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"Is there anybody else out there?" – First Insights from a Suspect Screening for Phytotoxins in Surface Water

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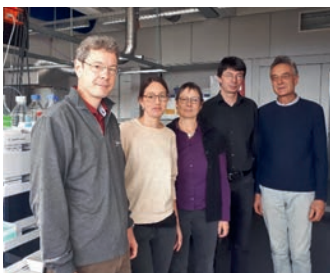
Abstract: To protect themselves, plants can produce toxic secondary metabolites (phytotoxins) that appear with widely varying structures and negative effects. These phytotoxins often show similar properties as known aquatic micropollutants in terms of mobility, persistence, toxicity, and possibly also ecotoxicity. However, their occurrence in surface waters remains largely unknown, which is also due to unknown ability of available screening approaches to detect them. Therefore, we performed a target and suspect screening based on a persistence-mobility prioritization for phytotoxins in small Swiss creeks using high resolution mass spectrometry. In total, three of 26 targets were detected, three of 78 suspects tentatively identified, and six suspects fully confirmed by reference standards. To the best of our knowledge, it is the first time that three different plant secondary metabolite classes are detected in the same surface water sample. Estrogenic isoflavones were detected at 73% of the sites with formononetin as main toxin, which is in agreement with previous studies. Furthermore, pyrrolizidine alkaloids and the indole alkaloid gramine were detected. Especially pyrrolizidine alkaloids might be critical due to their production by various plants including the invasive *Senecio inaequidens*, and their known importance in food and feed safety. Based on these first screening results, different phytotoxin classes should be assessed for their ecotoxicological effects and considered in future water monitoring.

Keywords: Environmental analytics · Isoflavones · Natural toxins · Pyrrolizidine alkaloids · Suspect screening



The team working on phytotoxins in the environment. Left to right: ***Thomas D. Bucheli*** (group leader Environmental Analytics Agroscope), ***Barbara F. Günthardt*** (Doctoral student), ***Juliane Hollender*** (Professor and group leader Environmental Chemistry Eawag), ***Martin Scheringer*** (Professor), and ***Konrad Hungerbühler*** (Professor emeritus); missing on the picture: ***Carina D. Schönsee*** (Doctoral student).

1. Introduction

Plants produce numerous different secondary metabolites (PSMs) structurally belonging to various classes, of which alkaloids and terpenes are the most important ones.[1] The concentration of individual PSMs ranges between just a few g kg^{-1} up to over 200 g kg^{-1} dry weight.[2] While many show beneficial effects, plants also produce toxic PSMs, named phytotoxins, with widely varying harmful impacts. In most cases, phytotoxins are produced by plants for protection against herbivores, pathogens, or other plant species.[1,3] Nevertheless, phytotoxins may also reach non-target organisms in terrestrial or possibly even aquatic environments. Indeed, the occurrence of phytotoxins in surface water has previously been demonstrated. For instance, ptaquiloside, a carcinogenic norsesquiterpene glucoside produced by bracken fern (*Pteridium aquilinum*), can leach into surface water and was even detected in groundwater with concentrations of up to 92 ng L^{-1}.[4,5] Clover (*Trifolium* spp.) produces estrogenic isoflavones that were regularly detected in Swiss rivers in the low ng L^{-1} range, but in some cases the concentrations were even above 200 ng L^{-1}.[6,7] However, environmental exposure or surface water monitoring studies have only been done for a few of the several thousand known phytotoxins.[8]

Recently, we published a freely accessible Toxic Plants–Phytotoxins (TPPT) database for Switzerland, accompanied by a preliminary aquatic risk assessment of phytotoxins.[9] All phytotoxins were defined following criteria for persistence, mobility and toxicity (PMT) on the basis of *in silico* predicted data using a method developed by Arp *et al.* for anthropogenic compounds.[10] Priority phytotoxins were required to i) show a half-life longer than 20 days, ii) have a soil organic carbon–water partition coefficient (log K_{oc}) or pH-dependent log K_{oc} (log D_{oc}) of ≤ 4.5 which indicates compound mobility, iii) be either acute rodent or acute aquatic toxic, and iv) be produced by plants with high abundance in Switzerland. The risk assessment showed that in total 516 phytotoxins have similar properties as anthropogenic compounds commonly found in aquatic monitoring studies, which is over 30% of all included phytotoxins. Therefore, many more potential phytotoxins of emerging concern might occur in the aqueous environment.

