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# Target screening of plant secondary metabolites in river waters by liquid chromatography coupled to high-resolution mass spectrometry (LC–HRMS)

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## Abstract

**Background:** Substantial efforts have been made to monitor potentially hazardous anthropogenic contaminants in surface waters while for plant secondary metabolites (PSMs) almost no data on occurrence in the water cycle are available. These metabolites enter river waters through various pathways such as leaching, surface run-off and rain sewers or input of litter from vegetation and might add to the biological activity of the chemical mixture. To reduce this data gap, we conducted a LC–HRMS target screening in river waters from two different catchments for 150 plant metabolites which were selected from a larger database considering their expected abundance in the vegetation, their potential mobility, persistence and toxicity in the water cycle and commercial availability of standards.

**Results:** The screening revealed the presence of 12 out of 150 possibly toxic PSMs including coumarins (bergapten, scopoletin, fraxidin, esculetin and psoralen), a flavonoid (formononetin) and alkaloids (lycorine and narciclasine). The compounds narciclasine and lycorine were detected at concentrations up to 3 µg/L while esculetin and fraxidin occurred at concentrations above 1 µg/L. Nine compounds occurred at concentrations above 0.1 µg/L, the Threshold for Toxicological Concern (TTC) for non-genotoxic and non-endocrine disrupting chemicals in drinking water.

**Conclusions:** Our study provides an overview of potentially biologically active PSMs in surface waters and recommends their consideration in monitoring and risk assessment of water resources. This is currently hampered by a lack of effect data including toxicity to aquatic organisms, endocrine disruption and genotoxicity and demands for involvement of these compounds in biotesting.

**Keywords:** Natural toxins, Bioactive compounds, Mixture toxicity, Surface water, Emerging contaminants

## Background

Plants produce a large variety of chemical compounds, which may be categorized as primary and secondary metabolites. Primary metabolites are necessary for growth and maintenance of cellular functions of the

plant while secondary metabolites play an important role, for example as defence (against herbivores, microbes, viruses or competing plants) and signal compounds to attract pollinating or seed dispersing animals [26, 36, 53]. Many PSMs can be seen as nature's own pesticides and have the potential to contribute to adverse effects of chemical mixtures in aquatic ecosystems together with anthropogenic chemicals [47]. Often, the production of PSMs is specific for taxonomic groups, species, genera or families. The amounts produced are typically lower than

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those of primary metabolites [36]. Secondary metabolites are diverse in their chemical nature. Most of them belong to four major classes of compounds, namely terpenoids, phenolic compounds, alkaloids and sulfur-containing compounds [13]. Due to their biological activity, PSMs have been used for drug development including anti-inflammatory, antioxidant and antiviral agents. However, many medicinally active PSMs also show toxic side effects [10, 11, 26, 39]. For instance, bergapten, a furanocoumarin, has shown antitumor effects in a variety of cell types, but is also a potential photosensitizer that can cause severe phytophotodermatitis after either skin contact or ingestion followed by sun UV exposure [9, 43, 51]. Some PSMs detected in the water cycle have been shown to cause severe impacts on human health such as aristolochic acids from *Aristolochia clematitis* causing Balkan endemic nephropathy [48] and the potent carcinogen ptaquiloside. The latter is produced by bracken fern and emitted into the water cycle particularly during rain events at toxicologically relevant concentrations [7, 35]. Recently, natural carboline alkaloids have been demonstrated to exhibit synergistic mutagenic effects with anthropogenic aromatic amines [31].

Plants produce toxic PSMs particularly under environmental stress and release these compounds to the environment through various means such as root exudates, volatilization and animal contact as part of their defence mechanism [2, 4, 10, 26]. Previous research demonstrated their pharmacological effect and toxicity by isolating them from plants [3, 42] and their contribution to mixture toxic risk in river water [5, 34]. In silico predictions suggest that many PSMs are persistent and mobile in the environment [14]. The authors identified priority phytotoxins characterized based on in silico-predicted values of half-life longer than 20 days, a log  $D_{OC}$  (organic carbon–water partition coefficient) below 4.5, rodent or aquatic toxicity and high abundance of the producing plant in Switzerland [14]. Assuming similar vegetation in Germany, these priority phytotoxins were used as a basis for target selection in the present study. PSMs may be transported to river water through leaching, rain sewers and surface run-off and might pose a risk not only to aquatic organisms, but also to human health in case of exposure, if the water is used for human consumption and recreational purposes. Recently, target and suspect screening of PSMs identified 12 compounds in Swiss small creeks from three compound classes including formononetin, an estrogenic isoflavone, the indole alkaloid gramine and several pyrrolizidine alkaloids [15]. Formononetin in concert with other isoflavones has been detected in Swiss and USA surface waters already earlier [16, 23]. Along with other organic matters the coumarins esculetin and umbelliferone were previously reported

in Suwannee River fulvic acid isolates, USA [41]. Thus, PSMs may add to the complex mixtures of anthropogenic organic micropollutants in water resources. Therefore, there are indications that some PSMs, which exhibit toxicity at environmental concentrations, may jeopardize water quality and affect aquatic ecosystems and human health in concert with anthropogenic compounds.

