Passive sampling of herbicides above sediments at sites with losses of submerged macrophytes in a mesotrophic lake

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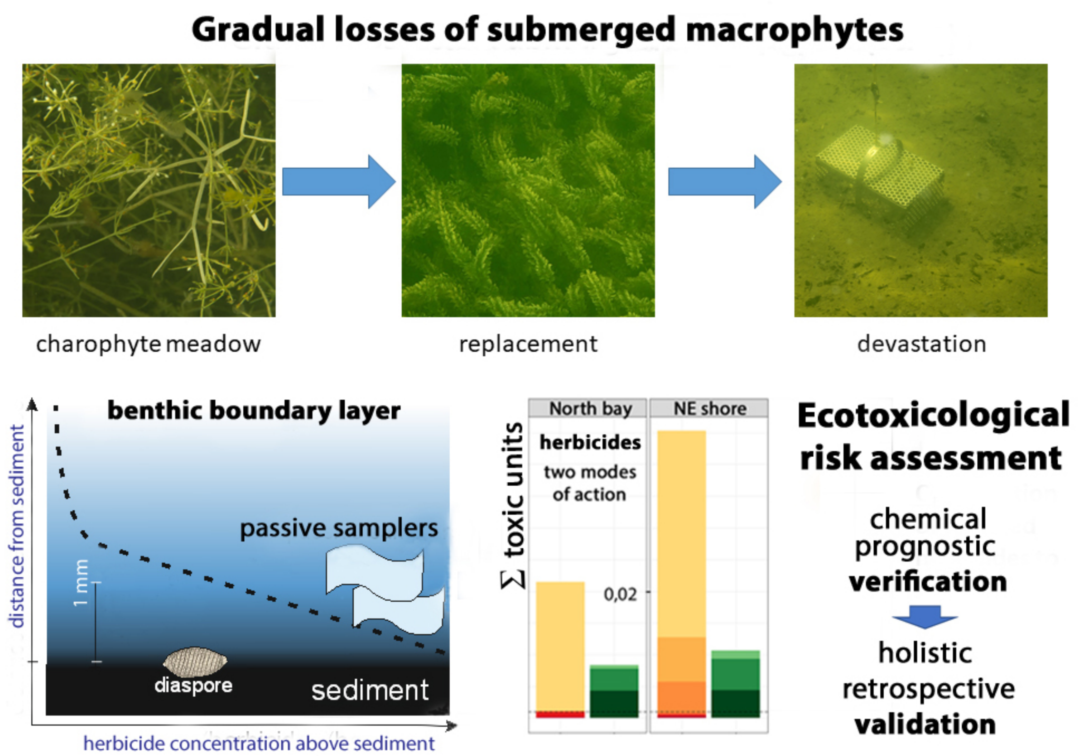
ABSTRACT. Declines of submerged macrophytes (SUM) were monitored in littoral zones of the deep, mesotrophic lake Suhrer See (Northern Germany) since 2017. Drastic losses coincided with intense agriculture in sandy sub-catchments and precipitation. All lines of evidence pointed to a causal connection with subsurface discharge indicating that herbicide application might have caused the effects. Passive sampling was applied in 2022 to elucidate, whether herbicides were really present at sites of losses and if so, in ecotoxicological relevant concentrations. Samplers were exposed on top of lake sediments in 2 m depth and under worst case conditions, i.e., at sites, known for losses of the whole functional group of SUM and at the beginning of the vegetation period. At this time, SUM diaspores were most vulnerable to repression of development and the subsurface discharge was high in the same instance. The potential ecotoxicological relevance of detected herbicide concentrations was assessed with a toxic units (TU) approach, with reference to acute effect concentrations (EC50 of green algae, 72h, growth). The TU ranged from 0.001 to 0.03. Most concentrations exceeded the threshold of relevance set by an assessment factor of 1000, i.e., TU > 0.001. Locally applied herbicides acted by suppressing developmental stages, and the sum of TU exceeded 0.02 at all sites, mainly due to diflufenican. Not applied locally, terbuthylazine and its relevant metabolites, including terbutryn, acted by inhibiting photosynthesis, and the sum of TU reached 0.005. On this base, diflufenican was assessed to be likely a main stressor, all other detected herbicides to be potentially relevant. Uncertainties and knowledge gaps were specified. The result of the chemical risk assessment was counterchecked for consistence with biological monitoring data within a whole lake perspective. Concepts of empirical and advanced causal attribution methodology were applied to get a grip to the ecological causal field and to protection.

Graphical Abstract



Highlights

- Monitoring of ecological stress responses of submerged macrophytes (SUM)
- Detection of herbicide residues at sites with missing SUM
- Identification of diflufenican and terbuthylazine as toxicity drivers
- Specification of uncertainty and knowledge gaps in chemical risk verification
- Risk validation by SUM responses, causal attribution and stressor concepts

1 Introduction

The deep, groundwater fed lake Suhrer See is a nature reserve with underwater meadows of charophytes (Schubert et al. 2018, lanaplan 2022, see also SM-1). In the extremely wet summer 2017, submerged macrophytes (SUM[1]) disappeared at some sites in the agricultural northeast, while charophyte meadows stayed vital in bays in the north and northwest, surrounded by terrain without agricultural production. In the subsequent extremely dry summer, charophytes recovered at some sites in the northeast and were replaced by dense stands of angiosperms at others. One site even remained bare. The circumstantial evidence pointed to an impact of agriculture, mediated by precipitation. Applied herbicides were the *prima facie* suspect, also in view of the coeval discussion of causal relations between pesticides and aquatic biodiversity losses (Schäffer et al. 2018, Schäfer et al. 2019). The suspicion was substantiated by a conspicuous decay of a before wintergreen charophyte meadow in 2020. The difference made a field next to the affected north bay, that was recultivated in autumn 2019, after it has been taken out of production for years.

The changes were documented by systematic, seasonal monitoring and underwater photography as part of care for the nature reserve in responsibility of the NABU Plön (branch of nature protection association). The local disturbances reached from replacements by less sensitive species to losses of the whole functional group. Thus, they

1 Abbreviations: AF: assessment factor; CC: Chemcatcher with SDB-RPS disk (passive sampler for polar compounds); CfS: candidates for substitution; DT50: half live; EC50: concentration causing an acute 50% effect in standardized tests; ERA: ecotoxicological risk assessment; Kow: octanol water partition coefficient; Ksw: sampler water partition coefficient; N, NE: north, northeast (exposure sites); PDMS: polydimethylsiloxane (passive sampler for nonpolar compounds); PPDB: pesticides properties data base; SUM: submerged macrophytes; TBZ, TBZ-: terbuthylazine, -metabolites; TBT: terbutryn; TU: toxic units; TWA: time-weighted average; WFD: water framework directive

displayed a classical response to increasing stress (Odum 1985, Krambeck 2022). Wintergreen charophyte stands were most sensitive and missing in the northeast with its larger agricultural subcatchment. The spring development of new SUM generations was regularly retarded at sites in the northeast, in wet summers more or less for the whole season. Recovery depended on summer dryness (for definition of wetness degrees by difference between precipitation and evaporation see SM 1.4).

All lines of evidence together made a causation of SUM declines by interflow from sandy sub-catchments with winter grain cultivation likely. The criteria of Hills framework for the evaluation of causation against a background of coincidences (Hill 1965, Köhler & Triebskorn 2013, Triebskorn et al. 2003) were consistent with the findings (see SM-3.1).

However, the possibility of ecotoxicological effects in lakes was previously excluded. Dilution in large water bodies seemed simply too high to reach concentration limits considered toxic. For example, an environmental agency screening in the federal state of Schleswig-Holstein (LLUR 2018) yielded detections of 121 organic xenobiotics in rivers, but hardly any in lakes. Monitoring according to the water framework directive (WFD) also revealed general improvements of lake phytoplankton with decreasing eutrophication, but on the other hand still deterioration of SUM. Therefore, the LLUR SH initiated analytical campaigns, extended its regular WFD macrophyte monitoring in the lake Suhrer See, supported a suspension of herbicide application on half a field in the northeast, and convened expert rounds. Eventually, proves were considered missing. Scientifically, the question was: Were herbicides really present at times and places of disturbances of SUM and if so, were concentrations high enough to be attributed to the observed responses?