With the development of novel analytical techniques, especially high resolution mass spectrometry (HRMS), improved screening methods have become available. They enable robust and sensitive detections over a wide mass range (up to 2000 Da)

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with high mass resolution (≥20'000 ($mz/\Delta mz$)) and accuracy (<5 ppm).[11] Nowadays, the coupling of liquid chromatography (LC) with electrospray ionization (ESI) to HRMS is a major routine method in environmental trace analysis of pesticides, pharmaceuticals, household or industrial chemicals.[12–14] Often, quantitative analysis with reference standards (target screening) is complemented with suspect screening, where a qualitative assessment of a high number of expected compounds is possible without the need for reference standards. A major requirement of these methods is a functional group ionizable with ESI through the formation of adducts: primarily $[M+H]^+$ in positive mode and $[M-H]^-$ in negative mode. Since phytotoxins show structural features comparable to those of anthropogenic compounds, LC-ESI-HRMS methods should also be largely applicable to phytotoxins.

The goal of this study was to test whether well-established screening approaches and methods for anthropogenic micropollutants are also applicable to phytotoxins of different PSM classes and whether the PMT prioritization identifies candidates that actually occur in surface waters. Furthermore, we aimed at a first overview of phytotoxin occurrence in Swiss surface waters to evaluate if these compounds may be considered as 'natural micropollutants' reaching non-target organisms.[8] To this end, an initial sampling campaign was carried out during high plant abundance followed by a target and suspect screening using HRMS.

2. Methods

For a first phytotoxin screening, 11 surface water sites in the canton of Zurich were sampled (Table 1). The selected sites showed typical vegetation for the Swiss Plateau as defined by InfoFlora.[15] Small creeks were selected based on the plants growing in their catchment areas: each plant known to produce phytotoxins prioritized by Günthardt *et al.*,[9] had to be present in the catchment area of at least one sampling site. Additionally, we ensured that, first, the sites covered different land uses in the catchments, including arable land, grassland and forest, and, second, that the impact of urban areas was low (≤15%). For each site, one grab sample (1 L) was taken in June 2018 during high plant population and stored at –20 °C until analysis.

Sample preparation and analysis followed a method described in detail elsewhere.[16,17] It has been successfully applied in broad screenings for pesticides, pharmaceuticals or their transformation products. Briefly, water samples were enriched with offline solid-phase extraction (SPE) performed on manually prepared cartridges containing five different materials (Oasis HLB, Isolute ENV+, Strata-X-AW, Strata-X-CV, and EnviCarb). Such cartridges achieved good enrichment for a broad range of compounds.[17] Therefore, they were also expected to capture phytotoxins of different PSM classes. The extracts were separated by HPLC on an Atlantis T3 C18 column (3 µm, 3.0 mm i.d. × 150 mm, Waters), employing an acidic methanol–water gradient. The analysis was performed with ESI, positive and negative mode in two separate runs, on a Q-Exactive Orbitrap (Thermo Fisher Scientific (San Jose, USA)) measuring full scans (for m/z = 100–1200 at resolution R: 140'000) and data-dependent MS/MS.

Targets and suspects were chosen following the PMT assessment performed by Günthardt *et al.*[9] From the resulting 516 priority phytotoxins belonging to 12 PSM classes, 26 targets were selected. For each of these classes one to three targets were chosen taking into account the expected measurement suitability and commercial availability of standards. Thus, the PSM classes and phytotoxin targets in this study were as follows: steroids (strophanthidin), terpenoid alkaloids (taxol, aconitine), pyrrolizidine alkaloids (senecionine, echimidine, lycopsamine), steroidal alkaloids (solasodine), isoquinoline alkaloids (berberine, isocorydine, protopine), quinolizidine alkaloids (cytisine, huperzine A, sparteine (Sigma Aldrich, Buchs, Switzerland)), sesquiterpenes (artemisinin, parthenolide), polyketides (daidzein, 8-prenylnaringenin), indole alkaloids (vincamine (Sigma Aldrich), reserpine, gramine (Sigma Aldrich)), triterpenes (cucurbitacin E), diterpenes (10-deacetylbaccatin III, lathyrol), amaryllidaceaea alkaloids (lycorine (Sigma Aldrich), galanthamine), and saponins (diosgenin). If not stated, standards were purchased from PhytoLab (Vestenbergsgreuth, Germany).

Table 1. Description of the surface water sites that were grab-sampled in June 2018.