The objective of the present study was to perform a first river water target screening of PSM selected from a larger database for their expected mobility, persistence and toxicity, their expected abundance and their commercial availability as standards in two selected catchments with primarily natural vegetation and agricultural land use, respectively. We focused particularly on sampling during or after rain events in the vegetation season to enhance the probability of detection of PSMs leaching to the river water. Since hardly any quantitative toxicity data for PSMs is available, we compared water concentrations with TTC suggested for drinking water contaminants for which no toxicity data exist for a preliminary estimate of risks [29].

## Experimental section

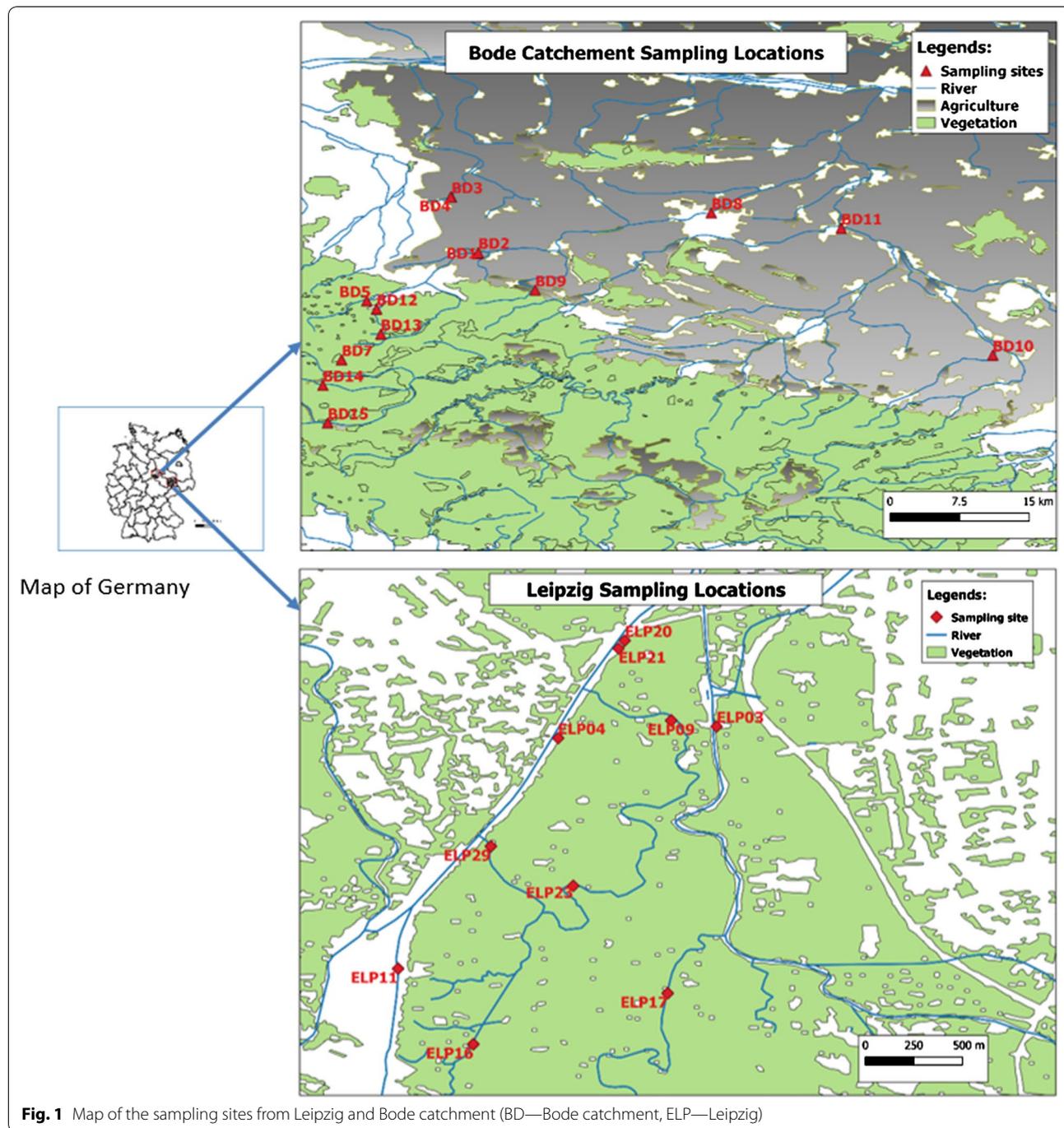
### Study site and water sampling

The investigated catchments are located in the north-west part of the federal state of Saxony (close to the city of Leipzig) and in Saxony-Anhalt (Bode catchment), Germany. Both catchments were selected due to their land coverage with natural and/or agricultural vegetation along the river banks. The 50 km<sup>2</sup> large floodplain forest in Leipzig reaches along the rivers Elster, Pleiße and Luppe (EPL catchment) together with some smaller tributaries. The natural old-growth deciduous forest was historically used for the extraction of coppice and clay. It is mainly characterized by *Quercus robur*, *Fraxinus excelsior*, *Acer pseudoplatanus*, *Ulmus minor*, *Alnus glutinosa*, *Tilia cordata*, *Carpinus betulus*, *Acer platanoides* and *Acer campestre*. In spring, the forest scrub is dominated by monocotyledonous and tuberous plants such as *Allium ursinum*, *Anemone nemorosa*, *Galanthus nivalis* and *Leucojum vernum* [22]. Thus, during their periodic growth and decay, they might leave their secondary metabolite footprints in the environmental components (e.g., soil, river). The Bode catchment is characterized by large diversity of natural and agricultural vegetation. Land use is dominated by forest (such as broad-leaved forest, coniferous forest and mixed forest) in the mountain areas and agriculture in the lowland areas. Main crops include winter wheat, triticale, winter barley, rye, rape, sugar beet and corn.