Passive sampling (Vrana et al. 2007, Schulze et al. 2011) is the method of choice to determine herbicides on top of sediment surfaces and appears as an appropriate and sufficiently sensitive approach (Grodtke et al. 2021). The most vulnerable charophyte diaspores (oospores, bulbillae) lie on the surface of sediments; and potentially wintergreen underwater meadows develop in around 2 m depth. Near zero flow and high vertical gradients are characteristic for the benthic boundary layers in this habitat. This poses a problem to determine real on-site concentrations. Nevertheless, passive sampling was applied successfully before to tackle gradients at the sediment/water interface (Mechelke et al. 2019). Additionally, passive samplers are known to have toxicokinetic and toxicodynamic properties similar to organisms (Vermeirssen et al. 2005), especially for nonpolar compounds (Bayen et al. 2009). Thus, samplers lying on top of sediments like diaspores of SUM promise best possible results. Tentatively, a pilot trial was started with passive samplers for nonpolar and polar compounds, covering the range of herbicides known to be applied locally.

Risk assessment required additional information on sensitivities of the affected developmental stages of SUM. Because corresponding ecotoxicological data were missing, standardized, short-term effect concentrations (EC50 green algae, 72h, growth) from data sets were used as a surrogate in order to approximate chronic toxic potentials of detected herbicide concentrations (EC 2018, Escher et al. 2021). Combined effects were assessed following the toxic units (TU) concept, which was developed for prognostic ecotoxicological risk assessments based on environmental concentrations when biological data are missing (Backhaus & Faust 2012). In the case given, the real risks are known *ex post*, and the task is to assess the probable contribution of herbicides to the manifested effects. Thus, uncertainties inherent to the TU concept have to be considered (ECHA 2012, EC 2018).

The chemical risk assessment verifies a risk posed by the measured herbicide concentrations in principle. A further confirmation of the hypothesis, that herbicides are a main cause of the observed stress responses of SUM, needs a risk validation, i.e. a countercheck of the prognosed risk by integration into the retrospective ecological risk assessment. To get a better grip to this task, we try to adopt concepts of advanced causal attribution methodology, in addition to the more accustomed multiple lines of evidence and Hill´s criteria. Principles of causal attribution and an application of formal logic to the emerging causal field (Hamilton et al. 2015, Minnerop and Otto 2020, Hill 1965, see SM-3) are in fact found useful to contextualize the findings within an ecological whole lake perspective and to assign protection priorities.

2 Materials and methods

Passive samplers were exposed from April to Mid-June 2022. PDMS sheets (SSP-M823, 150 x 55 x 0.5 mm^3 from Specialty Silicone Products Ballston Spa, NY, USA) were used as passive samplers for nonpolar compounds with log K_{ow} (octanol-water partition coefficient) ranging from 3.5 to 6.0. Chemcatchers® (CC, d=47 mm, from AT Engineering Technology, Tadley, UK) equipped with disks of styrene divinylbenzene reversed phase sulfonate (SDB-RPS from Affinisep, France) and diffusion limiting polyethersulfone membranes (PES, pore size 0.45 µm, from Pall, Port Washington/USA) were used as passive samplers for polar compounds (log K_{ow} < 4.0).

2.1 Selected sites for passive sampler exposure

Four sites with drastic losses of SUM were chosen for exposure of passive samplers, two at the northeast shore (NE-1 and NE-2) and two in the north bay (N-1 and N-2) of the lake Suhrer See in Schleswig-Holstein, Northern Germany (Figure 1, SM-1.1). PDMS sheets were exposed over two months at the northeast and north sites. Chemcatchers were exposed in two subsequent campaigns with a duration of 33 d and 29 d at NE-1, 15 and 29 d at NE-2 and 29 d and 36 d at N-1 and N-2. Chemcatchers were additionally exposed during the second field campaign (28 days) at a site in a bay in the northwest without agriculture in its subcatchment.

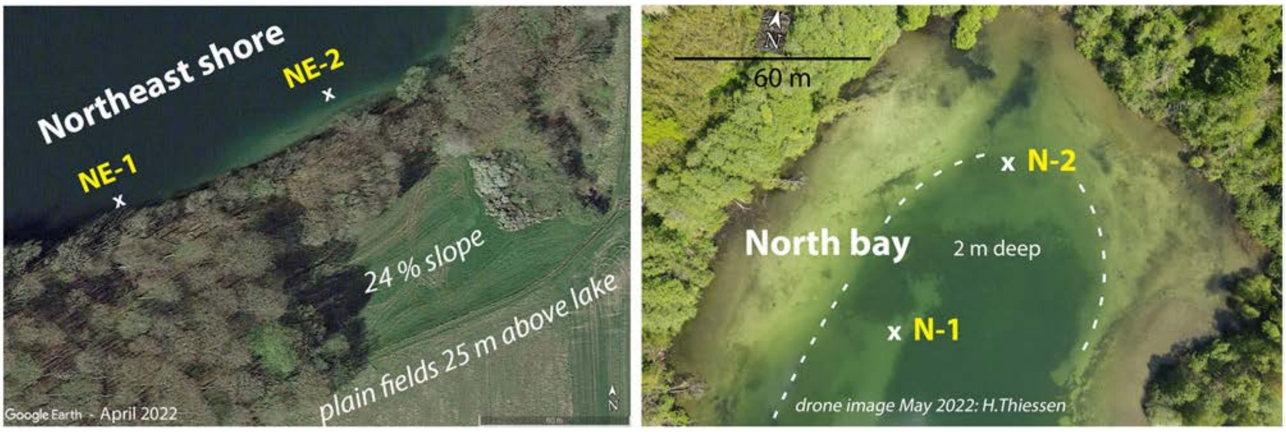


Figure 1: Left: Sampling sites at the north east shore. Right: Sampling sites in the north bay.

Duplicates of each kind of passive sampler were exposed in a perforated metal cage that was open towards the sediment surface and designed to sit on it (SM-2, Figure SM-2.1). The samplers were fastened to the cage with zip ties to lie on the sediment surface as close as possible. The cages were made retrievable from 2 m depth by a line with a buoy. One cage was exposed per site, snorkeling a distance of 10 m to 20 m from shore at the northeast sites and around 60 m in the north and northwest bay. Conditioned and assembled passive samplers were stored at 2°C and transported to exposure sites in Styrofoam boxes within one hour. Trip blanks were treated as the passive samplers in the cages, but stored at 2°C instead of being exposed. All samplers were handled with Latex gloves and on lint-free papers. After exposure, the samplers were disassembled, the PES-membranes were discarded, and the samples were stored frozen in glass vials.

2.2 Preparation, extraction and analysis of passive samplers

Before exposure, the passive samplers were conditioned. SDB-RPS disks were shaken in methanol for 30 min at 150 rpm on a rotary shaker and subsequently rinsed in bidistilled water (30 min on a rotary shaker). The PES membranes were treated similarly. The disks and the PES membranes were stored in bidistilled water until sampler assembly. PDMS sheets were soxhlet extracted with ethyl acetate for 40 h. Subsequently, the strips were stored in methanol until exposure.

SDB-RPS disks were extracted with 10 mL acetone for 30 min, 10 mL methanol (1% formic acid, 20 min), 10 mL methanol (20 min), and 10 mL methanol (with 5% ammonia solution, 20 min). The combined extracts were evaporated in an ExcelVap (Horizon Technologies) to a final volume of 1 mL. PDMS sheets were extracted thrice with 20 mL acetone for 1 h each. Combined extracts were evaporated to a final volume of 1 mL and dried with Na_2SO_4. Samples were stored frozen until analysis.

Chemcatchers were analyzed using a 1290 Infinity series HPLC system with a 6460 TripleQuad mass spectrometer (Agilent Technologies). The system was equipped with a Kinetex EVO C18-column (2.6 µm particle size, 50 mm x 3 mm) with a guard column (Phenomenex). Bidistilled water with 1% formic acid was used as eluent A and acetonitrile with 1% formic acid was used as eluent B. The analysis was performed in positive ionization mode. The eluent gradient, target list with transitions, and retention times can be found in Table SM-4 and SM-5.

The PDMS extracts were analyzed with a 7890A GC system, equipped with a HP-5SM UI column (30 m, d = 0.25 mm, 0.25 µm film), with a 5975 inert XL EI/CI mass spectrometer (Agilent Technologies) and a Gerstel Multipurpose autosampler. Helium was used as a carrier gas with a flow rate of 1.2 mL/min. The starting temperature was set to 50°C, which was held for 2 min. The temperature was increased to 200°C with a rate of 12°C/min. Subsequently, the temperature was raised to 250°C with a rate of 5°C/min. The final temperature was held for 10 min, yielding a total runtime of 34.5 min. Monitored masses and retention times can be found in Table SM-6.

2.3 Calculation of aqueous concentrations

Uptake kinetics of PS follow a saturation curve and can be described by simplified equations for the linear phase and the saturation phase (Grodtke 2021, Smedes and Booij 2012), facilitating the calculation of concentrations in water from amounts extracted with the passive sampler.