Site	Creek or closest larger creek[a]	Catchment size [km²][b]	Number of plant species producing priority phytotoxins in catchment[b]	Immediate vicinity of the sampling site	Catchment area[c]			
					Forest [%]	Agriculture [%]	Urban area [%]	Other [%]
I	Glatt[a]	4.26	69	Arable land	54	36	10	<1
II	Sihl[a]	1.96	83	Grassland	7	81	8	3
III	Chatzenbach	2.85	79	Arable land, grassland	13	70	15	3
IV	Teufenbach	1.80	84	Forest	19	74	4	4
V	Wildbach[a]	0.64	49	Forest	48	48	5	0
VI	Altbach[a]	0.76	51	Arable land	78	18	5	0
VII	Ruebisbach	2.93	51	Forest	29	57	14	<1
VIII	Töss[a]	<1	61	Forest	approx. 80	approx. 15	<5	0
IX	Aabach	5.34	87	Arable land, grassland	8	83	8	<1
X	Schlegeltobelbach	1.21	58	Forest	34	62	5	0
XI	Niederwiesenbach	16.0	45	Arable land	20	68	9	3

[a]Stands for the next larger creek in cases where the sampling site is a small tributary creek. [b]Source: Federal Office for the Environment (FOEN).[28] [c]Based on the Toxic Plants–Phytotoxins database.[9S]

Targets were verified by MS/MS fragments and quantified applying the XCalibur 4.1 (Thermo Scientific Corp.) software using an external calibration curve in tap water (0.25 ng L^{-1} to 500 ng L^{-1}) and the extracted ion chromatograms of the full scan (mass accuracy 5 ppm). Limit of detections (LoDs) laid between 0.1 ng L^{-1} and 4 ng L^{-1} for two thirds of the targets and only for three of them (cucurbitacin E, lathyrol, parthenolide) above 20 ng L^{-1}. Suspect peak lists were automatically generated with Compound Discoverer 2.1 (Thermo Scientific Corp., U.S.) including peak picking, retention time (RT) correction, grouping according to molecular weight and RT, background removal, exclusion below peak area threshold, and suspect annotation. The features were further reduced manually through expert peak evaluation, RT plausibility check and isotope pattern inspection as exemplified below.[11,18] For MS/MS fragment evaluation, priority was set on those suspects belonging to PSM classes for which targets were detected in this study, which resulted in 78 suspects of the total 516 priority phytotoxins. For newly detected suspect candidates, reference standards were purchased if available for final confirmation and quantification.

3. Results and Discussion

3.1 *Target and Suspect Confirmation*

Three of 26 target phytotoxins belonging to three different PSM classes were detected at least once in surface waters: the isoflavone daidzein, the indole alkaloid gramine and the pyrrolizidine alkaloid senecionine. In other words, over 10% of the targets occurred at least once. The highest concentrations were found for gramine with a maximum of 55 ng L^{-1} and an average concentration (of the detects) of 15 ng L^{-1}. Gramine was also detected at most sites, namely five out of 11. Daidzein was detected at four different sites in concentrations ranging from 1.4 ng L^{-1} to 5.5 ng L^{-1}. Senecionine was only detected at one site at a concentration of 12 ng L^{-1}.

Suspect candidates from the Compound Discoverer output list were manually reduced as briefly and exemplarily described in the following for the suspect seneciphylline. Using XCalibur, the exact mass trace showed a symmetric peak at a RT of 9.7 min with a mass accuracy below 5 ppm ($[C_{18}H_{23}NO_5+H]^+$). The isotope pattern further confirmed the molecular formula. The potential seneciphylline signal elutes before the target senecionine (ΔRT = –0.9), indicating a higher polarity of seneciphylline in reversed phase chromatography. EpiSuite estimated octanol–water partition coefficients (log K_{ow}) show that seneciphylline (log K_{ow} = –1.88) is more hydrophilic than senecionine (log K_{ow} = –0.39), which is structurally explained by the exchange of one of the methyl groups ($-CH_3$) in senecionine to a methylidene group ($=CH_2$) in seneciphylline. For structural identification, the MS/MS was inspected and the four most intense peaks evaluated manually: exact mass fragments *m/z* 306.1702 ($C_{17}H_{24}NO_4^+$ (–CO)), *m/z* 138.0915 ($C_8H_{12}ON^+$), *m/z* 120.0809 ($C_8H_{10}N^+$), and *m/z* 94.0653 ($C_6H_8N^+$). The three latter product ions were also found in the MS/MS of senecionine and were identified as common ions for all pyrrolizidine alkaloids. Further certainty was achieved through considering the toxin patterns of plant species that produce senecionine, for which seneciphylline was also a major toxin.[9] Finally, the standard was purchased and seneciphylline successfully confirmed.