Grab water samples were collected in the vegetation growing season of the years 2018 and 2019 in spring and summer during and after rain events when leaching of

PSMs was expected. A total of 38 rain event river samples were collected from both locations—23 samples from 6 streams situated in Leipzig and 15 samples from 15 streams in the Bode catchment (Fig. 1). In the ELP catchment, samples were taken repeatedly from the same spot at different rain event days. These samples were complemented with 18 dry weather samples (8 and 10 samples from ELP and Bode catchments, respectively) from

different seasons for comparison (for more information on samples see Table S1 in supplementary information (Additional file 1)). Water samples were taken with pre-cleaned glass beaker (500 mL) and solids were allowed to settle for about 2 min before transferring to sampling bottle. Aliquots of 1 mL were transferred to 2-mL autosampler vials for the chemical analysis. To minimize the interferences, all sampling bottles and laboratory vessels



were washed and rinsed with ethyl acetate, acetone and methanol before use. Field trip and laboratory blanks were also included to control interferences during the sampling campaign and transportation. Samples were chilled with ice packs during transportation to the UFZ laboratory, and then stored at  $-24\text{ }^{\circ}\text{C}$  until analysis.

#### Target secondary metabolite selection

Due to limited information on PSM, *in silico* evaluations were performed to assess their likelihood to occur in water. Prioritization for target screening was built on previous work by Gunthardt et al., identifying plant toxins with a high probability to reach the aqueous environment due to mobility and persistence [14]. The selected metabolites represent structurally diverse natural compounds from plant species. Thus, taking these PSM as the basic population of candidate compounds, we produced a shortlist of 150 metabolites also considering commercial availability and the probability of occurrence due to the abundance of the plants identified as the origin of these metabolites (for more information see Additional file 1: Table S2). Furthermore, only metabolites containing one or more of the elements nitrogen, oxygen and sulfur, in addition to carbon and hydrogen were selected to allow for a likely ionization by an electrospray ion source [30].

#### Chemical analysis

Water samples containing suspended matter were filtered using a glass fiber filter (Whatman GF/A, diameter 47 mm). Samples were prepared for direct injection by adding 25  $\mu\text{L}$  of an internal standard mixture (40 ng/L), 25  $\mu\text{L}$  of methanol (LC–MS grade) and 10  $\mu\text{L}$  of ammonium formate buffer (2 M, pH = 3.5) to each 1-mL sample aliquot (see Additional file 1: Table S4 for more information on internal standards). For the chemical analysis, 100  $\mu\text{L}$  of the sample was injected into a Thermo Ultimate 3000 LC system (consisting of a ternary pump, autosampler and column oven) coupled to a quadrupole-orbitrap instrument (Thermo QExactive Plus) equipped with a heated electrospray ionization (ESI) source.

#### Liquid chromatography

LC separation was performed on a Kinetex C18 EVO column ( $50 \times 2.1\text{ mm}$ ,  $2.6\text{ }\mu\text{m}$  particle size) using a gradient elution with 0.1% of formic acid (eluent A) and methanol containing 0.1% of formic acid (eluent B) at a flow rate of 300  $\mu\text{L}/\text{min}$ . After 1 min elution with 5% B, the fraction of B was linearly increased to 100% within 12 min and 100% B were kept for 11 min. Subsequently, the column was rinsed with a mixture of isopropanol + acetone 50:50/eluent B/eluent A (85%/10%/5%) to remove hydrophobic matrix constituents from the column. Finally,

the column was re-equilibrated to initial conditions for 5.7 min. The column was operated at  $40\text{ }^{\circ}\text{C}$ .

#### Mass spectrometry

The heated ESI source and the transfer capillary were both operated at  $300\text{ }^{\circ}\text{C}$ , with a spray voltage of 3.8 kV (pos. mode), a sheath gas flow rate of 45 a.u. and an auxiliary gas flow rate of 1 a.u. The full-scan MS1 was recorded in an  $m/z$  range from 100 to 1500 with a nominal resolving power of 140,000 (referenced to  $m/z$  200). For metabolite confirmation, data-dependent MS/MS acquisition was performed at a resolving power of 70,000 in additional runs (see Additional file 2: Table S5 for more information on MS setting). The MS was calibrated externally every 2 days using the calibration mixtures of the vendor, the mass accuracy was always below 5 ppm for all analyses. All MS analyses were performed in ESI positive mode (ESIpos) since we expected a better ionizability of SPMs than in/with ESI negative mode.