Uptake of CC-RPS with membranes was assumed to stay in the linear uptake phase within one month in standing water. Time-weighted average (TWA) concentrations can be calculated from amounts collected by sampler (m_s), uptake rate (R_s) and duration of exposure (t) by equation (1):

$$c_w^{twa} = \frac{m_s}{R_s \cdot t} \tag{1}$$

Under nearly quiescent condition, a sampling rate of 0.01 L/d was estimated to be the best available approximation (Römerscheid, 2023).

For PDMS, distribution equilibrium was assumed after two months in standing water. Calculation of concentrations in ambient water (c_w) from amounts per sampler (m_s) requires the volume of the sampler (V_s) and the sampler-water partition coefficient (K_{sw},(2)):

$$c_w = \frac{m_s}{V_s \cdot K_{sw}} \tag{2}$$

$K_{sw,\ PDMS}$ were calibrated by Grodtke (2021) for most of the compounds detected with standard deviations (sd) in the range of 10% to 20%. K_{sw} for diflufenican, pendimethalin and prosulfocarb were calculated from the linear fit between log K_{ow} and log K_{sw} ((3), rse: 0.451, r^2: 0.752)

$$\log K_{sw} = 0.79 \log K_{ow} + 0.36 \qquad (3)$$

Calibrations of K_{sw} were conducted for 20° C. Exposure temperature increased from early April to mid-June from 5 to 20 °C.

2.4 Relation to EC50 for assessment of toxic relevance

Because sensitivity data for diaspore development were missing, short-term toxicity data (EC50 for green algae, 72 h, growth) from standard data sets were taken as surrogate. An assessment factor (AF) of 1000 was inserted for extrapolation to potentially chronic effects (according to 3.3.1.1 in EC 2018). Herbicides with relations of detected concentrations to their EC50 near or over 1/1000 or a toxic unit (TU) of 0.001 were then identified as likely relevant. Relations of TU near or over 0.01 were interpreted as indication for likely main stressors. Multiple stress was assessed by addition of TU of compounds with a similar mode of action (Backhaus & Faust 2012). Toxic units are determined according to equation (4) from the aqueous concentration of an analyte (c_w) and its EC50 value.

$$TU = \frac{c_w}{EC50} \qquad (4)$$

3 Results and discussion

3.1 Timing and choice of exposure sites: landscape context and stress history

Spring was chosen for exposure because monitoring had revealed regular failings of SUM regrowth at this time of the year at sites with agricultural subcatchments (Krambeck 2020, 2022). After extraordinary precipitation in February 2022 (over 3 times the mean), high interflow was expected. SUM did in fact not develop up to June, neither at the exposure sites at the northeast shore, nor in the north bay.

The lake Suhrer See lies in a sandy morainal plain and is fed by groundwater. Subsurface discharge prevails due to the high permeability of soils and the small subcatchment. Traces of surface discharge are found at few places only, even after heavy rains.

The northeast shore with the exposure sites NE-1 and NE-2 (fig.1 left) is bordered by a steep, mainly forested slope ascending to 23 to 26 m above lake level over a distance of 100 m from shore. On top of the slope follows a sudden transition to a plain with long-standing intense agriculture. This relief of the landscape results in a high hydrological subsurface gradient towards the lake which feeds swamp springs at the base of the slope, accompanying the whole East side of the lake. The exposure site NE-2 was chosen because it was sometimes colonized by angiosperms and sometimes not, in dependence of precipitation. The site NE-1 was without SUM the last years and lies in between two drain creek outlets (see fig. SM-7).

The terrain around the north bay lies no more than 8 m above lake level and is surrounded by areas without intense agriculture in the east and west. In the north of the bay lies a swamp without visible hydrological connection that reaches to the limits of the catchment area in 100 m distance from the lake. A field north of the swamp was removed from production up to 2019 (see areal view, fig. SM-2). SUM responses to the recultivation (see also chapt.3.4) indicate a larger subsurface catchment. The two exposure sites in the north bay, N-1 and N-2 (fig. 1 right), were positioned in the western and inner realms of the bay known to be disturbed since the recultivation of the nearby field since 2020.

3.2 Chemical analysis of passive samplers

3.2.1 Detected compounds and local distributions

In the PDMS sheets, eight herbicides and one metabolite (desethyl-terbuthylazine, DE-TBZ) with log K_{ow} ranging from 2.0 to 5.4 could be quantified (Figure 2 A). All target analytes were present above quantification limits at all four exposure sites, except pendimethalin, flufenacet, and dimethachlor. The quantified concentrations range from 35 ng/sampler for DE-TBZ at the site N-2 to 724 ng/sampler for prosulfocarb at the site NE-2. The levels showed a tendency to higher concentrations at the sites in the northeast compared with the sites in the north bay.

Standard deviations of duplicates range from 0% to 83%, with a 90% quantile of 57%, implying that duplicates of single compounds rarely differed by more than a factor of 2, The scatter can be attributed to the exposure within benthic boundary layers with high concentration gradients and small-scale variability (Boudreau & Jörgensen 2001, Dade 1993, Roy et al. 2002). The thickness of the initial diffusive boundary layer may reach 1 mm to 2 mm on top of deep sediments and is followed by a zone of some cm, where laminar flow and Eddy diffusivity begin to rule the water exchange to the lake, thus that the very steep gradient in the initial millimeters diminishes up to balance with the overlaying lake water (Wehrli & Wüst 1996, Wetzel 1975). The samplers were mounted as close to the sediment surface as possible, which was in a range of 0 to 1 cm (cf. SM-2, Figure SM-10). Random proximity of bioturbation to duplicates may have increased the scatter. The uncertainty is comparable between both sampler types and a little higher than expected for deployments in turbulent running waters.

Fig 2B shows the aqueous concentrations c_w derived from PDMS sheets. Due to the increase of partition coefficients over three orders of magnitude (see tab SM-6, SM-2.2), the concentrations of the nonpolar compounds strongly decrease compared to c_s, while concentrations of the more polar compounds increase. Therefore, the highest aqueous concentration can be found for DE-TBZ at the site NE-2 (203 ng/L), while pendimethalin can only be detected at the site N-2 in a concentration as low as 2.8 ng/L.

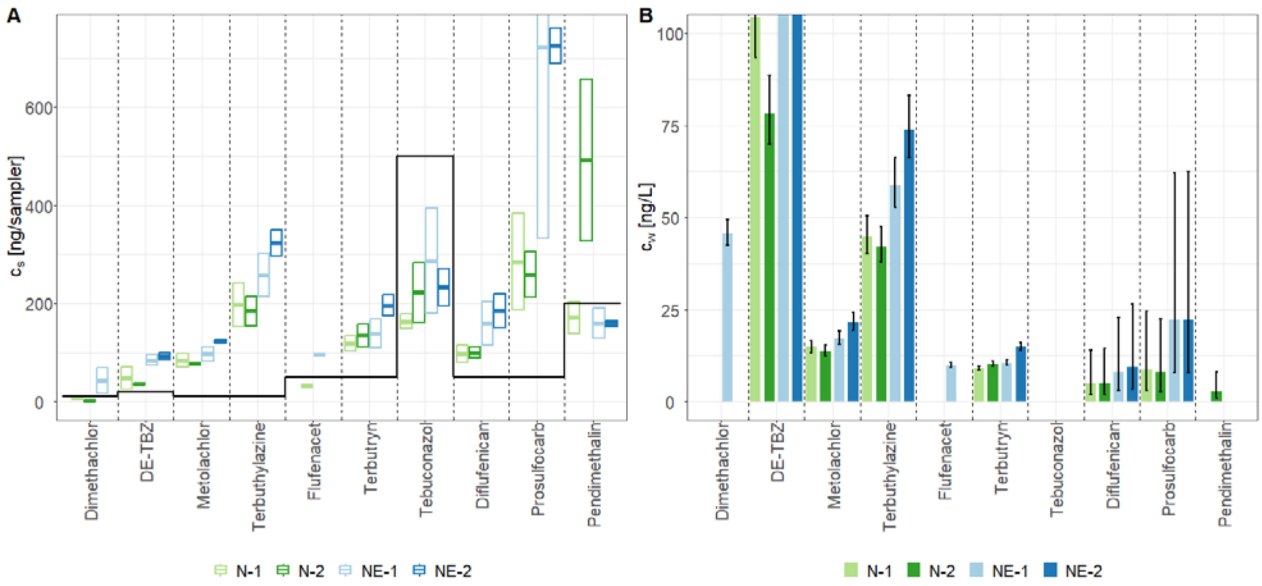


Figure 2 A: Detection of selected herbicides in PDMS strips m_s in [ng/sampler] (sorted by log K_{ow}). Length of the boxes represents the duplicate samples. Bold line represents the mean c_s. The black line shows the limit of quantification (derived by signal to noise ratio, see Table SM-4). B: Derived c_w concentrations from sampler-water partitioning coefficients (section 2.3) sorted by log K_{ow}. Uncertainties describe the error of the applied partition coefficients. DE-TBZ: desethyl terbuthylazine.