In total, suspect screening in the described way led to the detection of nine further phytotoxins. We tentatively identified the three isoflavones genistein, formononetin and biochanin A and six pyrrolizidine alkaloids, *i.e.* senecionine N-oxide, seneciphylline, seneciphylline N-oxide, retrorsine, retrorsine N-oxide. Besides the target gramine, no other indole alkaloid suspect was found. The isoflavones are known to occur (discussed below), therefore we remained on tentative identification level 2a according to Schymanski *et al.*[19] For all pyrrolizidine alkaloids, standards were purchased to fully confirm the structures since these molecules have not been described in surface waters until very recently.[20] Fig. 1 shows all structures of identified phytotoxins and Table 2 gives additional analytical information as well as maximum and average concentrations detected in sampled creeks.

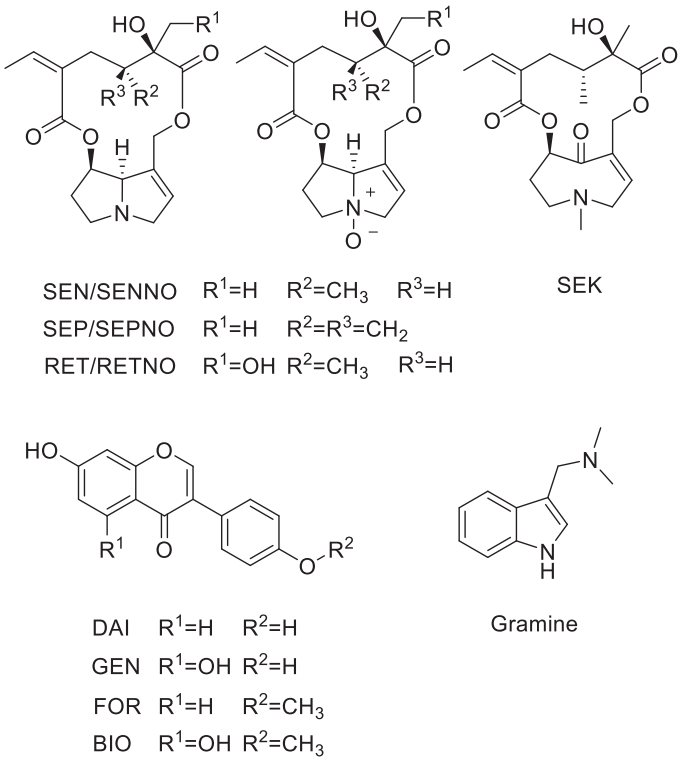

Fig. 1. Chemical structures of all targets and tentatively identified suspects: senecionine (SEN), senecionine N-oxide (SENNO), seneciphylline (SEP), seneciphylline N-oxide (SEPNO), retrorsine (RET), retrorsine N-oxide (RETNO), senkirkine (SEK), daidzein (DAI), genistein (GEN), formononetin (FOR), biochanin A (BIO), and gramine.

3.2 *Evaluation of the Screening Approach Using the Well-known Isoflavones*

Up to now, isoflavones are probably the best investigated phytotoxin class regarding their occurrence in Swiss surface waters.[6,7,21] As such, they can be used to evaluate the chosen screening approach including its analytical method and sampling strategy. Regarding the analytical method, the measured daidzein concentrations indicate that the chosen method is appropriate. The LoD of daidzein is 0.35 ng L^{-1}, which is very sensitive and below measured estrogenic effect concentrations.[7] Since formononetin, the main phytotoxin produced by *Trifolium spp.*, had even significantly bigger peak areas, the detection of isoflavones in the aquatic environment is well possible with the method presented here. Based on the detected daidzein concentrations and the method LoDs of all other phytotoxins (LoDs were between 0.1 ng L^{-1} and 4 ng L^{-1} for over 66% of targets), we also conclude that the chosen method is suitable for the screening of most phytotoxins in surface water. Only in the case of approximately 30% of the compounds, low concentrations (< 4 ng L^{-1}) might not be detected.

Isoflavones were detected at nine of the 11 sampled sites. Biochanin A shows a slightly increased occurrence compared with the quantified daidzein (Fig. 2a) in terms of average peak area (4.6×10^6 versus 2.3×10^6). While genistein has smaller peak areas in comparison with daidzein, formononetin always shows larger peak areas than daidzein. From this fact and due to the highly similar chemical structure, we can assume that the formononetin concentrations were higher than those of daidzein (between 1.4 ng L^{-1} and 5.5 ng L^{-1}). Additionally, formononetin was detected at a total of eight sites (*i.e.* in 73% of the samples) in contrast to daidzein

Table 2. Details of the detected phytotoxins in surface water samples (Table 1), including plant secondary metabolite (PSM) class, analytical information, identification confidence, and maximum as well as average detected concentrations.