#### Target screening

##### Qualitative target screening

The LC–HRMS raw data were converted to mzML format using ProteoWizard (version 2.1.0) [17]. The centroid data were subjected to MZmine (version 2.38) for peak detection followed by peak alignment and identification (target compound annotation) [20, 21, 38]. Settings for each step of the data processing are given in Additional file 2: Table S3. Further evaluation and visualization were performed using Excel 2013 (Microsoft office) and R (version 3.4.3).

Targets were identified by matching  $m/z$  and retention time between water samples and standard compounds with a mass and retention time tolerance of 5 ppm and  $\pm 0.1$  min, respectively. Prior to clearing of false positives from the annotated list, the cut-off intensity was set to  $10^4$  to exclude signals due to noise and background. For blank correction, seven blanks were analyzed together with the samples to remove noise and background contaminants. Duplicates resulting from multiple annotation were removed manually using peak resolution and intensity (for detailed steps on workflow see Additional file 2: Figure S1). For the tentatively identified target compounds, an inclusion list was developed for data-dependent acquisition (MS/MS). MS/MS experiments were conducted on authentic standard compounds and the samples to confirm the chemical structure. Diagnostic MS/MS fragments were matched with the MS/MS of reference standards. For the target compounds with low intensity in unresolved chromatograms, parallel reaction monitoring analysis was conducted for better chromatographic peaks visualization. The XCalibur v4.0.27.10 (Thermo Fisher Scientific) software was used

for analysis of extracted ion chromatograms (EICs) and mass spectra (MS1 and MS2).

#### Quantification of detected metabolites

TraceFinder 3.2 (ThermoFisher Scientific) was used for the quantification of the 12 confirmed target PSMs using extracted ion chromatograms of the full-scan data. In TraceFinder, the use of only one identifier mass (precursor ion) bares the risk of false-positive identification and quantification of contaminants. Thus, additional fragment ions were used to confirm the presence of target compounds and to eliminate errors in identification (see Additional file 2: Table S6). For some metabolites, ions used for confirmation were not clearly detectable due to low intensity. In such cases, confirmation was complemented using Xcalibur. A series of calibration standards ranging from 1 to 5000 ng/L were used. All the calibration standards were treated exactly the same way as river water samples. The target compounds were quantified using the internal standards with the nearest retention time. The method detection limit (MDLs) (Table 1) for the detected PSMs were determined following US-EPA procedure [49]. The calculated concentrations below the MDLs were excluded.

#### Risk estimates

Due to a lack of toxicity data for our target compounds, we based a tentative risk estimate on TTC for non-genotoxic and non-endocrine disrupting compounds of 0.1 µg/L. We defined the ratio between measured concentration of the compounds  $i$  ( $c_i$ ) and TTC as risk quotient (RQ), and calculated mixture RQs as the sum of

individual RQs (Eq. 1) assuming a mixture RQ below one as safe for exposed humans and aquatic organisms:

$$\left(\sum \text{RQ}\right) = \left(\sum c_i \text{TTC}\right) \quad (1)$$

## Results and discussion

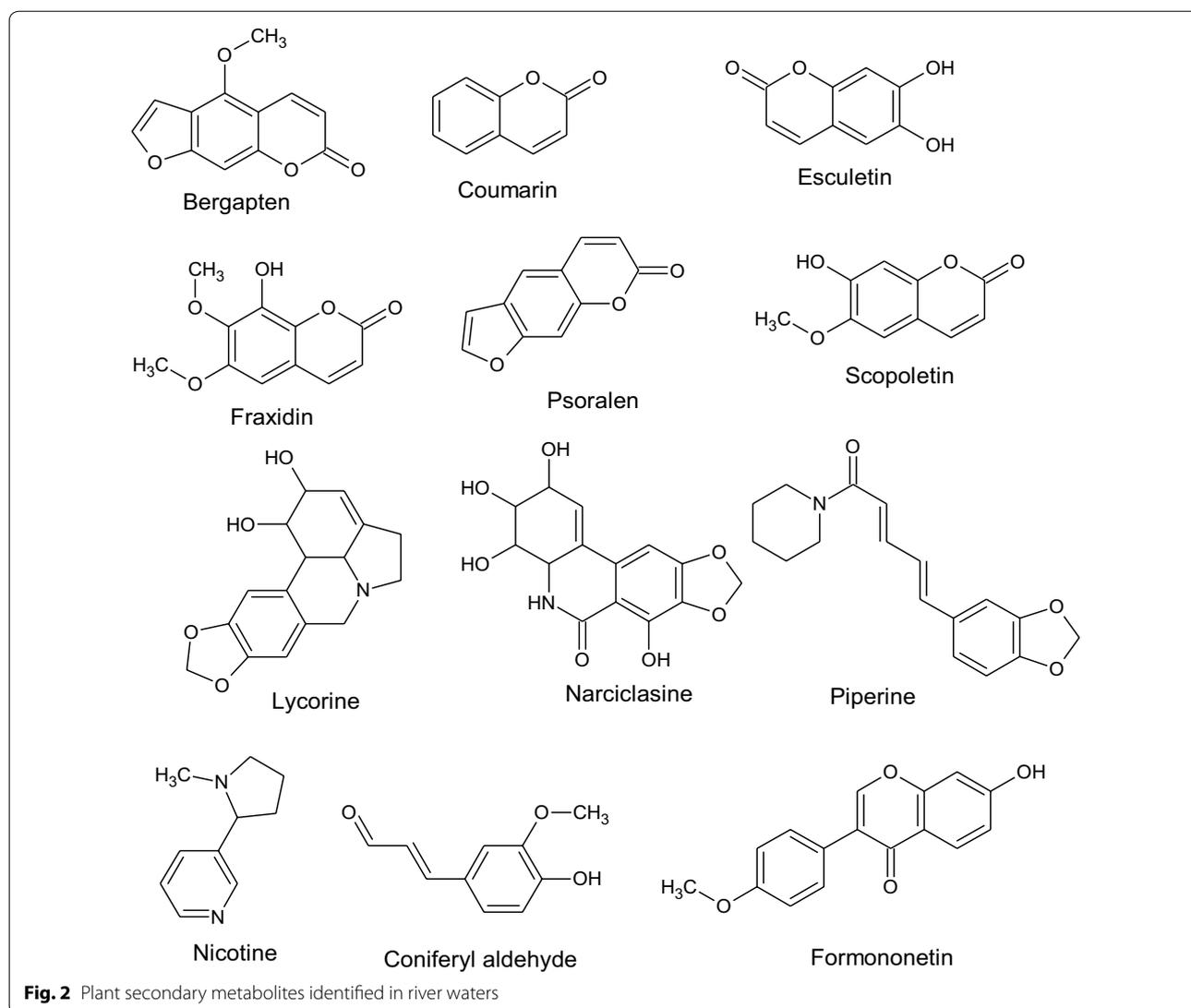
### Metabolites detected in river waters

