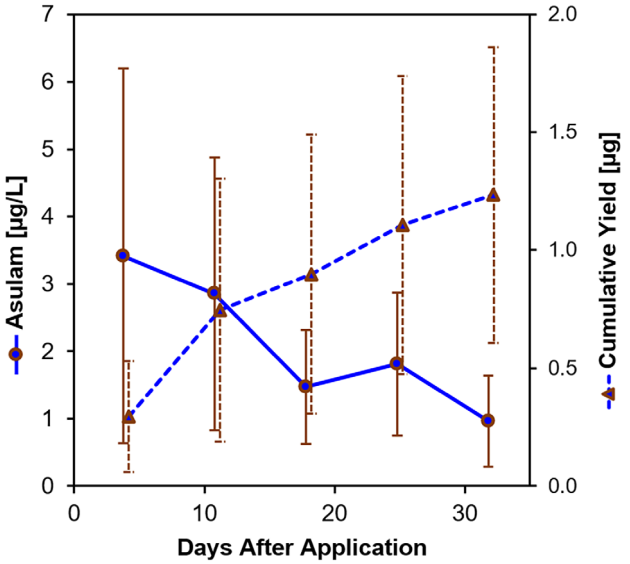


Figure 4. Concentration and cumulative yield of asulam in soil leachate after application to leaves of *Rumex obtusifolius* grown in pots under greenhouse conditions. Error bars denote the standard deviation of eight replicates.

parent compound contributed to these residues. Often, for the exudation experiment, plants were transferred to hydroponic systems.[6,13,25–29] Under such growth conditions, root exudation may differ from plants grown in natural soils.

In studies with solid substrates, root exudation was often determined by measuring residues in the substrate (e.g., refs. 3–5) and not, as in our experiments, in water leaching out of the substrate. The former design requires a proper separation of roots from the substrate in order not to overestimate root exudation. Finally, in many studies, inert media free of any organic matter were selected,[30] or when natural soils were used, they were sterilized.[3] The intention was to exclude possible degradation in soil after root exudation.

Our experiments were performed with a non-sterilized, agricultural soil to mimic natural growth conditions as well as possible. Therefore, in the excess leachate collected in the saucers, we determined a net yield of asulam, that is, the amount exuded by the roots into the soil minus the amount degraded in soil before it could leach to the saucers (or be taken up by the plants again[13]) and minus the amount stored in the soil. In agricultural soils, asulam was found to undergo fast degradation with DT_{50} values of 2–11 days (at 20 °C and field capacity).[8] Our study was carried out in a greenhouse in summer at rather high temperatures of 13–33 °C (mean, 21.6 °C), with regular watering of the soils, that is, optimum conditions for degradation. Furthermore, evapotranspiration was significant (91–96%). Upward transport of asulam in the pots with soil water most likely was a relevant process, except on the days of sampling, when excess water was applied so that leachate could be sampled from the saucers. Consequently, the residence time of asulam in soil and thus the time for degradation was rather high. This may at least partly be responsible for the low percentage of asulam found in the leachate (≈0.2‰) in comparison to other studies described above.

At an application rate of 3200 g/ha y^{-1}, this percentage in the leachate would correspond to an exudation rate of ≈0.7 g/ha y^{-1}. If we assume no further degradation in subsoils, minimal sorption, and a groundwater recharge rate of, for example, 350 L m^{-2} y^{-1},[31] this would result in a groundwater concentration of 0.2 µg/L. However, the concentrations actually expected in a monitoring well would be lower because asulam is not used every year and most likely not in the entire contributing recharge area.

3.3 Occurrence of asulam in groundwater

In the first two groundwater sampling campaigns, asulam was analyzed together with numerous other herbicides and metabolites which were included in the same MS/MS method, whereas the third campaign focused exclusively on asulam. Consequently, LOQs were different, ≈0.020 µg/L in the first, ≈0.007 µg/L in the second, and ≈0.003 µg/L in the third campaign (and LODs ≈0.006, ≈0.002, and ≈0.001 µg/L, respectively). Below, we report all concentrations above LOD, but it should be noted that values between LOD and LOQ are subject to higher errors. Replicate analysis of such samples on different days differed by 10–50% (in two samples with very low concentrations around LOD, the difference was even higher: 0.0007 *vs* 0.0012 µg/L and 0.0006 *vs* 0.0012 µg/L).

Asulam was found in groundwater samples at concentrations up to 0.017 µg/L and thus consistently below the Swiss parametric drinking water limit of 0.1 µg/L.[32] Fig. 5 shows a rank order plot of asulam concentrations detected during the three sampling campaigns. As a consequence of the differing LODs, the detection frequency differed in the three campaigns. Asulam was detectable in 9% (May) and 30% (November) of the monitoring sites in the Canton of Zurich, and in 36% of the monitoring sites in the Canton of Bern. We are not aware of any other groundwater monitoring studies, in Switzerland or elsewhere, with positive findings of asulam.