In the Chemcatchers, four herbicides (log K_{ow} ranging from 1.55 to 3.70), eight metabolites, and a fungicide (terbuconazole) could be quantified in the second sampling campaign (Figure 3A). Standard deviations of the duplicates range from 0.2% to 141%. The 90% quantile of the standard deviation is 65%.

Concentrations in the sorbent phase decrease with increasing log K_{ow}, which shows the mobility of more polar compounds on the one hand, and can additionally be ascribed to the limitations of the applied Chemcatcher design: With decreasing polarity, a larger amount might be retained in the diffusion limiting membrane. Time weighted average (TWA) concentrations ranged from 2.1 ng/L to 21 ng/L for terbuthylazine (TBZ, log K_{ow} 3.40), 5.4 ng/L to 68 ng/L for TBZ-DE (log K_{ow} 3.66), 4.2 ng/L to 257 ng/L for other metabolites and 2.3 ng/L to 6.1 ng/L for tebuconazole (log K_{ow} 3.70). All investigated fungicides and metabolites were detected in all samples. Parent compounds of herbicides besides TBZ where only detected in the northeast: flufenacet at NE-2 and metolachlor at NE-1 and NE-2. In the first sampling campaign, metolachlor was not detected, but dimethachlor, though only in concentrations near the limit of detection (0.5 ng/L to 0.8 ng/L). The result could be explained by the different application schedules (dimethachlor in autumn for rape and metolachlor for corn in May) The concentrations of all other compounds showed no trends between the two sampling campaigns. Concentration levels of single compounds were similar for the northeast sites and the northwest bay, whereas levels in the north bay were lower by comparison, except for the metabolite flufenacet-ESA which might be due to the application of flufenacet on the recultivated field the autumn before (see table SM-1).

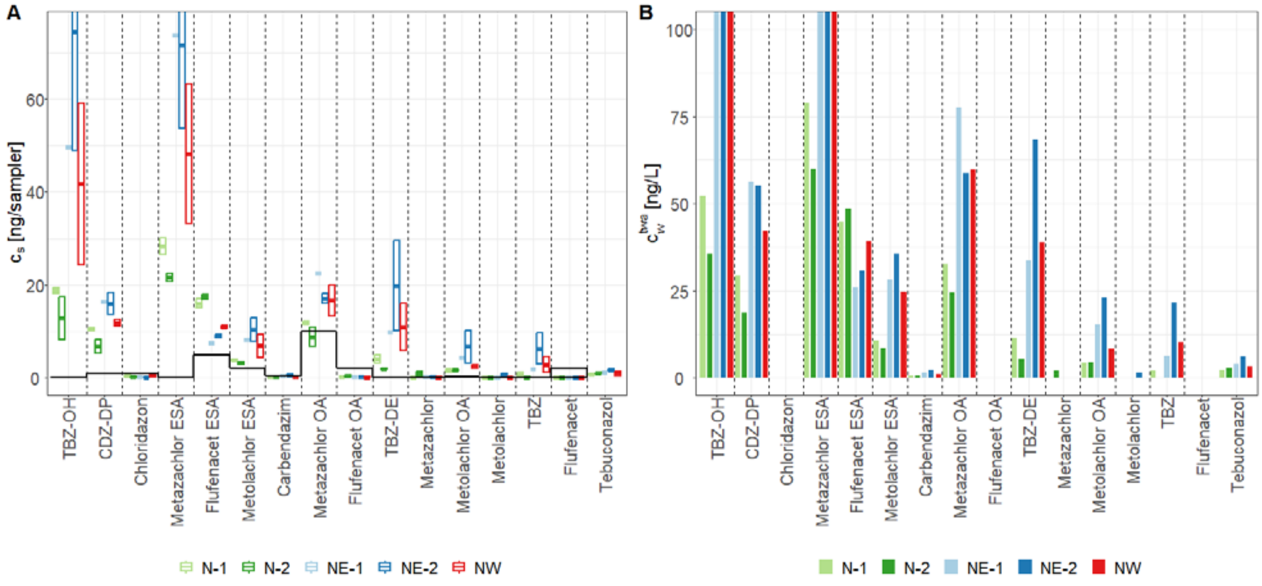


Figure 3: Detection of selected compounds in Chemcatchers, m_S in [ng/sampler] (sorted by log K_{ow}). Length of the boxes represents the duplicate samples. Bold line represents the mean c_S. The black line shows the limit of quantification (derived by signal to noise ratio, see Table SM-5). B: Derived c_w(TWA) concentrations from generic sampling rates (section 2.3), sorted by log K_{ow}. TBZ-OH: hydroxyterbuthylazine, CDZ-DP: diphenyl chloridazon, ESA: ethane oxosulfonic acid, OA: oxanilic acid, TBZ-DE: desethyl terbuthylazine, TBZ: terbuthylazine.

Time-weighted average concentrations calculated from Chemcatchers were generally lower than aqueous concentrations calculated for parallel PDMS samples and for compounds in the range of overlapping polarity, e.g., TBZ-DE. There are different potential reasons: Firstly, sampling rates needed for the calculation of TWA concentrations of RPS disk have to be adjusted for low flow conditions, but were seldom tested under stagnant conditions. Literature data point to values down to 0.01 L/d (Grodtke 2021, Münze et al 2015). This value was used for calculation as best available approximation as suggested by Römerscheid et al (2023). Secondly, for concentrations peaking shortly before passive sampler retrieval, equilibrium concentrations in PDMS may come out higher than TWA concentrations from CCs exposed in parallel.

Both samplers yielded the same distribution pattern, namely slightly elevated concentrations at the northeast shore compared to the north bay. Moreover, the pattern of the Chemcatcher results support the source allocation and conclusions on the origin and timing of polar contaminants.

All in all, PDMS results can be regarded more reliable in the given application. The exposure time of two months was rather long and the sheets floated directly over the sediment. The amounts collected per PDMS sheet were also a multiple of amounts collected per Chemcatcher, and the sheet thickness of 0.5 mm was similar to diaspore dimensions, a factor of importance for similar uptake kinetics.

The pilot trial conditions were already adjusted to experience with passive sampling. However, accounting for stagnant conditions and gradients in the diffusive boundary layer at the sediment-water interface was not trivial. For extended use under these extreme conditions, passive sampling could be further adapted to this very special application, e.g., by controlling uptake kinetics and adjusting exposure times, accurate positioning of passive samplers relative to sediment surfaces, adjusting passive sampler size to quantification needs, gathering application data to complete the target list and K_{sw} calibration for all compounds measured**.**

3.2.2 Source allocation: application and atmospheric deposition

A comparison of the detected local distributions and levels allows inferences to origins of interest for the risk interpretation and mitigation options. Lower herbicide levels in the north bay than at the northeast sites were expected, because of the different size of nearby agricultural areas. The distance of the lakeside edge of agricultural areas was about 100 m in both cases, but near the north bay there was only one intensely cultivated field of 3 ha, compared with an agricultural subcatchment of about 30 ha above the sites at the northeast shore, including fields at the catchment margins in 400 m to 800 m distance from shore.

The similarity of the levels of the Chemcatcher results at the northeast sites and in the northwest bay were surprising at the first glance, because herbicides were never applied in the northwest. The only herbicide detected in the northwest bay was terbuthylazine (TBZ), which is widely applied at corn cultivation in the region, but only outside the catchment of the lake. Parent compounds pointing to local application were only detected at the northeast sites. The otherwise similar patterns can be attributed to the fact that all compounds detected are known to be transported by atmospheric deposition and to be omnipresent in Europe. Concentrations in air depend on the distance from intensely used areas, and concentrations in rain water remain relatively high over longer distances (Dubus et al. 2000, Kreuger et al 2006, Kruse-Plaß et al.2021, Zaller et al. 2022, Machate et al. 2022). Thus, the findings in the northwest bay and a large part of corresponding detects at the other sites can be plausibly attributed to atmospheric deposition.

Terbuthylazine and metabolites detected by CC and PDMS show both a tendency to comparably lower levels in the north bay. This trend implies a dependence of benthic concentrations of deposited compounds also on subcatchment areas. The load of herbicides entering the lake with subsurface discharge at a given site depends in fact only on the presence in the respective subcatchment area, irrespective of the way of contamination. Only the load of applied herbicides per subcatchment area may be higher than the one due to deposition. A better differentiation then requires additional information on application. Deposition directly on the lake surface, on the other hand, has to be diluted fastly to non-detectability in the lake water, given concentrations in the ng/L range found in rain water (Kreuger et al 2006) and snow-melt (Maurer et al. 2023). In addition, the remobilization of herbicides from sediments to water is too slow to markedly increase pelagic concentrations. For instance, herbicides experimentally spiked to sediments remained near the detection limit in overlaying culture media for 4 weeks (Polst et al. 2023).