Peak picking followed by annotation (based on MS1 full-scan) resulted in 145 target peaks with  $m/z$  and retention time matching to the target metabolite with a tolerance of 5 ppm and  $\pm 0.1$  min, respectively. Some target metabolites were annotated multiple times due to picking multiple peaks at a single precursor ion mass with given retention time tolerance. Removal of false positives and peak filtering using intensity and resolution reduced the target list to 106 peaks. Based on additional MS/MS fragment comparison with reference standards, we confirmed the presence of 12 target metabolites in the river waters (see Additional file 2: Figure S2–S13 for MS spectra). For the rest (94), MS/MS fragment did not match between water sample and their respective reference standard, thus discarded. They could be isobaric compounds, annotated in the given retention time window. The identified compounds belong to different classes of natural compounds including coumarins, alkaloids, isoflavone and others. In general, the identified metabolites contain one or more phenolic groups representing a class of compounds found most abundantly in vegetation [1, 39]. The names and chemical structures of the identified metabolites are given in Fig. 2.

**Table 1** The concentration range (min–max, ng/L) of identified plant metabolites in river water

Plant secondary metabolite	Chemical formula	CAS no.	$m/z$ (M + H <sup>+</sup> )	Retention time (min)	MDL (ng/L)	Concentration range (min–max, ng/L)		Frequency of detection	
						Leipzig	Bode	Leipzig	Bode
Coumarin	C <sub>9</sub> H <sub>6</sub> O <sub>2</sub>	91-64-5	147.0441	7.3	11	12	43	1	1
Esculetin	C <sub>9</sub> H <sub>6</sub> O <sub>4</sub>	305-01-1	179.0336	4.2	50	116–1658	104–157	2	3
Fraxidin	C <sub>11</sub> H <sub>10</sub> O <sub>5</sub>	525-21-3	223.0600	7.8	4	56–1145	19–155	9	8
Scopoletin	C <sub>10</sub> H <sub>8</sub> O <sub>4</sub>	92-61-5	193.0496	7.1	2	9–47	7–49	9	9
Bergapten	C <sub>12</sub> H <sub>8</sub> O <sub>4</sub>	484-20-8	217.0495	10.1	4	510	541	1	1
Psoralen	C <sub>11</sub> H <sub>6</sub> O <sub>3</sub>	66-97-7	187.0388	9.1	3	ND	141–224	0	2
Lycorine	C <sub>16</sub> H <sub>17</sub> NO <sub>4</sub>	476-28-8	288.1225	1.0	3	1015–2331	11	2	1
Narciclasine	C <sub>14</sub> H <sub>13</sub> NO <sub>7</sub>	29477-83-6	308.0765	5.7	150	507–3353	ND	2	–
Nicotine	C <sub>10</sub> H <sub>14</sub> N <sub>2</sub>	54-11-5	163.1228	0.9	1.6	2–6	4–35	2	2
Piperine	C <sub>17</sub> H <sub>19</sub> NO <sub>3</sub>	94-62-2	286.1434	11.9	0.9	1–338	4–294	4	2
Formononetin	C <sub>16</sub> H <sub>12</sub> O <sub>4</sub>	485-72-3	269.0804	10.8	3	8–35	123	5	1
Coniferyl aldehyde	C <sub>10</sub> H <sub>10</sub> O <sub>3</sub>	458-36-6	179.0701	7.6	8	13–46	ND	2	–