Phytotoxin	PSM class	Retention time [min]	Precursor ion *m/z*[b]	Level of confidence[c]	Number of detects (total 11)	Maximum concentration [ng L^{-1}]	Average concentration (of detects) [ng L^{-1}]
Gramine[a]	Indole alkaloid	8.6	175.1230	1	5	55	15
Biochanin A	Isoflavone	18.6	285.0757	2a	4	not quantified	
Daidzein[a]	Isoflavone	15.8	255.0652	1	4	5.5	3.4
Formononetin	Isoflavone	17.7	269.0809	2a	8	not quantified	
Genistein	Isoflavone	17.5	271.0601	2a	4	not quantified	
Retrorsine	Pyrrolizidine alkaloid	9.3	352.1755	1	1	3.7	3.7
Retrorsine N-oxide	Pyrrolizidine alkaloid	9.5	368.1704	1	1	17	17
Senecionine[a]	Pyrrolizidine alkaloid	10.6	336.1806	1	1	12	12
Senecionine N-oxide	Pyrrolizidine alkaloid	10.8	352.1755	1	1	67	67
Seneciphylline	Pyrrolizidine alkaloid	9.7	334.1649	1	1	3.8	3.8
Seneciphylline N-oxide	Pyrrolizidine alkaloid	10	350.1598	1	1	22	22
Senkirkine	Pyrrolizidine alkaloid	10.7	366.1911	1	1	2.0	2.0

[a] targets; [b] used for quantification; [c] levels of confidence according to Schymanski *et al.*: 1 = confirmed structure, 2a = probable structure by library.[19]

that was only confirmed at four sites (*i.e.* in 36% of the samples). Therefore, formononetin was the dominating isoflavone within the sampled small creeks surrounded by natural vegetation. These results follow literature data, where Hoerger *et al.* also found formononetin to be the most prominent isoflavone detected in 90% of the samples with median concentrations ranging from detected to 9.0 ng L^{-1} and maximum concentrations of up to 217 ng L^{-1}.[7] Both in literature and in this study, genistein was also the least occurring and lowest concentrated isoflavone. Additionally, formononetin is the most stable of the four isoflavones, which could further explain its dominance.[7] Isoflavones are produced by clover, and especially red clover (*Trifolium pratense*) is a common pasture plant growing all over Switzerland and producing mainly formononetin and biochanin A. Hence, their regular occurrence in corresponding catchments is plausible. An exemplary sampling site is shown in Fig. 2b, where red clover can be seen between other plants growing in the close surroundings of the sampled creek.

3.3 *Relevance of Alkaloids*

To the best of our knowledge, it is the first time that phytotoxins from two different alkaloid classes, *i.e.* indole and pyrrolizidine alkaloids, were simultaneously detected in surface water. The indole alkaloid gramine is produced by several different plant species including certain grains and grasses (Graminae), which explains the broad occurrence of this phytotoxin. Moreover, at all five sites where gramine was detected, agricultural fields or grassland dominated the catchment area. Although gramine is toxic to insects, the concentrations were well below measured median le-