The poor soils of the sandy catchment of the lake Suhrer See are almost exclusively cultivated with winter crops, mainly with grains, on some fields in rotation with winter rape. The local farmers rely on agency recommendations for herbicide application (LWK-SH 2021). Most formulations recommended for autumn application with winter grain contain flufenacet, diflufenican, prosulfocarb, or pendimethalin (log K_{ow} between 3.5 and 5.4), about half thereof contain diflufenican. All four herbicides were detected by PDMS at the sampling sites.

The level of around 45 ng/L of dimethachlor at the site NE-1 might indicate a local application for winter rape cultivation. In the parallelly deployed Chemcatchers, the compound was found at all NE sites near limits of quantification, but only in the first sampling campaign in April which also points to a relationship to autumn application.

Terbuthylazine (TBZ) and metolachlor, both applied with corn and not locally, can be attributed to deposition only. The regularly detected terbutryn (TBT) is usually denoted as a biocide not approved for agricultural application, thus it would rather be expected in urban runoff. However, TBT can also be a metabolite of TBZ (CLH 2014, Lewis et al. 2016). Additionally, TBT is persistent under anoxic conditions (Muir & Yarechewski 1982). Therefore, it can be assumed, that TBT accumulates in sediments following subsurface discharge of TBZ and transformations in anaerobic sediment layers. CLH (2014) states, that the metabolite is more toxic than its mother substance, but considers the risk neglectable because TBT is not found in critical concentration in water containing TBZ and also not in sediments under water containing TBZ. The possibility, that TBT could accumulate in sediments when TBZ containing subsurface discharge passes through anaerobic layers, was not envisaged, the risk hence most likely underestimated. Moreover, TBT seems to be omnipresent, at least in Schleswig-Holstein, where it was found regularly far from biocide sources in other lake sediments (Machate et al. 2021) as well as in running waters (LLUR 2018).

Flufenacet was not detected at all sites in spring, where applied the autumn before. If detected, the concentrations were low, contrary to herbicides with higher log K_{ow}. Willkommen et al (2019) correspondingly found that subsurface losses of flufenacet responded quickly to application with high concentration peaks after extreme rainfalls in October 2017 and continued to respond to subsequent rain events on a low level over winter. By comparison, diflufenican and pendimethalin appeared with relatively low loads first, but steadily after every new rain event throughout the sampling campaign up to January. This timing supports a delay of subsurface transports of nonpolar compounds over winter and fits their presence in the lake littoral at the beginning of the vegetation period, i.e., at the time of highest vulnerability of SUM diaspores towards developmental inhibition. Conversely, the bulk of more polar compounds can be assumed to arrive earlier in winter in the littorals.

All in all, the passive sampler results prove the presence of locally applied and of deposited herbicides in the benthic boundary layer on lake sediments in 2 m depth with SUM losses in spring 2022 after extreme precipitation in February. The distribution patterns further suggest a subsurface transfer of applied and deposited herbicides alike from sub-catchments.

3.3 Chemical ecotoxicological risk assessment

3.3.1 Ecotoxicologically relevant compounds and properties

Sensitivity data for the inhibition of diaspore development in spring were missing. The evaluation was thus based on the nearest surrogate standard data sets of EC50 (green algae 72h, growth). The use of an assessment factor (AF) of 1000 on short-term toxicity data is recommended for the derivation of a protective threshold value (EC 2018, Escher et al. 2021). Measured concentrations of a compound to its EC50 or toxic units (TU) of 0.001 therefore have to be considered relevant. The verified risk or probability of adverse effects increases with the exceedance of this threshold for TU.

With a maximum detected concentration of 200 ng/l for DE-TBZ, compounds with EC50 over 1 mg/L yield TU below the threshold set by the AF of 1000. The fungicide tebuconazole and all metabolites except TBZ-DE were thus excluded from further consideration. On this base, the PDMS results comprised all potentially relevant compounds. Concentration ranges, EC50 and general information are listed in Table 1.

Table 1: Detected, ecotoxicologically relevant compounds with EC50 < 1 mg/L. Data were derived from PPDB, if not stated otherwise.

Compounds	properties					PDMS Results		
	log K_{ow}	Inhibition of	Crop or metabolite (MT)	DT50 [d]	EC50[1)] [µg/L]	$c_{w,\,min}$ [ng/L]	$c_{w,\,max}$ [ng/L]	detected n/8 sites
Dimethachlor	2.17	cell division	rape	3-30	6.5	4	70	5
TBZ-DE	2.30	photosynthesis	MT 1	54	140	57	218	8
Metolachlor[2)]	3.40	cell division	corn	20	57	12	22	8
Terbuthylazine	3.40	photosynthesis	corn	72	12	35	80	8

Flufenacet[3]	3.50	cell division	grain	20	2	3	10	2
Terbutryn[2]	3.66	photosynthesis	MT 26	52-74 (650)[4]	2.4	8	17	8
Diflufenican[3]	4.20	lipid synthesis	grain	65-95 (621)[5]	0.25	4	11	8
Prosulfocarb	4.48	lipid synthesis	grain	12	49	6	34	8
Pendimethalin[3]	5.40	cell division	grain	182	4	1	4	8

[1] EC50 green algae, 72h, growth; [2] not approved; [3] candidate for substitution; [4] Muir & Yarechewski 1982; [5] BVL Zulassungsbericht Falcon 2015

Three of the four nonpolar herbicides, that were mainly applied in autumn at local winter grain cultivation, are classified as "candidates for substitution" (CfS), according to the pesticides properties data base (PPDB). CfS fulfill two of three criteria for a classification as a persistent, bioaccumulative and toxic (PBT) substance and are subject to revocation by EC legislation (EU 2009, Annex II, 4). The detection on sediment surfaces falsifies the assumption of immobility of nonpolar compounds in soils, that underlies *per se* problematic approvals of formulations, that contain CfS like the very toxic diflufenican (BVL 2009).

The half-life time (DT50) stands for the risk of persistence. Sources besides standard data sets like PPDB (Lewis et al. 2016) may indicate a far higher persistence. Moreover, EC50 from different standard data sets may deviate within a range of one order of magnitude due to respective positions of test organisms in species sensitivity distributions. The consequences are discussed below in the context of risk verification and validation.

3.3.2 Local distribution of toxic units

Concentrations of detected compounds with similar modes of action related to their standard EC50 can be summarized to denote combined effects as they imply comparable acute effects (TU approach). For the assessment of chronic mixture toxicity, the same AF apply for summarized toxic units as for single substances (Backhaus & Faust 2012, EC 2018).

TBZ and metabolites inhibit photosynthesis. All other target compounds detected suppress developmental stages. The summarized TU for inhibition of developmental stages are in the range of 0.02 to 0.045. This was mainly due to diflufenican (from 75 to 97% over all exposure sites, when based on EC50 from PPDB, Figure 4). With the EC50 of 0.25 µg/L, TU around 0.02 to 0.03 were calculated for mean c_w per site. Dimethachlor and flufenacet contributed to the inhibition of developmental stages at NE-1 with a TU around 0.005 each. In addition, flucenacet was detected in the north bay with a TU near 0.001, i.e. still potentially relevant. Flufenacet was also applied at the field near the north bay the autumn before and is regularly used on fields above the NE-sites.

The potential for inhibition of photosynthesis was lower. The summarized TU of TBZ and its active metabolites were above 0.01 at NE-1 and 2 and below at N-1 and NE-2 mainly due to terbuthylazine and its metabolite terbutryn. Both contributed about equally to the summarized TU (fig.4)