ND not detected

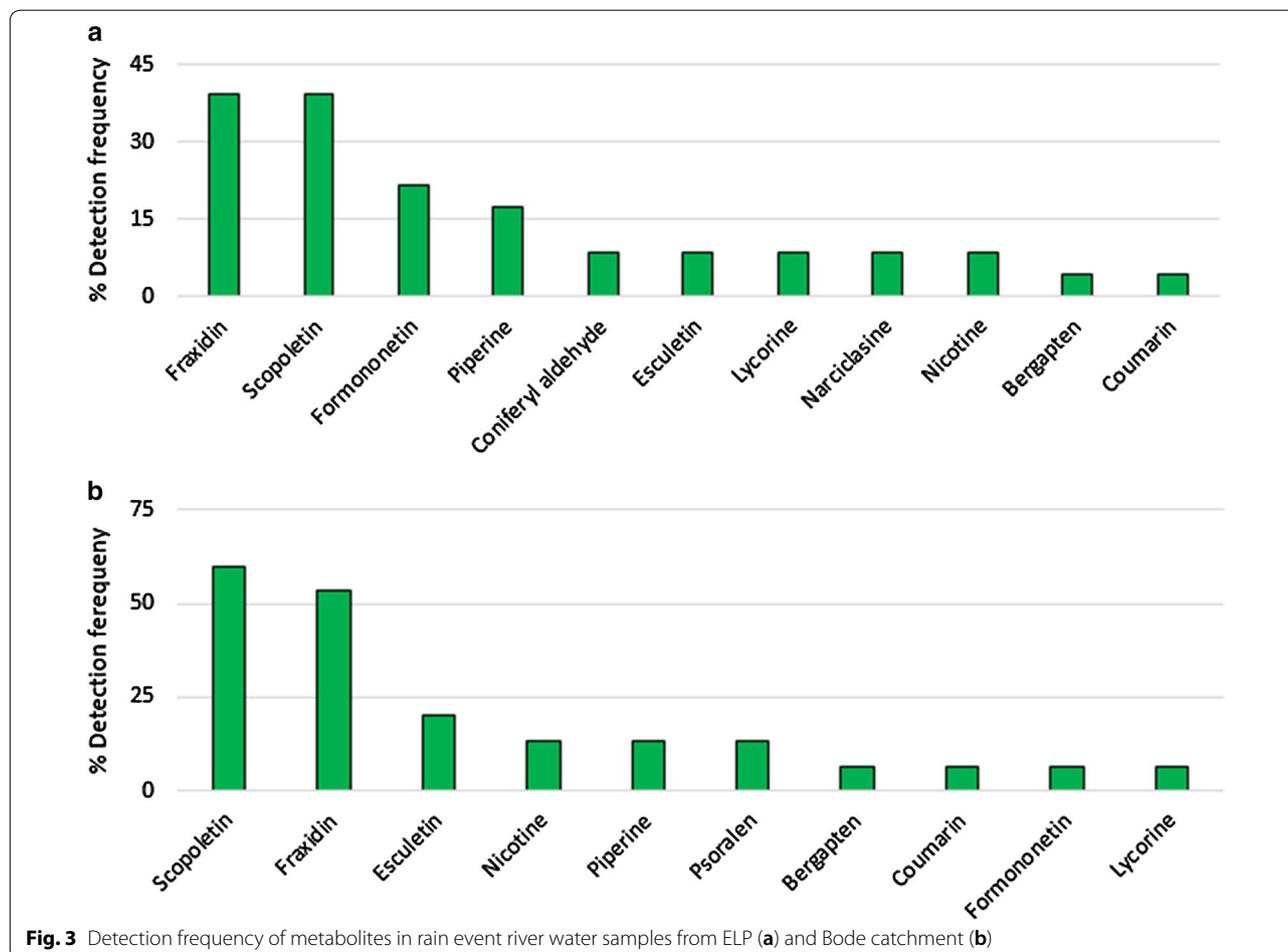


### Distribution of measured metabolites in river waters

In 18 out of 38 rain event samples PSMs were detected (9 samples from each catchment—Leipzig and Bode), while in none of the dry weather control samples any of the target PSMs were found. It is apparent that, in about 50% (20) rain event samples the target compounds were not detected. In the Bode catchment, larger numbers of metabolites were detected in rivers impacted by agricultural than natural vegetation (Fig. 1 and Additional file 2: Table S7).

Among the identified 12 metabolites, 11 were detected in samples from the ELP catchment and 10 were found in the Bode catchment. In both catchments, the coumarin derivatives scopoletin and fraxidin were detected with the highest frequency with 9 samples from each catchments (Fig. 3a and b). Esculetin, another coumarin derivative was the third most frequently detected PSM

in the Bode catchment with 20% while it was detected in 9% of the samples from the ELP catchment. The high detection frequency of fraxidin and esculetin is in good agreement with its formation by *Fraxinus excelsior*, a frequent tree in central European floodplains including the ones under investigation here. Scopoletin is produced by *Scopolia* species, but also the very frequently occurring stinging nettle *Urtica dioica*. However, all three compounds are present in a wide range of plants, which might contribute to emissions [52]. The isoflavone formononetin is the third most frequently occurring PSM in the ELP catchment with 22%, while it was found in 7% of the Bode catchment samples. Other compounds were detected only in specific samples from a specific season such as lycorine and narciclasine occurring in Amaryllidaceae, which show a high abundance within short



**Fig. 3** Detection frequency of metabolites in rain event river water samples from ELP (a) and Bode catchment (b)

growth periods such as *Galanthus* species in early spring. Although coniferyl aldehyde is a lignin component of many plants, it could be detected only in the EPL catchment and the coumarin psoralen only in Bode catchment. Natural compounds stemming likely from human consumption such as the piperidine alkaloid piperine as a component of pepper and nicotine from tobacco could be detected in both catchments.