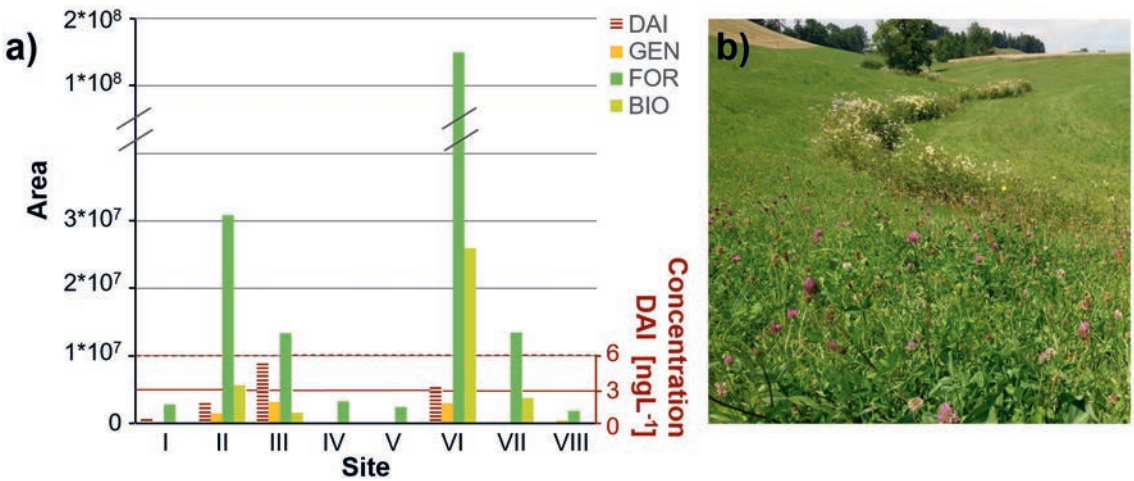

Fig. 2. a) Peak areas for the four isoflavones daidzein (DAI), genistein (GEN), formononetin (FOR), and biochanin A (BIO) as determined in surface waters (for site description, see Table 1). For the target daidzein concentrations are additionally given in red. b) Clover (*Trifolium* spp.) growing in the proximity of the sampled creek (Site II, foreground).

thal doses (LD_{50}) and thus should not show a pronounced effect.[22] In contrast to the wide occurrence of gramine, the detected pyrrolizidine alkaloids only occurred at one site, the Chatzenbach. However, with a sum concentration of 128 ng L^{-1}, the detected concentrations were more than twice as high as the maximum detected gramine concentration of 55 ng L^{-1} (Table 2). The pattern of all confirmed pyrrolizidine alkaloids in the Chatzenbach is shown in Fig. 3a. The N-oxides were present in significantly higher concentrations compared with the corresponding free bases, and senecionine N-oxide was the most dominant pyrrolizidine alkaloid followed by seneciphylline N-oxide and retrorsine N-oxide. Senecionine N-oxide was found to be the primary pyrrolizidine alkaloid produced in the roots of *Senecio* spp.,[23] which can explain its dominance. Due to different chemotypes, the toxin pattern varies strongly even within one species,[24,25] and therefore no single species can be allocated as primary source in this first study. Nevertheless, the most probable plants are of the genus *Senecio* spp., of which approximately 20 different species grow in Switzerland. According to the InfoFlora database, *S. aquaticus*, *S. errucifolius*, *S. inaequidens*, *S. jacobea*, *S. ovatus*, *S. paludosus*, *S. viscosus* and *S. vulgaris* can potentially be found in the sampling area.[15] Indeed, we were able to confirm the physical presence of *S. inaequidens* and *S. jacobea* by visual inspection (Fig. 3b). Whereas *S. jacobea* is a domestic plant growing along streets or edges of forests and meadows, *S. inaequidens* originates from South Africa. It is characterized as invasive in Switzerland and spreads along major traffic routes. Therefore, the canton of Zurich fights the spread of this plant, also in the sampling area.[26] Nevertheless, it remains unknown which *Senecio* spp. was the main source of the pyrrolizidine alkaloids found in this study and if the invasive species generally triggers increased levels in surface waters. In food and feed safety, pyrrolizidine alkaloids are considered as one of the PSM classes with the highest negative impact on livestock and human health.[27] Approximately 3% of all flowering plants produce them, resulting in contamination of teas, honey and to lesser extent animal products.[22-23] Therefore, occurrence in surface water could even increase their importance, particularly with regards to the safety of the environment and drinking water resources.

3.4 *Further PSM Classes Containing Priority Phytotoxins*

In this study, phytotoxins from different compound classes were detected in Swiss surface water. Not only do the results confirm the applicability of the chosen HRMS-based screening method, but they also confirm that the prioritization approach proposed by Günthardt *et al.*[9] provided reasonable candidates for subsequent suspect screening. In Fig. 4, the results of the prioritization approach are visualized by displaying the average Swiss plant frequency, the percentage of the Swiss land area in which a plant species is found as defined by Info Flora,[15] in connection with the different PSM classes and the phytotoxins average PM score. The PM score ranks organic compounds for being persistent and mobile based on the half-life of biodegradation and hydrolysis, and the *in silico* predicted pH-dependent soil-organic carbon water partition coefficient (D_{oc}).[9,10] The majority of phytotoxins detected in this study belong to PSM classes showing an average phytotoxin PM score higher than three and an average Swiss plant frequency over 40%. Thus, not only the intrinsic phytotoxin properties but also phytotoxin production indicated by plant abundance plays a major role in potential phytotoxin occurrence in surface waters. In the case of isoflavones (polyketides, Fig. 4/a), the average Swiss plant frequency exceeds 90% for all identified compounds, explaining why even genistein and daidzein can be detected although they are predicted to be rather unstable. In contrast, the major source of the indole alkaloid gramine (Fig. 4/b) is agricultural plants such as *Hordeum vulgare* or *Lupinus luteus* belonging to a category that is not accounted for in the average Swiss plant frequency.[9] Thus, gramine is found in catchments with substantial agricultural influence despite falling into a PM category that does not show high overall source-plant frequency. In such hotspots, characterized by a substantial toxic plant biomass in a restricted area, phytotoxins may frequently be detected. This is also shown for the pyrrolizidine alkaloids (Fig. 4/c) potentially originating from the invasive *Senecio* spp., or the well-described ptaquiloside (Fig. 4/d) from Bracken fern.[4,5] The general trend as seen in Fig. 4 suggests that particularly phytotoxins from the alkaloid and terpenoid PSM classes may be potential detects in other study areas in Switzerland as they show, on average, a higher PM score than phytotoxins from other PSM classes. When expanding the monitoring to regions outside of Switzerland, the overall prioritization needs to be performed again to account for the presence of other toxic plants and possibly other phytotoxins.