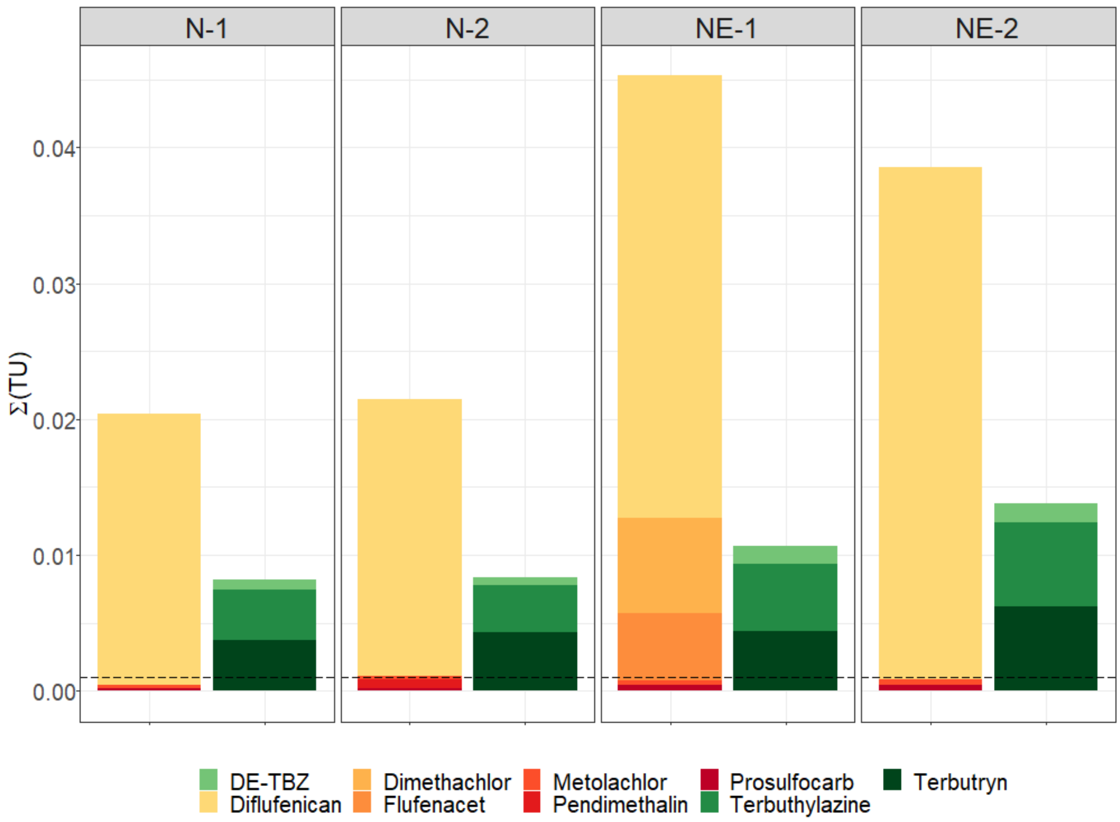


Figure 4: Summarized toxic units (cf. equation 4) with EC50 from PPDB for herbicides suppressing plant development (red/yellow columns) and herbicides inhibiting photosynthesis (green columns).

Different data bases may yield different lowest EC50 (green algae, 72h, growth). For instance, for diflufenican, 0.071 µg/L are denoted by the HSE risk report (2021) and 0.6 µg/L by ECHA (2019). The PPDB value lies in the middle of this range of the species sensitivity distribution. The sensitivity distribution of macrophyte diaspores may come close, especially for charophyte diaspores, judged by common properties (belonging to green algae and diaspore sizes). EC50 of macrophyte diaspores and their positions relative to the sensitivity distribution of standard test organisms are unknown, the mean value of PPDB can be considered as a best guess for congruence. Assuming, that the uncertainty of the real EC50 spans a range similar to the data base values, TU for diflufenican may range from 0.01 to 0.1, implying a high to even acute risk for adverse effects. The high error margin of diflufenican (due to the approximation of the K_{sw}) widens the uncertainty range further, indicating a likelihood for a still higher or lower risk.

So far, it has to be concluded from the TU approach: i) the detected herbicides are almost all relevant on base of TU around 0.001 or AF 1000 as threshold, ii) locally applied herbicides pose with AF between 50 and 25 a high risk for inhibition of plant development, diflufenican being the main driver, and iii) terbuthylazine, that is not locally applied, and its metabolite terbutryn, pose with AF around 100 a lower risk for photosynthesis inhibition.

The failure of the plant development observed in the field validates the high risk verified by the chemical risk assessment for herbicides with the fitting mode of action. But a contribution of other potential factors to the manifested biological effect cannot be excluded. An effect of photosynthesis inhibition in absence of green plants can be excluded. But switching from use of energy reserves in diaspores to photosynthesis is most likely a most vulnerable stage in the life cycle of SUM. In this stage, sensivities for photosynthetis inhibition should be higher than known for grown-up SUM. For angiosperms, EC50 near 10 to some 100 µg/l are reported (Lewis & Thursby 2018). In addition, uptake from sediments has been reported, for instance of terbutryn which is rather toxic (Turgut 2005). The concrete effect, that TBZ and terbutryn might provoke at the sediment surface, is thus unclear, but not neglectable with an AF around 100.

The recommendation of the high AF (EC 2018) pays tribute to the fact, that conclusions from acute chemical data to chronic toxicity and derived extrapolations used in risk verification are *per se* highly uncertain (Fent 2003, Van den Brink et al. 2008, Fairbrother et al. 2016, ECHA 2012). The recommendation (EC 2018) also integrates the experience that risk validations by monitoring data tend to indicate effects at lower concentrations than anticipated by prognostic extrapolations (Malaj et al 2014, Liess et al. 2019, Schäfer et al. 2019). The AF 1000 can thus be considered a feasible compromise. For concrete situations, the guideline (EC 2018) concedes the possibility of unusual risks for environmental effects, but requires a specification in such cases, especially when protection goals are at stake. Liess & Gröning (2024) demonstrated that "unusual risks" of ultra-low concentrations have in fact to be envisaged. For mayflies exposed to pulses of pyrethroid insecticides with a concentration of 1 ng/l, they found "that even at concentrations four orders of magnitude below the acute LC50, there was a pesticide-related mortality of 15 %."

In the given case, circumstantial evidences point in fact to additional risks missed by the TU approach. First of all, compounds with log $K_{ow} > 3$ (concerning most of the detected herbicides) generally tend to accumulate in sediments to a degree requiring more lines of evidence for deriving toxicity standards (sediment toxicity tests, aquatic toxicity tests in conjunction with equilibrium partitioning, bioassays with extracts from exposed passive samplers and field/mesocosm studies) (EC 2018, Chapman & Hollert 2006, Escher et al. 2021). The widely used pendimethalin was found at all four sites, barely reaching a TU of 0.001. But, its relatively large hydrophobicity (log $K_{ow} = 5.4$) implies a low partitioning to lake water in spite of a high presence and a very steep equilibrium gradient at the sediment interface. This poses a very high risk for time-cumulative toxicity at the sediment surface, due to increased binding of toxic molecules to enzymatic receptors (Escher et al. 2004, Liess & Gröning 2024, Sanchez-Bayo & Tennekes 2020), which is out of focus of the toxic units concept. With the very steep gradients in the diffusive boundary layer and diaspores with a size in the range of 0.5 mm lying in it, exposure concentrations may also systematically exceed concentrations around passive samplers floating near sediment surfaces. Discharge peaks of more mobile compounds might have been missed during the sampling in spring, because they peak rather shortly after application in autumn (Willkommen et al. 2019). However, short-term peaks can cause long lasting effects (Liess

& Beketov 2011). Especially wintergreen Chara meadows may be affected by such winter peaks.

Multiple effects beyond summarized TU are also out of focus of the chemical risk verification. Some herbicides potentially adding to the TU might have been missed for example by the measurements, though the target list was already adapted to main herbicides known to be applied locally. Combined effects of dissimilar acting chemicals are moreover known to be greatly underestimated by the TU approach (Shahid et al. 2019, Liess et al 2020). And there is an observation hinting to a potential combined effect, namely an abnormal occurrence of white instead red to black oogonia in late summer. The phenomenon could be due to photosynthetic inhibition leading to reduction of starch buildup in diaspores, thus decreasing their vitality and chance to develop in the next spring. Generally, organisms tend to reduce reproduction efforts under stress (Fent 2003). This tentative interpretation is of course speculative, but depicted to illustrate, that it cannot be excluded that a given TU sum might be more relevant in reality than anticipated, this or another unforeseen way. Indirect effects of xenobiotics besides herbicides are also not covered by TU, but important for regulations within the community associated with SUM (Jeppesen et al. 1998). For instance, bioturbation by chironomids is crucial especially for charophytes lacking aerenchyma (Wetzel 1975) and glyphosate, found in Suhrer See sediments (Machate et al. 2021), affects gut microbiomes of insects causing immunodeficiency (Motta et al. 2018). In addition, community effects alone like concurrence, in the case given between charophytes and angiosperm SUM, may increase sensitivities of more sensitive species by a factor of 10 (Liess et al. 2013).

Up to this point, the result consolidates the hypothesis, that subsurface transfers of herbicides from subcatchments affect SUM. The conclusion is already backed by the risk verification on grounds of TU, and further supported by additional risks in the given situation. Especially nonpolar herbicides applied for winter grain cultivation and the long-term exposure on lake sediments endorse an up-rating of risks. Extended passive sampling could contribute to clarification, best in connection with sediment toxicity testing by bio-analytical tools and extracts of exposed passive samplers (Escher et al. 2021).