#### Quantification of PSMs in river water

The target PSMs were detected in a concentration range of 1–3400 ng/L (Table 1). The concentrations of identified metabolites in individual samples are given in Additional file 2: Table S7. The highest concentrations were detected for lycorine and narciclasine with maximum concentrations of 2 and 3 µg/L during the times of high abundance of *Galanthus* sp. (snowdrop) and *Leucojum vernum* (spring snowflake). The concentrations of these phytotoxins strongly exceed the TTC of 0.1 µg/L for non-genotoxic and non-endocrine disrupting compounds and would be of concern, if these water resources would be

used for drinking water production. Both compounds are highly bioactive and toxic causing among others nausea and emesis in human and animals [24, 25]. Lycorine demonstrated acetylcholinesterase inhibition effects at  $IC_{50}$  of 213 µg/L [18, 32], while other authors reported above 1000 [6]. So, apart from its toxicity, lycorine also has more positive effects, as many SPMs, such as antibacterial, anti-viral, anti-malarial, anti-allergy effects, inhibits protein and DNA synthesis and has cardiovascular protection and antitumor effects [19].

The second group of PSMs exceeding the TTC of 0.1 µg/L is the coumarin derivatives with fraxidin and esculetin concentrations of 19 to 1145 ng/L and 116 to 1658 ng/L, respectively, while coumarin and scopoletin remained below 50 ng/L (Table 1). A maximum concentration of 300 µg/L esculetin was previously reported in Suwannee River fulvic acid isolates, USA [41]. In general, samples from the ELP catchment showed higher concentrations of coumarins than those from the Bode catchment. All four compounds have been isolated from *Fraxinus excelsior* [22, 40, 50, 52], a characteristic tree

along the rivers in both catchments. Coumarins comprise a very large class of substances, found in several higher plants and constitute fused benzene and pyrone rings [33, 45, 52]. Simple coumarins have been found to be biologically active with anti-stress, anti-fatigue, anti-gastric ulcer, anti-depressive, immuno-enhancing and anti-inflammatory effects [52, 54]. Scopoletin, isolated from *Scopolia carniolica* (Solanaceae), was shown to inhibit acetylcholinesterase at  $IC_{50}$  of 169  $\mu\text{g/L}$  in vitro assay [18].

Two furanocoumarins, bergapten and psoralen, have been detected in only two samples, each, from both catchments but in all cases above the TTC with 510 and 541 ng/L for bergapten and 141 and 224 ng/L for psoralen. They are synthesized by several plants, especially by those of the Apiaceae family [43]. They are generally known for their strong photosensitizing activity when applied topically or accidentally get in contact to the skin. The exposure to furanocoumarins combined with long UV radiation causes cytotoxic reactions (e.g., erythema) and genotoxic responses by binding to nucleobases in DNA [43, 51].

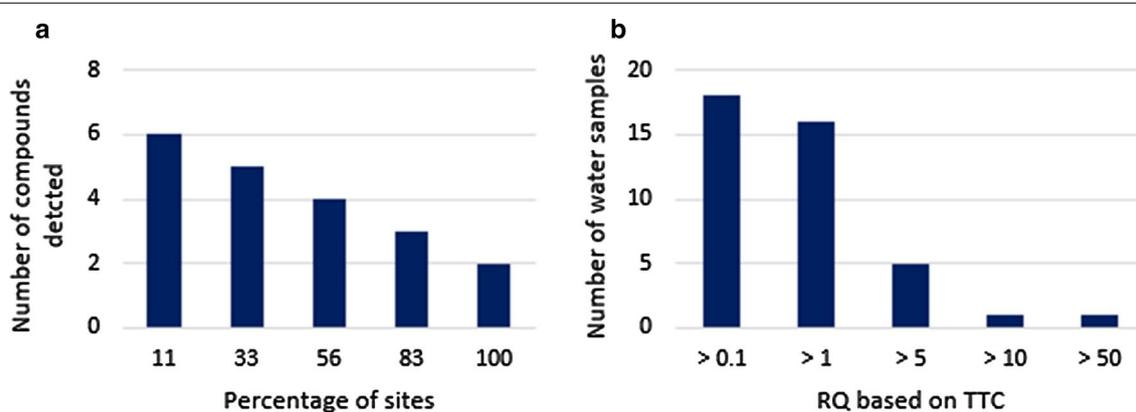
The flavonoid, formononetin was detected in five samples from the ELP catchment at a concentration range of 8–35 ng/L and in one sample from Bode catchment with 123 ng/L again exceeding the TTC. The latter was taken from an agricultural area [16, 23]. The PSM occurs in many leguminous plants such as clover (*Trifolium*), an abundant species in fertile meadows and pastures but also beans such as green beans, lima beans and soy [1, 33, 39]. Formononetin has been shown to display estrogenic properties with an  $IC_{50}$  of 104  $\mu\text{g/L}$  in vitro [28] and induce angiogenesis activities [27].