3.5 *Ecotoxicological Relevance and Future Research*

To assess the risk of phytotoxins, ecotoxicological effect data is needed. However, very few measured data are available and preliminary assessments were only provided by few individual

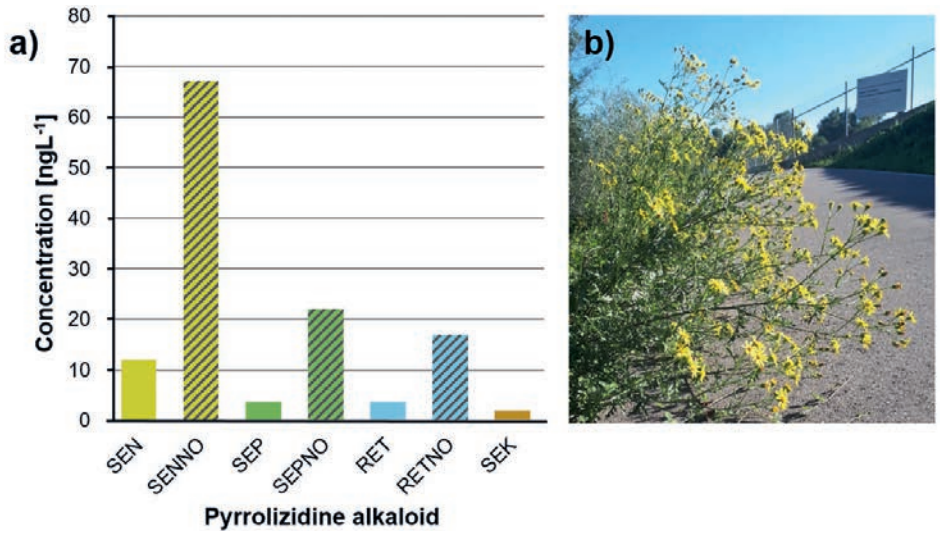

Fig. 3. a) Concentrations of the different pyrrolizidine alkaloids and their N-oxides detected in the Chatzenbach (site III): senecionine (SEN), senecionine N-oxide (SENNO), seneciphylline (SEP), seneciphylline N-oxide (SEPNO), retrorsine (RET), retrorsine N-oxide (RETNO) and senkirkine (SEK). b) *Senecio jacobea* in the proximity of the Chatzenbach (site III) as an exemplary plant producing pyrrolizidine alkaloids.