Transparency with respect to uncertainties is key where the severity of manifested effects requires clarity about potential influences, especially with respect to the necessarily subjective component of every assessment (ECHA 2012). Following the guideline, a qualitative assessment is compiled in Table 2. The overview assembles sources of uncertainty, estimated tendencies towards an under- or overestimation and possibilities to reduce respective uncertainties. The uncertainty inferred by extrapolations to chronic toxicity and by properties of benthic boundary layers is assessed to infer a possible underestimation of risks spanning two orders of magnitude. The uncertainty for unknown elements like sensitivities of diaspores is estimated to be very high in either direction. Overall, the risk assessment on base of TU is more likely under- than overestimated, which at least confirms the likely contribution of detected herbicides to the SUM losses at the exposure sites in spring 2022.

Table 2: Qualitative assessment of uncertainties in passive sampling and toxicity evaluation. Impact on toxicity interpretation and possibilities of reducing uncertainty. A tendency to a higher or lower risk, infered by an influence is denoted by plus and minus signs, respectively. The number of signs denote the estimated order of magnitude. After ECHA (2012, tab. R.19-1, 19-3)

source of uncertainty		direction & magnitude	reduction of uncertainty
general	specific		
K_{sw} calibration	available: batch experiment sd 10% to 20%		
K_{sw} interpolation	from calibration line fit, range: -50% to +200%	+/-	Calibration
detection	gradient in diffusive boundary layer	++	exact positioning
	different seasons and SUM stages	+ + **/ - -**	extended sampling,
	undetected compounds,	+	suspect /nontarget screenings
source allocation	local application vs. deposition	+/-	information from farmers
hydrology	timing and spatial extent of contaminated LDG plumes, preferential pathways	++/--	extended sampling, hydrolog. modeling
acute effects	EC50 green algae, 72h	+/-	data base
	sensitivity of diaspores	++/--	testing!
chronic effects	time-cumulative, combination, trans-generational and community effects	++	mesocosms, higher tiers, validation

sediment toxicity	accumulation of relevant herbicides (log K_{ow}>3), long lasting exposure	++	bioassays with passive sampler extracts

The uncertainty of the assessment could be reduced in a next step by subsequent experimental approaches. Passive sampling could further play a pivotal role, especially after implementation of the experience gained with the novel and challenging exposure in diffusive boundary layers. An extended sampling over seasons and in other than worst case situations would be revealing, best with target lists adapted to information on pesticide application and with biotests with passive sampler extracts (Escher et al. 2021), if possible. A nontarget screening for agricultural xenobiotics would be rewarding to disclose potential unexpected and indirect effects.

The so far open question of potential additional or even other main causes, that may contribute to the observed SUM responses, has to be answered independently. This task requires an extended validation, that is a recurrence to a holistic retrospective assessment.

3.4 Integration of the passive sampler result into the ecological context – consistency and implications for nature protection

To cure fatalities like losses of endangered charophytes in a nature protection area, one needs to know the cause. Multiple lines of evidence led to the postulate of a causal relationship with subsurface transfers from agricultural areas to lake sediments, that is to the biotope where SUM develop. Passive sampling confirmed the presence of herbicides on sediment surfaces. The prognostic chemical assessment verified a possible inhibition of SUM development by applied herbicides and an unexpected, albeit lower and unclear risk by deposited herbicides. The in fact missed SUM development validates the verified risk to a certain extent. It can be concluded, that inhibition of developmental stages by herbicides contributes to the observed effect, but not to what extent.

3.4.1 Causal field and stress addition: Implementation of passive sampling results

Causal attribution allows to identify the probability of the contribution of a main stressor to a manifested effect and is the method of choice for complex systems. The base of the probability consideration is a conceptual flow chart assembling observations and interpretation by expert knowledge in a logical manner, including semi-quantitative, qualitative and uncertain information (Hamilton et al. 2015, SM 3.2). Monitored SUM stages can be interpreted as stress responses in a causal field that represents the current state of knowledge (fig. 5) and is based on ecological stressor concepts (Liess et al. 2016, Odum 1985, Perujo et al. 2021).

The passive sampler results strengthened pivotal postulates and added the new aspect of a potential relevance of herbicide deposition. The results obtained in a worst case situation allow generalisations that can be integrated to local stress profiles in other situations and checked for plausibility with monitoring data (see below). Counterchecks with the whole lake experience enable an extended risk validation and outline of research and mitigation priorities.

First, it is to infer from the passive sampler results, that herbicides can be transported from subcatchments to sediments all around the lake. Second, application of herbicides in any subcatchment can be considered bearing a risk high enough to contribute to SUM losses in phases of high discharge. The question, to what extent, should thereby kept in mind. Likewise, a low risk for unspecified deposition effects can be assumed for sites without agricultural subcatchments in phases of high subsurface discharge. Third, phases with no discharge should imply no herbicide stress, even at sites with agricultural subcatchments.

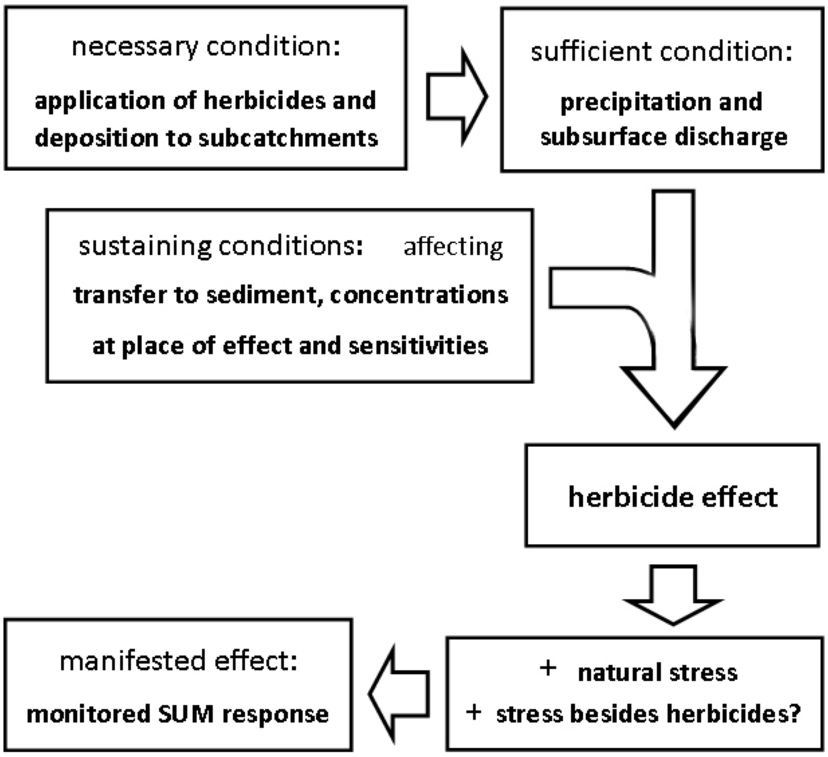


Figure 5: Conceptual flow chart and causal field for observed SUM responses. The terms "necessary, sufficient and sustaining conditions" highlight the use of formal logic. The concept of sustaining conditions, introduced to address causation in situations where cause and effect are distant in place and time (Minnerop & Otto 2020), is a key for causal attribution in complex local situations.

3.4.2 Plausibility of causation: matching site-specific stress profiles with known SUM responses in a whole lake context

SUM respond to all components of stress in a given situation. Most elements of man-made stress known to affect SUM in other lakes (fishery, boating, eutrophication, urban runoff, shore modifications) can be excluded for the virtually pristine lake Suhrer See. In 1991, Frenzel still found charophytes all around the lake except for one site with an exceptional steep underwater slope (30%, fig. SM-8) with *Potamogeton perfoliatus* only (Frenzel

1992). For the wide flats in the North, a few, later vanished *Chara tomentosa,* a typical species of wintergreen *Chara* meadows, were mentioned. The natural stress level is thus graded depending on locally prevailing underwater slopes and is correspondingly inserted into the stress profiles in table 3. If natural and herbicide stress are the main relevant stress components in a given situation, their sum should be comparable to the level of ecological stress indicated by SUM responses.

The summers 1997 and 2017 were both extremely wet (cf. table SM-2). The SUM responses (table 3, upper part) show a historical overall increase in man-made stress and the summation of natural stress and herbicide stress is plausible as reason at all locations. An unknown stress cannot be excluded, but there is no cogent necessity for one.