In both catchments, also the PSMs nicotine and piperine have been found in concentrations of 2 to 35 ng/L

and 1–338 ng/L again with two samples exceeding the TTC. The input of both metabolites to the river water is very likely due to human activities, while no plants containing these compounds in the catchments are known. Both PSMs are widely consumed by humans and related to tobacco smoking and food flavoring, respectively. Nicotine is highly addictive and acts as a receptor agonist at most nicotinic acetylcholine receptors (nAChRs) [12]. Piperine is a major component of Piper species (e.g., *Piper nigrum*, *Piper longum*, *Piper officinarum* and *Piper retrofractum*), which are globally marketed as flavoring agent and cooking spice with a long history of human health benefits and a wide consumption [44, 46]. Piperine has been found to have numerous medicinal applications such as antioxidant, antiplatelet, anti-inflammatory, anti-hypertensive, hepatoprotective, antithyroid, antitumor, antiasthmatic activity and has also been used as fertility enhancer [8]. Apart from its numerous benefits, it may also have adverse effects including hemorrhagic necrosis and edema in gastrointestinal tract, urinary bladder and adrenal glands observed in animal tests with rats [37]. Zwart et al. detected piperine in waste water treatment plant effluent and classified it as one of the most potent nonsteroidal estrogens at  $EC_{50}$  of 300 ng/L in vitro [55], which is in the same order of magnitude as the concentrations obtained in the present study.

#### Co-occurrence of PSMs

Similar to anthropogenic compounds, also PSMs occur in mixtures. In all of the samples, where we detected our target PSMs, we found at least two of them, at two sites (11% (2) of positive samples), we detected even six co-occurring PSMs (Fig. 4a). The compounds fraxidin and scopoletin were common to all samples, with only one exception in the Bode catchment. Based on TTC of



**Fig. 4** **a** Co-occurrence of detected PSMs in between sites (18 positive samples) and **b** the number of samples exceeding mixture risk quotient (RQ) levels of PSMs

0.1 µg/L, mixture risks exceeded a RQ of 1 at 16 out of 18 sites, at 5 sites mixture RQ was above 5. At one site each, even RQs of 10 and 50 were exceeded (Fig. 4b). This may indicate that toxic risks by frequently occurring PSMs may not be negligible and should be included in risk assessment of chemical mixtures in water resources.

## Conclusion

By target screening of 150 prioritized PSMs in river water from two small catchments in Germany, we were able to detect 12 compounds of different classes (e.g., coumarins, flavonoids, alkaloids and others), some of them occurring frequently. In many cases, concentrations of these compounds, which are known to exhibit substantial biological activity and possibly toxic effect, exceeded the concentrations of many anthropogenic chemicals in surface waters and TTC for drinking water individually and as mixture in almost all samples, in few cases by more than one order of magnitude. This finding clearly indicates that PSMs and other natural compounds should be included into monitoring and risk assessment and should be considered in the context of drinking water abstraction. A potential risk particularly during rain events promoting the leaching of PSMs to surface waters and massive occurrence of toxic plants in specific seasons may not be excluded. Large-scale seasonal target and suspect screening of PSMs together with toxicity testing of frequently occurring and high-concentration compounds is required to estimate the contribution of PSMs to overall water pollution and to identify seasons and situations potentially posing a risk to drinking water production. Toxic risks to aquatic ecosystems might be relevant, particularly in areas where vegetation undergoes drastic changes, for example by massive occurrence of toxic invasive species or by substantial changes in land use. Thus, we recommend to consider PSMs in monitoring and risk assessment of water resources. This is currently hampered by a lack of effect data including toxicity to aquatic organisms, endocrine disruption and genotoxicity and demands for biotesting of these compounds.

## Supplementary information

**Supplementary information** accompanies this paper at <https://doi.org/10.1186/s12302-020-00399-2>.

**Additional file 1.** Samples, target compounds and internal standards.

**Additional file 2.** Data evaluation and results.

## Abbreviations

LC–HRMS: Liquid chromatography coupled to high-resolution mass spectrometry; PSMs: Plant secondary metabolites; ELP: Elster, Luppe and Pleiße

catchment; BD: Bode catchment; TTC: Threshold for toxicological concern; MS: Mass spectrometry; RQ: Risk quotient; MDLs: Method detection limit.

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## Authors' contributions

MYN: conceptualization, investigation, experimental analysis, target compound selection, data evaluation and visualization, writing (original draft). MK: conceptualization, investigation, writing—review and editing; WB: conceptualization, supervision, writing—review and editing. CDS, BFG and TDB: in silico prediction and selection of target compounds, review and editing. All authors read and approved the final manuscript.

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## Availability of data and materials

The datasets obtained and analyzed in the current study are available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interest

The authors declare that they have no competing interests.

## Author details

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