For 18 monitoring sites in the Canton of Zurich, we have more detailed information on the extent of their contributing recharge areas and the land use within (Fig. 2). With an increasing proportion of meadows, pastures, and orchards, higher concentrations in groundwater may, in principle, be expected. In fact, asulam

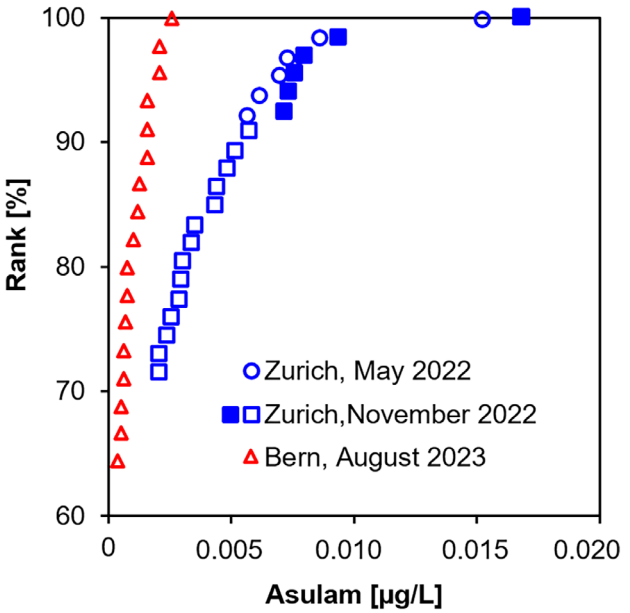


Figure 5. Rank order plot of asulam concentrations found in Swiss groundwater during three sampling campaigns. Full symbols denote concentrations above the limit of quantification (LOQ), empty symbols between the limit of detection (LOD) and LOQ. Note that LODs and LOQs differed in the three campaigns.

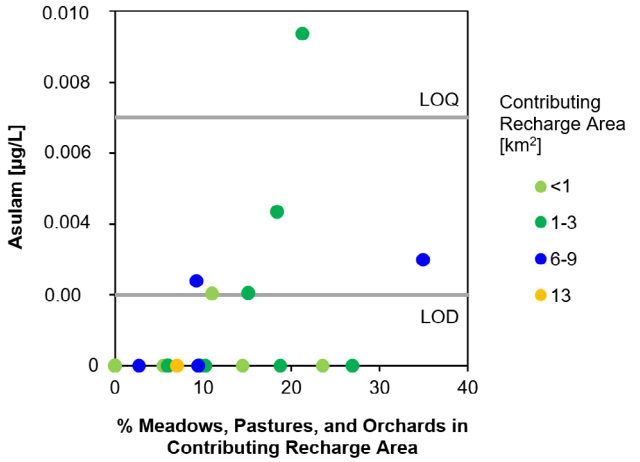


Figure 6. Asulam concentrations in those groundwater wells (second campaign, November 2022) where the contributing recharge areas and the land use are known. LOD is the limit of detection, LOQ the limit of quantification. Colors indicate the approximate size of the respective contributing recharge areas. Results below the LOD are depicted at the bottom of the plot.

was not detected in monitoring wells with less than 9% of the contributing recharge area occupied by meadows, pastures, and orchards. In wells with a higher proportion, asulam was partly detectable, but not in all wells (Fig. 6 and Supporting Information, Table S2). The data thus do not show a quantitative correlation between land use and concentration in groundwater (see statistical analysis in supporting information).

This finding may have different reasons. *Rumex obtusifolius* and *Rumex alpinus* prefer moist and nitrogen-rich soils. Differences in soil types and thus the occurrence of *Rumex* to be treated may partly explain, why asulam was found in certain wells, but not in others. Furthermore, the mean travel time for asulam to reach a well, and consequently the time available for degradation, is expected to differ depending on the local hydrogeological conditions. Finally, most contributing recharge areas are rather small (for 13 wells <3 km^2, for 5 wells 6–13 km^2; Fig. 6) and we do not have information on the actual use of asulam. Often, asulam is applied as single-plant treatment and farmers may, of course, use alternative herbicides against *Rumex*.

4 CONCLUSIONS

The field trial on the alpine pasture has shown that asulam is rapidly translocated from the aerial parts to the roots of *Rumex* plants, where it was found at concentrations of a few mg/kg, even weeks to months after application. In the greenhouse experiments, it could then be confirmed that asulam is also exuded from the roots and found in soil leachate at concentrations of a few µg/L. As a consequence of the optimum conditions in the greenhouse for degradation in soil after root exudation, the net yield eventually found in the saucers below the pots was rather low (≈0.2‰ of the applied amount).

Under real field conditions, root exudates may also be released into deeper soil layers, since roots of *Rumex* can reach more than 2 m into the subsoil.[9] Asulam translocated *via* this pathway may possibly exceed the amounts that may otherwise leach through the topsoil. Further inputs of asulam to subsoil may be expected after decomposition of roots. In subsoils, degradation of asulam is expected to be clearly slower than in topsoils and, due to the low organic carbon content, adsorption of asulam to soil and thus retention in the soil column is minimal. Consequently, further leaching to groundwater may be facilitated. Root exudation may thus be a plausible explanation for the detection of asulam in groundwater at low ng/L concentrations.

In pesticide fate models used for the assessment of leaching to groundwater in the context of authorization, root exudation into deeper soil layers currently is not taken into account, while the process of root uptake is. An adequate consideration of root exudation in the groundwater models, as stipulated, for example, in ref. 5, however, would require suitable test methods to quantify this process. The design of such studies is challenging and results from the laboratory or greenhouse are often difficult to transfer to natural conditions. Outdoor lysimeter studies may be a promising experimental approach.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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