Table 3: *Local stress profiles and plausibility with respect to manifested, gradual stress responses of SUM (A wintergreen Chara meadows, B summer only Chara, C angiosperm SUM, D devastation). Herbicide stress was inserted as being high or low at sites with or without agricultural subcatchments, respectively, and at times of high subsurface discharge (following the prognostic assessment on base of the passive sampling results).*

Historical increase of stress levels Comparison of local SUM responses in two extremely wet summers.				
sites	**1991**[a]	assessed stress profiles		**2017**[b]
	SUM	natural stress	herbicide stress	SUM
north bay	A	very low	low	A
NE-2	B	low	high	D
else	B	low to mean	no agricult.: low agricult.: high	C D
site with steep slope	C	high	high	D
Effects of deposited herbicides[c] Comparison of SUM responses in summer 2019 (dry) and summer 2021 (wet)				
sites	**2019** dry	assessed stress profiles		**2021** wet
	SUM	natural stress	herbicide stress	SUM
3 northern transects	B	very low to low	low	B to C
3 southern transects	B to C	low to mean	low	C
[a]after Frenzel (1992)	[b] data from Krambeck 2022	[c]after Ianaplan (WFD reports)		

The lower part of table 3 reveals the effect of deposited herbicides, since none of the six transects monitored are influenced by discharge from agricultural subcatchments. The data were adopted from Ianaplan (WFD reports) and translated into stress response stages (for original coverage grades see table SM-3). There is a tendency to switch to a state C under the influence of an additional low stress. In general, charophytes are widely replaced in southern parts of the lake by a small-leaved *Potamogeton* (fig. SM-9) and tend to disappear in wet summers at sites where they are still found in dry summers.

So far it can be concluded, that, if there should be some missed potential stressor besides herbicides it has to be bound to discharge as well (Hill´s criterion No. 1b, cf. SM 3.1). Acidification might be thinkable because it could modify the mobility of compounds in weakly buffered soils and entail toxic effects. Given the contemporary atmospheric deposition of ammonia from livestock rearing (Bittersohl et al. 2014), acidification should be kept in mind as a potential sustaining factor influencing risks by subsurface discharge.

However, the north bay experience (SM 1.2) is a strong independent line of evidence for herbicides being a main stressor, because SUM responded in a remarkably prompt and logical manner to changes in herbicide application (SM 1.2): A chemical removal of fallow vegetation on a nearby field (fig. SM-2) in autumn 2019 was followed by an extraordinary decay of the before wintergreen *Chara* meadow in the whole bay (fig. SM-3 and -4). In autumn 2020, an interculture was sowed instead of herbicide application. SUM partly recovered in 2021, but with an appearance of *Potamogeton crispus,* a disturbance indicator never noted before, in the inner part of the bay. That was especially alarming, because a residual stand of *Chara subspinosa,* a rare species and indicator of very good conditions, prospered up to 2019 at the same site (fig. SM-5 and -6). After application of flufenacet, diflufenican and chlortoluron on the critical field in autumn 2021, the inner and western parts of the bay stayed bare up to July 2022. The spatial patterns of SUM responses in the north bay hint to an arrangement of different subsurface discharges from the surroundings (fig. SM-5). The indicative value of SUM for groundwater entrances to lakes has long been recognized (Rosenberry et al. 2000).

One type of disturbance observed in front of the north and northwest bay cannot be subsumed in the causal field valid for the other monitored SUM losses. Sometimes, zones of one to forty meter diameter with sulphur fog appear in summer within vital *Chara* meadows, followed by decay and bare ground, recolonized later and firstly by angiosperms like *Ceratophyllum submersum*. The phenomenon is not understood. It might be related to a disturbance of biogeochemical sulphur cycles and of bioturbation normally oxygenating sediments (Jørgensen et al. 2019, Hupfer et al. 2019). The observed decay might be due to excess hydrogen sulphide being toxic, especially for charophytes lacking aerenchyma. The occurrence of dead zones in transition to deeper water suggests a connection with entrances of groundwater. A causal relation to acidification might be possible via mobilisation of aluminium sulphate in soils and transport to receiving waters (Bittersohl et al. 2016).

The northeast shore experience (SM 1.3) reveals small scale variations, comparably lower influence of surface discharge and a failure of a mitigation measure. The passive sampling result of similar TU at NE-1 and NE-2 is revealing in two respects. First, the different proximity to two short drain creeks is irrelevant (cf. fig. SM-7). Second, the SUM response indicates higher stress at NE-1 (permanent D) than at NE-1 (C-D in dependence of precipitation). The explication is a steady narrowing of the littoral from NE-2 towards NE-1 and a resulting higher natural stress at NE-1. Frenzel found a corresponding decrease of *Chara* sp. in 1991, from a mass occurrence at NE-2 to a mere presence at NE-1.

The contemporary SUM response to the creek inlet in between NE-1 and NE-2 is remarkable. A dense stand of angiosperms is found there in normal summers that almost mirrors the creek delta and with a sharp transition especially to the permanently bare ground towards NE-1. In front of the creek delta, devastation occurs at very high discharge only. The creek in fact delivers herbicides. For instance, diflufenican concentrations ranged from 13 to 23 ng/l in autumn 2020 (Höinghaus 2022). The dilution to lake water seems to confine effects to the immediate vicinity of the inflow. The turbulent inflow should also destroy otherwise high and stable partitioning gradients in diffusive boundary layers. A clay lens at the creek inlet might have blocked some subsurface discharge in addition.

The creek on the other side of NE-1 drains a depression within an area taken out of production since 2019. The mitigation measure worked insofar as mainly uncritical metabolites were still found in the outlet in autumn 2020 (Höinghaus 2022). But it failed with respect to the intention to cure the permanent loss of SUM. The equally high and relevant herbicide concentrations at NE-1 and NE-2 confirmed the after all self-evident explanation, that herbicide contaminated discharge from the surrounding subcatchment of around 30 ha must have passed underneath the 3 ha buffer zone installed.

3.4.3 Resulting options for biodiversity protection and evaluation by means of ecotoxicological indication

The example shows, that the protection of SUM against subsurface contamination requires longer-ranging measures than existing and familiar for protection of aquatic biota against surface runoff. Mitigation of atmospheric deposition is even further out of reach. But the so far assembled knowledge yields some starting points and priorities for concrete measures and pragmatic next steps. First, mitigation should not start at the worst but at the still best site, that is the north bay in this case. To reduce the stress in the north bay to a level on which a chance for a recurrence of rare charophytes is in sight, should be feasible. Not to apply the main risk driver diflufenican on the critical field could already suffice and would be worth a try. Responses of the SUM to what will be cultivated next should be anyway monitored, best in connection with information on applied herbicides. Experience with real environmental effects of herbicide application are of general interest and should be gained under different precipitation regimes if possible. The actual drone supported monitoring (current project on honorary base) should thus merge into a long-term routine.

To see and recognize, what is going right or wrong on the long run, is a most important precondition for further understanding and mitigation. But, current WFD routines are focused on eutrophication risks (Poikane et al.2020), and the German routine for SUM

(Schaumburg 2014) is also not designed to cope with SUM losses in clear lakes. Nevertheless, the data collection pays attention to charophytes, that is to the most sensitive group, and thereby allows ecotoxicologically meaningful interpretations (for instance in context with the north- south gradient, cf. table SM-3). In detail, the assembled data are of high interest, but would need a little upscaling for ecotoxicological indication.

4 Conclusion

Herbicides are present on lake sediments in ecotoxicological relevant concentrations. All lines of evidence point to an origin by application and deposition in subcatchments, followed by subsurface discharge and eventual accumulation in sediments, especially of nonpolar herbicides.

The result challenges current paradigms and concepts of risk assessment and environmental protection: i) Ecotoxicological effects cannot be excluded in lakes due to dilution to non-detectabilty (LLUR 2018). ii) WFD indices for eutrophication and risk assessment routines fail (Brown et al. 2016, Poikane et al 2020, Schaumburg et al. 2014, Weisner et al 2022). iii) Assumptions underlying approval routines for the detected risk drivers diflufenican and terbuthylazine (immobility in soils and no accumulation, cf. chapt. 3.2) are falsified. iv) Distances of fields 100m from shore and the ineffectiveness of taking a partial area out of production further render current buffer zone concepts inefficient. Subsidy elements like buffer stripes hence fall too short for mitigation, like in many other nature conservation areas where xenobiotics are omnipresent too (Wolfram et al. 2023).

For the time being, it remains to pragmatically concentrate efforts on sites with the best chances for a comeback of endangered species and to collect further experience with SUM responses to ecotoxicological stress. An extended application of passive sampling in other seasons and instances of lower stress responses of SUM could reduce uncertainties, best in combination with biotests with passive sampler extracts (Escher et al. 2021). Moreover, investigations of sediment processes and hydrological influences could further consolidate the causal field.

The holistic consideration of plausibility and probability of causation (see SM 3) turned out to be a workable approach to risk validation and to pragmatic recommendations for the protection of endangered species. The example shows: Biodiversity protection has to begin *ex post*, i.e., with recognition of biological indications for man-made stress, followed by an interplay between i) probabilistic causal attribution, that enables to pose the right questions, ii) answering the arising key questions by traditional mechanistic methods and iii) trying and evaluating measures on the base of the available state of knowledge.

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