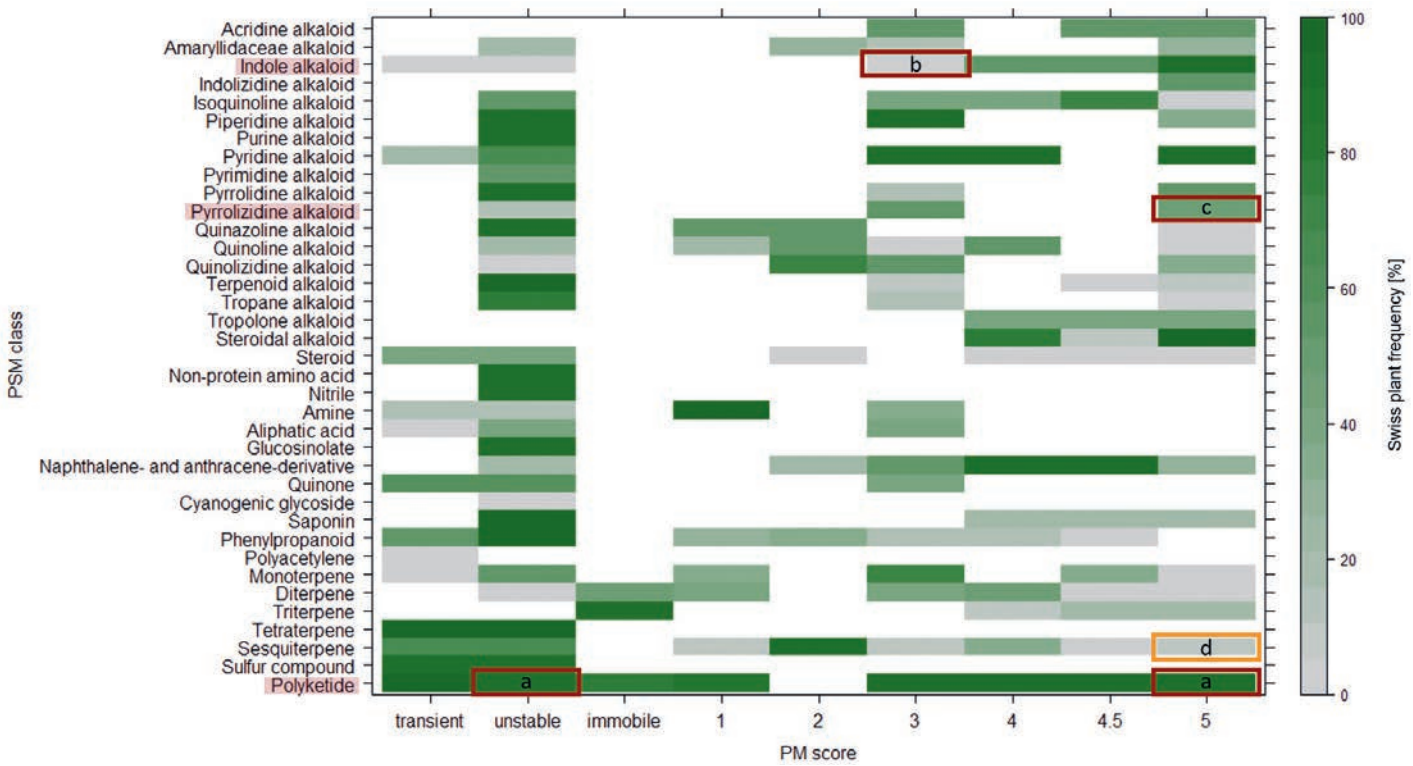

Fig. 4. Average Swiss plant frequency plotted according to the phytotoxins' respective PSM classes and predicted, average PM scores (persistence and mobility). Displayed data is taken from the Toxic Plants–Phytotoxins database by Günthardt *et al.* without any further amendment.[9] The Swiss plant frequency is the average percentage of the Swiss land area in which plant species producing phytotoxins of a certain PSM class are found as defined by Info Flora.[15] It gives a tendency on those PSM classes, of which phytotoxins are more likely to be produced in higher amounts. The higher the PM score, the more likely a compound can be found in the aqueous environment. Representatives of highlighted categories (a)–(c) were detected in this study, while category (d) includes the well-studied ptaquiloside from Bracken fern.

case studies for single compounds such as ptaquiloside, artemisinin, batatasin III, or the biopesticides rotenone and azadirachtin (for details, see Bucheli[8]). The regularly occurring isoflavones were shown to pose limited risk, primarily because their estrogenic activities are three to four orders of magnitude lower than those of 17β-estradiol.[7] The pyrrolizidine alkaloids' chronic effect concentrations in the aquatic environment as predicted by the U.S. EPA's ecological structure–activity relationships (ECOSAR) model[29] were in the low mg L^{-1} range, which was by far not reached in our studies. However, the occurrence of phytotoxins in general might still be of relevance in light of, *e.g.*, their potential mixture toxicity alone or in combination with anthropogenic compounds. In conclusion, based on both the prioritization results and the insights gained from the screening performed in this study, we suggest the following for future investigations on phytotoxins as 'natural micropollutants' in surface waters: 1) adapt the TPPT database and phytotoxin prioritization based on local plant abundance, 2) determine ecotoxicological effect data, and 3) include corresponding priority phytotoxins in the regular monitoring of surface waters to account for the exposure to potentially toxic natural products.

NaToxAq and PHYCROPOLL

Natural toxins, with the exception of cyanotoxins, are generally not yet regarded as environmental contaminants of concern for water quality. However, the occurrence of mycotoxins and phytotoxins in surface water bodies has indeed been demonstrated in studies of selected single compounds. The NaToxAq project (*http://natoxaq.eu*), in line with the Swiss project PHYCROPOLL, now aims to obtain a deep insight on natural toxins as environmental contaminants to ensure the production of clean drinking water. The focus is on origin, distribution and fate of natural toxins as emerging aquatic pollutants and potential remediation strategies.

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